PRODUCTION OF RECOMBINANT *ACINETOBACTER BAYLYI* RNA POLYMERASE HOLOENZYME AND ITS APPLICATION TO THE INVESTIGATION OF BACTERIAL TRANSCRIPTIONAL REGULATION OF SULFUR ASSIMILATION

by

JULIANA CALIL BRONDANI

(Under the Direction of Cory Momany)

ABSTRACT

The investigation of complex regulatory networks through *in vitro* transcription assays is critical to expand our understanding of genome expression and metabolism regulation in prokaryotes. However, the execution of such a project is dependent on the availability of the RNA polymerase holoenzyme ($RNAp^{holo}$), an essential element of transcription. Ideally, the $RNAp^{holo}$ applied to structural and/or biochemical assays should be obtained from the species being studied or, alternatively, from the closest available organism. *Acinetobacter baylyi* ADP1 is a non-pathogenic, easily cultured and genetically- manipulated bacterium with a vast repertoire of metabolic enzymes that make it a compelling model organism for the study of bacterial metabolism. Before this dissertation, no $RNAp^{holo}$ from *A. baylyi* ADP1 or from any other *Acinetobacter* species was recombinantly expressed and purified, thus limiting transcription studies. Here, we developed a method for the expression and purification of *A. baylyi* ADP1 $RNAp^{holo}$ that provided high yields of a highly pure and active enzyme that can be applied to regulatory studies. In this regard, the *A. baylyi* ADP1 $RNAp^{holo}$ was essential for

conducting experiments that characterized the biological function of ACIAD0746, a LysR-type transcriptional regulator (LTTR) from *A. baylyi*, and for identifying a second class of transcriptional regulators involved in the assimilation of sulfur. Aerobic bacteria rely on the assimilation of sulfur to make cysteine, which serves as a reservoir of bioavailable sulfur to be used in the synthesis of critical S-containing cell components, such as iron-sulfur clusters, siderophores, and co-factors. The transcriptional regulation of sulfur metabolism in prokaryotes has long been associated with the LTTRs CysB and Cbl, two proteins that are mainly found in *Betaproteobacteria* and *Gammaproteobacteria*. In this dissertation, the combination of X-ray crystallographic, transcriptional assays, and other supportive experiments revealed the existence of another set of proteins that participate in the metabolism of sulfur and are more diversely distributed in bacteria.

INDEX WORDS: Transcription, Gene expression, RNA polymerase, LysR-type transcriptional regulators, Sulfur assimilation, Cysteine biosynthesis.

PRODUCTION OF RECOMBINANT *ACINETOBACTER BAYLYI* RNA POLYMERASE HOLOENZYME AND ITS APPLICATION TO THE INVESTIGATION OF BACTERIAL TRANSCRIPTIONAL REGULATION OF SULFUR ASSIMILATION

by

JULIANA CALIL BRONDANI

B.S., Federal University of Santa Maria, Brazil, 2014

M.S., Federal University of Santa Maria, Brazil, 2016

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2023

© 2023

Juliana Calil Brondani

All Rights Reserved

PRODUCTION OF RECOMBINANT *ACINETOBACTER BAYLYI* RNA POLYMERASE HOLOENZYME AND ITS APPLICATION TO THE INVESTIGATION OF BACTERIAL TRANSCRIPTIONAL REGULATION OF SULFUR ASSIMILATION

by

JULIANA CALIL BRONDANI

Major Professor:	Cory Momany
Committee:	May Xiong
	J. Warren Beach
	Nicolle Lima Barbieri

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
May 2023

ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr. Cory Momany for the opportunity to be a member of his laboratory and for supporting me over the years. I also thank my committee members, Dr. May Xiong, Dr. Warren Beach, and Dr. Nicolle Barbieri, and the many individuals in the Department of Pharmaceutical and Biomedical Sciences that assisted me throughout my PhD studies. Finally, I thank my family and friends for the many roles they played in helping me complete my PhD.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS iv

LIST OF TABLES vii

LIST OF FIGURES viii

CHAPTER

1 INTRODUCTION AND LITERATURE REVIEW 1

1.1 Sulfur: An Essential Element of Life 1

1.2 Sulfur Metabolism in Bacteria 3

1.3 Transcription in Bacteria 9

1.4 LysR-Type Transcriptional Regulators 13

1.5 Introduction of Study 16

2 OVERPRODUCTION, PURIFICATION, AND TRANSCRIPTIONAL ACTIVITY OF RECOMBINANT *ACINETOBACTER BAYLYI* ADP1 RNA POLYMERASE HOLOENZYME 18

2.1 Abstract 19

2.2 Introduction 20

2.3 Materials and Methods 22

2.4 Results and Discussion 34

2.5 Conclusion 44

3 A SECOND CLASS OF BACTERIAL TRANSCRIPTIONAL REGULATORS INVOLVED IN SULFUR ASSIMILATION 45

3.1 Summary 46

3.2 Introduction 47

3.3 Results 50

3.4 Discussion 64

3.5 Figures 67

3.6 Star Methods 80

4 CONCLUSIONS 90

5 REFERENCES 96

APPENDICES

A Chapter 2 110

B Chapter 3 167

LIST OF TABLES

Page

Table 2.1: Bacterial strains and plasmids used in this work. 23

Table 2.2: Oligonucleotides used in building plasmids. 24

Table 2.3: Quantification and purity of $RNAp^{holo}$ at the different chromat.steps 36

Table A2.1: Oligonucleotides used for sequencing plasmids 110

Table A2.2: Alpha subunit query coverage, percent identity, and accession id 111

Table A2.3: Beta subunit query coverage, percent identity, and accession id 113

Table A2.4: Beta' subunit query coverage, percent identity, and accession id 114

Table A2.5: Sigma70 subunit query coverage, percent identity, and accession id 116

Table A2.6: Omega subunit query coverage, percent identity, and accession id 118

Table A3.1: Crystallographic statistics 167

Table A3.2: Representative orthologs in different bacterial phyla. 169

Table A3.3: Phyla in which orthologs are absent. 171

Table A3.4: Measurement of ACIAD0746- and YeiE sulfite affinity through Isothermal titration calorimetry (ITC). 173

Table A3.5: ACIAD0746 transcriptional regulation at different promoters associated with sulfur metabolism. 173

Table A3.6: Affinity of ACIAD0746 for promoters associated with sulfur metabolism. . 174

Table A3.7: Key resources table. 175

Table A3.8: Oligonucleotides. 178

LIST OF FIGURES

Page

Figure 1.1: Assimilatory sulfur pathway that leads to the production of L-cysteine 5

Figure 1.2: Basic bacterial RNA polymerase structure 11

Figure 1.3: Basic structure of LysR-type transcriptional regulators 15

Figure 2.1: Vectors built for $RNAp^{holo}$ expression 27

Figure 2.2: Protein purification approach 29

Figure 2.3: SDS Page gel showing the five subunits of *A. baylyi* ADP1 $RNAp^{holo}$ after each chromatographic separation step 38

Figure 2.4: *In-vitro* transcriptional studies 40

Figure 3.1: Crystal structures of the full-length ACIAD0746 from *A. baylyi* ADP1 and the effector binding domain of *E. coli*'s YeiE. 67

Figure 3.2: Sequence alignment of ACIAD0746 orthologs from different phyla. 69

Figure 3.3: Common genome arrangements of ACIAD0746 orthologs. 77

Figure 3.4: ACIAD0746, YeiE, and FinR functional studies. 78

Figure 3.5: FinR classic "sliding dimer" model of regulation. 79

Figure A2.1: Cloning strategy for plasmid pJB4 120

Figure A2.2: Cloning strategy for plasmid pDAP39 121

Figure A2.3: Sequence alignment of Alpha subunit (RpoA) 122

Figure A2.4: Sequence alignment of Beta subunit (RpoB) 126

Figure A2.5: Sequence alignment of Beta' subunit (RpoC) 140

Figure A2.6: Sequence alignment of Sigma70 subunit (RpoD) 159

Figure A2.7: Sequence alignment of Omega subunit (RpoZ) 166

Figure A3.1: ITC analysis of ACIAD0746 with several intermediates in the sulfur assimilation pathway. 180

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Sulfur: An Essential Element of Life

Sulfur is one of the most abundant elements on Earth, required for the synthesis of several biological compounds and commonly found in the atmosphere, oceans, plants, microorganisms, and animals. The presence of sulfur in two proteinogenic amino acids (cysteine and methionine) makes it an essential element to all forms of life, with critical involvement in a vast array of physiological processes [1], as discussed below.

Cysteine is involved in the maintenance of a protein's tertiary and quaternary structures. Due to the strong acidic nature of its thiols, cysteine forms a thiolate anion. In doing so, it forms inter- and intramolecular disulfide bridges with other cysteine residues, providing proteins with a better stability to thrive upon exposure to unfavorable settings [2]. Cysteine is also central in bacterial physiology by serving as a storage pool for bioavailable sulfur that is initially trafficked within the cells by cysteine desulfurase. Cysteine desulfurase can remove sulfur from cysteine for incorporation into the biosynthetic pathways of fundamental S-containing compounds, including iron-sulfur clusters, siderophores, and several co-factors (e.g., biotin, thiamine, lipoic acid, coenzyme A) [3, 4]. Moreover, cysteine can be condensed with O-succinylhomoserine to produce cystathionine, ultimately ending in the synthesis of methionine [5].

Iron-sulfur clusters act as sensors of the environment that enable organisms to adapt to different conditions. These clusters participate in a variety of metabolic reactions, regulate the expression of certain virulence genes, and directly link bacterial sulfur metabolism with survival due to their role as oxidative stress sensors [6]. For instance, the iron-sulfur cluster present in the transcription factor SoxR senses reactive species and leads to the up-regulation of the expression of superoxide dismutase, an antioxidant enzyme that works to mitigate oxidative damage [7, 8]. During bacterial infection, one of the host strategies to neutralize the invader is to minimize the circulating iron in the bloodstream, the so-called “nutritional immunity” [9]. The scant availability of iron ends up hampering iron-sulfur clusters production, thus attenuating the microbe’s pathogenicity, and increasing its sensitivity to the oxidative attack of macrophages [10]. In response, microorganisms increase the number of siderophores, small organic S-containing molecules secreted in the surrounding environment to scavenge iron. The iron-siderophore complex is then transported into the cell through specific cell receptors [11, 12]. Moreover, S-containing co-factors directly affect major metabolic and catabolic pathways. For example, biotin (vitamin B7) is involved with the synthesis of amino acids and fatty acids applied to cell membranes and virulence factors; Thiamine (vitamin B1) participates in carbohydrate metabolism to generate energy and in the biosynthesis of precursors for cellular macromolecules; Lipoic acid and coenzyme A are related to the catabolism of amino acids and fatty acids, respectively [13-16].

Methionine is the translation start site in nearly all eukaryotic proteins, while N-formylmethionine serves the same function in prokaryotes [17]. Most Met residues are

found in the internal hydrophobic core of globular proteins (such as enzymes, hormones, immunoglobulins) or interacting with the lipid bilayer in transmembrane proteins, thus helping in the transportation of ions and small molecules across biological membranes [1]. Also, surface-exposed methionine residues can protect the active site of proteins by behaving as an endogenous antioxidant due to its oxidation susceptibility [18]. During methionine metabolism, an important sulfur-containing intermediate is produced, S-adenosylmethionine (SAM). This compound is the major methyl donor in reactions catalyzed by methyltransferases that modify nucleic acids, proteins, and lipids. [19, 20].

1.2 Sulfur Metabolism in Bacteria

Several studies have demonstrated the capacity of bacteria to acquire S-amino acids directly from the environment. In *Bacillus subtilis*, for example, the YtmJK and MetQ transporters respectively introduce L-cystine and L-methionine [21, 22], as do homologues identified in *Lactococcus lactis* [23]. In *Listeria monocytogenes*, TcyKLMN works as a cystine/cysteine importer [24]. Methionine uptake transporters (MUT) are also verified in *Escherichia coli* and *Salmonella typhimurium* [25]. However, the efficacy of these transporters varies depending on the ecological niche. Pathogens may have easy access to these compounds when infecting their hosts, but soil and limnic organisms have limited accessibility to resources and need to synthesize their own S-amino acids utilizing the available sulfur [26].

In aerobic environments, inorganic sulfur is found in oxidized forms that must be reduced before incorporation into biological molecules. Two routes exist for bacteria to use sulfur; As shown in **Figure 1.1**, the assimilatory sulfur pathway involves a series of reduction steps that converts oxidized sulfur into L-cysteine, which serves as a reservoir of reduced sulfur for the synthesis of other S-containing molecules. Alternatively, the dissimilatory sulfur pathway uses oxidized sulfur as the terminal electron acceptor for energy generation [27]. This late pathway arose from the assimilatory route and is restricted to the anaerobic organisms [28].

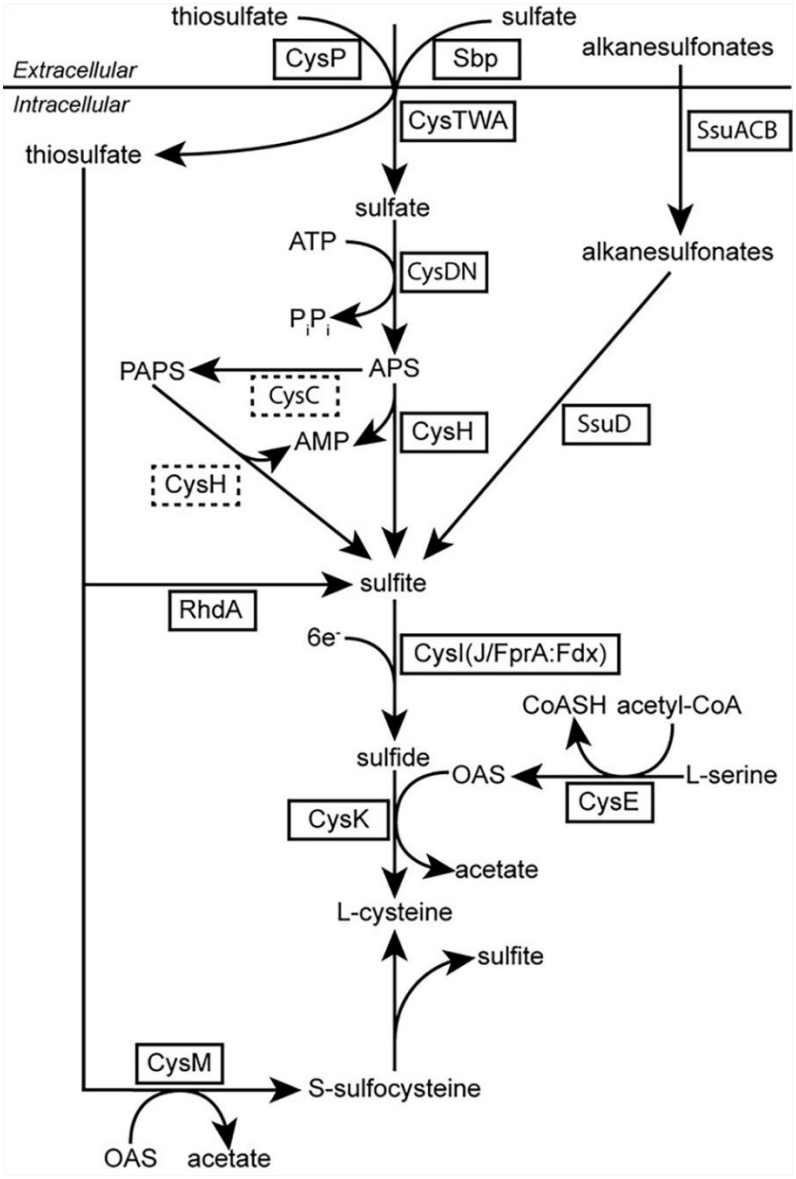


Figure 1.1. Assimilatory sulfur pathway that leads to the production of L-cysteine.

Cysteine biosynthesis starts with the cellular uptake of oxidized sulfur. Sulfate and thiosulfate, the preferential substrates for assimilatory sulfur reduction, are brought into the cells by the same carriers. This is mostly accomplished via an ABC-type transporter comprised of CysUWA coupled with Sbp or CysP. CysU (also called CysT)

and CysW form a membrane channel for the passage of sulfate and thiosulfate, while CysA is the cytoplasmic ATPase subunit responsible for the hydrolysis of ATP. Sbp and CysP are periplasmic substrate-binding proteins with overlapping functions that interact with membrane proteins, sulfate, and thiosulfate [29]. When the CysUWA + Sbp/CysP system is absent, a proton:sulfate symporter part of the SulP family that is related to plant transporters responsible for sulfate uptake can be used [30]. In *E. coli*, another sulfate transporter called CysZ is reported [31]. Once inside the cell, sulfate is combined with ATP to form adenosine 5'-phosphosulfate (APS) by the enzyme complex CysDN (ATP sulfurylase). In some species, such as *E. coli* and *Salmonella enterica*, APS is further phosphorylated to create adenosine 3'-phosphate 5'-phosphosulfate (PAPS) by the adenylyl-sulfate kinase CysC. Plants and some bacteria bypass the formation of PAPS. Either APS or PAPS are reduced by CysH to sulfite with a thioredoxin serving as the electron donor, releasing either adenosine-3',5'-bisphosphate or adenosine-5'phosphate in the process [32]. Structural differences are observed in CysH depending on the substrate it recognizes [33].

Sulfite is then reduced to sulfide through the sulfite reductase CysI + CysJ or Fpr. CysJ is a oxidoreductase flavoprotein that transfers electrons from NADPH to the siroheme contained in CysI, resulting in a six-electron transfer event that reduces sulfite to sulfide [34]. Plants do not have CysJ and rely on a ferredoxin reductase (Fpr) to transfer the electrons from NADPH to CysI [35]. Similarly, some bacteria such as species from the *Actinomycetales* and *Pseudomonadales* orders lack clear orthologs of CysJ and use a ferredoxin to facilitate the electrons transfer [36, 37]. *Acinetobacter baylyi* ADP1, for example, contain a gene annotated as *cysJ* (*aciad0799*). However,

besides being genomically located far from any other gene involved in sulfur assimilation, no orthologs of *aciad0799* are found in bacteria with characterized CysJ. Further, the gene's deletion does not lead to cysteine auxotrophy, indicating its noncorrelation with cysteine biosynthesis [38]. Two ferredoxin paralogs are present in *A. baylyi*, *fpr* and *fpr2*, which are potentially providing the electrons necessary for sulfite reduction in this organism.

Finally, sulfide is combined with O-acetyl-L-serine (OAS) to produce cysteine by the enzyme CysK. OAS is formed through the condensation of an acetyl group from acetyl CoA with the hydroxyl group of serine, in a reaction catalyzed by CysE (serine transacetylase) [39]. Thiosulfate can be combined directly with OAS by the enzyme CysM resulting in the formation of S-sulfocysteine, which is then reduced to cysteine, thereby bypassing the sulfate reduction steps [40]. Under sulfate starvation conditions, some species upregulate additional transporters to scavenge alternative sulfur sources from the environment, including sulfonates and sulfate esters. Both *E. coli* and *A. baylyi* ADP1 contain *ssuABC* homologues that fulfill this role. While *ssuBC* code for membrane ATP-binding proteins, *ssuA* codes for a periplasmic binding protein. Once inside the cell, these compounds are desulfonated by the alkanesulfonate monooxygenase SsuD, resulting in the release of sulfite that enters the cysteine biosynthesis pathway [41, 42]. Of note, *A. baylyi* ADP1 does not have CysZ (sulfate transporter) and CysC (adenylyl-sulfate kinase that forms PAPS). Also, a ferredoxin reductase (instead of CysJ) is potentially mediating the electrons transfer from NADPH to the siroheme in CysI.

All the steps described above that culminate with the synthesis of cysteine are extensively energy consuming. The oxidized sources of sulfur (sulfate, thiosulfate,

sulfonates) are strong acids completely ionized at physiological pH; therefore, their cellular uptake does not happen through passive diffusion and requires active transportation [43]. The subsequent reduction steps are also energy costly and need a tight regulation. The regulation of the genes involved in sulfur metabolism occurs by two interdependent mechanisms: 1) post-translational regulation happens through cysteine feedback inhibition of serine transacetylase (CysE), the protein responsible for generation of OAS. In the absence of OAS to combine with sulfide, cysteine is not made. However, this mechanism acts only in the last step of the cysteine biosynthetic pathway and must be complemented with 2) transcriptional regulation to control the previous steps. The transcriptional regulation of cysteine biosynthesis is well-characterized in *Gammaproteobacteria*, which rely on CysB and Cbl, two LysR-type transcriptional regulators (LTTRs) [44].

CysB has been reported to work as a sensor for the intracellular levels of cysteine biosynthesis intermediates [45, 46]. In *Enterobacteriaceae*, CysB responds to the inducer N-acetylserine (NAS), a constitutional isomer that spontaneously forms from OAS. On the other hand, sulfide and thiosulfate act as anti-inducers that keep the protein in its inactive state. Therefore, the intracellular levels of inducers and anti-inducers affect the functional state of CysB. Under sulfur limiting conditions, the lack of sulfide leads to the OAS accumulation and its isomerization into NAS, indicating that the cell is starved for sulfur. Then, NAS binds to CysB and activates the protein to up-regulate the transcription of the genes involved in sulfate transport and assimilation [47]. Cbl is a CysB-like LTTR that acts in concert with CysB to activate expression of genes involved in organosulfur metabolism [48]. In *E. coli*, this protein senses intracellular

levels of APS, which acts as an inhibitor. In the absence of APS, Cbl activates the transcription of the *ssu* and *tau* gene clusters to scavenge sulfur from alternative sources [49]. However, the genes encoding CysB and Cbl are absent from most bacteria, indicating the existence of another unidentified mechanism that works at the transcriptional level to fill this gap [50].

1.3 Transcription in Bacteria

As stated in the central dogma of molecular biology, the genetic information encoded in DNA is transcribed to mRNA by RNA polymerases (RNAp) before translation into proteins. Transcription in bacteria relies on a single multi-subunit DNA-dependent RNAp that dictates gene expression in individual living cells [51].

In terms of structure, the RNAp core complex (~400 kDa) (**Figure 1.2A**) is composed of five subunits encoded by four genes: β (*rpoB*), β' (*rpoC*), two copies of α (*rpoA*), and ω (*rpoZ*) containing approximately 151, 155, 37 (each), and 10 kDa, respectively [52, 53]. The bulk of the structure is made up by the β and β’ subunits that form the catalytic center for RNA synthesis with the protein’s active site found in their interface. The α subunits form a dimer at the periphery of the structure that is responsible for the assembly of the complex and for regulatory interactions. The ω subunit’s function is not fully understood, but studies have suggested its involvement with the structural stabilization of the β' subunit [54-56]. Moreover, the presence of channels supports the functioning of the protein. The downstream DNA channel directs dsDNA into the catalytic center, where a template channel guides the template DNA into

the active site and a non-template channel moves the non-template DNA strand away from the active site. Once the transcript separates from the RNA-DNA hybrid, it is extruded from the complex through the RNA exit channel. Also, the nucleoside 5′-triphosphate necessary for transcription enters the complex via a secondary channel [57].

The core RNAp (ββ'αω) is catalytically active but lacks specificity since it can only initiate transcription on nicked dsDNA and from DNA ends. This form of the enzyme is incapable of recognizing promoters and, therefore, only synthesizes RNA from non-specific DNA sequences. In order to initiate transcription at specific sites (e.g., target gene), the core enzyme must bind to a sigma factor to form the holoenzyme (ββ'αωσ) that can recognize promoters (**Figure 1.2B**) [58]. Multiple sigma factors, including σ70 (*rpoD*), σ32 (*rpoH*), σ38 (*rpoS*), and σ54 (*rpoN*), are encoded by bacterial cells and utilized under different environmental conditions [59]. While σ70 is a housekeeper that promotes the transcription of most of the genome, other sigma factors dictate the expression of specialized gene sets required for coping with stressful conditions [60]. For example, σ32 (heat shock protein) is present under exposure to elevated temperatures [61]. The σ70 protein contains four domains ($\sigma70_1$, $\sigma70_2$, $\sigma70_3$, and $\sigma70_4$) that interact with specific elements of promoters. The -10 element and the transcription start site (TSS) are always found on bacterial promoters and interact with $\sigma70_2$. The -35 element and the -35 to -10 spacing are usually present and interact with $\sigma70_4$. Of less common frequency are the extended -10 element that interacts with $\sigma70_3$, and the discriminator element that relates to $\sigma70_1$. The UP element is recognized by the C-terminal domain (CTD) of the RNAp α subunit (αCTD) [62]. Different promoters have

distinct elements that modulate the frequency of RNAp recruitment, since it is the combination of these interactions that act to localize the enzyme [63, 64].

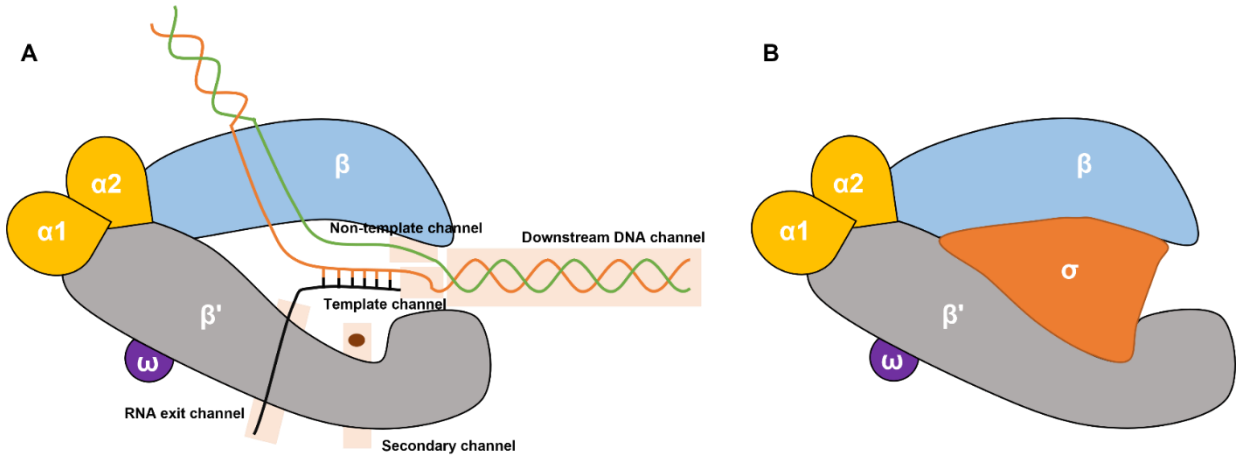


Figure 1.2. Basic bacterial RNA polymerase structure. **(A)** Core enzyme containing channels that support the functioning of the protein, as well as the β, β', α, and ω subunits. **(B)** Holoenzyme containing the basic core architecture plus the σ subunit.

The bacterial mechanism of transcription can be divided into three steps: initiation, elongation, and termination. The initiation step can be broken down further into 4 stages: 1) Closed Complex Formation involves the recruitment of the RNAp holoenzyme mediated by the aforementioned interactions between sigma factors and elements of the promoter. 2) Open Complex Formation is characterized by the initial melting and unwinding of the dsDNA, separating the template and the non-template strands. Unlike DNA replication, this process is not driven by helicases, but by changes in the RNAp-DNA interactions that stabilize the complex and make transcription almost certain from this point. 3) Unstable Ternary Complex formation is when RNA synthesis

begins in the active site, with the first rNTP being a purine (A/G). The first fragments of RNA synthetized are not full-length, containing only 8-10 nucleotides. The same interactions that allow promoter recognition by the holoenzyme interfere with the promoter clearance, which is the release of the RNAp to do elongation. In consequence, about 50-100 abortive transcription initiations happen before the RNAp moves to the next stage. 4) Stable Ternary Complex is the stage where the free RNAp can start processive RNA synthesis and elongation [65, 66].

Shortly after the elongation step begins, the sigma factor is released from the enzyme complex to start another cycle of initiation. Therefore, the elongation step is completed by the core RNAp and does not require a sigma factor. The ssDNA template is continuously transcribed into a ssRNA, and the two together form an RNA-DNA helix that is separated by an RNA helicase, with the RNA exiting the complex through its channel [67, 68]. Finally, transcription stops when the RNAp reaches a terminator that signals the end of the process. It can happen via two mechanisms; Rho Independent Termination is an intrinsic mechanism that requires a GC rich hairpin to form in the RNA and a U rich region located in the RNA 7 to 9 nucleotides 3' of the hairpin which blocks the RNA from moving in the RNA exit channel, leading to its detachment from the DNA. The Rho Dependent Termination is an extrinsic mechanism that requires an extra protein called Rho 5'-3' RNA helicase that pulls the RNA out of the RNAp active site [69, 70].

1.4 LysR-Type Transcriptional Regulators

LysR-type transcriptional regulators (LTTRs) are proteins that regulate the expression of other genes, by activation or repression of their transcription. The name derives from LysR, the first well-characterized protein in the group, responsible for the transcriptional activation of *lysA*, a diaminopimelate decarboxylase involved in the lysine production [71]. Despite their importance in bacterial survival through interference in critical processes, such as metabolism, virulence and resistance, the discovery and characterization of LTTRs, as well as their study as therapeutic targets are considered relatively new [72-74]. In 1988, Henikoff *et al*. documented LTTRs for the first time through the identification of nine structurally and functionally similar proteins in *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, *Rhizobium* spp., and *Enterobacter cloacae*. Those proteins presented DNA-Binding-Domain conservation, different from any other known bacterial regulatory protein, and constituted the novel family of regulators [75].

Considered the largest family of transcriptional regulators present in the prokaryotic kingdom, orthologs of LTTRs are found in numerous species of bacteria with conserved function and structure [76]. Classical LTTRs regulate their own expression and the genes they are divergent from. They usually work as tetramers (two dimers) and require the presence of a ligand/co-inducer to shift into an “active conformation” that regulates the transcription of single or operonic genes [77]. Exceptions do exist, such as LTTRs that do not require a specific co-inducer and respond to stimuli from the environment (e.g., OxyR), those that respond to more than one ligand (e.g., BenM), and proteins lacking self-regulation (e.g., AlsR) [78-80]. LTTRs able to regulate multiple

genes and interfere in several bacterial processes are called global regulators. AmpR, for example, is known to affect the expression of over 500 genes in *P. aeruginosa* and to have an impact on the infection progression and prognosis. It positively regulates the expression of *ampC*, *lasB*, and *rhlR*; while negatively regulating the expression of *poxB*, *lasA*, *lasI*, and *larR*. Consequently, AmpR drives the production of β-lactamases, extracellular proteases, pyocyanin, biofilm, efflux pumps, and quorum sensing responses [81-83].

In relation to their structure (**Figure 1.3**), LTTRs contain between 280 to 350 amino acids divided into two domains: an N-terminal DNA-Binding Domain (DBD) with a Helix–Turn–Helix (HTH) motif and a C-terminal Effector-Binding Domain (EBD) / Regulatory Domain (RD), both connected by a Linker Helix (LH) [77]. The DBD presents a highly conserved amino acid sequence among all LTTRs and has been proven to be critical for DNA interactions (such as promoter recognition and binding), as well as for transcriptional activation mediated by interactions with the RNA polymerase [84, 85]. The DBD binds a palindromic DNA sequence called the "LTTR box", which typically consists of T-N_{11}-A sequence, but can vary in both base pair composition and length [86]. On the other hand, the EBD/RD has little conservation at the amino acid level, which explains the different ligand-affinity identified in those proteins [77]. The EDB typically comprises two distinct subdomains (RD1 and RD2) connected by two cross-over regions that form a hinge to accommodate the ligand and promote conformational changes to the tertiary structure of the protein [87, 88].

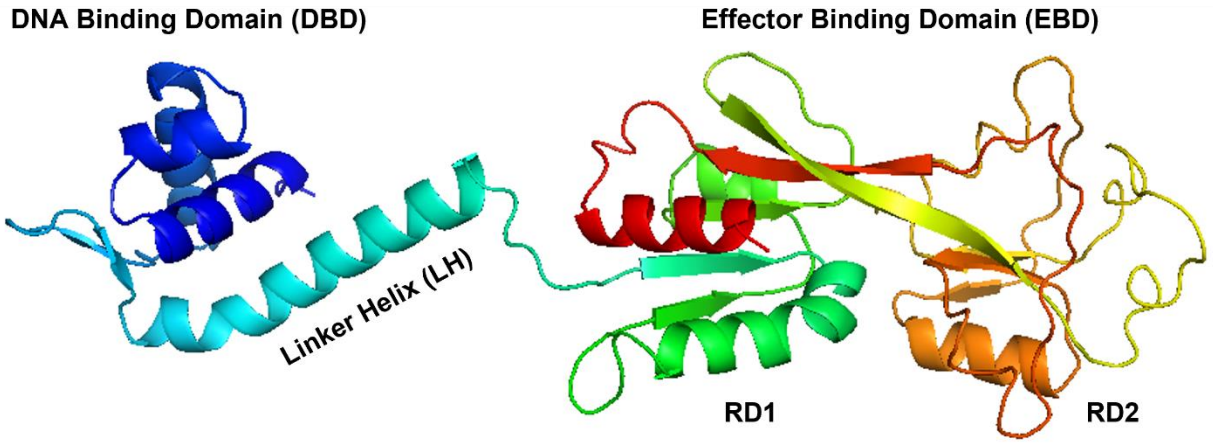


Figure 1.3. Basic structure of LysR-type transcriptional regulators. The X-ray structure shows a monomer unit of ACIAD0746 from *A. baylyi*.

Several crystal structures of EBDs have been resolved, however, due to solubility issues and the tremendous flexibility of the DBD-LH-EBD arrangement, the full-length proteins are particularly difficult to crystalize [77, 85]. Although bioinformatic analysis reveals individual genomes encoding more than 100 LTTRs [89], only 10 well-characterized full-length structures have been reported within all bacterial species [90]. CbnR was the first LTTR to have its full-length crystal structure solved, in 2003, presenting (2-chloro)cis, cis-muconate and cis, cis-muconate as co-inducers and activating the transcription of the *cbnABCD* operon, which encodes a series of enzymes involved in the catabolism of chlorocatechols [91, 92]. This protein, as well as BenM, TsaR, DntR and OxyR, forms a tetramer in the crystalline state, and for this reason has been used as a representative model to study the structure and molecular mechanism of transcription regulation of LTTRs [93]. Other reported full-length LTTR structures include: AphB (PDB ID: 3SZP), HypT (PDB ID: 5YDW), OxyR (PDB ID: 4X6G) and TsaR (PDB ID: 3FXQ) [94-97]. The first resolved EBD structure was of CysB in 1997, a

protein responsive to N-acetylserine (co-factor) and related to the L-cysteine synthesis [98, 99]. Since then, other LTTR-EBD structures were reported, either bound to their ligands/inhibitors or adopting an inducing state conformation, such as AmpR (PDB ID: 5MMH) and PqsR (PDB IDs: 4JVC, 4JVD and 4JVI) [100, 101].

1.5 Introduction of Study

Bacteria depend on the reduction of environmental sulfur to produce the cysteine that will further be the source of bioavailable sulfur for the synthesis of many critical compounds. The cysteine biosynthetic pathway involves the active transportation of oxidized sulfur and several reduction steps that utilize a substantial amount of energy. Therefore, it must be tightly regulated at the post-translational and transcriptional levels. In *Gammaproteobacteria*, the transcriptional regulation is done by CysB and Cbl. However, most bacteria lack these proteins and must rely on other regulatory protein(s) to control cysteine production that are yet to be identified, which is the scope of this work.

The research described here expands our understanding of cysteine biosynthesis by identifying a secondary class of regulators through the combination of structural and biochemical analysis. Previous studies performed in Dr. Momany's laboratory by Melesse Nune resulted in the determination of the full-length crystal structure of ACIAD0746, an LTTR from *Acinetobacter baylyi* ADP1, with unknown biological functions. Studies showing homology between ACIAD0746 and FinR, a protein found in pseudomonads and associated with the synthesis of siderophores,

oxidative stress response, and sulfite reduction, suggested that ACIAD0746 was involved in cysteine biosynthesis regulation. However, due to the unavailability of *A. baylyi* RNA polymerase (RNAp) we could neither conclusively verify this hypothesis nor test for potential natural ligands.

This dissertation begins (Chapter 2) with the cloning, production, and purification of the RNA polymerase holoenzyme ($RNAp^{holo}$) from *A. baylyi* ADP1, since RNAp is an essential element of transcription. We also validated the enzyme activity in all three stages of transcription (initiation, elongation, and termination) through kinetic studies. The successful production of ADP1 $RNAp^{holo}$ allowed us to perform transcription assays with different possible ligands, and conclusively demonstrate the involvement of ACIAD0746 in the regulation of sulfur assimilation, which is supported by additional biological assays found in Chapter 3. Moreover, we identified multiple ACIAD0746 orthologs across the bacterial phyla, differentiating this class of regulators from the previously characterized CysB and Cbl. Two of these orthologs, FinR from *Pseudomonas aeruginosa* and YeiE from *Escherichia coli*, were also object of study in this project.

CHAPTER 2

OVERPRODUCTION, PURIFICATION, AND TRANSCRIPTIONAL ACTIVITY OF RECOMBINANT *ACINETOBACTER BAYLYI* ADP1 RNA POLYMERASE HOLOENZYME[1]

[1] Juliana Calil Brondani, Derrick Afful, Hanna Nune, Jesse Hart, Shelby Cook, and Cory Momany. 2023. *Protein Expression and Purification*. 206:106254.
Reprinted here with permission of the publisher.

2.1 Abstract

Acinetobacter baylyi is an interesting model organism to investigate bacterial metabolism due to its vast repertoire of metabolic enzymes and ease of genetic manipulation. However, the study of gene expression *in vitro* is dependent on the availability of its RNA polymerase (RNAp), an essential enzyme in transcription. In this work, we developed a convenient method of producing the recombinant *A. baylyi* ADP1 RNA polymerase holoenzyme ($RNAp^{holo}$) in *E. coli* that yields 22 mg of a >96% purity protein from a 1-liter shake flask culture. We further characterized the *A. baylyi* ADP1 $RNAp^{holo}$ kinetic profile using T7 Phage DNA as template and demonstrated that it is a highly transcriptionally active enzyme with an elongation rate of 24 nt/sec and a termination efficiency of 94%. Moreover, the *A. baylyi* ADP1 $RNAp^{holo}$ has a substantial sequence identity (~95%) with the $RNAp^{holo}$ from the human pathogen *Acinetobacter baumannii*. This protein can serve as a source of material for structural and biological studies towards advancing our understanding of genome expression and regulation in *Acinetobacter* species.

Keywords: RNA polymerase, *Acinetobacter baylyi*, *Acinetobacter baumannii*, Transcription, Genome expression.

2.2 Introduction

In contrast to eukaryotes, which have multiple RNA polymerases tasked to make different RNA products, transcription of all classes of RNAs in bacteria is mediated by a single DNA-dependent RNA polymerase (RNAp). This key enzyme in gene expression has a large complex structure and exists in two forms, as a core complex lacking a sigma factor and a holoenzyme consisting of the core and sigma factor ($RNAp^{holo}$). The bacterial RNAp core is composed of five subunits codified by four genes: β (*rpoB*), β' (*rpoC*), two copies of α (*rpoA*), and ω (*rpoZ*) with relatively conserved masses across the species. For instance, in *Escherichia coli,* the approximate sizes of the subunits are 151 kDa, 155 kDa, 37 kDa, and 10 kDa, respectively. While in *Acinetobacter baylyi*, the slightly larger ω subunit (11 kDa) is the only notable difference. The β and β' subunits form the bulk of the structure and comprise the catalytic center and several channels where the interactions with nucleic acids happen. The α subunits dimerize and mediate the assembly of the complex, while the ω subunit's role is not fully understood [51, 52]. Although the RNAp core is a catalytically proficient enzyme, it is unable to initiate transcription from a promoter unless bound to a σ factor, making the $RNAp^{holo}$. Multiple σ factors direct the expression of specific groups of promoters, but σ70 (*rpoD* ≅ 70 kDa in *E. coli*; 72 kDa in *A. baylyi*) is considered a housekeeper protein that promotes the transcription of most of the genome [63, 64].

The conventional methods for the $RNAp^{holo}$ purification involve the cultivation of large volumes of bacterial cultures followed by the isolation of the protein complex in a species-specific manner. This approach has major drawbacks especially when dealing

with slow growing and pathogenic species, demanding an extensively time-consuming process and a compatible laboratory biosafety level [102]. Therefore, the application of recombinant protein expression systems using non-pathogenic and fast-growing bacteria (*e.g.*, *E. coli*) represents an advantageous approach to obtain RNAp^{holo} for transcriptional assays [103]. However, methods for the recombinant bacterial RNAp^{holo} purification are only described from *Mycobacterium tuberculosis*, *Bacillus subtilis*, *Thermus aquaticus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Aquifex aeolicus [102-107]*.

Acinetobacter baylyi ADP1 is a non-pathogenic member of the *Pseudomonadota* phylum. This organism is capable of utilizing a wide range of compounds, is easily cultured and well-characterized, making it a compelling model organism for the study of bacterial metabolism regulation [108]. More remarkable, the organism is naturally transformable with a particularly efficient DNA uptake and recombination system [109, 110]. Despite the ability to easily perform genetic manipulations of ADP1, *in vitro* transcription assays can simplify the breakdown of complex regulatory networks. Fine tuning of an organism's interactions between RNAp and its own regulatory proteins dictates that the RNAp used should match species for species as close as possible. However, to date, there are no reports regarding the expression and purification of *Acinetobacter* species RNAp^{holo}, which limits *in vitro* transcriptional studies in this species and other closely related organisms. Studies in the related human pathogen, *Acinetobacter baumannii*, would be simplified with a ready source of RNAp^{holo}. Here, we developed a method for a rapid production of the recombinant *A. baylyi* ADP1 RNAp^{holo} (with σ70), utilizing a two-vector system expressed in *E. coli* with *in vivo* assembly of the

subunits and a multi-step purification approach that produced high yields of a highly pure and active enzyme.

2.3 Materials and Methods

Cloning and Construction of Plasmids

The bacterial strains and plasmids used in this work are listed in **Table 2.1**. Oligonucleotides, synthesized by Integrated DNA Technologies, are listed in **Table 2.2**. The cloning strategies are shown in **Appendix Figures A2.1-2**. Each *rpo* gene was amplified by PCR from *A. baylyi* ADP1 genomic DNA isolated with a Zymo Quick-DNA kit. Phusion Hot Start High-Fidelity DNA Polymerase or Q5 DNA Hot Start polymerase were used for all PCR reactions following manufacturer's directions. Purification of PCR amplicons and DNA minipreps were performed using Epoch EconoSpin columns with buffer compositions reported at https://openwetware.org/wiki/Qiagen_Buffers. All cloning steps involved electroporation of DNA intermediates into XL-1 Blue cells. When carry-over template DNA could interfere with cloning, the PCR products were treated with DpnI for 1 hour at 37 °C. The *rpoA* PCR amplicon was produced using primers ADP1_rpoA-F/-R; the *rpoB* amplicon was produced using primers ADP1_rpoB-F/-R for insertion into pET28b and primers ADP1_rpoB-F/-LR for construction of plasmid pJB4; *rpoC* was produced using primers ADP1_rpoC-F/-R; and *rpoD* as two reactions with primers ADP1_rpoD5'-F/-R and ADP1_rpoD3'-F/-R. Genomic amplicons were introduced into plasmids pET28b(+).SapKO and pET28b.SapKO.BsaI following [111]

and sequenced. These plasmids were then used as PCR templates for making the amplicons that were combined into the final plasmids. A T7 promoter region containing a N-terminal 10X histidine followed by a TEV cleavage site was made by PCR amplification of pDAP18 using primers T7-TEV-BspQI-F and T7-TEV-NcoI-R.

Table 2.1. Bacterial strains and plasmids used in this work.

Name	Description	Source
E. coli strain		
XL1-Blue	F^+ *recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F′ proAB laclq ZΔM15 Tn10 (Tet^R)]	Agilent
BL21(DE3)	E. coli str. B, F^- *fhuA2 lon ompT gal (λ DE3) [dcm] ΔhsdSλ DE3=λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5*	New England Biolabs
Plasmids		
pDAP18	Contains T7 promoter and N-terminal 10xHis-TEV-site	[107]
pET28b(+)	T7lac promoter, Kan^R, pBR322 ori, 6x-His	Novagen
pCDFDuet-1	T7lac promoter, Str/Spc^R, CloDF13 ori, 6x-His, S-Tag	Novagen
pET28b-SapKO-WT	pET28b(+) containing dual BspQI cloning sites inserted between the start codon and two stop codons.	[111]
pET28b-SapKO-CH	pET28b(+) containing dual BspQI cloning sites inserted between the start codon and a C-terminal 5X-His with two stop codons.	[111]
pET28b-SapKO-BsaI	pET28b(+) containing dual BsaI cloning sites inserted between the start codon and a C-terminal 5X-His with two stop codons.	[111]
pRARE2.T7.BspQI-WT	pRARE2 containing the T7 promoter to T7 terminator region of pET28b-SapKO-WT	[107]
pDAP39	Derived from pCDFDuet-1, contains *rpoA*, *rpoZ*, and N-terminal 11xHis-TEV-*rpoD*	This work
pJB4	Derived from pRARE2.T7.BspQI-WT, contains *rpoB* and N-terminal 10xHis-TEV-*rpoC*	This work
T7 Phage DNA	T7 bacteriophage genomic DNA	Boca Scientific

Table 2.2. Oligonucleotides used in building plasmids.

Name	Sequence[a]	Cloning Enzyme
ADP1_rpoA-F	GGGGTCTCACATGACGCGTACTGCAAACGAG	BsaI
ADP1_rpoA-R	GGGGTCTCAATTATTAACGGCTGCGATAGGC	BsaI
ADP1_rpoB-F	GGGCTCTTCGATGGCATACTCATATACCGAAAAG	BspQI
ADP1_rpoB-R	GGGCTCTTCCTTAGTCACCATTTTTCAGTTC	BspQI
ADP1_rpoB-LR	GGGCTCTTCCGGCTTATTAGTCACCATTTTTCAGTTC	BspQI
ADP1_rpoC-F	GGATCATGACCCAGCCGAGTGAGC	BspHI
ADP1_rpoC-R	GCTCCTTAGGTTATTAGAACTGTTCTTGGTTCAATGC	Bsu36I
ADP1_RpoD5'-F	GGGCTCTTCGATGAGCGATATGCATTCCC	BspQI
ADP1_RpoD5'-R	GGGGTCTCAGTTCTTCTGGTGTTGGTTCACG	BsaI
ADP1_RpoD3'-F	GGGGTCTCAGAACTTGGTGAACGTCTGG	BsaI
ADP1_RpoD3'-R	GGGCTCTTCAGTGGTCGTTTTCGAGGAATGAAC	BspQI
T7-TEV-BspQI-F	GCGCTCTTCACCCGGGGATTTAAATCGTATTGTACAC	BspQI
T7-TEV-NcoI-R	GGCCATGGATTGGAAGTAGAGATTCTCGTG	NcoI
pRARE_TAA-F	GGGCTCTTCTTAATCTCACTAATTCGAGCTCCG	BspQI
pRARE_ATG-R	GAGCTCTTCTCATGGTATATCTCCTTC	BspQI
rpoC_extblunt_F	ATGAAAGACTTGCTCGATATCATGCGTAAGAAAACGGACTC	None
rpoC_extblunt_R	GGATTGGAAGTAGAGATTCTCGTGATGATGATGGTGGTGATGATGATGGTGCATATG	None
BspQI_into_BsaI-FR[b]	GGGGTCTCGTAATAACAAGAAGGAGATATACCATGAGAAGAGCTCTTCGCACCAGAGACCGG	BsaI
BsaI_into_BspQI-FR[b]	GGGCTCTTCCATGGGAGACCGGTCTCGCACTGAAGAGCTG	BspQI
ADP1_rpoCW-Gblock[c]	ATGGCACGCGTAACCGTTGAAGATTGTTTAGACCATGTAGACAACCGCTTTGAGCTTGTACTAGTGGCAAGCAAGCGCGCGCGTCAACTTGCTCGTCAAGGCATTGAGCCAACTGTAGAGTGGGACAACGACAAACCTACTGTTGTTTCACTTCGTGAAATTGCAGAAGGTCATGTGACTAAAGATATTTTAAAACAACGCGATCAAGATTATCAAACCTCTTCACTTGATCTGGCATTGTCTGCAAATAGCTTAAATCTGGAAGGTTTTTCATTTCAATAACAAGAAGGAGATATACCATGCACCATCATCATCACGAGAATCTCTACTTCCAAGGCATGAGAAGAGCTCTTCTTAATAACACCAGAGACCGGACTG	BsaI

WGblock-BsaI-F	GGGGTCTCACATGGCACGCGTAACCGTTG	BsaI
WGblock-R	CAGTCCGGTCTCTGGTGTTATTAAG	BsaI
Omega-Vector-F	*CT*TAATTAACCTAGGCTGCTGCCACCGC[d]	HiFi
Sigma-Vector-R	GTGGTGGTGATGATGGTGATGGCTGC	HiFi
Vector-Sigma-F	GCCATCACCATCATCACCACCACCATCATCATCAC GAGAATCTC	HiFi
Stuffer-Sigma-R	TTAAGCATTATTAGTCGTTTTCGAGGAATGAACG	HiFi
Sigma-Stuffer-F	AAACGACTAATAATGCTTAAGTCGAACAGAAAG	HiFi
Alpha-Stuffer-R	TACGCGTCATATGTATATCTCCTTCTTATACTTAACT AATATAC	HiFi
Stuffer-Alpha-F	AGATATACATATGACGCGTACTGCAAACGAGTTTC	HiFi
Vector-Omega-R	GCAGCAGCCTAGGTTAATTATTATTGAAATGAAAAA CCTTCCAG	HiFi
Sequencing Primers	**Appendix Table A2.1**	

[a] Restriction endonuclease sites used in cloning have dark underline, overhangs generated have squiggle underline.

[b] Double-stranded DNA synthesized by IDT as a Gblock. Only the relevant sequence used in the cloning steps for the ADP1 RNAp is shown.

[c] Double-stranded DNA as annealed complementary oligonucleotides.

[d] The italicized nucleotides in the primer are incorrectly designed, but do not occur in the final construct.

To create plasmid pJB4 (**Figure 2.1A**), a 4 piece ligation (FastLink™ Ligase) was performed with the following PCR products: 1) *rpoB* amplicon (primers ADP1_rpoB-F and ADP1_rpoB-LR) digested with BspQI, 2) *rpoC* amplicon digested with BspHI and Bsu36I, 3) the PCR amplicon derived from the T7 promoter/10XHis-TEV site region of plasmid pDAP18 digested with BspQI and NcoI, and 4) the plasmid pRARE.T7.BspQI amplicon (primers pRARE_BspQI_TAA-F and pRARE_BspQI_ATG-R) digested with

BspQI. After identification of relevant clones by sequencing, we identified that the *rpoC* start codon of this intermediate (pRARE2.rpoBC) was mis-assigned and required removal of a 48bp fragment at the N-terminus of the encoded protein. To correct this error, the plasmid pRARE2.rpoBC was amplified by PCR (the annealing and extension PCR steps were combined at 69 °C) utilizing primers rpoC_extblunt_F and rpoC_extblunt_R. The PCR product was treated with DpnI, purified, phosphorylated with T4 Polynucleotide Kinase, and ligated with Fast-Link™ DNA ligase to produce plasmid pJB4.

To create plasmid pDAP39 (**Figure 2.1B**), The *rpoA* PCR amplicon was BsaI cut/ligated with the double-stranded oligonucleotide BspQI_into_BsaI-FR and the plasmid pET28b.SapKPO.BsaI-CH to make pET28b.rpoA.qq. The resulting plasmid was cut/ligated with BspQI and the oligonucleotide BsaI_into_BspQI-FR to make pET28b.rpoA.bb. The plasmid pET28b.rpoA.bb was cut/ligated with BsaI and the rpoW-Gblock amplicon from PCR amplification with primers WGblock-BsaI-F and WGblock-R to make pET28b.rpoAW.qq. The rpoW-Gblock contains the *rpoW* gene as well as a 5X-His-TEV cleavage site followed by BspQI sites. To make plasmid pET28b.rpoAWD, the two PCR amplicons of *rpoD* were cut/ligated with BspQI and plasmid pET28b.rpoAW.qq. The internal BspQI site of *rpoD* is removed and replaced with a silent mutation in this process making plasmid pET28b.*rpoAWD*. The final pDAP39 plasmid used in these studies was made by performing a HiFi DNA Assembly (New England Biolabs) of four PCR amplicons. Plasmid pCDFDuet-1 was PCR amplified using primers Omega-Vector-F & Sigma-Vector-R. Plasmid pET28b.*rpoAWD* was amplified in three separate reactions with PCR primer sets: 1) Vector-Sigma-F &

Stuffer-Sigma-R, 2) Sigma-Stuffer-F & Alpha-Stuffer-R and 3) Stuffer-Alpha-F & Vector-Omega-R. The four PCR products were combined following the manufacturer's protocol for DNA assembly.

Plasmids were sequenced fully by multi-step Sanger sequencing by GeneWiz or Eton Biosciences (sequencing primers are listed in **Appendix Table A2.2**). The final plasmids pJB4 and pDAP39 were further verified by single plasmid sequencing using Oxford NanoPore technology by PlasmidSaurus.

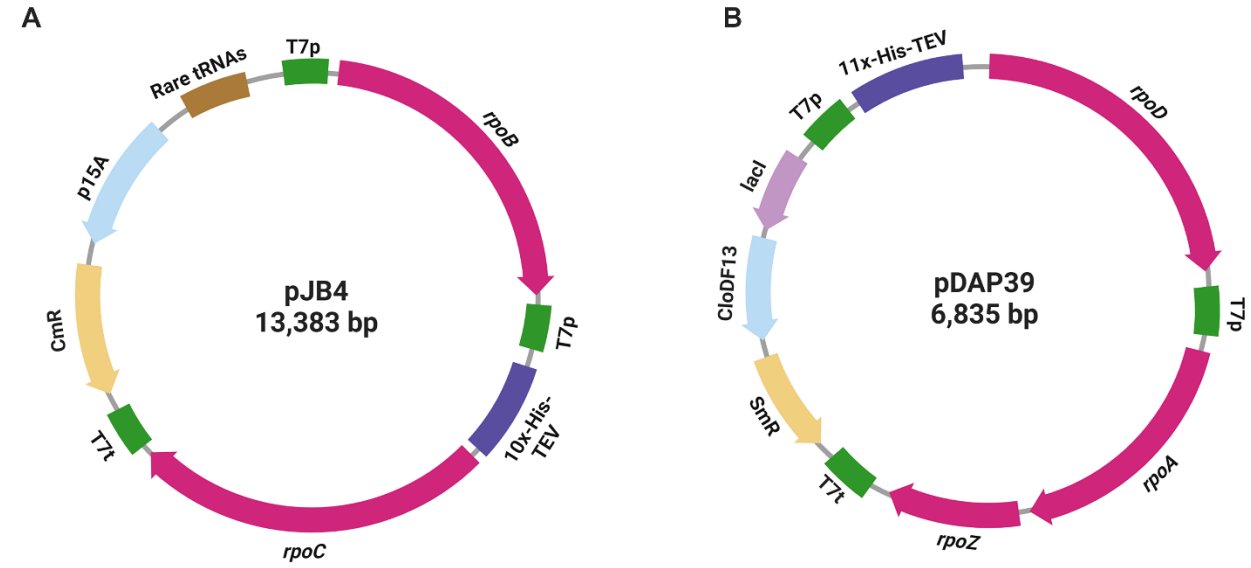


Figure 2.1. Vectors built for RNAp^{holo} expression. (A) pJB4 is a pRARE2-based vector encoding *rpoB* and *rpoC*. (B) pDAP39 is a pCDFDuet-1-based vector that encodes *rpoA*, *rpoZ*, and *rpoD*. All genes are under expression of the T7 RNA polymerase. Both vectors contain two T7 promoters (T7p) and one T7 terminator (T7t). N-terminal 10x-His or 11x-His tags with TEV protease cleavage sites were encoded adjacent to *rpoC* and *rpoD* as shown. Created with BioRender.com.

Protein Expression and Purification

To express the RNAp^{holo}, plasmids pDAP39 and pJB4 were co-transformed into electrocompetent *E. coli* BL21 (DE3) cells, and LB agar plates with chloramphenicol (33 µg/mL) and streptomycin/spectinomycin (50 µg/mL each) were used for selection. An inoculum was prepared by transferring 10 colonies from a plate into 10 mL of LB broth containing the same antibiotics. After shaking at 300 rpm for 5 hours at 37 °C until OD_{600} of 0.5-0.8, the culture was introduced into 490 mL of PA-5052 autoinduction media containing the appropriate antibiotics and supplemented with 1% tryptone and 2% yeast extract, following the Studier method [112]. The culture was incubated at 25 °C until saturation (OD_{600} = 11; ~30 hours). The cells were then harvested by centrifugation at 6,000 x *g* for 10 min. The pellet (approximately 10 g wet weight per 500 mL culture) was resuspended in 40 mL of lysis buffer (50 mM Tris, 500 mM NaCl, 20 mM imidazole, 5% v/v glycerol, 10 mM 2-mercaptoethanol, pH 8.0) supplemented with PMSF in ethanol (1 mM final) and a protease inhibitor cocktail (EDTA-free) mix (1:100 v/v), and the cells were broken using a French Press at 16,000 psi. The crude cell-free extract was then centrifuged at 60,000 x *g* for 30 min, at 4 °C. Finally, the cell-free supernatant was subjected to a multi-step purification process in an ÄKTA (Pharmacia) system following the method described by [107], with modifications as shown in **Figure 2.2**.

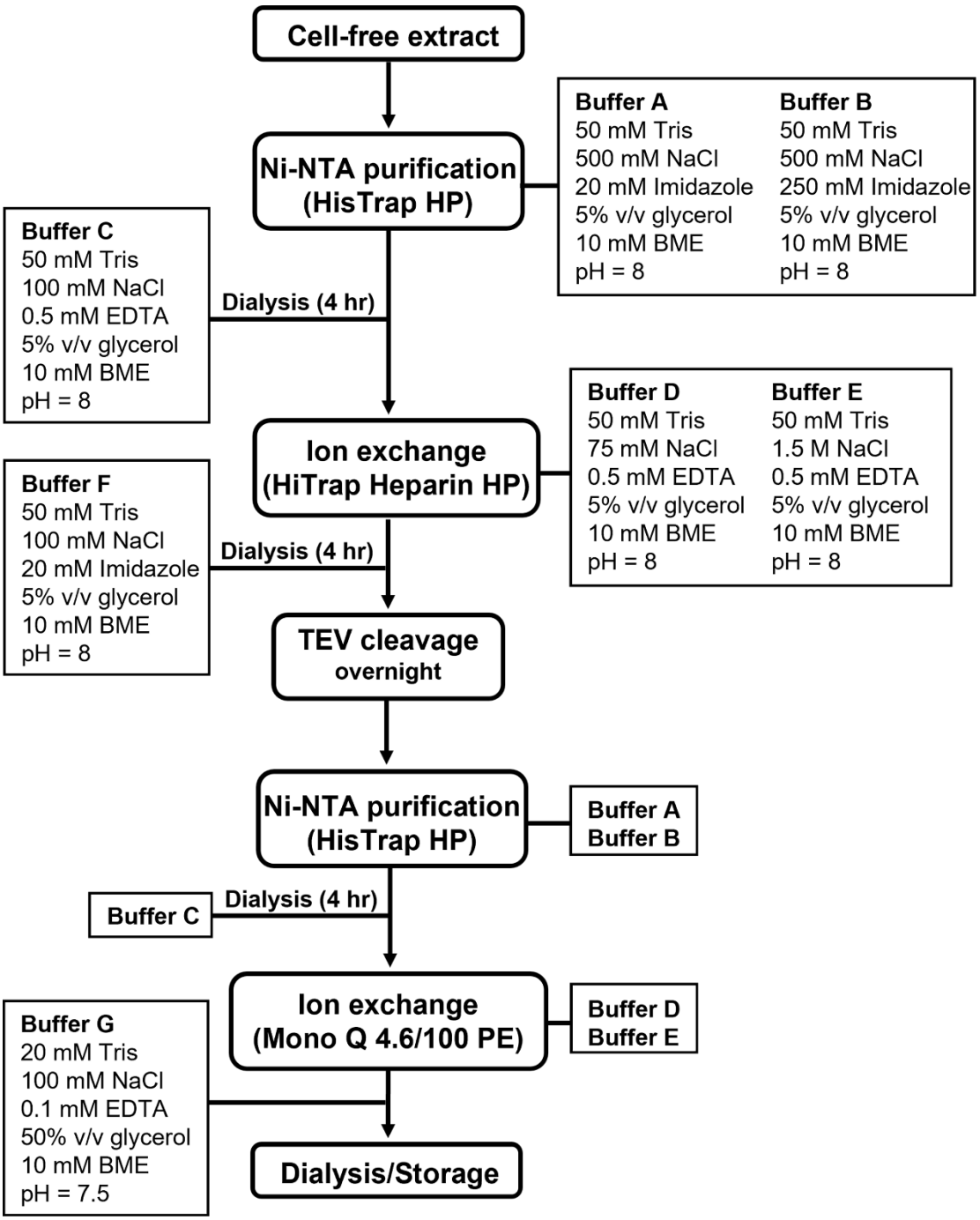


Figure 2.2. Protein purification approach. Schematic of the four chromatographic purification steps to which the cell-free extract was subjected. Chromatography

columns are in parenthesis, HPLC and dialysis buffers are boxed. BME represents 2-mercaptoethanol.

The first step in purifying recombinant $RNAp^{holo}$ involved metal-chelate chromatography using a 5 mL GeBiosciences HisTrap HP column pre-equilibrated with 3 column volumes (CV) of buffer A and utilizing a linear gradient of 0% to 100% buffer B over 30 CV at 3 mL/min. The peak fractions were collected and analyzed by 10% SDS-PAGE gel electrophoresis with Coomassie brilliant blue G250 staining to identify the ones corresponding to the $RNAp^{holo}$. The correct fractions were combined and dialyzed in buffer C for 4 hours at 4 °C, renewing the buffer after 2 hours. Next, chromatography was performed using a 5 mL HiTrap Heparin HP column pre-equilibrated with 3 column volumes (CV) of buffer D and using a gradient of 0% to 100% buffer E over 30 CV at 3 mL/min. The correct fractions were analyzed and pooled together following the same procedures described before and dialyzed for 4 hours in buffer F at 4 °C, with buffer renewal after 2 hours. Following this step, a Bradford assay (ThermoFisher Scientific) was performed for protein quantification according to the manufacturer's instructions and using bovine serum albumin as a standard. Based on the protein concentration, TEV protease purified in house (as described by [113]) was added in a 1:10 ratio TEV:protein mass and left to proceed overnight at 4 °C in order to remove the N-terminal polyhistidine tags. Then, a second metal-chelate purification was performed using buffers A and B to separate the TEV protease and the His tag fragments from the protein sample. In this step, the His-tag free $RNAp^{holo}$ became incapable of binding to the column and was found in the flow-through fractions. The fractions were collected,

analyzed, pooled together, and dialyzed in buffer C for 4 hours at 4 °C, renewing the buffer after 2 hours. The final step of purification involved a 4.6/100 PE MonoQ column using buffers (D and E) prepared with components and glassware free of RNases. To minimize RNAse contamination, the ÄKTA (Pharmacia) system was thoroughly cleaned with NaOH 0.5 M before the MonoQ column. A 0% to 20% linear gradient (buffer D to buffer E) was applied over 5 CV, followed by a gradient segment of 20% to 30% applied over 20 CV, and then a gradient from 30% to 100% applied over 5 CV, at a flow rate of 1 mL/min. Fractions were analyzed and combined before dialysis in buffer G for 12 hours, followed by storage at -20 °C. The protein purity and quantification at each stage of purification was performed by densitometry analysis of SDS-PAGE gels using NIH's ImageJ software and Bradford assay, respectively.

In vitro transcription assays

A modification of the quantitative assay described by [114] applying T7 Phage DNA as template was used to evaluate the kinetic activity of the purified *A. baylyi* ADP1 $RNAp^{holo}$. All assays were performed under conditions to minimize RNAse contamination. Each 200 μL transcription assay contained 40 mM Tris, 150 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, 0.01% Triton X-100, pH 7.5 (from a 5X buffer stock), 10 μg/mL BSA, 20 U RNase Inhibitor Murine (New England Biolabs), 5 nM T7 Phage DNA (Boca Scientific), 2 nM purified *A. baylyi* ADP1 $RNAp^{holo}$, and nuclease-free water to complete the final volume. The reaction was initiated by the addition of 10 μL ribonucleotide

triphosphates (New England Biolabs Ribonucleotide Solution Mix, 25 mM of each) to reach a final concentration of 1.2 mM of each NTP in the final reaction.

The first transcriptional experiment (performed in duplicate) determined the lag time associated with the initiation phase. In this case, a 200 µL reaction was prepared with the components described above. Immediately following the addition of the rNTPs, a 20 µL aliquot was taken and incubated at 75 °C for 10 min to heat inactivate the $RNAp^{holo}$. This sample represents the time zero point. The remaining reaction mixture was incubated at 37 °C and 20 µL aliquots were taken at the following time points: 10 sec, 30 sec, 45 sec, 1 min, 2 min, 3 min, 4 min, and 5 min. Aliquots were immediately heat treated. Then, 5 µL of a mixture of DNase I reaction buffer plus 10 U of DNase I was added to each aliquot with the final reaction volume adjusted to 50 µL using nuclease-free water and the sample allowed to digest for 45 min at 37 °C. For each 50 µL reaction, 250 µL of TE buffer (20 mM Tris, 1 mM EDTA, pH 7.5) was added and the mixture transferred to Microcon-100 concentration columns (Merck Millipore Ltd.) to concentrate the RNA transcripts and remove the degraded DNA fragments that would interfere with the RNA quantification. The columns were spun at 14,000 × *g* in a microcentrifuge for 10 min. For sample recovery, 20 µL of TE buffer was added and the columns inverted into new RNase-free vials that were spun at 1,000 x *g* for 3 min. The recovered 20 µL RNA samples were pipetted into an opaque 96-well microtiter plate containing 80 µL of TE buffer. Finally, 100 µL of Quant-iT™ RiboGreen® RNA Reagent (diluted 200-fold in TE buffer) was added and, after 3 min incubation in the dark, the RiboGreen fluorescence was determined using a Varioskan Flash microplate reader set with the standard fluorescein wavelengths (excitation 480 nm and emission 520 nm).

The time zero fluorescence measurement was used to correct the background fluorescence of the other time points. The standard curve generated using the RNA standards provided in the Quant-iT™ RiboGreen® RNA Reagent and Kit allowed for the quantification of total RNA synthesized.

A second transcription experiment (performed in triplicate) determined the other $RNAp^{holo}$ kinetic parameters using the same procedure described in the initiation study, with a few modifications. Again, a 200 µL reaction was prepared with the components previously mentioned, a time zero aliquot (20 µL) was taken for background correction and the remaining mixture incubated at 37 °C. However, to ensure only one round of transcription and prevent RNAp re-initiation, heparin (0.1 mg/mL) was added after 2 minutes of incubation. The other 20 µL aliquots were taken at the following time points: 1.5 min, 3 min, 4 min, 5 min, 10 min, 15 min, 20 min. and 25 min. The $RNAp^{holo}$ heat inactivation, DNAse I treatment, RNA transcripts concentration and purification steps, as well as the fluorescence measurement were done as before.

Analysis of protein sequence diversity among *Acinetobacter* species and other representative organisms

$RNAp^{holo}$ subunit protein sequences, selected from the NCBI database (https://www.ncbi.nlm.nih.gov/protein), were multiple aligned using CLUSTALW within the BioEdit software version 7.2.5 [115]. Percent sequence identity of *A. baylyi* subunits against other species were calculated individually using the BLASTP program (version $2.13.0^{+}$) [116]. Fifteen species of *Acinetobacter* were included in the analysis: *A. ihumii,*

A. johnsonii, A. ursingii, A. portensis, A. guerrae, A. rudis, A. baumannii, A. guillouiae, A. sichuanensis, A. seifertii, A. pittii, A. defluvii, A. terrestris, A. pragensis, and *A. indicus*. Moreover, thirteen other species were evaluated, each being the dominant phyla found in the environment and in the human microbiota [117-119]: *Bacteroides fragilis* (*Bacteroidota*), *Staphylococcus aureus* and *Bacillus subtilis* (*Bacillota*), *Mycobacterium tuberculosis* (*Actinomycetota*), *Pseudomonas aeruginosa* and *Escherichia coli* (*Pseudomonadota*), *Fusobacterium nucleatum* (*Fusobacteriota*), *Thermus aquaticus* (*Deinococcota*), *Aquifex aeolicus* (*Aquificota*), *Thermotoga maritima* (*Thermotogota*), *Blastopirellula marina* (*Planctomycetes*), *Chlamydia abortus* (*Chlamydiota*), and *Cyanobacteria bacterium CYA* (*Cyanobacteria*).

2.4 Results and Discussion

$RNAp^{holo}$ cloning, expression, and purification

Two plasmids (pJB4 and pDAP39) were constructed that led to the successful production of *A. baylyi* ADP1 $RNAp^{holo}$ (**Figure 2.1**). The choice for *A. baylyi* was based on our concurrent work of the $RNAp^{holo}$ with LysR-type transcriptional regulators in this species, as well as due to its metabolic versatility which makes it a compelling model organism for the study of bacterial metabolism regulation. Most cloning steps entailed the use of simultaneous cut/ligations with using Type II restriction endonucleases which cut outside their recognition sites [111]. We have found this method of cloning to be more efficient than any other cloning approaches. Plasmid pJB4 is based on the

pRARE2 plasmid that was isolated from Rosetta™ 2 (DE3) cells into which the *rpoB* and *rpoC* genes were cloned under one and two T7 promoters, respectively. Our work making recombinant *P. aeruginosa* RNAp [107] showed that a pET28-based plasmid design was inferior for producing high quality RNAp. So, the final plasmid used here to express the α, ω, and σ proteins is based on the pCDFDuet-1 backbone, in which *rpoD* is expressed from one T7 promoter. Expression of *rpoA* and *rpoZ* are influenced by two T7 promoters since there is no T7 terminator after the *rpoD*. The production of complex proteins such as RNAp requires the use of compatible plasmids generating adequate expression of all subunits in a numerically balanced way. In this work, we relied on the T7 gene expression system and encoded long N-terminal polyhistidine tags in the *rpoC* and *rpoD* genes (x10 and x11, respectively) to improve the holoenzyme yield [120, 121]. To allow the polyHis-tag to be removed leaving N-terminal glycines, we introduced TEV protease sites. The choice of placing *rpoA* and *rpoZ* under control of two T7 promoters was made to prevent the co-expression of the *E. coli* α subunits as a compensatory mechanism to overcome a potential misbalance of the expression of *A. baylyi*'s *rpoA*, since 2 mol of α subunit is required for every 1 mole of $RNAp^{holo}$. This problem was observed in the work of [107] when producing the $RNAp^{holo}$ from *P. aeruginosa*. Although we did not visualize any contaminants in our gels, we do not claim that the final product is completely free of *E. coli* contamination.

The expression of multi-subunit proteins comprises two major challenges: (1) the maintenance of the subunit's stability after production and (2) the correct assembly of the subunits to originate the holoenzyme. In this work, we utilized an auto-induction approach at room temperature, which improves the protein's solubility and stability in

comparison to growth conditions at higher temperatures. [112, 122]. Also, the supplementation of the standard PA-5052 auto-induction media with 1% tryptone and 2% yeast extract ensured high mass density (>20 g/L) and a high final protein yield (>20 mg/L) [123]. In fact, we obtained ~21 g/L of dry mass and 22 mg/L of purified $RNAp^{holo}$ (**Table 2.3**). Moreover, the *in vivo* assembly of the $RNAp^{holo}$ was adopted as a strategy to facilitate the correct protein complex formation, since previous studies have demonstrated that the purification of each $RNAp^{holo}$ subunit separately followed by *in vitro* assembly can negatively impact the enzyme's transcriptional activity, possibly due to mistakes acquired during assembly of the subunits [107, 124].

Table 2.3. Quantification and purity of $RNAp^{holo}$ at the different chromatographic steps.

Sample	Total protein (mg)[a]	Purity (%)[b]
Cell-free extract	830	13
Ni-NTA	64	72
Ion exchange-Heparin	48	80
TEV cleavage + Ni-NTA	24	85
Ion exchange-MonoQ	22	96

[a] The data shown here are from a 1 L culture.

[b] Purity was determined by densitometry from SDS-PAGE gel using the ImageJ program [125].

Our chromatographic purification approach comprised the use of four columns with different goals. The first HisTrap HP column aimed to bind to the 10x/11x-His-

tagged σ and β' proteins, removing most of the undesired proteins present in the crude extract, resulting in a 72% pure product. The second step applied a HiTrap Heparin HP column that acts as both an ion exchange and an affinity column for DNA-binding proteins such as $RNAp^{holo}$, resulting in a product with 80% purity. The third column utilized was also a HisTrap HP but now the objective was to remove the His-tags that could interfere with the subsequent biological assays [126-128]. The TEV protease overnight reaction removed the His-tags and rendered a $RNAp^{holo}$ product incapable of binding to the HisTrap HP column, which was collected in the flow-through, giving a $RNAp^{holo}$ product with 85% purity. Finally, a MonoQ column was applied due to its high sensitivity and resolution. This column eliminated potential DNA contaminants that could be present since $RNAp^{holo}$ is a DNA-binding protein. After this last step of purification, we obtained the *A. baylyi* ADP1 $RNAp^{holo}$ with 96% purity (**Figure 2.3**). From our preliminary transcription assays (data not shown), the absence of transcription in the control reactions without the addition of DNA template demonstrate that if the final product is contaminated with DNA, it is not in a concentration that affects the transcription assays.

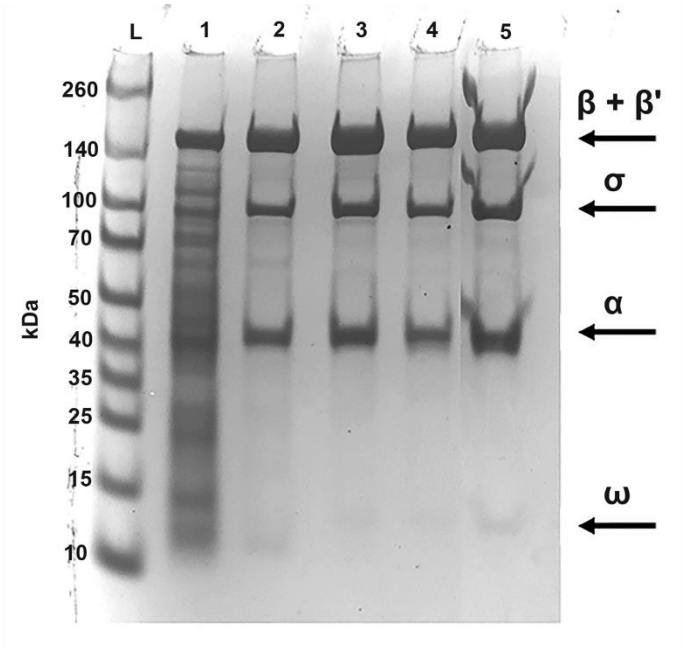


Figure 2.3. SDS Page gel showing the five subunits of *A. baylyi* ADP1 RNAp^{holo} after each chromatographic separation step. The MW marker (Spectra™ Multicolor Broad Range Protein Ladder) ranging from 10 to 260 kDa is shown as Lane L. Lane 1: cell-free extract. Lane 2: Ni-NTA fractions. Lane 3: ion exchange using heparin affinity. Lane 4: Ni-NTA after TEV cleavage. Lane 5: final ion exchange using a MonoQ column. β (151 kDa), β' (155 kDa), σ70 (72 kDa), α (37 kDa), and ω (11 kDa).

RNAp^{holo} characterization in transcriptional studies

The *A. baylyi* ADP1 RNAp^{holo} kinetic profile was determined following the calculations stipulated by [114]. The T7 Phage DNA template has only one transcriptional unit composed by 3 promoters (located at positions 460, 580, and 720 bp) and one strong terminator (at position 7720 bp) [129-131]. Studies have shown that bacterial RNAp initiate transcription exclusively from these promoters [132], resulting in

a prediction for T7 RNA synthesis, as shown in **Figure 2.4A** [114]. Phase I represents initiation which covers the lag time (T_0) associated with promoter recognition, dsDNA melting/unwinding, and abortive cycles until the start of processive RNA synthesis. Phase II assumes that all $RNAp^{holo}$ molecules are engaged in RNA chain elongation with the ssDNA template being constantly transcribed into ssRNA. Heparin is a polysulfate polysaccharide resembling ssDNA that "kidnaps" free $RNAp^{holo}$ and inhibits binding to promoter, while not affecting the molecules engaged in transcription [133]. Therefore, by adding excess of heparin in the beginning of elongation, we prevent $RNAp^{holo}$ re-initiation and ensure measurements from only one round of transcription. Transcription stops when the RNAp reaches the terminator at a certain time (T_i), graphically represented by the substantial decrease in the rate of ribonucleotide incorporation in Phase III. Around 80% of the RNAp molecules end RNA synthesis when hitting the terminator, while the remaining 20% bypass it and continue the incorporation of ribonucleotides up to the full length of the template, which is represented by the slight increase in RNA concentration on Phase IV [114].

In this work, we found that the purified *A. baylyi* ADP1 $RNAp^{holo}$ is active and follows the predicted kinetics for T7 RNA synthesis (**Figure 2.4B**), showing a T_0 = 0.5 min ± 0.03 and a T_i = 5.5 min ± 0.07. Considering an average transcript size of 7133 bp (initiation from one of the three promoters and termination at position 7720 bp), the RNAp elongation rate can be estimated using the equation: $7133 / (T_i - T_0)$. Thus, for *A. baylyi* $RNAp^{holo}$, the synthesis rate is 24 nucleotides per second ± 0.38, which is considered a high rate of transcription elongation for *in vitro* assays using bacterial polymerases (>20 nt/sec) [134, 135]. The linear progression observed in Phase II is not

altered by the addition of heparin (0.1 mg/mL) at minute 2, demonstrating that the RNAp molecules engaged in transcription are not affected by it. Moreover, we obtained a termination efficiency of 94% ± 2.86 through the equation: 1 – (slope of IV/slope of II).

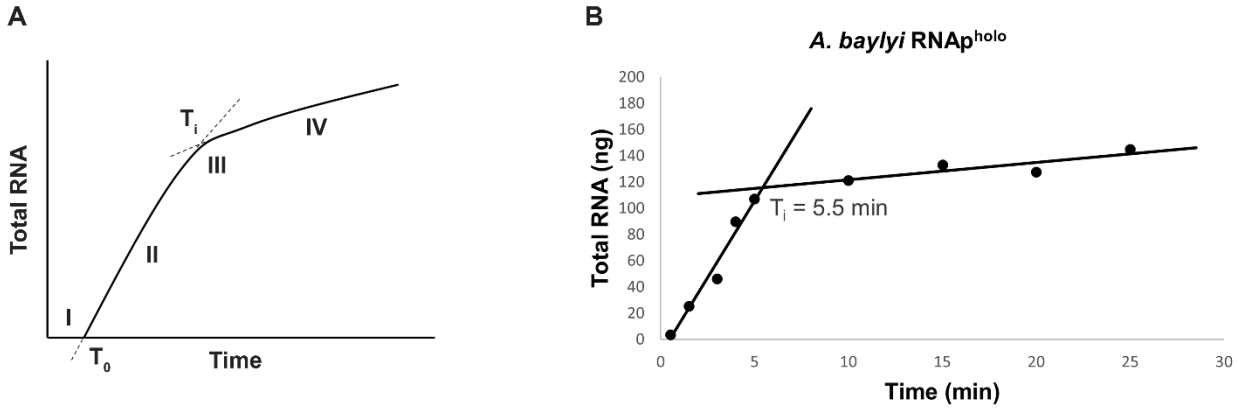


Figure 2.4. *In-vitro* transcriptional studies. (A) Model kinetics for bacterial RNAp on T7 Phage DNA based on Ref. [114]. I = initiation; II = elongation; III = termination; IV = terminator bypass; T_0 = lag time; T_i = time to reach the terminator. (B) Kinetics from *A. baylyi* ADP1 RNAp^{holo} with T_0 = 0.5 min ± 0.03, T_i = 5.5 min ± 0.07, elongation rate = 24 nt/sec ± 0.38, and termination efficiency = 94% ± 2.86. Calculations are based on duplicate or triplicate experiments using the same batch of purified RNAp^{holo}.

The general kinetic profile of the RNAp^{holo} from *A. baylyi* is similar to what was observed in other *Pseudomonadota* [107]. However, the specific RNAp activity obtained in an assay depends upon a variety of factors including template, enzyme, and exact methodological procedures. Even minor modifications in reaction conditions can lead to significant changes in the transcriptional behavior of bacterial RNA polymerases [132]. Therefore, direct comparisons of the RNAp activity are inadvisable when analyzing

results obtained from distinct experiments. For this reason, we propose that the method developed in this work is suitable to acquire an enzymatically active *A. baylyi* $RNAp^{holo}$, but we do not claim that this enzyme is more or less efficient than RNA polymerases characterized in previous publications.

Disparities in activity are expected according to the species-specific intrinsic characteristics of RNAp, as observed in the work of [114] applying T7 Phage DNA, in which a range of T_0 = 0.5 - 2.2 min, T_i = 7.5 – 11 min, elongation rate = 12 - 17.5 nt/sec, and termination efficiency = 45 - 85% values were obtained for the RNAp's from *Caulobacter crescentus*, *Rhodospirillum rubrum*, *Azotobacter vinelandii*, *Mycobacterium smegmatis*, *Bacillus subtilis,* and *E. coli*. Depending on the interactions between the $RNAp^{holo}$ and elements of the promoter, various T_0 are observed within different species, reflecting the time needed for each enzyme to form complexes at the promoters [132]. Also, obstructions during the RNAp progression in the DNA directly affect the elongation rate. *In vitro*, these are usually caused by random backtracking of RNAp, a process that mostly occurs because of the relative inaccuracy of the enzyme. In consequence, some nucleotides are misincorporated in the RNA chain and do not base pair correctly, which puts the 3' hydroxyl in a sub-optimal position to form a phosphodiester bond with the next nucleotide, temporarily pausing RNA synthesis. This phenomenon is inherent to all RNAp, but individual molecules have an asynchronous behavior that is smeared into an ensemble population average. Since backtracking is reversible, the elongation chain can be reactivated spontaneously [136-139]. Variations in the frequency and duration of such transient pauses among the RNAp obtained from different organisms can justify the variability in the elongation rates verified within

species. Moreover, the disparities in the termination efficiency are potentially related to distinct responses of each RNAp to regulatory nucleic acid signals [140].

RNAp^{holo} sequence identity among *Acinetobacter* species and representative organisms

The subunits of the RNAp^{holo} from *A. baylyi* have high sequence identities with other *Acinetobacter* species (**Appendix Figure A2.3-7** and **Appendix Table A2.3-7**), suggesting that the protein complex obtained here can be interchangeably applied in *in vitro* transcription studies of the related organisms. Interestingly, the σ70 subunit shows the most diversity and might require care in using the *A. baylyi* RNAp^{holo} in studies with some species. Of note, excepting the σ70 subunit, the sequence identity of *A. baylyi* with the WHO critical priority pathogen *Acinetobacter baumannii* is 95% or greater. The availability of an effective RNAp^{holo} can support studies to better understand *A. baumannii*'s expression profile and guide the development of novel targeted therapeutics [141]. After the *Acinetobacter* species, the RNAp^{holo} proteins from *A. baylyi* have the highest sequence identities with *P. aeruginosa* (~ 70%) and *E. coli* (~ 65%) as one would expect since they are members of the same phylum (*Pseudomonadota*). The overall sequence identity decreases to 35-50% when compared to other phyla, which argues against the interchangeable use of RNA polymerases from evolutionarily distant bacteria. Most sequence variations are observed in the ω subunit and in the N-terminus of the σ70 subunit. While low sequence identities were found when comparing the ω subunit from *A. baylyi* to *T. maritima*, *B. marina*, or *C. bacterium CYA* individually using

BLASTP, the multiple alignment of the ω subunit is consistent with other studies demonstrating the distant relationship among the functional ω homologs [53].

Despite the conservation observed within the bacterial RNA polymerases' catalytic centers, certain lineage-specific amino acid sequences are inserted within the domains of the β and β' subunits. These sequence insertions (SI) are characteristic of different evolutionary lineages and their frequency increases towards the exposed surface of the RNAp, likely mediating interactions with regulatory proteins [142, 143]. Here, we were able to verify that all *Acinetobacter* species, as well as *P. aeruginosa* and *E. coli*, have the three SI that protrude from the surface of the RNAp in *Pseudomonadota* [144, 145]: SI1 at position β 222-355, SI2 at position β 947-1060, and SI3 at position β' 948-1128. Partial or none of these insertions were observed in *S. aureus*, *B. subtilis*, *M. tuberculosis*, and *T. aquaticus*, which is expected since gram-positive bacteria and *Deinococci* do not have insertions at those positions [146, 147]. Interestingly, in the work of [114], from a set of six species tested under the same experimental conditions, the RNA polymerases showing the longest T_0 were from the only gram-positive species being tested (*B. subtilis*: T_0 = 2.2 min; *Mycobacterium smegmatis*: T_0 = 1.2 min), while the RNAp from *E. coli* presented T_0 = 0.5 min. Similarly, in the work of [132], the RNA polymerases from *B. subtilis* and *Lactobacillus curvatus*, both gram-positive species, showed a weaker interaction with the T7 phage promoter and needed a longer time to form starting complexes. It is known that SI3, and SI1 to a lesser extent, have been implicated in the stabilization of the open initiation complex [146] and, therefore, the absence of these insertions in some species may justify the differences in the initiation phase verified at the T7 promoters. Besides stabilization of

the open complex formation, SI3 also increases RNAp pausing, directly affecting the elongation rate [140]. Several papers demonstrate a considerably higher elongation rate from the RNAp of *B. subtilis* in comparison to *E. coli* [148, 149]. However, this pattern is not verified in *in vitro* transcriptional studies using T7 DNA [114, 132]. Thus, more studies are necessary to better understand the underlying differences associated with this template.

2.5 Conclusion

Despite the challenges associated with the production of complex proteins, we successfully developed a method to obtain the RNA polymerase holoenzyme from *A. baylyi* that provides high yield of a pure enzyme. We also identified, through transcriptional studies, that the purified protein is active. Besides being applied to the study of *A. baylyi* genome expression and regulation, this protein may also be suitable for *in vitro* transcription experiments in other species of *Acinetobacter*, including the multi-drug resistant human pathogen, *A. baumannii*.

CHAPTER 3

A SECOND CLASS OF BACTERIAL TRANSCRIPTIONAL REGULATORS INVOLVED IN SULFUR ASSIMILATION[2]

[2] Juliana Calil Brondani, Melesse Nune, Nickolaus Galloway, Derrick Afful, Ellen Neidle, and Cory Momany. To be submitted to *Cell*.

3.1 Summary

Regulation of sulfur metabolism in prokaryotes has long been associated with the LysR-type transcriptional regulators CysB and Cbl. However, these proteins are absent in most bacteria. In this work, the crystal structures of the full-length ACIAD0746 from *Acinetobacter baylyi* and the effector binding domain of YeiE from *Escherichia coli* show a conserved belt of amino acids associated with sulfite binding, a central intermediate in sulfur assimilation. Motif-based sequence searches reveal orthologs (variously named FinR, GigC, and CysL) widely distributed throughout bacteria. Isothermal titration calorimetry demonstrates ACIAD0746 and YeiE interaction with sulfite. Electrophoretic mobility shift assays show ACIAD0746 high affinity with promoters involved in sulfur metabolism. Transcriptional studies indicate activation at the *cysI*, *cysDN, fpr2* (with ACIAD0746) and *fprA* (with FinR, from *P. aeruginosa*) promoters, in the presence of sulfite. Our results reveal that these proteins represent a 2nd class of regulators involved in sulfur assimilation in prokaryotes.

Keywords: sulfur metabolism, cysteine biosynthesis, sulfite detoxification, LysR-type transcriptional regulators, FinR, GigC, CysL, YeiE, CysB, Cbl.

3.2 Introduction

Sulfur is essential to all forms of life and is directly incorporated into proteins through two amino acids – L-cysteine and L-methionine. Cysteine also serves as a storage pool for bioavailable sulfur for use in cellular processes. Cysteine desulfurase can remove the sulfur from cysteine and donate it to a variety of pathways such as iron-sulfur cluster formation and the biosynthesis of biotin, thiamine, siderophores, and lipoic acid [150]. Two pathways exist for bacteria to use sulfur; Cysteine is synthesized through assimilatory sulfur reduction which converts sulfur-containing compounds to cysteine through multiple reduction steps (**Figure 1.1**). Alternatively, dissimilatory sulfur reduction uses oxidized sulfur as the terminal electron acceptor for energy generation. The dissimilatory pathway arose from the assimilatory pathway and is only found in anaerobic organisms [28].

Sulfate, thiosulfate, or organosulfur compounds serve as the entry point for assimilatory sulfur reduction. Sulfate and thiosulfate are transported via an ABC-type transporter comprised of CysPUWA and Sbp. When this system is absent, a proton:sulfate symporter can be found that is related to plant transporters responsible for sulfate uptake [151]. Once in the cell, sulfate is combined with ATP to form adenosine 5’-phosphosulfate (APS) by the enzyme complex CysDN. In some organisms, such as *Escherichia coli*, APS is phosphorylated to create adenosine 3’-phosphate 5’-phosphosulfate (PAPS). In both cases, the enzyme CysH reduces the activated sulfate to sulfite with a thioredoxin serving as the electron donor, releasing either adenosine-3',5'-bisphosphate or adenosine-5’phosphate in the process [152].

Sulfite is then reduced to sulfide by the flavoprotein CysJ and the siroheme containing protein CysI [153]. Although, not all bacteria contain CysJ and must rely on reduced ferredoxin as a source of electrons for this process, the same method used by plants [36]. Once sulfide is produced, it is combined with *O*-acetyl-L-serine (OAS) to make cysteine by the enzyme CysK. Thiosulfate can be combined directly with OAS by the enzyme CysM resulting in the formation of S-sulfocysteine, which is then reduced to cysteine [154]. Finally, organosulfur compounds can be used as an alternative sulfur source when extracellular sulfate or thiosulfate concentrations are low (Kredich, 2008).

The high energy cost of the assimilatory pathway requires a tight regulation. The feedback inhibition of serine transacetylase, the protein responsible for generation of OAS, by cysteine occurs at the post-translational level and therefore must act in concert with either transcriptional or translational regulation [155]. The transcriptional regulation of sulfur metabolism is largely uncharacterized among the prokaryotes except for the *Gammaproteobacteria*, which rely on CysB and Cbl, two LysR-type transcriptional regulators (LTTRs). CysB has been reported to serve as the master regulator for cysteine biosynthesis, regulating the sulfite reductase gene cluster [156], as well as the genes responsible for bringing sulfate into the cell and for the synthesis of cysteine [157]. In *Enterobacteriaceae*, CysB senses *N*-acetylserine (NAS) [156], a compound that spontaneously forms from OAS [158]. A buildup of intracellular NAS indicates that the cell is starved for sulfur since OAS is not being applied to make cysteine before it spontaneously converts to NAS, thus leading to transcription of the genes involved in sulfate transport and assimilation. Cbl is a CysB-like LTTR that acts in concert with CysB to activate expression of genes involved in organosulfur metabolism [159]. Cbl

senses intracellular levels of APS [160] and activates transcription of the *ssu* and *tau* gene clusters to scavenge sulfur from alternative sources.

While CysB and Cbl are the best characterized transcriptional regulators that control sulfur assimilation, genes encoding these proteins are absent from the majority of bacteria [50]. Several regulatory elements other than CysB and Cbl have been reported in the literature but these elements seem to be relegated to small groups of bacteria in the same way as CysB and Cbl [23, 161]. The absence of these two proteins and the lack of other universal regulatory mechanisms like riboswitches, suggest that other unidentified regulatory mechanisms exist to fill this gap.

ACIAD0746 is one of five essential LTTRs in *Acinetobacter baylyi* ADP1 identified in gene knockout studies [38]. At the time the structure of ACIAD0746 was determined, no known function was assigned to this protein. Early sequence analysis identified a relationship of ACIAD0746 with FinR in pseudomonads, a protein associated with oxidative stress [162, 163]. The association of ACIAD0746 with cysteine biosynthesis evolved from a study connecting FinR with siderophore biosynthesis, which requires cysteine as a synthetic precursor. Genetic knockouts of *finR*, *cysI*, and *fprA* genes in *Pseudomonas putida* resulted in loss of pyridine-2,6-bis(thiocarboxylate) siderophore synthesis. Moreover, FinR controls expression of *fprA,* which takes the place of CysJ in sulfite reduction [36]. Since this study began, ACIAD0746 has been annotated as having a CysL-like motif. CysL, an LTTR first described in *Bacillus subtilis*, regulates the expression of *cysJI* [164]. The studies outlined above when combined with the work presented here using X-ray crystallography, phylogenetic analyses, isothermal titration calorimetry, electrophoresis mobility shift assays, and transcription assays

suggest that ACIAD0746 (as well as its orthologs CysL, GigC, FinR, and YeiE) represents a 2nd class of regulators involved in sulfur assimilation in prokaryotes and thus respond to intermediates in the cysteine biosynthetic pathway.

3.3 Results

Full-length crystal structure of ACIAD0746 from *A. baylyi*

Crystals were obtained of the apo form of the protein (**Table A3.1**). Attempts to produce sulfate or sulfite complexes of the full-length protein did not yield diffraction-grade crystals. LTTRs consist of a DNA-binding domain (DBD) that uses a winged helix-turn-helix motif, a long linker helix (LH), and an effector binding domain (EBD). As with most other LTTRs, ACIAD0746 is a homotetramer with internal two-fold symmetry, resulting in a dimer of dimers. We observed dimerization of ACIAD0746 monomers through the effector binding domains (EBDs). Further dimerization occurs between the linker helices which forms the functional tetramer (**Figure 3.1A**). The DBD/LH domains assume two conformations relative to the effector binding domains. The positioning of the DBDs in the functional tetramer appears to be optimal for DNA contact, with all the DBDs aligned on one face of the tetramer (**Figure 3.1B**). The structure of ACIAD0746 is most likely representative of the unliganded tetrameric complex since no strongly bound ions other than water are present in the predicted effector binding sites (**Figure 3.1C**).

Identification of multiple ACIAD0746 orthologs across bacterial phyla

Several representative orthologs of ACIAD0746 were identified across the bacterial phyla and evaluated using ClustalW (**Table A3.2**), a subset of which can be seen in **Figure 3.2**. Among the orthologs identified were named proteins: CysL from *B. subtilis,* FinR from *P. aeruginosa* and *P. putida*, and YeiE from *E. coli.* Once named proteins with gene designations were located, ACIAD0746 orthologs were identified in the OrthoDB database [165]. Gene designations present in the orthologous group include *finR*, *yeiE*, c*ysL*- but also *cmpR*, *cbbR*, *rbcR*, *aaeR*, *rscR*, and others. Thus, the OrthoDB entry is populated with LTTRs that have highly similar binding pockets, but lack distinctive features identified as critical to sulfite interaction and are functionally regulating different pathways. One example of these homologs is CbbR, the master regulator of carbon fixation, which also contains the REXGSGT motif present in the ACIAD0746 orthologs (**Figure 3.2**), along with three additional loops containing highly conserved sequences [166]. The amino acid sequences in these loops differ from the loop sequences found nearby in ACIAD0746 and other identified orthologs. In particular, and critically important, CbbR orthologs lack Glu147 of ACIAD0746. CbbR from *Xanthobacter flavus* was shown to interact with NADPH via DNA binding assays [167], though Dangel and Tabita state that different CbbR family members may recognize alternative effectors.

The phyla in which no representative orthologs were identified are described in **Table A3.3***.* Some of these phyla do not encode LTTRs, while others lack full metabolic pathways for sulfate to sulfide conversion. However, absence of sulfite metabolism or

cysteine biosynthesis is not always correlated with lack of ACIAD0476 orthologs. The Lactobacilli lack cysteine biosynthesis, but in several species, have YeiH divergent from YeiE. We hypothesize that these and other acid-loving organisms must be able to deal with the toxic sulfur dioxide, the predominant form of sulfite at low pH. Whereas CysB orthologs are often found clearly associated with cysteine biosynthetic genes, the ACIAD0746 orthologs fall into four categories of genome organization **(Figure 3.3)**.

Orthologous genes annotated as *finR* are found primarily in the *Proteobacteria*, but also some *Actinobacteria* and the lone Archaeal representative. These orthologs are divergent from ferredoxin:NADPH reductases (*fpr*, *fprA*). ACIAD0746 orthologs encoded by *yeiE* are usually divergently associated with *yeiH*, which is predicted to encode a membrane protein that is a member of the putative sulfate exporter family of proteins. We postulate that this is an exporter of sulfite and may be important for detoxification. Genes encoding the sulfite reduction step (*cysJI*) are occasionally found associated with CysL encoding genes in the *Bacillota*. Desulfitobacterium hafniense Y51 contains two ortholog instances – CysL and YeiE. *cysL* is divergently bracketed by *cysJI* and *cysK* while *yeiE* is found closely associated with a gene cluster encoding a dissimilatory sulfite reductase. Finally, there are a range of organisms that place the orthologs in random locations in genomes. The best characterized of these is the namesake, CysL, from the *Bacillota*, *Bacillus subtilis*. *Mycobacterium tuberculosis* follows this trend as well.

Effector binding domain crystal structure of YeiE from *E. coli*

Following recognition that *E. coli's* YeiE protein is an ACIAD0746 ortholog, the protein was produced and crystallized for structural studies (**Table A3.1**). Three sulfate ions were found in the crystal structure: two were in the conserved, predicted sulfite-binding pocket of each monomer and one was found coordinated at the interface between monomers (**Figure 3.1D**). The first conserved amino acid sequence motif, ASST in residues 95 to 98, appears to orient the conserved glutamic acid, Glu145 where the charged amino acid will encounter a positive dipole moment from the N-terminus of helix α9. Glu145 is also oriented by Thr197 and in a salt bridge with conserved Arg263 which further interacts with conserved Asp161, which is distant from the sulfate binding site. Asn126 and Ser127 coordinate with the sulfate via backbone amide of Ser127 and the serine hydroxyl group as well as a water molecule. The amino acid sequence, RExGSGT in residues 191 to 197 contribute several interactions with the bound sulfate. Arg191 forms a salt bridge with Glu192 but coordinates with the sulfate through two hydrogen bonds. Two backbone amides from Gly196 and Thr197, and the hydroxyl of Thr197, hydrogen bond to the sulfate. The positive dipole of their helix (α9) is oriented toward the sulfate. The amino acids Asn126, Glu145, and Arg263 were also found to be involved in the coordination of sulfate. Of note is the presence of Glu145 in the binding site (**Figure 3.1E**). This residue is positioned directly where sulfate is bound in CysB and Cbl structures. The cluster of serine and threonine residues surrounding Glu145 are the principal players in sulfate binding of CysB and Cbl [168]. The structure contained a modified cysteine residue at position 148 on both chains. These cysteines were found to

have a β-mercaptoethanol adduct linked through a disulfide bond. Previous work with FinR found in *P*. *putida* and *P*. *aeruginosa* suggests the protein takes part in oxidative stress responses [169]. The observation that Cys148 can be covalently modified by a reducing agent lends credence to that hypothesis, although that idea is not thoroughly explored in this body of work.

Sulfite strongly interacts with ACIAD0746 and YeiE

With the sequence analysis and functional evidence of FinR and CysL pointing to the likelihood that ACIAD0746 is involved with cysteine biosynthesis, the focus of isothermal titration calorimetry (ITC) analysis revolved around sulfur assimilation. The genes regulated by both CysL and FinR are involved in the conversion of sulfite to sulfide; as such, sulfite was the first molecule assayed. Sulfite was found to tightly interact with ACIAD0746 (**Figure 3.4A**). Statistics (**Table A3.4**) indicated a two-site binding model fit the data the best with Chi^2 values near 0.1. Site one exhibited tight binding with a K_d of 566 nM and a stoichiometry of 0.5 binding sites per tetramer. Site two had a K_d of 14 μM (approximately 28 times higher than site one) with four binding sites per tetramer. The low number of binding sites reported for the high affinity site may be due to rapid saturation leading to an undervaluation for the number of moles of sulfite that interact with site one. The lack of a double sigmoidal curve suggests that binding site occupation is non-sequential which may favor the rapid saturation of site one in the first few ligand injections. Since binding site type two is comprised of four binding sites per tetramer, it is likely that this site is the binding cleft containing the

RXGSGT sequence found in each monomer in the tetramer. Binding site type one is likely to have a stoichiometry of either one or two molecules per tetramer. In the case of a stoichiometry of one, the EBD interfaces between dimers would create one such site for sulfite to interact with. More likely is an interface between monomers that make up each dimer, resulting in two molecules of sulfite binding per functional tetramer. Favorable interaction of sulfite with ACIAD0746 is reflected in the ΔG, ΔH, and ΔS of both types of sites. Binding thermodynamics of both sites are driven by large favorable changes in enthalpy, possibly indicating an induced fit binding model [170].

LTTRs can recognize multiple effector molecules [171] [172], so additional small molecules from the sulfur assimilation pathway were screened (**Figure A3.1A-N**). Sulfate, thiosulfate, APS, and sulfide showed no interaction with ACIAD0746. Thus, the only inorganic sulfur molecule ACIAD0746 senses is sulfite. *A. baylyi* ADP1 does not have an ortholog of CysC which coverts APS into PAPS, so PAPS was not assayed. It is important to point out that *A. baylyi* ADP1 has a Cbl ortholog, which would presumably handle these ligands. Since sulfide is not sensed by ACIAD0746, a downstream product of the sulfur assimilation pathway was hypothesized to be sensed as an indicator for whether the cells were starved for sulfur. Cysteine, the final product of the assimilatory sulfur reduction pathway showed no evidence of interaction with ACIAD0746. NAS, the effector molecule CysB senses [158], showed no interaction with ACIAD0746. Considering the lack of binding to any of these secondary ligands, ACIAD0746 is a separate and distinct regulator from CysB. Serine is the precursor molecule to OAS, but it too showed no interaction. Methionine, another sulfur containing amino acid was also tested for interaction with ACIAD0746, but none was detected.

Alanine, the byproduct of cysteine/cysteine desulfurase interaction, was not found to interact with ACIAD0746. Our findings go counter to the study of [173] using differential scanning fluorimetry (DSF), which identified a shift in the thermal denaturation of GigC (ACIAD0746-ortholog) from *Acinetobacter baumannii* in the presence of cysteine, while no effect was observed when testing sulfite. From that, the authors suggested that cysteine could work as the ligand of GigC. Although DSF can be applied to ligand screening, this method provides many false positive and negative results and is considered less reliable in the evaluation of protein-ligand interactions than ITC [174]. It is unlikely that the intracellular presence of cysteine would trigger sulfur metabolism to make even more cysteine, given the toxicity associated with its excess mainly due to uncontrolled Fenton reactions [175]. Nonetheless, we decided to include cysteine in our transcription assays to verify this hypothesis.

With no evidence that any intermediates in the sulfur assimilation pathway aside from sulfite interact with ACIAD0746, energy carrying intermediates were assayed. NADPH is used as the reductant by both CysJ and FprA. Both NADPH and $NADP^+$ exhibited no interaction with ACIAD0746. NADH can act as reductant for ferredoxins, so NADH and NAD^+ were assayed and found not to interact. Finally, Fe(III) was assayed and found not to interact with ACIAD0746. Iron is important in electron transfer reactions and therefore the cytosolic iron concentration may hint at the availability of iron-sulfur containing enzymes which are necessary for the reduction of sulfite to sulfide. Additionally, FinR was shown to be involved in oxidative stress in several pseudomonads. Iron is an important indicator of oxidative stress, so the lack of Fe(III) interaction with ACIAD0746 indicates it likely does not function as SoxR, a MerR-type

transcriptional regulator that has a 2Fe-2S cluster on the C-terminal end of both monomers that make up the SoxR functional dimer. Additionally, no color was present in fractions containing ACIAD0746 during purification which further supports the hypothesis that ACIAD0746 is not an iron sensor and does not function as a redox sensor through the monitoring of iron. The important oxidative protectant glutathione was not assayed as *A. baylyi* ADP1 lacks the pathway to synthesize glutathione.

YeiE was also assayed for sulfate and sulfite binding via ITC (**Figure 3.4B**). As with ACIAD0746, YeiE was found to exhibit two-site binding with sulfite. The crystal structure of YeiE-EBD revealed two different sets of sites occupied with sulfate. The first binding site consists of the ligand binding pocket found in each YeiE monomer while the second site was at the interface between EBD monomers. It is likely the interfacial binding site is the second type of site sulfite interacts with. Both types of binding sites exhibited an affinity in the single digit μM range. As with ACIAD0746, the stoichiometry of binding site type one was found to be much less than 1, likely due to the rapid saturation of the sites as well as the non-sequential binding nature of both types of sites. Unlike ACIAD0746, sulfite interaction does not appear to be enthalpically driven with binding site type one. While the Gibbs free energy remained favorable the enthalpy was positive meaning that sulfite binding is largely entropically driven. Binding site type two shows extremely high enthalpy and entropy changes, but both are favorable unlike with binding site type one. The sign associated with the enthalpy of binding site type one along with the large entropic and enthalpic values of binding site type two differs greatly from ACIAD0746. Additionally, while sulfate was not found to interact with

ACIAD0746, it is found in the binding pocket of the crystal structure of YeiE-EBD (**Figure 3.1E**).

The ability of YeiE to bind sulfate was assayed via ITC to further distinguish the differences between YeiE and ACIAD0746. Sulfate did interact with YeiE but caused light precipitation of the protein in the ITC cuvette so trustworthy thermodynamic data was not obtained. In the study of [176], a similar ITC pattern was observed from *C. sakazakii*, with YeiE binding to sulfite (K_d = 1.38 µM) with a stoichiometry of 0.5. However, they did not identify binding affinity to sulfate. Our observation that YeiE precipitates upon addition of sulfate is distinctly different from that of ACIAD0746 which tolerates titration with sulfate. It is unsurprising that YeiE precipitates in the presence of sulfate since the crystallization condition for the EBD of YeiE contained a high concentration of ammonium sulfate. Sulfite was not found to cause any precipitation of either ACIAD0746 or YeiE.

Sulfite activates the ACIAD0746 and FinR transcriptional regulation at different promoters

The genome arrangement of sulfur assimilation genes in *A. baylyi* ADP1 is vastly different from that of *E. coli* and *Salmonella*, the two organisms where sulfur associated transcriptional regulation has been largely characterized [157]. Genome organization of sulfur assimilating genes are largely operonic in these two organisms while most of the same genes are scattered throughout the genome of *A. baylyi* ADP1. Moreover, *aciad0799*, the *A. baylyi* ADP1 gene annotated as *cysJ* and predicted to function as a

sulfite reductase, does not have an ortholog in bacteria with characterized CysJ and its deletion does not lead to cysteine auxotrophy [38]. Consequently, we postulate that a ferredoxin reductase (*fpr* or *fpr2*) is providing the electrons necessary for sulfite reduction in this organism. The ACIAD0746 transcriptional regulation of promoters controlling expression of these genes were examined by transcription assays including sulfite, sulfate, and cysteine as potential ligands and are summarized in **Table A3.5**.

The ABC type transporter that imports sulfate and thiosulfate is encoded by the *cysP* operon as well as *sbp*. CysP interacts with thiosulfate while Sbp captures sulfate [177]. The *ssu* gene cluster encodes a second ABC transporter that is specific for alternative sulfur sources such as aliphatic sulfonates [159]. No transcriptional regulation was observed at the *cysP*, *sbp*, and *ssu* promoters. CysDN is responsible for converting sulfate to APS which is then reduced to sulfite by CysH. Sulfite is further reduced to sulfide via a six-electron transfer mediated by CysI using CysJ (or a reduced ferredoxin) as the source of electrons. As shown in **Figure 3.4C**, ACIAD0746 activated the transcription of *cysDN*, *cysI,* and *fpr2*, but only in the presence of sulfite (1 mM). No regulation was observed when sulfate or cysteine were tested as inducers. The reactions containing sulfite but without ACIAD0746 did not activate transcription, demonstrating that the results obtained here were not due a direct effect of sulfite on the RNA polymerase. Our results corroborate with the transcriptional *lacZ* reporter data from [173], which suggests that GigC (ACIAD0746 ortholog from *A. baumannii*) acts as an activator of *cysI* and *cysDN*. However, the proposed role of cysteine as an effector was not confirmed by us. Also, [176] hypothesized that YeiE (from *C. sakazakii*) acts in

the defense against sulfite stress by transcriptionally activating the sulfite reductase *cysJI*, and proposed that sulfite could be the natural ligand of this protein.

CysK and CysM are responsible for fusing sulfide with OAS to form cysteine, but no transcriptional regulation was observed at these promoters. Moreover, our results suggest that ACIAD0746 does not regulate expression of *fpr* or expression of itself since *aciad0746* is divergent from *fpr*. This is an interesting finding because LTTRs classically regulate both themselves and the genes they are divergent from [178]. In *P. aeruginosa* PAO1 and *P. putida*, FinR does regulate expression of *finR* and the divergent gene *fprA* upon exposure to oxidative stress, although no FinR ligand was established in that study [163]. In our experiments, we verified *P. aeruginosa* PAO1's FinR transcriptional regulation in the presence of sulfite. As seen in **Figure 3.4D**, in the reactions absent from FinR (either with or without sulfite), a 90 bp fragment was detected, corresponding to *finR*. In the presence of FinR but without sulfite, no transcription activation was observed, and in the reactions containing FinR and sulfite (1 mM) a 270 bp fragment was identified, corresponding to *fprA*. No transcription regulation was observed when sulfate or cysteine were tested as potential ligands. LTTRs generally self-regulate their expression, however there are examples where other factors control expression of an LTTR [80], which seems to be the case of ACIAD0746. The results presented here strongly suggest that sulfite is the physiological ligand of ACIAD0746 (and ortholog proteins) and conclusively demonstrate for the first time its effect on the transcriptional regulation at the *cysDN*, *cysI, fpr2* (*A. baylyi* ADP1), and *finR-fprA* (*P. aeruginosa* PAO1) promoters, thereby the involvement of these proteins on cysteine biosynthesis.

ACIAD0746 strongly binds to *cysDN* and *cysI* promoters

We further evaluated the ACIAD0746 affinity for nine promoters involved in sulfur assimilation (*cysP*, *sbp*, *ssuA*, *cysDN*, *cysH*, *cysI*, *cysK*, *cysM*, and *fpr*), either with or without sulfite (1 mM). The *fpr2* promoter was not tested. As shown in **Figure 3.4E**, ACIAD0746 strongly interacted with the *cysDN* promoter, with K_d values of 9 nM in the absence of sulfite and 49 nM in the presence of sulfite. Stronger interactions were observed with the *cysI* promoter, with a K_d of 3 nM without sulfite and 5 nM with sulfite. Even with the apparent affinity decrease with sulfite present, the K_d's obtained here are similar to those reported for other LTTRs with regulatory function, in the presence of their effectors [179-181]. A considerably lower affinity was obtained for ACIAD0746 and the *fpr* promoter, with K_d values of 155 nM in the absence of sulfite and 121 nM in the presence of sulfite. These results corroborate with our transcription assays, supporting the idea that ACIAD0746 does not regulate itself and *fpr*, but activate the transcription at the *cysDN* and *cysI* promoters. The ACIAD0746 affinity for the other promoters showed no evident correlation with the transcription assays. As mentioned previously, the open conformation of the DBDs suggests that ACIAD0746 will readily interact with DNA, even non-specifically. Therefore, it is unsurprising that ACIAD0746 can also show high affinities for promoters it does not regulate (**Table A3.6**).

No common motifs have been identified among the promoters ACIAD0746 has high affinity for. Most LTTR DNA binding sites consist of dyad symmetry containing the consensus sequence motif T-N_{11}-A [178]. The CysL binding motif previously described was not found in any of the promoter sequences examined and was not useful as a

predictive tool for identifying potential promoters ACIAD0746 may interact with [164]. Previous work revealed that CysB orthologs do not share common regulatory sequence motifs between organisms. The lack of regulatory motifs identified here suggests that orthologs of ACIAD0746 may behave like CysB and therefore may not recognize one conserved pattern. The lack of patterns found associated within the promoters ACIAD0746 interacts with in this study further supports the idea that ACIAD0746 orthologs have evolved to recognize a slew of different DNA features.

FinR regulates *fprA* transcription through the classic “sliding dimer” model

Since FinR presented traditional LTTRs characteristics, we hypothesized that it should follow the classic LTTR regulatory mechanism called the ‘sliding dimer’ model, in which an inactive LTTR binds to the DNA blocking RNA polymerase access to the –35 region (or other sigma factor binding sites), therefore preventing transcription of the downstream gene. By binding to its inducer, the LTTR gets in its active state by undergoing conformational changes that result in the ‘sliding’ of the LTTR dimer to a secondary binding position, exposing the –35 element for RNA polymerase-mediated transcription [77, 93, 182]. This idea is supported by our identification of potential primary and secondary FinR boxes in the *finR-fprA* intergenic region (**Figure 3.5A**). The FinR box located at the -35 element of *finR* consists of a perfect TATC/GATA palindrome, suggesting that one of the dimers will strongly bind at that position. Another two FinR boxes located at and next to the -35 element of *fprA* contain imperfect

TATC/GATA palindromes, indicating that the second dimer loosely binds at those positions and is potentially moving between them depending on its activation state.

To verify this hypothesis, oligonucleotides MP1 and MP2 were designed to contain nucleotides that encompassed the identified primary and secondary FinR boxes, allowing FinR binding depending on whether the LTTR was induced or not. The 45 bp MP1 fragment contains the FinR box located at the *finR* -35 element (Recognition Binding Site or RBS) and the FinR box located next to the *fprA* -35 element (Activation Binding Site 2 or ABS2), but it lacks the FinR box that stretches across the *fprA* -35 element (Activation Binding Site 1 or ABS1). On the other hand, the 54 bp MP2 fragment contains all 3 FinR boxes (RBS, ABS1, and ABS2). As expected, our results show that in the absence of sulfite (**Figure 3.5B**) FinR binds very poorly to MP1 whereas strong binding is observed for MP2, corroborating the idea that the first dimer is anchored in the perfect palindromic sequence found at the RBS (blocking the -35 *finR* element) and the second dimer is weakly bound to the ABS1 (blocking the -35 *fprA* element). With both -35 elements blocked, no transcription is allowed, which was confirmed in our transcription assays. In the presence of 1 mM sulfite (**Figure 3.5C**), FinR affinity for MP1 substantially increased, while the binding for MP2 remained unaltered, suggesting that conformational changes move the second dimer from ABS1 to ABS2, exposing the *fprA* -35 element for RNA polymerase recognition and transcription, which was also verified in our transcription assays.

3.4 Discussion

The structures of *A. baylyi* ADP1's ACIAD0746 and *E. coli*'s YeiE proteins have resulted in the identification of an important subgroup of LTTRs involved in sulfur metabolism. Previously described members of this family include CysL from *B. subtilis* and FinR from *P. putida* and *P. aeruginosa*. Most members of this ortholog group went overlooked due to low amino acid sequence conservation between members. The crystal structures obtained in this study allowed for the identification of the amino acids critical for sulfite interaction. In some cases, these amino acids are the only conserved residues between orthologs. While CysB and Cbl are well characterized transcriptional regulators of sulfur metabolic genes, they are only largely present in the *Gammaproteobacteria*. The identification of proteins related to ACIAD0746 across most known phyla of bacteria strongly suggests this family is the master regulator of cysteine biosynthesis.

Sulfite has a long and storied history as a preservative in food and wine [183]. In wine making, sulfur dioxide is added as a preservative at a low pH [184]. Sulfur dioxide spontaneously converts to bisulfite, a neutrally charged molecule that can freely enter the cell. Once inside, bisulfite converts to sulfite due to the pH of the cell and can react with disulfide bonds resulting in oxidative damage [185]. Neutrophils produce sulfite in response to lipopolysaccharide as part of an oxidative burst meant to kill microbial invaders [186]. When *M. tuberculosis* is engulfed by a macrophage, it activates transcription of *cysD* and *cysNC* [187] [188]. *E. coli* contains YeiE (ACIAD0746-ortholog) divergent from *yeiH*. While the function of YeiH remains unknown, it is

annotated as a member of the putative sulfate exporter (PSE) family of proteins. In the case of *E. coli* and other organisms, YeiE may control transcription of *yeiH* which acts as an exporter to rid the cell of either sulfate or sulfite to survive. The ability of *E. coli's* YeiE to sense both sulfite and sulfate may allow the protein to control when the potential export apparatus *yeiH* is transcribed. Sulfate interaction with *E. coli's* YeiE leads to the affinity for sulfite to increase in the first binding site but decrease in the second. The synergistic binding of both sulfate and sulfite to YeiE may signal that ratio of sulfite to sulfate is within tolerable limits. However, when sulfite reaches a critical concentration that can displace the sulfate in the second binding site, YeiE becomes fully saturated with sulfite leading to the transcription of *yeiH* to rid the cell of the excess sulfite before damage can be done. The activation of sulfite reductase by YeiE may lead to temporary detoxification of sulfite through the conversion of sulfite to sulfide, thereby depleting the sulfite pool and preventing oxidative damage.

Bacterial sulfur metabolism and regulation are important targets for antibiotic development. The mechanisms used to convert sulfate and other sulfur sources into cysteine differ between bacteria and humans. New antibiotics against *M. tuberculosis* targeting sulfur metabolism are especially intriguing. Disruption of *cysH* not only caused *M. tuberculosis* to become auxotrophic for cysteine and methionine, it prevented infection in several immunocompetent mice strains [189]. Disruption of cysteine metabolism through the targeted inhibition of a transcriptional regulator is an appealing target as multiple steps in the metabolic pathway can be targeted in one fell swoop. The identification of ACIAD0746 orthologs in the majority of known bacterial phyla presents

an exciting target for the development of new broad-spectrum antibiotics and has wide ranging implications to sulfur metabolism.

3.5 Figures

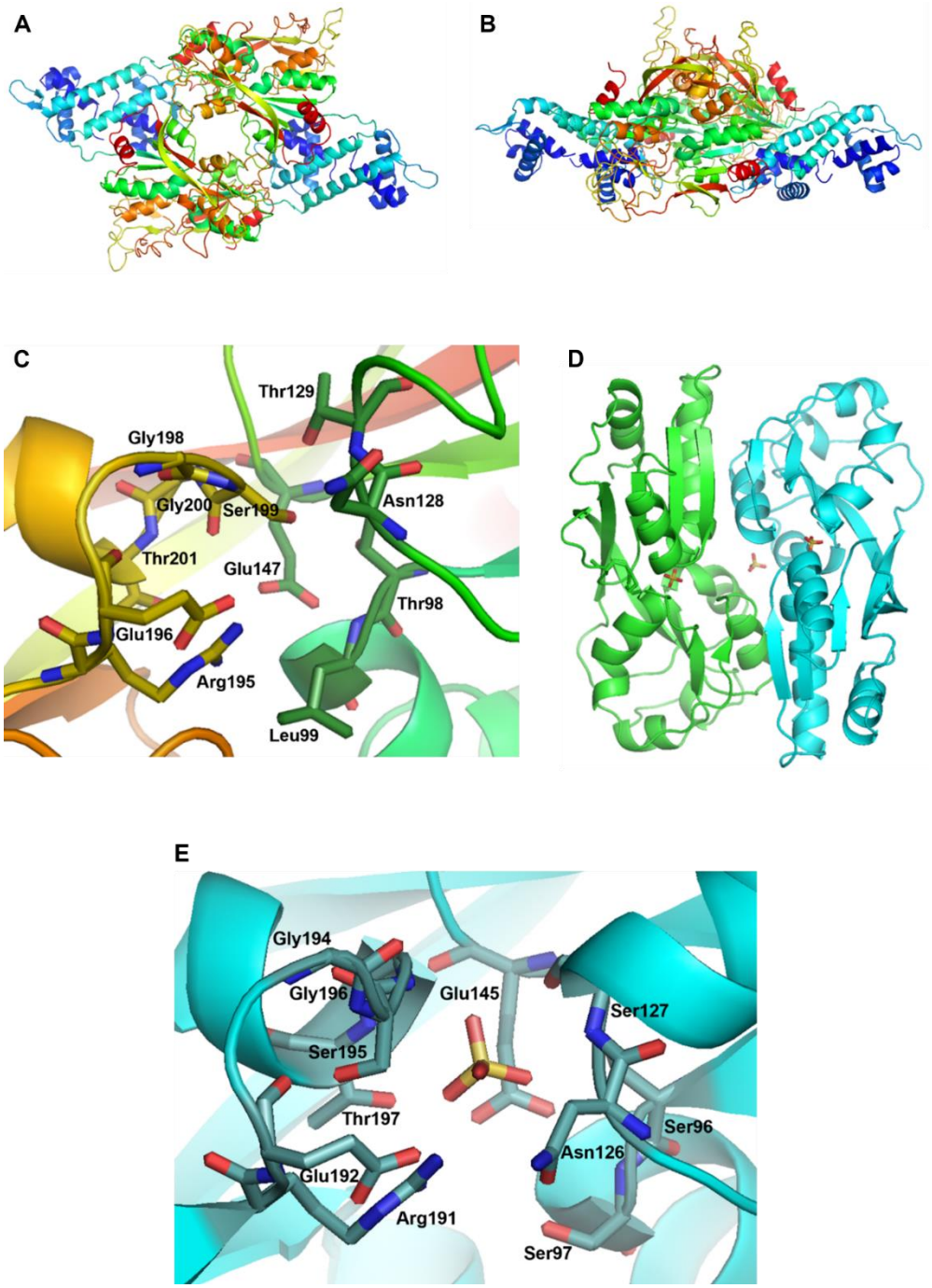
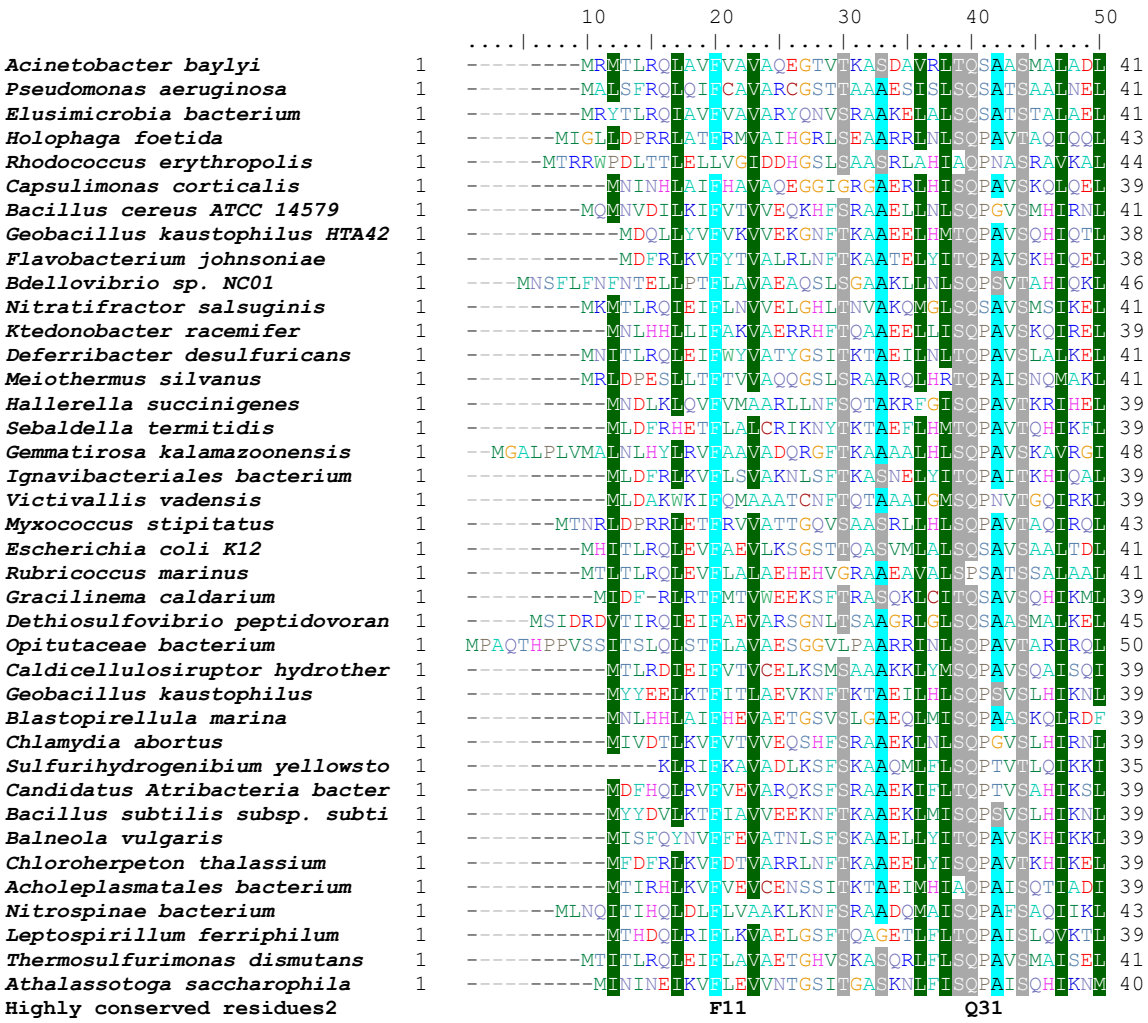
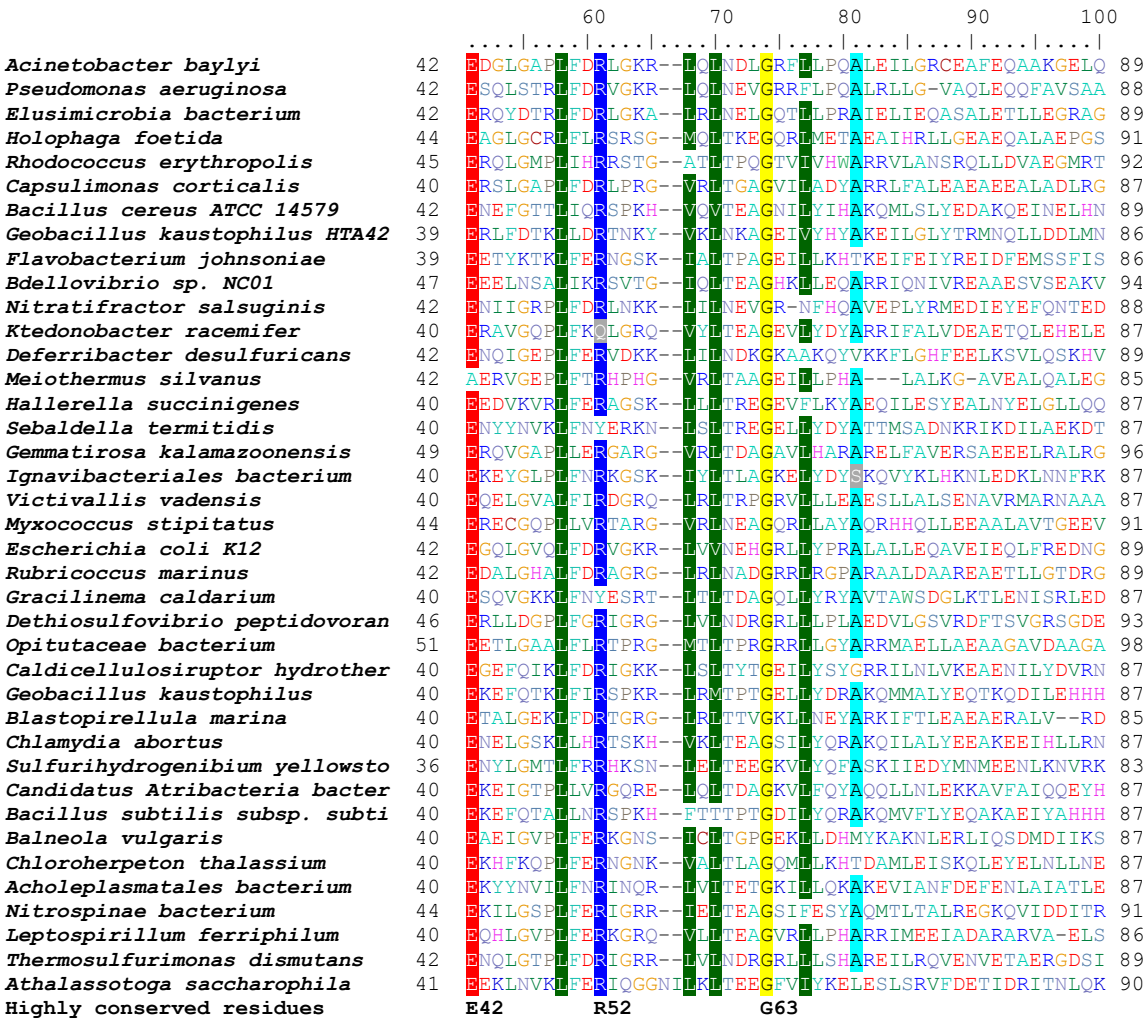
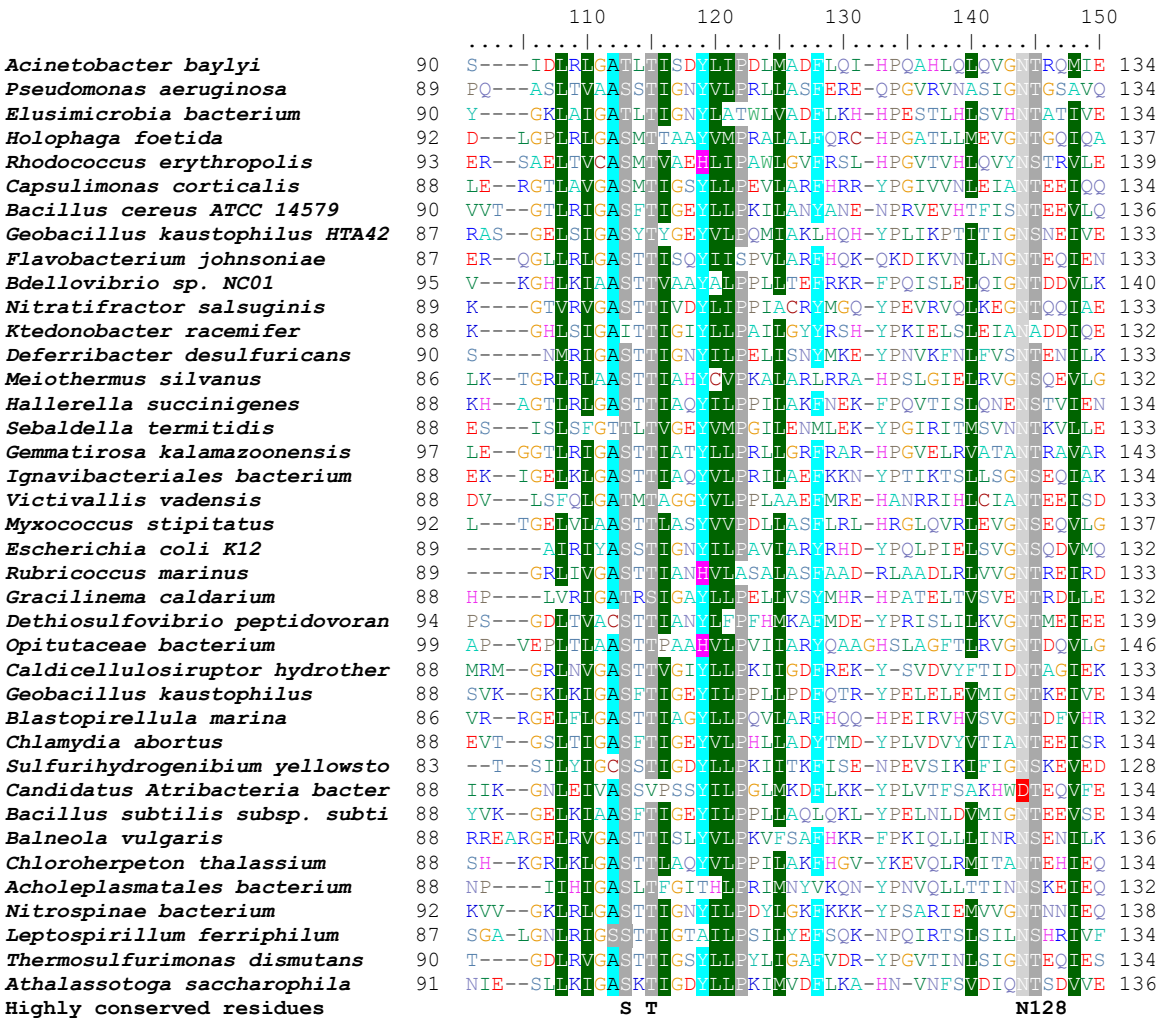


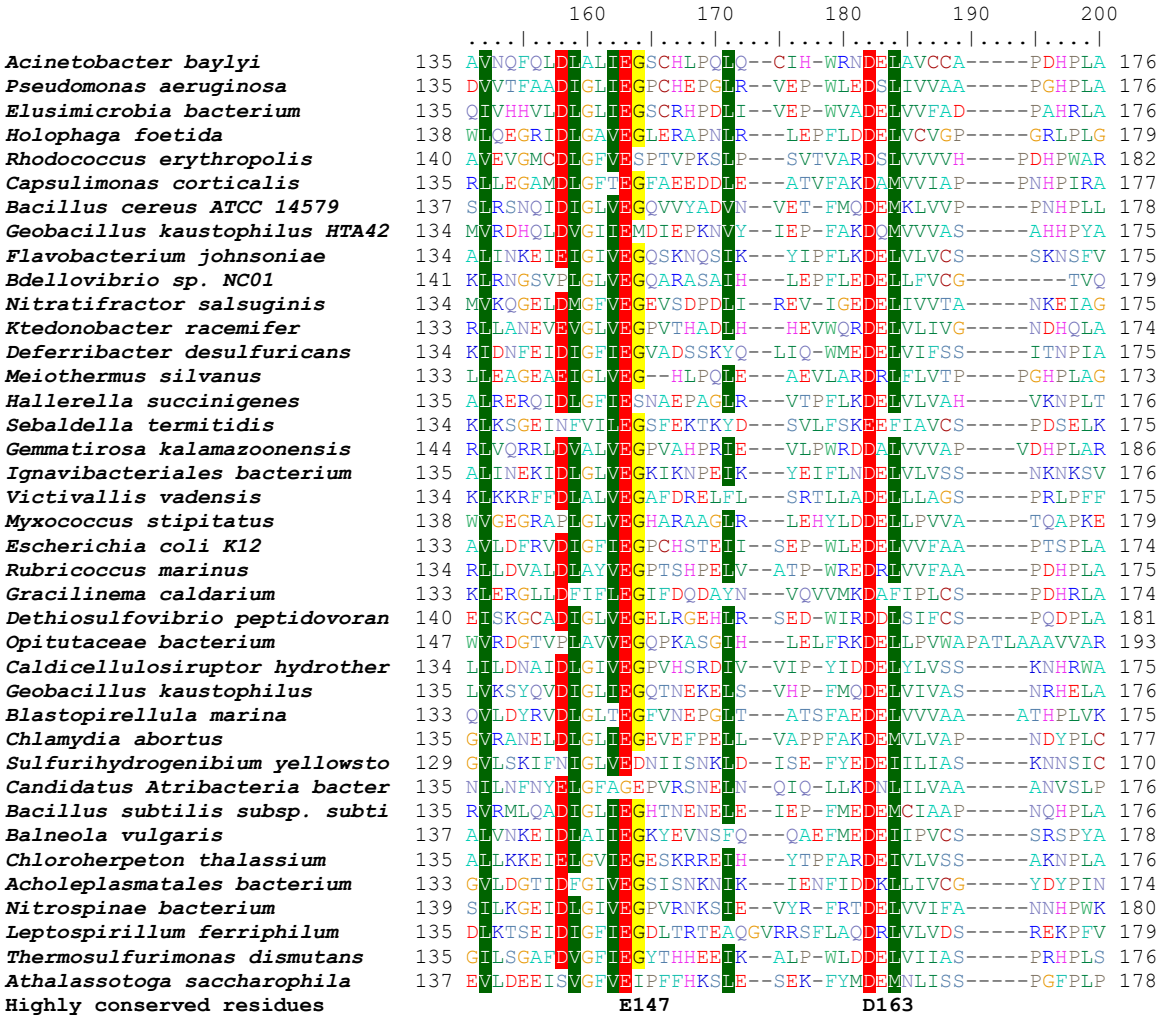
Figure 3.1. Crystal structures of the full-length ACIAD0746 from *A. baylyi* ADP1 and the effector binding domain of *E. coli's* YeiE. (A) Top view of the ACIAD0746 tetramer

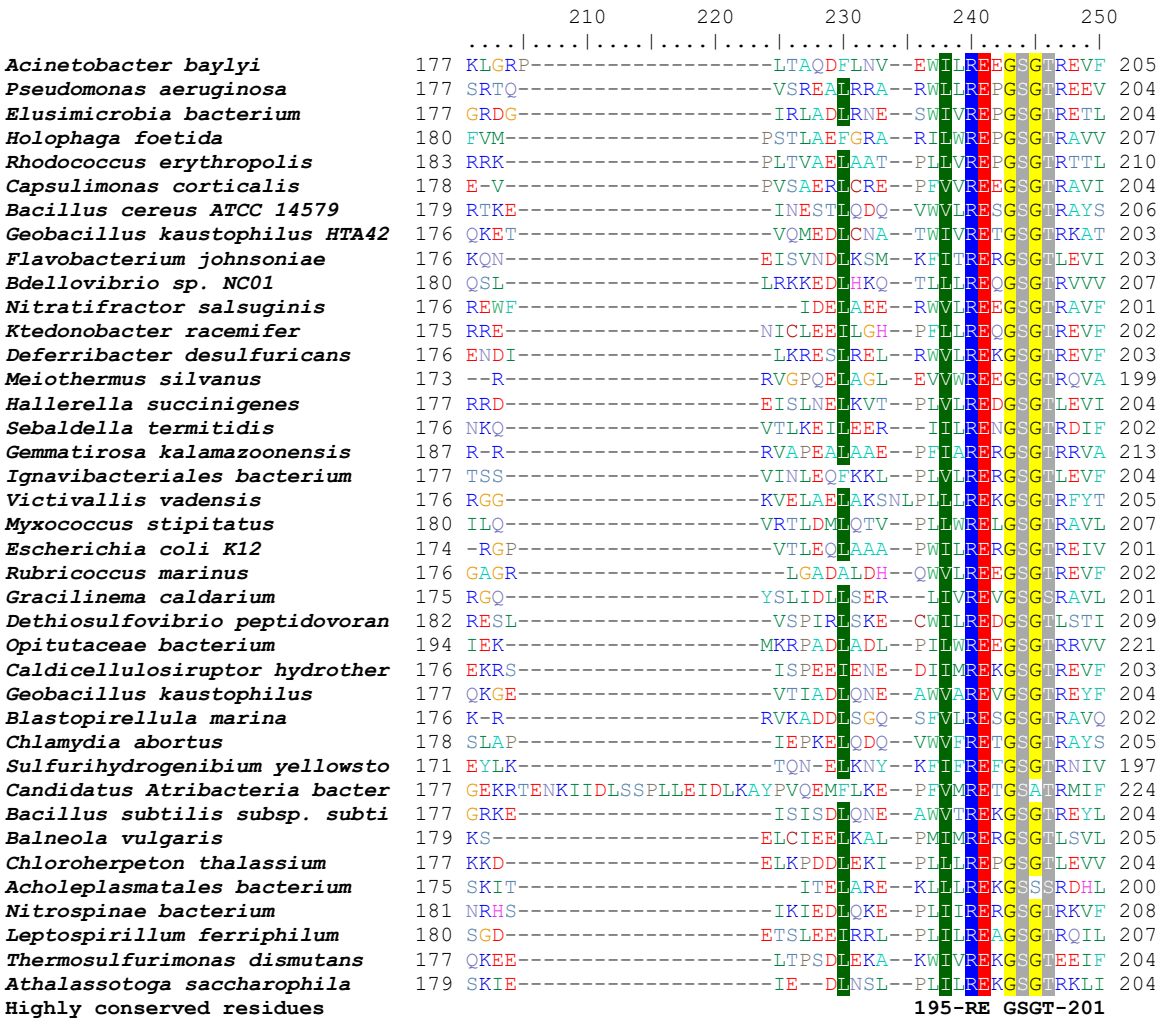
oligomerization. (B) Side view of the ACIAD0746 tetramer showing the DNA-binding domains (DBDs) extended in a conformation that is likely optimal for DNA contact. (C) View of the effector binding pocket with residues critical for sulfite interaction shown. (D) View of a YeiE-EBD dimer with two sulfate molecules in the binding pocket and a third sulfate at an interface between the two EBD molecules. (E) YeiE effector binding pocket showing residues important for ligand interaction.

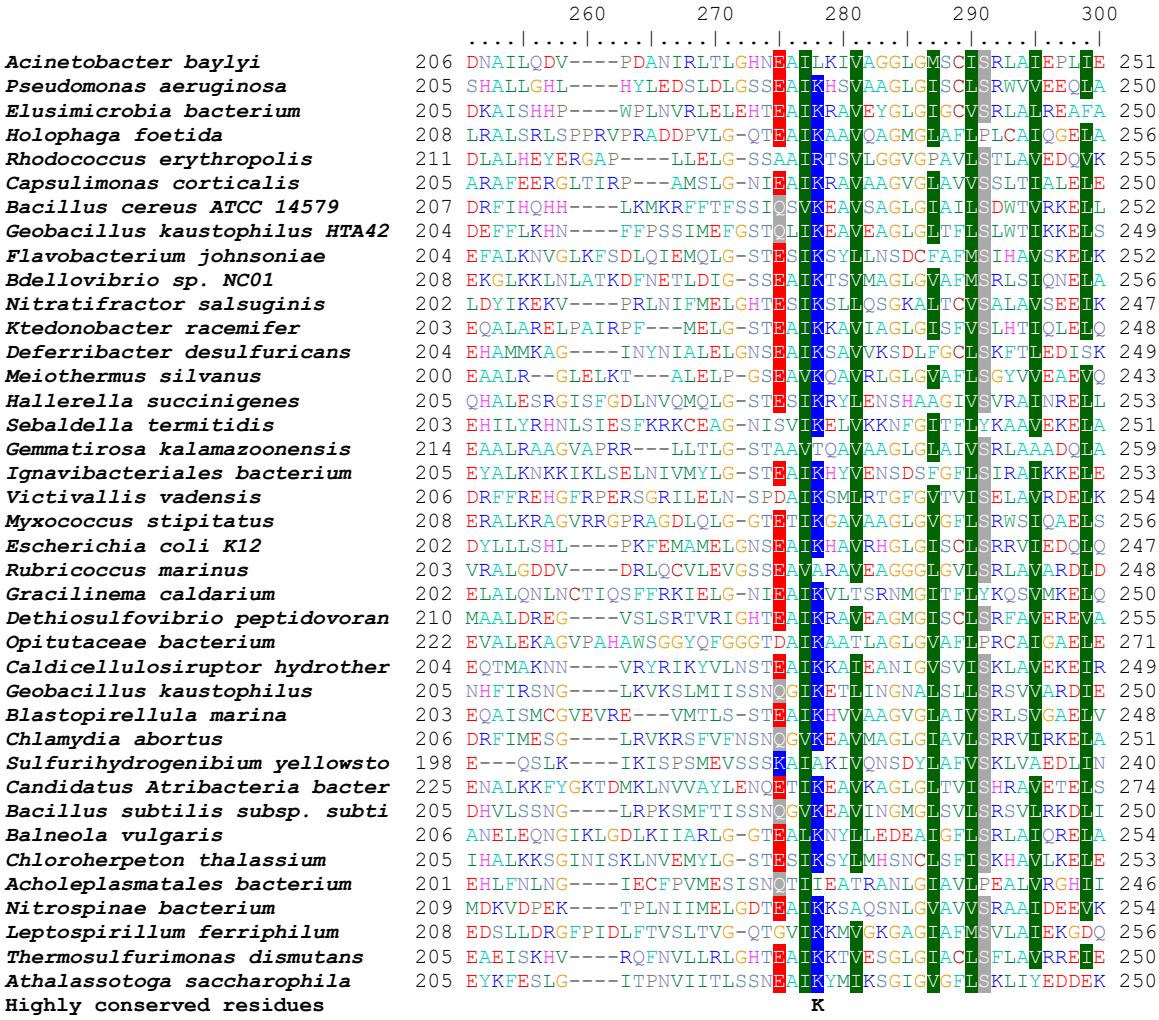

10 20 30 40 50
Acinetobacter baylyi 1 41
Pseudomonas aeruginosa 1 41
Elusimicrobia bacterium 1 41
Holophaga foetida 1 43
Rhodococcus erythropolis 1 44
Capsulimonas corticalis 1 39
Bacillus cereus ATCC 14579 1 41
Geobacillus kaustophilus HTA42 1 38
Flavobacterium johnsoniae 1 38
Bdellovibrio sp. NC01 1 46
Nitratifractor salsuginis 1 41
Ktedonobacter racemifer 1 39
Deferribacter desulfuricans 1 41
Meiothermus silvanus 1 41
Hallerella succinigenes 1 39
Sebaldella termitidis 1 39
Gemmatirosa kalamazoonensis 1 48
Ignavibacteriales bacterium 1 39
Victivallis vadensis 1 39
Myxococcus stipitatus 1 43
Escherichia coli K12 1 41
Rubricoccus marinus 1 41
Gracilinema caldarium 1 39
Dethiosulfovibrio peptidovoran 1 45
Opitutaceae bacterium 1 50
Caldicellulosiruptor hydrother 1 39
Geobacillus kaustophilus 1 39
Blastopirellula marina 1 39
Chlamydia abortus 1 39
Sulfurihydrogenibium yellowsto 1 35
Candidatus Atribacteria bacter 1 39
Bacillus subtilis subsp. subti 1 39
Balneola vulgaris 1 39
Chloroherpeton thalassium 1 39
Acholeplasmatales bacterium 1 39
Nitrospinae bacterium 1 43
Leptospirillum ferriphilum 1 39
Thermosulfurimonas dismutans 1 41
Athalassotoga saccharophila 1 40
Highly conserved residues2
F11
Q31

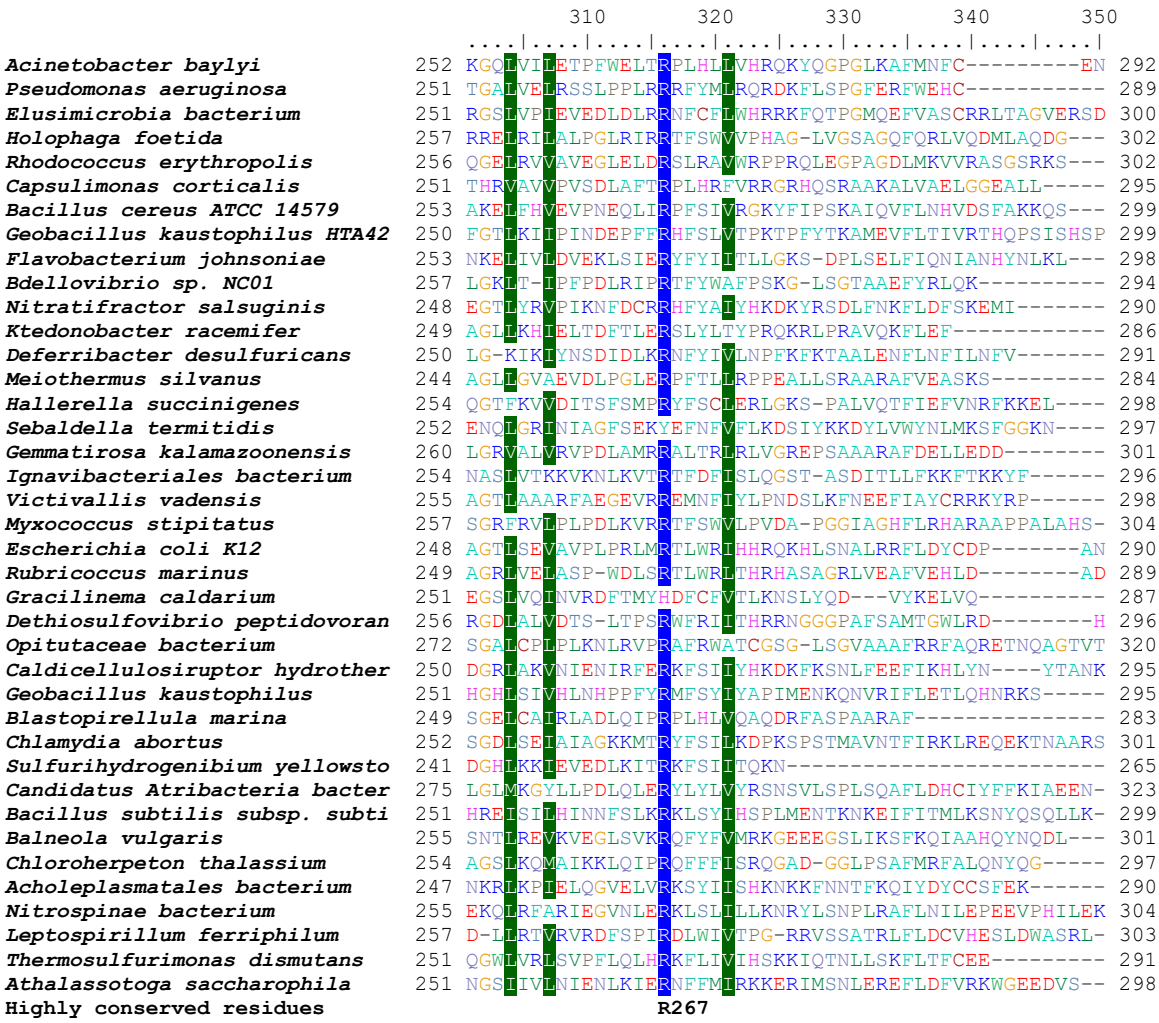

60 70 80 90 100
Acinetobacter baylyi 42 EDGLGAPLFDRLGKR--LQLNDLGRFLLPQALEILGRCEAFEQAAKGELQ 89
Pseudomonas aeruginosa 42 ESQLSTRLFDRVGKR--LQLNEVGRRFLPQALRLLG-VAQLEQQFAVSAA 88
Elusimicrobia bacterium 42 ERQYDTRLFDRLGKA--LRLNELGQTLLPRAIELIEQASALETLLEGRAG 89
Holophaga foetida 44 EAGLGCRLFLRSRSG--MQLTKEGQRLMETAEAIHRLLGEAEQALAEPGS 91
Rhodococcus erythropolis 45 ERQLGMPLIHRRSTG--ATLTPQGTVIVHWARRVLANSRQLLDVAEGMRT 92
Capsulimonas corticalis 40 ERSLGAPLFDRLPRG--VRLTGAGVILADYARRLFALEAEAEEALADLRG 87
Bacillus cereus ATCC 14579 42 ENEFGTTLIQRSPKH--VQVTEAGNILYIHAKQMLSLYEDAKQEINELHN 89
Geobacillus kaustophilus HTA42 39 ERLFDTKLLDRTNKY--VKLNKAGEIVYHYAKEILGLYTRMNQLLDDLMN 86
Flavobacterium johnsoniae 39 EETYKTKLFERNGSK--IALTPAGEILLKHTKEIFEIYREIDFEMSSFIS 86
Bdellovibrio sp. NC01 47 EEELNSALIKRSVTG--IQLTEAGHKLLEQARRIQNIVREAAESVSEAKV 94
Nitratifractor salsuginis 42 ENIIGRPLFDRLNKK--LILNEVGR-NFHQAVEPLYRMEDIEYEFQNTED 88
Ktedonobacter racemifer 40 ERAVGQPLFKQLGRQ--VYLTEAGEVLYDYARRIFALVDEAETQLEHELE 87
Deferribacter desulfuricans 42 ENQIGEPLFERVDKK--LILNDKGKAAKQYVKKFLGHFEELKSVLQSKHV 89
Meiothermus silvanus 42 AERVGEPLFTRHPHG--VRLTAAGEILLPHA---LALKG-AVEALQALEG 85
Hallerella succinigenes 40 EEDVKVRLFERAGSK--LLLTREGEVFLKYAEQILESYEALNYELGLLQQ 87
Sebaldella termitidis 40 ENYYNVKLFNYERKN--LSLTREGELLYDYATTMSADNKRIKDILAEKDT 87
Gemmatirosa kalamazoonensis 49 ERQVGAPLLERGARG--VRLTDAGAVLHARARELFAVERSAEEELRALRG 96
Ignavibacteriales bacterium 40 EKEYGLPLFNRKGSK--IYLTLAGKELYDYSKQVYKLHKNLEDKLNNFRK 87
Victivallis vadensis 40 EQELGVALFIRDGRQ--LRLTRPGRVLLLEAESLLALSENAVRMARNAAA 87
Myxococcus stipitatus 44 ERECGQPLLVRTARG--VRLNEAGQRLLAYAQRHHQLLEEAALAVTGEEV 91
Escherichia coli K12 42 EGQLGVQLFDRVGKR--LVVNEHGRLLYPRALALLEQAVEIEQLFREDNG 89
Rubricoccus marinus 42 EDALGHALFDRAGRG--LRLNADGRRLRGPARAALDAARERAETLLGTDRG 89
Gracilinema caldarium 40 ESQVGKKLFNYESRT--LTLTDAGQLLYRYAVTAWSDGLKTLENISRLED 87
Dethiosulfovibrio peptidovoran 46 ERLLDGPLFGRIGRG--LVLNDRGRLLLPLAEDVLGSVRDFTSVGRSGDE 93
Opitutaceae bacterium 51 EETLGAALFLRTPRG--MTLTPRGRRLLGYARRMAELLAEAAGAVDAAGA 98
Caldicellulosiruptor hydrother 40 EGEFQIKLFDRIGKK--LSLTYTGEILYSYGRRILNLVKEAENILYDVRN 87
Geobacillus kaustophilus 40 EKEFQTKLFIRSPKR--LRMTPTGELLYDRAKQMMALYEQTKQDILEHHH 87
Blastopirellula marina 40 ETALGEKLFDRTGRG--LRLTTVGKLLNEYARKIFTLEAEAERALV--RD 85
Chlamydia abortus 40 ENELGSKLLHRTSKH--VKLTEAGSILYQRAKQILALYEEAKEEIHLLRN 87
Sulfurihydrogenibium yellowsto 36 ENYLGMTLFRRHKSN--LELTEEGKVLYQFASKIIEDYMNMEENLKNVRK 83
Candidatus Atribacteria bacter 40 EKEIGTPLLVRGQRE--LQLTDAGKVLFQYAQQLLNLEKKAVFAIQQEYH 87
Bacillus subtilis subsp. subti 40 EKEFQTALLNRSPKH--FTTTPTGDILYQRAKQMVFLYEQAKAEIYAHHH 87
Balneola vulgaris 40 EAEIGVPLFERKGNS--ICLTGPGEKLLDHMYKAKNLERLIQSDMDIIKS 87
Chloroherpeton thalassium 40 EKHFKQPLFERNGNK--VALTLAGQMLLKHTDAMLEISKQLEYELNLLNE 87
Acholeplasmatales bacterium 40 EKYYNVILFNRINQR--LVITETGKILLQKAKEVIANFDEFENLAIATLE 87
Nitrospinae bacterium 44 EKILGSPLFERIGRR--IELTEAGSIFESYAQMTLTALREGKQVIDDITR 91
Leptospirillum ferriphilum 40 EQHLGVPLFERKGRQ--VLLTEAGVRLLPHARRIMEEIADARARVA-ELS 86
Thermosulfurimonas dismutans 42 ENQLGTPLFDRIGRR--LVLNDRGRLLLSHAREILRQVENVETAERGDSI 89
Athalassotoga saccharophila 41 EEKLNVKLFERIQGGNILKLTEEGFVLYKELESLSRVFDETIDRITNLQK 90
Highly conserved residues E42 R52 G63


110 120 130 140 150
Acinetobacter baylyi 90 S----IDLRLGATLTISDYLIPDLMADFLQI-HPQAHLQLQVGNTRQMIE 134
Pseudomonas aeruginosa 89 PQ---ASLTVAASSTIGNYVLPRLLASFERE-QPGVRVNASIGNTGSAVQ 134
Elusimicrobia bacterium 90 Y----GKLAIGATLTIGNYLATWLVADFLKH-HPESTLHLSVHNTATIVE 134
Holophaga foetida 92 D---LGPLRLGASMTTAAYVMPRALALFQRC-HPGATLLMEVGNTGQIQA 137
Rhodococcus erythropolis 93 ER--SAELTVCASMTVAEHLIPAWLGVFRSL-HPGVTVHLQVYNSTRVLE 139
Capsulimonas corticalis 88 LE--RGTLAVGASMTIGSYLLPEVLARFHRR-YPGIVVNLEIANTEEIQQ 134
Bacillus cereus ATCC 14579 90 VVT--GTLRIGASFTIGEYLLPKILANYANE-NPRVEVHTFISNTEEVLQ 136
Geobacillus kaustophilus HTA42 87 RAS--GELSIGASYTYGEYVLPQMIAKLHQH-YPLIKPTITIGNSNEIVE 133
Flavobacterium johnsoniae 87 ER--QGLLRLGASTTISQYIISPVLARFHQK-QKDIKVNLLNGNTEQIEN 133
Bdellovibrio sp. NC01 95 V---KGHLKIAASTTVAAYALPPLLTEFRKR-FPQISLELQIGNTDDVLK 140
Nitratifractor salsuginis 89 K----GTVRVGASTTIVDYLIPPIACRYMGQ-YPEVRVQLKEGNTQQIAE 133
Ktedonobacter racemifer 88 K----GHLSIGAITTIGIYLLPAILGYYRSH-YPKIELSLEIANADDIQE 132
Deferribacter desulfuricans 90 S-----NMRIGASTTIGNYILPELISNYMKE-YPNVKFNLFVSNTENILK 133
Meiothermus silvanus 86 LK--TGRLRLAASTTIAHYCVPKALARLRRA-HPSLGIELRVGNSQEVLG 132
Hallerella succinigenes 88 KH--AGTLRLGASTTIAQYILPPILAKFNEK-FPQVTISLQNENSTVIEN 134
Sebaldella termitidis 88 ES---ISLSFGTTLTVGEYVMPGILENMLEK-YPGIRITMSVNNTKVLLE 133
Gemmatirosa kalamazoonensis 97 LE--GGTLRIGASTTIATYLLPRLLGRFRAR-HPGVELRVATANTRAVAR 143
Ignavibacteriales bacterium 88 EK--IGELKLGASTTIAQYVLPRILAEFKKN-YPTIKTSLLSGNSEQIAK 134
Victivallis vadensis 88 DV---LSFQLGATMTAGGYVLPPLAAEFMRE-HANRRIHLCIANTEEISD 133
Myxococcus stipitatus 92 L---TGELVLAASTTLASYVVPDLLASFLRL-HRGLQVRLEVGNSEQVLG 137
Escherichia coli K12 89 ------AIRIYASSTIGNYILPAVIARYRHD-YPQLPIELSVGNSQDVMQ 132
Rubricoccus marinus 89 -----GRLIVGASTTIANHVLASALASFAAD-RLAADLRLVVGNTREIRD 133
Gracilinema caldarium 88 HP----LVRIGATRSIGAYLLPELLVSYMHR-HPATELTVSVENTRDLLE 132
Dethiosulfovibrio peptidovoran 94 PS---GDLTVACSTTIANYLFPFHMKAFMDE-YPRISLILKVGNTMEIEE 139
Opitutaceae bacterium 99 AP--VEPLTLAASTTPAAHVLPVIIARYQAAGHSLAGFTLRVGNTDQVLG 146
Caldicellulosiruptor hydrother 88 MRM--GRLNVGASTTVGIYLLPKIIGDFREK-Y-SVDVYFTIDNTAGIEK 133
Geobacillus kaustophilus 88 SVK--GKLKIGASFTIGEYILPPLLPDFQTR-YPELELEVMIGNTKEIVE 134
Blastopirellula marina 86 VR--RGELFLGASTTIAGYLLPQVLARFHQQ-HPEIRVHVSVGNTDFVHR 132
Chlamydia abortus 88 EVT--GSLTIGASFTIGEYVLPHLLADYTMD-YPLVDVYVTIANTEEISR 134
Sulfurihydrogenibium yellowsto 83 --T--SILYIGCSSTIGDYLLPKIITKFISE-NPEVSIKIFIGNSKEVED 128
Candidatus Atribacteria bacter 88 IIK--GNLEIVASSVPSSYILPGLMKDFLKK-YPLVTFSAKHWDTEQVFE 134
Bacillus subtilis subsp. subti 88 YVK--GELKIAASFTIGEYILPPLLAQLQKL-YPELNLDVMIGNTEEVSE 134
Balneola vulgaris 88 RREARGELRVGASTTISLYVLPKVFSAFHKR-FPKIQLLLINRNSENILK 136
Chloroherpeton thalassium 88 SH--KGRLKLGASTTLAQYVLPPILAKFHGV-YKEVQLRMITANTEHIEQ 134
Acholeplasmatales bacterium 88 NP-----IIHIGASLTFGITHLPRIMNYVKQN-YPNVQLLTTINNSKEIEQ 132
Nitrospinae bacterium 92 KVV--GKLRLGASTTIGNYILPDYLGKFKKK-YPSARIEMVVGNTNNIEQ 138
Leptospirillum ferriphilum 87 SGA-LGNLRIGSSTTIGTAILPSILYEFSQK-NPQIRTSLSILNSHRIVF 134
Thermosulfurimonas dismutans 90 T----GDLRVGASTTIGSYLLPYLIGAFVDR-YPGVTINLSIGNTEQIES 134
Athalassotoga saccharophila 91 NIE--SLLKIGAKTIGDYLLPKIMVDFLKA-HN-VNFSVDIQNTSDVVE 136
Highly conserved residues S T N128


160 170 180 190 200
Acinetobacter baylyi 135 AVNQFQLDLALIEGSCHLPQLQ--CIH-WRNDELAVCCA-----PDHPLA 176
Pseudomonas aeruginosa 135 DVVTFAADIGLIEGPCHEPGLR--VEP-WLEDSLIVVAA-----PGHPLA 176
Elusimicrobia bacterium 135 QIVHHVLDLGLIEGSCRHPDLI--VEP-WVADELVVFAD-----PAHRLA 176
Holophaga foetida 138 WLQEGRIDLGAVEGLERAPNLR---LEPFLDDELVCVGP-----GRLPLG 179
Rhodococcus erythropolis 140 AVEVGMCDLGFVESPTVPKSLP---SVTVARDSLVVVVH----PDHPWAR 182
Capsulimonas corticalis 135 RLLEGAMDLGFTEGFAEEDDLE---ATVFAKDAMVVIAP----PNHPIRA 177
Bacillus cereus ATCC 14579 137 SLRSNQIDIGLVEGQVVYADVN--VET-FMQDEMKLVVP-----PNHPLL 178
Geobacillus kaustophilus HTA42 134 MVRDHQLDVGIIEMDIEPKNVY--IEP-FAKDQMVVVAS-----AHHPYA 175
Flavobacterium johnsoniae 134 ALINKEIEIGIVEGQSKNQSIK---YIPFLKDELVLVCS-----SKNSFV 175
Bdellovibrio sp. NC01 141 KLRNGSVPLGLVEGQARASAIH---LEPFLEDELLFVCG--------TVQ 179
Nitratifractor salsuginis 134 MVKQGELDMGFVEGEVSDPDLI--REV-IGEDELIVVTA-----NKEIAG 175
Ktedonobacter racemifer 133 RLLANEVEVGLVEGPVTHADLH---HEVWQRDELVLIVG-----NDHQLA 174
Deferribacter desulfuricans 134 KIDNFEIDIGFIEGVADSSKYQ--LIQ-WMEDELVIFSS-----ITNPIA 175
Meiothermus silvanus 133 LLEAGEAEIGLVEG--HLPQLE---AEVLARDRLFLVTP----PGHPLAG 173
Hallerella succinigenes 135 ALRERQIDLGFIESNAEPAGLR---VTPFLKDELVLVAH-----VKNPLT 176
Sebaldella termitidis 134 KLKSGEINFVILEGSFEKTKYD---SVLFSKEEFIAVCS-----PDSELK 175
Gemmatirosa kalamazoonensis 144 RLVQRRLDVALVEGPVAHPRIE---VLPWRDDALVVVAP----VDHPLAR 186
Ignavibacteriales bacterium 135 ALINEKIDLGLVEGKIKNPEIK---YEIFLNDELVLVSS-----NKNKSV 176
Victivallis vadensis 134 KLKKRFFDLALVEGAFDRELFL---SRTLLADELLLAGS-----PRLPFF 175
Myxococcus stipitatus 138 WVGEGRAPLGLVEGHARAAGLR---LEHYLDDELLPVVA-----TQAPKE 179
Escherichia coli K12 133 AVLDFRVDIGFIEGPCHSTEII--SEP-WLEDELVVFAA-----PTSPLA 174
Rubricoccus marinus 134 RLLDVALDLAYVEGPTSHPELV--ATP-WREDRLVVFAA-----PDHPLA 175
Gracilinema caldarium 133 KLERGLLDFIFLEGIFDQDAYN---VQVVMKDAFIPLCS-----PDHRLA 174
Dethiosulfovibrio peptidovoran 140 EISKGCADIGLVEGELRGEHLR--SED-WIRDDLSIFCS-----PQDPLA 181
Opitutaceae bacterium 147 WVRDGTVPLAVVEGQPKASGIH---LELFRKDELLPVWAPATLAAAVVAR 193
Caldicellulosiruptor hydrother 134 LILDNAIDLGIVEGPVHSRDIV--VIP-YIDDELYLVSS-----KNHRWA 175
Geobacillus kaustophilus 135 LVKSYQVDIGLIEGQTNEKELS--VHP-FMQDELVIVAS-----NRHELA 176
Blastopirellula marina 133 QVLDYRVDLGLTEGFVNEPGLT---ATSFAEDELVVVAA----ATHPLVK 175
Chlamydia abortus 135 GVRANELDLGLIEGEVEFPELL--VAPPFAKDEMVLVAP-----NDYPLC 177
Sulfurihydrogenibium yellowsto 129 GVLSKIFNIGLVEDNIISNKLD--ISE-FYEDEIILIAS-----KNNSIC 170
Candidatus Atribacteria bacter 135 NILNFNYELGFAGEPVRSNELN--QIQ-LLKDNLILVAA-----ANVSLP 176
Bacillus subtilis subsp. subti 135 RVRMLQADIGLIEGHTNENELE--IEP-FMEDEMCIAAP-----NQHPLA 176
Balneola vulgaris 137 ALVNKEIDLAIIEGKYEVNSFQ---QAEFMEDEIIPVCS-----SRSPYA 178
Chloroherpeton thalassium 135 ALLKKEIELGVIEGESKRREIH---YTPFARDEIVLVSS-----AKNPLA 176
Acholeplasmatales bacterium 133 GVLDGTIDFGIVEGSISNKNIK---IENFIDDKLLIVCG-----YDYPIN 174
Nitrospinae bacterium 139 SILKGEIDLGIVEGPVRNKSIE--VYR-FRTDELVVIFA-----NNHPWK 180
Leptospirillum ferriphilum 135 DLKTSEIDIGFIEGDLTRTEAQGVRRSFLAQDRLVLVDS-----REKPFV 179
Thermosulfurimonas dismutans 135 GILSGAFDVGFIEGYTHHEEIK--ALP-WLDDELVIIAS-----PRHPLS 176
Athalassotoga saccharophila 137 EVLDEEISVGFVEIPFFHKSLE--SEK-FYMDEMNLISS-----PGFPLP 178
Highly conserved residues E147 D163


210 220 230 240 250
Acinetobacter baylyi 177 KLGRP----------LTAQDFLNV--EWILREEGSGTREVF 205
Pseudomonas aeruginosa 177 SRTQ----------VSREALRRA--RWLLREPGSGTREEV 204
Elusimicrobia bacterium 177 GRDG----------IRLADLRNE--SWIVREPGSGTRETL 204
Holophaga foetida 180 FVM----------PSTLAEFGRA--RILWREPGSGTRAVV 207
Rhodococcus erythropolis 183 RRK----------PLTVAELAAT--PLLVREPGSGTRTTL 210
Capsulimonas corticalis 178 E-V----------PVSAERLCRE--PFVVREEGSGTRAVI 204
Bacillus cereus ATCC 14579 179 RTKE----------INESTLQDQ--VWVLRESGSGTRAYS 206
Geobacillus kaustophilus HTA42 176 QKET----------VQMEDLCNA--TWIVRETGSGTRKAT 203
Flavobacterium johnsoniae 176 KQN----------EISVNDLKSM--KFITRERGSGTLEVI 203
Bdellovibrio sp. NC01 180 QSL----------LRKKEDLHKQ--TLLLREQGSGTRVVV 207
Nitratifractor salsuginis 176 REWF----------IDELAEE--RWVLREEGSGTRAVF 201
Ktedonobacter racemifer 175 RRE----------NICLEEILGH--PFLLREQGSGTREVF 202
Deferribacter desulfuricans 176 ENDI----------LKRESLREL--RWVLREKGSGTREVF 203
Meiothermus silvanus 173 --R----------RVGPQELAGL--EVVWREEGSGTRQVA 199
Hallerella succinigenes 177 RRD----------EISLNELKVT--PLVLREDGSGTLEVI 204
Sebaldella termitidis 176 NKQ----------VTLKEILEER---IILRENGSGTRDIF 202
Gemmatirosa kalamazoonensis 187 R-R----------RVAPEALAAE--PFIARERGSGTRRVA 213
Ignavibacteriales bacterium 177 TSS----------VINLEQFKKL--PLVLREKGSGTLEVF 204
Victivallis vadensis 176 RGG----------KVELAELAKSNLPLLLREKGSGTRFYT 205
Myxococcus stipitatus 180 ILQ----------VRTLDMLQTV--PLLWRELGSGTRAVL 207
Escherichia coli K12 174 -RGP----------VTLEQLAAA--PWILREKGSGTREIV 201
Rubricoccus marinus 176 GAGR----------LGADALDH--QWVLREEGSGTREVF 202
Gracilinema caldarium 175 RGQ----------YSLIDLLSER---LIVREVGSGSRAVL 201
Dethiosulfovibrio peptidovoran 182 RESL----------VSPIRLSKE--CWILREDGSGTLSTI 209
Opitutaceae bacterium 194 IEK----------MKRPADLADL--PILWREEGSGTRRVV 221
Caldicellulosiruptor hydrother 176 EKRS----------ISPEEIENE--DIIMREKGSGTREVF 203
Geobacillus kaustophilus 177 QKGE----------VTIADLQNE--AWVAREVGSGTREYF 204
Blastopirellula marina 176 K-R----------RVKADDLSGQ--SFVLRESGSGTRAVQ 202
Chlamydia abortus 178 SLAP----------IEPKELQDQ--VWVFRETGSGTRAYS 205
Sulfurihydrogenibium yellowsto 171 EYLK----------TQN-ELKNY--KFIFREFGSGTRNIV 197
Candidatus Atribacteria bacter 177 GEKRTENKIIDLSSPLLEIDLKAYPVQEMFLKE--PFVMRETGSATRMIF 224
Bacillus subtilis subsp. subti 177 GRKE----------ISISDLQNE--AWVTREKGSGTREYL 204
Balneola vulgaris 179 KS----------ELCIEELKAL--PMIMRERGSGTLSVL 205
Chloroherpeton thalassium 177 KKD----------ELKPDDLEKI--PLLLREPGSGTLEVV 204
Acholeplasmatales bacterium 175 SKIT----------ITELARE--KLLLREKGSSSRDHL 200
Nitrospinae bacterium 181 NRHS----------IKIEDLQKE--PLIIRERGSGTRKVF 208
Leptospirillum ferriphilum 180 SGD----------ETSLEEIRRL--PLILREAGSGTRQIL 207
Thermosulfurimonas dismutans 177 QKEE----------LTPSDLEKA--KWIVREKGSGTEEIF 204
Athalassotoga saccharophila 179 SKIE----------IE--DLNSL--PLILREKGSGTRKLI 204
Highly conserved residues
195-RE GSGT-201


260 270 280 290 300
Acinetobacter baylyi 206 DNAILQDV----PDANIRLTLGHNEAILKIVAGGLGMSCISRLAIEPIIE 251
Pseudomonas aeruginosa 205 SHALLGHL----HYLEDSLDLGSSEAIKHSVAAGLGISCLSRWVVEEQLA 250
Elusimicrobia bacterium 205 DKAISHHP----WPLNVRLELEHTEAIKRAVEYGLGIGGVSRLALREAFA 250
Holophaga foetida 208 LRALSRLSPPRVPRADDPVLG-QTEAIKAAVQAGMGLAFLPLCAIQGELA 256
Rhodococcus erythropolis 211 DLALHEYERGAP----LLELG-SSAAIRTSVLGGVGPAVLSTLAVEDQVK 255
Capsulimonas corticalis 205 ARAFEERGLTIRP---AMSLG-NIEAIKRAVAAGVGLAVVSSLTIALELE 250
Bacillus cereus ATCC 14579 207 DRFIHQHH----LKMKRFFTFSSIQSVKEAVSAGLGIAILSDWTVRKELL 252
Geobacillus kaustophilus HTA42 204 DEFFLKHN----FFPSSIMEFGSTQLIKEAVEAGLGLTFLSLWTIKKELS 249
Flavobacterium johnsoniae 204 EFALKNVGLKFSDLQIEMQLG-STESIKSYLLNSDCFAFMSIHAVSKELK 252
Bdellovibrio sp. NC01 208 EKGLKKLNLATKDFNETLDIG-SSEAIKTSVMAGLGVAFMSRLSIQNELA 256
Nitratifractor salsuginis 202 LDYIKEKV----PRLNIFMELGHTESIKSLLQSGKALTCVSALAVSEEIK 247
Ktedonobacter racemifer 203 EQALARELPAIRPF---MELG-STEAIKKAVIAGLGISFVSLHTIQLELQ 248
Deferribacter desulfuricans 204 EHAMMKAG----INYNIALELGNSEAIKSAVVKSDLFGCLSKFTLEDISK 249
Meiothermus silvanus 200 EAALR--GLELKT---ALELP-GSEAVKQAVRLGLGVAFLSGYVVEAEVQ 243
Hallerella succinigenes 205 QHALESRGISFGDLNVQMQLG-STESIKRYLENSHAAGIVSVRAINRELL 253
Sebaldella termitidis 203 EHILYRHNLSIESFKRKCEAG-NISVIKELVKKNFGTTFLYKAAVEKELA 251
Gemmatirosa kalamazoonensis 214 EAALRAAGVAPRR---LLTLG-STAAVTQAVAAGLGLAIVSRLAAADQLA 259
Ignavibacteriales bacterium 205 EYALKNKKIKLSELNIVMYLG-STEAIKHYVENSDSFGFLSIRAIKKELE 253
Victivallis vadensis 206 DRFFREHGFRPERSGRILELN-SPDAIKSMLRTGFGVTVISELAVRDELK 254
Myxococcus stipitatus 208 ERALKRAGVRRGPRAGDLQLG-GTETIKGAVAAGLGVGFLSRWSIQAELS 256
Escherichia coli K12 202 DYLLLSHL----PKFEMAMELGNSEAIKHAVRHGLGISCLSRRVIEDQLQ 247
Rubricoccus marinus 203 VRALGDDV----DRLQCVLEVGSSEAVARAVEAGGGLGVLSRLAVARDLD 248
Gracilinema caldarium 202 ELALQNLNCTIQSFFRKIELG-NIEAIKVLTSRNMGITFLYKQSVMKELQ 250
Dethiosulfovibrio peptidovoran 210 MAALDREG----VSLSRTVRIGHTEAIKRAVEAGMGISCLSRFAVEREVA 255
Opitutaceae bacterium 222 EVALEKAGVPAHAWSGGYQFGGGTDAIKAATLAGLGVAFLPRCAIGAELE 271
Caldicellulosiruptor hydrother 204 EQTMAKNN----VRYRIKYVLNSTEAIKKAIEANIGVSVISKLAVEKEIR 249
Geobacillus kaustophilus 205 NHFIRSNG----LKVKSLMIISSNQGIKETLINGNALSLLSRSVVARDIE 250
Blastopirellula marina 203 EQAISMCGVEVRE---VMTLS-STEAIKHVVAAGVGLAIVSRLSVGAELV 248
Chlamydia abortus 206 DRFIMESG----LRVKRSFVFNSNQGVKEAVMAGLGIAVLSRRVIRKELA 251
Sulfurihydrogenibium yellowsto 198 E---QSLK----IKISPSMEVSSSKAIAKIVQNSDYLAFVSKLVAEDLIN 240
Candidatus Atribacteria bacter 225 ENALKKFYGKTDMKLNVVAYLENQETIKEAVKAGLGLTVISHRAVETELS 274
Bacillus subtilis subsp. subti 205 DHVLSSNG----LRPKSMFTISSNQGVKEAVINGMGLSVLSRSVLRKDLI 250
Balneola vulgaris 206 ANELEQNGIKLGDLKIIARLG-GTEALKNYLLEDEAIGFLSRLAIQRELA 254
Chloroherpeton thalassium 205 IHALKKSGININSKLNVEMYLG-STESIKSYLMHSNCLSFISKHAVLKELE 253
Acholeplasmatales bacterium 201 EHLFNLNG----IECFPVMESISNQTIIEATRANLGIAVLPEALVRGHII 246
Nitrospinae bacterium 209 MDKVDPEK----TPLNIIMELGDTEAIKKSAQSNLGVAVVSRAAIDEEVK 254
Leptospirillum ferriphilum 208 EDSLLDRGFPIDLFTVSLTVG-QTGVIKKMVGKGAGIAFMSVLAIEKGDQ 256
Thermosulfurimonas dismutans 205 EAEISKHV----RQFNVLLRLGHTEAIKKTVESGLGIACLSFLAVRREIE 250
Athalassotoga saccharophila 205 EYKFESLG----ITPNVIITLSSNEAIKYMIKSGIGVGFLSKLIYEDDEK 250
Highly conserved residues K


310 320 330 340 350
Acinetobacter baylyi 252 KGQLVILETPFWELTRPLHLLVHRQKYQGPGLKAFMNFC--------EN 292
Pseudomonas aeruginosa 251 TGALVELRSSLPPLRRRFYMLRQRDKFLSPGFERFWEHC---------- 289
Elusimicrobia bacterium 251 RGSLVPIEVEDLDLRRNFCFLWHRRKFQTPGMQEFVASCRRLTAGVERSD 300
Holophaga foetida 257 RRELRILALPGLRIRRTFSWVVPHAG-LVGSAGQFQRLVQDMLAQDG--- 302
Rhodococcus erythropolis 256 QGELRVVAVEGLELDRSLRAVWRPPRQLEGPAGDLMKVVRASGSRKS--- 302
Capsulimonas corticalis 251 THRVAVVPVSDLAFTRPLHRFVRRGRHQSRAAKALVAELGGEALL----- 295
Bacillus cereus ATCC 14579 253 AKELFHVEVPNEQLIRPFSIVRGKYFIPSKAIQVFLNHVDSFAKKQS--- 299
Geobacillus kaustophilus HTA42 250 FGTLKIIPINDEPFFRHFSLVTPKTPFYTKAMEVFLTIVRTHQPSISHSP 299
Flavobacterium johnsoniae 253 NKELIVLDVEKLSIERYFYIITLLGKS-DPLSELFIQNIANHYNLKL--- 298
Bdellovibrio sp. NC01 257 LGKLT-IPFPDLRIPRTFYWAFPSKG-LSGTAAEFYRLQK---------- 294
Nitratifractor salsuginis 248 EGTLYRVPIKNFDCRRHFYAIYHKDKYRSDLFNKFLDFSKEMI------- 290
Ktedonobacter racemifer 249 AGLLKHIELTDFTLERSLYLTYPRQKRLPRAVQKFLEF------------ 286
Deferribacter desulfuricans 250 LG-KIKIYNSDIDLKRNFYIVLNPFKFKTAALENFLNFILNFV------- 291
Meiothermus silvanus 244 AGLIGVAEVDLPGLERPFTLLRPPEALLSRAARAFVEASKS--------- 284
Hallerella succinigenes 254 QGTFKVVDITSFSMPRYFSCLERLGKS-PALVQTFIEFVNRFKKEL---- 298
Sebaldella termitidis 252 ENQLGRINIAGFSEKYEFNFVFLKDSIYKKDYLVWYNLMKSFGGKN---- 297
Gemmatirosa kalamazoonensis 260 LGRVALVRVPDLAMRRALTRLRLVGREPSAAARAFDELLEDD-------- 301
Ignavibacteriales bacterium 254 NASIVTKKVKNLKVTRTFDFISLQGST-ASDITLLFKKFTKKYF------ 296
Victivallis vadensis 255 AGTLAAARFAEGEVRREMNFIYLPNDSLKFNEEFIAYCRRKYRP------ 298
Myxococcus stipitatus 257 SGRFRVLPLPDLKVRRTFSWVLPVDA-PGGIAGHFLRHARAAPPALAHS- 304
Escherichia coli K12 248 AGTLSEVAVPLPRLMRTLWRIHHRQKHLSNALRRFLDYCDP-------AN 290
Rubricoccus marinus 249 AGRLVELASP-WDLSRTLWRLTHRHASAGRLVEAFVEHLD--------AD 289
Gracilinema caldarium 251 EGSLVQINVRDFTMYHDFCFVTLKNSLYQD---VYKELVQ---------- 287
Dethiosulfovibrio peptidovoran 256 RGDLALVDTS-LTPSRWFRIITHRRNGGGPAFSAMTGWLRD--------H 296
Opitutaceae bacterium 272 SGALCPLPLKNLRVPRAFRWATCGSG-LSGVAAAFRRFAQRETNQAGTVT 320
Caldicellulosiruptor hydrother 250 DGRLAKVNIENIRFERKFSIIYHKDKFKSNLFEEFIKHLYN----YTANK 295
Geobacillus kaustophilus 251 HGHLSIVHLNHPPFYRMFSYIYAPIMENKQNVRIFLETLQHNRKS----- 295
Blastopirellula marina 249 SGELCAIRLADLQIPRPLHLVQAQDRFASPAARAF--------------- 283
Chlamydia abortus 252 SGDLSEIAIAGKKMTRYFSILKDPKSPSTMAVNTFIRKLREQEKTNAARS 301
Sulfurihydrogenibium yellowsto 241 DGHLKKIEVEDLKITRKFSIITQKN------------------------ 265
Candidatus Atribacteria bacter 275 LGLMKGYLLPDLQLERYLYLVYRSNSVLSPLSQAFLDHCIYFFKIAEEN- 323
Bacillus subtilis subsp. subti 251 HREISILHINNFSLKRKLSYIHSPLMENTKNKEIFITMLKSNYQSQLLK- 299
Balneola vulgaris 255 SNTLREVKVEGLSVKRQFYFVMRKGEEEGSLIKSFKQIAAHQYNQDL--- 301
Chloroherpeton thalassium 254 AGSLKQMAIKKLQIPRQFFFISRQGAD-GGLPSAFMRFALQNYQG----- 297
Acholeplasmatales bacterium 247 NKRLKPIELQGVELVRKSYIISHKNKKFNNTFKQIYDYCCSFEK------ 290
Nitrospinae bacterium 255 EKQLRFARIEGVNLERKLSLILLKNRYLSNPLRAFLNILEPEEVPHILEK 304
Leptospirillum ferriphilum 257 D-LLRTVRVRDFSPIRDLWIVTPG-RRVSSATRLFLDCVHESLDWASRL- 303
Thermosulfurimonas dismutans 251 QGWLVRLSVPFLQLHRKFLIVIHSKKIQTNLLSKFLTFCEE--------- 291
Athalassotoga saccharophila 251 NGSIIVLNIENLKIERNFFMIRKKERIMSNLEREFLDFVRKWGEEDVS-- 298
Highly conserved residues R267

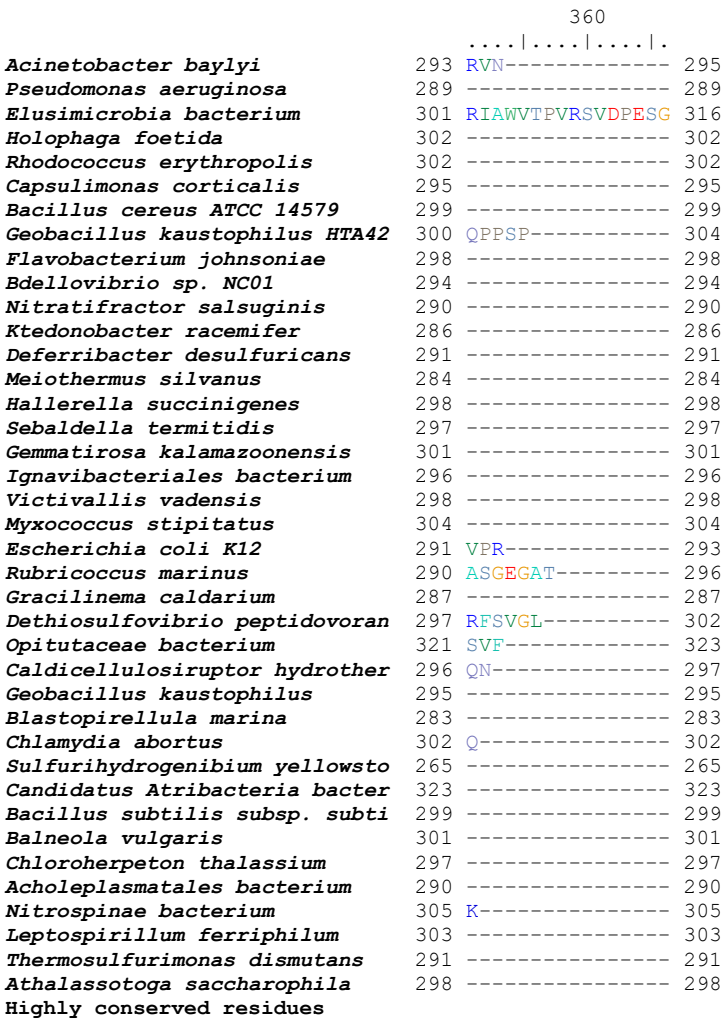


Figure 3.2. Sequence alignment[1] of ACIAD0746 orthologs from different phyla.

[1] Alignment generated using CLUSTALW in BioEdit with a threshold for shading at 85%.

[2] Highly conserved residues discussed in the text, primarily associated with sulfite binding, are numbered according to the ADP1 sequence position.

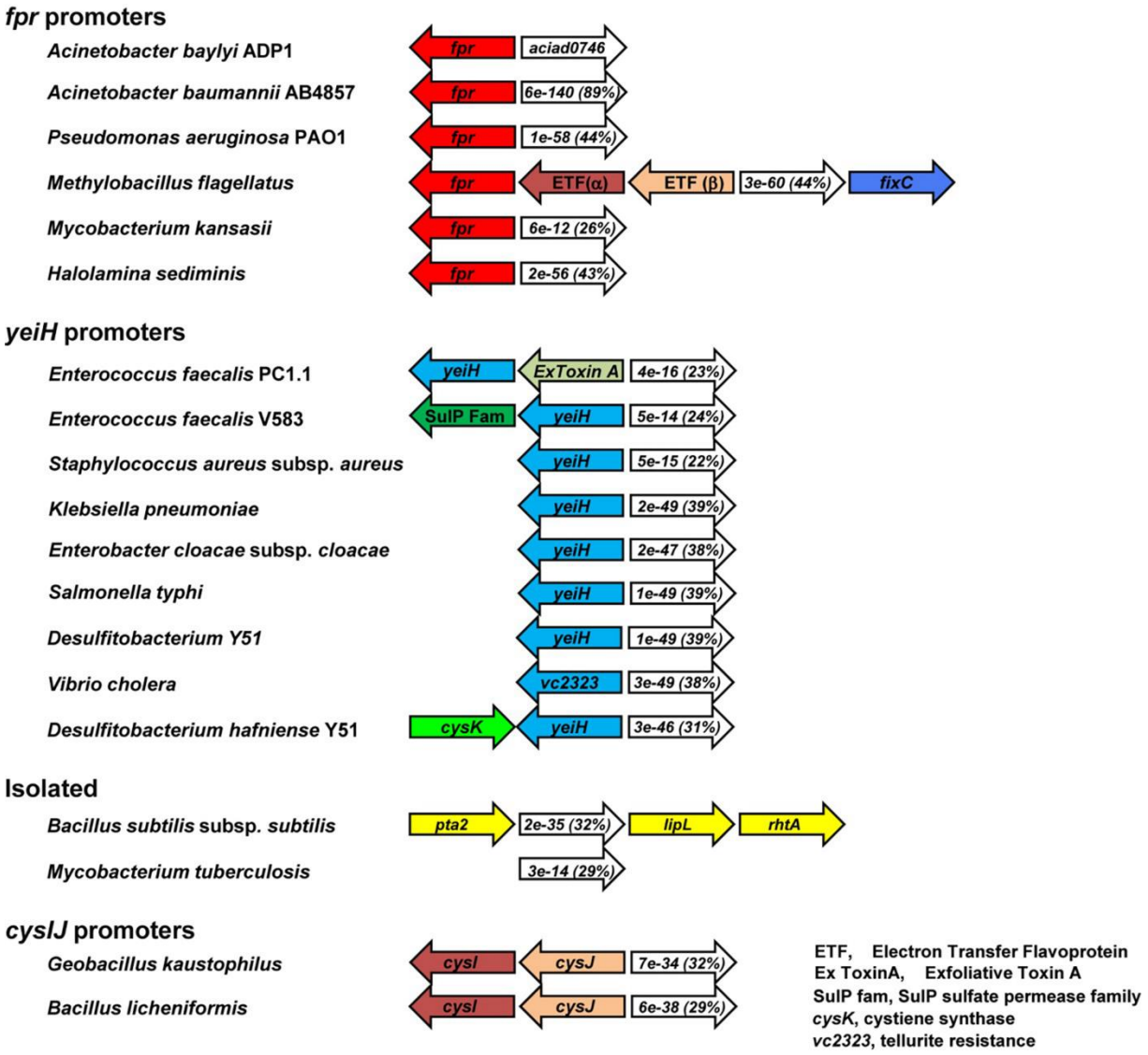


Figure 3.3. Common genome arrangements of ACIAD0746 orthologs. White arrows indicate ACIAD0746 orthologs while the numbers inside the arrows indicate the expect value of the protein compared to ACIAD0746. The percent identity is in parenthesis.

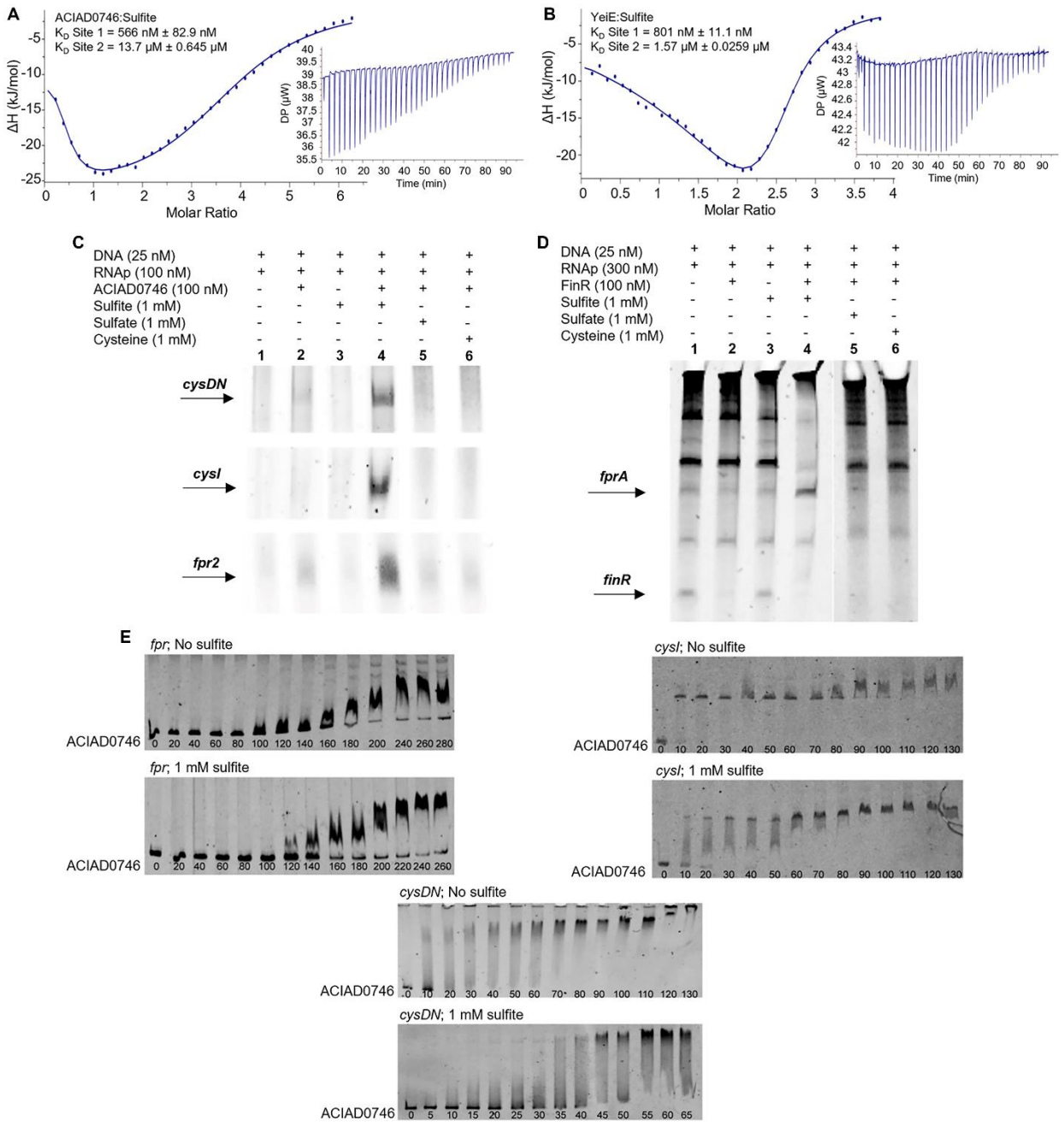


Figure 3.4. ACIAD0746, YeiE, and FinR functional studies. (AB) Interaction of sulfite with ACIAD0746 and YeiE. (CD) ACIAD0746 and FinR transcriptional regulation at different promoters involved in sulfur assimilation. (E) ACIAD0746 DNA interaction at the *fpr*, *cysI*, and *cysDN* promoters.

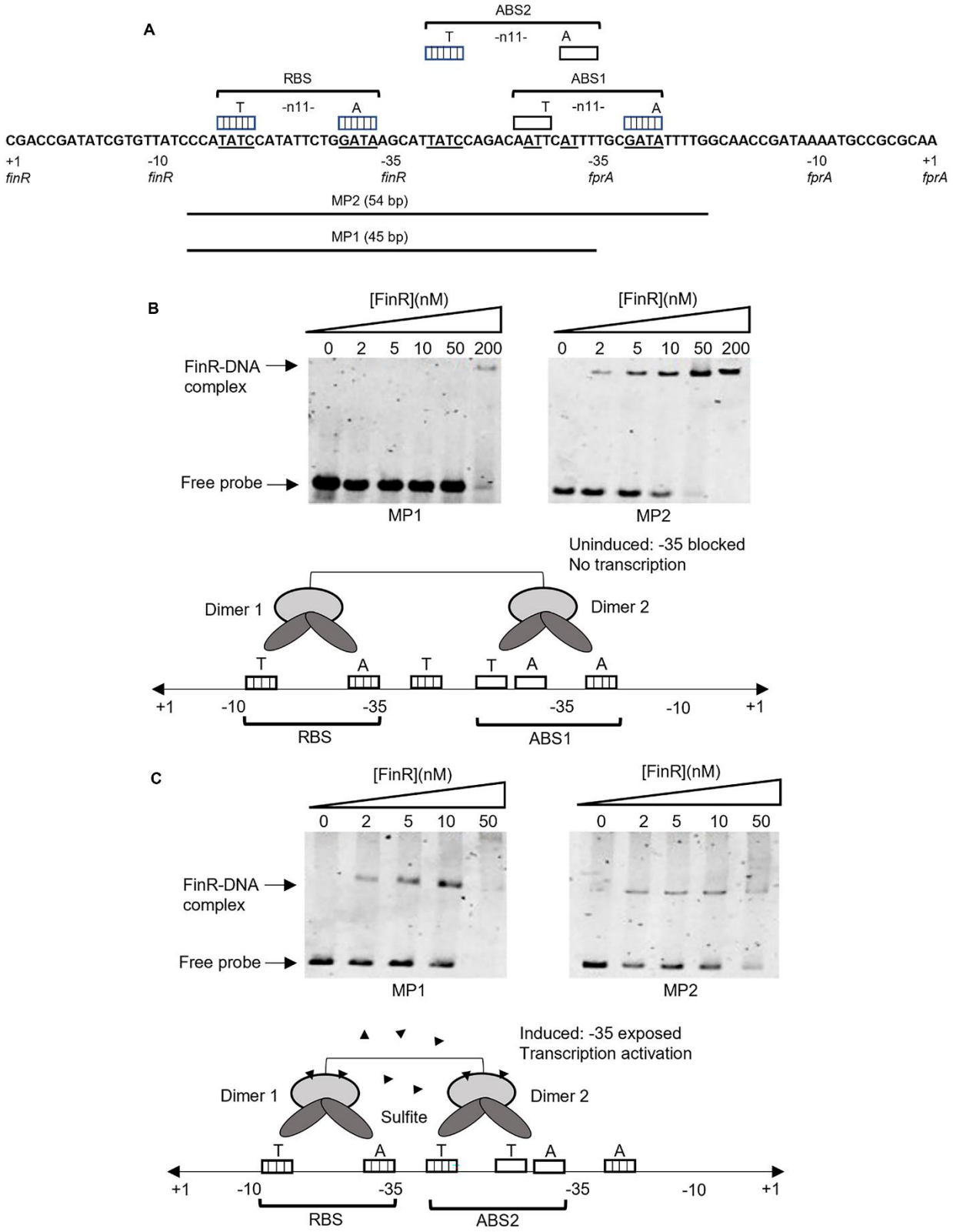


Figure 3.5. FinR classic “sliding dimer” model of regulation. (A) Classical T-N11-A

motifs with a TATC/GATA consensus sequence as potential FinR primary and secondary binding sites in the *finR–fprA* intergenic region of pseudomonads. (B) FinR affinity for MP1 and MP2, in the absence of sulfite. (C) FinR affinity for MP1 and MP2, in the presence of sulfite 1 mM. Striped rectangles indicate perfect TATC/GATA repeat sequences, whereas empty rectangles indicate imperfect TATC/GATA palindromic sequences.

3.6 Start Methods

Cloning, protein production and purification

All bacterial strains, plasmids, primers, and DNA used in the study are listed in the key resources table (**Table A3.7**). *A. baylyi* ADP1, *E. coli* XL1-Blue, and *P. aeruginosa* PAO1 genomic DNA were extracted from cultures using the Qiagen DNEasy blood and tissue kit. Most promoter regions used in transcriptional assays and electrophoretic mobility shift assays (EMSAs) in this study were amplified from *A. baylyi* ADP1 and *P. aeruginosa* PAO1 genomic DNA and subcloned into pUC18. The clones were subsequently amplified by PCR for the aforementioned analysis. Exceptionally, the *cysM*, *ssu*, *sbp,* and *fpr2* promoters used in the experiments were obtained directly from genome amplification. To create the pUC18 promoter constructs, reactions containing 1 µL (50 ng/µL) pUC18 plasmid DNA, 2 µL target promoter PCR product (various concentrations), 2 µL 10 mM ATP, 2 µL Fast-Link™ ligase buffer, and 1 µL ZraI were assembled then incubated at 37˚C for 1 hr. After reactions were cooled to room

temperature for 5 min, 1 µL T4 DNA ligase was added and the ligation allowed to proceed for 1 hour at room temperature. The reaction was incubated at 65˚C for 15 min to heat denature the T4 DNA ligase, cooled to room temperature for 5 min. Finally, an additional 1 µL ZraI was added and incubated for 1 hr at 37 ˚C. The LTTRs expression constructs were created via BspQI cloning using the protocol and vectors outlined in Galloway *et al*. [111] except that T4 ligase was used in place of Fast-link™ ligase. PCR products encoding the *aciad0746* (full-length), *yeiE* (full length and EBD), and *finR* (full length) were introduced into the pET28b(+).SapKO-CH.BspQI vector which encodes an in-frame C-terminal 5X poly histidine sequence. For all cloning, XL1-Blue cells (Agilent) were transformed by electroporation and colonies screened for the appropriate sequences by analysis of restriction digests (XbaI/XhoI for expression constructs; StuI/EcoRI or XbaI/SspI for promoter constructs). All constructs were sequenced by Genewiz using the T7 and T7-terminal universal primers or Plasmidsaurus.

The expression and purification of ACIAD0746, YeiE, and FinR were as previously described for the LTTRs BenM [190] and MdcR [191]. Briefly, BL21(DE3) RIPL cells were overexpressed in Studier autoinduction medium [112], then broken in a French Pressure cell, and the proteins purified by metal-chelate (HisTrap) and anion-exchange chromatography (HiTrap Q HP). The selenomethionyl-labelling of the protein was done by transforming the pET28 plasmid containing *aciad0746* into *E. coli* B834 (DE3) cells. The cells were grown in PA-5052 autoinduction media [112] supplemented with 50 µg/mL kanamycin, and selenomethionine was used in place of methionine. Incorporation of selenomethionine was nearly complete as verified by LC-MS/MS at the University of Georgia Proteomics and Mass Spectrometry Core facility

(https://pams.uga.edu/). The ACIAD0746 used in crystallizations was dialyzed against 1 L 20 mM TRIS, 500 mM NaCl, 10 mM βME, 10% glycerol, pH adjusted to 8.0 with hydrochloric acid then passed through a 5 mL HiTrap Q HP anion exchange column and the flow-through used directly in crystallization screens. The LTTRs used in EMSAs and ITC studies were purified by HiTrap Q HP anion exchange with a sodium chloride gradient and exhaustively dialyzed against 4 L volumes of 20 mM TRIS, 10 mM BME, pH adjusted to 8.0 with hydrochloric acid a total of 5 times for a total dialysis volume of 20 L. Each 4 L dialysis was allowed to equilibrate for at least 6 hours at 4˚C. Protein purity was assessed by running all samples on 12% SDS-PAGE gels stained with the colloidal Coomassie Brilliant Blue staining protocol [192], modified to use ethanol instead of methanol. Protein samples were quantified using the Bio-Rad Protein Assay Dye Reagent concentrate using BSA (Pierce) as the standard.

The LTTRs used in the transcription assays were purified as described before, with some modifications. The purification approach added a final 4.6/100 PE MonoQ column. To minimize RNAse contamination, the ÄKTA (Pharmacia) system was thoroughly cleaned with NaOH 0.5M before the MonoQ column. MonoQ buffers (A: 20 mM TRIS, 0.5 mM EDTA, 5% glycerol, and 10 mM BME, pH 8.0; B: 20 mM TRIS, 0.5 mM EDTA, 1 M NaCl, 5% glycerol, and 10 mM BME, pH 8.0) were prepared using components and glassware free of RNases. Protein purity and quantification were performed as previously. Also, the *A. baylyi* ADP1 and the *P. aeruginosa* PAO1 RNA polymerase holoenzymes were preciously obtained in our laboratory [107, 193].

Crystallization of ACIAD0746 and YeiE-EBD

ACIAD0746 protein was concentrated to 119 mg/mL using a GE Vivaspin protein concentrator. YeiE-EBD was dialyzed into 20 mM TRIS, 10 mM BME, pH adjusted to 8.0 with HCl and concentrated to 7.6 mg/mL. Crystallization trials were performed using the microbatch method (2 µL protein with 2 µL reagent) in 96-well microbatch trays with Hampton Research reagent kits: Crystal Screen I & II, Index, SaltRx, and the Grid Screens Ammonium Sulfate, PEG 6000, PEG/LiCl, and Sodium Malonate. Microbatch crystallizations for data collection were prepared under Al's oil (a 1:1 v/v mix of paraffin oil and silicon oil) by mixing 2 µL protein with 2 µL Hampton Research Crystal Screen I cocktail number 23 (HR-CSI-23, 0.2 M $MgCl_2$, 0.1 M HEPES pH 7.5, 30% v/v PEG 400). Crystals were also readily obtained from Hampton Research Crystal Screen I (HR-CSI-14, 0.2 M calcium chloride dihydrate, 0.1 M HEPES sodium pH 7.5, 28% v/v polyethylene glycol 400). Crystals were harvested after one week. The best crystals used for data collection were grown in HR-CSI-23 as above, then transferred to an artificial mother liquor containing a cryo protectant (15% 2,3-butanediol, 50% HR-CSI-14, 35% protein buffer). Crystals were soaked in cryoprotectant and then flash frozen and stored in liquid nitrogen for later data collection. The highest resolution data set used as the native was obtained from crystals that had been presoaked in 0.02 mM mercury benzoate dissolved in cryosolvents for 10 minutes. BME in the solution prevented derivatization (no Hg atoms were found), but crystals that were not similarly soaked in the mercury benzoate diffracted to lower resolution. YeiE crystals were prepared by microbatch crystallizations underoil as above with Hampton Research

Index cocktail 30 (HR-IN-30, 0.1 M sodium chloride, 0.1 M BIS-TRIS pH 6.5, 1.5 M ammonium sulfate). Crystallization reactions were incubated at 18˚C for one week. Crystals were transferred to artificial mother liquor containing a cryoprotectant (75% HR-IN-30, 25% glycerol) before being frozen in liquid nitrogen for subsequent data collection.

Structure determination and refinement

Data were collected from frozen crystals shipped to the Southeast Regional Collaborative Access Team (SER-CAT) 22-ID beamline at the Advanced Photon Source, Argonne National Laboratory. Data were collected using a Rayonix 300HS detector (wavelength 1 Å) and processed using HKL3000 [194] and scaled with Scalepack. X-ray data were collected from one crystal of selenomethionyl-ACIAD0746 were collected at the selenium peak (□=0.97932Å, f' -8.36; f'' 5.35 from Se scan). The selenomethionyl-derivative ACIAD0746 structure was solved by SAD phasing using the AutoSolve routine of the PHENIX suite [195]. A challenge in the phasing process was that the *b* and *c* cell dimensions were very close to one another in the SeMet analog (109.9 Å and 110.4 Å) and the assignment of the cell constants with *mmm* Laue symmetry, which automatically would place *a*<*b*<*c*, had the *b* and *c* axes of the SeMet data set mis-assigned relative to the native data set (110.7 Å and 111.5Å). The native data set was temporarily reindexed to match the SeMet cell for the purposes of initial phasing. Autosolve identified 24 Se sites out of an expected 27 and built 809 residues of the possible 1204 (301 residues are in the monomer including the C-terminal

polyhistidine tag) with an initial R_{work} 42% and R_{free} 49%. The overall topology of the full-length tetrameric protein was very clear; so the chains were largely built at this point using secondary structure features common to LTTRs as guides. To place the model into the native cell (space group $P2_12_12_1$, $a<b<c$), a rotation/translation function of an intermediate model was performed against the high-resolution data set. Several rounds of PHENIX Autobuild, coupled with manual building in Coot [196], and refinement PHENIX Refine completed the structure. The model was refined as described previously. Three electron density features were clearly sulfate molecules. A poorly ordered density feature extending from Cys 148 was modelled in the YeiE-EBD structure as a mercaptoethanol adduct (CME).

Phylogenetic analysis

The critical residues within the ligand binding pocket were identified using the ACIAD0746 crystal structure. PSI-BLAST searches [197] with the ACIAD0746 amino acid sequence identified proteins containing the conserved residues. The identified proteins were used to construct the following general motif for the EBD region of ACIAD0746: <x(89,110)-[AVCSGYL]-[STI]-x-[TSL]-x(6,7)-[PT]-x(19,27)-[NDSHY]-[TSA]-x(17)-[EDG]-[GVNTSARDE]-x(14,17)-[DE]-x(26,38)-R-E-x-[GNYRH]-S-[GA]-[TSIL]-x(60,80)-[RH]. This motif was used with ScanProsite [198] to search the Prosite database for further LTTRs. BLAST [199] was also used to identify additional LTTRs that contain the binding pocket residues in specific phyla. Subsets of aligned protein sequences, selected for the conserved amino acid features, were extracted using a

python script and realigned with CLUSTALW. Genome organizations of putative orthologs were evaluated using the SEED database (http://theseed.org) [200], and the presence or absence of sulfur metabolic pathways was evaluated for different organisms using the KEGG database (http://www.genome.jp/) [201].

Isothermal titration calorimetry

ITC assays were performed using a Malvern MicroCal PEAQ-ITC system. Potential ligands working solutions were prepared using the protein dialysis buffer. Assays were performed using either 19 or 37 injections of ligand. The 19 injection experiments had an initial injection of 0.4 µL of ligand over 0.8 s, followed by 18 injections of 2 µL- each over the course of 4 s. Experiments performed with 37 injections likewise had an initial injection of 0.4 µL over 0.8 s, followed by 36 injections of 2 µL volume. All experiments were performed in triplicate with the system set to 25˚C, with 750 RPM stirring, and 150 s intervals between injections. Controls were performed for each ligand where the ligand being assayed was titrated into protein dialysis buffer.

All ligands were initially screened against 33.5 µM ACIAD0746 tetramer with 19 injections except for APS. Since the amount of APS available for experiments was limiting, ACIAD0746 was diluted to 20 uM tetramer for the assays. Once sulfite was found to interact, ACIAD0746 was concentrated to 77.6 µM tetramer and was titrated with 2.5 mM sodium sulfite using 37 injections. The *E. coli* ortholog YeiE was assayed for sulfite and sulfate binding. YeiE was concentrated to 30.5 µM tetramer and was titrated with 600 µM sodium sulfite over 37 injections. Sodium sulfate binding was

assayed by titrating 30.5 μM YeiE tetramer with 1.5 mM sodium sulfate over the course of 37 injections.

All data were analyzed with the MicroCal PEAQ-ITC Analysis Software. As mentioned before, ligand was titrated into dialysis buffer for each molecule being assayed. These titrations were assigned as controls to their corresponding protein-ligand titrations using the “line” setting within the “assign controls” window of the software such that a line was drawn between all points of the control data and then subtracted from the experimental data.

Electrophoretic mobility shift assays

Target promoters were amplified using the previously mentioned primers and the vectors they were cloned into as templates. All experiments were performed in triplicate. Native polyacrylamide gels (PAGE) were prepared by mixing 2 mL Protogel 30% with 0.5 mL 10X TAE (400 mM TRIS, 200 mM acetic acid, 10 mM EDTA, pH adjusted to 8.0 with HCl), and 7.5 mL Milli-Q water (6% polyacrylamide final concentration). The solution was degassed for 30 min then spiked with 100 μL 10% ammonium persulfate and 10 μL N,N,N’,N-tetramethylethane-1,2-diamine (TEMED). Gels were poured and allowed to solidify for two hours prior to use.

Each electrophoretic mobility shift assay (EMSA) reaction contained 0.8 μL 125 mM TRIS-acetate, 0.1 μL 100 mM potassium acetate, 0.5 μL 25 mM ammonium acetate, 0.5 μL 0.1 M magnesium acetate, 1 μL 100 mM βME, 0.1 μL 100 mM calcium chloride, 1 μL 20 nM DNA, and 4 μL nuclease free water. Reactions containing sulfite

used 3.9 µL nuclease free water and 0.1 µL of 100 mM sodium sulfite for a final concentration of 1 mM sodium sulfite in each reaction. ACIAD0746 and FinR were diluted in dialysis buffer supplemented with 10 µg/mL bovine serum albumin. Each EMSA reaction was combined with 2 µL of diluted protein to generate a 10 µL final reaction volume. Reactions were incubated at 25˚C for 1 hr. Samples (5 µL) were electrophoresed for 1 hr at 4˚C at 200 V and 20 mA. Gels were stained in 0.5X TAE with 1X Sybr Green I and then scanned with a GE Storm 865 Scanner. GelQuant.NET software provided by biochemlabsolutions.com was used to obtain band intensities. GraphPad Prism 7 was used to calculate K_d values as detailed by [202].

In vitro transcription assays

Target promoters were amplified using the previously mentioned primers and the vectors they were cloned into as templates. All experiments were performed in triplicate and under conditions to minimize RNAse contamination. Each *in vitro* transcription assay had a 20 µL reaction volume initially containing the transcription buffer (40 mM TRIS, 150 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, 0.01% Triton X-100, pH 7.5), BSA (10 µg/mL), RNase Inhibitor Murine (10 U), respective DNA template (25 nM), respective ligand when applicable (sodium sulfite, sodium sulfate, or cysteine, at 1 mM), purified ACIAD0746 or FinR (100 nM), and nuclease free water to adjust the final volume. The reactions were maintained at 25°C for 10 minutes to allow protein binding. Then, *A. baylyi* ADP1 or *P. aeruginosa* PAO1 RNA polymerase (100 and 300 nM, respectively) were added and the reactions incubated again at 25°C for 10 minutes. Finally, the

transcription reactions were initiated by adding ribonucleotide triphosphate (1.2 mM of each) and placing the reactions in a water bath set to 37°C for 30 minutes. After this time, the RNA polymerase was denatured at 75°C for 10 min and a mixture of 1x DNase I reaction buffer plus 5U of DNase I was added, followed by another 15 minutes incubation in a water bath set to 37°C. To visualize the RNA transcripts on gel, 10 µL aliquots of each sample were mixed with 10 µL of 2x RNA loading dye (95% formamide, 0.02% SDS, 0.02% bromophenol blue, 0.001% xylene cyanol, 1 mM EDTA), and the mixtures were heated at 90°C for 2 min. The samples were run on a 6% Urea PAGE gel. The gels were stained with SYBR™ Gold Nucleic Acid Gel Stain and imaged on a GE Storm 865 Scanner Imaging System.

CHAPTER 4

CONCLUSIONS

Evolutionary success by bacteria is directly linked to the remarkable ability of their cells to adapt to changes in the environment through both temporary and permanent modifications. Upon exposure to antibiotics, hosts, competitors, and niches with an abundance or scarcity of nutrients, bacteria modulate their genome expression as a strategy to survive and evade defenses [203-206]. For example, the subinhibitory concentration of erythromycin and rifampicin alter global transcription in *Salmonella typhimurium* by activation or inhibition of 5% of its promoters [207]. The soil bacterium *Pseudomonas fluorescens* Pf0-1 transcriptionally modifies its metabolic responses in the presence of bacterial competitors, which is critical for their growth and survival in the nutrient-limited soil environment [208]. Also, a study with cystic fibrosis patients connected the host microflora with *Pseudomonas aeruginosa*'s gene expression towards the production of virulence factors [209]. Gene expression is a complex system that can be regulated at multiple steps, including transcriptional initiation and elongation, RNA stability, and rate of translation [210]. In this dissertation, we focused on the study of the regulation of transcription to better understand the sulfur metabolism in bacteria, using *Acinetobacter baylyi* ADP1 as the primary model organism. However, the execution of such a project required the presence of the RNA polymerase (RNAp) from the species being studied, which is a limiting factor since the only RNAp commercially

available is from *Escherichia coli,* and methods for the laboratory production of this protein using recombinant protein technology have only been published for six species [102-107]. Therefore, this dissertation began with the development of a cloning and purification strategy to obtain a functional *A. baylyi* RNAp holoenzyme. Once this obstacle was overcome, we could further perform transcriptional studies that revealed a new family of transcription regulators working on the assimilation of sulfur.

The central dogma of molecular biology directs genome replication, DNA transcription, and RNA translation. In prokaryotes, transcription is mediated by only one RNA polymerase (RNAp). Despite being catalytically active, the core RNAp (ββ'αω) is incapable of recognizing promoters and must be associated with a sigma factor to originate the RNAp holoenzyme (ββ'αωσ) that will participate in the transcription of specific genes [211]. Thus, the presence of the $RNAp^{holo}$ is fundamental for the execution of studies that aim to investigate bacterial gene expression at the transcriptional level. As one of the most structurally complicated enzymes in cells, the development of methods for its production and purification remains a challenge. The use of recombinant protein is a steadily growing field due to the benefits associated with this technology, such as the non-cultivation of large volumes of bacterial cultures, which is especially important when dealing with pathogenic and slow growing species. Among the various types of expression organisms, *E. coli* dominates the bacterial systems due to its inherent advantages, including rapid growth, rapid expression, ease of culture and high product yields [212]. In Chapter 2, we developed a method of producing the recombinant *A. baylyi* ADP1 $RNAp^{holo}$ in *E. coli* using auto-induction that rendered a

high yield (>20 mg) of a highly active (>20 nt/sec elongation rate), pure (>95%), and stable protein.

Moreover, the identification of a substantial sequence identity (~95%) between the RNAp^{holo} from *A. baylyi* ADP1 and several other *Acinetobacter* species suggests that besides being applied to the study of *A. baylyi* genome expression and regulation, this protein may also be suitable for *in vitro* transcription studies, structural biology, and drug discovery in other species of *Acinetobacter*. This includes the human pathogen *Acinetobacter baumannii*, a leading cause of nosocomial infections and global outbreaks [213]. This organism is part of the ESKAPE group of pathogens (along with *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterobacter* spp) that have a remarkable capacity to modulate their genome expression and acquire resistance determinants, being a threat to the current antibiotic era [214, 215]. Some strains of *A. baumannii* have been reported to be resistant to all antimicrobials available and, for this reason, this species is considered priority 1 (critical) by the World Health Organization for which the development of novel treatment strategies is urgent [216, 217].

In this regard, the availability of the *A. baylyi*'s RNAp^{holo} allowed us to identify potential new therapeutic targets involved in sulfur assimilation. As shown in chapter 3, bacteria rely on several reduction steps to assimilate the oxidized sulfur present in the environment. The reduced/bioavailable sulfur is required to produce cysteine and other S-containing molecules essential for survival [218]. Previous studies with *Gammaproteobacteria* have shown the involvement of two proteins, CysB and Cbl, in the regulation of sulfur assimilation, but the fact that most bacteria do not contain these

proteins indicated the existence of other regulatory protein(s), yet to be identified, to fill this void [50]. Structural, phylogenetic, and biochemical studies previously performed by students in Dr. Momany's lab pointed in the direction of ACIAD0746, an LTTR found in *A. baylyi* ADP1, with multiple orthologs identified in nearly all bacterial phyla (including FinR in *P. aeruginosa*, YeiE in *E. coli* and *Cronobacter sakazakii*, and GigC *in A. baumannii*) [173, 176]. The transcriptional studies performed here conclusively showed that ACIAD0746 activates the transcription at three promoters involved in cysteine biosynthesis (*cysDN, cysI*, and *fpr2*), but only in the presence of sulfite. Besides participating in the synthesis of cysteine, the activation of *cysI* and *fpr2* also have roles in detoxification by directly acting in the reduction of sulfite to sulfide, since the intracellular accumulation of sulfite is toxic to bacteria. Also, the activation of *cysDN* might be linked to oxidative stress defenses. Therefore, our results suggested the identification of a secondary class of regulators that can be the target of new antimicrobial therapies aiming to disrupt sulfur assimilation, promote sulfite accumulation to toxic levels, and make bacteria more sensitive to the attack of macrophages [219, 220].

From this work we could also draw two important observations regarding studies using LTTRs. First, the need for conclusive experiments when investigating gene expression. The structural disposition of LTTR tetramers with all DNA-Binding Domains aligned in one face makes these proteins prone to binding to any given DNA, even without regulatory implications. Their promiscuous nature directly affects protein-DNA binding tests, such as EMSAs, and can lead to erroneously inferred biological functions. In our work, several promoters part of the sulfur assimilation pathway showed high

affinities with ACIAD0746, but only three of them were proven to be regulated by this protein. Second, while most characterized LTTRs regulate the expression of itself and the gene they are divergently transcribed from, exceptions do exist [77]. In our studies, ACIAD0746 showed a non-classical behavior by positively regulating the transcription of *fpr2* instead of *fpr* (the divergent gene) and by not regulating its own expression. On the other hand, FinR showed a classical behavior by activating the transcription at both the *finR* and the *fprA* (divergent gene) promoters.

Most of the antibiotics currently available in the market target the cell wall (e.g., beta-lactams, alanine racemase/ligase inhibitors, D-Ala-D-Ala terminus antibacterials, and C55-isoprenyl pyrophosphate antibacterials) and the protein synthesis (e.g., tetracyclines, aminoglycosides, macrolides, amphenicols, oxazolidinones, pleuromutilins, streptogramins, and lincosamides). Some classes exert their effects by disrupting the bacterial membrane structure (e.g., polymyxin, lipopeptides, and daptomycin), by interfering with metabolic pathways such as the synthesis of folic acid (e.g., sulfonamide and trimethroprim) and fatty acids (Triclosan), or by causing damage to macromolecules such as DNA and ribosomes (Nitrofurantoin and Metronidazole) [221]. The production of nucleic acids remains a poorly explored field that can be a valuable source of new drugs to combat emerging multi-resistant species. Quinolones are the only FDA-approved class of antibiotics targeting DNA synthesis by inhibiting topoisomerases II and IV, while Rifamycins and Lipiarmycins are the only options to disrupt RNA synthesis by hampering the RNA polymerase functioning [222]. To date, there are no drugs targeting transcriptional regulators being used in therapeutics. It is known that the majority of newly approved antibiotics are derivatives of existing classes,

providing limited clinical advantages due to an expected cross-resistance [223]. In this regard, the inhibition of transcriptional regulators involved in the assimilation of sulfur represents an interesting novel approach to combat bacterial infections. The fact that ACIAD0746 orthologs were found in nearly all bacterial phyla suggests that inhibitors would possess a broad spectrum of action. Moreover, the significant differences in sulfur metabolism between prokaryotes and eukaryotes indicate that a treatment based on the sulfite reduction inhibition would exhibit negligeable side effects in humans.

CHAPTER 5

REFERENCES

1. Brosnan, J.T. and M.E. Brosnan, *The sulfur-containing amino acids: an overview.* The Journal of nutrition, 2006. **136**(6): p. 1636S-1640S.
2. Burns, A., P. Olszowy, and P. Ciborowski, *2 - Biomolecules*, in *Proteomic Profiling and Analytical Chemistry (Second Edition)*, P. Ciborowski and J. Silberring, Editors. 2016, Elsevier: Boston. p. 7-24.
3. Mihara, H. and N. Esaki, *Bacterial cysteine desulfurases: their function and mechanisms.* Applied microbiology and biotechnology, 2002. **60**(1): p. 12-23.
4. Roche, B., et al., *Reprint of: Iron/sulfur proteins biogenesis in prokaryotes: Formation, regulation and diversity.* Biochimica et Biophysica Acta (BBA) - Bioenergetics, 2013. **1827**(8): p. 923-937.
5. Ferla, M.P. and W.M. Patrick, *Bacterial methionine biosynthesis.* Microbiology, 2014. **160**(8): p. 1571-1584.
6. Kiley, P.J. and H. Beinert, *The role of Fe–S proteins in sensing and regulation in bacteria.* Current opinion in microbiology, 2003. **6**(2): p. 181-185.
7. Hidalgo, E. and B. Demple, *An iron-sulfur center essential for transcriptional activation by the redox-sensing SoxR protein.* The EMBO journal, 1994. **13**(1): p. 138-146.
8. Kobayashi, K., M. Fujikawa, and T. Kozawa, *Oxidative stress sensing by the iron–sulfur cluster in the transcription factor, SoxR.* Journal of Inorganic Biochemistry, 2014. **133**: p. 87-91.
9. Hood, M.I. and E.P. Skaar, *Nutritional immunity: transition metals at the pathogen–host interface.* Nature Reviews Microbiology, 2012. **10**(8): p. 525-537.
10. Ganz, T., *Iron and infection.* International journal of hematology, 2018. **107**(1): p. 7-15.
11. Golonka, R., B. San Yeoh, and M. Vijay-Kumar, *The iron tug-of-war between bacterial siderophores and innate immunity.* Journal of innate immunity, 2019. **11**(3): p. 249-262.
12. Runyen-Janecky, L., et al., *Role and regulation of iron-sulfur cluster biosynthesis genes in Shigella flexneri virulence.* Infection and immunity, 2008. **76**(3): p. 1083-1092.
13. Begley, T.P., C. Kinsland, and E. Strauss, *The biosynthesis of coenzyme A in bacteria.* Vitamins & Hormones, 2001. **61**: p. 157-171.
14. Du, Q., H. Wang, and J. Xie, *Thiamin (vitamin B1) biosynthesis and regulation: a rich source of antimicrobial drug targets?* International journal of biological sciences, 2011. **7**(1): p. 41.

15. Salaemae, W., G.W. Booker, and S.W. Polyak, *The role of biotin in bacterial physiology and virulence: a novel antibiotic target for Mycobacterium tuberculosis.* Virulence mechanisms of bacterial pathogens, 2016: p. 797-822.
16. Spalding, M.D. and S.T. Prigge, *Lipoic acid metabolism in microbial pathogens.* Microbiology and Molecular Biology Reviews, 2010. **74**(2): p. 200-228.
17. Sherman, F., J.W. Stewart, and S. Tsunasawa, *Methionine or not methionine at the beginning of a protein.* Bioessays, 1985. **3**(1): p. 27-31.
18. Levine, R.L., et al., *Methionine residues as endogenous antioxidants in proteins.* Proceedings of the National Academy of Sciences, 1996. **93**(26): p. 15036-15040.
19. Fontecave, M., M. Atta, and E. Mulliez, *S-adenosylmethionine: nothing goes to waste.* Trends in biochemical sciences, 2004. **29**(5): p. 243-249.
20. North, J.A., et al., *A bifunctional salvage pathway for two distinct S-adenosylmethionine by-products that is widespread in bacteria, including pathogenic Escherichia coli.* Molecular microbiology, 2020. **113**(5): p. 923-937.
21. Burguiere, P., et al., *Three different systems participate in L-cystine uptake in Bacillus subtilis.* Journal of bacteriology, 2004. **186**(15): p. 4875-4884.
22. Hullo, M.-F., et al., *The metNPQ operon of Bacillus subtilis encodes an ABC permease transporting methionine sulfoxide, D-and L-methionine.* Research in microbiology, 2004. **155**(2): p. 80-86.
23. Sperandio, B., et al., *Sulfur amino acid metabolism and its control in Lactococcus lactis IL1403.* Journal of bacteriology, 2005. **187**(11): p. 3762-3778.
24. Brenner, M., et al., *Listeria monocytogenes TcyKLMN cystine/cysteine transporter facilitates glutathione synthesis and virulence gene expression.* Mbio, 2022: p. e00448-22.
25. Zhang, Z., et al., *A transporter of Escherichia coli specific for L-and D-methionine is the prototype for a new family within the ABC superfamily.* Archives of Microbiology, 2003. **180**(2): p. 88-100.
26. Kertesz, M.A., *Bacterial transporters for sulfate and organosulfur compounds.* Research in Microbiology, 2001. **152**(3-4): p. 279-290.
27. Rabus, R., T.A. Hansen, and F. Widdel, *Dissimilatory sulfate-and sulfur-reducing prokaryotes.* The prokaryotes, 2006. **2**: p. 659-768.
28. Dhillon, A., et al., *Domain evolution and functional diversification of sulfite reductases.* Astrobiology, 2005. **5**(1): p. 18-29.
29. Sirko, A., et al., *Sulfate and thiosulfate transport in Escherichia coli K-12: evidence for a functional overlapping of sulfate-and thiosulfate-binding proteins.* Journal of bacteriology, 1995. **177**(14): p. 4134-4136.
30. Aguilar-Barajas, E., et al., *Bacterial transport of sulfate, molybdate, and related oxyanions.* Biometals, 2011. **24**(4): p. 687-707.
31. Zhang, L., et al., *The Escherichia coli CysZ is a pH dependent sulfate transporter that can be inhibited by sulfite.* Biochimica et Biophysica Acta (BBA)-Biomembranes, 2014. **1838**(7): p. 1809-1816.
32. Kredich, N.M., *The molecular basis for positive regulation of cys promoters in Salmonella typhimurium and Escherichia coli.* Molecular microbiology, 1992. **6**(19): p. 2747-2753.

33. Carroll, K.S., et al., *Investigation of the iron− sulfur cluster in Mycobacterium tuberculosis APS reductase: implications for substrate binding and catalysis.* Biochemistry, 2005. **44**(44): p. 14647-14657.
34. Ostrowski, J., et al., *Characterization of the Flavoprotein Moieties of NADPH-Sulfite Reductase from Salmonella typhimurium and Escherichia coli: Physico-chemical and catalytic properties, amino acid sequence deduced from dna sequence of cysJ, and comparison with nadph-cytochrome P-450 reductase.* Journal of Biological Chemistry, 1989. **264**(27): p. 15796-15808.
35. Hell, R., *Molecular physiology of plant sulfur metabolism.* Planta, 1997. **202**(2): p. 138-148.
36. Lewis, T.A., et al., *Role for ferredoxin: NAD (P) H oxidoreductase (FprA) in sulfate assimilation and siderophore biosynthesis in Pseudomonads.* Journal of bacteriology, 2013. **195**(17): p. 3876-3887.
37. Rückert, C., et al., *Functional genomics and expression analysis of the Corynebacterium glutamicum fpr2-cysIXHDNYZ gene cluster involved in assimilatory sulphate reduction.* BMC genomics, 2005. **6**(1): p. 1-18.
38. De Berardinis, V., et al., *A complete collection of single-gene deletion mutants of Acinetobacter baylyi ADP1.* Molecular systems biology, 2008. **4**(1): p. 174.
39. Johnson, C.M., S.L. Roderick, and P.F. Cook, *The serine acetyltransferase reaction: acetyl transfer from an acylpantothenyl donor to an alcohol.* Archives of biochemistry and biophysics, 2005. **433**(1): p. 85-95.
40. Nakamura, T., H. Iwahashi, and Y. Eguchi, *Enzymatic proof for the identity of the S-sulfocysteine synthase and cysteine synthase B of Salmonella typhimurium.* Journal of bacteriology, 1984. **158**(3): p. 1122-1127.
41. Eichhorn, E., J.R. van der Ploeg, and T. Leisinger, *Characterization of a two-component alkanesulfonate monooxygenase from Escherichia coli.* Journal of Biological Chemistry, 1999. **274**(38): p. 26639-26646.
42. van der Ploeg, J.R., E. Eichhorn, and T. Leisinger, *Sulfonate-sulfur metabolism and its regulation in Escherichia coli.* Archives of microbiology, 2001. **176**: p. 1-8.
43. Cypionka, H., *Uptake of sulfate, sulfite and thiosulfate by proton-anion symport in Desulfovibrio desulfuricans.* Archives of microbiology, 1987. **148**: p. 144-149.
44. Kredich, N.M., *Biosynthesis of cysteine.* EcoSal Plus, 2008. **3**(1).
45. Hryniewicz, M.M. and N.M. Kredich, *Stoichiometry of binding of CysB to the cysJIH, cysK, and cysP promoter regions of Salmonella typhimurium.* Journal of bacteriology, 1994. **176**(12): p. 3673-3682.
46. Ostrowski, J. and N.M. Kredich, *Molecular characterization of the cysJIH promoters of Salmonella typhimurium and Escherichia coli: regulation by cysB protein and N-acetyl-L-serine.* Journal of bacteriology, 1989. **171**(1): p. 130-140.
47. Lynch, A., et al., *Characterization of the CysB protein of Klebsiella aerogenes: direct evidence that N-acetylserine rather than O-acetylserine serves as the inducer of the cysteine regulon.* Biochemical journal, 1994. **299**(1): p. 129-136.
48. Iwanicka-Nowicka, R. and M.M. Hryniewicz, *A new gene, cbl, encoding a member of the LysR family of transcriptional regulators belongs to Escherichia coli cys regulon.* Gene, 1995. **166**(1): p. 11-17.

49. Bykowski, T., et al., *The switch from inorganic to organic sulphur assimilation in Escherichia coli: adenosine 5'-phosphosulphate (APS) as a signalling molecule for sulphate excess.* Molecular microbiology, 2002. **43**(5): p. 1347-1358.
50. Park, C., B. Shin, and W. Park, *Protective role of bacterial alkanesulfonate monooxygenase under oxidative stress.* Applied and environmental microbiology, 2020. **86**(15): p. e00692-20.
51. Li, G.-W. and X.S. Xie, *Central dogma at the single-molecule level in living cells.* Nature, 2011. **475**(7356): p. 308-315.
52. Parker, J., *RNA Polymerase*, in *Encyclopedia of Genetics*, S. Brenner and J.H. Miller, Editors. 2001, Academic Press: New York. p. 1746-1747.
53. Minakhin, L., et al., *Bacterial RNA polymerase subunit ω and eukaryotic RNA polymerase subunit RPB6 are sequence, structural, and functional homologs and promote RNA polymerase assembly.* Proceedings of the National Academy of Sciences, 2001. **98**(3): p. 892-897.
54. Mathew, R. and D. Chatterji, *The evolving story of the omega subunit of bacterial RNA polymerase.* Trends in microbiology, 2006. **14**(10): p. 450-455.
55. Murakami, K.S. and S.A. Darst, *Bacterial RNA polymerases: the wholo story.* Current opinion in structural biology, 2003. **13**(1): p. 31-39.
56. Sutherland, C. and K.S. Murakami, *An introduction to the structure and function of the catalytic core enzyme of Escherichia coli RNA polymerase.* EcoSal Plus, 2018. **8**(1).
57. Santangelo, T.J. and I. Artsimovitch, *Termination and antitermination: RNA polymerase runs a stop sign.* Nature Reviews Microbiology, 2011. **9**(5): p. 319-329.
58. Gross, C., et al. *The functional and regulatory roles of sigma factors in transcription*. in *Cold Spring Harbor symposia on quantitative biology*. 1998. Cold Spring Harbor Laboratory Press.
59. Potvin, E., F. Sanschagrin, and R.C. Levesque, *Sigma factors in Pseudomonas aeruginosa.* FEMS microbiology reviews, 2008. **32**(1): p. 38-55.
60. Lonetto, M., M. Gribskov, and C.A. Gross, *The sigma 70 family: sequence conservation and evolutionary relationships.* Journal of bacteriology, 1992. **174**(12): p. 3843-3849.
61. Arsène, F., T. Tomoyasu, and B. Bukau, *The heat shock response of Escherichia coli.* International journal of food microbiology, 2000. **55**(1-3): p. 3-9.
62. Vuthoori, S., et al., *Domain 1.1 of the σ70 subunit of Escherichia coli RNA polymerase modulates the formation of stable polymerase/promoter complexes.* Journal of molecular biology, 2001. **309**(3): p. 561-572.
63. Feklístov, A., et al., *Bacterial sigma factors: a historical, structural, and genomic perspective.* Annual review of microbiology, 2014. **68**: p. 357-376.
64. Vassylyev, D.G., et al., *Crystal structure of a bacterial RNA polymerase holoenzyme at 2.6 Å resolution.* Nature, 2002. **417**(6890): p. 712-719.
65. Lee, D.J., S.D. Minchin, and S.J. Busby, *Activating transcription in bacteria.* Annual review of microbiology, 2012. **66**(1): p. 125-152.
66. Ruff, E.F., M.T. Record Jr, and I. Artsimovitch, *Initial events in bacterial transcription initiation.* Biomolecules, 2015. **5**(2): p. 1035-1062.

67. Roberts, J.W., S. Shankar, and J.J. Filter, *RNA polymerase elongation factors.* Annu. Rev. Microbiol., 2008. **62**: p. 211-233.
68. Washburn, R.S. and M.E. Gottesman, *Regulation of transcription elongation and termination.* Biomolecules, 2015. **5**(2): p. 1063-1078.
69. Roberts, J.W., *Mechanisms of bacterial transcription termination.* Journal of molecular biology, 2019. **431**(20): p. 4030-4039.
70. Vassylyev, D.G., et al., *Structural basis for transcription elongation by bacterial RNA polymerase.* Nature, 2007. **448**(7150): p. 157-162.
71. Stragier, P., et al., *Regulation of diaminopimelate decarboxylase synthesis in Escherichia coli: I. Identification of a lysR gene encoding an activator of the lysA gene.* Journal of molecular biology, 1983. **168**(2): p. 307-320.
72. Zhang, Y., et al., *A putative LysR-type transcriptional regulator PrhO positively regulates the type III secretion system and contributes to the virulence of Ralstonia solanacearum.* Molecular plant pathology, 2018. **19**(8): p. 1808-1819.
73. Fu, Y., et al., *Four LysR-type transcriptional regulator family proteins (LTTRs) involved in antibiotic resistance in Aeromonas hydrophila.* World Journal of Microbiology and Biotechnology, 2019. **35**(8): p. 127.
74. Mandal, R.S., et al., *Ribavirin suppresses bacterial virulence by targeting LysR-type transcriptional regulators.* Scientific reports, 2016. **6**: p. 39454.
75. Henikoff, S., et al., *A large family of bacterial activator proteins.* Proceedings of the National Academy of Sciences, 1988. **85**(18): p. 6602-6606.
76. Schell, M.A., *Molecular biology of the LysR family of transcriptional regulators.* Annual review of microbiology, 1993. **47**(1): p. 597-626.
77. Maddocks, S.E. and P.C. Oyston, *Structure and function of the LysR-type transcriptional regulator (LTTR) family proteins.* Microbiology, 2008. **154**(12): p. 3609-3623.
78. Storz, G., L.A. Tartaglia, and B.N. Ames, *Transcriptional regulator of oxidative stress-inducible genes: direct activation by oxidation.* Science, 1990. **248**(4952): p. 189-194.
79. Collier, L.S., G.L. Gaines, and E.L. Neidle, *Regulation of benzoate degradation inAcinetobacter sp. strain ADP1 by BenM, a LysR-type transcriptional activator.* Journal of bacteriology, 1998. **180**(9): p. 2493-2501.
80. Renna, M.C., et al., *Regulation of the Bacillus subtilis alsS, alsD, and alsR genes involved in post-exponential-phase production of acetoin.* Journal of Bacteriology, 1993. **175**(12): p. 3863-3875.
81. Kong, K.-F., et al., *Pseudomonas aeruginosa AmpR is a global transcriptional factor that regulates expression of AmpC and PoxB β-lactamases, proteases, quorum sensing, and other virulence factors.* Antimicrobial Agents and Chemotherapy, 2005. **49**(11): p. 4567-4575.
82. Balasubramanian, D., et al., *The regulatory repertoire of Pseudomonas aeruginosa AmpC ß-lactamase regulator AmpR includes virulence genes.* PloS one, 2012. **7**(3).
83. Balasubramanian, D., H. Kumari, and K. Mathee, *Pseudomonas aeruginosa AmpR: an acute–chronic switch regulator.* Pathogens and disease, 2015. **73**(2): p. 1.

84. Lochowska, A., et al., *Identification of activating region (AR) of Escherichia coli LysR-type transcription factor CysB and CysB contact site on RNA polymerase alpha subunit at the cysP promoter.* Molecular microbiology, 2004. **53**(3): p. 791-806.
85. Momany, C. and E.L. Neidle, *Defying stereotypes: the elusive search for a universal model of LysR-type regulation.* Molecular microbiology, 2012. **83**(3): p. 453-456.
86. Parsek, M.R., et al., *Critical nucleotides in the interaction of a LysR-type regulator with its target promoter region. catBC promoter activation by CatR.* Journal of Biological Chemistry, 1994. **269**(15): p. 11279-11284.
87. Park, N., et al., *Crystal structure of the regulatory domain of AphB from Vibrio vulnificus, a virulence gene regulator.* Molecules and cells, 2017. **40**(4): p. 299.
88. Stec, E., et al., *Structural basis of the sulphate starvation response in E. coli: crystal structure and mutational analysis of the cofactor-binding domain of the Cbl transcriptional regulator.* Journal of molecular biology, 2006. **364**(3): p. 309-322.
89. Pareja, E., et al., *ExtraTrain: a database of Extragenic regions and Transcriptional information in prokaryotic organisms.* BMC microbiology, 2006. **6**(1): p. 1-10.
90. Berman, H.M., et al., *The protein data bank.* Nucleic acids research, 2000. **28**(1): p. 235-242.
91. Muraoka, S., et al., *Crystal structure of a full-length LysR-type transcriptional regulator, CbnR: unusual combination of two subunit forms and molecular bases for causing and changing DNA bend.* Journal of molecular biology, 2003. **328**(3): p. 555-566.
92. Ogawa, N., et al., *Transcriptional activation of the chlorocatechol degradative genes of Ralstonia eutropha NH9.* Journal of bacteriology, 1999. **181**(21): p. 6697-6705.
93. Koentjoro, M.P. and N. Ogawa, *Structural studies of transcriptional regulation by LysR-type transcriptional regulators in bacteria.* Reviews in Agricultural Science, 2018. **6**: p. 105-118.
94. Taylor, J.L., et al., *The crystal structure of AphB, a virulence gene activator from Vibrio cholerae, reveals residues that influence its response to oxygen and pH.* Molecular microbiology, 2012. **83**(3): p. 457-470.
95. Jo, I., et al., *Structural details of the OxyR peroxide-sensing mechanism.* Proceedings of the National Academy of Sciences, 2015. **112**(20): p. 6443-6448.
96. Monferrer, D., et al., *Structural studies on the full-length LysR-type regulator TsaR from Comamonas testosteroni T-2 reveal a novel open conformation of the tetrameric LTTR fold.* Molecular microbiology, 2010. **75**(5): p. 1199-1214.
97. Jo, I., et al., *Structural basis for HOCl recognition and regulation mechanisms of HypT, a hypochlorite-specific transcriptional regulator.* Proceedings of the National Academy of Sciences, 2019. **116**(9): p. 3740-3745.
98. Kredich, N.M., *Regulation of L-Cysteine Biosynthesis in Salmonella Typhimurium I. effects of growth on varying sulfur sources and o-acetyl-l-serine on gene expression.* Journal of Biological Chemistry, 1971. **246**(11): p. 3474-3484.

99. Tyrrell, R., et al., *The structure of the cofactor-binding fragment of the LysR family member, CysB: a familiar fold with a surprising subunit arrangement.* Structure, 1997. **5**(8): p. 1017-1032.
100. Dik, D.A., et al., *Muropeptide binding and the X-ray structure of the effector domain of the transcriptional regulator AmpR of Pseudomonas aeruginosa.* Journal of the american chemical society, 2017. **139**(4): p. 1448-1451.
101. Ilangovan, A., et al., *Structural basis for native agonist and synthetic inhibitor recognition by the Pseudomonas aeruginosa quorum sensing regulator PqsR (MvfR).* PLoS pathogens, 2013. **9**(7).
102. Banerjee, R., et al., *Optimization of recombinant Mycobacterium tuberculosis RNA polymerase expression and purification.* Tuberculosis, 2014. **94**(4): p. 397-404.
103. Tang, H., et al., *Rapid RNA polymerase genetics: one-day, no-column preparation of reconstituted recombinant Escherichia coli RNA polymerase.* Proceedings of the National Academy of Sciences, 1995. **92**(11): p. 4902-4906.
104. Kuznedelov, K. and K. Severinov, *Recombinant bacterial RNA polymerase: preparation and applications.* Methods, 2009. **47**(1): p. 44-52.
105. Minakhin, L., et al., *Recombinant Thermus aquaticus RNA polymerase, a new tool for structure-based analysis of transcription.* Journal of bacteriology, 2001. **183**(1): p. 71-76.
106. Yang, X. and P.J. Lewis, *Overproduction and purification of recombinant Bacillus subtilis RNA polymerase.* Protein expression and purification, 2008. **59**(1): p. 86-93.
107. Afful, D., L. Cai, and C. Momany, *Overproduction and purification of highly active recombinant Pseudomonas aeruginosa str. PAO1 RNA polymerase holoenzyme complex.* Protein expression and purification, 2019. **163**: p. 105448.
108. Elliott, K.T. and E.L. Neidle, *Acinetobacter baylyi ADP1: transforming the choice of model organism.* IUBMB life, 2011. **63**(12): p. 1075-1080.
109. Young, D.M., D. Parke, and L.N. Ornston, *Opportunities for genetic investigation afforded by Acinetobacter baylyi, a nutritionally versatile bacterial species that is highly competent for natural transformation.* Annu. Rev. Microbiol., 2005. **59**: p. 519-551.
110. Palmen, R. and K.J. Hellingwerf, *Uptake and processing of DNA by Acinetobacter calcoaceticus–a review.* Gene, 1997. **192**(1): p. 179-190.
111. Galloway, N.R., et al., *Rapid Cloning For Protein Crystallography Using Type IIS Restriction Enzymes.* Crystal Growth & Design, 2013. **13**(7): p. 2833-2839.
112. Studier, F.W., *Protein production by auto-induction in high-density shaking cultures.* Protein expression and purification, 2005. **41**(1): p. 207-234.
113. Blommel, P.G., et al., *Enhanced bacterial protein expression during auto-induction obtained by alteration of lac repressor dosage and medium composition.* Biotechnology progress, 2007. **23**(3): p. 585-598.
114. Chamberlin, M.J., et al., *A quantitative assay for bacterial RNA polymerases.* Journal of Biological Chemistry, 1979. **254**(20): p. 10061-10069.
115. Hall, T.A. *BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT*. in *Nucleic acids symposium series*. 1999. [London]: Information Retrieval Ltd., c1979-c2000.

116. Altschul, S.F., et al., *Gapped BLAST and PSI-BLAST: a new generation of protein database search programs.* Nucleic acids research, 1997. **25**(17): p. 3389-3402.
117. Bik, E.M., et al., *Molecular analysis of the bacterial microbiota in the human stomach.* Proceedings of the National Academy of Sciences, 2006. **103**(3): p. 732-737.
118. Fierer, N., M.A. Bradford, and R.B. Jackson, *Toward an ecological classification of soil bacteria.* Ecology, 2007. **88**(6): p. 1354-1364.
119. Jin, D., et al., *Bacterial communities and potential waterborne pathogens within the typical urban surface waters.* Scientific reports, 2018. **8**(1): p. 1-9.
120. Rajan, S.S., et al., *Presence of an N-Terminal Polyhistidine Tag Facilitates Stable Expression of an Otherwise Unstable N-Terminal Domain of Mouse Tissue Inhibitor of Metalloproteinase-1 inEscherichia coli.* Protein expression and purification, 1998. **13**(1): p. 67-72.
121. Sun, Q.M., et al., *An Improved Strategy for High-Level Production of Human Vasostatin120–180.* Biotechnology progress, 2005. **21**(4): p. 1048-1052.
122. Studier, F.W., *Stable expression clones and auto-induction for protein production in E. coli*, in *Structural genomics*. 2014, Springer. p. 17-32.
123. Olmos-Dichara, A., et al., *Growth and lactic acid production by Lactobacillus casei ssp. rhamnosus in batch and membrane bioreactor: influence of yeast extract and tryptone enrichment.* Biotechnology Letters, 1997. **19**(8): p. 709-714.
124. Jacques, J.-F., et al., *A recombinant Mycobacterium tuberculosis in vitro transcription system.* FEMS microbiology letters, 2006. **255**(1): p. 140-147.
125. Schneider, C.A., W.S. Rasband, and K.W. Eliceiri, *NIH Image to ImageJ: 25 years of image analysis.* Nature methods, 2012. **9**(7): p. 671-675.
126. Carson, M., et al., *His-tag impact on structure.* Acta Crystallographica Section D: Biological Crystallography, 2007. **63**(3): p. 295-301.
127. Kapust, R.B. and D.S. Waugh, *Controlled intracellular processing of fusion proteins by TEV protease.* Protein expression and purification, 2000. **19**(2): p. 312-318.
128. Majorek, K.A., et al., *Double trouble—Buffer selection and H is-tag presence may be responsible for nonreproducibility of biomedical experiments.* protein science, 2014. **23**(10): p. 1359-1368.
129. Dunn, J.J. and F.W. Studier, *T7 early RNAs and Escherichia coli ribosomal RNAs are cut from large precursor RNAs in vivo by ribonuclease III.* Proceedings of the National Academy of Sciences, 1973. **70**(12): p. 3296-3300.
130. Hsieh, T.-S. and J.C. Wang, *Physicochemical studies on interactions between DNA and RNA polymerase. Isolation and mapping of a T7 DNA fragment containing the early promoters for Escherichia coli RNA polymerase.* Biochemistry, 1976. **15**(26): p. 5776-5783.
131. Koller, T., et al., *High resolution physical mapping of specific binding sites of Escherichia coli RNA polymerase on the DNA of bacteriophage T7.* Journal of Molecular Biology, 1978. **120**(1): p. 121-131.
132. Lee, W.J., J.W. Bush, and M.J. Chamberlin, *Utilization of promoter and terminator sites on bacteriophage T7 DNA by RNA polymerases from a variety of bacterial orders.* Cell, 1979. **16**(1): p. 97-109.

133. Mishra, S. and A.R. Horswill, *Heparin mimics extracellular DNA in binding to cell surface-localized proteins and promoting Staphylococcus aureus biofilm formation.* Msphere, 2017. **2**(3): p. e00135-17.
134. Epshtein, V. and E. Nudler, *Cooperation between RNA polymerase molecules in transcription elongation.* Science, 2003. **300**(5620): p. 801-805.
135. Uptain, S., C. Kane, and M. Chamberlin, *Basic mechanisms of transcript elongation and its regulation.* Annual review of biochemistry, 1997. **66**(1): p. 117-172.
136. Adelman, K., et al., *Single molecule analysis of RNA polymerase elongation reveals uniform kinetic behavior.* Proceedings of the National Academy of Sciences, 2002. **99**(21): p. 13538-13543.
137. Davenport, R.J., et al., *Single-molecule study of transcriptional pausing and arrest by E. coli RNA polymerase.* Science, 2000: p. 2497-2500.
138. Mosaei, H. and N. Zenkin, *Two distinct pathways of RNA polymerase backtracking determine the requirement for the Trigger Loop during RNA hydrolysis.* Nucleic Acids Research, 2021. **49**(15): p. 8777-8784.
139. Shaevitz, J.W., et al., *Backtracking by single RNA polymerase molecules observed at near-base-pair resolution.* Nature, 2003. **426**(6967): p. 684-687.
140. Artsimovitch, I., et al., *RNA polymerases from Bacillus subtilis and Escherichia coli differ in recognition of regulatory signals in vitro.* Journal of Bacteriology, 2000. **182**(21): p. 6027-6035.
141. Murray, G.L., et al., *Global gene expression profile of Acinetobacter baumannii during bacteremia.* The Journal of infectious diseases, 2017. **215**(suppl_1): p. S52-S57.
142. Lane, W.J. and S.A. Darst, *Molecular evolution of multisubunit RNA polymerases: sequence analysis.* Journal of molecular biology, 2010. **395**(4): p. 671-685.
143. Nudler, E., *RNA polymerase active center: the molecular engine of transcription.* Annual review of biochemistry, 2009. **78**: p. 335-361.
144. Darst, S.A., et al., *Conformational flexibility of bacterial RNA polymerase.* Proceedings of the National Academy of Sciences, 2002. **99**(7): p. 4296-4301.
145. Opalka, N., et al., *Direct localization of a β-subunit domain on the three-dimensional structure of Escherichia coli RNA polymerase.* Proceedings of the National Academy of Sciences, 2000. **97**(2): p. 617-622.
146. Artsimovitch, I., et al., *Co-overexpression of Escherichia coliRNA Polymerase Subunits Allows Isolation and Analysis of Mutant Enzymes Lacking Lineage-specific Sequence Insertions.* Journal of Biological Chemistry, 2003. **278**(14): p. 12344-12355.
147. Zhang, G., et al., *Crystal structure of Thermus aquaticus core RNA polymerase at 3.3 Å resolution.* Cell, 1999. **98**(6): p. 811-824.
148. Johnson, G.E., et al., *Functionally uncoupled transcription–translation in Bacillus subtilis.* Nature, 2020. **585**(7823): p. 124-128.
149. Proshkin, S., et al., *Cooperation between translating ribosomes and RNA polymerase in transcription elongation.* Science, 2010. **328**(5977): p. 504-508.
150. Mihara, H. and N. Esaki, *Bacterial cysteine desulfurases: their function and mechanisms.* Appl Microbiol Biotechnol, 2002. **60**(1-2): p. 12-23.

151. Kertesz, M.A., *Bacterial transporters for sulfate and organosulfur compounds.* Res Microbiol, 2001. **152**(3-4): p. 279-90.
152. Tsang, M.L. and J.A. Schiff, *Sulfate-reducing pathway in Escherichia coli involving bound intermediates.* J Bacteriol, 1976. **125**(3): p. 923-33.
153. Ostrowski, J., et al., *Characterization of the flavoprotein moieties of NADPH-sulfite reductase from Salmonella typhimurium and Escherichia coli. Physicochemical and catalytic properties, amino acid sequence deduced from DNA sequence of cysJ, and comparison with NADPH-cytochrome P-450 reductase.* J Biol Chem, 1989. **264**(27): p. 15796-808.
154. Nakamura, T., H. Iwahashi, and Y. Eguchi, *Enzymatic proof for the identity of the S-sulfocysteine synthase and cysteine synthase B of Salmonella typhimurium.* J Bacteriol, 1984. **158**(3): p. 1122-7.
155. Kredich, N., *Biosynthesis of Cysteine.* EcoSal Plus, 2008.
156. Lynch, A.S., et al., *Characterization of the CysB protein of Klebsiella aerogenes: direct evidence that N-acetylserine rather than O-acetylserine serves as the inducer of the cysteine regulon.* Biochem J, 1994. **299 (Pt 1)**: p. 129-36.
157. Colyer, T.E. and N.M. Kredich, *In vitro characterization of constitutive CysB proteins from Salmonella typhimurium.* Mol Microbiol, 1996. **21**(2): p. 247-56.
158. Flavin, M. and C. Slaughter, *Enzymatic synthesis of homocysteine or methionine directly from O-succinyl-homoserine.* Biochimica et Biophysica Acta (BBA)-Enzymology, 1967. **132**(2): p. 400-405.
159. van Der Ploeg, J.R., et al., *The Escherichia coli ssuEADCB gene cluster is required for the utilization of sulfur from aliphatic sulfonates and is regulated by the transcriptional activator Cbl.* J Biol Chem, 1999. **274**(41): p. 29358-65.
160. Bykowski, T., et al., *The switch from inorganic to organic sulphur assimilation in Escherichia coli: adenosine 5'-phosphosulphate (APS) as a signalling molecule for sulphate excess.* Mol Microbiol, 2002. **43**(5): p. 1347-58.
161. Rey, D.A., et al., *The McbR repressor modulated by the effector substance S-adenosylhomocysteine controls directly the transcription of a regulon involved in sulphur metabolism of Corynebacterium glutamicum ATCC 13032.* Molecular microbiology, 2005. **56**(4): p. 871-887.
162. Yeom, S., J. Yeom, and W. Park, *Molecular characterization of FinR, a novel redox-sensing transcriptional regulator in Pseudomonas putida KT2440.* Microbiology, 2010. **156**(Pt 5): p. 1487-96.
163. Boonma, S., et al., *The FinR-regulated essential gene fprA, encoding ferredoxin NADP+ reductase: roles in superoxide-mediated stress protection and virulence of Pseudomonas aeruginosa.* PLoS One, 2017. **12**(2): p. e0172071.
164. Guillouard, I., et al., *Identification of Bacillus subtilis CysL, a regulator of the cysJI operon, which encodes sulfite reductase.* J Bacteriol, 2002. **184**(17): p. 4681-9.
165. Zdobnov, E.M., et al., *OrthoDB v9. 1: cataloging evolutionary and functional annotations for animal, fungal, plant, archaeal, bacterial and viral orthologs.* Nucleic acids research, 2016. **45**(D1): p. D744-D749.
166. Dangel, A.W. and F.R. Tabita, *CbbR, the master regulator for microbial carbon dioxide fixation.* Journal of bacteriology, 2015. **197**(22): p. 3488-3498.

167. Van Keulen, G., et al., *The LysR-type transcriptional regulator CbbR controlling autotrophic CO2 fixation by Xanthobacter flavus is an NADPH sensor.* Journal of bacteriology, 1998. **180**(6): p. 1411-1417.
168. Mittal, M., A.K. Singh, and S. Kumaran, *Structural and biochemical characterization of ligand recognition by CysB, the master regulator of sulfate metabolism.* Biochimie, 2017. **142**: p. 112-124.
169. Kim, J. and W. Park, *Oxidative stress response in Pseudomonas putida.* Applied microbiology and biotechnology, 2014. **98**(16): p. 6933-6946.
170. Du, X., et al., *Insights into Protein–Ligand Interactions: Mechanisms, Models, and Methods.* International Journal of Molecular Sciences, 2016. **17**(2): p. 144.
171. Xiao, G., et al., *MvfR, a key Pseudomonas aeruginosa pathogenicity LTTR-class regulatory protein, has dual ligands.* Molecular microbiology, 2006. **62**(6): p. 1689-1699.
172. Ezezika, O.C., et al., *Distinct Effector-binding Sites Enable Synergistic Transcriptional Activation by BenM, a LysR-type Regulator.* Journal of Molecular Biology, 2007. **367**(3): p. 616-629.
173. Gebhardt, M.J., et al., *GigC, a LysR family transcription regulator, is required for cysteine metabolism and virulence in Acinetobacter baumannii.* Infection and immunity, 2020. **89**(1): p. e00180-20.
174. Gao, K., R. Oerlemans, and M.R. Groves, *Theory and applications of differential scanning fluorimetry in early-stage drug discovery.* Biophysical reviews, 2020. **12**(1): p. 85-104.
175. Park, S. and J.A. Imlay, *High levels of intracellular cysteine promote oxidative DNA damage by driving the Fenton reaction.* Journal of bacteriology, 2003. **185**(6): p. 1942-1950.
176. Hong, S., et al., *Crystal structures of YeiE from Cronobacter sakazakii and the role of sulfite tolerance in gram-negative bacteria.* Proceedings of the National Academy of Sciences, 2022. **119**(11): p. e2118002119.
177. Hryniewicz, M.M., et al., *Sulfate and thiosulfate transport in Escherichia coli K-12 – Identification of a gene encoding a novel protein involved in thiosulfate binding.* J Bacteriol, 1990. **172**.
178. Schell, M.A., *Molecular biology of the LysR family of transcriptional regulators.* Annu Rev Microbiol, 1993. **47**: p. 597-626.
179. Sperandio, B., et al., *Control of methionine synthesis and uptake by MetR and homocysteine in Streptococcus mutans.* Journal of bacteriology, 2007. **189**(19): p. 7032-7044.
180. Kamimura, N., et al., *Regulatory system of the protocatechuate 4, 5-cleavage pathway genes essential for lignin downstream catabolism.* Journal of bacteriology, 2010. **192**(13): p. 3394-3405.
181. Porrúa, O., et al., *The LysR-type regulator AtzR binding site: DNA sequences involved in activation, repression and cyanuric acid-dependent repositioning.* Molecular microbiology, 2007. **66**(2): p. 410-427.
182. Craven, S., et al., *LysR homologs in Acinetobacter: insights into a diverse and prevalent family of transcriptional regulators, p 163–202.* Acinetobacter molecular biology. Horizon Press, Norwich, United Kingdom, 2008.

183. Taylor, S.L., N.A. Higley, and R.K. Bush, *Sulfites in foods: uses, analytical methods, residues, fate, exposure assessment, metabolism, toxicity, and hypersensitivity.* Adv Food Res, 1986. **30**: p. 1-76.
184. Gould, G., *The use of other chemical preservatives: sulfite and nitrite.* The Microbiological Safety and Quality of Foods, 2000: p. 200-213.
185. Cecil, R. and R. Wake, *The reactions of inter-and intra-chain disulphide bonds in proteins with sulphite.* Biochemical Journal, 1962. **82**(3): p. 401.
186. Mitsuhashi, H., et al., *Sulfite is released by human neutrophils in response to stimulation with lipopolysaccharide.* J Leukoc Biol, 1998. **64**(5): p. 595-9.
187. Schnappinger, D., et al., *Transcriptional adaptation of Mycobacterium tuberculosis within macrophages.* Journal of Experimental Medicine, 2003. **198**(5): p. 693-704.
188. Fontán, P., et al., *Global transcriptional profile of Mycobacterium tuberculosis during THP-1 human macrophage infection.* Infection and immunity, 2008. **76**(2): p. 717-725.
189. Senaratne, R.H., et al., *5′-Adenosinephosphosulphate reductase (CysH) protects Mycobacterium tuberculosis against free radicals during chronic infection phase in mice.* Molecular microbiology, 2006. **59**(6): p. 1744-1753.
190. Ruangprasert, A., et al., *Full-length structures of BenM and two variants reveal different oligomerization schemes for LysR-type transcriptional regulators.* Journal of molecular biology, 2010. **404**(4): p. 568-586.
191. Stoudenmire, J.L., et al., *Malonate degradation in Acinetobacter baylyi ADP1: Operon organization and regulation by MdcR.* Microbiology, 2017. **163**(5): p. 789.
192. Candiano, G., et al., *Blue silver: a very sensitive colloidal Coomassie G-250 staining for proteome analysis.* Electrophoresis, 2004. **25**(9): p. 1327-33.
193. Calil Brondani, J., et al., *Overproduction, purification, and transcriptional activity of recombinant Acinetobacter baylyi ADP1 RNA polymerase holoenzyme.* Protein Expression and Purification, 2023. **206**: p. 106254.
194. Minor, W., et al., *HKL-3000: the integration of data reduction and structure solution–from diffraction images to an initial model in minutes.* Acta Crystallographica Section D: Biological Crystallography, 2006. **62**(8): p. 859-866.
195. Adams, P.D., et al., *PHENIX: a comprehensive Python-based system for macromolecular structure solution.* Acta Crystallographica Section D: Biological Crystallography, 2010. **66**(2): p. 213-221.
196. Emsley, P., et al., *Features and development of Coot.* Acta Crystallographica Section D: Biological Crystallography, 2010. **66**(4): p. 486-501.
197. Altschul, S.F., et al., *Gapped BLAST and PSI-BLAST: a new generation of protein database search programs.* Nucleic Acids Res, 1997. **25**(17): p. 3389-402.
198. de Castro, E., et al., *ScanProsite: detection of PROSITE signature matches and ProRule-associated functional and structural residues in proteins.* Nucleic Acids Res, 2006. **34**(Web Server issue): p. W362-5.
199. Altschul, S.F., et al., *Basic local alignment search tool.* J Mol Biol, 1990. **215**(3): p. 403-10.

200. Overbeek, R., et al., *The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes.* Nucleic acids research, 2005. **33**(17): p. 5691-5702.
201. Kanehisa, M. and S. Goto, *KEGG: kyoto encyclopedia of genes and genomes.* Nucleic acids research, 2000. **28**(1): p. 27-30.
202. Heffler, M.A., R.D. Walters, and J.F. Kugel, *Using electrophoretic mobility shift assays to measure equilibrium dissociation constants: GAL4-p53 binding DNA as a model system.* Biochem Mol Biol Educ, 2012. **40**(6): p. 383-7.
203. Chaguza, C., *Bacterial survival: evolve and adapt or perish.* Nature Reviews Microbiology, 2020. **18**(1): p. 5-5.
204. Fajardo, A. and J.L. Martínez, *Antibiotics as signals that trigger specific bacterial responses.* Current opinion in microbiology, 2008. **11**(2): p. 161-167.
205. Qian, Y., et al., *Resource competition shapes the response of genetic circuits.* ACS synthetic biology, 2017. **6**(7): p. 1263-1272.
206. Shi, A., F. Fan, and J.R. Broach, *Microbial adaptive evolution.* Journal of Industrial Microbiology and Biotechnology, 2022. **49**(2): p. kuab076.
207. Goh, E.-B., et al., *Transcriptional modulation of bacterial gene expression by subinhibitory concentrations of antibiotics.* Proceedings of the National Academy of Sciences, 2002. **99**(26): p. 17025-17030.
208. Garbeva, P., et al., *Transcriptional and antagonistic responses of Pseudomonas fluorescens Pf0-1 to phylogenetically different bacterial competitors.* The ISME journal, 2011. **5**(6): p. 973-985.
209. Duan, K., et al., *Modulation of Pseudomonas aeruginosa gene expression by host microflora through interspecies communication.* Molecular microbiology, 2003. **50**(5): p. 1477-1491.
210. He, B. and K. Tan, *Understanding transcriptional regulatory networks using computational models.* Current opinion in genetics & development, 2016. **37**: p. 101-108.
211. Bustamante, C., W. Cheng, and Y.X. Mejia, *Revisiting the central dogma one molecule at a time.* Cell, 2011. **144**(4): p. 480-497.
212. Chen, R., *Bacterial expression systems for recombinant protein production: E. coli and beyond.* Biotechnology advances, 2012. **30**(5): p. 1102-1107.
213. Perez, F., et al., *Global challenge of multidrug-resistant Acinetobacter baumannii.* Antimicrobial agents and chemotherapy, 2007. **51**(10): p. 3471-3484.
214. Peleg, A.Y., H. Seifert, and D.L. Paterson, *Acinetobacter baumannii: emergence of a successful pathogen.* Clinical microbiology reviews, 2008. **21**(3): p. 538-582.
215. Mulani, M.S., et al., *Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review.* Frontiers in microbiology, 2019. **10**: p. 539.
216. Antunes, L., P. Visca, and K.J. Towner, *Acinetobacter baumannii: evolution of a global pathogen.* Pathogens and disease, 2014. **71**(3): p. 292-301.
217. Eliopoulos, G.M., L.L. Maragakis, and T.M. Perl, *Acinetobacter baumannii: epidemiology, antimicrobial resistance, and treatment options.* Clinical infectious diseases, 2008. **46**(8): p. 1254-1263.
218. Romero, L.C., et al., *Cysteine and cysteine-related signaling pathways in Arabidopsis thaliana.* Molecular plant, 2014. **7**(2): p. 264-276.

219. Davis, K.M., et al., *Iron-sulfur cluster repair contributes to Yersinia pseudotuberculosis survival within deep tissues.* Infection and immunity, 2019. **87**(10): p. e00533-19.
220. Miller, H.K. and V. Auerbuch, *Bacterial iron–sulfur cluster sensors in mammalian pathogens.* Metallomics, 2015. **7**(6): p. 943-956.
221. Thomas, D. and C. Wessel, *The state of innovation in antibacterial therapeutics.* Biotechnology Innovation Organization, 2022.
222. Ma, C., X. Yang, and P.J. Lewis, *Bacterial transcription as a target for antibacterial drug development.* Microbiology and Molecular Biology Reviews, 2016. **80**(1): p. 139-160.
223. Hutchings, M.I., A.W. Truman, and B. Wilkinson, *Antibiotics: past, present and future.* Current opinion in microbiology, 2019. **51**: p. 72-80.

APPENDICES

Appendix A (Chapter 2)

Table A2.1. Oligonucleotides used for sequencing plasmids.

Name	Sequence	Company
Plasmid pJB4		
rpob-1029-F	CATGGTTCATTCGTTGCGGA	Eton Biosciences
rpob-3434-F	GGGTGAGCAAATCGACAAAA	Eton Biosciences
rpob-1131-R	TCTCAGCCGCTTCTTTCGTT	Eton Biosciences
rpob-2059-R	CGGTTGGCATCATCGTGTT	Eton Biosciences
rpob-3002-R	ACGTTTTGTGGTTCCGCC	Eton Biosciences
rpob-flanking-F	ATGCCAGGTCTCCGATCCC	Eton Biosciences
rpob-flanking-R	CGCAAGACCAATACGAATACGG	Eton Biosciences
rpob-176-R	CGAACGAAATGCGGCTTG	Eton Biosciences
rpob-1809-F	GTTGATGGTCGCGTGAGTG	Eton Biosciences
rpob-2576-F	TACTGCCGATATTCCTAACGTC	Eton Biosciences
rpobc-4925-R	GGACGTAGATCTGGCGGAAG	Eton Biosciences
rpoc-660-F	CGTCTACGTGAAGAAATTCCACA	Eton Biosciences
rpoc-1440-F	GACTTCGACGGTGACCAAAT	Eton Biosciences
rpoc-2233-F	TGATGTCTGACTCGGGTG	Eton Biosciences
rpoc-3021-F	GAGTTAGGTCGTGAGCGTGA	Eton Biosciences
rpoc-1794-R	GCAGCGACCTGGTGTAGT	Eton Biosciences
rpoc-2603-R	GTCTTCTGCCGTTACACGAC	Eton Biosciences
rpoc-3704-R	CAAACGCAAGATGTCATGTGGG	Eton Biosciences
rpoc-flanking-R	ACTCAGCTTCCTTTCGGGC	Eton Biosciences
rpoc-flanking-F	CACGGCCGCATAATCGAA	Eton Biosciences
rpoc-3760-F	GTCTCCAAGGTGTAAAAATCAACG	Eton Biosciences
rpoB_seq2-R	ACGAAATGCGGCTTGGAGG	IDT DNA
rpoB_seq3-F	GCCAATACCTTGCTGAGCCATG	IDT DNA
rpoB_seq4-R	TCTGCACGCAAAGTATCCGC	IDT DNA
rpoB_seq5-F	TGGAAGAGATGGTTTCTGTCCG	IDT DNA
rpoB_seq6-R	CCGCGACAGATACAACCTGC	IDT DNA
rpoB_seq7-F	CTGCGGGTGATATCCTTGTAGG	IDT DNA
rpoB_seq7-R	GTTCCGCCGCCATTTGACTC	IDT DNA
rpoB_seq8-F	ATGCTCACGGTGAAATCGGATG	IDT DNA

rpoC_seq9-R	CGCAAGACCAATACGAATACGG	IDT DNA
rpoC_seq10-F	TCGAACAACAAGCCAGAATGG	IDT DNA
rpoC_seq11-R	CGCTTCAAACGGTTGTTACGG	IDT DNA
rpoC_seq12-F	CAGGTCGCTGCTTACTTTGGG	IDT DNA
rpoC_seq13-R	CTTGCTTCTGCGGTGGAATGAC	IDT DNA
rpoC_seq14-F	GTCCGTTCAGTCATTGCATGTG	IDT DNA
rpoC_seq15-R	CTGTTTGCAGCAGAAGTTCGG	IDT DNA
Plasmid pDAP39		
DuetUP1-F	GGATCTCGACGCTCTCCCT	Eton Biosciences
T7Term-R	GCTAGTTATTGCTCAGCGG	Eton Biosciences
DuetDOWN1-R	GATTATGCGGCCGTGTACAA	Eton Biosciences
DuetUP2-F	TTGTACACGGCCGCATAATC	Eton Biosciences
rpoA_seq1-F	TGACCCAATCTTGCTTCGTCC	IDT DNA

Table A2.2. Alpha subunit query coverage, percent identity, and accession identifiers[1].

Description	Query Cover	Per. ident	Accession
DNA-directed RNA polymerase subunit alpha [Acinetobacter baylyi]	100%	100	UXJ60277.1
DNA-directed RNA polymerase subunit alpha [Acinetobacter ihumii]	100%	99.7	WP_130803402.1
DNA-directed RNA polymerase subunit alpha [Acinetobacter johnsonii]	100%	99.7	WP_205667570.1
DNA-directed RNA polymerase subunit alpha [Acinetobacter ursingii]	100%	99.7	WP_151832561.1
DNA-directed RNA polymerase subunit alpha [Acinetobacter portensis]	100%	99.1	WP_152874598.1
DNA-directed RNA polymerase subunit alpha [Acinetobacter guerrae]	100%	99.7	WP_120370455.1
DNA-directed RNA polymerase subunit alpha [Acinetobacter rudis]	100%	99.1	WP_016654899.1
DNA-directed RNA polymerase subunit alpha [Acinetobacter baumannii]	100%	99.1	WP_119890479.1
DNA-directed RNA polymerase subunit alpha [Acinetobacter guillouiae]	100%	98.81	WP_004725727.1
DNA-directed RNA polymerase subunit alpha [Acinetobacter sichuanensis]	100%	99.1	WP_107007588.1
DNA-directed RNA polymerase subunit alpha [Acinetobacter seifertii]	100%	98.81	WP_216134586.1
DNA-directed RNA polymerase subunit alpha [Acinetobacter pittii]	100%	98.81	WP_250733767.1

DNA-directed RNA polymerase subunit alpha [Acinetobacter defluvii]	100%	98.51	WP_065994111.1
DNA-directed RNA polymerase subunit alpha [Acinetobacter terrestris]	100%	98.51	WP_131319106.1
DNA-directed RNA polymerase subunit alpha [Acinetobacter pragensis]	100%	98.51	WP_067669347.1
DNA-directed RNA polymerase subunit alpha [Acinetobacter indicus]	100%	98.21	WP_168416616.1
DNA-directed RNA polymerase subunit alpha [Pseudomonas aeruginosa]	98%	71.52	ARU37076.1
DNA-directed RNA polymerase subunit alpha [Escherichia coli]	96%	66.87	UJY26219.1
DNA-directed RNA polymerase subunit alpha [Staphylococcus aureus]	88%	41.81	AIU86156.1
DNA-directed RNA polymerase subunit alpha [Bacillus subtilis]	86%	42.81	SPY10807.1
DNA-directed RNA polymerase subunit alpha [Mycobacterium tuberculosis]	89%	42.38	WP_003418351.1
DNA-directed RNA polymerase subunit alpha [Bacteroides fragilis]	93%	37.8	PJY87546.1
DNA-directed RNA polymerase subunit alpha [Thermotoga maritima]	91%	39.1	Q9X1I2.1
RNA polymerase, alpha subunit [Thermus aquaticus]	86%	39.66	CAB65464.2
DNA-directed RNA polymerase subunit alpha [Fusobacterium nucleatum]	89%	36.42	VTX55421.1
DNA-directed RNA polymerase subunit alpha [Aquifex aeolicus]	93%	37.78	WP_010879981.1
DNA-directed RNA polymerase subunit alpha [Blastopirellula marina]	92%	38.12	WP_002655267.1
DNA-directed RNA polymerase subunit alpha [Chlamydia abortus]	93%	37.32	ASD30301.1
DNA-directed RNA polymerase subunit alpha [Cyanobacteria bacterium]	88%	39.74	KAA0216054.1

[1] Query coverages and percent identities comparing the sequence of *Acinetobacter baylyi* against other sequences were calculated by BLASTP program (version 2.13.0^{+}) using the “Align two or more sequences mode” [116].

Table A2.3. Beta subunit query coverage, percent identity, and accession identifiers[1].

Description	Query Cover	Per. ident	Accession
DNA-directed RNA polymerase subunit beta [Acinetobacter baylyi]	100%	100	WP_011182019.1
DNA-directed RNA polymerase subunit beta [Acinetobacter ihumii]	100%	97.06	WP_130803509.1
DNA-directed RNA polymerase subunit beta [Acinetobacter johnsonii]	100%	94.86	UIP94786.1
DNA-directed RNA polymerase subunit beta [Acinetobacter ursingii]	100%	97.06	WP_262458689.1
DNA-directed RNA polymerase subunit beta [Acinetobacter portensis]	100%	93.69	WP_152875432.1
DNA-directed RNA polymerase subunit beta [Acinetobacter guerrae]	100%	97.21	WP_151981197.1
DNA-directed RNA polymerase subunit beta [Acinetobacter rudis]	100%	93.98	WP_016657952.1
DNA-directed RNA polymerase subunit beta [Acinetobacter baumannii]	100%	95.08	WP_114190161.1
DNA-directed RNA polymerase subunit beta [Acinetobacter guillouiae]	100%	93.47	BAP35189.1
DNA-directed RNA polymerase subunit beta [Acinetobacter sichuanensis]	100%	93.83	RFC82650.1
DNA-directed RNA polymerase subunit beta [Acinetobacter seifertii]	100%	94.64	OUC58002.1
DNA-directed RNA polymerase subunit beta [Acinetobacter pittii]	100%	94.86	SSP13026.1
DNA-directed RNA polymerase subunit beta [Acinetobacter defluvii]	100%	94.2	AWL27883.1
DNA-directed RNA polymerase subunit beta [Acinetobacter terrestris]	100%	94.79	WP_131383137.1
DNA-directed RNA polymerase subunit beta [Acinetobacter pragensis]	100%	93.83	KYQ71704.1
DNA-directed RNA polymerase subunit beta [Acinetobacter indicus]	100%	94.27	QSG84854.1
DNA-directed RNA polymerase subunit beta [Pseudomonas aeruginosa]	99%	73.28	OHQ72289.1
DNA-directed RNA polymerase subunit beta [Escherichia coli]	99%	70.3	KIG93869.1
DNA-directed RNA polymerase subunit beta [Staphylococcus aureus]	81%	59.66	BBA23185.1
DNA-directed RNA polymerase subunit beta [Bacillus subtilis]	82%	57.88	SPY10843.1
DNA-directed RNA polymerase subunit beta [Mycobacterium tuberculosis]	81%	58.13	WP_003900992.1

DNA-directed RNA polymerase subunit beta [Bacteroides fragilis]	98%	44.48	UBF16585.1
DNA-directed RNA polymerase subunit beta [Thermotoga maritima]	91%	39.76	WP_004081508.1
DNA-directed RNA polymerase subunit beta [Thermus aquaticus]	81%	51	WP_053767537.1
DNA-directed RNA polymerase subunit beta [Fusobacterium nucleatum]	91%	46.64	PZA03558.1
DNA-directed RNA polymerase subunit beta [Aquifex aeolicus]	99%	46.06	WP_010881267.1
DNA-directed RNA polymerase subunit beta [Blastopirellula marina]	99%	44.15	WP_040353010.1
DNA-directed RNA polymerase subunit beta [Chlamydia abortus]	98%	46.97	ASD30785.1
DNA-directed RNA polymerase subunit beta [Cyanobacteria bacterium]	99%	43.88	KAA0216509.1

[1] Query coverages and percent identities comparing the sequence of *Acinetobacter baylyi* against other sequences were calculated by BLASTP program (version 2.13.0+) using the “Align two or more sequences mode” [116].

Table A2.4. Beta’ subunit query coverage, percent identity, and accession identifiers[1].

Description	Query Cover	Per. ident	Accession
DNA-directed RNA polymerase subunit beta' [Acinetobacter baylyi]	100%	100	WP_026056991.1
DNA-directed RNA polymerase subunit beta' [Acinetobacter ihumii]	100%	98	WP_130803508.1
DNA-directed RNA polymerase subunit beta' [Acinetobacter johnsonii]	99%	94.84	UIP94785.1
DNA-directed RNA polymerase subunit beta' [Acinetobacter ursingii]	100%	97.71	WP_004985071.1
DNA-directed RNA polymerase subunit beta' [Acinetobacter portensis]	99%	94.34	UPO23045.1
DNA-directed RNA polymerase subunit beta' [Acinetobacter guerrae]	100%	98.14	WP_120371113.1
DNA-directed RNA polymerase subunit beta' [Acinetobacter rudis]	100%	95.63	WP_016657951.1
DNA-directed RNA polymerase subunit beta' [Acinetobacter baumannii]	100%	97.57	WP_111811412.1
DNA-directed RNA polymerase subunit beta' [Acinetobacter guillouiae]	100%	95.78	BAP35190.1

DNA-directed RNA polymerase subunit beta' [Acinetobacter sichuanensis]	100%	94.92	RFC82651.1
DNA-directed RNA polymerase subunit beta' [Acinetobacter seifertii]	100%	97.49	WP_200028190.1
DNA-directed RNA polymerase subunit beta' [Acinetobacter pittii]	100%	97.57	WP_167564197.1
DNA-directed RNA polymerase subunit beta' [Acinetobacter defluvii]	100%	95.49	AWL27884.1
DNA-directed RNA polymerase subunit beta' [Acinetobacter terrestris]	99%	93.76	WP_131319945.1
DNA-directed RNA polymerase subunit beta' [Acinetobacter pragensis]	99%	93.84	WP_067669563.1
DNA-directed RNA polymerase subunit beta' [Acinetobacter indicus]	99%	95.56	QSG84855.1
DNA-directed RNA polymerase subunit beta' [Pseudomonas aeruginosa]	99%	74.47	PWU34336.1
DNA-directed RNA polymerase subunit beta' [Escherichia coli]	98%	71.94	UJY26961.1
DNA-directed RNA polymerase subunit beta' [Staphylococcus aureus]	91%	52.63	UOO31366.1
DNA-directed RNA polymerase subunit beta' [Bacillus subtilis]	89%	53.28	SPY10842.1
DNA-directed RNA polymerase subunit beta' [Mycobacterium tuberculosis]	90%	47.88	WP_003917327.1
DNA-directed RNA polymerase subunit beta' [Bacteroides fragilis]	95%	49.47	QCQ43800.1
DNA-directed RNA polymerase subunit beta' [Thermotoga maritima]	83%	45.7	WP_046380550.1
DNA-directed RNA polymerase subunit beta' [Thermus aquaticus]	87%	48.46	WP_053767538.1
DNA-directed RNA polymerase subunit beta' [Fusobacterium nucleatum]	95%	49.7	VTX48731.1
DNA-directed RNA polymerase subunit beta' [Aquifex aeolicus]	96%	49.88	WP_010881268.1
DNA-directed RNA polymerase subunit beta' [Blastopirellula marina]	96%	49.82	WP_002655480.1
DNA-directed RNA polymerase subunit beta' [Chlamydia abortus]	97%	50.25	ASD30784.1
DNA-directed RNA polymerase subunit beta' [Cyanobacteria bacterium]	97%	48.24	KAA0216510.1

[1] Query coverages and percent identities comparing the sequence of *Acinetobacter baylyi* against other sequences were calculated by BLASTP program (version 2.13.0^{+}) using the “Align two or more sequences mode” [116].

Table A2.5. Sigma70 subunit query coverage, percent identity, and accession identifiers[1].

Description	Query Cover	Per. ident	Accession
RNA polymerase sigma factor RpoD [Acinetobacter baylyi]	100%	100	WP_004929482.1
RNA polymerase sigma factor RpoD [Acinetobacter ihumii]	100%	90.95	WP_130803269.1
RNA polymerase sigma factor RpoD [Acinetobacter johnsonii]	100%	90.32	QSE46611.1
RNA polymerase sigma factor RpoD [Acinetobacter ursingii]	100%	91.11	WP_262545831.1
RNA polymerase sigma factor RpoD [Acinetobacter portensis]	100%	87.46	WP_152874413.1
RNA polymerase sigma factor RpoD [Acinetobacter guerrae]	100%	91.43	WP_120368727.1
RNA polymerase sigma factor RpoD [Acinetobacter rudis]	100%	89.05	WP_228128724.1
RNA polymerase sigma factor RpoD [Acinetobacter baumannii]	100%	90.79	EIL1911795.1
RNA polymerase sigma factor RpoD [Acinetobacter guillouiae]	100%	89.21	KAB0625444.1
RNA polymerase sigma factor RpoD [Acinetobacter sichuanensis]	100%	89.84	RFC85393.1
RNA polymerase sigma factor RpoD [Acinetobacter seifertii]	100%	91.27	WP_151741557.1
RNA polymerase sigma factor RpoD [Acinetobacter pittii]	99%	90.75	KCX61699.1
RNA polymerase sigma factor RpoD [Acinetobacter defluvii]	100%	89.21	AWL29575.1
RNA polymerase sigma factor RpoD [Acinetobacter terrestris]	100%	86.21	WP_131316464.1
RNA polymerase sigma factor RpoD [Acinetobacter pragensis]	100%	88.89	WP_264753348.1
RNA polymerase sigma factor RpoD [Acinetobacter indicus]	100%	89.52	QSG85334.1
RNA polymerase sigma factor RpoD [Pseudomonas aeruginosa]	99%	62.68	ARU36945.1
RNA polymerase sigma factor RpoD [Escherichia coli]	99%	59.84	UKM89695.1
RNA polymerase sigma factor rpoD [Staphylococcus aureus]	59%	64.62	BBA24206.1
RNA polymerase sigma factor RpoD [Bacillus subtilis]	56%	70.59	QGU23987.1

RNA polymerase sigma factor [Mycobacterium tuberculosis]	43%	59.83	WP_003413944.1
RNA polymerase sigma factor rpoD [Bacteroides fragilis]	46%	40.54	OCR40175.1
RNA polymerase sigma factor rpoD [Thermotoga maritima]	63%	55.91	AKE31082.1
RNA polymerase sigma factor RpoD [Thermus aquaticus]	55%	59.56	WP_003045010.1
RNA polymerase sigma factor RpoD [Fusobacterium nucleatum]	60%	58.48	WCB32758.1
RNA polymerase sigma factor RpoD [Aquifex aeolicus]	63%	63.39	WP_243694522.1
RNA polymerase sigma factor rpoD [Blastopirellula marina]	44%	57.66	EAQ82296.1
RNA polymerase sigma factor RpoD [Chlamydia abortus]	62%	66.12	AUS60452.1
sigma-70 family RNA polymerase sigma factor [Cyanobacteria bacterium]	53%	53.21	KAA0214283.1

[1] Query coverages and percent identities comparing the sequence of *Acinetobacter baylyi* against other sequences were calculated by BLASTP program (version 2.13.0^{+}) using the “Align two or more sequences mode” [116].

Table A2.6. Omega subunit query coverage, percent identity, and accession identifiers[1].

Description	Query Cover	Per. ident	Accession
DNA-directed RNA polymerase subunit omega [Acinetobacter baylyi]	100%	100	WP_004923811.1
DNA-directed RNA polymerase subunit omega [Acinetobacter ihumii]	100%	96.77	WP_130803491.1
DNA-directed RNA polymerase subunit omega [Acinetobacter johnsonii]	100%	92.47	WP_004691468.1
DNA-directed RNA polymerase subunit omega [Acinetobacter ursingii]	100%	97.85	VTX92829.1
DNA-directed RNA polymerase subunit omega [Acinetobacter portensis]	100%	95.7	UPO23507.1
DNA-directed RNA polymerase subunit omega [Acinetobacter guerrae]	100%	97.85	RKG31375.1
DNA-directed RNA polymerase subunit omega [Acinetobacter rudis]	98%	95.65	WP_016654792.1
DNA-directed RNA polymerase subunit omega [Acinetobacter baumannii]	98%	94.57	WP_016653489.1
DNA-directed RNA polymerase subunit omega [Acinetobacter guillouiae]	100%	96.77	BAP35275.1
DNA-directed RNA polymerase subunit omega [Acinetobacter sichuanensis]	100%	94.62	WP_107007128.1
DNA-directed RNA polymerase subunit omega [Acinetobacter seifertii]	98%	93.48	QPV59598.1
DNA-directed RNA polymerase subunit omega [Acinetobacter pittii]	98%	94.57	SSP20178.1
DNA-directed RNA polymerase subunit omega [Acinetobacter defluvii]	100%	94.62	AWL30196.1
DNA-directed RNA polymerase subunit omega [Acinetobacter terrestris]	100%	93.55	WP_171537988.1
DNA-directed RNA polymerase subunit omega [Acinetobacter pragensis]	100%	94.62	KYQ71885.1
DNA-directed RNA polymerase subunit omega [Acinetobacter indicus]	100%	93.55	WP_045795272.1
DNA-directed RNA polymerase subunit omega [Pseudomonas aeruginosa]	73%	70.59	ARU36122.1
DNA-directed RNA polymerase subunit omega [Escherichia coli]	68%	54.69	WP_069372558.1
DNA-directed RNA polymerase subunit omega [Staphylococcus aureus]	51%	30.61	BBA23759.1
DNA-directed RNA polymerase subunit omega [Bacillus subtilis]	59%	66.1	MCF7752482.1

DNA-directed RNA polymerase subunit omega [Mycobacterium tuberculosis]	68%	56.25	SGD51522.1
DNA-directed RNA polymerase subunit omega [Bacteroides fragilis]	47%	31.82	UBF15270.1
DNA-directed RNA polymerase subunit omega [Thermotoga maritima]	62%	22.03	AKE31315.1
DNA-directed RNA polymerase subunit omega [Thermus aquaticus]	54%	31.37	WP_053767593.1
DNA-directed RNA polymerase subunit omega [Fusobacterium nucleatum]	60%	30.16	WP_150152587.1
DNA-directed RNA polymerase subunit omega [Aquifex aeolicus]	59%	35.09	WP_010880068.1
DNA-directed RNA polymerase subunit omega [Blastopirellula marina]	81%	28.57	WP_105353498.1
DNA-directed RNA polymerase subunit omega [Chlamydia abortus]	74%	26.09	SHE11626.1
DNA-directed RNA polymerase subunit omega [Cyanobacteria bacterium]	53%	25.42	KAA0217347.1

[1] Query coverages and percent identities comparing the sequence of *Acinetobacter baylyi* against other sequences were calculated by BLASTP program (version 2.13.0^{+}) using the “Align two or more sequences mode” [116].

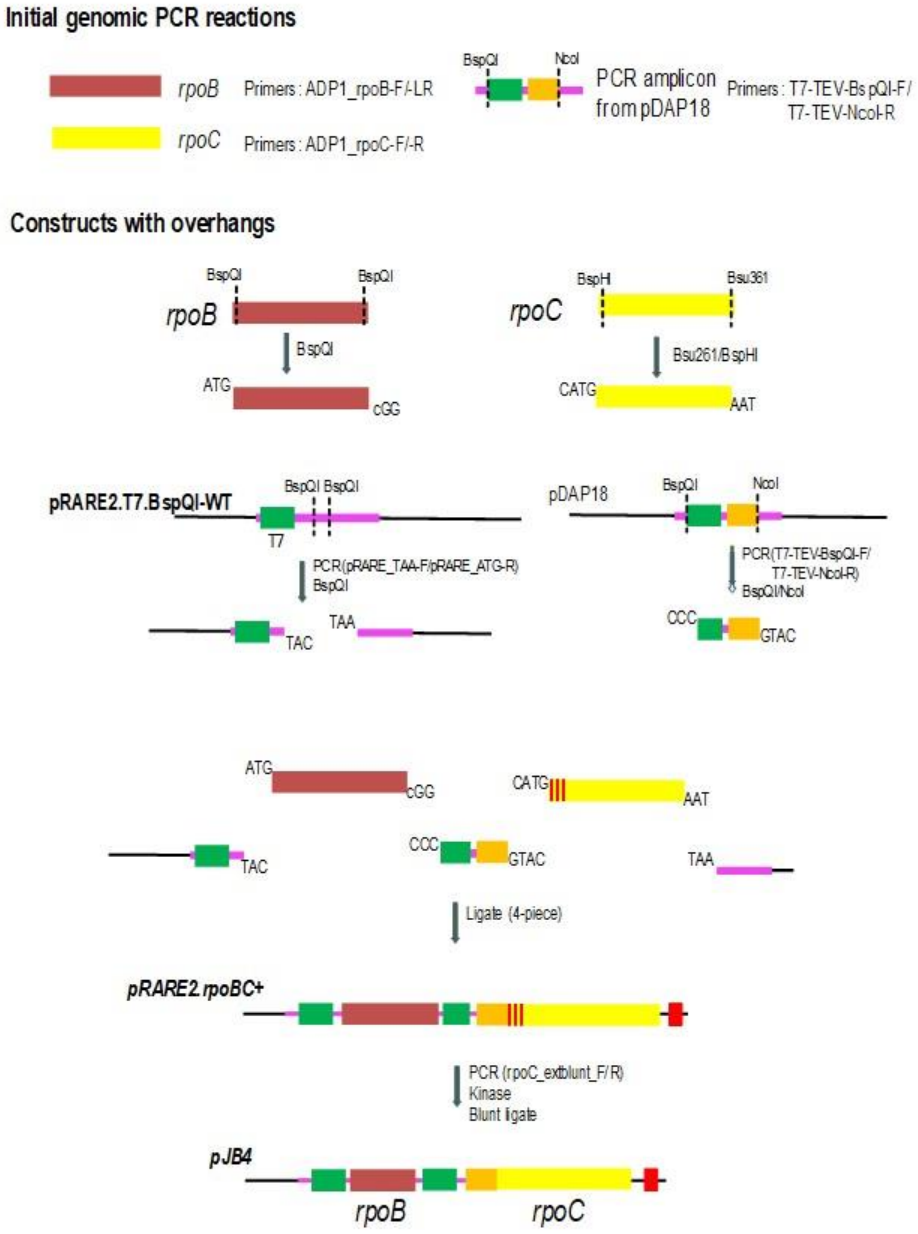


Figure A2.1. Cloning strategy for plasmid pJB4. T7 promoters are shown as green blocks. Red blocks denote the T7 terminator sequence. Lines represent plasmid backbones that should be considered continuously circular except at the junctions shown. The region removed from *rpoC* is shown as red vertical lines. Individual reactions are shown with resulting 5' or 3' overhangs.

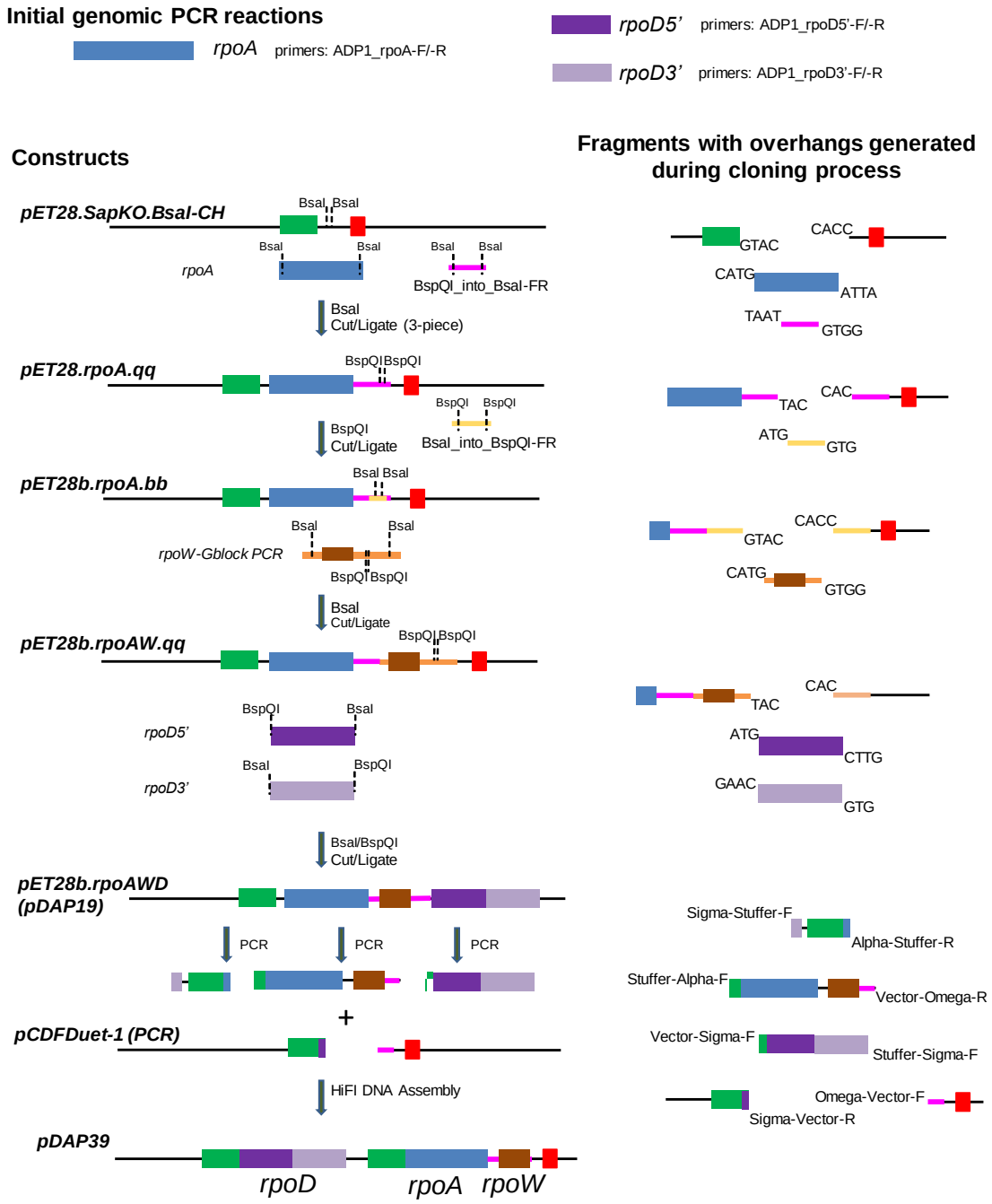
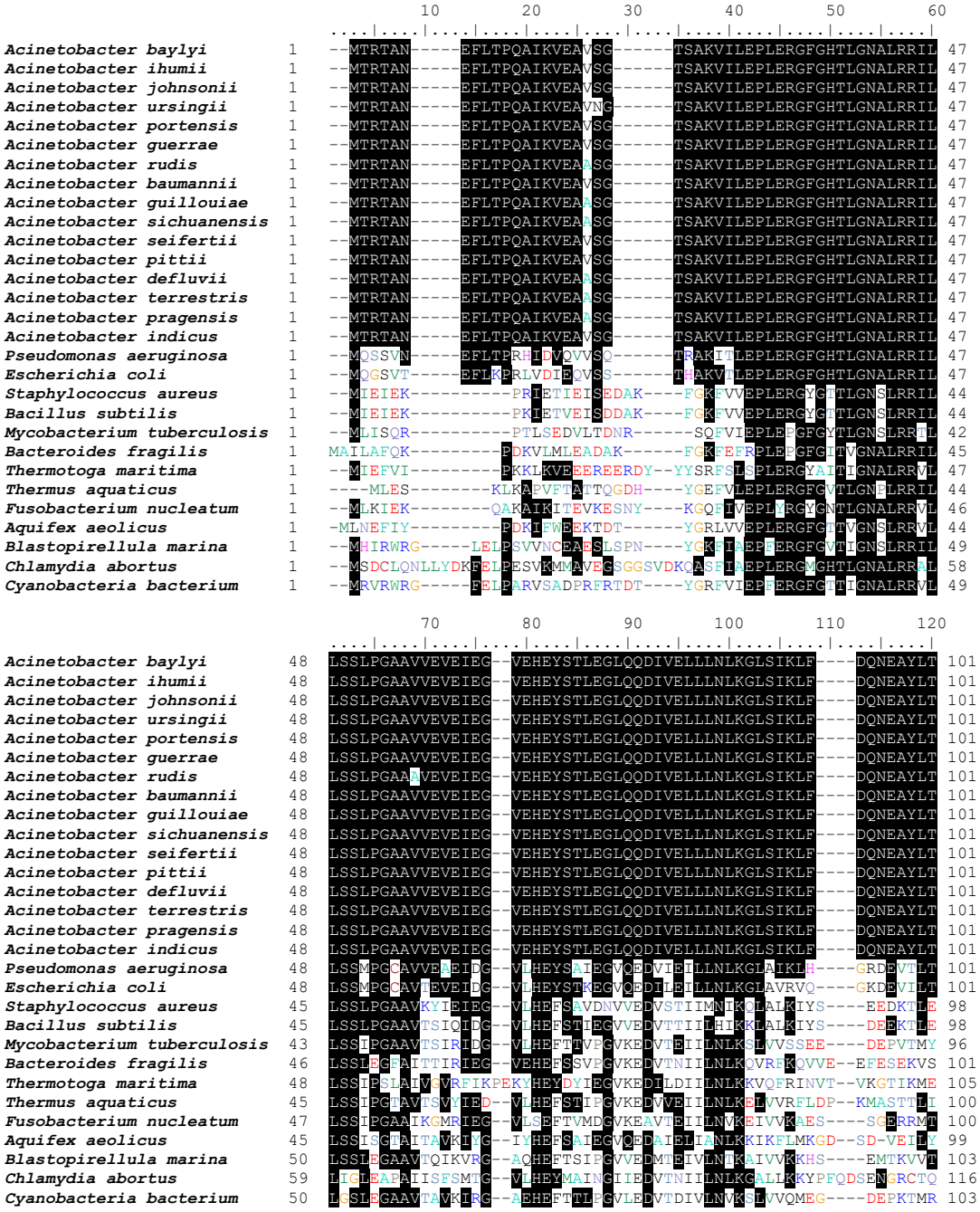
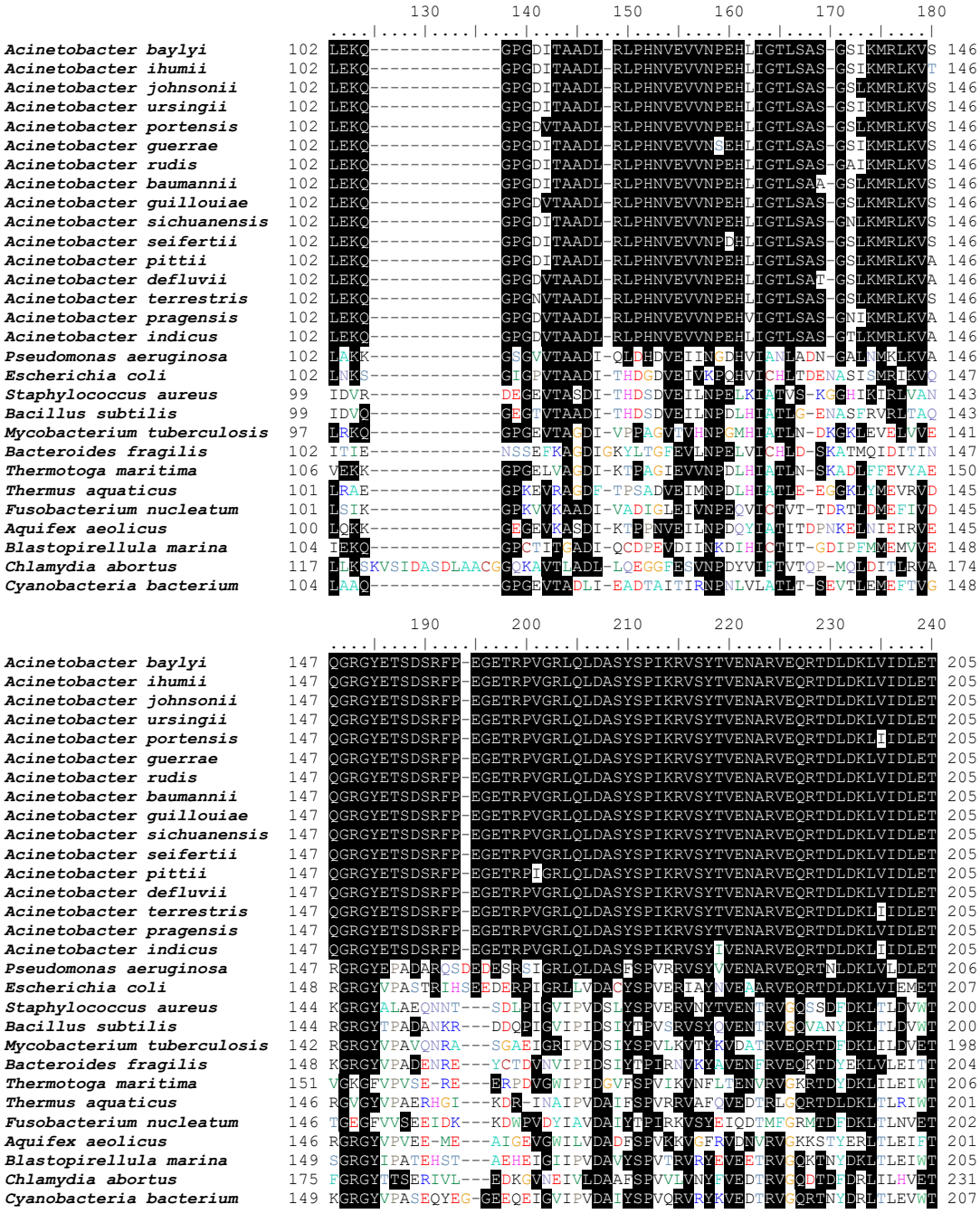
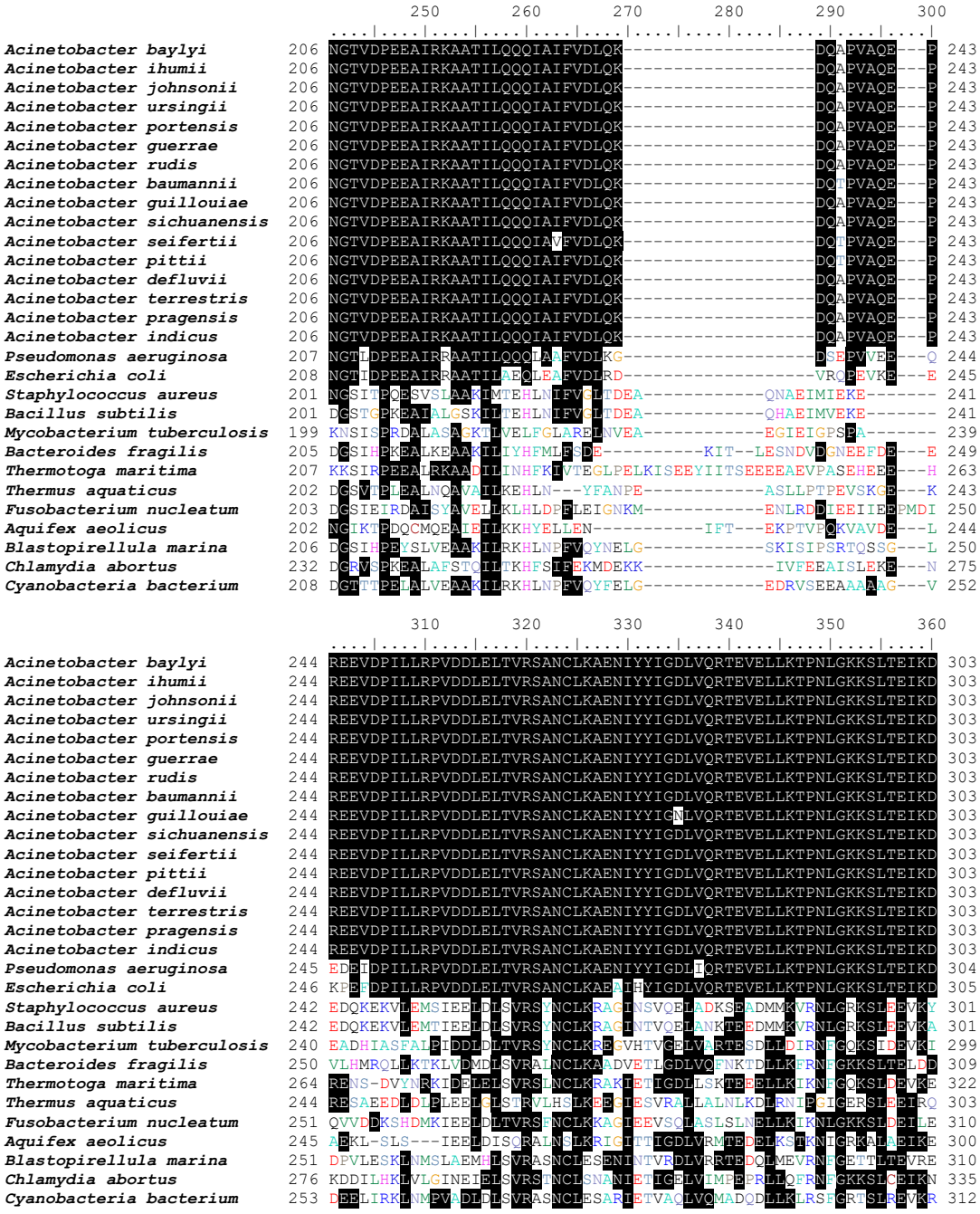


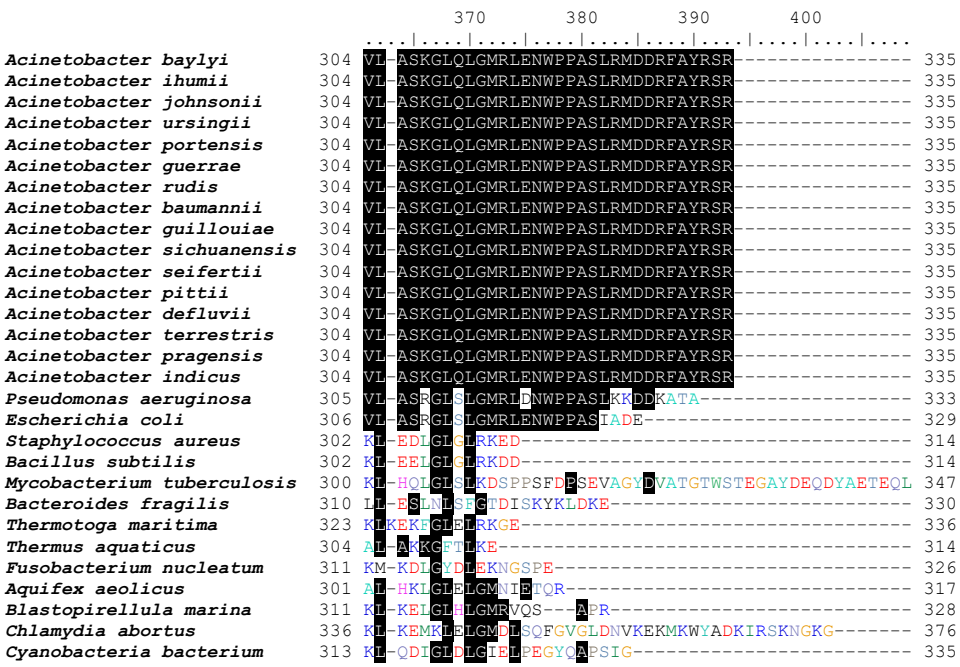
Figure A2.2. Cloning strategy for plasmid pDAP39. T7 promoters are shown as green blocks. Red blocks denote the T7 terminator sequence. Lines represent plasmid backbones that should be considered continuously circular except at the junctions shown. Reactions are shown on the left, while the resulting 5' or 3' overhangs within each reaction are shown on the right.

Figure A2.3. Sequence alignment of Alpha subunit (RpoA)[1].



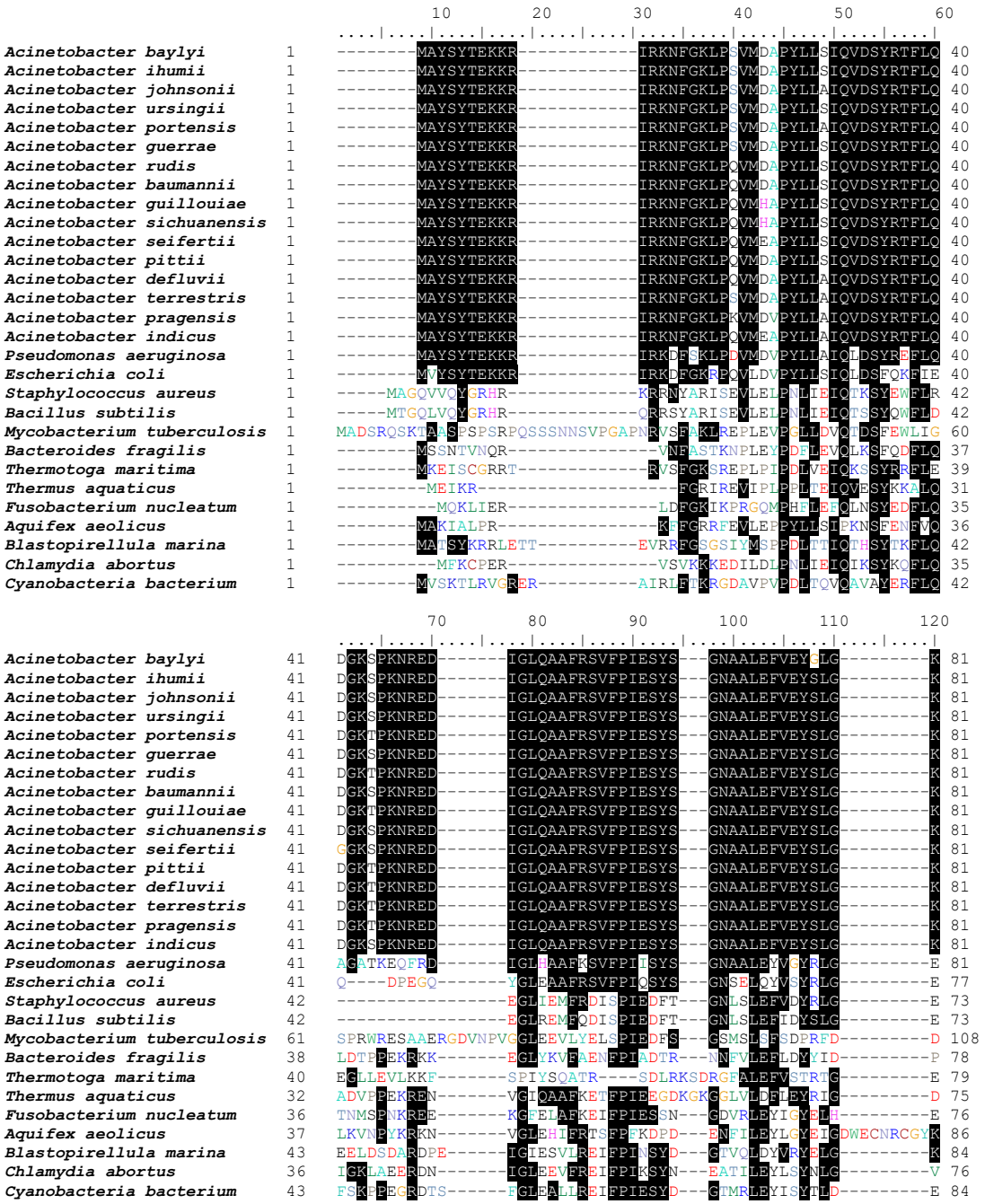

130 140 150 160 170 180
Acinetobacter baylyi 102 LEKQ----------GPGDITAADL-RLPHNVEVVNPEHLIGTLSAS-GSIKMRLKVS 146
Acinetobacter ihumii 102 LEKQ----------GPGDITAADL-RLPHNVEVVNPEHLIGTLSAS-GSIKMRLKVT 146
Acinetobacter johnsonii 102 LEKQ----------GPGDITAADL-RLPHNVEVVNPEHLIGTLSAS-GSLKMRLKVS 146
Acinetobacter ursingii 102 LEKQ----------GPGDITAADL-RLPHNVEVVNPEHLIGTLSAS-GSIKMRLKVS 146
Acinetobacter portensis 102 LEKQ----------GPGDVTAADL-RLPHNVEVVNPEHLIGTLSAS-GSLKMRLKVS 146
Acinetobacter guerrae 102 LEKQ----------GPGDITAADL-RLPHNVEVVNSEHLIGTLSAS-GSIKMRLKVS 146
Acinetobacter rudis 102 LEKQ----------GPGDITAADL-RLPHNVEVVNPEHLIGTLSAS-GAIKMRLKVS 146
Acinetobacter baumannii 102 LEKQ----------GPGDITAADL-RLPHNVEVVNPEHLIGTLSAA-GSLKMRLKVS 146
Acinetobacter guillouiae 102 LEKQ----------GPGDVTAADL-RLPHNVEVVNPEHLIGTLSAS-GSLKMRLKVS 146
Acinetobacter sichuanensis 102 LEKQ----------GPGDITAADL-RLPHNVEVVNPEHLIGTLSAS-GNLKMRLKVS 146
Acinetobacter seifertii 102 LEKQ----------GPGDITAADL-RLPHNVEVVNPDHLIGTLSAS-GSLKMRLKVS 146
Acinetobacter pittii 102 LEKQ----------GPGDITAADL-RLPHNVEVVNPEHLIGTLSAS-GSLKMRLKVA 146
Acinetobacter defluvii 102 LEKQ----------GPGDVTAADL-RLPHNVEVVNPEHLIGTLSAT-GSLKMRLKVA 146
Acinetobacter terrestris 102 LEKQ----------GPGNVTAADL-RLPHNVEVVNPEHLIGTLSAS-GSLKMRLKVS 146
Acinetobacter pragensis 102 LEKQ----------GPGDVTAADL-RLPHNVEVVNPEHVIGTLSAS-GNIKMRLKVA 146
Acinetobacter indicus 102 LEKQ----------GPGDVTAADL-RLPHNVEVVNPEHLIGTLSAS-GTLKMRLKVA 146
Pseudomonas aeruginosa 102 LAKK----------GSGVVTAADI-QLDHDVEIINGDHVIANLADN-GALNMKLKVA 146
Escherichia coli 102 LNKS----------GIGFVTAADI-THDGDVEIVKPQHVICHLTDENASISMRIKVQ 147
Staphylococcus aureus 99 IDVR----------DEGEVTASDI-THDSDVEILNPELKIATVS-KGGHIKTRLVAN 143
Bacillus subtilis 99 IDVQ----------GEGIVTAADI-THDSDVEILNPDLHIATLG-ENASFRVRLTAQ 143
Mycobacterium tuberculosis 97 LRKQ----------GPGEVTASDI-VPPAGVTVHNPGMHIATLN-DKGKLEVELVVE 141
Bacteroides fragilis 102 ITIE----------NSSEFKASDIGKYLTGPEVLNPELVICHLD-SKATMQIDITIN 147
Thermotoga maritima 106 VEKK----------GPGELVASDI-KTPAGIEVVNPDLHIATLN-SKADLFFEVYAE 150
Thermus aquaticus 101 LRAE----------GPKEVRASDF-TPSADVEIMNPDLHIATLE-EGGKLVMEVRVD 145
Fusobacterium nucleatum 101 LSIK----------GPKVVKAADI-VADIGLEIVNPEQVICTVT-TDRTLDMEFIVD 145
Aquifex aeolicus 100 LQKK----------GEGEVKASDI-KTPPNVEILNPDQYIATITDPNKELNIEIRVE 145
Blastopirellula marina 104 IEKQ----------GPCTITCADI-QCDPEVDIINKDIHICTIT-GDIPFMMEMVVE 148
Chlamydia abortus 117 LLKSKVSIDASDLAACGGDKAVTLADL-LQEGGFFSVNPDYVIPTVTQP-MQLDITLRVA 174
Cyanobacteria bacterium 104 LAAQ----------GPGEVTADLI-EADTAITIRNPNLVLATLT-SEVTLEMEFTVG 148
190 200 210 220 230 240
Acinetobacter baylyi 147 QGRGYETSDSRFP-EGETRPVGRLQLDASYSPIKRVSYTVENARVEQRTDLDKLVIDLET 205
Acinetobacter ihumii 147 QGRGYETSDSRFP-EGETRPVGRLQLDASYSPIKRVSYTVENARVEQRTDLDKLVIDLET 205
Acinetobacter johnsonii 147 QGRGYETSDSRFP-EGETRPVGRLQLDASYSPIKRVSYTVENARVEQRTDLDKLVIDLET 205
Acinetobacter ursingii 147 QGRGYETSDSRFP-EGETRPVGRLQLDASYSPIKRVSYTVENARVEQRTDLDKLVIDLET 205
Acinetobacter portensis 147 QGRGYETSDSRFP-EGETRPVGRLQLDASYSPIKRVSYTVENARVEQRTDLDKLIIDLET 205
Acinetobacter guerrae 147 QGRGYETSDSRFP-EGETRPVGRLQLDASYSPIKRVSYTVENARVEQRTDLDKLVIDLET 205
Acinetobacter rudis 147 QGRGYETSDSRFP-EGETRPVGRLQLDASYSPIKRVSYTVENARVEQRTDLDKLVIDLET 205
Acinetobacter baumannii 147 QGRGYETSDSRFP-EGETRPVGRLQLDASYSPIKRVSYTVENARVEQRTDLDKLVIDLET 205
Acinetobacter guillouiae 147 QGRGYETSDSRFP-EGETRPVGRLQLDASYSPIKRVSYTVENARVEQRTDLDKLVIDLET 205
Acinetobacter sichuanensis 147 QGRGYETSDSRFP-EGETRPVGRLQLDASYSPIKRVSYTVENARVEQRTDLDKLVIDLET 205
Acinetobacter seifertii 147 QGRGYETSDSRFP-EGETRPVGRLQLDASYSPIKRVSYTVENARVEQRTDLDKLVIDLET 205
Acinetobacter pittii 147 QGRGYETSDSRFP-EGETRPIGRLQLDASYSPIKRVSYTVENARVEQRTDLDKLVIDLET 205
Acinetobacter defluvii 147 QGRGYETSDSRFP-EGETRPVGRLQLDASYSPIKRVSYTVENARVEQRTDLDKLVIDLET 205
Acinetobacter terrestris 147 QGRGYETSDSRFP-EGETRPVGRLQLDASYSPIKRVSYTVENARVEQRTDLDKLIIDLET 205
Acinetobacter pragensis 147 QGRGYETSDSRFP-EGETRPVGRLQLDASYSPIKRVSYTVENARVEQRTDLDKLVIDLET 205
Acinetobacter indicus 147 QGRGYETSDSRFP-EGETRPVGRLQLDASYSPIKRVSYTVENARVEQRTDLDKLIIDLET 205
Pseudomonas aeruginosa 147 RGRGYEPADARQSDEDESRSIGRLQLDASFSPVRRVSYVVENARVEQRTNLDKLVLDLET 206
Escherichia coli 148 RGRGYVPASTRIHSEDERFIGRLLVDACYSPVERIAYTVEAARVEQRTDLDKLVIEMET 207
Staphylococcus aureus 144 KGRGYALAEQNNT---SDLPIGVIPVDSIYSPVERVNYTVENTRVGQSSDFDKLTLDVWT 200
Bacillus subtilis 144 RGRGYTPADANKR---DDQPIGVIPIDSIYTPVSRVSYQVENTRVGQVANYDKLTLDVWT 200
Mycobacterium tuberculosis 142 RGRGYVPAVQNRA---SGAEIGRIPVDSIYSPVLKVTYKVDATRVEQRTDFDKLILDVET 198
Bacteroides fragilis 148 KGRGYVPADENRE---YCTDVNVIPIDSIYTPIRNVKYAVENFRVEQKTDYEKLVLEITT 204
Thermotoga maritima 151 VGKGFVPVSE-RE---ERPDVGWIPIDGVFSPVIKVNFLTENVRVGKRTDYDKLILEIWT 206
Thermus aquaticus 146 RGVGYVPAERHGI---KDR-INAIPVDAIFSPVRRVAFQVEDTRLGQRTDLDKLTLRIWT 201
Fusobacterium nucleatum 146 TGEGFVVSEEIDK---KDWPVDYIAVDAIYTPIRKVSYEIQDTMFGRMTDFDKLTLNVET 202
Aquifex aeolicus 146 RGRGYVPVEE-ME---AIGEVGWILVDADFSPVKKVGFRVDNVRVGKKSTYERLTLEIFT 201
Blastopirellula marina 149 SGRGYIPATEHST---AEHEIGIIPVDAVYSPVTRVRYEVEETRVGQKTNYDKLTLEIWT 205
Chlamydia abortus 175 FGRGYTTSERIVL---EDKGVNEIVLDAAHSPVVIVNYEVDTRVGQDTDFDRLILHVET 231
Cyanobacteria bacterium 149 KGRGYVPASEQYEG-GEEQEIGVIPVDAIYSPVQRVRYKVEDTRVGQRTNYDRLTLEVWT 207

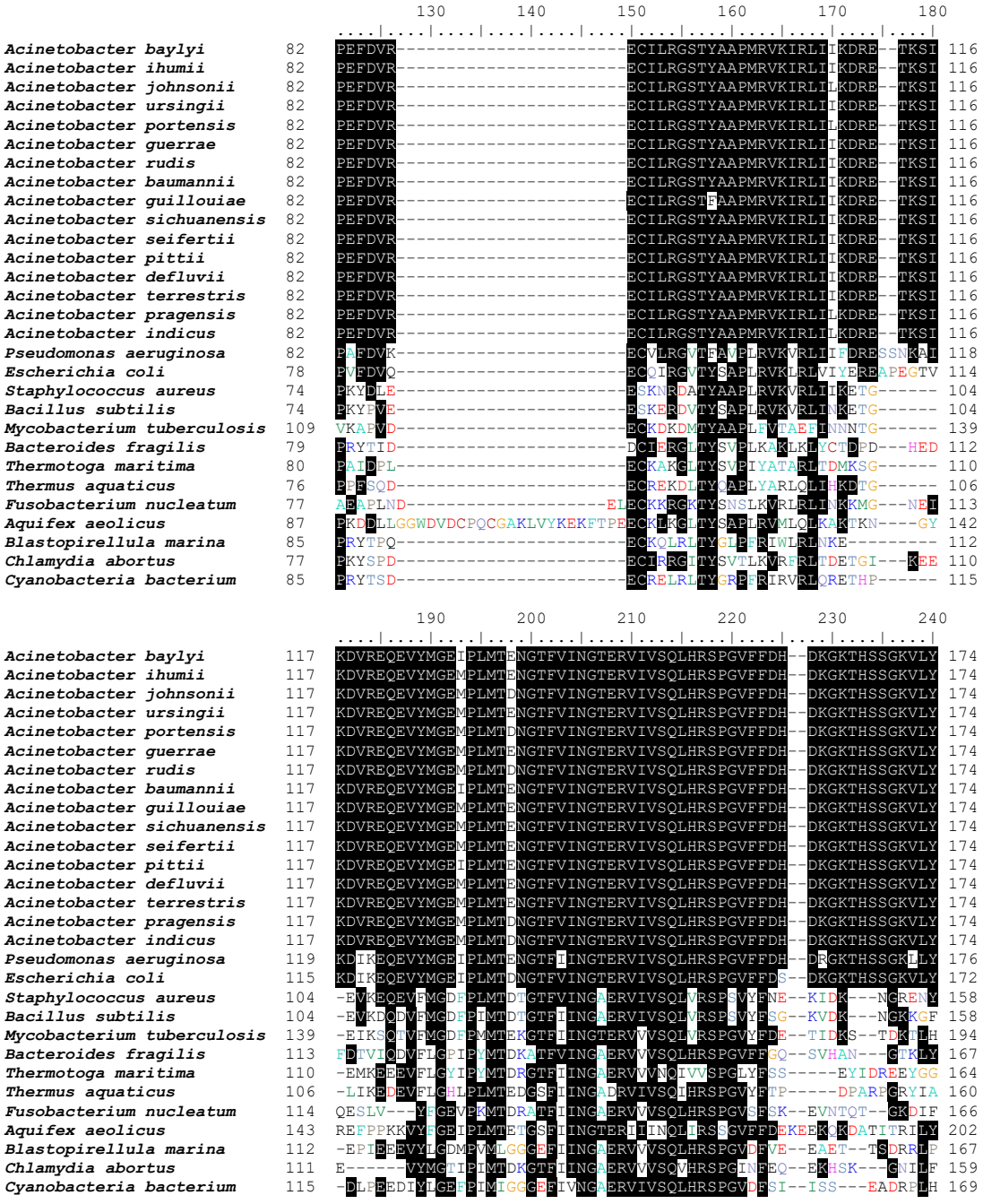

250 260 270 280 290 300
Acinetobacter baylyi 206 NGTVDPEEAIRKAATILQQQIAIFVDLQK----------DQAPVAQE---P 243
Acinetobacter ihumii 206 NGTVDPEEAIRKAATILQQQIAIFVDLQK----------DQAPVAQE---P 243
Acinetobacter johnsonii 206 NGTVDPEEAIRKAATILQQQIAIFVDLQK----------DQAPVAQE---P 243
Acinetobacter ursingii 206 NGTVDPEEAIRKAATILQQQIAIFVDLQK----------DQAPVAQE---P 243
Acinetobacter portensis 206 NGTVDPEEAIRKAATILQQQIAIFVDLQK----------DQAPVAQE---P 243
Acinetobacter guerrae 206 NGTVDPEEAIRKAATILQQQIAIFVDLQK----------DQAPVAQE---P 243
Acinetobacter rudis 206 NGTVDPEEAIRKAATILQQQIAIFVDLQK----------DQAPVAQE---P 243
Acinetobacter baumannii 206 NGTVDPEEAIRKAATILQQQIAIFVDLQK----------DQTPVAQE---P 243
Acinetobacter guillouiae 206 NGTVDPEEAIRKAATILQQQIAIFVDLQK----------DQAPVAQE---P 243
Acinetobacter sichuanensis 206 NGTVDPEEAIRKAATILQQQIAIFVDLQK----------DQAPVAQE---P 243
Acinetobacter seifertii 206 NGTVDPEEAIRKAATILQQQIAVFVDLQK----------DQTPVAQE---P 243
Acinetobacter pittii 206 NGTVDPEEAIRKAATILQQQIAIFVDLQK----------DQTPVAQE---P 243
Acinetobacter defluvii 206 NGTVDPEEAIRKAATILQQQIAIFVDLQK----------DQAPVAQE---P 243
Acinetobacter terrestris 206 NGTVDPEEAIRKAATILQQQIAIFVDLQK----------DQAPVAQE---P 243
Acinetobacter pragensis 206 NGTVDPEEAIRKAATILQQQIAIFVDLQK----------DQAPVAQE---P 243
Acinetobacter indicus 206 NGTVDPEEAIRKAATILQQQIAIFVDLQK----------DQAPVAQE---P 243
Pseudomonas aeruginosa 207 NGTLDPEEAIRAATILQQQLAAFVDLKG----------DSEPVVEE---Q 244
Escherichia coli 208 NGTIDPEEAIRAATILAEQLEAFVDLRD----------VRQPEVKE---E 245
Staphylococcus aureus 201 NGSITPQESVSLAAKIMTEHLNIFVGLTDEA----------QNAEIMIEKE------- 241
Bacillus subtilis 201 DGSTGPKEAIALGSKILTEHLNIFVGLTDEA----------QHAEIMVEKE------- 241
Mycobacterium tuberculosis 199 KNSISPRDALASAGKTLVELFGLARELNVEA----------EGIEIGPSPA------- 239
Bacteroides fragilis 205 DGSIHPKEALKEAAKILIYHFMLFSDE----------KIT--LESNDVDGNEEFDE---E 249
Thermotoga maritima 207 KKSIRPEEALRKAADILINHFKIVTEGLPELKISEEYIITSEEEEAEVPASEHEEE---H 263
Thermus aquaticus 202 DGSVTPLEALNQAVAILKEHLN---YFANPE----------ASLLPTPEVSKGE---K 243
Fusobacterium nucleatum 203 DGSIEIRDAISYAVELLKLHLDPFLEIGNKM----------ENLRDDIEEIIEEPMDI 250
Aquifex aeolicus 202 NGIKTPDQCMQEAIELLKKHYELLEN----------IFT---EKPTVPQKVAVDE---L 244
Blastopirellula marina 206 DGSIHPEYSLVEAAKILRKHLNPFVQYNELG----------SKISIPSRTQSSG---L 250
Chlamydia abortus 232 DGRVSPKEALAFSTQILTKHFSIFEKMDEKK----------IVFEEAISLEKE---N 275
Cyanobacteria bacterium 208 DGTTTPELALVEAAKILRKHLNPFVQYFELG----------EDRVSEEAAAAAG---V 252
310 320 330 340 350 360
Acinetobacter baylyi 244 REEVDPILLRPVDDLELTVRSANCLKAENIYYIGDLVQRTEVELLKTPNLGKKSLTEIKD 303
Acinetobacter ihumii 244 REEVDPILLRPVDDLELTVRSANCLKAENIYYIGDLVQRTEVELLKTPNLGKKSLTEIKD 303
Acinetobacter johnsonii 244 REEVDPILLRPVDDLELTVRSANCLKAENIYYIGDLVQRTEVELLKTPNLGKKSLTEIKD 303
Acinetobacter ursingii 244 REEVDPILLRPVDDLELTVRSANCLKAENIYYIGDLVQRTEVELLKTPNLGKKSLTEIKD 303
Acinetobacter portensis 244 REEVDPILLRPVDDLELTVRSANCLKAENIYYIGDLVQRTEVELLKTPNLGKKSLTEIKD 303
Acinetobacter guerrae 244 REEVDPILLRPVDDLELTVRSANCLKAENIYYIGDLVQRTEVELLKTPNLGKKSLTEIKD 303
Acinetobacter rudis 244 REEVDPILLRPVDDLELTVRSANCLKAENIYYIGDLVQRTEVELLKTPNLGKKSLTEIKD 303
Acinetobacter baumannii 244 REEVDPILLRPVDDLELTVRSANCLKAENIYYIGDLVQRTEVELLKTPNLGKKSLTEIKD 303
Acinetobacter guillouiae 244 REEVDPILLRPVDDLELTVRSANCLKAENIYYIGNLVQRTEVELLKTPNLGKKSLTEIKD 303
Acinetobacter sichuanensis 244 REEVDPILLRPVDDLELTVRSANCLKAENIYYIGDLVQRTEVELLKTPNLGKKSLTEIKD 303
Acinetobacter seifertii 244 REEVDPILLRPVDDLELTVRSANCLKAENIYYIGDLVQRTEVELLKTPNLGKKSLTEIKD 303
Acinetobacter pittii 244 REEVDPILLRPVDDLELTVRSANCLKAENIYYIGDLVQRTEVELLKTPNLGKKSLTEIKD 303
Acinetobacter defluvii 244 REEVDPILLRPVDDLELTVRSANCLKAENIYYIGDLVQRTEVELLKTPNLGKKSLTEIKD 303
Acinetobacter terrestris 244 REEVDPILLRPVDDLELTVRSANCLKAENIYYIGDLVQRTEVELLKTPNLGKKSLTEIKD 303
Acinetobacter pragensis 244 REEVDPILLRPVDDLELTVRSANCLKAENIYYIGDLVQRTEVELLKTPNLGKKSLTEIKD 303
Acinetobacter indicus 244 REEVDPILLRPVDDLELTVRSANCLKAENIYYIGDLVQRTEVELLKTPNLGKKSLTEIKD 303
Pseudomonas aeruginosa 245 EDEIDPILLRPVDDLELTVRSANCLKAENIYYIGDLIQRTEVELLKTPNLGKKSLTEIKD 304
Escherichia coli 246 KPEFDPILLRPVDDLELTVRSANCLKAEAIHYIGDLVQRTEVELLKTPNLGKKSLTEIKD 305
Staphylococcus aureus 242 EDQKEKVLEMSIEELDLSVRSYNCLKRAGINSVQELADKSEADMMKVRNLGRKSLEEVKY 301
Bacillus subtilis 242 EDQKEKVLEMTIEELDLSVRSYNCLKRAGINTVQELANKTEEDMMKVRNLGRKSLEEVKA 301
Mycobacterium tuberculosis 240 EADHIASFAIPIDDLDLTVRSYNCLKREGVHTVGELVARTESDLLDIRNFGQKSIDEVKI 299
Bacteroides fragilis 250 VLHMRQLLKTKLVDMDLSVRALNCLKAADVETLGDLVQFNKTDLLKFRNFGKKSLTELDD 309
Thermotoga maritima 264 RENS-DVYNFKIDELELSVRSLNCLKRAKIETIGDLLSKTEEELLKIKNFGQKSIDEVKE 322
Thermus aquaticus 244 RESAEEDLDIPLEELGLSTRVLHSLKEEGIESVRALLALNLKDLRNIPGIGERSLEEIRQ 303
Fusobacterium nucleatum 251 QVVDDKSHDMKIEELDLTVRSFNCLKKAGIEEVSQLASLSLNELLKIKNLGKKSLDEILE 310
Aquifex aeolicus 245 AEKL-SLS---IEELDISQRALNSLKRIGITTIGDLVRMTEDELKSTKNIGRKALAEIKE 300
Blastopirellula marina 251 DPVLESKLNMSLAEMHLSVRASNCLESENINTVRDLVRRTEDQLMEVRNFGETTLTEVRE 310
Chlamydia abortus 276 KDDILHKLVLGINEIELSVRSTNCLSNANIETIGDLVIMEPRLLQFRNFGKKSLCEIKN 335
Cyanobacteria bacterium 253 DEELIRKLNMPVADLDLSVRASNCLESARIETVAQLVQMADQDLLKLRSFGRTSIREVKR 312

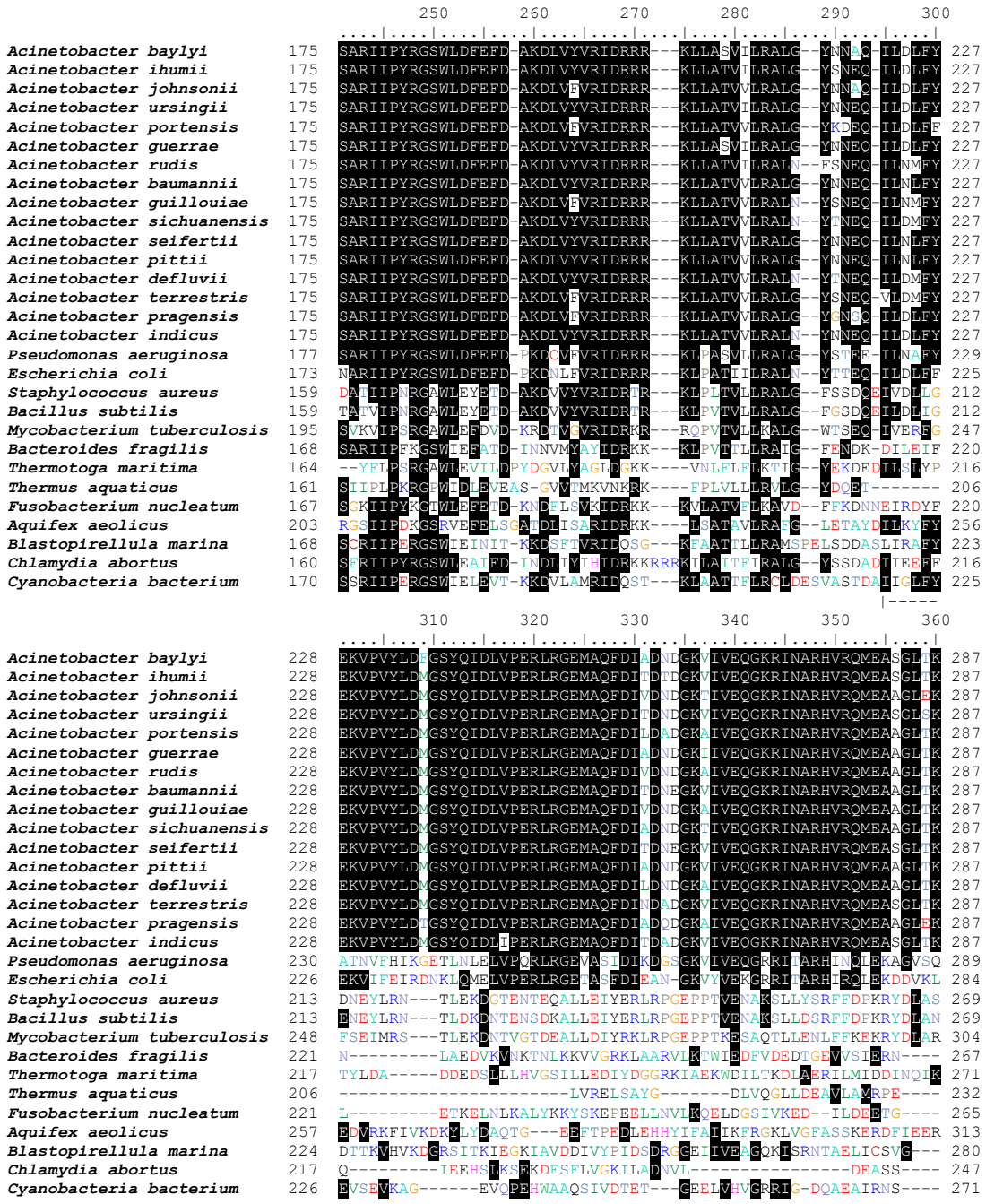


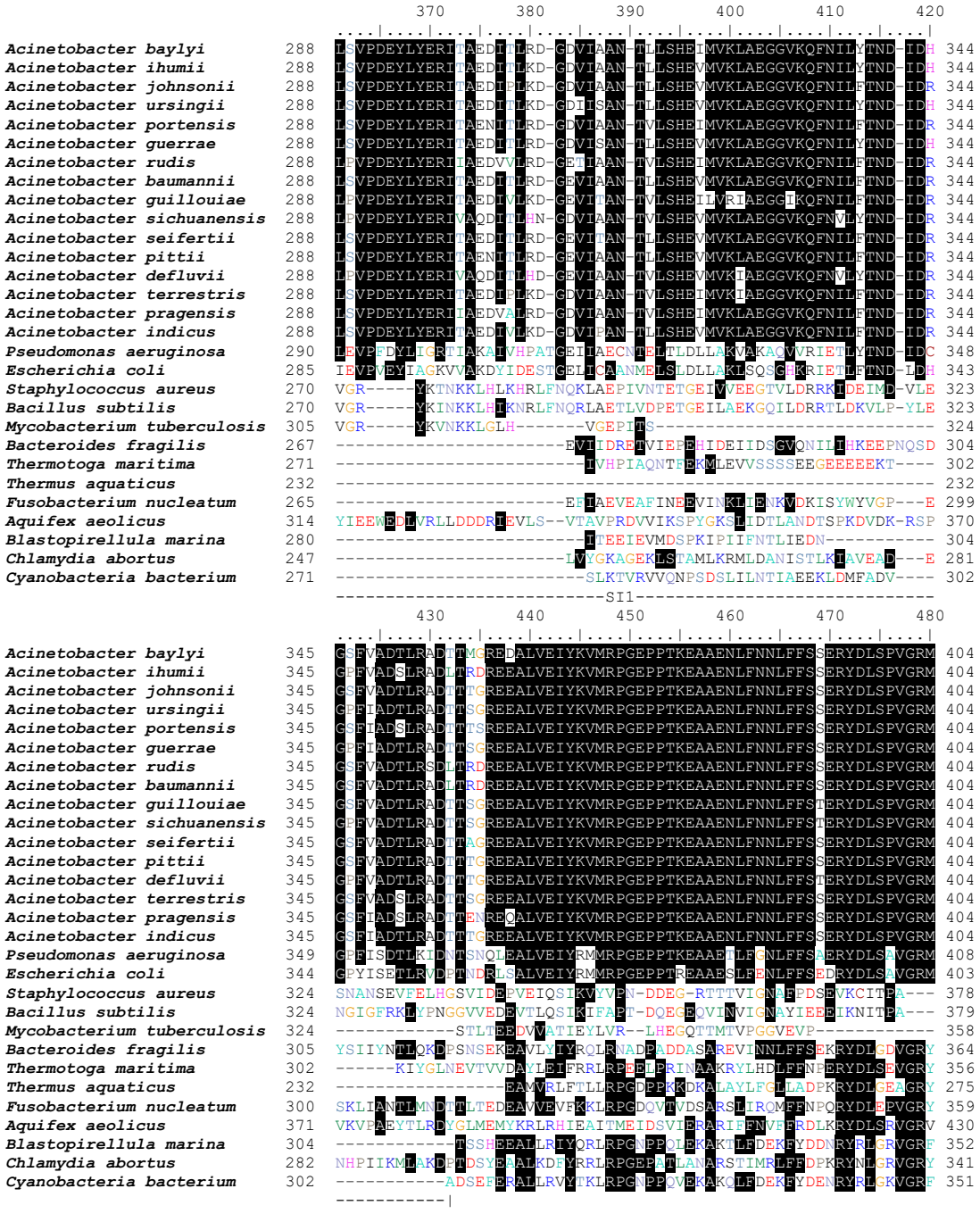
[1] Protein sequences were selected from the NCBI database (https://www.ncbi.nlm.nih.gov/protein) and aligned using CLUSTALW implemented within BioEdit using default parameters [115]. Black background denotes 55% identity, which highlights the high sequence identity within the *Acinetobacter* species.

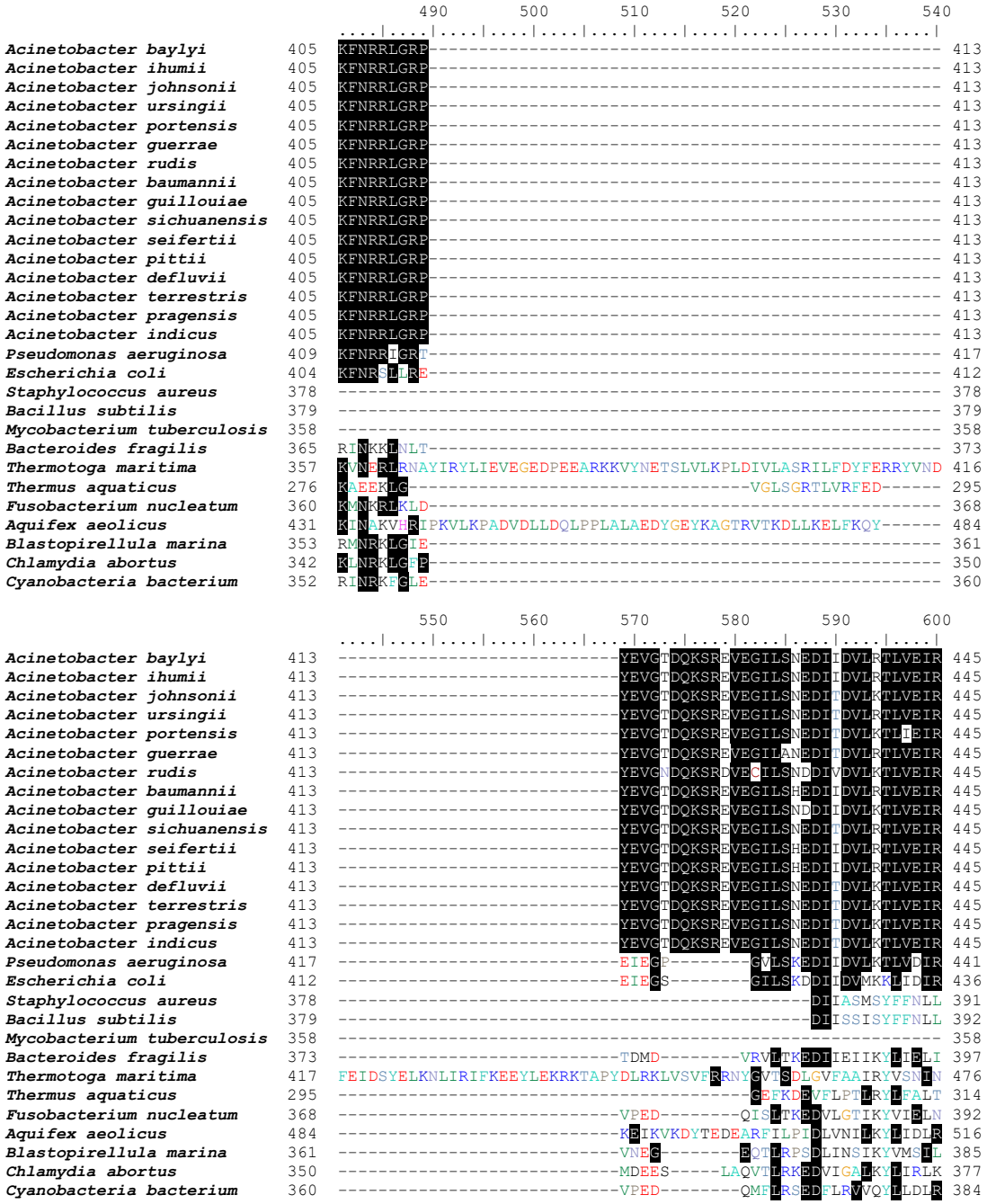
Figure A2.4. Sequence alignment of Beta subunit (RpoB)[1].

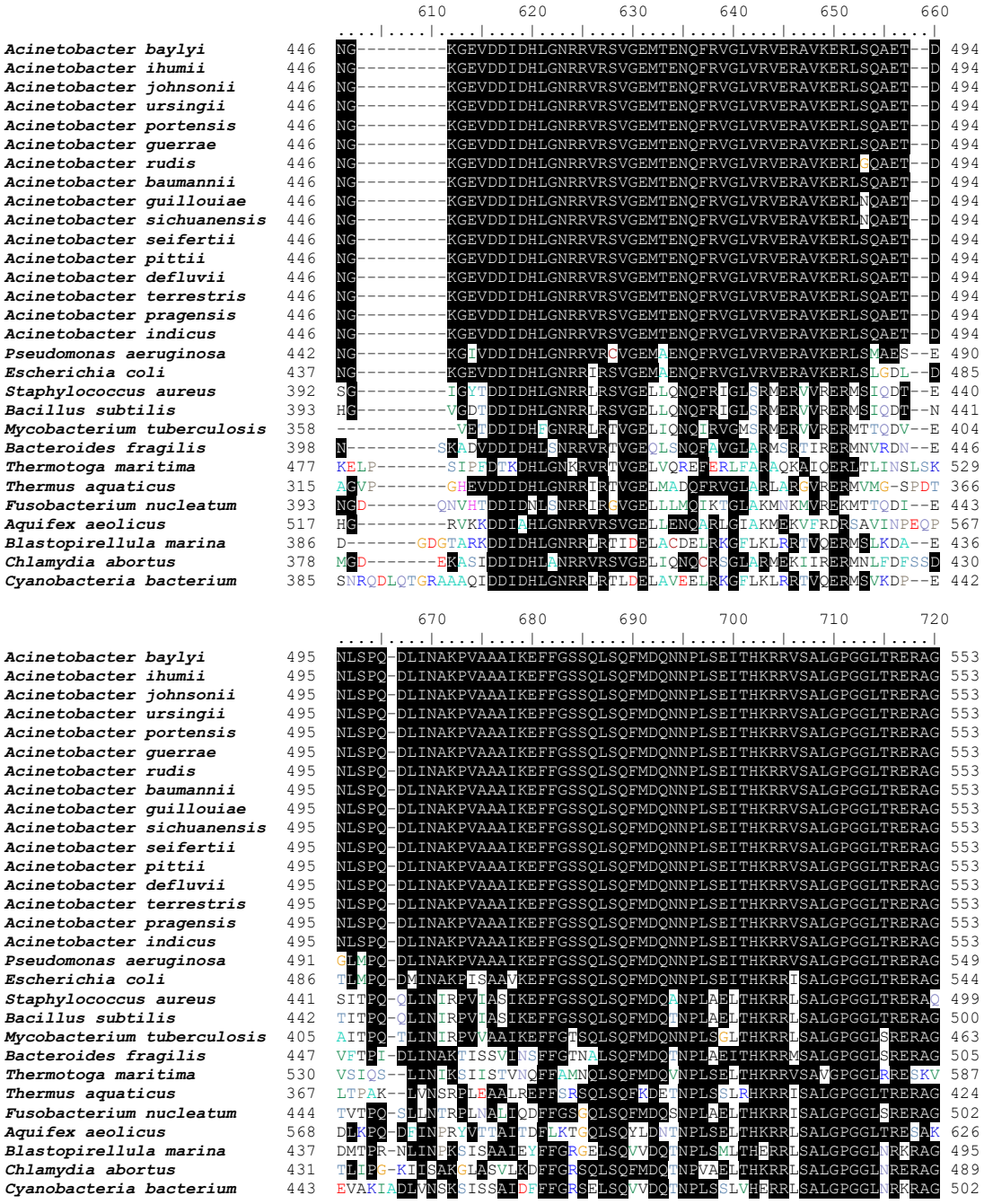


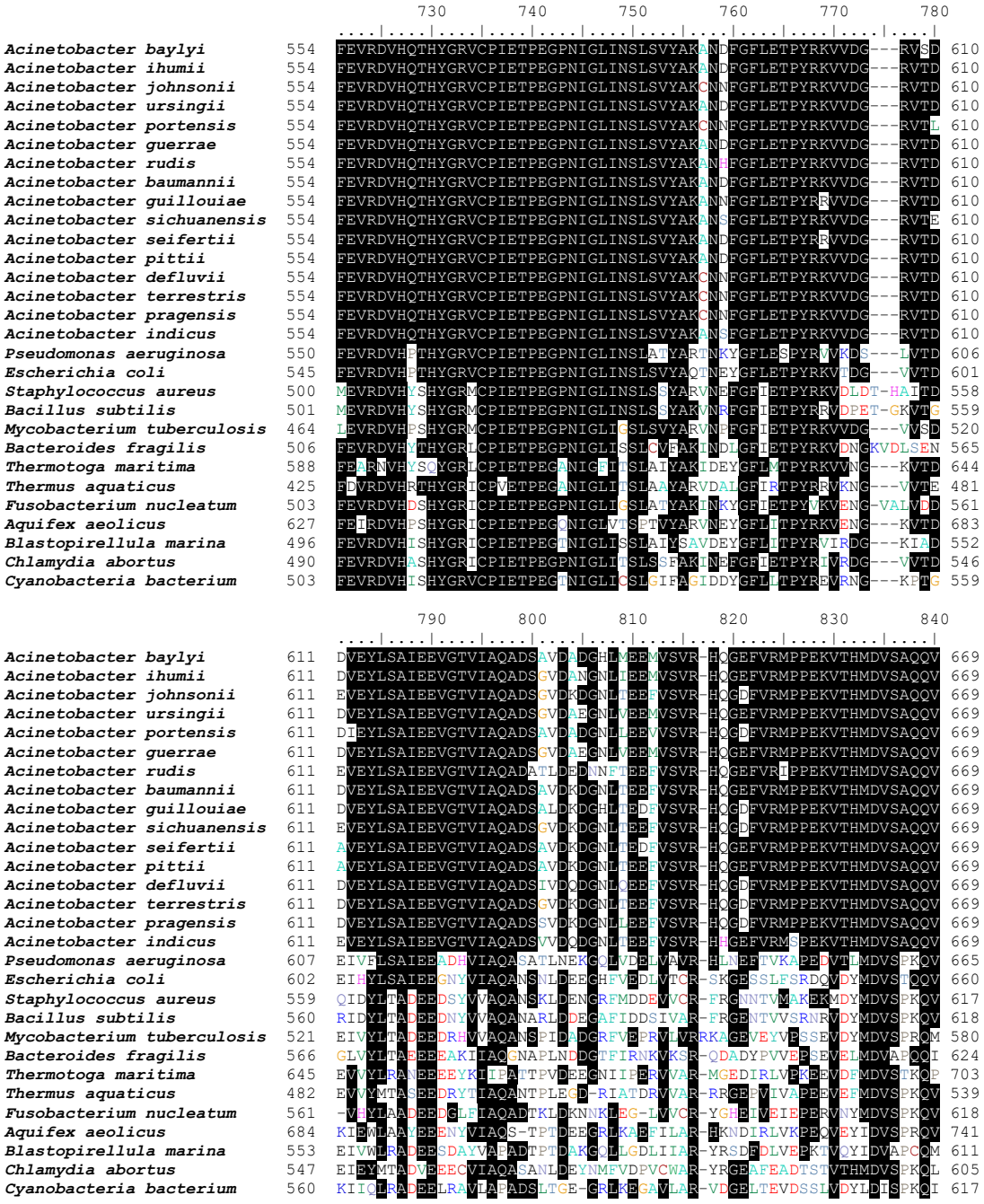

130 140 150 160 170 180
Acinetobacter baylyi 82 PEFDVR----------ECILRGSTYAAPMRVKIRLIIKDRE--TKSI 116
Acinetobacter ihumii 82 PEFDVR----------ECILRGSTYAAPMRVKIRLIIKDRE--TKSI 116
Acinetobacter johnsonii 82 PEFDVR----------ECILRGSTYAAPMRVKIRLILKDRE--TKSI 116
Acinetobacter ursingii 82 PEFDVR----------ECILRGSTYAAPMRVKIRLIIKDRE--TKSI 116
Acinetobacter portensis 82 PEFDVR----------ECILRGSTYAAPMRVKIRLILKDRE--TKSI 116
Acinetobacter guerrae 82 PEFDVR----------ECILRGSTYAAPMRVKIRLIIKDRE--TKSI 116
Acinetobacter rudis 82 PEFDVR----------ECILRGSTYAAPMRVKIRLIIKDRE--TKSI 116
Acinetobacter baumannii 82 PEFDVR----------ECILRGSTYAAPMRVKIRLIIKDRE--TKSI 116
Acinetobacter guillouiae 82 PEFDVR----------ECILRGSTFAAPMRVKIRLIIKDRE--TKSI 116
Acinetobacter sichuanensis 82 PEFDVR----------ECILRGSTYAAPMRVKIRLIIKDRE--TKSI 116
Acinetobacter seifertii 82 PEFDVR----------ECILRGSTYAAPMRVKIRLIIKDRE--TKSI 116
Acinetobacter pittii 82 PEFDVR----------ECILRGSTYAAPMRVKIRLIIKDRE--TKSI 116
Acinetobacter defluvii 82 PEFDVR----------ECILRGSTYAAPMRVKIRLIIKDRE--TKSI 116
Acinetobacter terrestris 82 PEFDVR----------ECILRGSTYAAPMRVKIRLIIKDRE--TKSI 116
Acinetobacter pragensis 82 PEFDVR----------ECILRGSTYAAPMRVKIRLILKDRE--TKSI 116
Acinetobacter indicus 82 PEFDVR----------ECILRGSTYAAPMRVKIRLILKDRE--TKSI 116
Pseudomonas aeruginosa 82 PAFDVK----------ECVLRGVTFAVPLRVKVRLIIFDRESSNKAI 118
Escherichia coli 78 PVFDVQ----------ECQIRGVTYSAPLRVKLRLVIYERESAPEGTV 114
Staphylococcus aureus 74 PKYDLE----------ESKNRDATYAAPLRVKVRLIIKETG------ 104
Bacillus subtilis 74 PKYFVE----------ESKERDVTYSAPLRVKVRLINKETG------ 104
Mycobacterium tuberculosis 109 VKAFVD----------ECKDKDMTYAAPLFVTAEFINNNTG------ 139
Bacteroides fragilis 79 PRYTID----------DCIERGLTYSVPLKAKLKLYCTDPD---HED 112
Thermotoga maritima 80 PAIDPL----------ECKAKGLTYSVPIYATARLTDMKSG------ 110
Thermus aquaticus 76 PPFSQD----------ECREKDLTYSAPLYARLQLLHKDTG------ 106
Fusobacterium nucleatum 77 AEAPLND----------ELECKKRGKTYSNSLKVRLRLINKKMG---NEI 113
Aquifex aeolicus 87 PKDDLLGGWDVDCPQCGAKLVYKEKFTPEECKLKGLTYSAPLRVMLQLKAKTKN----GY 142
Blastopirellula marina 85 PRYTPQ----------ECKQLRLTYSLPFRIWLRLNKE--------- 112
Chlamydia abortus 77 PKYSPD----------ECIRRGITYSVTLKVRFRLTDETGI---KEE 110
Cyanobacteria bacterium 85 PRYTSD----------ECRELRLTYSRPFRIRVRLQRETHP------ 115
190 200 210 220 230 240
Acinetobacter baylyi 117 KDVREQEVYMGEIPLMTENGTFVINGTERVIVSQLHRSPGVFFDH--DKGKTHSSGKVLY 174
Acinetobacter ihumii 117 KDVREQEVYMGEMPLMTENGTFVINGTERVIVSQLHRSPGVFFDH--DKGKTHSSGKVLY 174
Acinetobacter johnsonii 117 KDVREQEVYMGEMPLMTDNGTFVINGTERVIVSQLHRSPGVFFDH--DKGKTHSSGKVLY 174
Acinetobacter ursingii 117 KDVREQEVYMGEMPLMTENGTFVINGTERVIVSQLHRSPGVFFDH--DKGKTHSSGKVLY 174
Acinetobacter portensis 117 KDVREQEVYMGEMPLMTDNGTFVINGTERVIVSQLHRSPGVFFDH--DKGKTHSSGKVLY 174
Acinetobacter guerrae 117 KDVREQEVYMGEMPLMTENGTFVINGTERVIVSQLHRSPGVFFDH--DKGKTHSSGKVLY 174
Acinetobacter rudis 117 KDVREQEVYMGEMPLMTDNGTFVINGTERVIVSQLHRSPGVFFDH--DKGKTHSSGKVLY 174
Acinetobacter baumannii 117 KDVREQEVYMGEIPLMTENGTFVINGTERVIVSQLHRSPGVFFDH--DKGKTHSSGKVLY 174
Acinetobacter guillouiae 117 KDVREQEVYMGEMPLMTENGTFVINGTERVIVSQLHRSPGVFFDH--DKGKTHSSGKVLY 174
Acinetobacter sichuanensis 117 KDVREQEVYMGEMPLMTENGTFVINGTERVIVSQLHRSPGVFFDH--DKGKTHSSGKVLY 174
Acinetobacter seifertii 117 KDVREQEVYMGEMPLMTENGTFVINGTERVIVSQLHRSPGVFFDH--DKGKTHSSGKVLY 174
Acinetobacter pittii 117 KDVREQEVYMGEIPLMTENGTFVINGTERVIVSQLHRSPGVFFDH--DKGKTHSSGKVLY 174
Acinetobacter defluvii 117 KDVREQEVYMGEMPLMTENGTFVINGTERVIVSQLHRSPGVFFDH--DKGKTHSSGKVLY 174
Acinetobacter terrestris 117 KDVREQEVYMGEMPLMTENGTFVINGTERVIVSQLHRSPGVFFDH--DKGKTHSSGKVLY 174
Acinetobacter pragensis 117 KDVREQEVYMGEMPLMTDNGTFVINGTERVIVSQLHRSPGVFFDH--DKGKTHSSGKVLY 174
Acinetobacter indicus 117 KDVREQEVYMGEMPLMTDNGTFVINGTERVIVSQLHRSPGVFFDH--DKGKTHSSGKVLY 174
Pseudomonas aeruginosa 119 KDIKEQEVYMGEIPLMTENGTFIINGTERVIVSQLHRSPGVFFDH--DRGKTHSSGKLLY 176
Escherichia coli 115 KDIKEQEVYMGEIPLMTDNGTFVINGTERVIVSQLHRSPGVFFDS--DKGKTHSSGKVLY 172
Staphylococcus aureus 104 -EVKEQEVFMGDFPLMTDTGTFVINGAERVIVSQLVRSPSVYFNE--KIDK---NGRENY 158
Bacillus subtilis 104 -EVKDQDVFMGDFPIMTDTGTFIINGAERVIVSQLVRSPSVYFSG--KVDK---NGKKGF 158
Mycobacterium tuberculosis 139 -EIKSQTVFMGDFPMMTEKGTFIINGTERVVVSQLVRSPGVYFDE--TIDKS--TDKTLH 194
Bacteroides fragilis 113 FDTVIQDVFLGPIPYMTDKATFVINGAERVVVSQLHRSPGVFFSQ--SVHAN---GTKLY 167
Thermotoga maritima 110 -EMKEEEVFLGYIPYMTDRGTFIINGAERVVVNQIVVSPGLYFSS-----EYIDREEYGG 164
Thermus aquaticus 106 -LIKEDEVFLGHLPLMTEDGSFIINGADRVIVSQIHRSPGVYFTP-----DPARPGRYIA 160
Fusobacterium nucleatum 114 QESLV---YEGEVPKMTDRATFIINGAERVVVSQLHRSPGVSFSK--EVNTQT--GKDIF 166
Aquifex aeolicus 143 REFPPKKVYEGEIPLMTETGSFIINGTERIIINQLIRSSGVFFDEKEEKQKDATITRILY 202
Blastopirellula marina 112 -EPIEEEVYLGDMPVMLGGGEFIINGAERVVVSQLHRSPGVDFVE--EAET--TSDRRLP 167
Chlamydia abortus 111 E------VYMGTIPIMTDKGTFIINGAERVVVSQVHRSPGINFEQ--EKHSK---GNIIF 159
Cyanobacteria bacterium 115 -DLEEEDIYLGEFPIMIGGGEFIVNGAERVIVSQLHRSPGVDFSI--ISS---EADRPLH 169

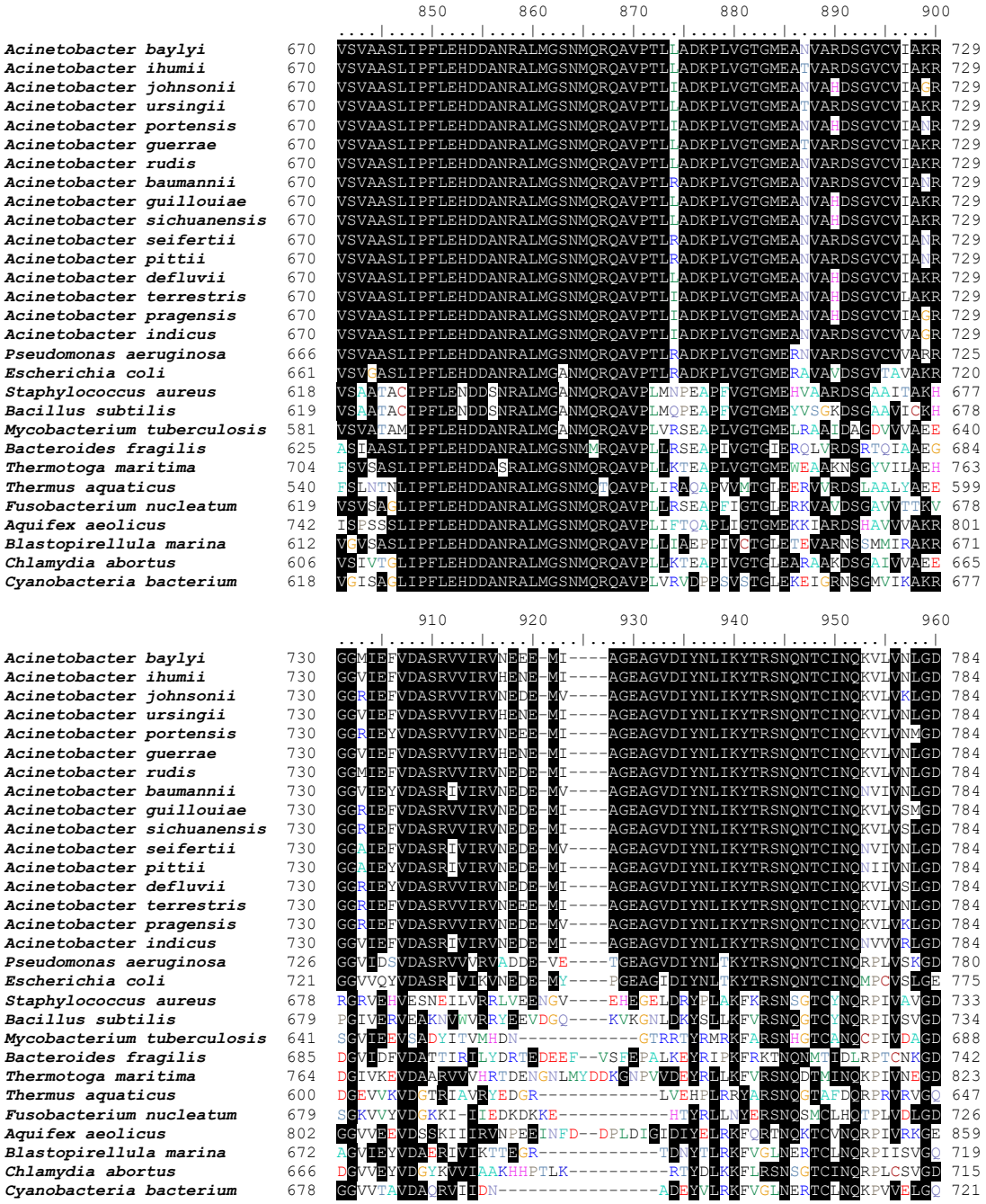

250 260 270 280 290 300
Acinetobacter baylyi 175 SARIIPYRGSWLDFEFD-AKDLVYVRIDRRR---KLLASVILRALG--YNNAQ-ILDLFY 227
Acinetobacter ihumii 175 SARIIPYRGSWLDFEFD-AKDLVYVRIDRRR---KLLATVILRALG--YSNEQ-ILDLFY 227
Acinetobacter johnsonii 175 SARIIPYRGSWLDFEFD-AKDLVEVRIDRRR---KLLATVVLRALG--YNNAQ-ILDLFY 227
Acinetobacter ursingii 175 SARIIPYRGSWLDFEFD-AKDLVYVRIDRRR---KLLATVILRALG--YNNEQ-ILDLFY 227
Acinetobacter portensis 175 SARIIPYRGSWLDFEFD-AKDLVEVRIDRRR---KLLATVVLRALG--YKDEQ-ILDLFF 227
Acinetobacter guerrae 175 SARIIPYRGSWLDFEFD-AKDLVYVRIDRRR---KLLASVILRALG--YNNEQ-ILDLFY 227
Acinetobacter rudis 175 SARIIPYRGSWLDFEFD-AKDLVYVRIDRRR---KLLATVILRALN--FSNEQ-ILNMFY 227
Acinetobacter baumannii 175 SARIIPYRGSWLDFEFD-AKDLVYVRIDRRR---KLLATVVLRALG--YNNEQ-ILNLFY 227
Acinetobacter guillouiae 175 SARIIPYRGSWLDFEFD-AKDLVEVRIDRRR---KLLATVVLRALN--YSNEQ-ILNMFY 227
Acinetobacter sichuanensis 175 SARIIPYRGSWLDFEFD-AKDLVYVRIDRRR---KLLATVVLRALN--YTNEQ-ILDMFY 227
Acinetobacter seifertii 175 SARIIPYRGSWLDFEFD-AKDLVYVRIDRRR---KLLATVVLRALG--YNNEQ-ILNLFY 227
Acinetobacter pittii 175 SARIIPYRGSWLDFEFD-AKDLVYVRIDRRR---KLLATVVLRALG--YNNEQ-ILNLFY 227
Acinetobacter defluvii 175 SARIIPYRGSWLDFEFD-AKDLVYVRIDRRR---KLLATVVLRALN--YTNEQ-ILDMFY 227
Acinetobacter terrestris 175 SARIIPYRGSWLDFEFD-AKDLVEVRIDRRR---KLLATVVLRALG--YSNEQ-VLDMFY 227
Acinetobacter pragensis 175 SARIIPYRGSWLDFEFD-AKDLVEVRIDRRR---KLLATVVLRALG--YGNSQ-ILDLFY 227
Acinetobacter indicus 175 SARIIPYRGSWLDFEFD-AKDLVYVRIDRRR---KLLATVVLRALN--YNNEQ-ILDLFY 227
Pseudomonas aeruginosa 177 SARIIPYRGSWLDFEFD-EKDCVEVRIDRRR---KLPASVLLRALG--YSTEE-ILNAFY 229
Escherichia coli 173 NARIIPYRGSWLDFEFD-EKDNLEVRIDRRR---KLPATIILRALN--YTTEQ-ILDLFF 225
Staphylococcus aureus 159 DATIIPNRGAWLEYETD-AKDVVYVRIDRIR---KLPLTVLLRALG--FSSDQEIVDLLG 212
Bacillus subtilis 159 TATVIPNRGAWLEYETD-AKDVVYVRIDRIR---KLPVTVLLRALG--FGSDQEILDLIG 212
Mycobacterium tuberculosis 195 SVKVIPSRGAWLEEDVD-KRDTVEVRIDRKR---RQPVTVLLKALG--WTSEQ-IVERFG 247
Bacteroides fragilis 168 SARIIPFKGSWIEEATD-INNVMVAYIDRKK---KLPVTTLLRAIG--FENDK-DILEIF 220
Thermotoga maritima 164 --YFLPSRGAWLEVILDPYDGVLVAGLDGKK----VNLFLFLKTIG--YEKDEDILSLYP 216
Thermus aquaticus 161 SIIPLPKRGEWIDLEVEAS-GVVTMKVNKRK----FPLVLLLRVLG--YDQET------- 206
Fusobacterium nucleatum 167 SGKIIPYKGTWLEEFETD-KNDFLSVKIDRKK---KVLATVFLKAVD--FFKDNNEIRDYF 220
Aquifex aeolicus 203 RGSIIPDKGSRVEFELSGADDLISARIDRKK----LSATAVLRAFG--LETAYDILKYFY 256
Blastopirellula marina 168 SCRIIPERGSWIEINIT-KKDSFTVRIDQSG---KFAATTLLRAMSPELSDDASLIRAFY 223
Chlamydia abortus 160 SFRIIPYRGSWLEAIFD-INDLIYIHIDRKKRRRKILALTFIRALG--YSSDADLIEEFF 216
Cyanobacteria bacterium 170 SSRIIPERGSWIELEVT-KKDVLAMRIDQST---KLAATTFLRGLDESVASTDAIIGLFY 225
310 320 330 340 350 360
Acinetobacter baylyi 228 EKVPVYLDFGSYQIDLVPERLRGEMAQFDIADNDGKVIVEQGKRINARHVRQMEASGLIK 287
Acinetobacter ihumii 228 EKVPVYLDMGSYQIDLVPERLRGEMAQFDITDTDGKVIVEQGKRINARHVRQMEASGLIK 287
Acinetobacter johnsonii 228 EKVPVYLDMGSYQIDLVPERLRGEMAQFDIVDNDGKTIVEQGKRINARHVRQMEAAGLEK 287
Acinetobacter ursingii 228 EKVPVYLDMGSYQIDLVPERLRGEMAQFDITDNDGKVIVEQGKRINARHVRQMEASGLSK 287
Acinetobacter portensis 228 EKVPVYLDMGSYQIDLVPERLRGEMAQFDILDADGKAIVEQGKRINARHVRQMEAAGLIK 287
Acinetobacter guerrae 228 EKVPVYLDMGSYQIDLVPERLRGEMAQFDIADNDGKIIVEQGKRINARHVRQMEAAGLIK 287
Acinetobacter rudis 228 EKVPVYLDMGSYQIDLVPERLRGEMAQFDIVDNDGKAIVEQGKRINARHVRQMEAAGLIK 287
Acinetobacter baumannii 228 EKVPVYLDMGSYQIDLVPERLRGEMAQFDITDNEGKVIVEQGKRINARHVRQMEAAGLIK 287
Acinetobacter guillouiae 228 EKVPVYLDMGSYQIDLVPERLRGEMAQFDIVDNDGKAIVEQGKRINARHVRQMEAAGLIK 287
Acinetobacter sichuanensis 228 EKVPVYLDMGSYQIDLVPERLRGEMAQFDIADNDGKTIVEQGKRINARHVRQMEAAGLIK 287
Acinetobacter seifertii 228 EKVPVYLDMGSYQIDLVPERLRGEMAQFDITDNEGKVIVEQGKRINARHVRQMEASGLIK 287
Acinetobacter pittii 228 EKVPVYLDMGSYQIDLVPERLRGEMAQFDIADNDGKVIVEQGKRINARHVRQMEAAGLIK 287
Acinetobacter defluvii 228 EKVPVYLDMGSYQIDLVPERLRGEMAQFDILDNDGKAIVEQGKRINARHVRQMEAAGLIK 287
Acinetobacter terrestris 228 EKVPVYLDMGSYQIDLVPERLRGEMAQFDINDADGKVIVEQGKRINARHVRQMEASGLIK 287
Acinetobacter pragensis 228 EKVPVYLDMGSYQIDLVPERLRGEMAQFDIADQDGKAIVEQGKRINARHVRQMEAAGLEK 287
Acinetobacter indicus 228 EKVPVYLDMGSYQIDLIPERLRGEMAQFDITDADGKVIVEQGKRINARHVRQMEASGLIK 287
Pseudomonas aeruginosa 230 ATNVFHIKGETLNLELVPQRLRGEVASIDIKDGSGKVIVEQGRRITARHINQLEKAGVSQ 289
Escherichia coli 226 EKVIFEIRDNKLQMELVPERLRGETASFDIEAN-GKVVVEKGRRITARHIRQLEKDDVKL 284
Staphylococcus aureus 213 DNEYLRN---TLEKDGTENTEQALLEIYERLRPGEPPTVENAKSLLYSRFFDPKRYDLAS 269
Bacillus subtilis 213 ENEYLRN---TLDKDNTENSDKALLEIYERLRPGEPPTVENAKSLLDSRFFDPKRYDLAN 269
Mycobacterium tuberculosis 248 FSEIMRS---TLEKDNTVGTDEALLDIYRKLRPGEPPTKESAQTLLENLFFKEKRYDLAR 304
Bacteroides fragilis 221 N---------LAEDVKVNKTNLKKVVGRKLAARVLKTWIEDFVDEDTGEVVSIERN---- 267
Thermotoga maritima 217 TYLDA-----DDEDSLLLHVGSILLEDIYDGGRKIAEKWDILTKDLAERILMIDDINQIK 271
Thermus aquaticus 206 ----------LVRELSAYG-------DLVQGLLDEAVLAMRPE---- 232
Fusobacterium nucleatum 221 L---------ETKELNLKALYKKYSKEPEELLNVLKQELDGSIVKED--ILDEETG---- 265
Aquifex aeolicus 257 EDVRKFIVKDKYLYDAQTG---EEFTPEDLEHHYIFAIIKFRGKLVGFASSKERDFIEER 313
Blastopirellula marina 224 DTTKKVHVKDGRSITKIEGGKIAVDDIVYPIDSDRGGEIIVEAGQKISRNTAELICSVG--- 280
Chlamydia abortus 217 Q---------IEEHSLKSEKDFSFLVGKILADNVL----------DEASS---- 247
Cyanobacteria bacterium 226 EVSEVKAG------EVQPEHWAAQSIVDTET---GEEIVHVGRRIG-DQAEAIRNS---- 271

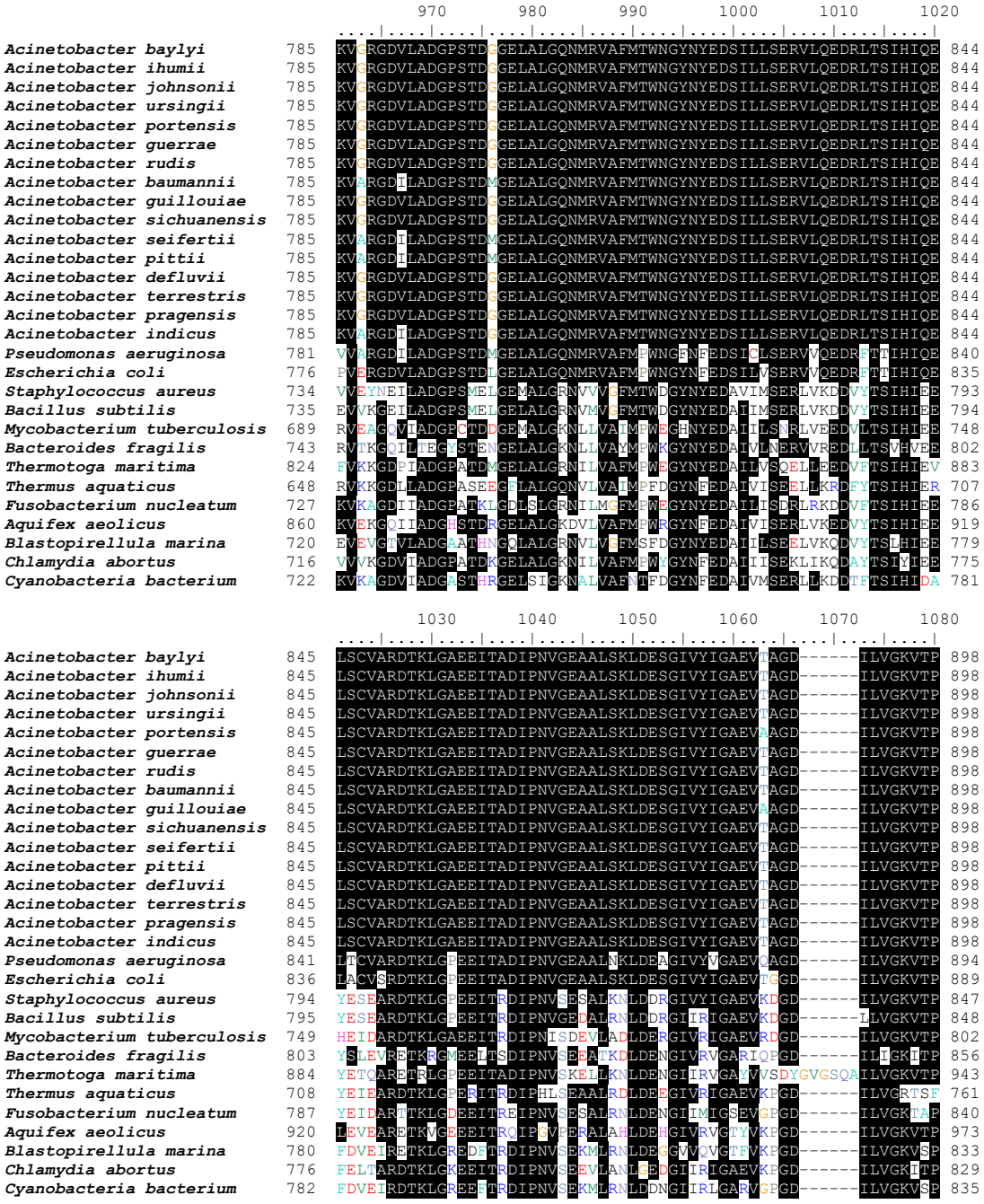

370 380 390 400 410 420
Acinetobacter baylyi 288 LSVPDEYLYERITAEDITIRD-GDVIAAN-TLLSHEIMVKLAEGGVKQFNILYTND-IDH 344
Acinetobacter ihumii 288 LSVPDEYLYERITAEDITKD-GDVIAAN-TLLSHEVMVKLAEGGVKQFNILYTND-IDH 344
Acinetobacter johnsonii 288 LSVPDEYLYERITAEDIPIKD-GDVIAAN-TLLSHEVMVKLAEGGVKQFNILFTND-IDR 344
Acinetobacter ursingii 288 LSVPDEYLYERITAEDITIKD-GDIISAN-TLLSHEVMVKLAEGGVKQFNILYTND-IDH 344
Acinetobacter portensis 288 LSVPDEYLYERITAENITIRD-GDVIAAN-TVLSHEIMVKLAEGGVKQFNILFTND-IDR 344
Acinetobacter guerrae 288 LSVPDEYLYERITAEDITIRD-GDVISAN-TLLSHEVMVKLAEGGVKQFNILYTND-IDH 344
Acinetobacter rudis 288 LPVPDEYLYERILAEDVVIRD-GETIAAN-TVLSHEIMVKLAEGGVKQFNILFTND-IDR 344
Acinetobacter baumannii 288 LSVPDEYLYERITAEDITIRD-GEVIAAN-TLLSHEVMVKLAEGGVKQFNILFTND-IDR 344
Acinetobacter guillouiae 288 LPVPDEYLYERITAEDIVLKD-GEVITAN-TVLSHEILVRIAEGGIKQFNILFTND-IDR 344
Acinetobacter sichuanensis 288 LPVPDEYLYERIVAQDITLHN-GDVIAAN-TVLSHEVMVKLAEGGVKQFNVLYTND-IDR 344
Acinetobacter seifertii 288 LSVPDEYLYERITAEDITIRD-GEVITAN-TLLSHEVMVKLAEGGVKQFNILFTND-IDR 344
Acinetobacter pittii 288 LSVPDEYLYERITAENITIRD-GEVIAAN-TLLSHEVMVKLAEGGVKQFNILFTND-IDR 344
Acinetobacter defluvii 288 LPVPDEYLYERIVAQDITLHD-GEVIAAN-TVLSHEVMVKIAEGGVKQFNVLYTND-IDR 344
Acinetobacter terrestris 288 LSVPDEYLYERITAEDIPLKD-GDVIAAN-TVLSHEIMVKIAEGGVKQFNILFTND-IDR 344
Acinetobacter pragensis 288 LSVPDEYLYERITAEDVAIRD-GDVIAAN-TVLSHEIMVKLAEGGVKQFNILFTND-IDR 344
Acinetobacter indicus 288 LSVPDEYLYERITAEDIVLKD-GDVIPAN-TLLSHEVMVKLAEGGVKQFNILFTND-IDR 344
Pseudomonas aeruginosa 290 LEVPFDYLIGRTIAKAIVHPATGEIIAECNTELTLDLLAKVAKAQVVRIETLYTND-IDC 348
Escherichia coli 285 IEVPVEYIAGKVVAKDYIDESTGELICAANMELSLDLLAKLSQSGHKRIETLFTND-LDH 343
Staphylococcus aureus 270 VGR-----YKTNKKLHLKHRLFNQKLAEPIVNTETGEIVVEEGTVLDRRKIDEIMD-VLE 323
Bacillus subtilis 270 VGR-----YKINKKLHIKNRLFNQRLAETLVDPETGEILAEKGQILDRRTLDKVLP-YLE 323
Mycobacterium tuberculosis 305 VGR-----YKVNKKLGLH-------VGEPITS---------- 324
Bacteroides fragilis 267 ----------EVIIDRETVIEPEHIDEIIDSGVQNILIHKEEPNQSD 304
Thermotoga maritima 271 ----------IVHPIAQNTFEKMLEVVSSSSEEGEEEEEKT---- 302
Thermus aquaticus 232 ---------- 232
Fusobacterium nucleatum 265 ----------EFIAEVEAFINEEVINKLIENKVDKISYWYVGP---E 299
Aquifex aeolicus 314 YIEEWEDLVRLLDDDRIEVLS--VTAVPRDVVIKSPYGKSLIDTLANDTSPKDVDK-RSP 370
Blastopirellula marina 280 ----------ITEEIEVMDSPKIPIIFNTLIEDN---------- 304
Chlamydia abortus 247 ----------IVYGKAGEKLSTAMLKRMLDANISTLKIAVEAD---E 281
Cyanobacteria bacterium 271 ----------SLKTVRVVQNPSDSLILNTIAEEKLDMFADV---- 302
----------SI1----------
430 440 450 460 470 480
Acinetobacter baylyi 345 GSFVADTLRADTTMGREDALVEIYKVMRPGEPPTKEAAENLFNNLFFSSERYDLSPVGRM 404
Acinetobacter ihumii 345 GPFVADSLRADLTRDREEALVEIYKVMRPGEPPTKEAAENLFNNLFFSSERYDLSPVGRM 404
Acinetobacter johnsonii 345 GSFVADTLRADTTGREEALVEIYKVMRPGEPPTKEAAENLFNNLFFSSERYDLSPVGRM 404
Acinetobacter ursingii 345 GPFIADTLRADTTSGREEALVEIYKVMRPGEPPTKEAAENLFNNLFFSSERYDLSPVGRM 404
Acinetobacter portensis 345 GSFIADSLRADTTSREEALVEIYKVMRPGEPPTKEAAENLFNNLFFSSERYDLSPVGRM 404
Acinetobacter guerrae 345 GPFIADTLRADTTSGREEALVEIYKVMRPGEPPTKEAAENLFNNLFFSSERYDLSPVGRM 404
Acinetobacter rudis 345 GSFVADTLRSDLTRDREEALVEIYKVMRPGEPPTKEAAENLFNNLFFSSERYDLSPVGRM 404
Acinetobacter baumannii 345 GSFVADTLRADLTRDREEALVEIYKVMRPGEPPTKEAAENLFNNLFFSSERYDLSPVGRM 404
Acinetobacter guillouiae 345 GSFVADTLRADTTSGREEALVEIYKVMRPGEPPTKEAAENLFNNLFFSTERYDLSPVGRM 404
Acinetobacter sichuanensis 345 GPFVADTLRADTTSGREEALVEIYKVMRPGEPPTKEAAENLFNNLFFSTERYDLSPVGRM 404
Acinetobacter seifertii 345 GSFVADTLRADTTAGREEALVEIYKVMRPGEPPTKEAAENLFNNLFFSSERYDLSPVGRM 404
Acinetobacter pittii 345 GSFVADTLRADTTTGREEALVEIYKVMRPGEPPTKEAAENLFNNLFFSSERYDLSPVGRM 404
Acinetobacter defluvii 345 GPFVADTLRADTTTGREEALVEIYKVMRPGEPPTKEAAENLFNNLFFSTERYDLSPVGRM 404
Acinetobacter terrestris 345 GSFVADSLRADTTSGREEALVEIYKVMRPGEPPTKEAAENLFNNLFFSSERYDLSPVGRM 404
Acinetobacter pragensis 345 GSFIADSLRADTTENREQALVEIYKVMRPGEPPTKEAAENLFNNLFFSSERYDLSPVGRM 404
Acinetobacter indicus 345 GSFIADTLRADTTTGREEALVEIYKVMRPGEPPTKEAAENLFNNLFFSSERYDLSPVGRM 404
Pseudomonas aeruginosa 349 GPFISDTLKIDNTSNQLEALVEIYRMMRPGEPPTKEAABTLFGNLFFSAERYDLSAVGRM 408
Escherichia coli 344 GPYISETLRVDPTNDRLSALVEIYRMMRPGEPPTREAABSLFENLFFSEDRYDLSAVGRM 403
Staphylococcus aureus 324 SNANSEVFELHGSVIDEPVEIQSIKVYVPN-DDEG-RTTTVIGNAFPDSEVKCITPA--- 378
Bacillus subtilis 324 NGIGFRKLYPNGGVVEDEVTLQSIKIFAPT-DQEGEQVINVICNAYIEEEIKNITPA--- 379
Mycobacterium tuberculosis 324 ----------STLTEEDVVATIEYLVR--LHEGQTTMTVPGGVEVP---------- 358
Bacteroides fragilis 305 YSIIYNTLQKDPSNSEKEAVLYIYRQLRNADPADDASAREVINNLFFSEKRYDLSDVGRY 364
Thermotoga maritima 302 ------KIYGLNEVTVVDAYLEIFRRLRPEELPRINAAKRYLHDLFFNPERYDLSEVGRY 356
Thermus aquaticus 232 ----------EAMVRLFTLLRPGDPPKKDKALAYLFGLLADPKRYDLSEAGRY 275
Fusobacterium nucleatum 300 SKLIANTLMNDTTLTEDEAVVEVEKKLRPGDQVTVDSARSLIRQMFFNPQRYDLEPVGRY 359
Aquifex aeolicus 371 VKVPAEYTLRDYGLMEMYKRLRHIEAITMEIDSVIERARIFFNVFFRDLKRYDLSRVGRV 430
Blastopirellula marina 304 ----------TSSHEEALLRIYQRLRPGNPPQLEKAKTLFDEKFYDDNRYRLGRVGRF 352
Chlamydia abortus 282 NHPIIKMLAKDPTDSYESALKDFYRRLRPGEPATLANARSTIMRLFFDPKRYNLGRVGRY 341
Cyanobacteria bacterium 302 ----------ADSEFERALLRVYTKLRPGNPPQVEKAKQLFDEKFYDENRYRLGKVGRF 351
----------|

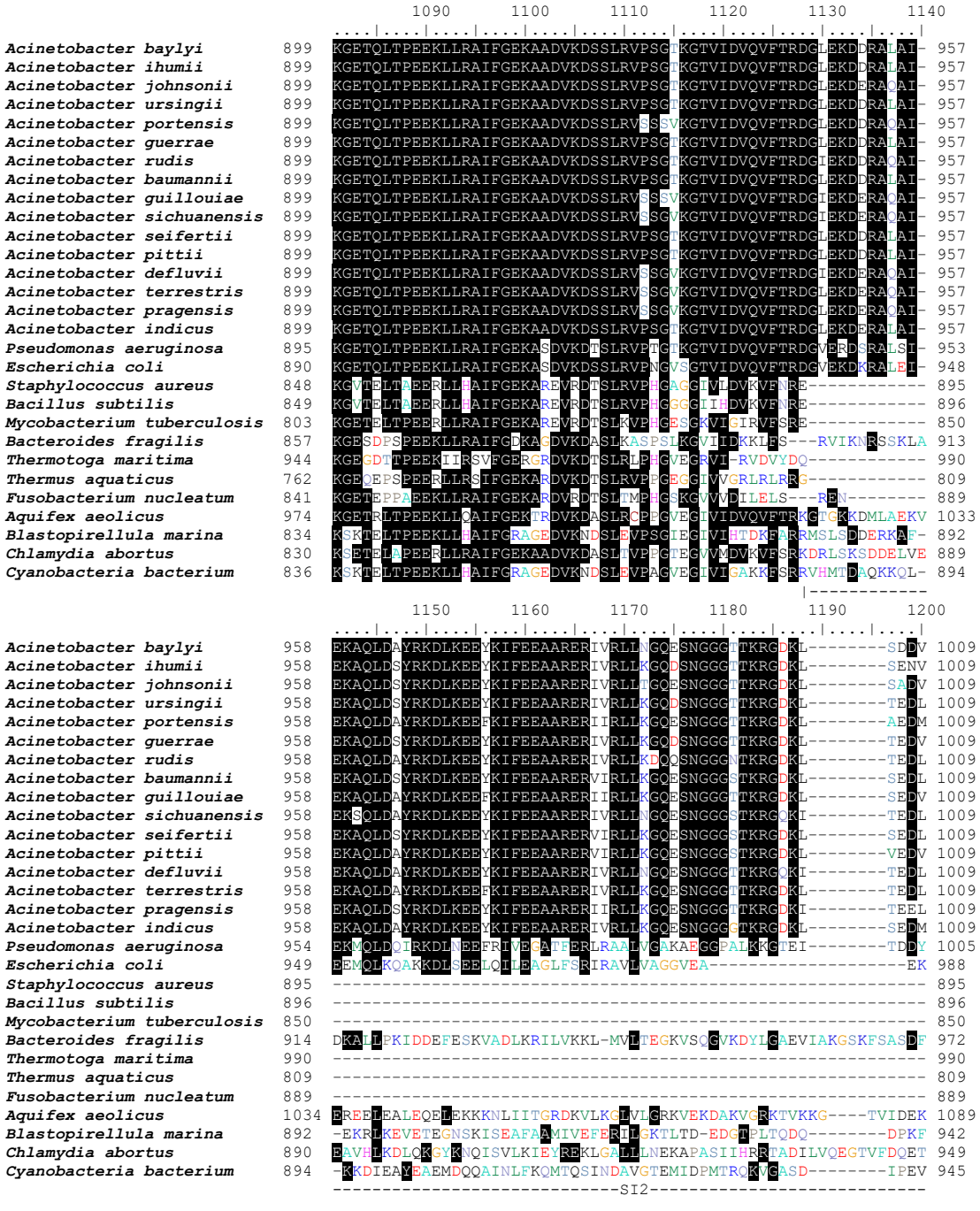

490 500 510 520 530 540
Acinetobacter baylyi 405 KFNRRLGRP---------- 413
Acinetobacter ihumii 405 KFNRRLGRP---------- 413
Acinetobacter johnsonii 405 KFNRRLGRP---------- 413
Acinetobacter ursingii 405 KFNRRLGRP---------- 413
Acinetobacter portensis 405 KFNRRLGRP---------- 413
Acinetobacter guerrae 405 KFNRRLGRP---------- 413
Acinetobacter rudis 405 KFNRRLGRP---------- 413
Acinetobacter baumannii 405 KFNRRLGRP---------- 413
Acinetobacter guillouiae 405 KFNRRLGRP---------- 413
Acinetobacter sichuanensis 405 KFNRRLGRP---------- 413
Acinetobacter seifertii 405 KFNRRLGRP---------- 413
Acinetobacter pittii 405 KFNRRLGRP---------- 413
Acinetobacter defluvii 405 KFNRRLGRP---------- 413
Acinetobacter terrestris 405 KFNRRLGRP---------- 413
Acinetobacter pragensis 405 KFNRRLGRP---------- 413
Acinetobacter indicus 405 KFNRRLGRP---------- 413
Pseudomonas aeruginosa 409 KFNRRIGRT---------- 417
Escherichia coli 404 KFNRSLLRE---------- 412
Staphylococcus aureus 378 ---------- 378
Bacillus subtilis 379 ---------- 379
Mycobacterium tuberculosis 358 ---------- 358
Bacteroides fragilis 365 RINKKLNLT---------- 373
Thermotoga maritima 357 KVNRRLRNAYIRYLIEVEGEDPEEARKKVYNETSLVLKPLDIVLASRILFDYFERRYVND 416
Thermus aquaticus 276 KAEEKLG----------VGLSGRTLVRFED------ 295
Fusobacterium nucleatum 360 KMNKRLKLD---------- 368
Aquifex aeolicus 431 KINAKVHRIPKVLKPADVDLLDQLPPLALAEDYGEYKAGTRVTKDLLKELFKQY------ 484
Blastopirellula marina 353 RMNRKLGIE---------- 361
Chlamydia abortus 342 KLNRKLGRE---------- 350
Cyanobacteria bacterium 352 RINRKFGLE---------- 360
550 560 570 580 590 600
Acinetobacter baylyi 413 ----------YEVGTDQKSREVEGILSNEDIIDVLRTLVEIR 445
Acinetobacter ihumii 413 ----------YEVGTDQKSREVEGILSNEDIIDVLRTLVEIR 445
Acinetobacter johnsonii 413 ----------YEVGTDQKSREVEGILSNEDITDVLRTLVEIR 445
Acinetobacter ursingii 413 ----------YEVGTDQKSREVEGILSNEDITDVLRTLVEIR 445
Acinetobacter portensis 413 ----------YEVGTDQKSREVEGILSNEDITDVLRTLIEIR 445
Acinetobacter guerrae 413 ----------YEVGTDQKSREVEGILANEDITDVLRTLVEIR 445
Acinetobacter rudis 413 ----------YEVGNDQKSRDVECILSNDDIVDVLRTLVEIR 445
Acinetobacter baumannii 413 ----------YEVGTDQKSREVEGILSHEDIIDVLRTLVEIR 445
Acinetobacter guillouiae 413 ----------YEVGTDQKSREVEGILSNDDIIDVLRTLVEIR 445
Acinetobacter sichuanensis 413 ----------YEVGTDQKSREVEGILSNEDITDVLRTLVEIR 445
Acinetobacter seifertii 413 ----------YEVGTDQKSREVEGILSHEDIIDVLRTLVEIR 445
Acinetobacter pittii 413 ----------YEVGTDQKSREVEGILSHEDIIDVLRTLVEIR 445
Acinetobacter defluvii 413 ----------YEVGTDQKSREVEGILSNEDITDVLKTLVEIR 445
Acinetobacter terrestris 413 ----------YEVGTDQKSREVEGILSNEDITDVLKTLVEIR 445
Acinetobacter pragensis 413 ----------YEVGTDQKSREVEGILSNEDITDVLKTLVEIR 445
Acinetobacter indicus 413 ----------YEVGTDQKSREVEGILSNEDITDVLKTLVEIR 445
Pseudomonas aeruginosa 417 ----------EIEGP-------GVLSKEDIDVLKTLVDIR 441
Escherichia coli 412 ----------EIEGS-------GILSKDDIIDVMKKLIDIR 436
Staphylococcus aureus 378 ----------DIIASMSYFFNLL 391
Bacillus subtilis 379 ----------DIISSISYFFNLL 392
Mycobacterium tuberculosis 358 ---------- 358
Bacteroides fragilis 373 ----------TDMD--------VRVLIKEDIIEIIKYLIELI 397
Thermotoga maritima 417 FEIDSYELKNLIRIFKEEYLEKRKTAPYDLRKLVSVFRRNYGVTSDLGVFAAIRYVSNIN 476
Thermus aquaticus 295 ----------GEFKDEVFLPTLRYLFALT 314
Fusobacterium nucleatum 368 ----------VPED--------QISLIKEDVLGTIKYVIELN 392
Aquifex aeolicus 484 ----------KEIKVKDYTEDEARFILPIDLVNILKYLIDIR 516
Blastopirellula marina 361 ----------VNEG--------EQTIRPSDLINSIKYVMSIL 385
Chlamydia abortus 350 ----------MDEES-----LAQVTIRKEDVIGALKYLIRLK 377
Cyanobacteria bacterium 360 ----------VPED--------QMFIRSEDFLRVVQYLLDIR 384

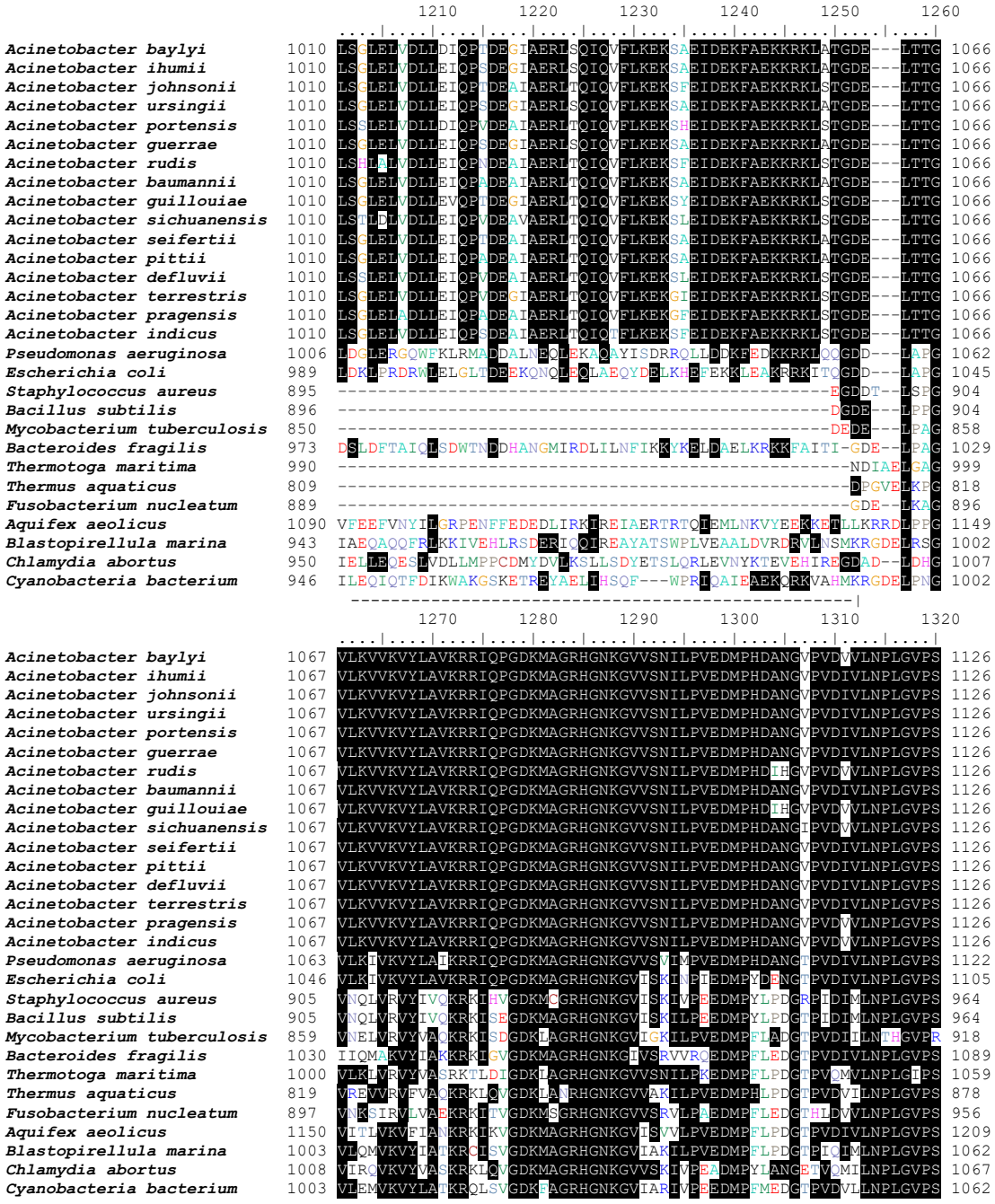

610 620 630 640 650 660
Acinetobacter baylyi 446 NG---------KGEVDDIDHLGNRRVRSVGEMTENQFRVGLVRVERAVKERLSQAET--D 494
Acinetobacter ihumii 446 NG---------KGEVDDIDHLGNRRVRSVGEMTENQFRVGLVRVERAVKERLSQAET--D 494
Acinetobacter johnsonii 446 NG---------KGEVDDIDHLGNRRVRSVGEMTENQFRVGLVRVERAVKERLSQAET--D 494
Acinetobacter ursingii 446 NG---------KGEVDDIDHLGNRRVRSVGEMTENQFRVGLVRVERAVKERLSQAET--D 494
Acinetobacter portensis 446 NG---------KGEVDDIDHLGNRRVRSVGEMTENQFRVGLVRVERAVKERLSQAET--D 494
Acinetobacter guerrae 446 NG---------KGEVDDIDHLGNRRVRSVGEMTENQFRVGLVRVERAVKERLSQAET--D 494
Acinetobacter rudis 446 NG---------KGEVDDIDHLGNRRVRSVGEMTENQFRVGLVRVERAVKERLSQAET--D 494
Acinetobacter baumannii 446 NG---------KGEVDDIDHLGNRRVRSVGEMTENQFRVGLVRVERAVKERLSQAET--D 494
Acinetobacter guillouiae 446 NG---------KGEVDDIDHLGNRRVRSVGEMTENQFRVGLVRVERAVKERLNQAET--D 494
Acinetobacter sichuanensis 446 NG---------KGEVDDIDHLGNRRVRSVGEMTENQFRVGLVRVERAVKERLNQAET--D 494
Acinetobacter seifertii 446 NG---------KGEVDDIDHLGNRRVRSVGEMTENQFRVGLVRVERAVKERLSQAET--D 494
Acinetobacter pittii 446 NG---------KGEVDDIDHLGNRRVRSVGEMTENQFRVGLVRVERAVKERLSQAET--D 494
Acinetobacter defluvii 446 NG---------KGEVDDIDHLGNRRVRSVGEMTENQFRVGLVRVERAVKERLSQAET--D 494
Acinetobacter terrestris 446 NG---------KGEVDDIDHLGNRRVRSVGEMTENQFRVGLVRVERAVKERLSQAET--D 494
Acinetobacter pragensis 446 NG---------KGEVDDIDHLGNRRVRSVGEMTENQFRVGLVRVERAVKERLSQAET--D 494
Acinetobacter indicus 446 NG---------KGEVDDIDHLGNRRVRSVGEMTENQFRVGLVRVERAVKERLSQAET--D 494
Pseudomonas aeruginosa 442 NG---------KGEVDDIDHLGNRRVRSVGEMAENQFRVGLVRVERAVKERLSMAES--E 490
Escherichia coli 437 NG---------KGEVDDIDHLGNRRIRSVGEMAENQFRVGLVRVERAVKERLSLGDL--D 485
Staphylococcus aureus 392 SG---------IGYTDDIDHLGNRRLRSVGELLQNQFRIGLSRMERVVRERMSIQDT--E 440
Bacillus subtilis 393 HG---------VGDTDDIDHLGNRRLRSVGELLQNQFRIGLSRMERVVRERMSIQDT--N 441
Mycobacterium tuberculosis 358 ------------VETDDIDHFGNRRLRTVGELIQNQIRVGMSRMERVVRERMTTQDV--E 404
Bacteroides fragilis 398 N---------SKADVDDIDHLSNRRVRTVGEQLSNQFAVGLARMSRTIRERMNVRDN--E 446
Thermotoga maritima 477 KELP-------SIPFDTKDHLGNKRVRTVGELVQREFERLFARAQKAIQERLTLINSLSK 529
Thermus aquaticus 315 AGVP-------GHEVDDIDHLGNRRIRTVGELMADQFRVGLARLARGVRERMVMG-SPDT 366
Fusobacterium nucleatum 393 NGD-------QNVHTDDIDNLSNRRIRSVGELLLMQIKTGLAKMNKMVREKMTTQDI--E 443
Aquifex aeolicus 517 HG---------RVKKDDIEHLGNRRVRSVGELLENQARLGIAKMEKVFRDRSAVINPEQP 567
Blastopirellula marina 386 D-------GDGTARKDDIDHLGNRRLRTIDELACDELRKGFLKLRRTVQERMSLKDA--E 436
Chlamydia abortus 378 MGD-------EKASIDDIDHLANRRVRSVGELIQNQCRGLARMEKIIRERMNLFDFSSD 430
Cyanobacteria bacterium 385 SNRQDLQTGRAAAQIDDIDHLGNRRLRTLDELAVEELRKGFLKLRRTVQERMSVKDP--E 442
670 680 690 700 710 720
Acinetobacter baylyi 495 NLSPQ-DLINAKPVAAAIKEFFGSSQLSQFMDQNNPLSEITHKRRVSALGPGGLTRERAG 553
Acinetobacter ihumii 495 NLSPQ-DLINAKPVAAAIKEFFGSSQLSQFMDQNNPLSEITHKRRVSALGPGGLTRERAG 553
Acinetobacter johnsonii 495 NLSPQ-DLINAKPVAAAIKEFFGSSQLSQFMDQNNPLSEITHKRRVSALGPGGLTRERAG 553
Acinetobacter ursingii 495 NLSPQ-DLINAKPVAAAIKEFFGSSQLSQFMDQNNPLSEITHKRRVSALGPGGLTRERAG 553
Acinetobacter portensis 495 NLSPQ-DLINAKPVAAAIKEFFGSSQLSQFMDQNNPLSEITHKRRVSALGPGGLTRERAG 553
Acinetobacter guerrae 495 NLSPQ-DLINAKPVAAAIKEFFGSSQLSQFMDQNNPLSEITHKRRVSALGPGGLTRERAG 553
Acinetobacter rudis 495 NLSPQ-DLINAKPVAAAIKEFFGSSQLSQFMDQNNPLSEITHKRRVSALGPGGLTRERAG 553
Acinetobacter baumannii 495 NLSPQ-DLINAKPVAAAIKEFFGSSQLSQFMDQNNPLSEITHKRRVSALGPGGLTRERAG 553
Acinetobacter guillouiae 495 NLSPQ-DLINAKPVAAAIKEFFGSSQLSQFMDQNNPLSEITHKRRVSALGPGGLTRERAG 553
Acinetobacter sichuanensis 495 NLSPQ-DLINAKPVAAAIKEFFGSSQLSQFMDQNNPLSEITHKRRVSALGPGGLTRERAG 553
Acinetobacter seifertii 495 NLSPQ-DLINAKPVAAAIKEFFGSSQLSQFMDQNNPLSEITHKRRVSALGPGGLTRERAG 553
Acinetobacter pittii 495 NLSPQ-DLINAKPVAAAIKEFFGSSQLSQFMDQNNPLSEITHKRRVSALGPGGLTRERAG 553
Acinetobacter defluvii 495 NLSPQ-DLINAKPVAAAIKEFFGSSQLSQFMDQNNPLSEITHKRRVSALGPGGLTRERAG 553
Acinetobacter terrestris 495 NLSPQ-DLINAKPVAAAIKEFFGSSQLSQFMDQNNPLSEITHKRRVSALGPGGLTRERAG 553
Acinetobacter pragensis 495 NLSPQ-DLINAKPVAAAIKEFFGSSQLSQFMDQNNPLSEITHKRRVSALGPGGLTRERAG 553
Acinetobacter indicus 495 NLSPQ-DLINAKPVAAAIKEFFGSSQLSQFMDQNNPLSEITHKRRVSALGPGGLTRERAG 553
Pseudomonas aeruginosa 491 GLMPQ-DLINAKPVAAAIKEFFGSSQLSQFMDQNNPLSEITHKRRVSALGPGGLTRERAG 549
Escherichia coli 486 TLMPQ-DMINAKPISAAVKEFFGSSQLSQFMDQNNPLSEITHKRRISALGPGGLTRERAG 544
Staphylococcus aureus 441 SITPQ-QLINIRPVIASIKEFFGSSQLSQFMDQANPLAELTHKRRLSALGPGGLTRERAQ 499
Bacillus subtilis 442 TITPQ-QLINIRPVIASIKEFFGSSQLSQFMDQINPLAELTHKRRLSALGPGGLTRERAG 500
Mycobacterium tuberculosis 405 AITPQ-TLINIRPVVAAIKEFFGISQLSQFMDQNNPLSGLTHKRRLSALGPGGISRERAG 463
Bacteroides fragilis 447 VFTPI-DLINAKTISSVINSFFGTNALSQFMDQINPLAEITHKRRMSALGPGGLSRERAG 505
Thermotoga maritima 530 VSIQS--LINIKSIISTVNQFFAMNQLSQFMDQVNPLSELTHKRRVSAVGPGGIRRESKV 587
Thermus aquaticus 367 LTPAK--LVNSRPLEAALREFFSRSQLSQFKDEINPLSSLRHKRRISALGPGGLTRERAG 424
Fusobacterium nucleatum 444 TVTPQ-SLLNTRPLNALIQDFFGSSQLSQFMDQSNPLAELTHKRRISALGPGGISRERAG 502
Aquifex aeolicus 568 DLKPQ-DSINPRYVTTAITDFLKTGQLSQYLDNINPLSELTHKRRLSALGPGGLTRESAK 626
Blastopirellula marina 437 DMTPR-NLINPKSISAAIEYFFGRGELSQVVDQSNPLSMLTHERRLSALGPGGINRKRAG 495
Chlamydia abortus 431 TLIPG-KISAKGLASVLKDFFGRSQLSQFMDQINPVAELTHKRRLSALGPGGLNRERAG 489
Cyanobacteria bacterium 443 EVAKIADLVNSKSISSAIDFFFGRSELSQVVDQSNPLSSLVHERRLSALGPGGLNRKRAG 502

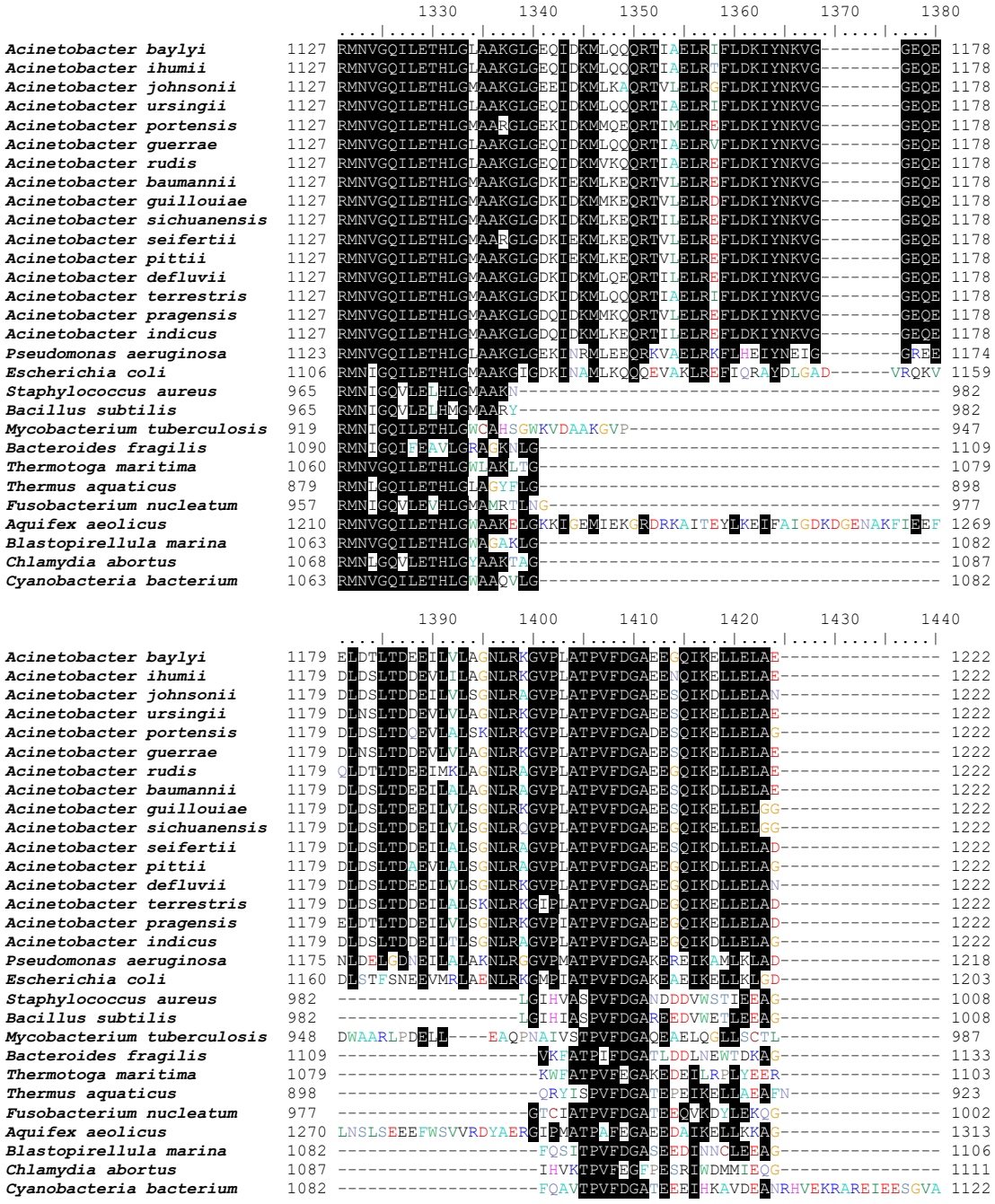

730 740 750 760 770 780
Acinetobacter baylyi 554 FEVRDVHQTHYGRVCPIETPEGPNIGLINSLSVYAKANDFGFLETPYRKVVDG---RVSD 610
Acinetobacter ihumii 554 FEVRDVHQTHYGRVCPIETPEGPNIGLINSLSVYAKANDFGFLETPYRKVVDG---RVTD 610
Acinetobacter johnsonii 554 FEVRDVHQTHYGRVCPIETPEGPNIGLINSLSVYAKCNNFGFLETPYRKVVDG---RVTD 610
Acinetobacter ursingii 554 FEVRDVHQTHYGRVCPIETPEGPNIGLINSLSVYAKANDFGFLETPYRKVVDG---RVTD 610
Acinetobacter portensis 554 FEVRDVHQTHYGRVCPIETPEGPNIGLINSLSVYAKCNNFGFLETPYRKVVDG---RVTL 610
Acinetobacter guerrae 554 FEVRDVHQTHYGRVCPIETPEGPNIGLINSLSVYAKANDFGFLETPYRKVVDG---RVTD 610
Acinetobacter rudis 554 FEVRDVHQTHYGRVCPIETPEGPNIGLINSLSVYAKANNFGFLETPYRKVVDG---RVTD 610
Acinetobacter baumannii 554 FEVRDVHQTHYGRVCPIETPEGPNIGLINSLSVYAKANDFGFLETPYRKVVDG---RVTD 610
Acinetobacter guillouiae 554 FEVRDVHQTHYGRVCPIETPEGPNIGLINSLSVYAKANNFGFLETPYRRVVDG---RVTD 610
Acinetobacter sichuanensis 554 FEVRDVHQTHYGRVCPIETPEGPNIGLINSLSVYAKANSFGFLETPYRKVVDG---RVTE 610
Acinetobacter seifertii 554 FEVRDVHQTHYGRVCPIETPEGPNIGLINSLSVYAKANDFGFLETPYRRVVDG---RVTD 610
Acinetobacter pittii 554 FEVRDVHQTHYGRVCPIETPEGPNIGLINSLSVYAKANDFGFLETPYRKVVDG---RVTD 610
Acinetobacter defluvii 554 FEVRDVHQTHYGRVCPIETPEGPNIGLINSLSVYAKCNNFGFLETPYRKVVDG---RVTD 610
Acinetobacter terrestris 554 FEVRDVHQTHYGRVCPIETPEGPNIGLINSLSVYAKCNNFGFLETPYRKVVDG---RVTD 610
Acinetobacter pragensis 554 FEVRDVHQTHYGRVCPIETPEGPNIGLINSLSVYAKCNNFGFLETPYRKVVDG---RVTD 610
Acinetobacter indicus 554 FEVRDVHQTHYGRVCPIETPEGPNIGLINSLSVYAKANSFGFLETPYRKVVDG---RVTD 610
Pseudomonas aeruginosa 550 FEVRDVHPTHYGRVCPIETPEGPNIGLINSLATYARTNKYGFLESPYRVVKDS---LVTD 606
Escherichia coli 545 FEVRDVHPTHYGRVCPIETPEGPNIGLINSLSVYAQTNEYGFLETPYRKVTDG---VVTD 601
Staphylococcus aureus 500 MEVRDVHYSHYGRMCPIETPEGPNIGLINSLSSYARVNEFGFIETPYRKVDLDT-HAITD 558
Bacillus subtilis 501 MEVRDVHYSHYGRMCPIETPEGPNIGLINSLSSYAKVNRFGFIETPYRRVDPET-GKVTG 559
Mycobacterium tuberculosis 464 LEVRDVHPSHYGRMCPIETPEGPNIGLICSLSVYARVNEFGFLETPYRKVVDG---VVSD 520
Bacteroides fragilis 506 FEVRDVHYTHYGRICPIETPEGPNIGLISSLCVEAKINDLGFIETPYRKVDNGKVDLSEN 565
Thermotoga maritima 588 FEARNVHYSQYGRICPIETPEGANIGFISSLAIYAKIDEYGFLMTPYRKVVNG---KVTD 644
Thermus aquaticus 425 FDVRDVHRTHYGRICPVETPEGANIGLIGSLAAYARVDALGFIRTPYRRVKNG---VVTE 481
Fusobacterium nucleatum 503 FEVRDVHDSHYGRICPIETPEGPNIGLICSLATYAKINKYGFLETPYVKVENG-VALVDD 561
Aquifex aeolicus 627 FEIRDVHPSHYGRICPIETPEGCNIGLVTSPTVYARVNEYGFLITPYRKVENG---KVTD 683
Blastopirellula marina 496 FEVRDVHISHYGRICPIETPEGTNIGLISSLAIYSAVDEYGFLITPYRVIRDG---KIAD 552
Chlamydia abortus 490 FEVRDVHASHYGRICPIETPEGPNIGLIGSLSSFAKINPFGFIETPYRIVRDG---VVTD 546
Cyanobacteria bacterium 503 FEVRDVHISHYGRVCPIETPEGTNIGLIGSLGIFAGIDDYGFLITPYRRVRNG---KPTG 559
790 800 810 820 830 840
Acinetobacter baylyi 611 DVEYLSAIEEVGTVIAQADSAVDADGHLVEEMVSVR-HQGEFVRMPPEKVTHMDVSAQQV 669
Acinetobacter ihumii 611 DVEYLSAIEEVGTVIAQADSGVDANGNLIEEMVSVR-HQGEFVRMPPEKVTHMDVSAQQV 669
Acinetobacter johnsonii 611 EVEYLSAIEEVGTVIAQADSGVDKDGNLIEEFVSVR-HQGDFVRMPPEKVTHMDVSAQQV 669
Acinetobacter ursingii 611 DVEYLSAIEEVGTVIAQADSGVDAEGNLVEEMVSVR-HQGEFVRMPPEKVTHMDVSAQQV 669
Acinetobacter portensis 611 DIEYLSAIEEVGTVIAQADSAVDADGNLIEEVVSVR-HQGDFVRMPPEKVTHMDVSAQQV 669
Acinetobacter guerrae 611 DVEYLSAIEEVGTVIAQADSGVDAEGNLVEEMVSVR-HQGEFVRMPPEKVTHMDVSAQQV 669
Acinetobacter rudis 611 EVEYLSAIEEVGTVIAQADATLDEDNNFTEEFVSVR-HQGEFVRIPPEKVTHMDVSAQQV 669
Acinetobacter baumannii 611 DVEYLSAIEEVGTVIAQADSAVDKDGNLIEEFVSVR-HQGEFVRMPPEKVTHMDVSAQQV 669
Acinetobacter guillouiae 611 DVEYLSAIEEVGTVIAQADSALDKDGHLIEDFVSVR-HQGDFVRMPPEKVTHMDVSAQQV 669
Acinetobacter sichuanensis 611 EVEYLSAIEEVGTVIAQADSGVDKDGNLIEEFVSVR-HQGDFVRMPPEKVTHMDVSAQQV 669
Acinetobacter seifertii 611 AVEYLSAIEEVGTVIAQADSAVDKDGNLIEEDFVSVR-HQGEFVRMPPEKVTHMDVSAQQV 669
Acinetobacter pittii 611 AVEYLSAIEEVGTVIAQADSAVDKDGNLIEEFVSVR-HQGEFVRMPPEKVTHMDVSAQQV 669
Acinetobacter defluvii 611 DVEYLSAIEEVGTVIAQADSIVDQDGNLIEEFVSVR-HQGDFVRMPPEKVTHMDVSAQQV 669
Acinetobacter terrestris 611 DVEYLSAIEEVGTVIAQADSGVDKDGNLIEEFVSVR-HQGDFVRMPPEKVTHMDVSAQQV 669
Acinetobacter pragensis 611 DVEYLSAIEEVGTVIAQADSSVDKDGNLIEEFVSVR-HQGDFVRMPPEKVTHMDVSAQQV 669
Acinetobacter indicus 611 EVEYLSAIEEVGTVIAQADSVVDQDGNLIEEFVSVR-HPGEFVRMSPEKVTHMDVSAQQV 669
Pseudomonas aeruginosa 607 EIVELSAIEEADEVIAQASATLNEKGQLVDELVAVR-HLNEFTVKAPEEVTLMDVSPKQV 665
Escherichia coli 602 EIEYLSAIEEGNYVIAQANSNLDEEGHFVEDLVTCR-SKGESSLFSRDQVDYMDVSTQQV 660
Staphylococcus aureus 559 QIDYLTAEEDSYVVAQANSKLDENGRFMDDEVVCR-FRGNNTVMAKEKMDYMDVSPKQV 617
Bacillus subtilis 560 RIDYLTAEEDNYVVAQANARLDDEGAFIDDSIVAR-FRGENTVVSRNRVDYMDVSPKQV 618
Mycobacterium tuberculosis 521 EIVYLTAEEDRHVVAQANSPIDADGRFVEPRVLVRRKAGEVEYVPSSEVDYMDVSPRQM 580
Bacteroides fragilis 566 GLVYLTAEEEAKIIAQGNAPLNLDGTFIRNKVKSR-QDADYPVVEPSEVELMDVAPQQI 624
Thermotoga maritima 645 EVVYLRAEEEEYKIIPATTPVDEEGNIIPERVVAR-MGEDIRLVPKEEVDFMDVSTKQP 703
Thermus aquaticus 482 EVVYMTASEEDRYTIAQANTPLEGD-RIATDRVVAR-RRGEPVIVAPEEVEFMDVSPKQV 539
Fusobacterium nucleatum 561 -VEYLAAEEDGLEIAQADTKLDKNNKLEG-LVVCR-YGHEIVEIEPERVNYMDVSPKQV 618
Aquifex aeolicus 684 KIEWLAAYEENYVIAQS-TPTDEEGRLKAEFILAR-HKNDIRLVKPEQVEYIDVSPRQV 741
Blastopirellula marina 553 EIVWLRAEEESDAYVAPADTPTDAKGQLLGDLIIAR-YRSDFDLVEPKTVQYIDVAPCQM 611
Chlamydia abortus 547 EIEYMTADVEEECVIAQASANLDEYNMFVDPVCWAR-YRGEAFEADTSTVTHMDVSPKQL 605
Cyanobacteria bacterium 560 KIIQLRAEEELRAVLAPADSLTGE-GRLKEGAVLAR-VDGELTEVDSSIVDYLDISPKQI 617

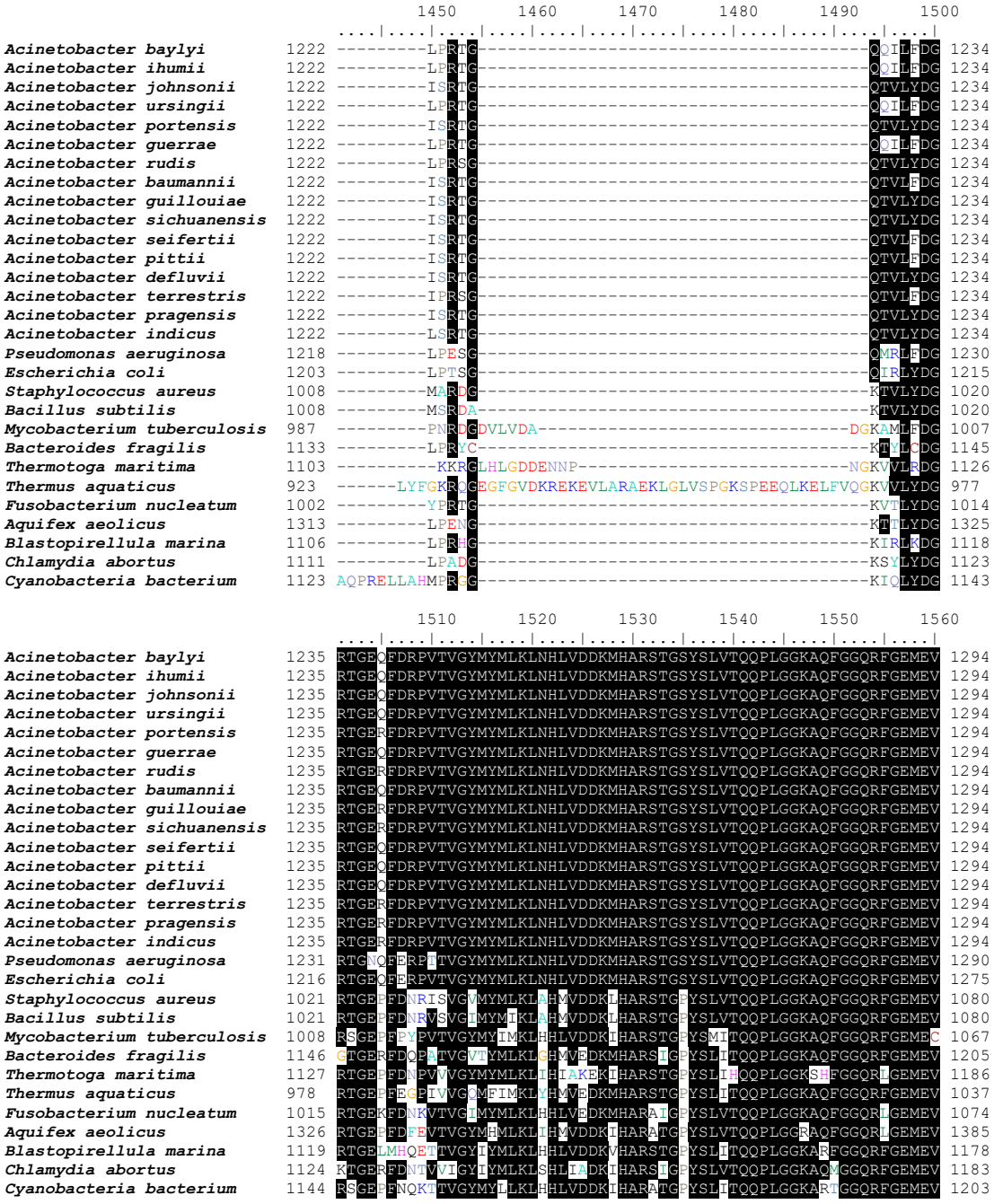

850 860 870 880 890 900
Acinetobacter baylyi 670 729
Acinetobacter ihumii 670 729
Acinetobacter johnsonii 670 729
Acinetobacter ursingii 670 729
Acinetobacter portensis 670 729
Acinetobacter guerrae 670 729
Acinetobacter rudis 670 729
Acinetobacter baumannii 670 729
Acinetobacter guillouiae 670 729
Acinetobacter sichuanensis 670 729
Acinetobacter seifertii 670 729
Acinetobacter pittii 670 729
Acinetobacter defluvii 670 729
Acinetobacter terrestris 670 729
Acinetobacter pragensis 670 729
Acinetobacter indicus 670 729
Pseudomonas aeruginosa 666 725
Escherichia coli 661 720
Staphylococcus aureus 618 677
Bacillus subtilis 619 678
Mycobacterium tuberculosis 581 640
Bacteroides fragilis 625 684
Thermotoga maritima 704 763
Thermus aquaticus 540 599
Fusobacterium nucleatum 619 678
Aquifex aeolicus 742 801
Blastopirellula marina 612 671
Chlamydia abortus 606 665
Cyanobacteria bacterium 618 677
910 920 930 940 950 960
Acinetobacter baylyi 730 784
Acinetobacter ihumii 730 784
Acinetobacter johnsonii 730 784
Acinetobacter ursingii 730 784
Acinetobacter portensis 730 784
Acinetobacter guerrae 730 784
Acinetobacter rudis 730 784
Acinetobacter baumannii 730 784
Acinetobacter guillouiae 730 784
Acinetobacter sichuanensis 730 784
Acinetobacter seifertii 730 784
Acinetobacter pittii 730 784
Acinetobacter defluvii 730 784
Acinetobacter terrestris 730 784
Acinetobacter pragensis 730 784
Acinetobacter indicus 730 784
Pseudomonas aeruginosa 726 780
Escherichia coli 721 775
Staphylococcus aureus 678 733
Bacillus subtilis 679 734
Mycobacterium tuberculosis 641 688
Bacteroides fragilis 685 742
Thermotoga maritima 764 823
Thermus aquaticus 600 647
Fusobacterium nucleatum 679 726
Aquifex aeolicus 802 859
Blastopirellula marina 672 719
Chlamydia abortus 666 715
Cyanobacteria bacterium 678 721

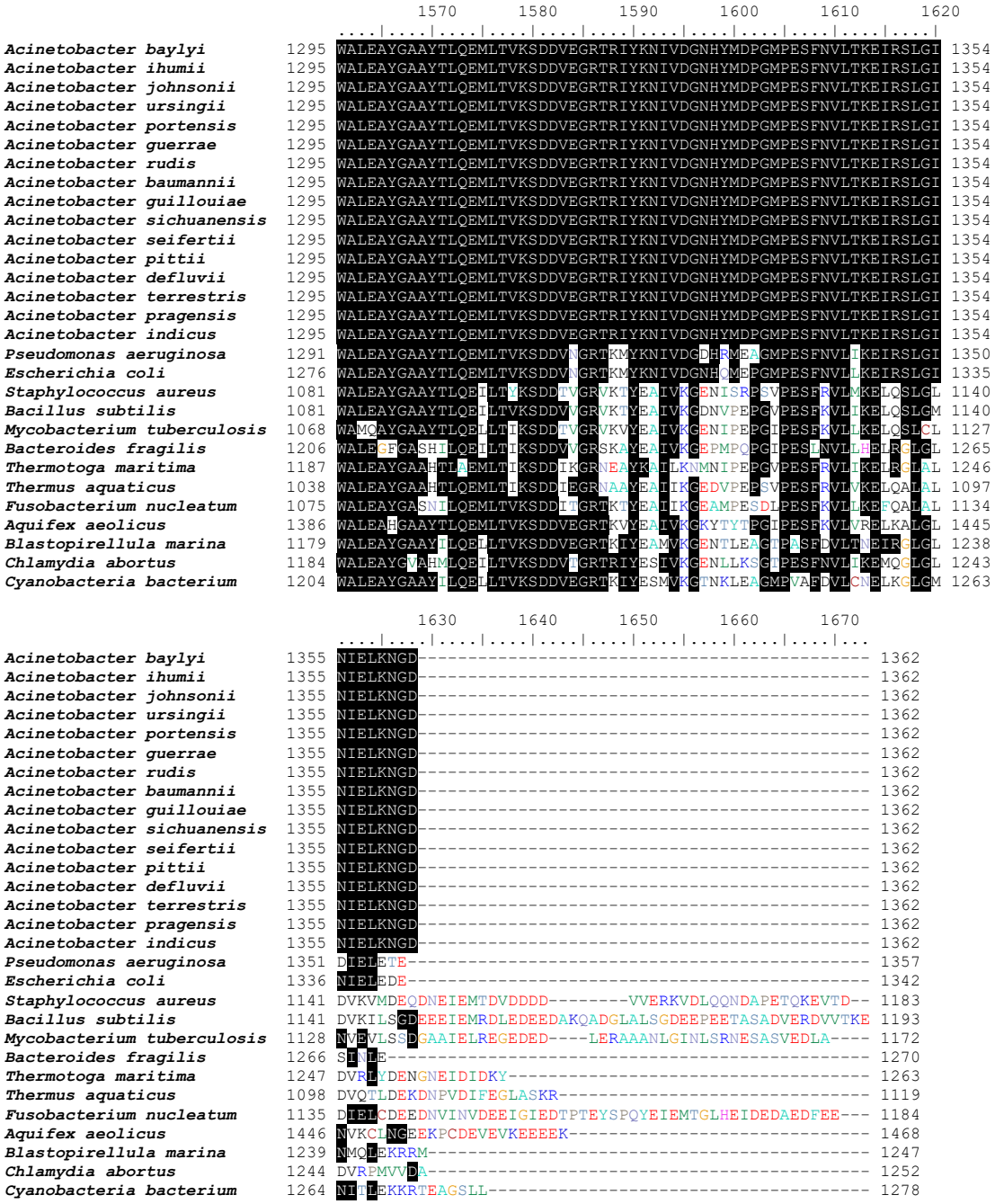

970 980 990 1000 1010 1020
Acinetobacter baylyi 785 844
Acinetobacter ihumii 785 844
Acinetobacter johnsonii 785 844
Acinetobacter ursingii 785 844
Acinetobacter portensis 785 844
Acinetobacter guerrae 785 844
Acinetobacter rudis 785 844
Acinetobacter baumannii 785 844
Acinetobacter guillouiae 785 844
Acinetobacter sichuanensis 785 844
Acinetobacter seifertii 785 844
Acinetobacter pittii 785 844
Acinetobacter defluvii 785 844
Acinetobacter terrestris 785 844
Acinetobacter pragensis 785 844
Acinetobacter indicus 785 844
Pseudomonas aeruginosa 781 840
Escherichia coli 776 835
Staphylococcus aureus 734 793
Bacillus subtilis 735 794
Mycobacterium tuberculosis 689 748
Bacteroides fragilis 743 802
Thermotoga maritima 824 883
Thermus aquaticus 648 707
Fusobacterium nucleatum 727 786
Aquifex aeolicus 860 919
Blastopirellula marina 720 779
Chlamydia abortus 716 775
Cyanobacteria bacterium 722 781
1030 1040 1050 1060 1070 1080
Acinetobacter baylyi 845 898
Acinetobacter ihumii 845 898
Acinetobacter johnsonii 845 898
Acinetobacter ursingii 845 898
Acinetobacter portensis 845 898
Acinetobacter guerrae 845 898
Acinetobacter rudis 845 898
Acinetobacter baumannii 845 898
Acinetobacter guillouiae 845 898
Acinetobacter sichuanensis 845 898
Acinetobacter seifertii 845 898
Acinetobacter pittii 845 898
Acinetobacter defluvii 845 898
Acinetobacter terrestris 845 898
Acinetobacter pragensis 845 898
Acinetobacter indicus 845 898
Pseudomonas aeruginosa 841 894
Escherichia coli 836 889
Staphylococcus aureus 794 847
Bacillus subtilis 795 848
Mycobacterium tuberculosis 749 802
Bacteroides fragilis 803 856
Thermotoga maritima 884 943
Thermus aquaticus 708 761
Fusobacterium nucleatum 787 840
Aquifex aeolicus 920 973
Blastopirellula marina 780 833
Chlamydia abortus 776 829
Cyanobacteria bacterium 782 835


1090 1100 1110 1120 1130 1140
Acinetobacter baylyi 899 KGETQLTPEEKLLRAIFGEKAADVKDSSLRVPSGIKGTVIDVQVFTRDGLEKDDRALAI- 957
Acinetobacter ihumii 899 KGETQLTPEEKLLRAIFGEKAADVKDSSLRVPSGIKGTVIDVQVFTRDGLEKDDRALAI- 957
Acinetobacter johnsonii 899 KGETQLTPEEKLLRAIFGEKAADVKDSSLRVPSGIKGTVIDVQVFTRDGLEKDERAQAI- 957
Acinetobacter ursingii 899 KGETQLTPEEKLLRAIFGEKAADVKDSSLRVPSGIKGTVIDVQVFTRDGLEKDERALAI- 957
Acinetobacter portensis 899 KGETQLTPEEKLLRAIFGEKAADVKDSSLRVSSSVKGTVIDVQVFTRDGLEKDDRAQAI- 957
Acinetobacter guerrae 899 KGETQLTPEEKLLRAIFGEKAADVKDSSLRVPSGIKGTVIDVQVFTRDGLEKDDRALAI- 957
Acinetobacter rudis 899 KGETQLTPEEKLLRAIFGEKAADVKDSSLRVPSGIKGTVIDVQVFTRDGIEKDDRAQAI- 957
Acinetobacter baumannii 899 KGETQLTPEEKLLRAIFGEKAADVKDSSLRVPSGIKGTVIDVQVFTRDGLEKDERALAI- 957
Acinetobacter guillouiae 899 KGETQLTPEEKLLRAIFGEKAADVKDSSLRVSSSVKGTVIDVQVFTRDGIEKDERAQAI- 957
Acinetobacter sichuanensis 899 KGETQLTPEEKLLRAIFGEKAADVKDSSLRVSSGVKGTVIDVQVFTRDGLEKDERAQAI- 957
Acinetobacter seifertii 899 KGETQLTPEEKLLRAIFGEKAADVKDSSLRVPSGIKGTVIDVQVFTRDGLEKDDRALAI- 957
Acinetobacter pittii 899 KGETQLTPEEKLLRAIFGEKAADVKDSSLRVPSGIKGTVIDVQVFTRDGLEKDDRALAI- 957
Acinetobacter defluvii 899 KGETQLTPEEKLLRAIFGEKAADVKDSSLRVSSGVKGTVIDVQVFTRDGIEKDERAQAI- 957
Acinetobacter terrestris 899 KGETQLTPEEKLLRAIFGEKAADVKDSSLRVSSGVKGTVIDVQVFTRDGLEKDERAQAI- 957
Acinetobacter pragensis 899 KGETQLTPEEKLLRAIFGEKAADVKDSSLRVSSGVKGTVIDVQVFTRDGLEKDERAQAI- 957
Acinetobacter indicus 899 KGETQLTPEEKLLRAIFGEKAADVKDSSLRVPSGIKGTVIDVQVFTRDGLEKDERALAI- 957
Pseudomonas aeruginosa 895 KGETQLTPEEKLLRAIFGEKASDVKDTSLRVPTGIKGTVIDVQVFTRDGVERDSRALSI- 953
Escherichia coli 890 KGETQLTPEEKLLRAIFGEKASDVKDSSLRVPNGVSGTVIDVQVFTRDGVEKDKRALEI- 948
Staphylococcus aureus 848 KGVTELTAEERLLRAIFGEKAREVRDTSLRVPAGAGGIVLDVKVFNRE---------- 895
Bacillus subtilis 849 KGVTELTAEERLLRAIFGEKAREVRDTSLRVPHGGGGIIHDVKVFNRE---------- 896
Mycobacterium tuberculosis 803 KGETELTPEERLLRAIFGEKAREVRDTSLKVPHGESGKVIGIRVFSRE---------- 850
Bacteroides fragilis 857 KGESDPSPEEKLLRAIFGKAGDVKDASLKASPSLKGVIIDKKIFS---RVIKNRSSKLA 913
Thermotoga maritima 944 KGESDTTPEEKIIRSVFGERGRDVKDTSLRLPHGVEGRVI-RVDVYDQ---------- 990
Thermus aquaticus 762 KGEQEPSPEERLLRSIFGEKARDVKDTSLRVPPGEGGIVVGRLRLRRG---------- 809
Fusobacterium nucleatum 841 KGETEPPAEEKLLRAIFGEKARDVRDTSLIMPHGSKGVVVDILELS---REN-------- 889
Aquifex aeolicus 974 KGETRLTPEEKLLQAIFGEKTRDVKDASLRCPPGVEGIVIDVQVFTRKGTGKKDMLAEKV 1033
Blastopirellula marina 834 KSKTELTPEEKLLHAIFGRAGEDVKNDSIEVPSGIEGIVIHTDKFARRMSLSDDERKAF- 892
Chlamydia abortus 830 KSETELAPEERLLRAIFGEKAADVKDASLTVPPGTEGVVMDVKVFSRKDRLSKSDDELVE 889
Cyanobacteria bacterium 836 KSKTELTPEEKLLHAIFGRAGEDVKNDSIEVPAGVEGIVIGAKKFSRRVHMTDAQKKQL- 894
1150 1160 1170 1180 1190 1200
Acinetobacter baylyi 958 EKAQLDAYRKDLKEEYKIFEEAARERIVRLLNGQESNGGGTTKRGDKL--------SDDV 1009
Acinetobacter ihumii 958 EKAQLDSYRKDLKEEYKIFEEAARERIVRLLKGQDSNGGGTTKRGDKL--------SENV 1009
Acinetobacter johnsonii 958 EKAQLDSYRKDLKEEYKIFEEAARERIVRLLTGQESNGGGTTKRGDKL--------SADV 1009
Acinetobacter ursingii 958 EKAQLDSYRKDLKEEYKIFEEAARERIVRLLKGQDSNGGGTTKRGDKL--------TEDL 1009
Acinetobacter portensis 958 EKAQLDAYRKDLKEEFKIFEEAARERIIRLLKGQESNGGGTTKRGDKL--------AEDM 1009
Acinetobacter guerrae 958 EKAQLDSYRKDLKEEYKIFEEAARERIVRLLKGQDSNGGGTTKRGDKL--------TEDV 1009
Acinetobacter rudis 958 EKAQLDAYRKDLKEEYKIFEEAARERIVRLLKDQQSNGGGNTKRGDKL--------TEDL 1009
Acinetobacter baumannii 958 EKAQLDSYRKDLKEEYKIFEEAARERVIRLLKGQESNGGGSTKRGDKL--------SEDL 1009
Acinetobacter guillouiae 958 EKAQLDAYRKDLKEEFKIFEEAARERIIRLLKGQESNGGGTTKRGDKL--------SEDV 1009
Acinetobacter sichuanensis 958 EKSQLDAYRKDLKEEYKIFEEAARERIVRLLNGQESNGGGSTKRGQKI--------TEDL 1009
Acinetobacter seifertii 958 EKAQLDSYRKDLKEEYKIFEEAARERVIRLLKGQESNGGGSTKRGDKL--------SEDL 1009
Acinetobacter pittii 958 EKAQLDAYRKDLKEEYKIFEEAARERVIRLLKGQESNGGGSTKRGDKL--------VEDV 1009
Acinetobacter defluvii 958 EKAQLDAYRKDLKEEYKIFEEAARERIVRLLNGQESNGGGTTKRGQKI--------TEDL 1009
Acinetobacter terrestris 958 EKAQLDAYRKDLKEEFKIFEEAARERIVRLLKGQESNGGGTTKRGDKL--------TEDL 1009
Acinetobacter pragensis 958 EKAQLDSYRKDLKEEYKIFEEAARERIIRLLKGQESNGGGTTKRGDKI--------TEEL 1009
Acinetobacter indicus 958 EKAQLDAYRKDLKEEYKIFEEAARERIVRLLKGQESNGGGGTKRGDKL--------SEDM 1009
Pseudomonas aeruginosa 954 EKMQLDQIRKDLNEEFRIVEGATFERLRAALVGAKAEGGPALKKGTEI--------TDDV 1005
Escherichia coli 949 EEMQLKQAKKDLSEELQIIEAGLFSRIRAVLVAGGVEA--------------------EK 988
Staphylococcus aureus 895 ---------- 895
Bacillus subtilis 896 ---------- 896
Mycobacterium tuberculosis 850 ---------- 850
Bacteroides fragilis 914 DKALLPKIDDEFESKVADLKRILVKKL-MVLTEGKVSQGVKDYLGAEVIAKGSKFSASDF 972
Thermotoga maritima 990 ---------- 990
Thermus aquaticus 809 ---------- 809
Fusobacterium nucleatum 889 ---------- 889
Aquifex aeolicus 1034 EREELEALEQELEKKKNLIITGRDKVLKGLVLGRKVEKDAKVGRKTVKKG----TVIDEK 1089
Blastopirellula marina 892 -EKRLKEVETEGNSKISEAFAAMIVEFERILGKTLTD-EDGTPLTQDQ--------DPKF 942
Chlamydia abortus 890 EAVHLKDLQKGYKNQISVLKIEYREKLGALLLNEKAPASIIHRRTADILVQEGTVFDQET 949
Cyanobacteria bacterium 894 -KKDIEAYEAEMDQQAINLFKQMTQSINDAVGTEMIDPMTRQKVGASD--------IPEV 945
SI2


1210 1220 1230 1240 1250 1260
Acinetobacter baylyi 1010 LSGLELVDLLDIQPTDEGIAERLSQIQVFLKEKSAEIDEKFAEKKRKLATGDE---LTTG 1066
Acinetobacter ihumii 1010 LSGLELVDLLEIQPSDEGIAERLSQIQVFLKEKSAEIDEKFAEKKRKLATGDE---LTTG 1066
Acinetobacter johnsonii 1010 LSGLELVDLLEIQPTDEAIAERLTQIQVFLKEKSFEIDEKFAEKKRKLSTGDE---LTTG 1066
Acinetobacter ursingii 1010 LSGLELVDLLEIQPSDEGIAERLSQIQVFLKEKSAEIDEKFAEKKRKLATGDE---LTTG 1066
Acinetobacter portensis 1010 LSSLELVDLLDIQPVDEAIAERLTQIQVFLKEKSHEIDEKFAEKKRKLSTGDE---LTTG 1066
Acinetobacter guerrae 1010 LSGLELVDLLEIQPSDEGIAERLSQIQVFLKEKSAEIDEKFAEKKRKLATGDE---LTTG 1066
Acinetobacter rudis 1010 LSHLALVDLLEIQPNDEAIAERLTQIQVFLKEKSFEIDEKFAEKKRKLSTGDE---LTTG 1066
Acinetobacter baumannii 1010 LSGLELVDLLEIQPADEAIAERLTQIQVFLKEKSAEIDEKFAEKKRKLATGDE---LTTG 1066
Acinetobacter guillouiae 1010 LSGLELVDLLEVQPTDEGIAERLTQIQVFLKEKSYEIDEKFAEKKRKLSTGDE---LTTG 1066
Acinetobacter sichuanensis 1010 LSTLDLVDLLEIQPVDEAVAERLTQIQVFLKEKSLEIDEKFAEKKRKLSTGDE---LTTG 1066
Acinetobacter seifertii 1010 LSGLELVDLLEIQPTDEAIAERLTQIQVFLKEKSAEIDEKFAEKKRKLATGDE---LTTG 1066
Acinetobacter pittii 1010 LSGLELVDLLEIQPADEAIAERLTQIQVFLKEKSAEIDEKFAEKKRKLATGDE---LTTG 1066
Acinetobacter defluvii 1010 LSSLELVDLLEIQPVDEAIAERLTQIQVFLKEKSLEIDEKFAEKKRKLSTGDE---LTTG 1066
Acinetobacter terrestris 1010 LSGLELVDLLEIQPVDEGIAERLTQIQVFLKEKGIEIDEKFAEKKRKLSTGDE---LTTG 1066
Acinetobacter pragensis 1010 LSGLELADLLEIQPADEAIAERLTQIQVFLKEKGFEIDEKFAEKKRKLSTGDE---LTTG 1066
Acinetobacter indicus 1010 LSGLELVDLLEIQPSDEAIAERLTQIQTFLKEKSFEIDEKFAEKKRKLSTGDE---LTTG 1066
Pseudomonas aeruginosa 1006 LDGLERGQWFKLRMADDALNEQLEKAQAYISDRRQLLDPKFEDKKRKLQQGDD---LAPG 1062
Escherichia coli 989 LDKLPRDRWLELGLTDEEKQNQLEQLAEQYDELKHEFEKKLEAKRRKITQGDD---LAPG 1045
Staphylococcus aureus 895 ----------EGDDT--LSPG 904
Bacillus subtilis 896 ----------DGDE---LPPG 904
Mycobacterium tuberculosis 850 ----------DEDE---LPAG 858
Bacteroides fragilis 973 DSLDFTAIQLSDWTNDDHANGMIRDLILNFIKKYKELDAELKRKKFAITI-GDE--LPAG 1029
Thermotoga maritima 990 ----------NDIAELGAG 999
Thermus aquaticus 809 ----------DPGVELKPG 818
Fusobacterium nucleatum 889 ----------GDE--LKAG 896
Aquifex aeolicus 1090 VFEEFVNYILGRPENFFEDEDLIRKIREIAERTRTQIEMLNKVYEEKKPLLKRRDLPPG 1149
Blastopirellula marina 943 IAEQAQQPFRLKKIVEHLRSDERIQQIREAYATSWPLVEAALDVRDRVLNSMKRGDELRSG 1002
Chlamydia abortus 950 IELLEQESLVDLLMPPCDMYDVLKSLLSDYETSLQRLEVNYKTEVEHIREGDAD--LDHG 1007
Cyanobacteria bacterium 946 ILEQIQTFDIKWAKGSKETREYAELIHSQF---WPRIQAIEAEKQRKVAHMKRGDELPNG 1002
1270 1280 1290 1300 1310 1320
Acinetobacter baylyi 1067 VLKVVKVYLAVKRRIQPGDKMAGRHGNKGVVSNILPVEDMPHDANGVPVDVVLNPLGVPS 1126
Acinetobacter ihumii 1067 VLKVVKVYLAVKRRIQPGDKMAGRHGNKGVVSNILPVEDMPHDANGVPVDIVLNPLGVPS 1126
Acinetobacter johnsonii 1067 VLKVVKVYLAVKRRIQPGDKMAGRHGNKGVVSNILPVEDMPHDANGVPVDIVLNPLGVPS 1126
Acinetobacter ursingii 1067 VLKVVKVYLAVKRRIQPGDKMAGRHGNKGVVSNILPVEDMPHDANGVPVDIVLNPLGVPS 1126
Acinetobacter portensis 1067 VLKVVKVYLAVKRRIQPGDKMAGRHGNKGVVSNILPVEDMPHDANGVPVDIVLNPLGVPS 1126
Acinetobacter guerrae 1067 VLKVVKVYLAVKRRIQPGDKMAGRHGNKGVVSNILPVEDMPHDANGVPVDIVLNPLGVPS 1126
Acinetobacter rudis 1067 VLKVVKVYLAVKRRIQPGDKMAGRHGNKGVVSNILPVEDMPHDIHGVPVDVVLNPLGVPS 1126
Acinetobacter baumannii 1067 VLKVVKVYLAVKRRIQPGDKMAGRHGNKGVVSNILPVEDMPHDANGVPVDIVLNPLGVPS 1126
Acinetobacter guillouiae 1067 VLKVVKVYLAVKRRIQPGDKMAGRHGNKGVVSNILPVEDMPHDIHGVPVDVVLNPLGVPS 1126
Acinetobacter sichuanensis 1067 VLKVVKVYLAVKRRIQPGDKMAGRHGNKGVVSNILPVEDMPHDANGIPVDVVLNPLGVPS 1126
Acinetobacter seifertii 1067 VLKVVKVYLAVKRRIQPGDKMAGRHGNKGVVSNILPVEDMPHDANGVPVDIVLNPLGVPS 1126
Acinetobacter pittii 1067 VLKVVKVYLAVKRRIQPGDKMAGRHGNKGVVSNILPVEDMPHDANGVPVDIVLNPLGVPS 1126
Acinetobacter defluvii 1067 VLKVVKVYLAVKRRIQPGDKMAGRHGNKGVVSNILPVEDMPHDANGVPVDIVLNPLGVPS 1126
Acinetobacter terrestris 1067 VLKVVKVYLAVKRRIQPGDKMAGRHGNKGVVSNILPVEDMPHDANGVPVDIVLNPLGVPS 1126
Acinetobacter pragensis 1067 VLKVVKVYLAVKRRIQPGDKMAGRHGNKGVVSNILPVEDMPHDANGVPVDVVLNPLGVPS 1126
Acinetobacter indicus 1067 VLKVVKVYLAVKRRIQPGDKMAGRHGNKGVVSNILPVEDMPHDANGVPVDVVLNPLGVPS 1126
Pseudomonas aeruginosa 1063 VLKIVKVYLAIKRRIQPGDKMAGRHGNKGVVSVIMPVEDMPHDANGTPVDIVLNPLGVPS 1122
Escherichia coli 1046 VLKIVKVYLAVKRRIQPGDKMAGRHGNKGVISKINPIEDMPYDPNGTPVDIVLNPLGVPS 1105
Staphylococcus aureus 905 VNQIVRVYIVQKRKIHVGDKMCGRHGNKGVISKIVPEEDMPYLPDGRPIDIMLNPLGVPS 964
Bacillus subtilis 905 VNQLVRVYIVQKRKISEGDKMAGRHGNKGVISKILPEEDMPYLPDGTPIDIMLNPLGVPS 964
Mycobacterium tuberculosis 859 VNELVRVYVAQKRKISDGDKLAGRHGNKGVIGKILPVEDMPFLADGTPVDIILNTHGVPR 918
Bacteroides fragilis 1030 IIQMAKVYIAKKRKIGVGDKMAGRHGNKGIVSRVVRQEDMPFLEDGTPVDIVLNPLGVPS 1089
Thermotoga maritima 1000 VLKLVRVYVASRKTLDIGDKLAGRHGNKGVVSNILPKEDMPFLPDGTPVQMVLNPLGIPS 1059
Thermus aquaticus 819 VREVVRVFVAQKRKLQVGDKLAARHGNKGVVAKILPVEDMPHLPDGTPVDVILNPLGVPS 878
Fusobacterium nucleatum 897 VNKSIRVLVAEKRKITVGDKMSGRHGNKGVVSRVLPAEDMPFLEDGTHIDVVLNPLGVPS 956
Aquifex aeolicus 1150 VITLVKVFIANKRKIKVGDKMAGRHGNKGVISVVLPVEDMPFLPDGTPVDIVLNPLGVPS 1209
Blastopirellula marina 1003 VLQMVKVYIATKRQISVGDKMAGRHGNKGVIAKILPVEDMPFLPDGTPIQIMLNPLGVPS 1062
Chlamydia abortus 1008 VIRQVKVYVASKRKLQVGDKMAGRHGNKGVVSKIVPEADMPYLANGETVQMILNPLGVPS 1067
Cyanobacteria bacterium 1003 VLEMVKVYLATKRQLSVGDKFAGRHGNKGVIARIVPEEDMPFMEDGTPVDVLLNPLGVPS 1062

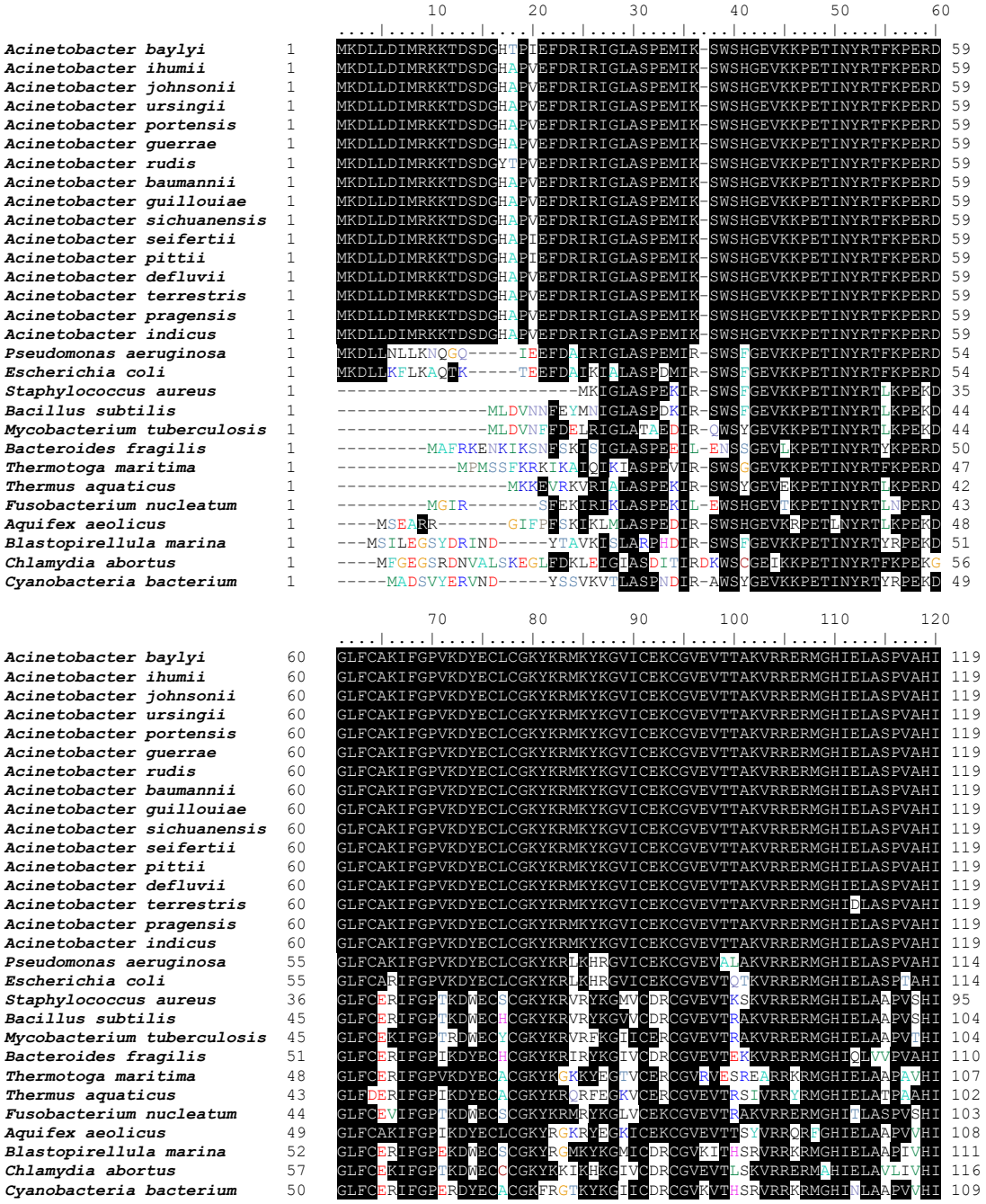

1330 1340 1350 1360 1370 1380
Acinetobacter baylyi 1127 RMNVGQILETHLGLAAKGLGEQIDKMLQQQRTIAELRIFLDKIYNKVG-------GEQE 1178
Acinetobacter ihumii 1127 RMNVGQILETHLGLAAKGLGEQIDKMLQQQRTIAELRTFLDKIYNKVG-------GEQE 1178
Acinetobacter johnsonii 1127 RMNVGQILETHLGMAAKGLGEEIDKMLKAQRTVLELRGFLDKIYNKVG-------GEQE 1178
Acinetobacter ursingii 1127 RMNVGQILETHLGLAAKGLGEQIDKMLQQQRTIAELRIFLDKIYNKVG-------GEQE 1178
Acinetobacter portensis 1127 RMNVGQILETHLGMAARGLGEKIDKMMQEQRTIMELREFLDKIYNKVG-------GEQE 1178
Acinetobacter guerrae 1127 RMNVGQILETHLGLAAKGLGEQIDKMLQQQRTIAELRVFLDKIYNKVG-------GEQE 1178
Acinetobacter rudis 1127 RMNVGQILETHLGLAAKGLGEQIDKMVKQQRTIAELREFLDKIYNKVG-------GEQE 1178
Acinetobacter baumannii 1127 RMNVGQILETHLGMAAKGLGDKIDKMLKEQRTVLELREFLDKIYNKVG-------GEQE 1178
Acinetobacter guillouiae 1127 RMNVGQILETHLGMAAKGLGDKIDKMMKEQRTVLELREFLDKIYNKVG-------GEQE 1178
Acinetobacter sichuanensis 1127 RMNVGQILETHLGMAAKGLGDKIDKMLKEQRTILELREFLDKIYNKVG-------GEQE 1178
Acinetobacter seifertii 1127 RMNVGQILETHLGMAARGLGDKIDKMLKEQRTVLELREFLDKIYNKVG-------GEQE 1178
Acinetobacter pittii 1127 RMNVGQILETHLGMAAKGLGDKIDKMLKEQRTVLELREFLDKIYNKVG-------GEQE 1178
Acinetobacter defluvii 1127 RMNVGQILETHLGMAAKGLGDKIDKMLQEQRTILELREFLDKIYNKVG-------GEQE 1178
Acinetobacter terrestris 1127 RMNVGQILETHLGMAAKGLGDKIDKMLQQQRTIAELRIFLDKIYNKVG-------GEQE 1178
Acinetobacter pragensis 1127 RMNVGQILETHLGMAAKGLGDQIDKMMKQQRTVLELREFLDKIYNKVG-------GEQE 1178
Acinetobacter indicus 1127 RMNVGQILETHLGMAAKGLGDQIDKMLKEQRTILELREFLDKIYNKVG-------GEQE 1178
Pseudomonas aeruginosa 1123 RMNVGQILETHLGLAAKGLGEKINRMLEEQRKVAELRKFLHEIYNEIG-------GREE 1174
Escherichia coli 1106 RMNIGQILETHLGMAAKGIGDKINAMLKQQQEVAKLREFIQRAYDLGAD------VRQKV 1159
Staphylococcus aureus 965 RMNIGQVLELHLGMAAKN---------- 982
Bacillus subtilis 965 RMNIGQVLELHMGMAARY---------- 982
Mycobacterium tuberculosis 919 RMNIGQILETHLGWCAHSGWKVDAAKGVP---------- 947
Bacteroides fragilis 1090 RMNIGQIFEAVLGRAGKNLG---------- 1109
Thermotoga maritima 1060 RMNVGQILETHLGWLAKLTG---------- 1079
Thermus aquaticus 879 RMNIGQILETHLGLAGYFLG---------- 898
Fusobacterium nucleatum 957 RMNIGQVLEVHLGMAMRTLNG---------- 977
Aquifex aeolicus 1210 RMNVGQILETHLGMAAKELGKKIGEMIEKGRDRKAITEYLKEIFAIGDKDGENAKFIEEF 1269
Blastopirellula marina 1063 RMNVGQILETHLGMAGAKLG---------- 1082
Chlamydia abortus 1068 RMNIGQVLETHLGMAAKTAG---------- 1087
Cyanobacteria bacterium 1063 RMNVGQILETHLGMAAQVLG---------- 1082
1390 1400 1410 1420 1430 1440
Acinetobacter baylyi 1179 ELDTLTDEEILVLAGNLRKGVPLATPVFDGAEEEQIKELLELAE---------- 1222
Acinetobacter ihumii 1179 DLDSLTDDEVLILAGNLRKGVPLATPVFDGAEEEQIKELLELAE---------- 1222
Acinetobacter johnsonii 1179 DLDSLTDDEILVLSGNLRAGVPLATPVFDGAEEEQIKELLELAN---------- 1222
Acinetobacter ursingii 1179 DLNSLTDDEVLVLAGNLRKGVPLATPVFDGAEEEQIKELLELAE---------- 1222
Acinetobacter portensis 1179 DLDSLTDEEVLALSKNLRKGVPLATPVFDGADEEQIKELLELAG---------- 1222
Acinetobacter guerrae 1179 DLNSLTDDEVLVLAGNLRKGVPLATPVFDGAEEEQIKELLELAE---------- 1222
Acinetobacter rudis 1179 QLDTLTDEEIMKLAGNLRAGVPLATPVFDGAEEEQIKELLELAE---------- 1222
Acinetobacter baumannii 1179 DLDSLTDEEILALAGNLRAGVPLATPVFDGAEEEQIKDLLELAE---------- 1222
Acinetobacter guillouiae 1179 DLDSLTDEEILVLSGNLRKGVPLATPVFDGAEEEQIKELLELGG---------- 1222
Acinetobacter sichuanensis 1179 DLDSLTDDEILVLSGNLRAGVPLATPVFDGAEEEQIKELLELGG---------- 1222
Acinetobacter seifertii 1179 DLDSLTDDEILALSGNLRAGVPLATPVFDGAEEEQIKDLLELAD---------- 1222
Acinetobacter pittii 1179 DLDSLTDAEVLALSGNLRAGVPLATPVFDGAEEEQIKDLLELAG---------- 1222
Acinetobacter defluvii 1179 DLDSLTDEEILVLSGNLRKGVPLATPVFDGAEEEQIKDLLELAN---------- 1222
Acinetobacter terrestris 1179 DLDSLTDDEILALSKNLRKGIPLATPVFDGADEEQIKELLELAD---------- 1222
Acinetobacter pragensis 1179 ELDTLTDDEILVLSGNLRKGVPLATPVFDGAEEEQIKELLELAD---------- 1222
Acinetobacter indicus 1179 DLDSLTDDEILTLSGNLRAGVPLATPVFDGAEEEQIKDLLELAG---------- 1222
Pseudomonas aeruginosa 1175 NLDELGDAEILALAKNLRSGVPMATPVFDGAKEREIKAMLKLAD---------- 1218
Escherichia coli 1160 DLSTFSNEEVMRLAENLRKGMPLATPVFDGAKEAEIKELLKLGD---------- 1203
Staphylococcus aureus 982 ----------IGIHVASPVFDGANDDDVWSTIEEAG---------- 1008
Bacillus subtilis 982 ----------LGIHIASPVFDGAREEDVWETLEEAG---------- 1008
Mycobacterium tuberculosis 948 DWAARLPDELL----EAQPNAIVSTPVFDGAQEAELQGLLSCTL---------- 987
Bacteroides fragilis 1109 ----------VKEATPIFDGATLDDLNEWTDKAG---------- 1133
Thermotoga maritima 1079 ----------KWEATPVFEGAKEDEILREIYEER---------- 1103
Thermus aquaticus 898 ----------QRYISPVFDGATEPEIKELLAEAFN---------- 923
Fusobacterium nucleatum 977 ----------GTCIATPVFDGATEEQVKDYLEKQG---------- 1002
Aquifex aeolicus 1270 LNSLSEEEFWSVVRDYAERGIPMATPAFEGAEEDAIKELLKKAG---------- 1313
Blastopirellula marina 1082 ----------FQSITPVFDGASEEDINNGLEEAG---------- 1106
Chlamydia abortus 1087 ----------IHVKTPVFEGFPESRIWDMMIEQG---------- 1111
Cyanobacteria bacterium 1082 ----------FQAVTPVFDGATEEEIHKAVDEANRHVEKRAREIEESGVA 1122

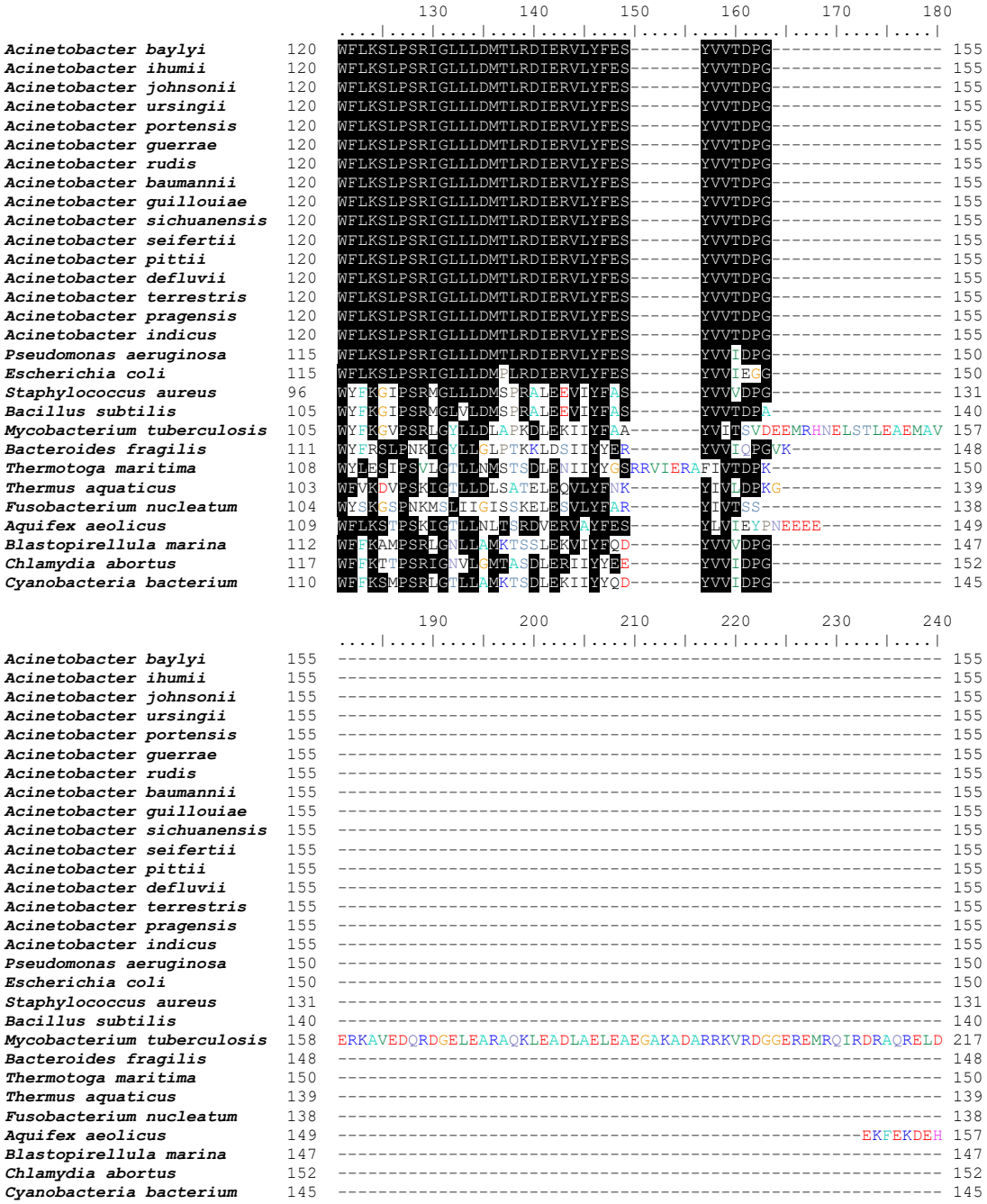

1450 1460 1470 1480 1490 1500
Acinetobacter baylyi 1222 ---------LPRTG----------QQILFDG 1234
Acinetobacter ihumii 1222 ---------LPRTG----------QQILFDG 1234
Acinetobacter johnsonii 1222 ---------ISRTG----------QTVLYDG 1234
Acinetobacter ursingii 1222 ---------LPRTG----------QQILFDG 1234
Acinetobacter portensis 1222 ---------ISRTG----------QTVLYDG 1234
Acinetobacter guerrae 1222 ---------LPRTG----------QQILFDG 1234
Acinetobacter rudis 1222 ---------LPRSG----------QTVLYDG 1234
Acinetobacter baumannii 1222 ---------ISRTG----------QTVLFDG 1234
Acinetobacter guillouiae 1222 ---------ISRTG----------QTVLYDG 1234
Acinetobacter sichuanensis 1222 ---------ISRTG----------QTVLYDG 1234
Acinetobacter seifertii 1222 ---------ISRTG----------QTVLFDG 1234
Acinetobacter pittii 1222 ---------ISRTG----------QTVLFDG 1234
Acinetobacter defluvii 1222 ---------ISRTG----------QTVLYDG 1234
Acinetobacter terrestris 1222 ---------IPRSG----------QTVLFDG 1234
Acinetobacter pragensis 1222 ---------ISRTG----------QTVLYDG 1234
Acinetobacter indicus 1222 ---------LSRTG----------QTVLYDG 1234
Pseudomonas aeruginosa 1218 ---------LPESG----------QMRLFDG 1230
Escherichia coli 1203 ---------LPTSG----------QIRLYDG 1215
Staphylococcus aureus 1008 ---------MARDG----------KTVLYDG 1020
Bacillus subtilis 1008 ---------MSRDA----------KTVLYDG 1020
Mycobacterium tuberculosis 987 ---------PNRDGDVLVDA----------DGKAMLFDG 1007
Bacteroides fragilis 1133 ---------LPRYC----------KTVLCDG 1145
Thermotoga maritima 1103 ----------KKRGLHLGDDENNP----------NGKVVLRDG 1126
Thermus aquaticus 923 ------LYFGKRQGEGFGVDKREKEVLARAEKLGLVSPGKSPEEQLKELFVQGKVVLYDG 977
Fusobacterium nucleatum 1002 ---------YPRTG----------KVTLYDG 1014
Aquifex aeolicus 1313 ---------LPEMG----------KTTLYDG 1325
Blastopirellula marina 1106 ---------LPRHG----------KIRLKDG 1118
Chlamydia abortus 1111 ---------LPADG----------KSYLYDG 1123
Cyanobacteria bacterium 1123 AQPRELLAHMPRGG----------KIQLYDG 1143
1510 1520 1530 1540 1550 1560
Acinetobacter baylyi 1235 RTGEQFDRPVTVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEV 1294
Acinetobacter ihumii 1235 RTGEQFDRPVTVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEV 1294
Acinetobacter johnsonii 1235 RTGEQFDRPVTVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEV 1294
Acinetobacter ursingii 1235 RTGEQFDRPVTVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEV 1294
Acinetobacter portensis 1235 RTGERFDRPVTVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEV 1294
Acinetobacter guerrae 1235 RTGEQFDRPVTVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEV 1294
Acinetobacter rudis 1235 RTGERFDRPVTVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEV 1294
Acinetobacter baumannii 1235 RTGEQFDRPVTVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEV 1294
Acinetobacter guillouiae 1235 RTGERFDRPVTVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEV 1294
Acinetobacter sichuanensis 1235 RTGERFDRPVTVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEV 1294
Acinetobacter seifertii 1235 RTGEQFDRPVTVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEV 1294
Acinetobacter pittii 1235 RTGEQFDRPVTVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEV 1294
Acinetobacter defluvii 1235 RTGEQFDRPVTVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEV 1294
Acinetobacter terrestris 1235 RTGERFDRPVTVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEV 1294
Acinetobacter pragensis 1235 RTGERFDRPVTVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEV 1294
Acinetobacter indicus 1235 RTGERFDRPVTVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEV 1294
Pseudomonas aeruginosa 1231 RTGNQFERPITVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEV 1290
Escherichia coli 1216 RTGEQFERPVTVGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEV 1275
Staphylococcus aureus 1021 RTGEPFDNRISVGYMYMLKLAHMVDDKLHARSTGPYSLVTQQPLGGKAQFGGQRFGEMEV 1080
Bacillus subtilis 1021 RTGEPFDNRVSVGYMYMIKLAHMVDDKLHARSTGPYSLVTQQPLGGKAQFGGQRFGEMEV 1080
Mycobacterium tuberculosis 1008 RSGEPFPYPVTVGYMYIMKLHHLVDDKIHARSTGPYSMITQQPLGGKAQFGGQRFGEMEC 1067
Bacteroides fragilis 1146 GTGERFDQPATVGYTYMLKLGHMVEDKMHARSIGPYSLITQQPLGGKAQFGGQRFGEMEV 1205
Thermotoga maritima 1127 RTGEPFDNPVVVGYMYMLKLIHIAKEKIHARSTGPYSLIHQQPLGGKSHFGGQRLGEMEV 1186
Thermus aquaticus 978 RTGEPFEQPIVVGYMFIMKLYHMVEDKMHARSTGPYSLITQQPLGGKAQFGGQRFGEMEV 1037
Fusobacterium nucleatum 1015 RTGEKFDNKVTVGYMYMLKLHHLVEDKMHARAIGPYSLVTQQPLGGKAQFGGQRLGEMEV 1074
Aquifex aeolicus 1326 RTGEPFDFEVTVGYMHMLKLIHMVDDKIHARATGPYSLVTQQPLGGRAQFGGQRLGEMEV 1385
Blastopirellula marina 1119 RTGELMHQETTVGYIYMLKLHHLVDDKVHARSTGPYSLITQQPLGGKARFGGQRFGEMEV 1178
Chlamydia abortus 1124 KTGERFDNTVVTGYIYMLKLSHLIADKIHARSIGPYSLVTQQPLGGKAQMGGQRFGEMEV 1183
Cyanobacteria bacterium 1144 RSGEPFNQKTTVGYMYLLKLHHLVDDKIHARATGPYSLITQQPLGGKARTGGQRFGEMEV 1203

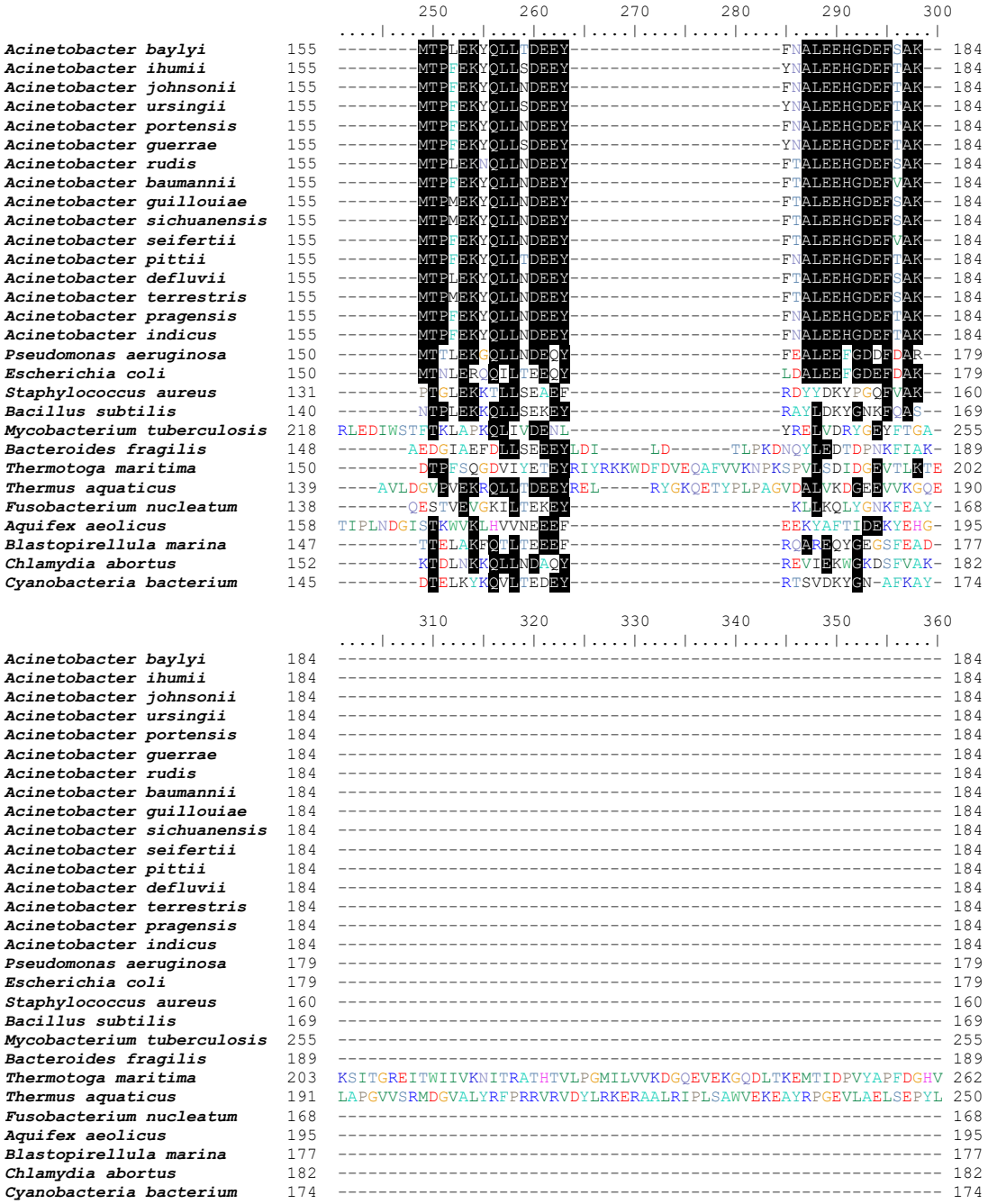


[1] Protein sequences were selected from the NCBI database (https://www.ncbi.nlm.nih.gov/protein) and aligned using CLUSTALW implemented within BioEdit using default parameters [115]. Black background denotes 55% identity, which highlights the high sequence identity within the *Acinetobacter* species.

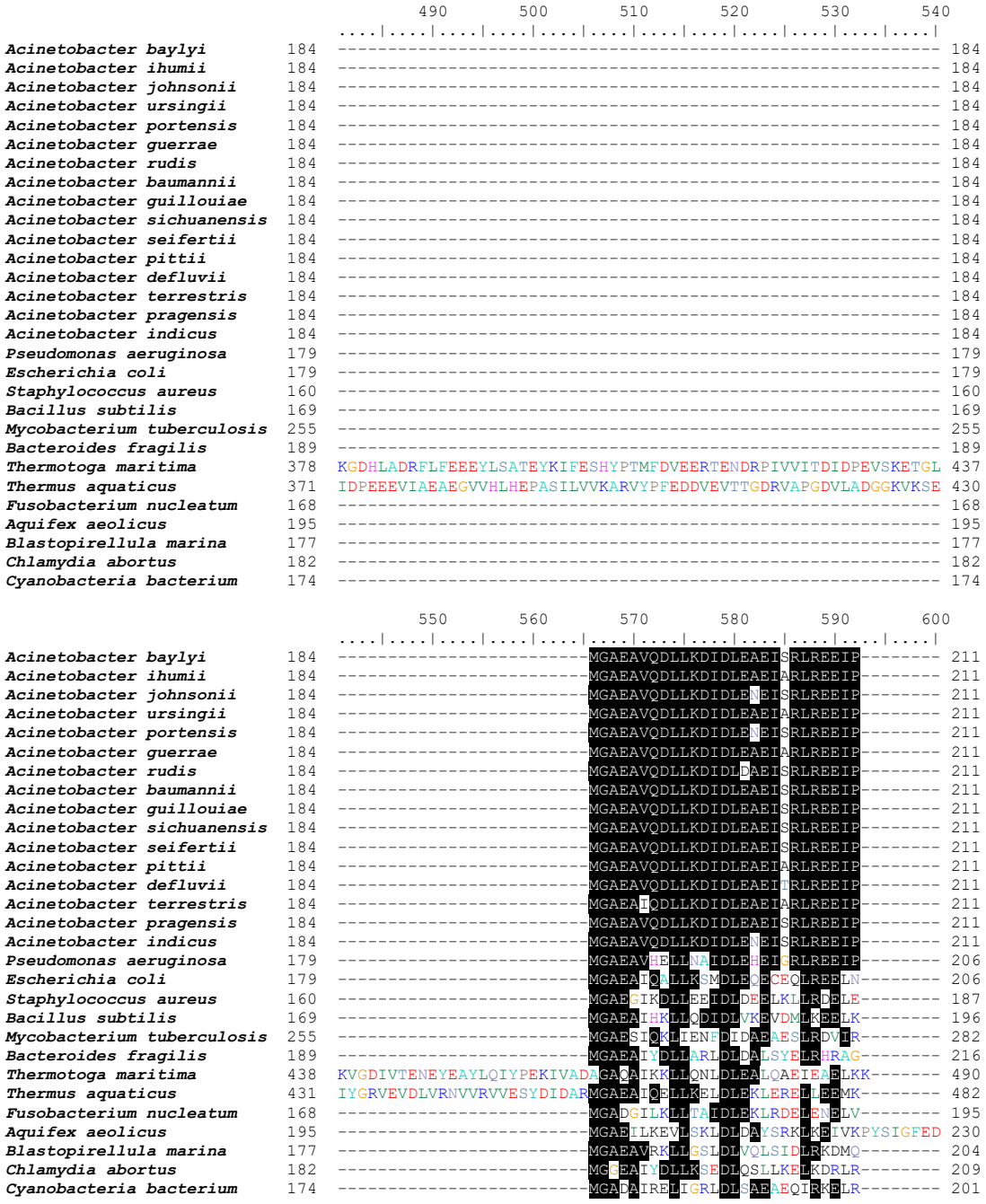
Figure A2.5. Sequence alignment of Beta' subunit (RpoC)[1].

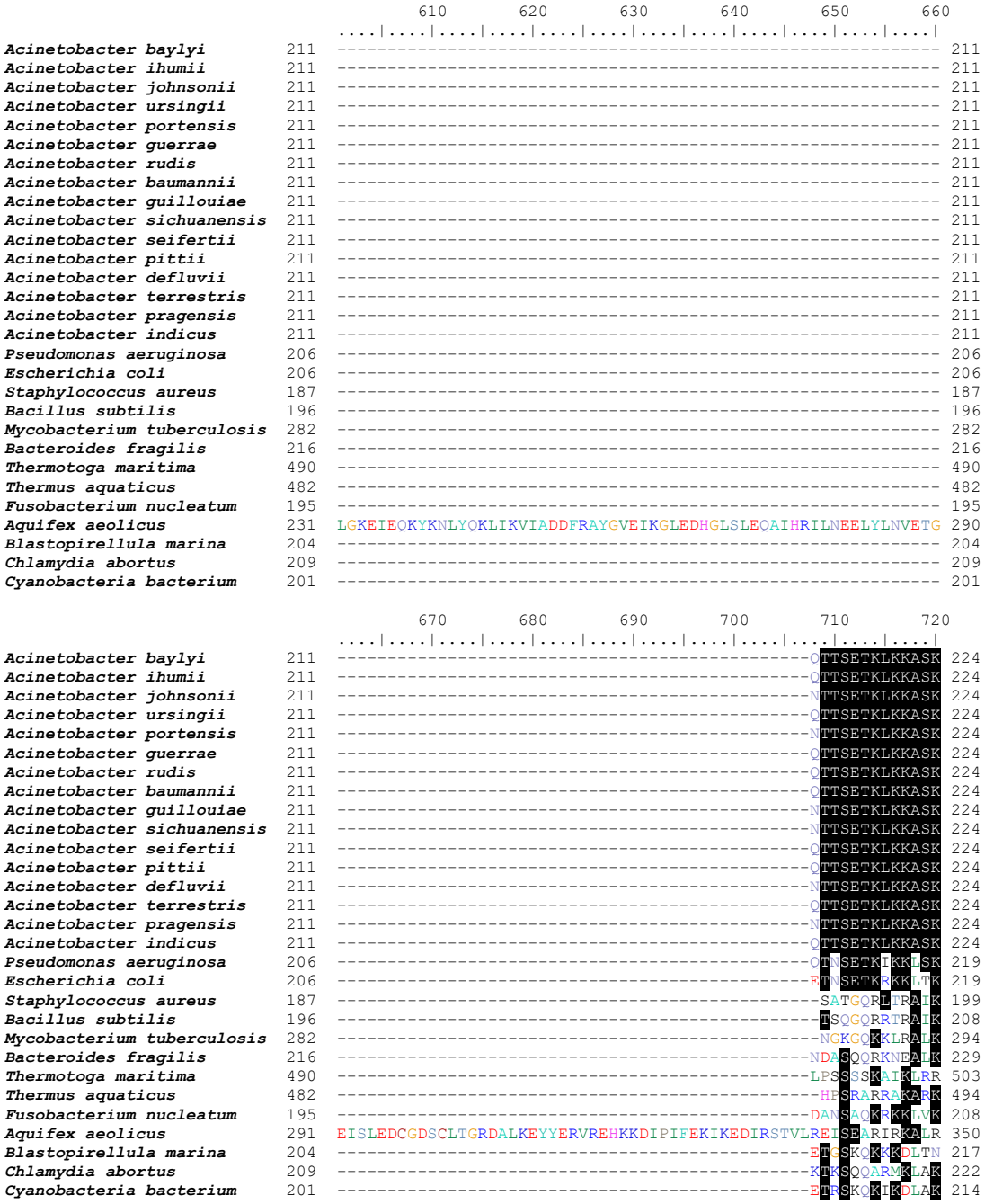


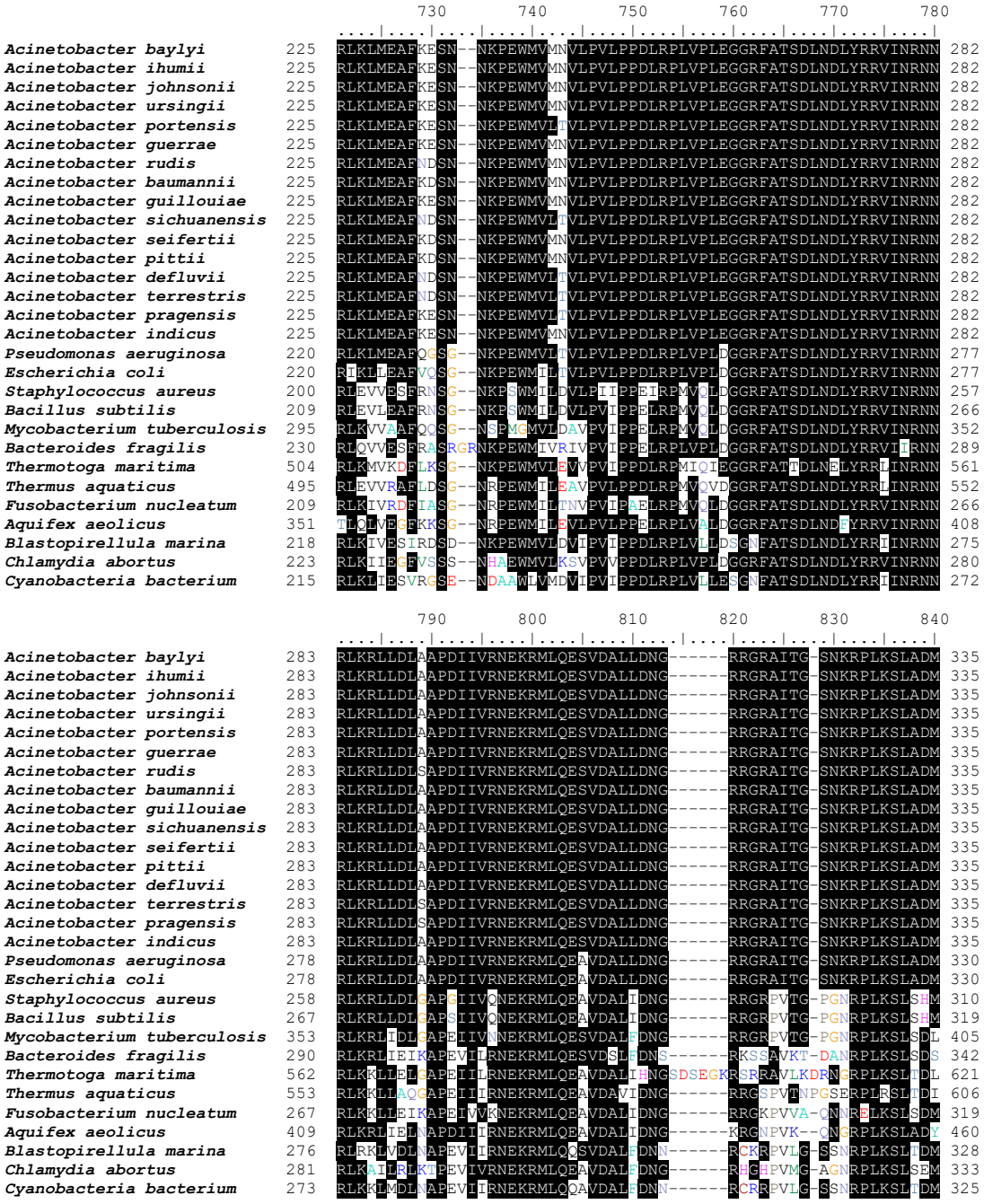

130 140 150 160 170 180
Acinetobacter baylyi 120 WFLKSLPSRIGLLLDMTLRDIERVLYFES-------YVVTDPG---------- 155
Acinetobacter ihumii 120 WFLKSLPSRIGLLLDMTLRDIERVLYFES-------YVVTDPG---------- 155
Acinetobacter johnsonii 120 WFLKSLPSRIGLLLDMTLRDIERVLYFES-------YVVTDPG---------- 155
Acinetobacter ursingii 120 WFLKSLPSRIGLLLDMTLRDIERVLYFES-------YVVTDPG---------- 155
Acinetobacter portensis 120 WFLKSLPSRIGLLLDMTLRDIERVLYFES-------YVVTDPG---------- 155
Acinetobacter guerrae 120 WFLKSLPSRIGLLLDMTLRDIERVLYFES-------YVVTDPG---------- 155
Acinetobacter rudis 120 WFLKSLPSRIGLLLDMTLRDIERVLYFES-------YVVTDPG---------- 155
Acinetobacter baumannii 120 WFLKSLPSRIGLLLDMTLRDIERVLYFES-------YVVTDPG---------- 155
Acinetobacter guillouiae 120 WFLKSLPSRIGLLLDMTLRDIERVLYFES-------YVVTDPG---------- 155
Acinetobacter sichuanensis 120 WFLKSLPSRIGLLLDMTLRDIERVLYFES-------YVVTDPG---------- 155
Acinetobacter seifertii 120 WFLKSLPSRIGLLLDMTLRDIERVLYFES-------YVVTDPG---------- 155
Acinetobacter pittii 120 WFLKSLPSRIGLLLDMTLRDIERVLYFES-------YVVTDPG---------- 155
Acinetobacter defluvii 120 WFLKSLPSRIGLLLDMTLRDIERVLYFES-------YVVTDPG---------- 155
Acinetobacter terrestris 120 WFLKSLPSRIGLLLDMTLRDIERVLYFES-------YVVTDPG---------- 155
Acinetobacter pragensis 120 WFLKSLPSRIGLLLDMTLRDIERVLYFES-------YVVTDPG---------- 155
Acinetobacter indicus 120 WFLKSLPSRIGLLLDMTLRDIERVLYFES-------YVVTDPG---------- 155
Pseudomonas aeruginosa 115 WFLKSLPSRIGLLLDMTLRDIERVLYFES-------YVVIDPG---------- 150
Escherichia coli 115 WFLKSLPSRIGLLLDMPLRDIERVLYFES-------YVVIEGG---------- 150
Staphylococcus aureus 96 WYEKGIPSRMGLLLDMSPRALEEVIYFAS-------YVVVDPG---------- 131
Bacillus subtilis 105 WYEKGIPSRMGLVLDMSPRALEEVIYFAS-------YVVTDPA---------- 140
Mycobacterium tuberculosis 105 WYEKGVPSRLGYLLDLAPKDLEKIIYFAA-------YVITSVDEEMRHNELSTLEAEMAV 157
Bacteroides fragilis 111 WYFRSLPNKIGYLLGLPTKKLDSIIYYER-------YVVIQPGVK---------- 148
Thermotoga maritima 108 WYLESIPSVLGTLLNMSTSDLENIIYYGSRRVIERAFIVTDPK---------- 150
Thermus aquaticus 103 WFVKDVPSKIGTLLDLSATELEQVLYFNK-------YIVLDPKG---------- 139
Fusobacterium nucleatum 104 WYSKGSPNKMSLIIGISSKELESVLYFAR-------YIVTSS---------- 138
Aquifex aeolicus 109 WFLKSTPSKIGTLLNLTSRDVERVAYFES-------YLVIEYPNEEEE---------- 149
Blastopirellula marina 112 WFEKAMPSRLGNLLAMKTSSLEKVIYFQD-------YVVVDPG---------- 147
Chlamydia abortus 117 WFEKTTPSRIGNVLGMTASDLERIIYYEE-------YVVIDPG---------- 152
Cyanobacteria bacterium 110 WFEKSMPSRLGTLLAMKTSDLEKIIYYQD-------YVVIDPG---------- 145
190 200 210 220 230 240
Acinetobacter baylyi 155 ---------- 155
Acinetobacter ihumii 155 ---------- 155
Acinetobacter johnsonii 155 ---------- 155
Acinetobacter ursingii 155 ---------- 155
Acinetobacter portensis 155 ---------- 155
Acinetobacter guerrae 155 ---------- 155
Acinetobacter rudis 155 ---------- 155
Acinetobacter baumannii 155 ---------- 155
Acinetobacter guillouiae 155 ---------- 155
Acinetobacter sichuanensis 155 ---------- 155
Acinetobacter seifertii 155 ---------- 155
Acinetobacter pittii 155 ---------- 155
Acinetobacter defluvii 155 ---------- 155
Acinetobacter terrestris 155 ---------- 155
Acinetobacter pragensis 155 ---------- 155
Acinetobacter indicus 155 ---------- 155
Pseudomonas aeruginosa 150 ---------- 150
Escherichia coli 150 ---------- 150
Staphylococcus aureus 131 ---------- 131
Bacillus subtilis 140 ---------- 140
Mycobacterium tuberculosis 158 ERKAVEDQRDGELEARAQKLEADLAELEAEGAKADARRKVRDGGEREMRQIRDRAQRELD 217
Bacteroides fragilis 148 ---------- 148
Thermotoga maritima 150 ---------- 150
Thermus aquaticus 139 ---------- 139
Fusobacterium nucleatum 138 ---------- 138
Aquifex aeolicus 149 ----------EKFEKDEH 157
Blastopirellula marina 147 ---------- 147
Chlamydia abortus 152 ---------- 152
Cyanobacteria bacterium 145 ---------- 145

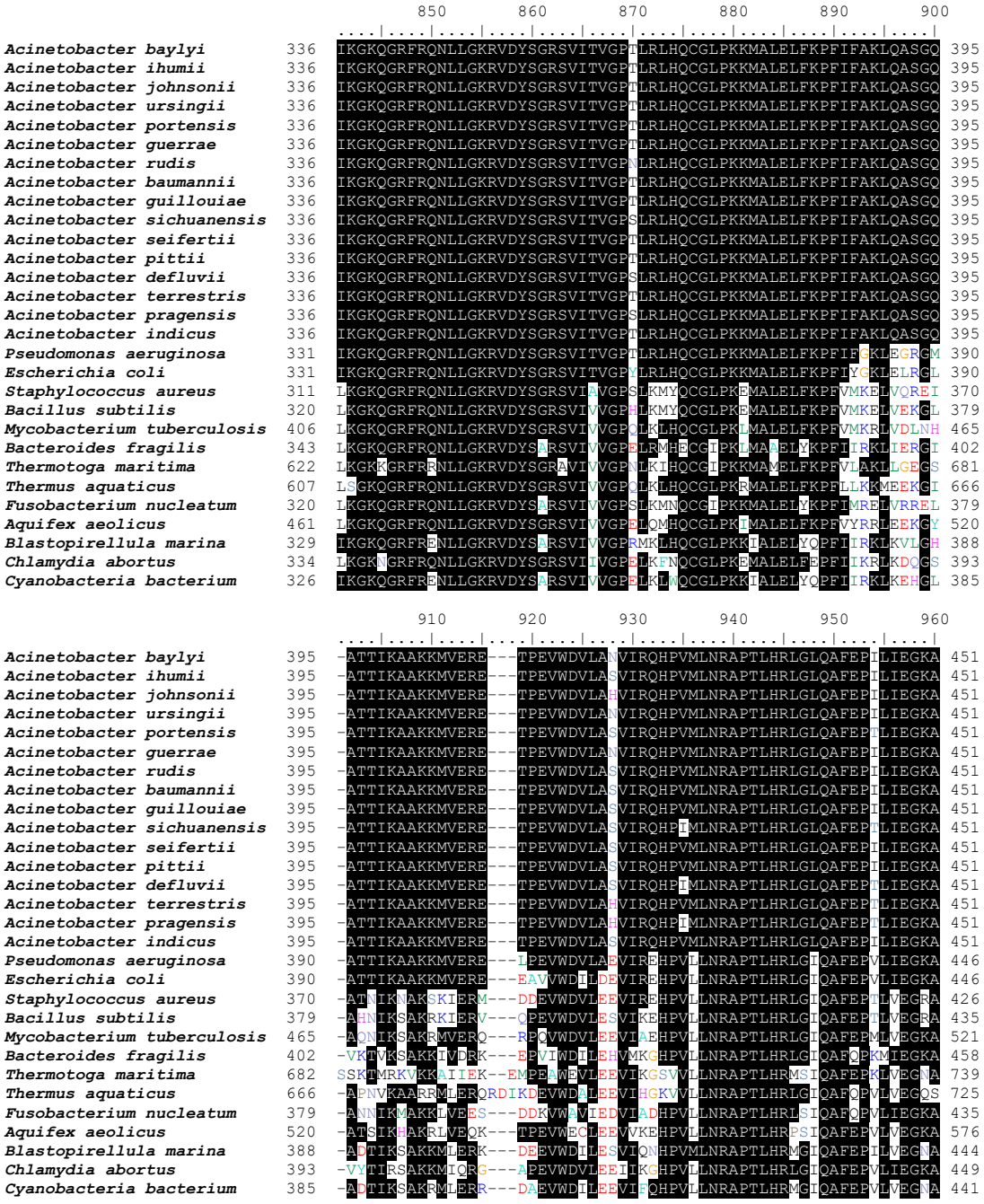

250 260 270 280 290 300
Acinetobacter baylyi 155 --------MTPLEKYQLLTDEEY--------------------FNALEEHGDEFSAK-- 184
Acinetobacter ihumii 155 --------MTPFEKYQLLSDEEY--------------------YNALEEHGDEFTAK-- 184
Acinetobacter johnsonii 155 --------MTPFEKYQLLNDEEY--------------------FNALEEHGDEFTAK-- 184
Acinetobacter ursingii 155 --------MTPFEKYQLLSDEEY--------------------YNALEEHGDEFTAK-- 184
Acinetobacter portensis 155 --------MTPFEKYQLLNDEEY--------------------FNALEEHGDEFTAK-- 184
Acinetobacter guerrae 155 --------MTPFEKYQLLSDEEY--------------------YNALEEHGDEFTAK-- 184
Acinetobacter rudis 155 --------MTPLEKNQLLNDEEY--------------------FTALEEHGDEFSAK-- 184
Acinetobacter baumannii 155 --------MTPFEKYQLLNDEEY--------------------FTALEEHGDEFVAK-- 184
Acinetobacter guillouiae 155 --------MTPMEKYQLLNDEEY--------------------FTALEEHGDEFSAK-- 184
Acinetobacter sichuanensis 155 --------MTPMEKYQLLNDEEY--------------------FTALEEHGDEFSAK-- 184
Acinetobacter seifertii 155 --------MTPFEKYQLLNDEEY--------------------FTALEEHGDEFVAK-- 184
Acinetobacter pittii 155 --------MTPFEKYQLLTDEEY--------------------FNALEEHGDEFTAK-- 184
Acinetobacter defluvii 155 --------MTPLEKYQLLNDEEY--------------------FTALEEHGDEFSAK-- 184
Acinetobacter terrestris 155 --------MTPMEKYQLLNDEEY--------------------FTALEEHGDEFSAK-- 184
Acinetobacter pragensis 155 --------MTPFEKYQLLNDEEY--------------------FNALEEHGDEFTAK-- 184
Acinetobacter indicus 155 --------MTPFEKYQLLNDEEY--------------------FNALEEHGDEFTAK-- 184
Pseudomonas aeruginosa 150 --------MTTLEKGQLLNDEQY--------------------FEALEEFGDLFDAR-- 179
Escherichia coli 150 --------MTNLERQQILTEEQY--------------------LDALEEFGDEFDAK-- 179
Staphylococcus aureus 131 --------PTGLEKKTLLSEAEF--------------------RDYYDKYPGQFVAK-- 160
Bacillus subtilis 140 --------NTPLEKKQLLSEKEY--------------------RAYLDKYGNKFQAS-- 169
Mycobacterium tuberculosis 218 RLEDIWSTPTKLAPKQLIVDENL--------------------YREIVDRYGEYFTGA- 255
Bacteroides fragilis 148 -------AEDGIAEFDLLSEEEYLDI-----LD------TLPKDNQYLEDTDPNKFIAK- 189
Thermotoga maritima 150 --------DTPFSQGDVIYETEYRIYRKKWDFDVEQAFVVKNPKSPVLSDIDGEVTLKTE 202
Thermus aquaticus 139 ----AVLDGVPVEKRQLLTDEEYREL-----RYGKQETYPLPAGVDALVKDGEEVVKGQE 190
Fusobacterium nucleatum 138 -------QESTVEVGKILTEKEY--------------------KLLKQLYGNKFEAY- 168
Aquifex aeolicus 158 TIPLNDGISTKWVKLHVVNEEEF--------------------EEKYAFTIDEKYEHG- 195
Blastopirellula marina 147 --------TTELAKFQTLTEEEF--------------------RQAREQYGEGSFEAD- 177
Chlamydia abortus 152 --------KTDLNKKQLLNDAQY--------------------REVIEKWGKDSFVAK- 182
Cyanobacteria bacterium 145 --------DTELKYKQVLTEDEY--------------------RTSVDKYGN-AFKAY- 174
310 320 330 340 350 360
Acinetobacter baylyi 184 ---------- 184
Acinetobacter ihumii 184 ---------- 184
Acinetobacter johnsonii 184 ---------- 184
Acinetobacter ursingii 184 ---------- 184
Acinetobacter portensis 184 ---------- 184
Acinetobacter guerrae 184 ---------- 184
Acinetobacter rudis 184 ---------- 184
Acinetobacter baumannii 184 ---------- 184
Acinetobacter guillouiae 184 ---------- 184
Acinetobacter sichuanensis 184 ---------- 184
Acinetobacter seifertii 184 ---------- 184
Acinetobacter pittii 184 ---------- 184
Acinetobacter defluvii 184 ---------- 184
Acinetobacter terrestris 184 ---------- 184
Acinetobacter pragensis 184 ---------- 184
Acinetobacter indicus 184 ---------- 184
Pseudomonas aeruginosa 179 ---------- 179
Escherichia coli 179 ---------- 179
Staphylococcus aureus 160 ---------- 160
Bacillus subtilis 169 ---------- 169
Mycobacterium tuberculosis 255 ---------- 255
Bacteroides fragilis 189 ---------- 189
Thermotoga maritima 203 KSITGREITWIIVKNITRATHTVLPGMILVVKDGQEVEKGQDLTKEMTIDPVYAPFDGHV 262
Thermus aquaticus 191 LAPGVVSRMDGVALYRFPRRVRVDYLRKERAALRIPLSAWVEKEAYRPGEVLAELSEPYL 250
Fusobacterium nucleatum 168 ---------- 168
Aquifex aeolicus 195 ---------- 195
Blastopirellula marina 177 ---------- 177
Chlamydia abortus 182 ---------- 182
Cyanobacteria bacterium 174 ---------- 174

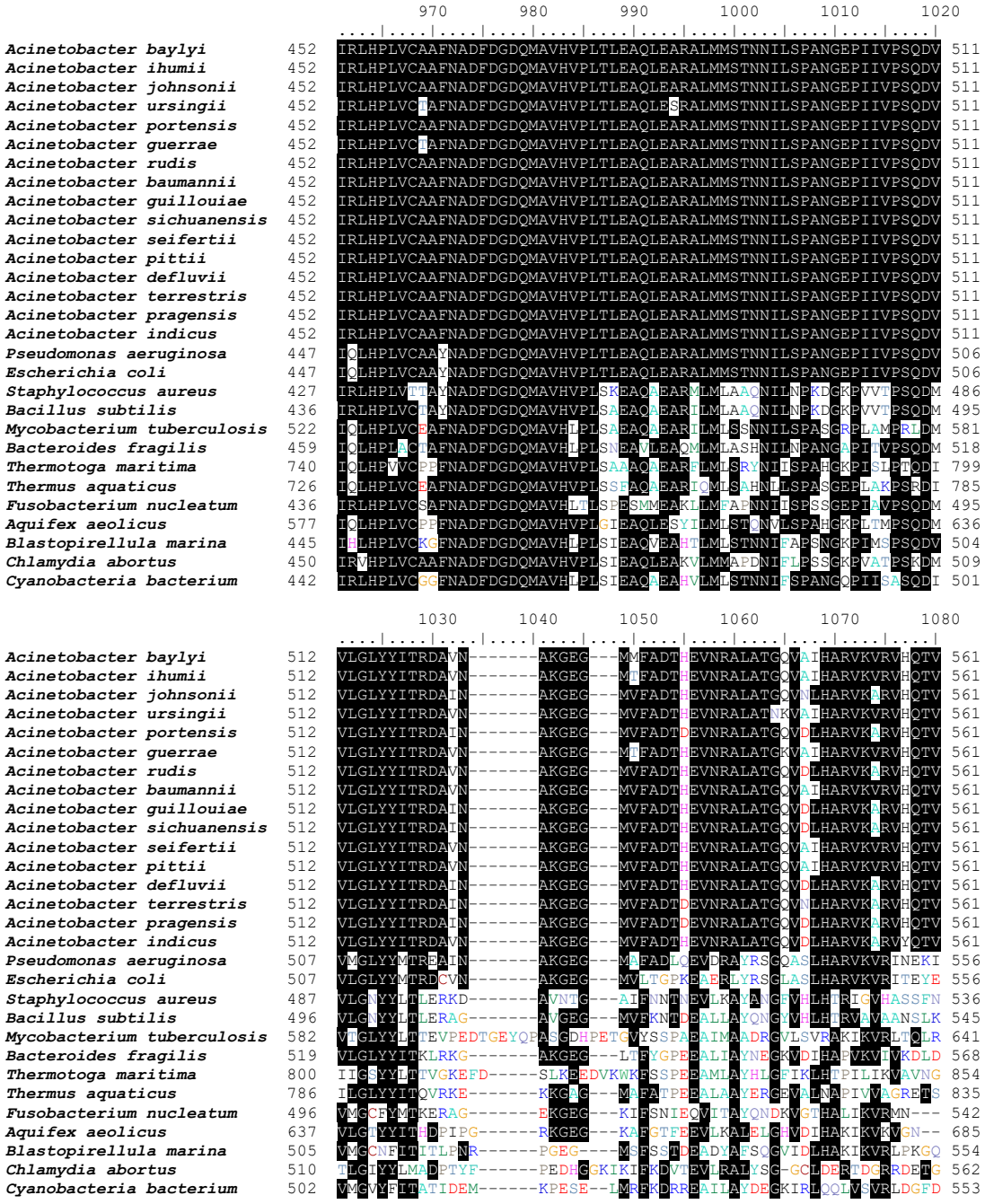
```
                                        370       380       390       400       410       420
                                   ....|....|....|....|....|....|....|....|....|....|....|....|
Acinetobacter baylyi          184  ------------------------------------------------------------ 184
Acinetobacter ihumii          184  ------------------------------------------------------------ 184
Acinetobacter johnsonii       184  ------------------------------------------------------------ 184
Acinetobacter ursingii        184  ------------------------------------------------------------ 184
Acinetobacter portensis       184  ------------------------------------------------------------ 184
Acinetobacter guerrae         184  ------------------------------------------------------------ 184
Acinetobacter rudis           184  ------------------------------------------------------------ 184
Acinetobacter baumannii       184  ------------------------------------------------------------ 184
Acinetobacter guillouiae      184  ------------------------------------------------------------ 184
Acinetobacter sichuanensis    184  ------------------------------------------------------------ 184
Acinetobacter seifertii       184  ------------------------------------------------------------ 184
Acinetobacter pittii          184  ------------------------------------------------------------ 184
Acinetobacter defluvii        184  ------------------------------------------------------------ 184
Acinetobacter terrestris      184  ------------------------------------------------------------ 184
Acinetobacter pragensis       184  ------------------------------------------------------------ 184
Acinetobacter indicus         184  ------------------------------------------------------------ 184
Pseudomonas aeruginosa        179  ------------------------------------------------------------ 179
Escherichia coli              179  ------------------------------------------------------------ 179
Staphylococcus aureus         160  ------------------------------------------------------------ 160
Bacillus subtilis             169  ------------------------------------------------------------ 169
Mycobacterium tuberculosis    255  ------------------------------------------------------------ 255
Bacteroides fragilis          189  ------------------------------------------------------------ 189
Thermotoga maritima           263  EIDELSNTITLKPLTTSKDQPVVFTIPYGAKILVSNGQKVKKGDQITTSTSLPAVKASIS 322
Thermus aquaticus             251  FRAEESGVVELKDLAEGHLIYLRQEEEVVARYFLPAGMTPLVVEGEIVEVGQPLAEGKGL 310
Fusobacterium nucleatum       168  ------------------------------------------------------------ 168
Aquifex aeolicus              195  ------------------------------------------------------------ 195
Blastopirellula marina        177  ------------------------------------------------------------ 177
Chlamydia abortus             182  ------------------------------------------------------------ 182
Cyanobacteria bacterium       174  ------------------------------------------------------------ 174

                                        430       440       450       460       470       480
                                   ....|....|....|....|....|....|....|....|....|....|....|....|
Acinetobacter baylyi          184  ------------------------------------------------------------ 184
Acinetobacter ihumii          184  ------------------------------------------------------------ 184
Acinetobacter johnsonii       184  ------------------------------------------------------------ 184
Acinetobacter ursingii        184  ------------------------------------------------------------ 184
Acinetobacter portensis       184  ------------------------------------------------------------ 184
Acinetobacter guerrae         184  ------------------------------------------------------------ 184
Acinetobacter rudis           184  ------------------------------------------------------------ 184
Acinetobacter baumannii       184  ------------------------------------------------------------ 184
Acinetobacter guillouiae      184  ------------------------------------------------------------ 184
Acinetobacter sichuanensis    184  ------------------------------------------------------------ 184
Acinetobacter seifertii       184  ------------------------------------------------------------ 184
Acinetobacter pittii          184  ------------------------------------------------------------ 184
Acinetobacter defluvii        184  ------------------------------------------------------------ 184
Acinetobacter terrestris      184  ------------------------------------------------------------ 184
Acinetobacter pragensis       184  ------------------------------------------------------------ 184
Acinetobacter indicus         184  ------------------------------------------------------------ 184
Pseudomonas aeruginosa        179  ------------------------------------------------------------ 179
Escherichia coli              179  ------------------------------------------------------------ 179
Staphylococcus aureus         160  ------------------------------------------------------------ 160
Bacillus subtilis             169  ------------------------------------------------------------ 169
Mycobacterium tuberculosis    255  ------------------------------------------------------------ 255
Bacteroides fragilis          189  ------------------------------------------------------------ 189
Thermotoga maritima           323  GTVRFGSNLNIRALEDGNFEVLSTG-----EVYVEQVIEERKYPVFEGALVYVNNGDQVK 377
Thermus aquaticus             311  LRLPRHMTAKEVEAEEEGDSVHLTLFLEWTEPKDYKVAPHMNVIVPEGAKVQAGEKIVAA 370
Fusobacterium nucleatum       168  ------------------------------------------------------------ 168
Aquifex aeolicus              195  ------------------------------------------------------------ 195
Blastopirellula marina        177  ------------------------------------------------------------ 177
Chlamydia abortus             182  ------------------------------------------------------------ 182
Cyanobacteria bacterium       174  ------------------------------------------------------------ 174
```

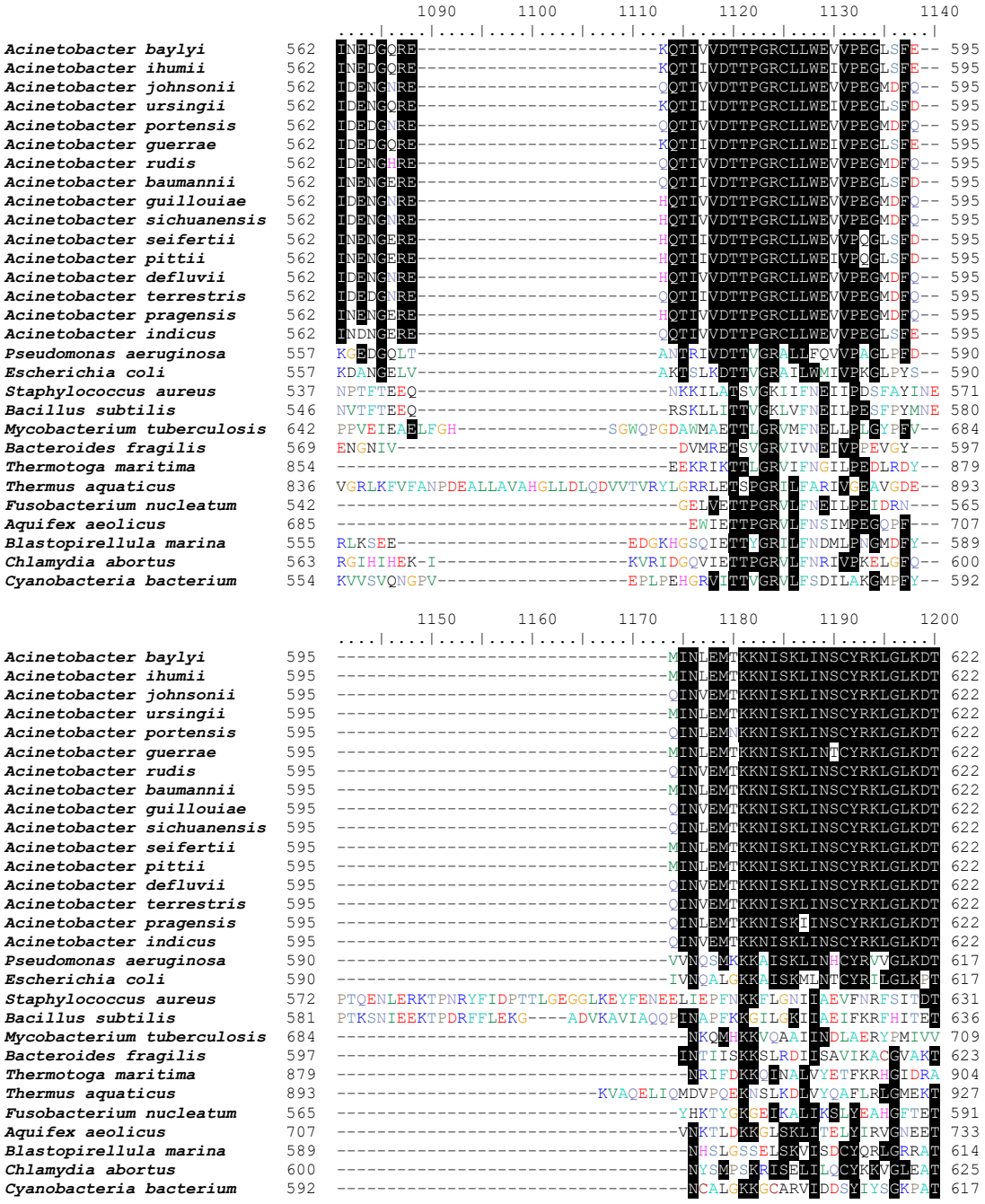

490 500 510 520 530 540
Acinetobacter baylyi 184 184
Acinetobacter ihumii 184 184
Acinetobacter johnsonii 184 184
Acinetobacter ursingii 184 184
Acinetobacter portensis 184 184
Acinetobacter guerrae 184 184
Acinetobacter rudis 184 184
Acinetobacter baumannii 184 184
Acinetobacter guillouiae 184 184
Acinetobacter sichuanensis 184 184
Acinetobacter seifertii 184 184
Acinetobacter pittii 184 184
Acinetobacter defluvii 184 184
Acinetobacter terrestris 184 184
Acinetobacter pragensis 184 184
Acinetobacter indicus 184 184
Pseudomonas aeruginosa 179 179
Escherichia coli 179 179
Staphylococcus aureus 160 160
Bacillus subtilis 169 169
Mycobacterium tuberculosis 255 255
Bacteroides fragilis 189 189
Thermotoga maritima 378 KGDHLADRFLFEEEYLSATEYKIFESHYPTMFDVEERTENDRPIVVITDIDPEVSKETGL 437
Thermus aquaticus 371 IDPEEEVIAEAEGVVHLHEPASILVVKARVYPFEDDVEVTTGDRVAPGDVLADGGKVKSE 430
Fusobacterium nucleatum 168 168
Aquifex aeolicus 195 195
Blastopirellula marina 177 177
Chlamydia abortus 182 182
Cyanobacteria bacterium 174 174
550 560 570 580 590 600
Acinetobacter baylyi 184 MGAEAVQDLLKDIDLEAEISRLREEIP 211
Acinetobacter ihumii 184 MGAEAVQDLLKDIDLEAEIARLREEIP 211
Acinetobacter johnsonii 184 MGAEAVQDLLKDIDLENEISRLREEIP 211
Acinetobacter ursingii 184 MGAEAVQDLLKDIDLEAEIARLREEIP 211
Acinetobacter portensis 184 MGAEAVQDLLKDIDLENEISRLREEIP 211
Acinetobacter guerrae 184 MGAEAVQDLLKDIDLEAEIARLREEIP 211
Acinetobacter rudis 184 MGAEAVQDLLKDIDLDAEISRLREEIP 211
Acinetobacter baumannii 184 MGAEAVQDLLKDIDLEAEISRLREEIP 211
Acinetobacter guillouiae 184 MGAEAVQDLLKDIDLEAEISRLREEIP 211
Acinetobacter sichuanensis 184 MGAEAVQDLLKDIDLEAEISRLREEIP 211
Acinetobacter seifertii 184 MGAEAVQDLLKDIDLEAEISRLREEIP 211
Acinetobacter pittii 184 MGAEAVQDLLKDIDLEAEIARLREEIP 211
Acinetobacter defluvii 184 MGAEAVQDLLKDIDLEAEITRLREEIP 211
Acinetobacter terrestris 184 MGAEAIQDLLKDIDLEAEIARLREEIP 211
Acinetobacter pragensis 184 MGAEAVQDLLKDIDLEAEISRLREEIP 211
Acinetobacter indicus 184 MGAEAVQDLLKDIDLENEISRLREEIP 211
Pseudomonas aeruginosa 179 MGAEAVHELLNAIDLEHEIGRLREEIP 206
Escherichia coli 179 MGAEAIQALLKSMDLEQECEQLREELN 206
Staphylococcus aureus 160 MGAEGIKDLLEEIDLDEELKLLRDELE 187
Bacillus subtilis 169 MGAEAIHKLLQDIDLVKEVDMLKEELK 196
Mycobacterium tuberculosis 255 MGAESIQKLIENFDIDAEAESLRDVIR 282
Bacteroides fragilis 189 MGAEAIYDLLARLDLDALSYELRHRAG 216
Thermotoga maritima 438 KVGDIVTENEYEAYLQIYPEKIVADAGAQAIKKLLQNLDLEALQAEIEAELKK 490
Thermus aquaticus 431 IYGRVEVDLVRNVVRVVESYDIDARMGAEAIQELLKELDLEKLERELLEEMK 482
Fusobacterium nucleatum 168 MGADGILKLLTAIDLEKLRDELENELV 195
Aquifex aeolicus 195 MGAEILKEVLSKLDLDAYSRKLKEIVKPYSIGFED 230
Blastopirellula marina 177 MGAEAVRKLLGSLDLVQLSIDLRKDMQ 204
Chlamydia abortus 182 MGEEAIYDLLKSEDLQSLLKELKDRLR 209
Cyanobacteria bacterium 174 MGADAIREELIGRLDLSAEAEQIRKELR 201

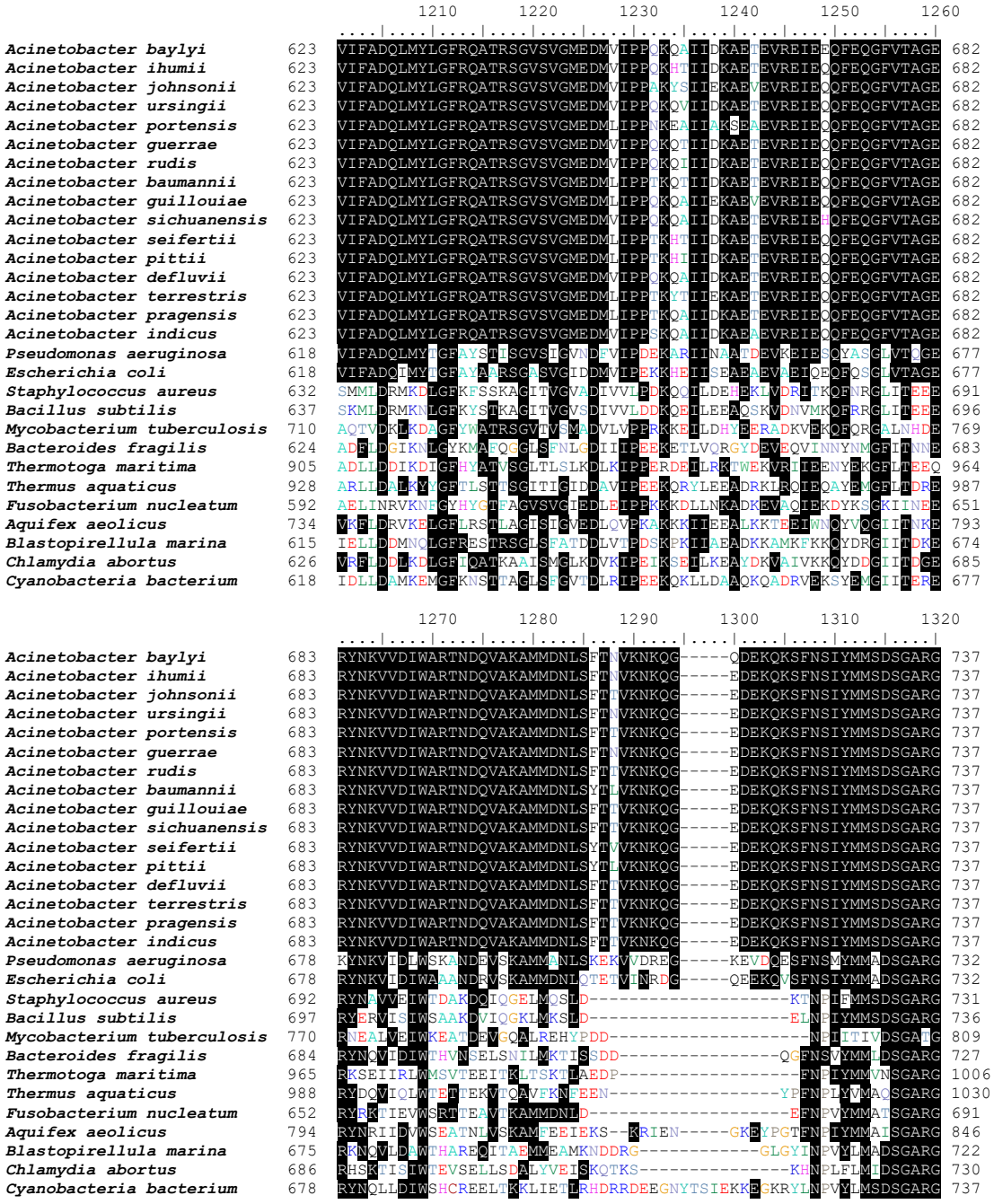

610 620 630 640 650 660
Acinetobacter baylyi 211 ---------- 211
Acinetobacter ihumii 211 ---------- 211
Acinetobacter johnsonii 211 ---------- 211
Acinetobacter ursingii 211 ---------- 211
Acinetobacter portensis 211 ---------- 211
Acinetobacter guerrae 211 ---------- 211
Acinetobacter rudis 211 ---------- 211
Acinetobacter baumannii 211 ---------- 211
Acinetobacter guillouiae 211 ---------- 211
Acinetobacter sichuanensis 211 ---------- 211
Acinetobacter seifertii 211 ---------- 211
Acinetobacter pittii 211 ---------- 211
Acinetobacter defluvii 211 ---------- 211
Acinetobacter terrestris 211 ---------- 211
Acinetobacter pragensis 211 ---------- 211
Acinetobacter indicus 211 ---------- 211
Pseudomonas aeruginosa 206 ---------- 206
Escherichia coli 206 ---------- 206
Staphylococcus aureus 187 ---------- 187
Bacillus subtilis 196 ---------- 196
Mycobacterium tuberculosis 282 ---------- 282
Bacteroides fragilis 216 ---------- 216
Thermotoga maritima 490 ---------- 490
Thermus aquaticus 482 ---------- 482
Fusobacterium nucleatum 195 ---------- 195
Aquifex aeolicus 231 LGKEIEQKYKNLYQKLIKVIADDFRAYGVEIKGLEDHGLSLEQAIHRILNEELYLNVETG 290
Blastopirellula marina 204 ---------- 204
Chlamydia abortus 209 ---------- 209
Cyanobacteria bacterium 201 ---------- 201
670 680 690 700 710 720
Acinetobacter baylyi 211 ----------QTTSETKLKKASK 224
Acinetobacter ihumii 211 ----------QTTSETKLKKASK 224
Acinetobacter johnsonii 211 ----------NTTSETKLKKASK 224
Acinetobacter ursingii 211 ----------QTTSETKLKKASK 224
Acinetobacter portensis 211 ----------NTTSETKLKKASK 224
Acinetobacter guerrae 211 ----------QTTSETKLKKASK 224
Acinetobacter rudis 211 ----------QTTSETKLKKASK 224
Acinetobacter baumannii 211 ----------QTTSETKLKKASK 224
Acinetobacter guillouiae 211 ----------NTTSETKLKKASK 224
Acinetobacter sichuanensis 211 ----------NTTSETKLKKASK 224
Acinetobacter seifertii 211 ----------QTTSETKLKKASK 224
Acinetobacter pittii 211 ----------QTTSETKLKKASK 224
Acinetobacter defluvii 211 ----------NTTSETKLKKASK 224
Acinetobacter terrestris 211 ----------QTTSETKLKKASK 224
Acinetobacter pragensis 211 ----------NTTSETKLKKASK 224
Acinetobacter indicus 211 ----------QTTSETKLKKASK 224
Pseudomonas aeruginosa 206 ----------QTNSETKIKKLSK 219
Escherichia coli 206 ----------ETNSETKRKKLTK 219
Staphylococcus aureus 187 ----------SATGQRLTRAIK 199
Bacillus subtilis 196 ----------TSQGQRRTRAIK 208
Mycobacterium tuberculosis 282 ----------NGKGQKKLRAIK 294
Bacteroides fragilis 216 ----------NDASQQRKNEAIK 229
Thermotoga maritima 490 ----------LPSSSSKAIKLRR 503
Thermus aquaticus 482 ----------HPSRARRAKARK 494
Fusobacterium nucleatum 195 ----------DANSAQKRKKLVK 208
Aquifex aeolicus 291 EISLEDCGDSCLTGRDALKEYYERVREHKKDIPIFEKIKEDIRSTVLREISEARIRKALR 350
Blastopirellula marina 204 ----------ETGSKQKKKDLTN 217
Chlamydia abortus 209 ----------KTKSQQARMKLAK 222
Cyanobacteria bacterium 201 ----------ETRSKQKIKDLAK 214

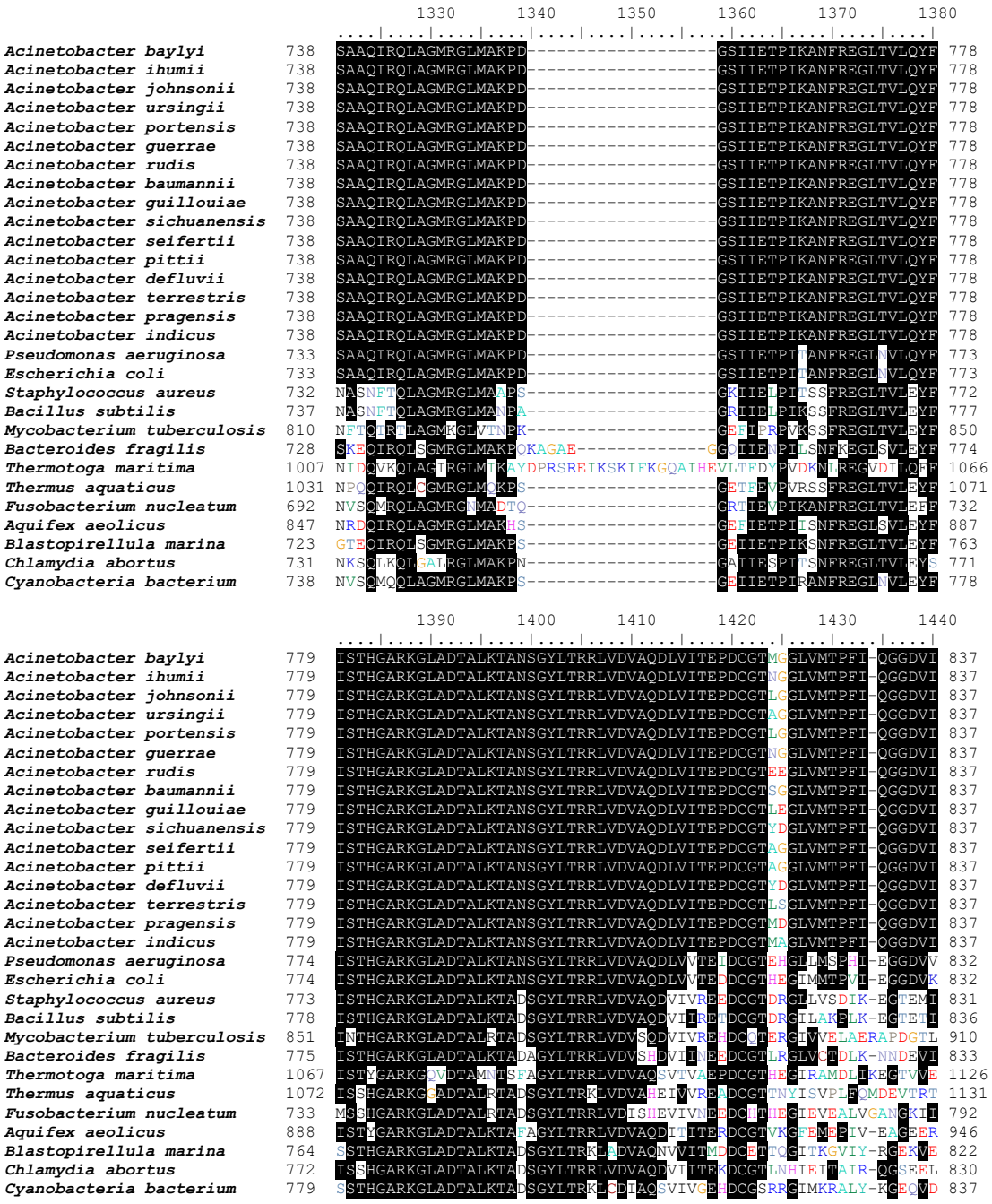

730 740 750 760 770 780
Acinetobacter baylyi 225 RLKLMEAFKESN--NKPEWMVMNVLPVLPPDLRPLVPLEGGRFATSDLNDLYRRVINRNN 282
Acinetobacter ihumii 225 RLKLMEAFKESN--NKPEWMVMNVLPVLPPDLRPLVPLEGGRFATSDLNDLYRRVINRNN 282
Acinetobacter johnsonii 225 RLKLMEAFKESN--NKPEWMVMNVLPVLPPDLRPLVPLEGGRFATSDLNDLYRRVINRNN 282
Acinetobacter ursingii 225 RLKLMEAFKESN--NKPEWMVMNVLPVLPPDLRPLVPLEGGRFATSDLNDLYRRVINRNN 282
Acinetobacter portensis 225 RLKLMEAFKESN--NKPEWMVLTVLPVLPPDLRPLVPLEGGRFATSDLNDLYRRVINRNN 282
Acinetobacter guerrae 225 RLKLMEAFKESN--NKPEWMVMNVLPVLPPDLRPLVPLEGGRFATSDLNDLYRRVINRNN 282
Acinetobacter rudis 225 RLKLMEAFNDSN--NKPEWMVMNVLPVLPPDLRPLVPLEGGRFATSDLNDLYRRVINRNN 282
Acinetobacter baumannii 225 RLKLMEAFKDSN--NKPEWMVMNVLPVLPPDLRPLVPLEGGRFATSDLNDLYRRVINRNN 282
Acinetobacter guillouiae 225 RLKLMEAFKESN--NKPEWMVMNVLPVLPPDLRPLVPLEGGRFATSDLNDLYRRVINRNN 282
Acinetobacter sichuanensis 225 RLKLMEAFNDSN--NKPEWMVLTVLPVLPPDLRPLVPLEGGRFATSDLNDLYRRVINRNN 282
Acinetobacter seifertii 225 RLKLMEAFKDSN--NKPEWMVMNVLPVLPPDLRPLVPLEGGRFATSDLNDLYRRVINRNN 282
Acinetobacter pittii 225 RLKLMEAFKDSN--NKPEWMVMNVLPVLPPDLRPLVPLEGGRFATSDLNDLYRRVINRNN 282
Acinetobacter defluvii 225 RLKLMEAFNDSN--NKPEWMVLTVLPVLPPDLRPLVPLEGGRFATSDLNDLYRRVINRNN 282
Acinetobacter terrestris 225 RLKLMEAFNDSN--NKPEWMVLTVLPVLPPDLRPLVPLEGGRFATSDLNDLYRRVINRNN 282
Acinetobacter pragensis 225 RLKLMEAFKESN--NKPEWMVLTVLPVLPPDLRPLVPLEGGRFATSDLNDLYRRVINRNN 282
Acinetobacter indicus 225 RLKLMEAFKESN--NKPEWMVMNVLPVLPPDLRPLVPLEGGRFATSDLNDLYRRVINRNN 282
Pseudomonas aeruginosa 220 RLKLMEAFQGSG--NKPEWMVLTVLPVLPPDLRPLVPLDGGRFATSDLNDLYRRVINRNN 277
Escherichia coli 220 RIKLLEAFVQSG--NKPEWMILTVLPVLPPDLRPLVPLDGGRFATSDLNDLYRRVINRNN 277
Staphylococcus aureus 200 RLEVVESFRNSG--NKPSWMILDVLPIIPPEIRPMVQLDGGRFATSDLNDLYRRVINRNN 257
Bacillus subtilis 209 RLEVLEAFRNSG--NKPSWMILDVLPVIPPELRPMVQLDGGRFATSDLNDLYRRVINRNN 266
Mycobacterium tuberculosis 295 RLKVVAAFQQSG--NSPMGMVLDAVPVIPPELRPMVQLDGGRFATSDLNDLYRRVINRNN 352
Bacteroides fragilis 230 RLQVVESFRASRGRNKPEWMIVRIVPVIPPELRPLVPLDGGRFATSDLNDLYRRVIRNN 289
Thermotoga maritima 504 RLKMVKDFLKSG--NKPEWMVLEVVPVIPPDLRPMIQIEGGRFATTDLNELYRRLINRNN 561
Thermus aquaticus 495 RLEVVRAFLDSG--NRPEWMILEAVPVLPPDLRPMVQVDGGRFATSDLNDLYRRLINRNN 552
Fusobacterium nucleatum 209 RLKIVRDFIASG--NRPEWMILTNVPVIPAELRPMVQLDGGRFATSDLNDLYRRVINRNN 266
Aquifex aeolicus 351 TLQIVEGFKKSG--NRPEWMILEVLPVLPPELRPLVALDGGRFATSDLNDFYRRVINRNN 408
Blastopirellula marina 218 RLKIVESIRDSD--NKPEWMVLDVIPVIPPDLRPLVLLDSGNFATSDLNDLYRRIINRNN 275
Chlamydia abortus 223 RLKIIEGFVSSS--NHAEWMVLKSVPVVPPDLRPLVPLDGGRFATSDLNDLYRRVINRNN 280
Cyanobacteria bacterium 215 RLKLIESVRGSE--NDAAWLVMDVIPVIPPDLRPLVLLESGNFATSDLNDLYRRIINRNN 272
790 800 810 820 830 840
Acinetobacter baylyi 283 RLKRLLDLAAPDIIVRNEKRMLQESVDALLDNG------RRGRAITG-SNKRPLKSLADM 335
Acinetobacter ihumii 283 RLKRLLDLAAPDIIVRNEKRMLQESVDALLDNG------RRGRAITG-SNKRPLKSLADM 335
Acinetobacter johnsonii 283 RLKRLLDLAAPDIIVRNEKRMLQESVDALLDNG------RRGRAITG-SNKRPLKSLADM 335
Acinetobacter ursingii 283 RLKRLLDLAAPDIIVRNEKRMLQESVDALLDNG------RRGRAITG-SNKRPLKSLADM 335
Acinetobacter portensis 283 RLKRLLDLAAPDIIVRNEKRMLQESVDALLDNG------RRGRAITG-SNKRPLKSLADM 335
Acinetobacter guerrae 283 RLKRLLDLAAPDIIVRNEKRMLQESVDALLDNG------RRGRAITG-SNKRPLKSLADM 335
Acinetobacter rudis 283 RLKRLLDLSAPDIIVRNEKRMLQESVDALLDNG------RRGRAITG-SNKRPLKSLADM 335
Acinetobacter baumannii 283 RLKRLLDLAAPDIIVRNEKRMLQESVDALLDNG------RRGRAITG-SNKRPLKSLADM 335
Acinetobacter guillouiae 283 RLKRLLDLAAPDIIVRNEKRMLQESVDALLDNG------RRGRAITG-SNKRPLKSLADM 335
Acinetobacter sichuanensis 283 RLKRLLDLAAPDIIVRNEKRMLQESVDALLDNG------RRGRAITG-SNKRPLKSLADM 335
Acinetobacter seifertii 283 RLKRLLDLAAPDIIVRNEKRMLQESVDALLDNG------RRGRAITG-SNKRPLKSLADM 335
Acinetobacter pittii 283 RLKRLLDLAAPDIIVRNEKRMLQESVDALLDNG------RRGRAITG-SNKRPLKSLADM 335
Acinetobacter defluvii 283 RLKRLLDLAAPDIIVRNEKRMLQESVDALLDNG------RRGRAITG-SNKRPLKSLADM 335
Acinetobacter terrestris 283 RLKRLLDLSAPDIIVRNEKRMLQESVDALLDNG------RRGRAITG-SNKRPLKSLADM 335
Acinetobacter pragensis 283 RLKRLLDLSAPDIIVRNEKRMLQESVDALLDNG------RRGRAITG-SNKRPLKSLADM 335
Acinetobacter indicus 283 RLKRLLDLAAPDIIVRNEKRMLQESVDALLDNG------RRGRAITG-SNKRPLKSLADM 335
Pseudomonas aeruginosa 278 RLKRLLDLAAPDIIVRNEKRMLQEAVDALLDNG------RRGRAITG-SNKRPLKSLADM 330
Escherichia coli 278 RLKRLLDLAAPDIIVRNEKRMLQEAVDALLDNG------RRGRAITG-SNKRPLKSLADM 330
Staphylococcus aureus 258 RLKRLLDLGAPGIIVQNEKRMLQEAVDALIDNG------RRGRPVTG-PGNRPLKSLSHM 310
Bacillus subtilis 267 RLKRLLDLGAPSIIVQNEKRMLQEAVDALIDNG------RRGRPVTG-PGNRPLKSLSHM 319
Mycobacterium tuberculosis 353 RLKRLIDLGAPEIIVNNEKRMLQESVDALEDNG------RRGRPVTG-PGNRPLKSLSDL 405
Bacteroides fragilis 290 RLKRLIEIKAPEVILRNEKRMLQESVDSLEDNS------RKSSAVKT-DANRPLKSLSDS 342
Thermotoga maritima 562 RLKKLLELGAPEIILRNEKRMLQEAVDALIHNGSDSEGKRSRRAVLKDRNGRPLKSLTDL 621
Thermus aquaticus 553 RLKKLLAQGAPEIILRNEKRMLQEAVDAVIDNG------RRGSPVTNPGSERPLRSLTDI 606
Fusobacterium nucleatum 267 RLKKLLEIKAPEIVVKNEKRMLQEAVDALIDNG------RRGKPVVA-QNNRPLKSLSDM 319
Aquifex aeolicus 409 RLKRLIELVAPDIIIRNEKRMLQEAVDALIDNG------KRGNPVK--QNGRPLKSLADY 460
Blastopirellula marina 276 RLRKLVDLVAPEVIIRNEKRMLQQSVDALEDNN------RCKRPVLG-SSNRPLKSLTDM 328
Chlamydia abortus 281 RLKKILRLKTPEVIVRNEKRMLQEAVDALEDNG------RHGHPVMG-AGNRPLKSLSEM 333
Cyanobacteria bacterium 273 RLKKLMDLVAPEVIIRNEKRMLQQAVDALEDNN------RCRRPVLG-SSNRPLKSLTDM 325

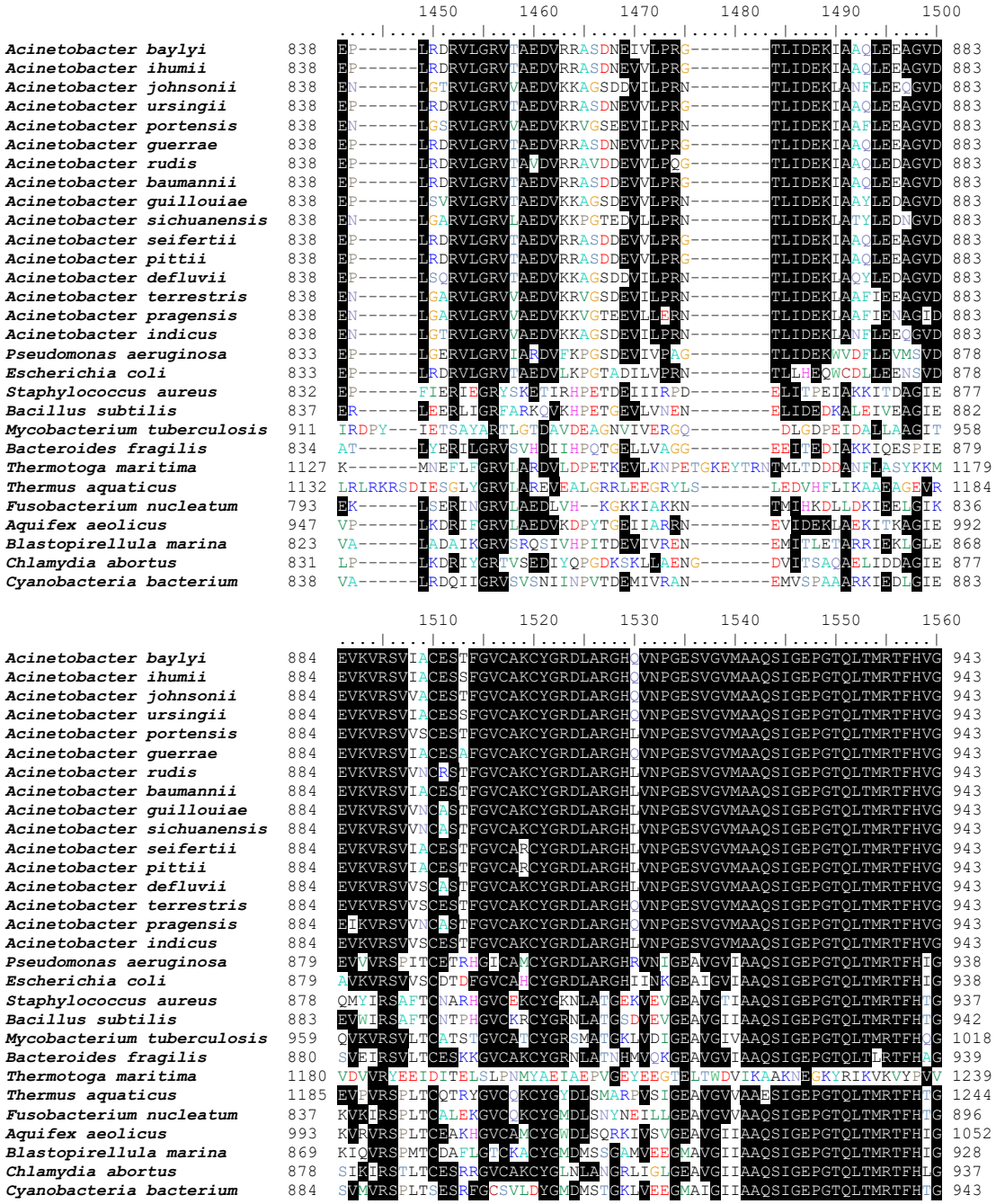

850 860 870 880 890 900
Acinetobacter baylyi 336 IKGKQGRFRQNLLGKRVDYSGRSVITVGPTLRLHQCGLPKKMALELFKPFIFAKLQASGQ 395
Acinetobacter ihumii 336 IKGKQGRFRQNLLGKRVDYSGRSVITVGPTLRLHQCGLPKKMALELFKPFIFAKLQASGQ 395
Acinetobacter johnsonii 336 IKGKQGRFRQNLLGKRVDYSGRSVITVGPTLRLHQCGLPKKMALELFKPFIFAKLQASGQ 395
Acinetobacter ursingii 336 IKGKQGRFRQNLLGKRVDYSGRSVITVGPTLRLHQCGLPKKMALELFKPFIFAKLQASGQ 395
Acinetobacter portensis 336 IKGKQGRFRQNLLGKRVDYSGRSVITVGPTLRLHQCGLPKKMALELFKPFIFAKLQASGQ 395
Acinetobacter guerrae 336 IKGKQGRFRQNLLGKRVDYSGRSVITVGPTLRLHQCGLPKKMALELFKPFIFAKLQASGQ 395
Acinetobacter rudis 336 IKGKQGRFRQNLLGKRVDYSGRSVITVGPNLRLHQCGLPKKMALELFKPFIFAKLQASGQ 395
Acinetobacter baumannii 336 IKGKQGRFRQNLLGKRVDYSGRSVITVGPTLRLHQCGLPKKMALELFKPFIFAKLQASGQ 395
Acinetobacter guillouiae 336 IKGKQGRFRQNLLGKRVDYSGRSVITVGPTLRLHQCGLPKKMALELFKPFIFAKLQASGQ 395
Acinetobacter sichuanensis 336 IKGKQGRFRQNLLGKRVDYSGRSVITVGPSLRLHQCGLPKKMALELFKPFIFAKLQASGQ 395
Acinetobacter seifertii 336 IKGKQGRFRQNLLGKRVDYSGRSVITVGPTLRLHQCGLPKKMALELFKPFIFAKLQASGQ 395
Acinetobacter pittii 336 IKGKQGRFRQNLLGKRVDYSGRSVITVGPTLRLHQCGLPKKMALELFKPFIFAKLQASGQ 395
Acinetobacter defluvii 336 IKGKQGRFRQNLLGKRVDYSGRSVITVGPSLRLHQCGLPKKMALELFKPFIFAKLQASGQ 395
Acinetobacter terrestris 336 IKGKQGRFRQNLLGKRVDYSGRSVITVGPTLRLHQCGLPKKMALELFKPFIFAKLQASGQ 395
Acinetobacter pragensis 336 IKGKQGRFRQNLLGKRVDYSGRSVITVGPSLRLHQCGLPKKMALELFKPFIFAKLQASGQ 395
Acinetobacter indicus 336 IKGKQGRFRQNLLGKRVDYSGRSVITVGPTLRLHQCGLPKKMALELFKPFIFAKLQASGQ 395
Pseudomonas aeruginosa 331 IKGKQGRFRQNLLGKRVDYSGRSVITVGPTLRLHQCGLPKKMALELFKPFIFCKLEGRGM 390
Escherichia coli 331 IKGKQGRFRQNLLGKRVDYSGRSVITVGPYLRLHQCGLPKKMALELFKPFIYGKLELRGL 390
Staphylococcus aureus 311 LKGKQGRFRQNLLGKRVDYSGRSVIAVGPSLKMYQCGLPKEMALELFKPFVMKELVQREI 370
Bacillus subtilis 320 LKGKQGRFRQNLLGKRVDYSGRSVIVVGPHLKMYQCGLPKEMALELFKPFVMKELVEKGL 379
Mycobacterium tuberculosis 406 LKGKQGRFRQNLLGKRVDYSGRSVIVVGPQLKLHQCGLPKLMALELFKPFVMKRLVDLNH 465
Bacteroides fragilis 343 LKGKQGRFRQNLLGKRVDYSARSVIVVGPELRMHECGIPKLMAELYKPFIRKLIERGI 402
Thermotoga maritima 622 LKGKKGRFRNLLGKRVDYSGRAVIVVGPNLKIHQCGIPKKMAELFKPFVLAKLLGEGS 681
Thermus aquaticus 607 LSGKQGRFRQNLLGKRVDYSGRSVIVVGPQLKLHQCGLPKRMALELFKPFLLKKMEEKGI 666
Fusobacterium nucleatum 320 LKGKQGRFRQNLLGKRVDYSARSVIVVGPSLKMNQCGIPKKMALELYKPFIMRELVRREL 379
Aquifex aeolicus 461 LKGKQGRFRQNLLGKRVDYSGRSVIVVGPELQMHQCGLPKIMALELFKPFVYRRLEEKGY 520
Blastopirellula marina 329 IKGKQGRFRENLLGKRVDYSARSVIVVGPRMKLHQCGLPKKIALELYQPFIIRKLKVLGH 388
Chlamydia abortus 334 LKGKKGRFRQNLLGKRVDYSGRSVIIVGPELKFNQCGLPKEMALELFEPFIIKRLKDQGS 393
Cyanobacteria bacterium 326 IKGKQGRFRENLLGKRVDYSARSVIVVGPELKLMQCGLPKKIALELYQPFIIRKLKEHGL 385
910 920 930 940 950 960
Acinetobacter baylyi 395 -ATTIKAAKKMVERE---TPEVWDVLANVIRQHPVMLNRAPTLHRLGLQAFEPILIEGKA 451
Acinetobacter ihumii 395 -ATTIKAAKKMVERE---TPEVWDVLASVIRQHPVMLNRAPTLHRLGLQAFEPILIEGKA 451
Acinetobacter johnsonii 395 -ATTIKAAKKMVERE---TPEVWDVLAAVIRQHPVMLNRAPTLHRLGLQAFEPILIEGKA 451
Acinetobacter ursingii 395 -ATTIKAAKKMVERE---TPEVWDVLANVIRQHPVMLNRAPTLHRLGLQAFEPILIEGKA 451
Acinetobacter portensis 395 -ATTIKAAKKMVERE---TPEVWDVLASVIRQHPVMLNRAPTLHRLGLQAFEPILIEGKA 451
Acinetobacter guerrae 395 -ATTIKAAKKMVERE---TPEVWDVLANVIRQHPVMLNRAPTLHRLGLQAFEPILIEGKA 451
Acinetobacter rudis 395 -ATTIKAAKKMVERE---TPEVWDVLASVIRQHPVMLNRAPTLHRLGLQAFEPILIEGKA 451
Acinetobacter baumannii 395 -ATTIKAAKKMVERE---TPEVWDVLASVIRQHPVMLNRAPTLHRLGLQAFEPILIEGKA 451
Acinetobacter guillouiae 395 -ATTIKAAKKMVERE---TPEVWDVLASVIRQHPVMLNRAPTLHRLGLQAFEPILIEGKA 451
Acinetobacter sichuanensis 395 -ATTIKAAKKMVERE---TPEVWDVLASVIRQHPIMLNRAPTLHRLGLQAFEPILIEGKA 451
Acinetobacter seifertii 395 -ATTIKAAKKMVERE---TPEVWDVLASVIRQHPVMLNRAPTLHRLGLQAFEPILIEGKA 451
Acinetobacter pittii 395 -ATTIKAAKKMVERE---TPEVWDVLASVIRQHPVMLNRAPTLHRLGLQAFEPILIEGKA 451
Acinetobacter defluvii 395 -ATTIKAAKKMVERE---TPEVWDVLASVIRQHPIMLNRAPTLHRLGLQAFEPILIEGKA 451
Acinetobacter terrestris 395 -ATTIKAAKKMVERE---TPEVWDVLAAVIRQHPVMLNRAPTLHRLGLQAFEPILIEGKA 451
Acinetobacter pragensis 395 -ATTIKAAKKMVERE---TPEVWDVLAAVIRQHPIMLNRAPTLHRLGLQAFEPILIEGKA 451
Acinetobacter indicus 395 -ATTIKAAKKMVERE---TPEVWDVLASVIRQHPVMLNRAPTLHRLGLQAFEPILIEGKA 451
Pseudomonas aeruginosa 390 -ATTIKAAKKMVERE---IPEVWDVLAEVIREHPVLLNRAPTLHRLGIQAFEPVLIEGKA 446
Escherichia coli 390 -ATTIKAAKKMVERE---EAVVWDILDEVIREHPVLLNRAPTLHRLGIQAFEPVLIEGKA 446
Staphylococcus aureus 370 -ATTIKNAKSKIERM---DDEVWDVLEEVIREHPVLLNRAPTLHRLGIQAFEPTLVEGRA 426
Bacillus subtilis 379 -AHNIKSAKRKIERV---QPEVWDVLESVIKEHPVLLNRAPTLHRLGIQAFEPTLVEGRA 435
Mycobacterium tuberculosis 465 -AQNIKSAKRMVERQ---RPQVWDVLEEVIAEHPVLLNRAPTLHRLGIQAFEPMLVEGKA 521
Bacteroides fragilis 402 -VKTVKSAKKIVDRK---EPVIWDILEHVMKGHPVLLNRAPTLHRLGIQAFQPKMIEGKA 458
Thermotoga maritima 682 SSKTMRKVKKAIIEK--EMPEAWEVLEEVIKGSVVLLNRAPTLHRMSIQAFEPKLVEGRA 739
Thermus aquaticus 666 -APNVKAARRMLERQRDIKDEVWDALEEVIHGKVVLLNRAPTLHRLGIQAFQPVLVEGQS 725
Fusobacterium nucleatum 379 -ANNIKMAKKLVEES---DDKVWAVIEDVIADHPVLLNRAPTLHRLSIQAFQPVLIEGKA 435
Aquifex aeolicus 520 -ATSIKHAKRLVEQK---TPEVWECLEEVVKEHPVLLNRAPTLHRPSIQAFEPVLVEGKA 576
Blastopirellula marina 388 -ADTIKSAKKMLERK---DEEVWDILESVIQNHPVMLNRAPTLHRMGIQAFEPTLVEGRA 444
Chlamydia abortus 393 -VYTIRSAKKMIQRG---APEVWDVLEEIIKGHPVLLNRAPTLHRLGIQAFEPVLIEGKA 449
Cyanobacteria bacterium 385 -ADTIKSAKRMLERR---DAEVWDILEEVIFQHPVLLNRAPTLHRMGIQAFEPVLVEGRA 441

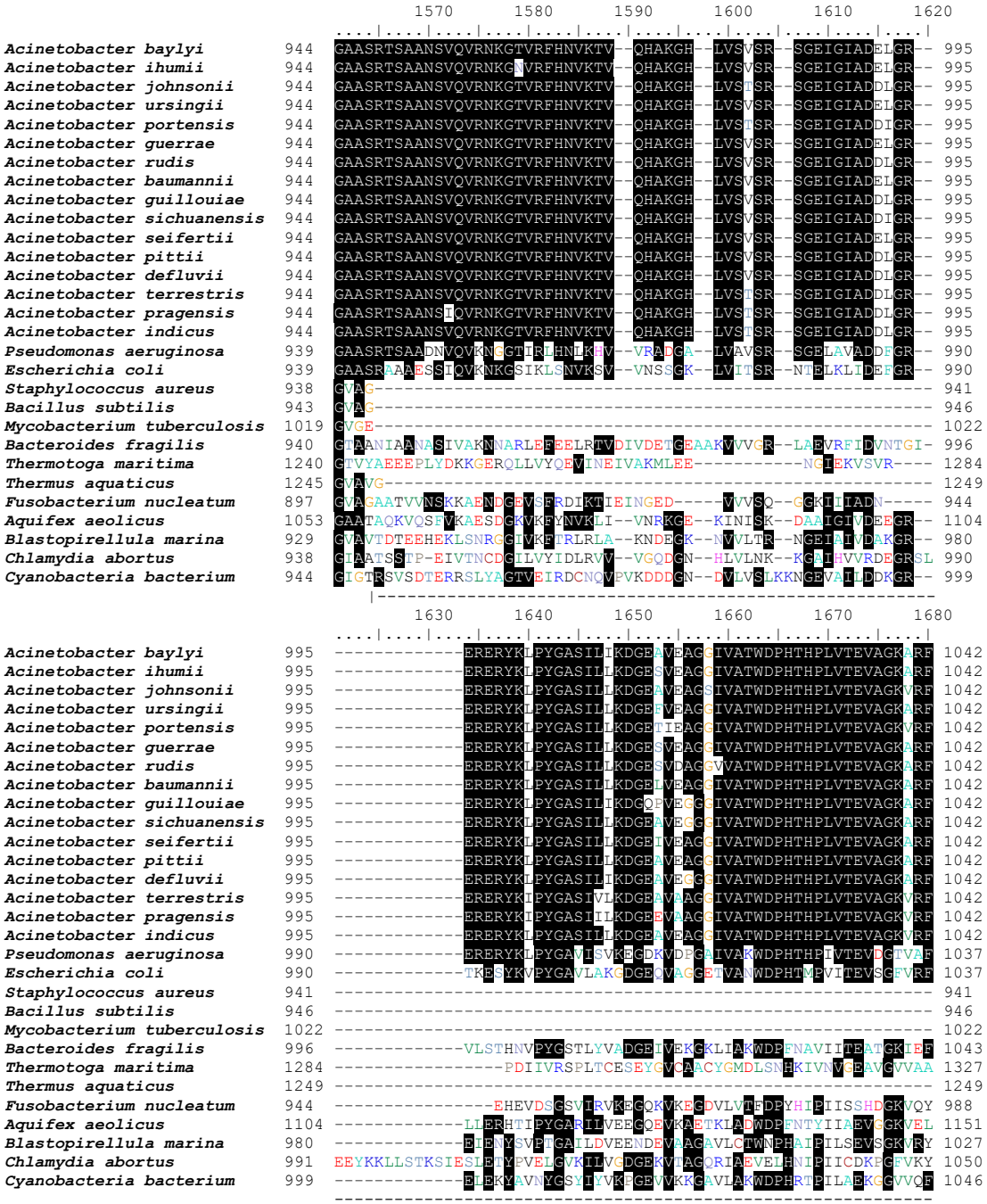

970 980 990 1000 1010 1020
Acinetobacter baylyi 452 IRLHPLVCAAFNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPIIVPSQDV 511
Acinetobacter ihumii 452 IRLHPLVCAAFNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPIIVPSQDV 511
Acinetobacter johnsonii 452 IRLHPLVCAAFNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPIIVPSQDV 511
Acinetobacter ursingii 452 IRLHPLVCIAFNADFDGDQMAVHVPLTLEAQLESRALMMSTNNILSPANGEPIIVPSQDV 511
Acinetobacter portensis 452 IRLHPLVCAAFNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPIIVPSQDV 511
Acinetobacter guerrae 452 IRLHPLVCIAFNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPIIVPSQDV 511
Acinetobacter rudis 452 IRLHPLVCAAFNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPIIVPSQDV 511
Acinetobacter baumannii 452 IRLHPLVCAAFNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPIIVPSQDV 511
Acinetobacter guillouiae 452 IRLHPLVCAAFNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPIIVPSQDV 511
Acinetobacter sichuanensis 452 IRLHPLVCAAFNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPIIVPSQDV 511
Acinetobacter seifertii 452 IRLHPLVCAAFNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPIIVPSQDV 511
Acinetobacter pittii 452 IRLHPLVCAAFNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPIIVPSQDV 511
Acinetobacter defluvii 452 IRLHPLVCAAFNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPIIVPSQDV 511
Acinetobacter terrestris 452 IRLHPLVCAAFNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPIIVPSQDV 511
Acinetobacter pragensis 452 IRLHPLVCAAFNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPIIVPSQDV 511
Acinetobacter indicus 452 IRLHPLVCAAFNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPIIVPSQDV 511
Pseudomonas aeruginosa 447 IQLHPLVCAAYNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPIIVPSQDV 506
Escherichia coli 447 IQLHPLVCAAYNADFDGDQMAVHVPLTLEAQLEARALMMSTNNILSPANGEPIIVPSQDV 506
Staphylococcus aureus 427 IRLHPLVTTAYNADFDGDQMAVHVPLSKEAQAEARMLMLAAQNILNPKDGKPVVTPSQDM 486
Bacillus subtilis 436 IRLHPLVCTAYNADFDGDQMAVHVPLSAEAQAEARILMLAAQNILNPKDGKPVVTPSQDM 495
Mycobacterium tuberculosis 522 IQLHPLVCEAFNADFDGDQMAVHIPLSAEAQAEARILMLSSNNILSPASGRPLAMPRLDM 581
Bacteroides fragilis 459 IQLHPLACTAFNADFDGDQMAVHIPLSNEAVLEAQMLMLASHNILNPANGAPITVPSQDM 518
Thermotoga maritima 740 IQLHPVVCPPFNADFDGDQMAVHVPLSAAAQAEARFLMLSRYNIISPAHGKPISLPTQDI 799
Thermus aquaticus 726 IQLHPLVCEAFNADFDGDQMAVHVPLSSEAQAEARIQMLSAHNILSPASGEPLAKPSRDI 785
Fusobacterium nucleatum 436 IRLHPLVCSAFNADFDGDQMAVHLTLSPESMMEAKLLMFAPNNIISPSSGEPIAVPSQDM 495
Aquifex aeolicus 577 IQLHPLVCPPFNADFDGDQMAVHVPLGIEAQLESYILMLSTQNVLSPAHGKPLTMPSQDM 636
Blastopirellula marina 445 IHLHPLVCKGFNADFDGDQMAVHIPLSIEAQVEAHTLMLSTNNIFAPSNGKPIMSPSQDV 504
Chlamydia abortus 450 IRVHPLVCAAFNADFDGDQMAVHVPLSIEAQLEAKVLMMAPDNIFLPSSGKPVATPSKDM 509
Cyanobacteria bacterium 442 IRLHPLVCGGFNADFDGDQMAVHIPLSIEAQAEAHVLMLSTNNIFSPANGQPIISASQDI 501
1030 1040 1050 1060 1070 1080
Acinetobacter baylyi 512 VLGLYYITRDAVN-------AKGEG---MMFADTHEVNRALATGQVAIHARVKVRVHQTV 561
Acinetobacter ihumii 512 VLGLYYITRDAVN-------AKGEG---MIFADTHEVNRALATGQVAIHARVKVRVHQTV 561
Acinetobacter johnsonii 512 VLGLYYITRDAIN-------AKGEG---MVFADTHEVNRALATGQVNLHARVKARVHQTV 561
Acinetobacter ursingii 512 VLGLYYITRDAVN-------AKGEG---MVFADTHEVNRALATNKVAIHARVKVRVHQTV 561
Acinetobacter portensis 512 VLGLYYITRDAIN-------AKGEG---MVFADTDEVNRALATGQVDLHARVKARVHQTV 561
Acinetobacter guerrae 512 VLGLYYITRDAVN-------AKGEG---MIFADTHEVNRALATGKVAIHARVKVRVHQTV 561
Acinetobacter rudis 512 VLGLYYITRDAVN-------AKGEG---MVFADTHEVNRALATGQVDLHARVKARVHQTV 561
Acinetobacter baumannii 512 VLGLYYITRDAVN-------AKGEG---MVFADTHEVNRALATGQVAIHARVKVRVHQTV 561
Acinetobacter guillouiae 512 VLGLYYITRDAIN-------AKGEG---MVFADTHEVNRALATGQVDLHARVKARVHQTV 561
Acinetobacter sichuanensis 512 VLGLYYITRDAIN-------AKGEG---MVFADTHEVNRALATGQVDLHARVKARVHQTV 561
Acinetobacter seifertii 512 VLGLYYITRDAVN-------AKGEG---MVFADTHEVNRALATGQVAIHARVKVRVHQTV 561
Acinetobacter pittii 512 VLGLYYITRDAVN-------AKGEG---MVFADTHEVNRALATGQVAIHARVKVRVHQTV 561
Acinetobacter defluvii 512 VLGLYYITRDAIN-------AKGEG---MVFADTHEVNRALATGQVDLHARVKARVHQTV 561
Acinetobacter terrestris 512 VLGLYYITRDAIN-------AKGEG---MVFADTDEVNRALATGQVNLHARVKARVHQTV 561
Acinetobacter pragensis 512 VLGLYYITRDAIN-------AKGEG---MVFADTDEVNRALATGQVDLHARVKARVHQTV 561
Acinetobacter indicus 512 VLGLYYITRDAVN-------AKGEG---MVFADTHEVNRALATGQVDLHARVKARVYQTV 561
Pseudomonas aeruginosa 507 VMGLYYMTREAIN-------AKGEG---MAFADLQEVDRAYRSGQASLHARVKVRINEKI 556
Escherichia coli 507 VLGLYYMTRDCVN-------AKGEG---MVLTGPKEAERLYRSGLASLHARVKVRITEYE 556
Staphylococcus aureus 487 VLGLYYLTLERKD-------AVNTG---AIFNNTNEVLKAYAGFVHLHTRIGVHASSFN 536
Bacillus subtilis 496 VLGLYYLTLERAG-------AVGEG---MVFKNTDEALLAYQNGYVHLHTRVAVAANSLK 545
Mycobacterium tuberculosis 582 VTGLYYLTTEVPEDTGEYQPASGDHPETGVYSSPAEAIMAADRGVLSVRAKIKVRLTQLR 641
Bacteroides fragilis 519 VLGLYYITKLRKG-------AKGEG---LTFYGPEEALIAYNEGKVDIHAPVKVIVKDLD 568
Thermotoga maritima 800 IIGSYYLTTVGKEFD-----SLKEEDVKWKFSSPEEAMLAYHICFIKLHTPILIKVAVNG 854
Thermus aquaticus 786 ILGLYYITQVRKE-------KKGAG---MAFATPEEALAAYERGEVALNAPIVVAGRETS 835
Fusobacterium nucleatum 496 VMGCFYMTKERAG-------EKGEG---KIFSNIEQVITAYQNDKVGTHALIKVRMN--- 542
Aquifex aeolicus 637 VLGYYYITPDPIPG------RKGEG---KAFGTFEEVLKALELGHVDIHAKIKVKVGN-- 685
Blastopirellula marina 505 VMGCNFITTTLPNR------PGEG----MSFSSTDEADYAFSQGVIDLHAKIKVRLPKGQ 554
Chlamydia abortus 510 TLGIYYLMADPTYF------PEDHGGKIKIFKDVTEVLRALYSG-GCLDERTDGRRDETG 562
Cyanobacteria bacterium 502 VMGVYFITATIDEM------KPESE--LMRFKDRREAIIAYDEGKIRLQQLVSVRLDGFD 553

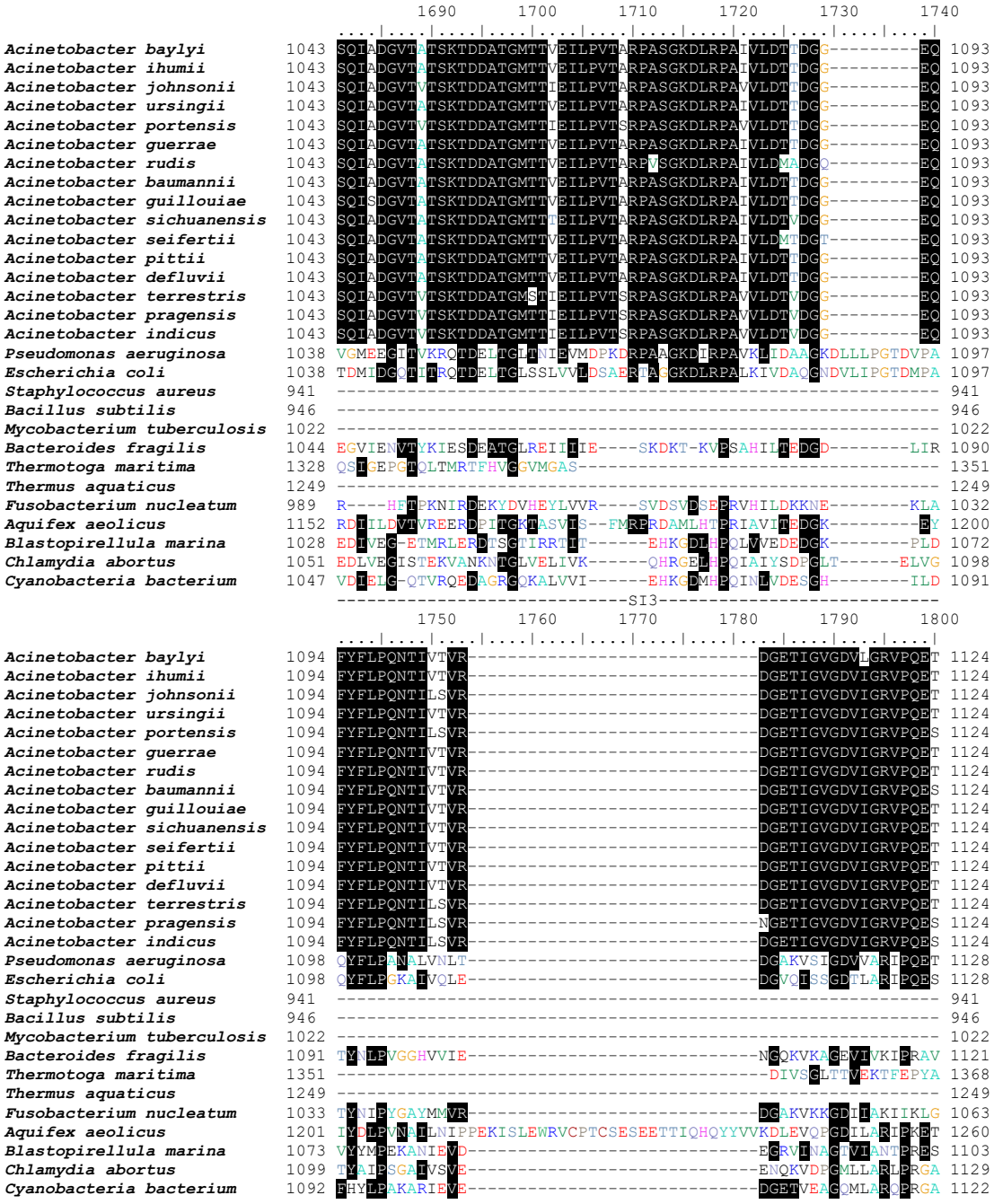

1090 1100 1110 1120 1130 1140
Acinetobacter baylyi 562 INEDGQRE----------KQTIVVDTTPGRCLLWEVVPEGLSFE-- 595
Acinetobacter ihumii 562 INEDGQRE----------KQTIIVDTTPGRCLLWEIVPEGLSFE-- 595
Acinetobacter johnsonii 562 IDENGNRE----------QQTIVVDTTPGRCLLWEVVPEGMDFQ-- 595
Acinetobacter ursingii 562 IDENGQRE----------KQTIIVDTTPGRCLLWEIVPEGLSFD-- 595
Acinetobacter portensis 562 IDEDGNRE----------QQTIVVDTTPGRCLLWEVVPEGMDFQ-- 595
Acinetobacter guerrae 562 IDEDGQRE----------KQTIIVDTTPGRCLLWEIVPEGLSFE-- 595
Acinetobacter rudis 562 IDENGHRE----------QQTIVVDTTPGRCLLWEVVPEGMDFQ-- 595
Acinetobacter baumannii 562 INENGERE----------QQTIIVDTTPGRCLLWEVVPEGLSFD-- 595
Acinetobacter guillouiae 562 IDENGNRE----------HQTIVVDTTPGRCLLWEVVPEGMDFQ-- 595
Acinetobacter sichuanensis 562 IDENGNRE----------HQTIVVDTTPGRCLLWEVVPEGMDFQ-- 595
Acinetobacter seifertii 562 INENGERE----------HQTIIVDTTPGRCLLWEVVPQGLSFD-- 595
Acinetobacter pittii 562 INENGERE----------HQTIIVDTTPGRCLLWEIVPQGLSFD-- 595
Acinetobacter defluvii 562 IDENGNRE----------HQTIVVDTTPGRCLLWEVVPEGMDFQ-- 595
Acinetobacter terrestris 562 IDEDGNRE----------QQTIIVDTTPGRCLLWEVVPEGMDFQ-- 595
Acinetobacter pragensis 562 INENGERE----------HQTIVVDTTPGRCLLWEVVPEGMDFQ-- 595
Acinetobacter indicus 562 INDNGERE----------QQTIVVDTTPGRCLLWEVVPEGLSFE-- 595
Pseudomonas aeruginosa 557 KGEDGQLT----------ANTRIVDTTVGRALLFQVVPAGLPFD-- 590
Escherichia coli 557 KDANGELV----------AKTSLKDTTVGRAILWMIVPKGLPYS-- 590
Staphylococcus aureus 537 NPTFTEEQ----------NKKILATSVGKIIFNEIIPDSFAYINE 571
Bacillus subtilis 546 NVTFTEEQ----------RSKLLITTVGKLVFNEILPESFPYMNE 580
Mycobacterium tuberculosis 642 PPVEIEAELFGH----------SGWQPGDAWMAETTLGRVMFNELLPLGYPFV-- 684
Bacteroides fragilis 569 ENGNIV----------DVMRETSVGRVIVNEIVPPEVGY--- 597
Thermotoga maritima 854 ----------EEKRIKTTLGRVIFNGILPEDLRDY-- 879
Thermus aquaticus 836 VGRLKFVFANPDEALLAVAHGLLDLQDVVTVRYLGRRLETSPGRILFARIVGEAVGDE-- 893
Fusobacterium nucleatum 542 ----------GELVETTPGRVLFNEILPEIDRN--- 565
Aquifex aeolicus 685 ----------EWIETTPGRVLFNSIMPEGQPF--- 707
Blastopirellula marina 555 RLKSEE----------EDGKHGSQIETTYGRILFNDMLPNGMDFY-- 589
Chlamydia abortus 563 RGIHIHEK-I----------KVRIDGQVIETTPGRVLFNRIVPKELGFQ-- 600
Cyanobacteria bacterium 554 KVVSVQNGPV----------EPLPEHGRVITTVGRVLFSDILAKGMPFY-- 592
1150 1160 1170 1180 1190 1200
Acinetobacter baylyi 595 ----------MINLEMTKKNISKLINSCYRKLGLKDT 622
Acinetobacter ihumii 595 ----------MINLEMTKKNISKLINSCYRKLGLKDT 622
Acinetobacter johnsonii 595 ----------QINVEMTKKNISKLINSCYRKLGLKDT 622
Acinetobacter ursingii 595 ----------MINLEMTKKNISKLINSCYRKLGLKDT 622
Acinetobacter portensis 595 ----------QINLEMNKKNISKLINSCYRKLGLKDT 622
Acinetobacter guerrae 595 ----------MINLEMTKKNISKLINICYRKLGLKDT 622
Acinetobacter rudis 595 ----------QINVEMTKKNISKLINSCYRKLGLKDT 622
Acinetobacter baumannii 595 ----------MINLEMTKKNISKLINSCYRKLGLKDT 622
Acinetobacter guillouiae 595 ----------QINVEMTKKNISKLINSCYRKLGLKDT 622
Acinetobacter sichuanensis 595 ----------QINLEMTKKNISKLINSCYRKLGLKDT 622
Acinetobacter seifertii 595 ----------MINLEMTKKNISKLINSCYRKLGLKDT 622
Acinetobacter pittii 595 ----------MINLEMTKKNISKLINSCYRKLGLKDT 622
Acinetobacter defluvii 595 ----------QINVEMTKKNISKLINSCYRKLGLKDT 622
Acinetobacter terrestris 595 ----------QINVEMTKKNISKLINSCYRKLGLKDT 622
Acinetobacter pragensis 595 ----------QINLEMTKKNISKIINSCYRKLGLKDT 622
Acinetobacter indicus 595 ----------QINVEMTKKNISKLINSCYRKLGLKDT 622
Pseudomonas aeruginosa 590 ----------VVNQSMKKKAISKLINHCYRVVGLKDT 617
Escherichia coli 590 ----------IVNQALGKKAISKMINTCYRLGLKST 617
Staphylococcus aureus 572 PTQENLERKTPNRYFIDPTTLGEGGLKEYFENEELIEPFNKKFLGNIIAEVFNRFSITDT 631
Bacillus subtilis 581 PTKSNIEEKTPDRFFLEKG----ADVKAVIAQQPINAPFKKGILGKIIAEIFKRFHITET 636
Mycobacterium tuberculosis 684 ----------NKQMHKKVQAAIINDLAERYPMIVV 709
Bacteroides fragilis 597 ----------INTIISKKSLRDIISAVIKACGVAKT 623
Thermotoga maritima 879 ----------NRIFDKKLINALVYETFKRHGIDRA 904
Thermus aquaticus 893 ----------KVAQELIQMDVPQEKNSLKDLVYQAFLRLGMEKT 927
Fusobacterium nucleatum 565 ----------YHKTYGKGEIKALIKSLYEAHGFTET 591
Aquifex aeolicus 707 ----------VNKTLDKKGISKLITELYIRVGNEET 733
Blastopirellula marina 589 ----------NHSLGSSEISKVISDCYQRLGRRAT 614
Chlamydia abortus 600 ----------NYSMPSKRISELILQCYKKVGLEAT 625
Cyanobacteria bacterium 592 ----------NCALGKKGCARVLDDSYIYSGKPAT 617


1210 1220 1230 1240 1250 1260
Acinetobacter baylyi 623 VIFADQLMYLGFRQATRSGVSVGMEDMVIPPQKQAIIDKAETEVREIEQQFEQGFVTAGE 682
Acinetobacter ihumii 623 VIFADQLMYLGFRQATRSGVSVGMEDMVIPPQKHIIDKAETEVREIEQQFEQGFVTAGE 682
Acinetobacter johnsonii 623 VIFADQLMYLGFRQATRSGVSVGMEDMVIPPAKYSIIEKAEVEVREIEQQFEQGFVTAGE 682
Acinetobacter ursingii 623 VIFADQLMYLGFRQATRSGVSVGMEDMVIPPQKQVIIDKAETEVREIEQQFEQGFVTAGE 682
Acinetobacter portensis 623 VIFADQLMYLGFRQATRSGVSVGMEDMLIPPNKEAIIAKSEAEVREIEQQFEQGFVTAGE 682
Acinetobacter guerrae 623 VIFADQLMYLGFRQATRSGVSVGMEDMVIPPQKQTIIDKAETEVREIEQQFEQGFVTAGE 682
Acinetobacter rudis 623 VIFADQLMYLGFRQATRSGVSVGMEDMVIPPQKQIIIDKAETEVREIEQQFEQGFVTAGE 682
Acinetobacter baumannii 623 VIFADQLMYLGFRQATRSGVSVGMEDMLIPPTKQTIIDKAETEVREIEQQFEQGFVTAGE 682
Acinetobacter guillouiae 623 VIFADQLMYLGFRQATRSGVSVGMEDMLIPPQKQAIIEKAEVEVREIEQQFEQGFVTAGE 682
Acinetobacter sichuanensis 623 VIFADQLMYLGFRQATRSGVSVGMEDMVIPPQKQAIIDKAETEVREIEQQFEQGFVTAGE 682
Acinetobacter seifertii 623 VIFADQLMYLGFRQATRSGVSVGMEDMLIPPTKHTIIDKAETEVREIEQQFEQGFVTAGE 682
Acinetobacter pittii 623 VIFADQLMYLGFRQATRSGVSVGMEDMLIPPTKHIIDKAETEVREIEQQFEQGFVTAGE 682
Acinetobacter defluvii 623 VIFADQLMYLGFRQATRSGVSVGMEDMVIPPQKQAIIDKAETEVREIEQQFEQGFVTAGE 682
Acinetobacter terrestris 623 VIFADQLMYLGFRQATRSGVSVGMEDMLIPPTKYTIIEKAETEVREIEQQFEQGFVTAGE 682
Acinetobacter pragensis 623 VIFADQLMYLGFRQATRSGVSVGMEDMLIPPTKQAIIDKAETEVREIEQQFEQGFVTAGE 682
Acinetobacter indicus 623 VIFADQLMYLGFRQATRSGVSVGMEDMVIPPSKQAIIDKAEAEVREIEQQFEQGFVTAGE 682
Pseudomonas aeruginosa 618 VIFADQLMYTGFAYSTISGVSIGVNDFVIPDEKARIINAATDEVKEIEQQYASGLVTLGE 677
Escherichia coli 618 VIFADQIMYTGFAYAARSGASVGIDDMVIPEKKHEIISEAEAEVAEIQEQFQSGLVTAGE 677
Staphylococcus aureus 632 SMMIDRMKDLGFKFSSKAGITVGVADIVVLPDKQQILDEHEKLVDRITKQFNRGLITEEE 691
Bacillus subtilis 637 SKMIDRMKNLGFKYSTKAGITVGVSDIVVLDDKQELLEEAQSKVDNVMKQFRRGLITEEE 696
Mycobacterium tuberculosis 710 AQTVDKLKDAGFYWATRSGVTVSMADVLVPPRKKELLDHYEERADKVEKQFQRGALNHDE 769
Bacteroides fragilis 624 ADFIDGIKNLGYKMAFQQGLSFNLGDIIIPEEKETLVQRGYDEVEQVINNYNMGFITNNE 683
Thermotoga maritima 905 ADLLDDIKDIGFHYATVSGLTLSLKDLKIPPERDELLRKIWEKVRIIEENYEKGFLTEEQ 964
Thermus aquaticus 928 ARLLDALKKYGFTLSTTSGITIGIDDAVIPEEKQRYLEEADRKIRQIEQAYEMGFLTDRE 987
Fusobacterium nucleatum 592 AELINRVKNFGYHYGTFAGVSVGIEDLEIPPKKKDLLNKADKEVAQIEKDYKSGKIINEE 651
Aquifex aeolicus 734 VKFIDRVKELGFLRSTLAGISIGWEDLQVPKAKKKIIEEALKKTEEIWNQYVQGIITNKE 793
Blastopirellula marina 615 IELLDDMNQLGFRESTRSGLSFATDDLVIPDSKPKIIAEADKKAMKFKKQYDRGIITDKE 674
Chlamydia abortus 626 VRFIDDLKDLGFTQATKAAISMGLKDVKIPEIKSELLKEAYDKVAIVKKQYDDGIITDGE 685
Cyanobacteria bacterium 618 IDLLDAMKEMGFKNSTTAGLSFGVTDLRIPEEKQKLLDAAQKQADRVEKSYEMGIITERE 677
1270 1280 1290 1300 1310 1320
Acinetobacter baylyi 683 RYNKVVDIWARTNDQVAKAMMDNLSFTVKNKQG-----QDEKQKSFNSIYMMSDSGARG 737
Acinetobacter ihumii 683 RYNKVVDIWARTNDQVAKAMMDNLSFTVKNKQG-----EDEKQKSFNSIYMMSDSGARG 737
Acinetobacter johnsonii 683 RYNKVVDIWARTNDQVAKAMMDNLSFTVKNKQG-----EDEKQKSFNSIYMMSDSGARG 737
Acinetobacter ursingii 683 RYNKVVDIWARTNDQVAKAMMDNLSFTVKNKQG-----EDEKQKSFNSIYMMSDSGARG 737
Acinetobacter portensis 683 RYNKVVDIWARTNDQVAKAMMDNLSFTVKNKQG-----EDEKQKSFNSIYMMSDSGARG 737
Acinetobacter guerrae 683 RYNKVVDIWARTNDQVAKAMMDNLSFTVKNKQG-----EDEKQKSFNSIYMMSDSGARG 737
Acinetobacter rudis 683 RYNKVVDIWARTNDQVAKAMMDNLSFTVKNKQG-----EDEKQKSFNSIYMMSDSGARG 737
Acinetobacter baumannii 683 RYNKVVDIWARTNDQVAKAMMDNLSYTLVKNKQG-----EDEKQKSFNSIYMMSDSGARG 737
Acinetobacter guillouiae 683 RYNKVVDIWARTNDQVAKAMMDNLSFTVKNKQG-----EDEKQKSFNSIYMMSDSGARG 737
Acinetobacter sichuanensis 683 RYNKVVDIWARTNDQVAKAMMDNLSFTVKNKQG-----EDEKQKSFNSIYMMSDSGARG 737
Acinetobacter seifertii 683 RYNKVVDIWARTNDQVAKAMMDNLSYTVVKNKQG-----EDEKQKSFNSIYMMSDSGARG 737
Acinetobacter pittii 683 RYNKVVDIWARTNDQVAKAMMDNLSYTLVKNKQG-----EDEKQKSFNSIYMMSDSGARG 737
Acinetobacter defluvii 683 RYNKVVDIWARTNDQVAKAMMDNLSFTVKNKQG-----EDEKQKSFNSIYMMSDSGARG 737
Acinetobacter terrestris 683 RYNKVVDIWARTNDQVAKAMMDNLSFTVKNKQG-----EDEKQKSFNSIYMMSDSGARG 737
Acinetobacter pragensis 683 RYNKVVDIWARTNDQVAKAMMDNLSFTVKNKQG-----EDEKQKSFNSIYMMSDSGARG 737
Acinetobacter indicus 683 RYNKVVDIWARTNDQVAKAMMDNLSFTVKNKQG-----EDEKQKSFNSIYMMSDSGARG 737
Pseudomonas aeruginosa 678 KYNKVIDLWSKANDEVSKAMMANLSKEKVVDREG-----KEVDQESFNSMYMMADSGARG 732
Escherichia coli 678 RYNKVIDIWAAANDRVSKAMMDNLQTETVINRDG-----QEEKQVSFNSIYMMADSGARG 732
Staphylococcus aureus 692 RYNAVVEIWTDAKDQIQGELMQSLD----------KTNEIFMMSDSGARG 731
Bacillus subtilis 697 RYERVISIWSAAKDVIQGKLMKSLD----------ELNEIYMMSDSGARG 736
Mycobacterium tuberculosis 770 RNEALVEIWKEATDEVGQALREHYPDD----------NEIITIVDSGAIG 809
Bacteroides fragilis 684 RYNQVIDIWTHVNSELSNILMKTISSDD----------QGFNSVYMMLDSGARG 727
Thermotoga maritima 965 RKSEIIRLWMSVTEEITKLTSKTLAEDP----------FNEIYMMVNSGARG 1006
Thermus aquaticus 988 RYDQVIQLWTETTEKVTQAVFKNFEEN----------YPFNPLYMMAQSGARG 1030
Fusobacterium nucleatum 652 RYRKTIEVWSRTTEAVTKAMMDNLD----------EFNPVYMMATSGARG 691
Aquifex aeolicus 794 RYNRIIDVWSEATNLVSKAMFEEIEKS--KRIEN-----GKEYPGTFNEIYMMAISGARG 846
Blastopirellula marina 675 RKNQVLDAWTHAREQITAEMMEAMKNDDRG----------GLGYINPVYLMADSGARG 722
Chlamydia abortus 686 RHSKTISIWTEVSELLSDALYVEISKQTKS----------KHNPLFLMADSGARG 730
Cyanobacteria bacterium 678 RYNQLLDIWSHCREELTKKLIETIRHDRRDEEGNYTSIEKKEGKRYLNPVYLMSDSGARG 737

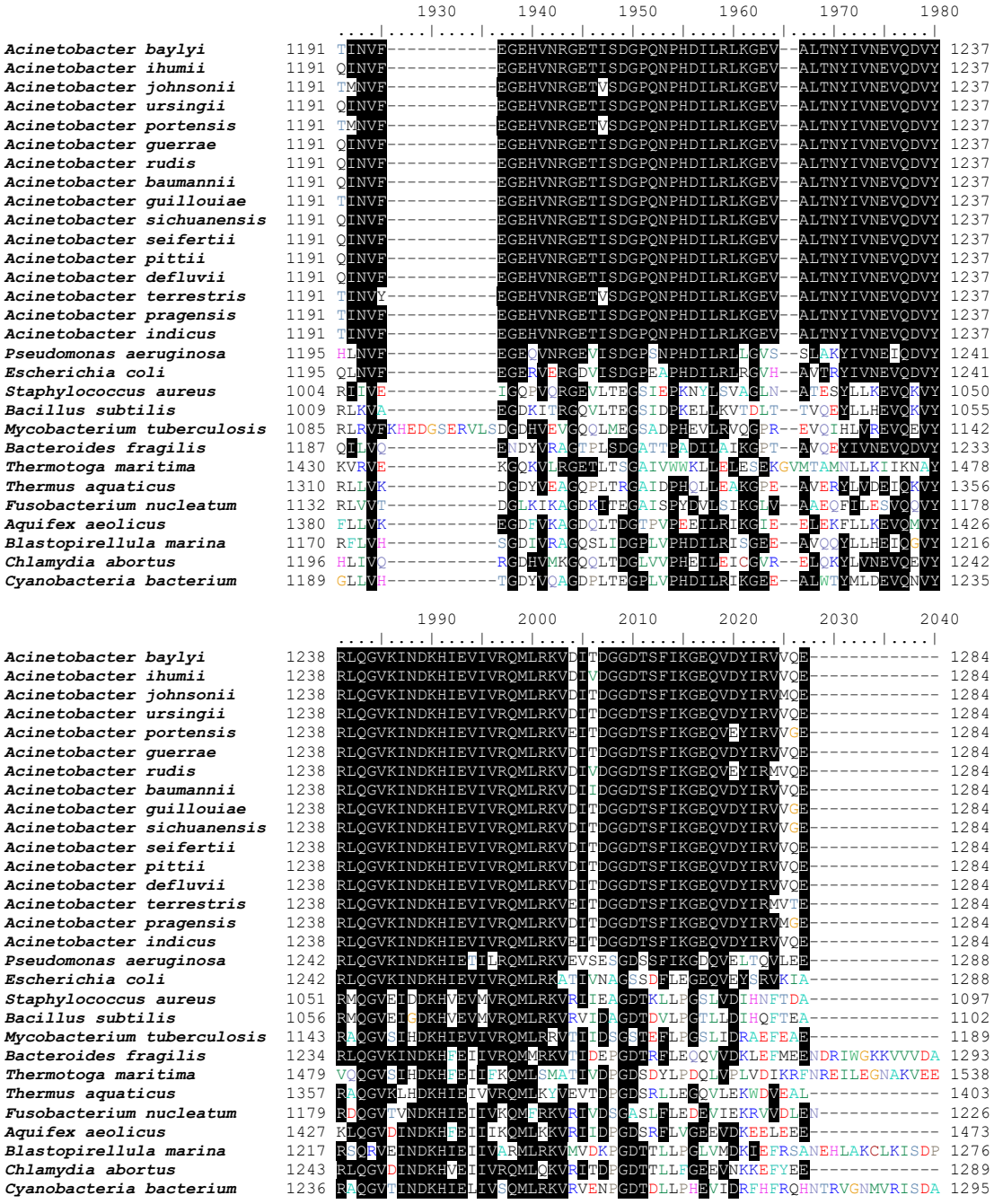

1330 1340 1350 1360 1370 1380
Acinetobacter baylyi 738 SAAQIRQLAGMRGLMAKPD----------GSIIETPIKANFREGLTVLQYF 778
Acinetobacter ihumii 738 SAAQIRQLAGMRGLMAKPD----------GSIIETPIKANFREGLTVLQYF 778
Acinetobacter johnsonii 738 SAAQIRQLAGMRGLMAKPD----------GSIIETPIKANFREGLTVLQYF 778
Acinetobacter ursingii 738 SAAQIRQLAGMRGLMAKPD----------GSIIETPIKANFREGLTVLQYF 778
Acinetobacter portensis 738 SAAQIRQLAGMRGLMAKPD----------GSIIETPIKANFREGLTVLQYF 778
Acinetobacter guerrae 738 SAAQIRQLAGMRGLMAKPD----------GSIIETPIKANFREGLTVLQYF 778
Acinetobacter rudis 738 SAAQIRQLAGMRGLMAKPD----------GSIIETPIKANFREGLTVLQYF 778
Acinetobacter baumannii 738 SAAQIRQLAGMRGLMAKPD----------GSIIETPIKANFREGLTVLQYF 778
Acinetobacter guillouiae 738 SAAQIRQLAGMRGLMAKPD----------GSIIETPIKANFREGLTVLQYF 778
Acinetobacter sichuanensis 738 SAAQIRQLAGMRGLMAKPD----------GSIIETPIKANFREGLTVLQYF 778
Acinetobacter seifertii 738 SAAQIRQLAGMRGLMAKPD----------GSIIETPIKANFREGLTVLQYF 778
Acinetobacter pittii 738 SAAQIRQLAGMRGLMAKPD----------GSIIETPIKANFREGLTVLQYF 778
Acinetobacter defluvii 738 SAAQIRQLAGMRGLMAKPD----------GSIIETPIKANFREGLTVLQYF 778
Acinetobacter terrestris 738 SAAQIRQLAGMRGLMAKPD----------GSIIETPIKANFREGLTVLQYF 778
Acinetobacter pragensis 738 SAAQIRQLAGMRGLMAKPD----------GSIIETPIKANFREGLTVLQYF 778
Acinetobacter indicus 738 SAAQIRQLAGMRGLMAKPD----------GSIIETPIKANFREGLTVLQYF 778
Pseudomonas aeruginosa 733 SAAQIRQLAGMRGLMAKPD----------GSIIETPITANFREGLNVLQYF 773
Escherichia coli 733 SAAQIRQLAGMRGLMAKPD----------GSIIETPITANFREGLNVLQYF 773
Staphylococcus aureus 732 NASNFTQLAGMRGLMAAPS----------GKIIETPITSSFREGLTVLEYF 772
Bacillus subtilis 737 NASNFTQLAGMRGLMANPA----------GRIIETPIKSSFREGLTVLEYF 777
Mycobacterium tuberculosis 810 NFTQIRTLAGMKGLVTNPK----------GEFIPRPVKSSFREGLTVLEYF 850
Bacteroides fragilis 728 SKEQIRQLSGMRGLMAKPQKAGAE----------GGQIIETPILSNFKEGLSVLEYF 774
Thermotoga maritima 1007 NIDQVKQLAGIRGLMIKAYDPRSREIKSKIFKGQAIHEVLTFDVPVDKNLREGVDILQFF 1066
Thermus aquaticus 1031 NPQQIRQLQGMRGLMQKPS----------GETFEVPVRSSFREGLTVLEYF 1071
Fusobacterium nucleatum 692 NVSQMRQLAGMRGNMADTQ----------GRTIEVPIKANFREGLTVLEEF 732
Aquifex aeolicus 847 NRDQIRQLAGMRGLMAKHS----------GEFIETPIISNFREGLSVLEYF 887
Blastopirellula marina 723 GTEQIRQLSGMRGLMAKPS----------GEIIETPIKSNFREGLTVLEYF 763
Chlamydia abortus 731 NKSQLKQLGALRGLMAKPN----------GAIIESPITSNFREGLTVLEYS 771
Cyanobacteria bacterium 738 NVSQMQQLAGMRGLMAKPS----------GEIIETPIRANFREGLNVLEYF 778
1390 1400 1410 1420 1430 1440
Acinetobacter baylyi 779 ISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVITEPDCGTMGGLVMTPFI-QGGDVI 837
Acinetobacter ihumii 779 ISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVITEPDCGTNGGLVMTPFI-QGGDVI 837
Acinetobacter johnsonii 779 ISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVITEPDCGTLGGLVMTPFI-QGGDVI 837
Acinetobacter ursingii 779 ISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVITEPDCGTAGGLVMTPFI-QGGDVI 837
Acinetobacter portensis 779 ISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVITEPDCGTLGGLVMTPFI-QGGDVI 837
Acinetobacter guerrae 779 ISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVITEPDCGTNGGLVMTPFI-QGGDVI 837
Acinetobacter rudis 779 ISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVITEPDCGTEEGLVMTPFI-QGGDVI 837
Acinetobacter baumannii 779 ISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVITEPDCGTSGGLVMTPFI-QGGDVI 837
Acinetobacter guillouiae 779 ISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVITEPDCGTLEGLVMTPFI-QGGDVI 837
Acinetobacter sichuanensis 779 ISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVITEPDCGTYDGLVMTPFI-QGGDVI 837
Acinetobacter seifertii 779 ISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVITEPDCGTAGGLVMTPFI-QGGDVI 837
Acinetobacter pittii 779 ISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVITEPDCGTAGGLVMTPFI-QGGDVI 837
Acinetobacter defluvii 779 ISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVITEPDCGTYDGLVMTPFI-QGGDVI 837
Acinetobacter terrestris 779 ISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVITEPDCGTLSGLVMTPFI-QGGDVI 837
Acinetobacter pragensis 779 ISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVITEPDCGTMDGLVMTPFI-QGGDVI 837
Acinetobacter indicus 779 ISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVITEPDCGTMAGLVMTPFI-QGGDVI 837
Pseudomonas aeruginosa 774 ISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVTEIDCGTEHGLLMSPHI-EGGDVV 832
Escherichia coli 774 ISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVTEIDCGTHEGIMMTPVI-EGGDVK 832
Staphylococcus aureus 773 ISTHGARKGLADTALKTADSGYLTRRLVDVAQDVIVREEDCGTDRGLLVSDIK-EGTEMI 831
Bacillus subtilis 778 ISTHGARKGLADTALKTADSGYLTRRLVDVAQDVIIREIDCGTDRGILAKPLK-EGTETI 836
Mycobacterium tuberculosis 851 INTHGARKGLADTALRTADSGYLTRRLVDVSQDVIVREADCGTERGIVVELAERAPDGTL 910
Bacteroides fragilis 775 ISTHGARKGLADTALKTADAGYLTRRLVDVSHDVIINEEDCGTLRGLVQTDLK-NNDEVI 833
Thermotoga maritima 1067 ISTYGARKGQVDTAMNTSFAGYLTRRLVDVAQSVTVAEPDCGTHEGIRAMDLIKEGTVVE 1126
Thermus aquaticus 1072 ISSHGARKGSADTALRTADSGYLTRKLVDVAHEIVVREADCGTNYISVPLFQMDEVTRT 1131
Fusobacterium nucleatum 733 MSSHGARKGLADTALRTADSGYLTRRLVDISHEVIVNEEDCHTHEGIEVEALVGANGKIL 792
Aquifex aeolicus 888 ISTYGARKGLADTALKTAPAGYLTRRLVDVAQDITITEEDCGTVKGFEMEPIV-EAGEER 946
Blastopirellula marina 764 SSTHGARKGLADTALKTADSGYLTRKLADVAQNVVITMDDCETTQGITKGVIY-RGEKVE 822
Chlamydia abortus 772 ISSHGARKGLADTALKTADSGYLTRRLVDVAQDVIITEKDCGTLNHIEITAIR-QGSEEL 830
Cyanobacteria bacterium 779 SSTHGARKGLADTALKTADSGYLTRKLCDIAQSVIVGEEDCGSRRGIMKRALY-KGEQVD 837

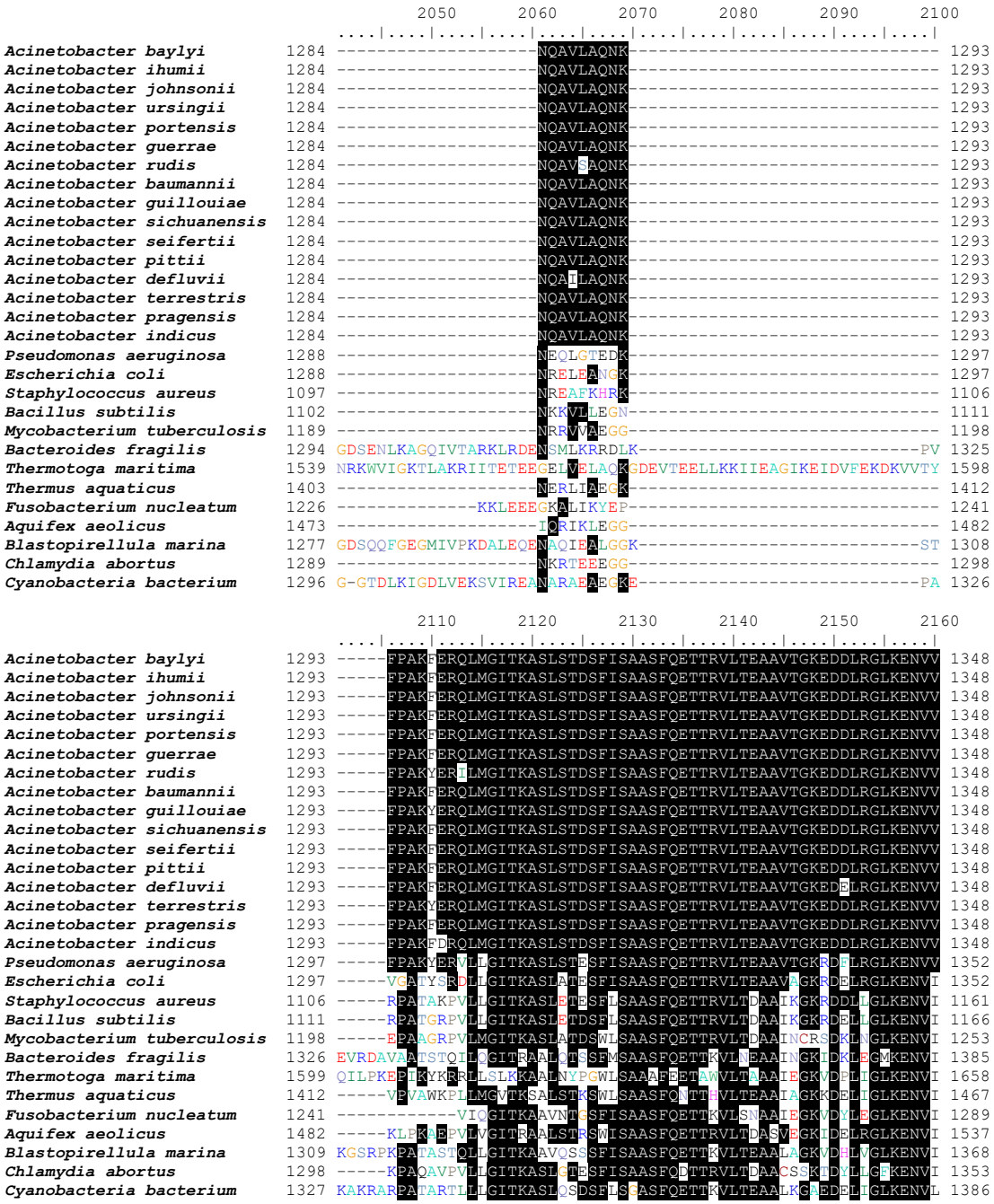

1450 1460 1470 1480 1490 1500
Acinetobacter baylyi 838 EP------IRDRVLGRVIAEDVRRASDNEIVLPRG--------TLIDEKIAAQLEEAGVD 883
Acinetobacter ihumii 838 EP------LRDRVLGRVIAEDVRRASDNEVVLPRG--------TLIDEKIAAQLEEAGVD 883
Acinetobacter johnsonii 838 EN------LSTRVLGRVVAEDVKKAGSDEVILPRN--------TLIDEKLANFLEEQGVD 883
Acinetobacter ursingii 838 EP------LRDRVLGRVIAEDVRRASDNEVVLPRG--------TLIDEKIAAQLEEAGVD 883
Acinetobacter portensis 838 EN------LSSRVLGRVVAEDVKRVGSEEVILPRN--------TLIDEKIAAFLEEAGVD 883
Acinetobacter guerrae 838 EP------LRDRVLGRVIAEDVRRASDNEVVLPRG--------TLIDEKIAAQLEEAGVD 883
Acinetobacter rudis 838 EP------LRDRVLGRVIAVDVRRAVDDEVVLPQG--------TLIDEKIAAQLEDAGVD 883
Acinetobacter baumannii 838 EP------LRDRVLGRVIAEDVRRASDDEVVLPRG--------TLIDEKIAAQLEEAGVD 883
Acinetobacter guillouiae 838 EP------LSVRVLGRVIAEDVKKAGSDEVVLPRN--------TLIDEKIAAYLEDAGVD 883
Acinetobacter sichuanensis 838 EN------LGARVLGRVIAEDVKKPGTEDVLLPRN--------TLIDEKLATYLEDNGVD 883
Acinetobacter seifertii 838 EP------LRDRVLGRVIAEDVRRASDDEVVLPRG--------TLIDEKIAAQLEEAGVD 883
Acinetobacter pittii 838 EP------LRDRVLGRVIAEDVRRASDDEVVLPRG--------TLIDEKIAAQLEEAGVD 883
Acinetobacter defluvii 838 EP------LSQRVLGRVIAEDVKKAGSDDVILPRN--------TLIDEKLAQYLEDAGVD 883
Acinetobacter terrestris 838 EN------LGARVLGRVVAEDVKRVGSDEVILPRN--------TLIDEKLAAFLEEAGVD 883
Acinetobacter pragensis 838 EN------LGARVLGRVVAEDVKKVGTEEVLLPRN--------TLIDEKLAAFIENAGID 883
Acinetobacter indicus 838 EN------LGTRVLGRVVAEDVKKAGSDEVILPRN--------TLIDEKLANFLEEQGVD 883
Pseudomonas aeruginosa 833 EP------LSERVLGRVIARDVFKPGSDEVIVPAG--------TLIDEKWVDFLEVMSVD 878
Escherichia coli 833 EP------LRDRVLGRVIAEDVLKPGTADILVPRN--------TLLHEQWCDLLEENSVD 878
Staphylococcus aureus 832 EP------FIERIEGRYSKETIRHPETDEIIIRPD--------ELITPEIAKKITDAGIE 877
Bacillus subtilis 837 ER------LEERLIGRFARKQVKHPETGEVLVNEN--------ELIDEDKALEIVEAGIE 882
Mycobacterium tuberculosis 911 IRDPY---IETSAYARTLGTDAVDEAGNVIVERGQ---------DLGDPEIDALLAAGIT 958
Bacteroides fragilis 834 AT------LYERILGRVSVHDIIHPQTGELLVAGG--------EEITEDIAKKIQESPIE 879
Thermotoga maritima 1127 K-------MNEFLEGRVLARDVLDPETKEVLKNPETGKEYTRNTMLTDDDANELASYKKM 1179
Thermus aquaticus 1132 LRLRKRSDIESGLYGRVLAREVEALGRRLEEGRYLS-------LEDVHFLIKAAEAGEVR 1184
Fusobacterium nucleatum 793 EK------LSERINGRVLAEDLVH--KGKKIAKKN--------TMIHKDLLDKIEELGIK 836
Aquifex aeolicus 947 VP------LKDRIEGRVLAEDVKDPYTGEIIARRN--------EVIDEKLAEKITKAGIE 992
Blastopirellula marina 823 VA------LADAIKGRVSRQSIVHPITDEVIVREN--------EMITLETARRIEKLGLE 868
Chlamydia abortus 831 LP------LKDRIYGRTVSEDIYQPGDKSKLLAENG-------DVITSAQAELIDDAGIE 877
Cyanobacteria bacterium 838 VA------LRDQIIGRVSVSNIINPVTDEMIVRAN--------EMVSPAAARKIEDLGIE 883
1510 1520 1530 1540 1550 1560
Acinetobacter baylyi 884 EVKVRSVIACESTFGVCAKCYGRDLARGHQVNPGESVGVMAAQSIGEPGTQLTMRTFHVG 943
Acinetobacter ihumii 884 EVKVRSVIACESSFGVCAKCYGRDLARGHQVNPGESVGVMAAQSIGEPGTQLTMRTFHVG 943
Acinetobacter johnsonii 884 EVKVRSVVACESTFGVCAKCYGRDLARGHQVNPGESVGVMAAQSIGEPGTQLTMRTFHVG 943
Acinetobacter ursingii 884 EVKVRSVIACESSFGVCAKCYGRDLARGHQVNPGESVGVMAAQSIGEPGTQLTMRTFHVG 943
Acinetobacter portensis 884 EVKVRSVVSCESTFGVCAKCYGRDLARGHLVNPGESVGVMAAQSIGEPGTQLTMRTFHVG 943
Acinetobacter guerrae 884 EVKVRSVIACESAFGVCAKCYGRDLARGHQVNPGESVGVMAAQSIGEPGTQLTMRTFHVG 943
Acinetobacter rudis 884 EVKVRSVVNCRSTFGVCAKCYGRDLARGHLVNPGESVGVMAAQSIGEPGTQLTMRTFHVG 943
Acinetobacter baumannii 884 EVKVRSVIACESTFGVCAKCYGRDLARGHLVNPGESVGVMAAQSIGEPGTQLTMRTFHVG 943
Acinetobacter guillouiae 884 EVKVRSVVNCASTFGVCAKCYGRDLARGHLVNPGESVGVMAAQSIGEPGTQLTMRTFHVG 943
Acinetobacter sichuanensis 884 EVKVRSVVNCASTFGVCAKCYGRDLARGHLVNPGESVGVMAAQSIGEPGTQLTMRTFHVG 943
Acinetobacter seifertii 884 EVKVRSVIACESTFGVCARCYGRDLARGHLVNPGESVGVMAAQSIGEPGTQLTMRTFHVG 943
Acinetobacter pittii 884 EVKVRSVIACESTFGVCARCYGRDLARGHLVNPGESVGVMAAQSIGEPGTQLTMRTFHVG 943
Acinetobacter defluvii 884 EVKVRSVVSCASTFGVCAKCYGRDLARGHLVNPGESVGVMAAQSIGEPGTQLTMRTFHVG 943
Acinetobacter terrestris 884 EVKVRSVVSCESTFGVCAKCYGRDLARGHQVNPGESVGVMAAQSIGEPGTQLTMRTFHVG 943
Acinetobacter pragensis 884 EIKVRSVVNCASTFGVCAKCYGRDLARGHQVNPGESVGVMAAQSIGEPGTQLTMRTFHVG 943
Acinetobacter indicus 884 EVKVRSVVSCESTFGVCAKCYGRDLARGHLVNPGESVGVMAAQSIGEPGTQLTMRTFHVG 943
Pseudomonas aeruginosa 879 EVVVRSPITCETRHGICAMCYGRDLARGHRVNIGEAVGVIAAQSIGEPGTQLTMRTFHIG 938
Escherichia coli 879 AVKVRSVVSCDTDFGVCAMCYGRDLARGHIINKGEAIGVIAAQSIGEPGTQLTMRTFHIG 938
Staphylococcus aureus 878 QMYIRSAFTCNARHGVCEKCYGKNLATGEKVEVGEAVGIIAAQSIGEPGTQLTMRTFHIG 937
Bacillus subtilis 883 EVWIRSAFTCNTPHGVCKRCYGRNLATGSDVEVGEAVGIIAAQSIGEPGTQLTMRTFHIG 942
Mycobacterium tuberculosis 959 QVKVRSVLTCATSTGVCAICYGRSMATGKLVDIGEAVGIVAAQSIGEPGTQLTMRTFHIG 1018
Bacteroides fragilis 880 SVEIRSVLTCESKKGVCAKCYGRNLATNKMVQKGEAVGVIAAQSIGEPGTQLTLRTFHIG 939
Thermotoga maritima 1180 VDVVRYEEIDITELSLPNMYAEIAEPVGEYEEGTELTWDVIKAAKNEGKYRIKVKVYFVV 1239
Thermus aquaticus 1185 EVEVRSPLTCQTRYGVCQKCYGWDLSMARPVSIGEAVGVVAAESIGEPGTQLTMRTFHIG 1244
Fusobacterium nucleatum 837 KVKIRSPLTCALEKGVCQKCYGMDLSNYNEILLGEAVGVVAAQSIGEPGTQLTMRTFHIG 896
Aquifex aeolicus 993 KVRVRSPLTCEAKHGVCAMCYGNDLSQRKIVSVGEAVGIIAAQSIGEPGTQLTMRTFHIG 1052
Blastopirellula marina 869 KIQVRSPMTCDAFIGTCKACYGMDMSSGAMVEEGMAVGIIAAQSIGEPGTQLTMRTFHIG 928
Chlamydia abortus 878 SIKIRSTLTCESRRGVCAKCYGLNLANGRLIGLGEAVGIIAAQSIGEPGTQLTMRTFHIG 937
Cyanobacteria bacterium 884 SVVVRSPLTSESRFGCSVLDYGMDMSTGKLVEEGMAIGIIAAQSIGEPGTQLTMRTFHIG 943

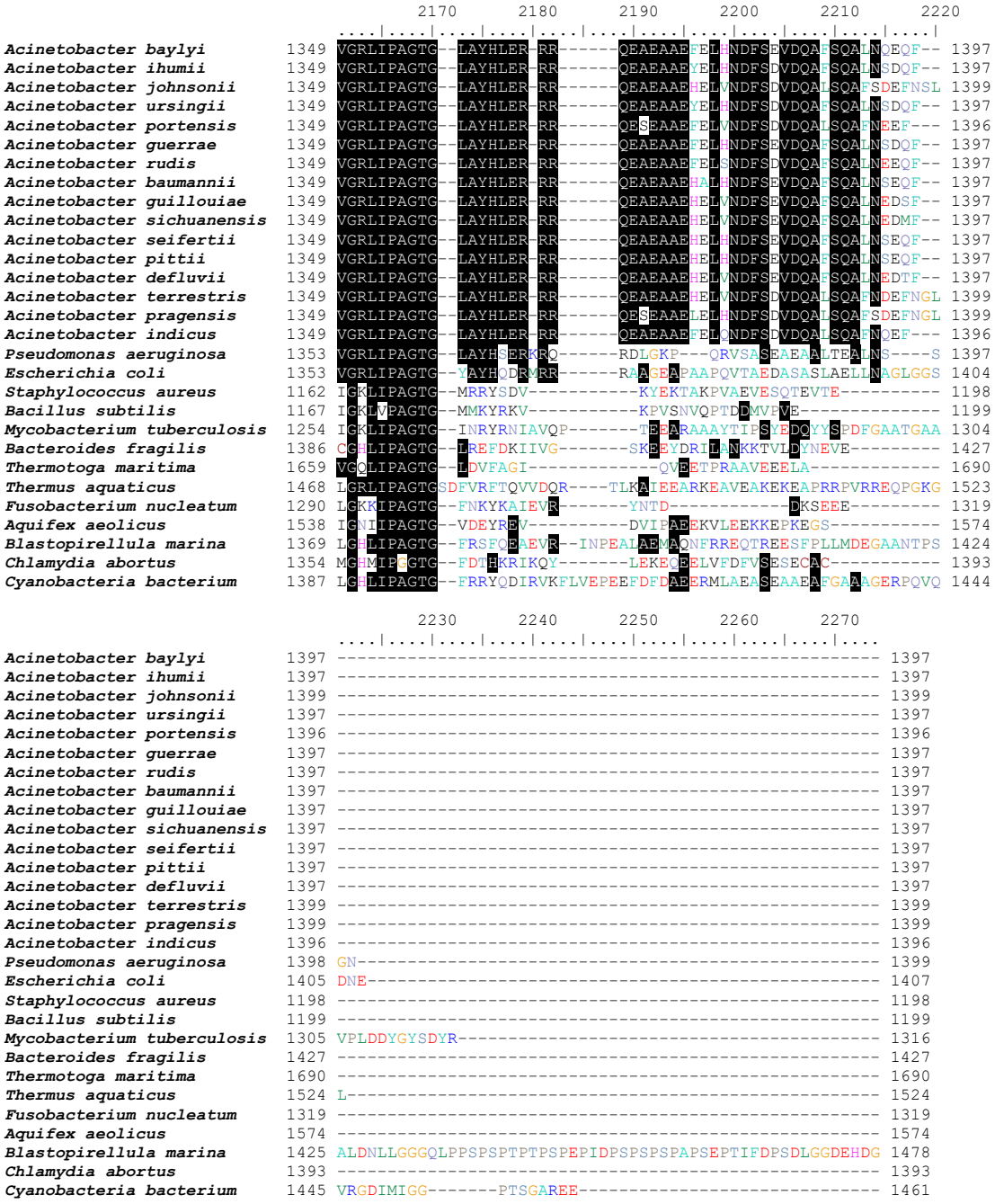

1570 1580 1590 1600 1610 1620
Acinetobacter baylyi 944 995
Acinetobacter ihumii 944 995
Acinetobacter johnsonii 944 995
Acinetobacter ursingii 944 995
Acinetobacter portensis 944 995
Acinetobacter guerrae 944 995
Acinetobacter rudis 944 995
Acinetobacter baumannii 944 995
Acinetobacter guillouiae 944 995
Acinetobacter sichuanensis 944 995
Acinetobacter seifertii 944 995
Acinetobacter pittii 944 995
Acinetobacter defluvii 944 995
Acinetobacter terrestris 944 995
Acinetobacter pragensis 944 995
Acinetobacter indicus 944 995
Pseudomonas aeruginosa 939 990
Escherichia coli 939 990
Staphylococcus aureus 938 941
Bacillus subtilis 943 946
Mycobacterium tuberculosis 1019 1022
Bacteroides fragilis 940 996
Thermotoga maritima 1240 1284
Thermus aquaticus 1245 1249
Fusobacterium nucleatum 897 944
Aquifex aeolicus 1053 1104
Blastopirellula marina 929 980
Chlamydia abortus 938 990
Cyanobacteria bacterium 944 999
1630 1640 1650 1660 1670 1680
Acinetobacter baylyi 995 1042
Acinetobacter ihumii 995 1042
Acinetobacter johnsonii 995 1042
Acinetobacter ursingii 995 1042
Acinetobacter portensis 995 1042
Acinetobacter guerrae 995 1042
Acinetobacter rudis 995 1042
Acinetobacter baumannii 995 1042
Acinetobacter guillouiae 995 1042
Acinetobacter sichuanensis 995 1042
Acinetobacter seifertii 995 1042
Acinetobacter pittii 995 1042
Acinetobacter defluvii 995 1042
Acinetobacter terrestris 995 1042
Acinetobacter pragensis 995 1042
Acinetobacter indicus 995 1042
Pseudomonas aeruginosa 990 1037
Escherichia coli 990 1037
Staphylococcus aureus 941 941
Bacillus subtilis 946 946
Mycobacterium tuberculosis 1022 1022
Bacteroides fragilis 996 1043
Thermotoga maritima 1284 1327
Thermus aquaticus 1249 1249
Fusobacterium nucleatum 944 988
Aquifex aeolicus 1104 1151
Blastopirellula marina 980 1027
Chlamydia abortus 991 1050
Cyanobacteria bacterium 999 1046


1690 1700 1710 1720 1730 1740
Acinetobacter baylyi 1043 SQIADGVTATSKTDDATGMTTVEILPVTARPASGKDLRPAIVLDTTDGS---------EQ 1093
Acinetobacter ihumii 1043 SQIADGVTATSKTDDATGMTTVEILPVTARPASGKDLRPAIVLDTTDGS---------EQ 1093
Acinetobacter johnsonii 1043 SQIADGVTVTSKTDDATGMTTIEILPVTARPASGKDLRPAVVLDTTDGS---------EQ 1093
Acinetobacter ursingii 1043 SQIADGVTATSKTDDATGMTTVEILPVTARPASGKDLRPAIVLDTTDGS---------EQ 1093
Acinetobacter portensis 1043 SQIADGVTVTSKTDDATGMTTIEILPVTSRPASGKDLRPAVVLDTTDGS---------EQ 1093
Acinetobacter guerrae 1043 SQIADGVTATSKTDDATGMTTVEILPVTARPASGKDLRPAIVLDTTDGS---------EQ 1093
Acinetobacter rudis 1043 SQIADGVTATSKTDDATGMTTVEILPVTARPVSGKDLRPAIVLDMADGQ---------EQ 1093
Acinetobacter baumannii 1043 SQIADGVTATSKTDDATGMTTVEILPVTARPASGKDLRPAIVLDTTDGS---------EQ 1093
Acinetobacter guillouiae 1043 SQISDGVTATSKTDDATGMTTVEILPVTARPASGKDLRPAIVLDTTDGS---------EQ 1093
Acinetobacter sichuanensis 1043 SQIADGVTATSKTDDATGMTTTEILPVTARPASGKDLRPAIVLDTVDGS---------EQ 1093
Acinetobacter seifertii 1043 SQIADGVTATSKTDDATGMTTVEILPVTARPASGKDLRPAIVLDMTDGT---------EQ 1093
Acinetobacter pittii 1043 SQIADGVTATSKTDDATGMTTVEILPVTARPASGKDLRPAIVLDTTDGS---------EQ 1093
Acinetobacter defluvii 1043 SQIADGVTATSKTDDATGMTTVEILPVTARPASGKDLRPAIVLDTTDGS---------EQ 1093
Acinetobacter terrestris 1043 SQIADGVTVTSKTDDATGMSTIEILPVTSRPASGKDLRPAVVLDTVDGS---------EQ 1093
Acinetobacter pragensis 1043 SQIADGVTVTSKTDDATGMTTIEILPVTSRPASGKDLRPAVVLDTVDGS---------EQ 1093
Acinetobacter indicus 1043 SQIADGVTVTSKTDDATGMTTIEILPVTSRPASGKDLRPAVVLDTVDGS---------EQ 1093
Pseudomonas aeruginosa 1038 VGMEEGITVKRQTDELTGITNIEVMDPKDRPAAGKDIRPAVKLIDAAGKDLLLPGTDVPA 1097
Escherichia coli 1038 TDMIDGQTITRQTDELTGLSSLVVLDSARTAGKDLRPALKIVDAQGNDVLIPGTDMPA 1097
Staphylococcus aureus 941 ---------- 941
Bacillus subtilis 946 ---------- 946
Mycobacterium tuberculosis 1022 ---------- 1022
Bacteroides fragilis 1044 EGVIENVTYKIESDEATGLREIIIIE----SKDKT-KVESAHIITEDGD--------LIR 1090
Thermotoga maritima 1328 QSIGEPGTQLTMRTFHVGGVMGAS---------- 1351
Thermus aquaticus 1249 ---------- 1249
Fusobacterium nucleatum 989 R----HFTPKNIRDEKYDVHEYLVVR----SVDSVDSEERVHILDKKNE--------KLA 1032
Aquifex aeolicus 1152 RDIILDVTVREERDPITGKTASVIS--FMRRRDAMLHTERIAVITEDGK--------EY 1200
Blastopirellula marina 1028 EDTVEG-ETMRLERDTSGTIRRTIP------EHKGDLHPQLVVEDEDGK--------PLD 1072
Chlamydia abortus 1051 EDLVEGISTEKVANKNTGLVELIVK------QHRGELHPQIAIYSDEGLT------ELVG 1098
Cyanobacteria bacterium 1047 VDIELG-QTVRQEDAGRGQKALVVI------EHKGDMHPQINLVDESGH--------ILD 1091
SI3
1750 1760 1770 1780 1790 1800
Acinetobacter baylyi 1094 FYFLPQNTIVTVR----------DGETIGVGDVLGRVPQET 1124
Acinetobacter ihumii 1094 FYFLPQNTIVTVR----------DGETIGVGDVIGRVPQET 1124
Acinetobacter johnsonii 1094 FYFLPQNTILSVR----------DGETIGVGDVIGRVPQET 1124
Acinetobacter ursingii 1094 FYFLPQNTIVTVR----------DGETIGVGDVIGRVPQET 1124
Acinetobacter portensis 1094 FYFLPQNTILSVR----------DGETIGVGDVIGRVPQES 1124
Acinetobacter guerrae 1094 FYFLPQNTIVTVR----------DGETIGVGDVIGRVPQET 1124
Acinetobacter rudis 1094 FYFLPQNTIVTVR----------DGETIGVGDVIGRVPQET 1124
Acinetobacter baumannii 1094 FYFLPQNTIVTVR----------DGETIGVGDVIGRVPQES 1124
Acinetobacter guillouiae 1094 FYFLPQNTIVTVR----------DGETIGVGDVIGRVPQET 1124
Acinetobacter sichuanensis 1094 FYFLPQNTIVTVR----------DGETIGVGDVIGRVPQET 1124
Acinetobacter seifertii 1094 FYFLPQNTIVTVR----------DGETIGVGDVIGRVPQET 1124
Acinetobacter pittii 1094 FYFLPQNTIVTVR----------DGETIGVGDVIGRVPQET 1124
Acinetobacter defluvii 1094 FYFLPQNTIVTVR----------DGETIGVGDVIGRVPQET 1124
Acinetobacter terrestris 1094 FYFLPQNTILSVR----------DGETIGVGDVIGRVPQET 1124
Acinetobacter pragensis 1094 FYFLPQNTILSVR----------NGETIGVGDVIGRVPQES 1124
Acinetobacter indicus 1094 FYFLPQNTILSVR----------DGETIGVGDVIGRVPQES 1124
Pseudomonas aeruginosa 1098 QYFLPANALVNLT----------DGAKVSIGDVVARIPQET 1128
Escherichia coli 1098 QYFLPGKAIVQLE----------DGVQISSGDTLARIPQES 1128
Staphylococcus aureus 941 ---------- 941
Bacillus subtilis 946 ---------- 946
Mycobacterium tuberculosis 1022 ---------- 1022
Bacteroides fragilis 1091 TYALPVGGHVVIE----------NGQKVKAGEVIVKIPRAV 1121
Thermotoga maritima 1351 ----------DIVSGLTTVEKTFEPYA 1368
Thermus aquaticus 1249 ---------- 1249
Fusobacterium nucleatum 1033 TYNIPYGAYMMVR----------DGAKVKKGDIIAKIIKLG 1063
Aquifex aeolicus 1201 IYDLPVNAILNIPPEKISLEWRVCPTCSESEETTIQHQYYVVKDLEVQPGDILARIPKET 1260
Blastopirellula marina 1073 VYYMPEKANIEVD----------EGRVINAGTVIANTPRES 1103
Chlamydia abortus 1099 TYAIPSGAIVSVE----------ENQKVDEGMLLARLPRGA 1129
Cyanobacteria bacterium 1092 FHYLPAKARIEVE----------DGETVEAGQMLARQPRGA 1122

1810 1820 1830 1840 1850 1860
Acinetobacter baylyi 1125 SRTRDITGGLPRVADLFEARKPKEHAILAEVSG---------IVSFGKET--------- 1165
Acinetobacter ihumii 1125 SRTRDITGGLPRVADLFEARKPKEHAILAEVSG---------IVSFGKET--------- 1165
Acinetobacter johnsonii 1125 SRTRDITGGLPRVADLFEARKPKEHAILAEISG---------VVSFGKET--------- 1165
Acinetobacter ursingii 1125 SRTRDITGGLPRVADLFEARKPKEHAILAEISG---------IVSFGKET--------- 1165
Acinetobacter portensis 1125 SRTRDITGGLPRVADLFEARKPKEHAILAEISG---------VVSFGKET--------- 1165
Acinetobacter guerrae 1125 SRTRDITGGLPRVADLFEARKPKEHAILAEVSG---------IVSFGKET--------- 1165
Acinetobacter rudis 1125 SRTRDITGGLPRVADLFEARKPKEHAILAEVSG---------VVSFGKET--------- 1165
Acinetobacter baumannii 1125 SRTRDITGGLPRVADLFEARKPKEHAILAEVSG---------IVSFGKET--------- 1165
Acinetobacter guillouiae 1125 SRTRDITGGLPRVADLFEARKPKEHAILAEISG---------VVSFGKET--------- 1165
Acinetobacter sichuanensis 1125 SRTRDITGGLPRVADLFEARKPKEHAILAEVSG---------IVSFGKET--------- 1165
Acinetobacter seifertii 1125 SRTRDITGGLPRVADLFEARKPKEHAILAEVSG---------IVSFGKET--------- 1165
Acinetobacter pittii 1125 SRTRDITGGLPRVADLFEARKPKEHAILAEVSG---------VVSFGKET--------- 1165
Acinetobacter defluvii 1125 SRTRDITGGLPRVADLFEARKPKEHAILAEVSG---------IVSFGKET--------- 1165
Acinetobacter terrestris 1125 SRTRDITGGLPRVADLFEARKPKEHAILAEISG---------VVSFGKET--------- 1165
Acinetobacter pragensis 1125 SRTRDITGGLPRVADLFEARKPKEHAILAEISG---------VVSFGKET--------- 1165
Acinetobacter indicus 1125 SRTRDITGGLPRVADLFEARKPKEHAILAEVSG---------IVSFGKET--------- 1165
Pseudomonas aeruginosa 1129 SKTRDITGGLPRVADLFEARRPKEPSILAEISG---------TISFGKET--------- 1169
Escherichia coli 1129 GGTKDITGGLPRVADLFEARRPKEPAILAEISG---------IVSFGKET--------- 1169
Staphylococcus aureus 941 ---SDITQGLPRIQEIFEARNPKSQAVITEIEG---------VVEDIKLAK--------- 980
Bacillus subtilis 946 ---DDITQGLPRIQELFEARNPKSQATITEIDG---------TVVEINEVR--------- 985
Mycobacterium tuberculosis 1022 ----DITGGLPRVQELFEARVPRGKAPIADVTG---------RVRLEDGE--------- 1059
Bacteroides fragilis 1122 GKAGDITGGLPRVTELFEARNPSNPAVVSEIDG---------EVTMGKVKR--------- 1163
Thermotoga maritima 1369 FLREEKSGGKKEIRKYYGS-----EAILQEVDG---------FVKDIATDE--------- 1405
Thermus aquaticus 1249 ---TDITQGLPRVIELFEARRPKAKAVISEIDG---------VVRIEEGE--------- 1287
Fusobacterium nucleatum 1064 EGTKDITGGLPRVQELFEARNPKGKATLSEIDG---------RIEILPTKKK------- 1106
Aquifex aeolicus 1261 AKVRDIVGGLPRVEELFEARKPKNPAILSEIDGYVKIYEDADEVIIFNPRTGETAKYSIK 1320
Blastopirellula marina 1104 GGIKDITGGLPRVTEIFEARKPKDPAVMAEIDG---------TVEILGEK--------- 1144
Chlamydia abortus 1130 IKTKDITGGLPRVAELVEARKPEDAADIAKIDG---------VVDFKGIQ--------- 1170
Cyanobacteria bacterium 1123 AGSQDIVGGLPRVTEIFEARKPKDPATMAEISG---------RIELHADK--------- 1163
1870 1880 1890 1900 1910 1920
Acinetobacter baylyi 1165 ---------------------------------KGKNRLVITPDDGSEIYEELIPKWR 1190
Acinetobacter ihumii 1165 ---------------------------------KGKNRLVITPDDGSEIYEELIPKWR 1190
Acinetobacter johnsonii 1165 ---------------------------------KGKNRLVITPDDGSEIYEELIPKWR 1190
Acinetobacter ursingii 1165 ---------------------------------KGKNRLVITPDDGSEIYEELIPKWR 1190
Acinetobacter portensis 1165 ---------------------------------KGKNRLVITPDDGSEIYEELIPKWR 1190
Acinetobacter guerrae 1165 ---------------------------------KGKNRLVITPDDGSEIYEELIPKWR 1190
Acinetobacter rudis 1165 ---------------------------------KGKNRLVITPDDGSEIYEELIPKWR 1190
Acinetobacter baumannii 1165 ---------------------------------KGKNRLVITPDDGSEIYEELIPKWR 1190
Acinetobacter guillouiae 1165 ---------------------------------KGKNRLVITPDDGSEIYEELIPKWR 1190
Acinetobacter sichuanensis 1165 ---------------------------------KGKNRLVITPDDGAEVYEELIPKWR 1190
Acinetobacter seifertii 1165 ---------------------------------KGKNRLVITPDDGSEIYEELIPKWR 1190
Acinetobacter pittii 1165 ---------------------------------KGKNRLVITPDDGSEIYEELIPKWR 1190
Acinetobacter defluvii 1165 ---------------------------------KGKNRLVITPDDGSEIYEELIPKWR 1190
Acinetobacter terrestris 1165 ---------------------------------KGKNRLVITPDDGSEIYEELIPKWR 1190
Acinetobacter pragensis 1165 ---------------------------------KGKNRLVITPDDGSEIYEELIPKWR 1190
Acinetobacter indicus 1165 ---------------------------------KGKNRLVITPDDGSEIYEELIPKWR 1190
Pseudomonas aeruginosa 1169 ---------------------------------KGKRRLVITPNDGSDPYEELIPKWR 1194
Escherichia coli 1169 ---------------------------------KGKPRLVITPVDGSDPYEEMIPKWR 1194
Staphylococcus aureus 980 ----------------------------------DRQQEIVVKGANETRSYLASGTS 1003
Bacillus subtilis 985 ----------------------------------DKQQEIVVQGAVETRSYTAPYNS 1008
Mycobacterium tuberculosis 1059 ---------------------------------RFYKITIVPDDGGPEVVYDKISKRQ 1084
Bacteroides fragilis 1163 ---------------------------------GNREIIVTSKTG--EVKKYLVPLSK 1186
Thermotoga maritima 1405 ---------------------------------SGRTVIYIEDYAGNIHAYKVPKRA 1429
Thermus aquaticus 1287 ----------------------------------DRLSVFVESEGFSKEYKLPKDA 1309
Fusobacterium nucleatum 1106 ---------------------------------QMRVVNVKSLENPEEFKEYLIPMGE 1131
Aquifex aeolicus 1321 KDELILVRHGQFVKKGQKITETKVAEIDGQVRIKGRGFKVIVNPETG-LQREYFVPKGK 1379
Blastopirellula marina 1144 ---------------------------------KRGKRTIIVRNEAGVEREFLVPHGK 1169
Chlamydia abortus 1170 ---------------------------------KNKRILVVRDEITGMEEEFLIPLTK 1195
Cyanobacteria bacterium 1163 ---------------------------------RKGKMTIRVISDAGVEKDHHVPSDK 1188

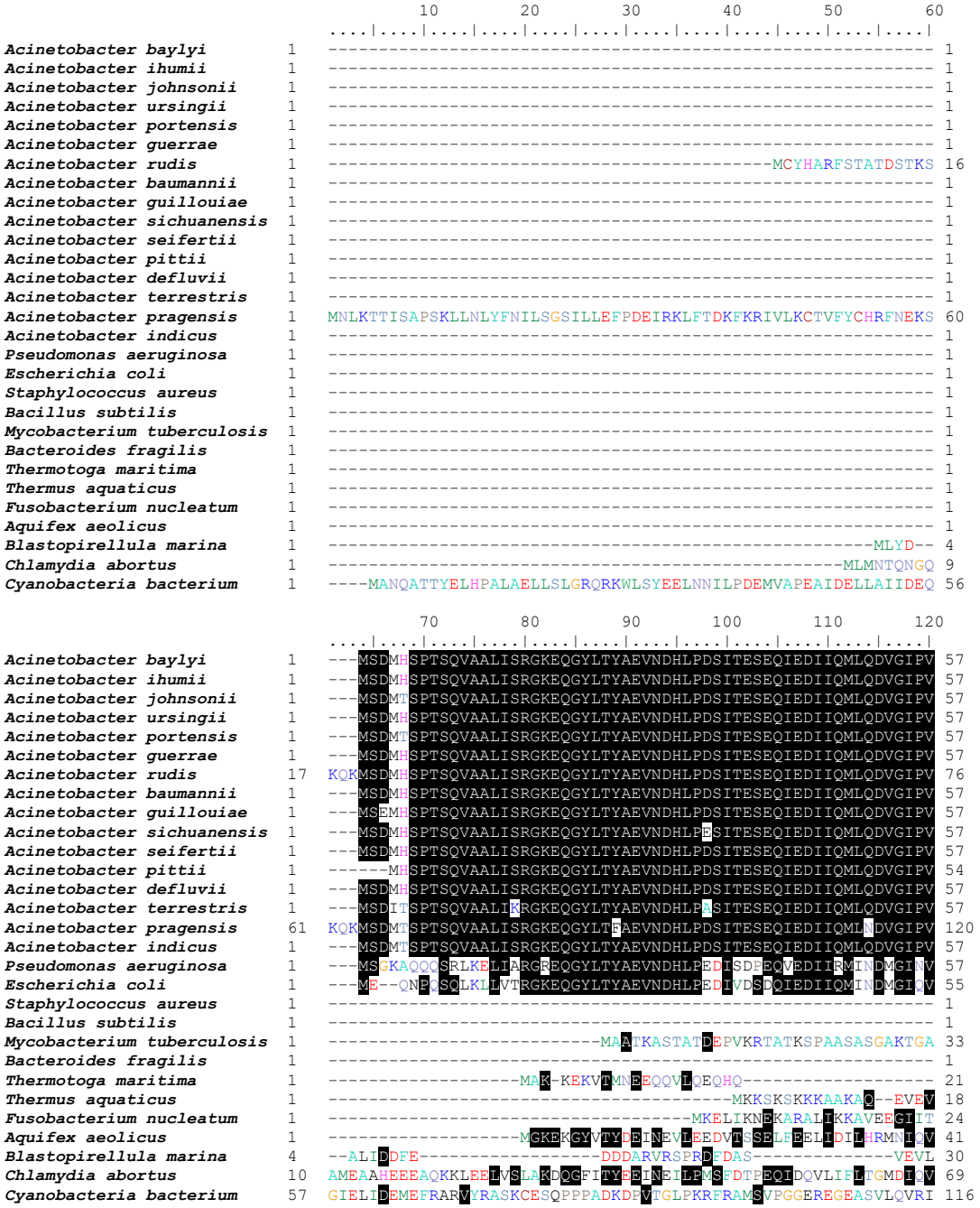

1930 1940 1950 1960 1970 1980
Acinetobacter baylyi 1191 TINVF----------EGEHVNRGETISDGPQNPHDILRLKGEV--ALTNYIVNEVQDVY 1237
Acinetobacter ihumii 1191 QINVF----------EGEHVNRGETISDGPQNPHDILRLKGEV--ALTNYIVNEVQDVY 1237
Acinetobacter johnsonii 1191 TMNVF----------EGEHVNRGETVSDGPQNPHDILRLKGEV--ALTNYIVNEVQDVY 1237
Acinetobacter ursingii 1191 QINVF----------EGEHVNRGETISDGPQNPHDILRLKGEV--ALTNYIVNEVQDVY 1237
Acinetobacter portensis 1191 TMNVF----------EGEHVNRGETVSDGPQNPHDILRLKGEV--ALTNYIVNEVQDVY 1237
Acinetobacter guerrae 1191 QINVF----------EGEHVNRGETISDGPQNPHDILRLKGEV--ALTNYIVNEVQDVY 1237
Acinetobacter rudis 1191 QINVF----------EGEHVNRGETISDGPQNPHDILRLKGEV--ALTNYIVNEVQDVY 1237
Acinetobacter baumannii 1191 QINVF----------EGEHVNRGETISDGPQNPHDILRLKGEV--ALTNYIVNEVQDVY 1237
Acinetobacter guillouiae 1191 TINVF----------EGEHVNRGETISDGPQNPHDILRLKGEV--ALTNYIVNEVQDVY 1237
Acinetobacter sichuanensis 1191 QINVF----------EGEHVNRGETISDGPQNPHDILRLKGEV--ALTNYIVNEVQDVY 1237
Acinetobacter seifertii 1191 QINVF----------EGEHVNRGETISDGPQNPHDILRLKGEV--ALTNYIVNEVQDVY 1237
Acinetobacter pittii 1191 QINVF----------EGEHVNRGETISDGPQNPHDILRLKGEV--ALTNYIVNEVQDVY 1237
Acinetobacter defluvii 1191 QINVF----------EGEHVNRGETISDGPQNPHDILRLKGEV--ALTNYIVNEVQDVY 1237
Acinetobacter terrestris 1191 TINVY----------EGEHVNRGETVSDGPQNPHDILRLKGEV--ALTNYIVNEVQDVY 1237
Acinetobacter pragensis 1191 TINVF----------EGEHVNRGETISDGPQNPHDILRLKGEV--ALTNYIVNEVQDVY 1237
Acinetobacter indicus 1191 TINVF----------EGEHVNRGETISDGPQNPHDILRLKGEV--ALTNYIVNEVQDVY 1237
Pseudomonas aeruginosa 1195 HLNVF----------EGEQVNRGEVISDGPSNPHDILRLLGVS--SLAKYIVNEIQDVY 1241
Escherichia coli 1195 QLNVF----------EGERVERGDVISDGPEAPHDILRLRGVH--AVTRYIVNEVQDVY 1241
Staphylococcus aureus 1004 RIIVE----------IGQPVQRGEVLTEGSIEEKNYLSVAGLN--ATESYLLKEVQKVY 1050
Bacillus subtilis 1009 RLKVA----------EGDKITRGQVLTEGSIDEKELLKVTDLT--TVQEYLLHEVQKVY 1055
Mycobacterium tuberculosis 1085 RLRVFKHEDGSERVLSDGDHVEVGQQLMEGSADPHEVLRVQGPR--EVQIHLVREVQEVY 1142
Bacteroides fragilis 1187 QLIVQ----------ENDYVRAGIPLSDGATTEADILAIKGPT--AVQEYIVNEVQDVY 1233
Thermotoga maritima 1430 KVRVE----------KGQKVLRGETLTSGAIVWWKLLELESEKGVMTAMNLLKIIKNAY 1478
Thermus aquaticus 1310 RLLVK----------DGDYVEAGQPLTFGAIDPHQLLEAKGPE--AVERYLVDEIQKVY 1356
Fusobacterium nucleatum 1132 RLVVT----------DGLKIKAGDKITEGAISEYDVLSIKGLV--AAEQFILESVQQVY 1178
Aquifex aeolicus 1380 FLIVK----------EGDFVKAGDQLTDGTPVPEEILRIKGIE--ELEKFLLKEVQMVY 1426
Blastopirellula marina 1170 RFIVH----------SGDIVRAGQSLIDGPLVPHDILRISGEE--AVQQYLLHEIQEVY 1216
Chlamydia abortus 1196 HLIVQ----------RGDHVMKGQQLTDGLVVPHEILEICGVR--ELQKYLVNEVQEVY 1242
Cyanobacteria bacterium 1189 GLLVH----------TGDYVQAGDPLTEGPLVPHDILRIKGEE--ALWTYMLDEVQNVY 1235
1990 2000 2010 2020 2030 2040
Acinetobacter baylyi 1238 RLQGVKINDKHIEVIVRQMLRKVDITDGGDTSFIKGEQVDYIRVVQE---------- 1284
Acinetobacter ihumii 1238 RLQGVKINDKHIEVIVRQMLRKVDIVDGGDTSFIKGEQVDYIRVVQE---------- 1284
Acinetobacter johnsonii 1238 RLQGVKINDKHIEVIVRQMLRKVDITDGGDTSFIKGEQVDYIRVMQE---------- 1284
Acinetobacter ursingii 1238 RLQGVKINDKHIEVIVRQMLRKVDITDGGDTSFIKGEQVDYIRVVQE---------- 1284
Acinetobacter portensis 1238 RLQGVKINDKHIEVIVRQMLRKVEITDGGDTSFIKGEQVEYIRVVGE---------- 1284
Acinetobacter guerrae 1238 RLQGVKINDKHIEVIVRQMLRKVDITDGGDTSFIKGEQVDYIRVVQE---------- 1284
Acinetobacter rudis 1238 RLQGVKINDKHIEVIVRQMLRKVDIVDGGDTSFIKGEQVEYIRMVQE---------- 1284
Acinetobacter baumannii 1238 RLQGVKINDKHIEVIVRQMLRKVDIIDGGDTSFIKGEQVDYIRVVQE---------- 1284
Acinetobacter guillouiae 1238 RLQGVKINDKHIEVIVRQMLRKVDITDGGDTSFIKGEQVDYIRVVGE---------- 1284
Acinetobacter sichuanensis 1238 RLQGVKINDKHIEVIVRQMLRKVDITDGGDTSFIKGEQVDYIRVVGE---------- 1284
Acinetobacter seifertii 1238 RLQGVKINDKHIEVIVRQMLRKVDITDGGDTSFIKGEQVDYIRVVQE---------- 1284
Acinetobacter pittii 1238 RLQGVKINDKHIEVIVRQMLRKVDITDGGDTSFIKGEQVDYIRVVQE---------- 1284
Acinetobacter defluvii 1238 RLQGVKINDKHIEVIVRQMLRKVDITDGGDTSFIKGEQVDYIRVVQE---------- 1284
Acinetobacter terrestris 1238 RLQGVKINDKHIEVIVRQMLRKVEITDGGDTSFIKGEQVDYIRMVTE---------- 1284
Acinetobacter pragensis 1238 RLQGVKINDKHIEVIVRQMLRKVDITDGGDTSFIKGEQVDYIRVMGE---------- 1284
Acinetobacter indicus 1238 RLQGVKINDKHIEVIVRQMLRKVEITDGGDTSFIKGEQVDYIRVVQE---------- 1284
Pseudomonas aeruginosa 1242 RLQGVKINDKHIEIILRQMLRKVEVSESGDSSFIKGDQVELTQVLEE---------- 1288
Escherichia coli 1242 RLQGVKINDKHIEVIVRQMLRKATIVNAGSSDFLEGEQVEYSRVKIA---------- 1288
Staphylococcus aureus 1051 RMQGVEIDDKHVEVMVRQMLRKVRIIEAGDTKLLPGSLVDIHNFTDA---------- 1097
Bacillus subtilis 1056 RMQGVEIGDKHVEVMVRQMLRKVRVIDAGDTDVLPGTLLDIHQFTEA---------- 1102
Mycobacterium tuberculosis 1143 RAQGVSIHDKHIEVIVRQMLRRVTIIDSGSTEFLPGSLIDRAEFEAE---------- 1189
Bacteroides fragilis 1234 RLQGVKINDKHEEIIVRQMMRKVIIDEPGDTRFLEQQVVDKLEFMEENDRIWGKKVVVDA 1293
Thermotoga maritima 1479 VQQGVSIHDKHFEIIFKQMLSMATIVDSGDSDYLPDQIVPLVDIKRFNREILEGNAKVEE 1538
Thermus aquaticus 1357 RAQGVKLHDKHIEIVVRQMLKYVEVTDPGDSRLLEGQVLEKWDVEAL---------- 1403
Fusobacterium nucleatum 1179 RDQGVTVNDKHIEIIVKQMERKVRIVDSGASLFLEDEVIEKRVVDLEN---------- 1226
Aquifex aeolicus 1427 KLQGVDINDKHEEIIIKQMLKKVRIIDPGDSRFLVGEEVDKEELEEE---------- 1473
Blastopirellula marina 1217 RSQGVEINDKHIEIIVARMLRKVMVDKPGDTTLLPGLVMDKIEFRSANEHLAKCLKISDP 1276
Chlamydia abortus 1243 RLQGVDINDKHVEIIVRQMLQKVRITDPGDTTLLFGEEVNKKEFYEE---------- 1289
Cyanobacteria bacterium 1236 RAQGVTINDKHIEIIVSQMLRKVRVENPGDTDLLPHEVIDRFHFRQHNTRVGNMVRISDA 1295

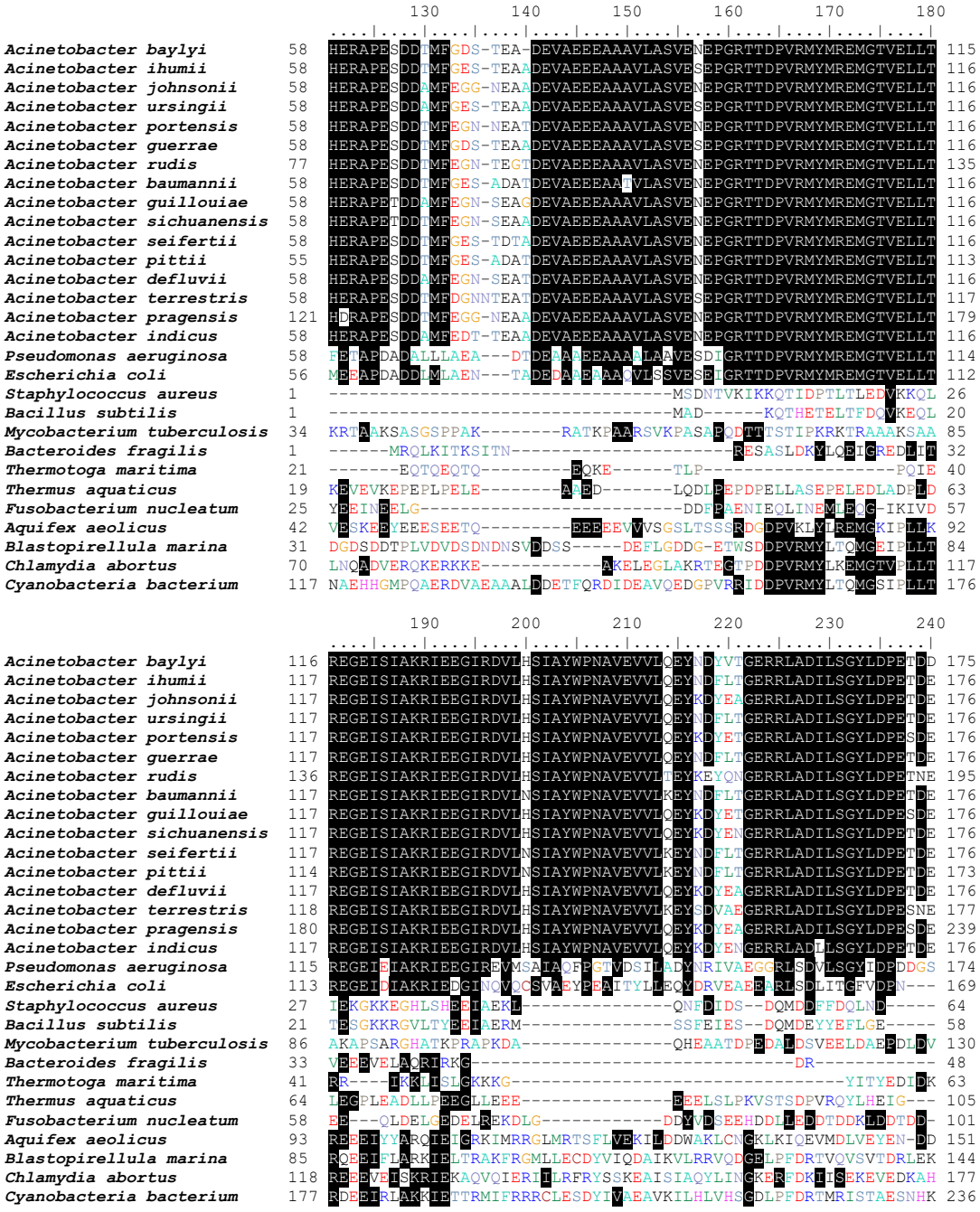

2050 2060 2070 2080 2090 2100
Acinetobacter baylyi 1284 NQAVLAQNK 1293
Acinetobacter ihumii 1284 NQAVLAQNK 1293
Acinetobacter johnsonii 1284 NQAVLAQNK 1293
Acinetobacter ursingii 1284 NQAVLAQNK 1293
Acinetobacter portensis 1284 NQAVLAQNK 1293
Acinetobacter guerrae 1284 NQAVLAQNK 1293
Acinetobacter rudis 1284 NQAVSAQNK 1293
Acinetobacter baumannii 1284 NQAVLAQNK 1293
Acinetobacter guillouiae 1284 NQAVLAQNK 1293
Acinetobacter sichuanensis 1284 NQAVLAQNK 1293
Acinetobacter seifertii 1284 NQAVLAQNK 1293
Acinetobacter pittii 1284 NQAVLAQNK 1293
Acinetobacter defluvii 1284 NQATLAQNK 1293
Acinetobacter terrestris 1284 NQAVLAQNK 1293
Acinetobacter pragensis 1284 NQAVLAQNK 1293
Acinetobacter indicus 1284 NQAVLAQNK 1293
Pseudomonas aeruginosa 1288 NEQLGTEDK 1297
Escherichia coli 1288 NRELEANGK 1297
Staphylococcus aureus 1097 NREAFKHRK 1106
Bacillus subtilis 1102 NKKVLLEGN 1111
Mycobacterium tuberculosis 1189 NRRVVAEGG 1198
Bacteroides fragilis 1294 GDSENLKAGQIVTARKLRDENSMLKRRDLK PV 1325
Thermotoga maritima 1539 NRKWVIGKTLAKRIITETEEGELVELAQKGDEVTEELLKKIIEAGIKEIDVFEKDKVVTY 1598
Thermus aquaticus 1403 NERLIAEGK 1412
Fusobacterium nucleatum 1226 KKLEEEGKALIKYEP 1241
Aquifex aeolicus 1473 IQRIKLEGG 1482
Blastopirellula marina 1277 GDSQQFGEGMIVPKDALEQENAQIEALGGK ST 1308
Chlamydia abortus 1289 NKRTEEEGG 1298
Cyanobacteria bacterium 1296 G-GTDLKIGDLVEKSVIREANARAEAEGKE PA 1326
2110 2120 2130 2140 2150 2160
Acinetobacter baylyi 1293 FPAKFERQLMGITKASLSTDSFISAASFQETTRVLTEAAVTGKEDDLRGLKENVV 1348
Acinetobacter ihumii 1293 FPAKFERQLMGITKASLSTDSFISAASFQETTRVLTEAAVTGKEDDLRGLKENVV 1348
Acinetobacter johnsonii 1293 FPAKFERQLMGITKASLSTDSFISAASFQETTRVLTEAAVTGKEDDLRGLKENVV 1348
Acinetobacter ursingii 1293 FPAKFERQLMGITKASLSTDSFISAASFQETTRVLTEAAVTGKEDDLRGLKENVV 1348
Acinetobacter portensis 1293 FPAKFERQLMGITKASLSTDSFISAASFQETTRVLTEAAVTGKEDDLRGLKENVV 1348
Acinetobacter guerrae 1293 FPAKFERQLMGITKASLSTDSFISAASFQETTRVLTEAAVTGKEDDLRGLKENVV 1348
Acinetobacter rudis 1293 FPAKYERILMGITKASLSTDSFISAASFQETTRVLTEAAVTGKEDDLRGLKENVV 1348
Acinetobacter baumannii 1293 FPAKFERQLMGITKASLSTDSFISAASFQETTRVLTEAAVTGKEDDLRGLKENVV 1348
Acinetobacter guillouiae 1293 FPAKYERQLMGITKASLSTDSFISAASFQETTRVLTEAAVTGKEDDLRGLKENVV 1348
Acinetobacter sichuanensis 1293 FPAKFERQLMGITKASLSTDSFISAASFQETTRVLTEAAVTGKEDDLRGLKENVV 1348
Acinetobacter seifertii 1293 FPAKFERQLMGITKASLSTDSFISAASFQETTRVLTEAAVTGKEDDLRGLKENVV 1348
Acinetobacter pittii 1293 FPAKFERQLMGITKASLSTDSFISAASFQETTRVLTEAAVTGKEDDLRGLKENVV 1348
Acinetobacter defluvii 1293 FPAKFERQLMGITKASLSTDSFISAASFQETTRVLTEAAVTGKEDELRGLKENVV 1348
Acinetobacter terrestris 1293 FPAKYERQLMGITKASLSTDSFISAASFQETTRVLTEAAVTGKEDDLRGLKENVV 1348
Acinetobacter pragensis 1293 FPAKFERQLMGITKASLSTDSFISAASFQETTRVLTEAAVTGKEDDLRGLKENVV 1348
Acinetobacter indicus 1293 FPAKFDRQLMGITKASLSTDSFISAASFQETTRVLTEAAVTGKEDDLRGLKENVV 1348
Pseudomonas aeruginosa 1297 FPAKYERVLLGITKASLSTESFISAASFQETTRVLTEAAVTGKRDFLRGLKENVV 1352
Escherichia coli 1297 VGATYSRQLLGITKASLATESFISAASFQETTRVLTEAAVAGKRDELRGLKENVI 1352
Staphylococcus aureus 1106 RPATAKPVLLGITKASLETESFLSAASFQETTRVLTDAAIKGKRDDLLGLKENVI 1161
Bacillus subtilis 1111 RPATGRPVLLGITKASLETDSFLSAASFQETTRVLTDAAIKGKRDELLGLKENVI 1166
Mycobacterium tuberculosis 1198 EPAAGRPVLMGITKASLATDSWLSAASFQETTRVLTDAAINCRSDKLNGLKENVI 1253
Bacteroides fragilis 1326 EVRDAVAATSTQILQGITRAALDTSSFMSAASFQETTKVLNEAAINGKTDKLEGMKENVI 1385
Thermotoga maritima 1599 QILPKEPTKYKRRLLSLKKAALNYPGWLSAAAFEETAWVLTAAAIEGKVDPLIGLKENVI 1658
Thermus aquaticus 1412 VPVAWKPLLMGVTKSALSTKSWLSAASFQNTTHVLTEAAIAGKKDELIGLKENVI 1467
Fusobacterium nucleatum 1241 VIQGITKAAVNTGSFISAASFQETTKVLSNAAIEGKVDYLEGLKENVI 1289
Aquifex aeolicus 1482 KLPKAEPVIVGITRAALSTRSWISAASFQETTRVLTDASVEGKTDELRGLKENVI 1537
Blastopirellula marina 1309 KGSRPKPATASTQLLGITKAAVQSSSFISAASFQETTKVLTEAALAGKVDHLVGLKENVI 1368
Chlamydia abortus 1298 KPAQAVPVLLGITKASLSTESFISAASFQDTTRVLTDAACSSKTDYLLGEKENVI 1353
Cyanobacteria bacterium 1327 KAKRARPATARTLLGITKASLQSDSFLSGASFQETTKVLTEAALKGAEDELIGLKENVL 1386

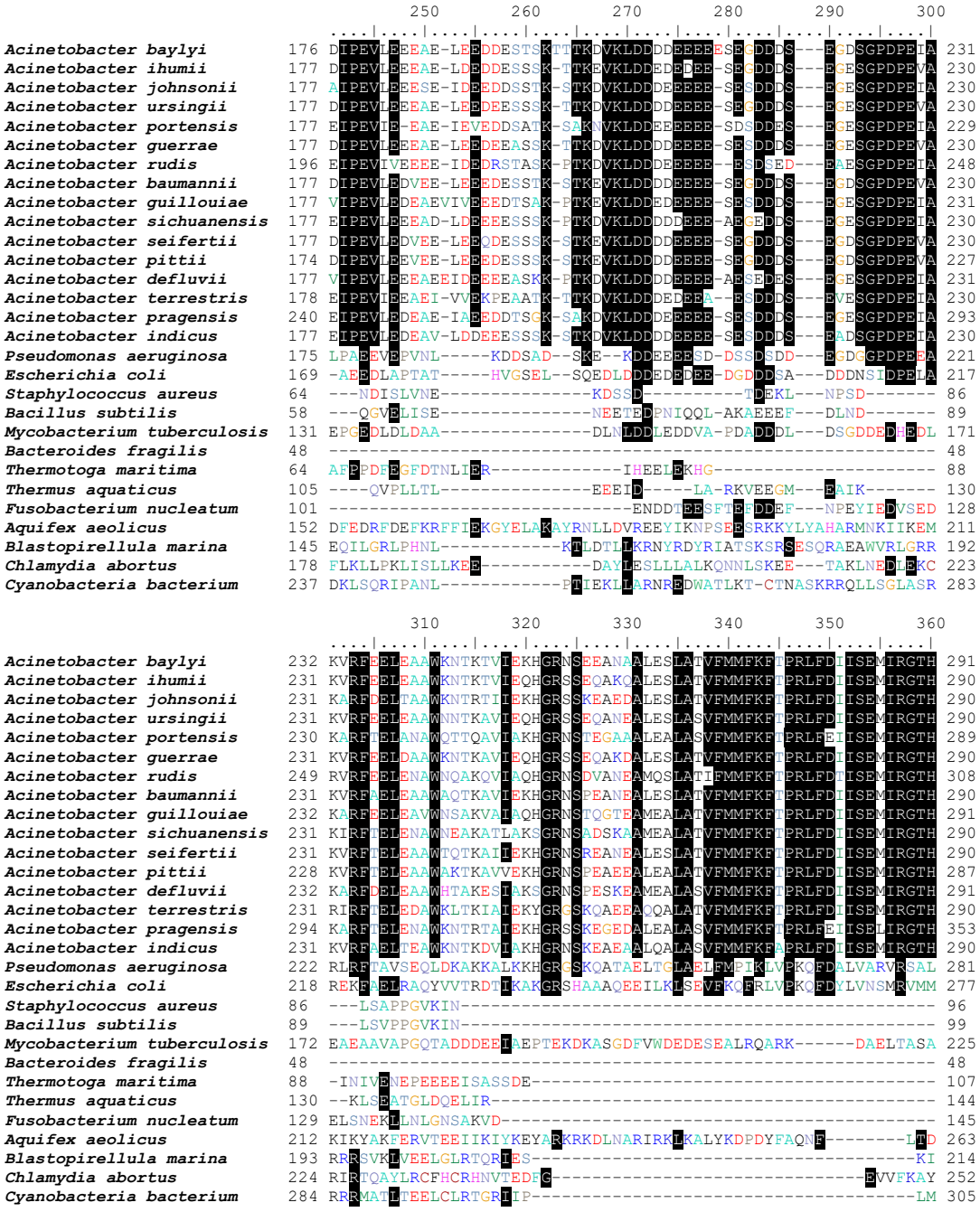


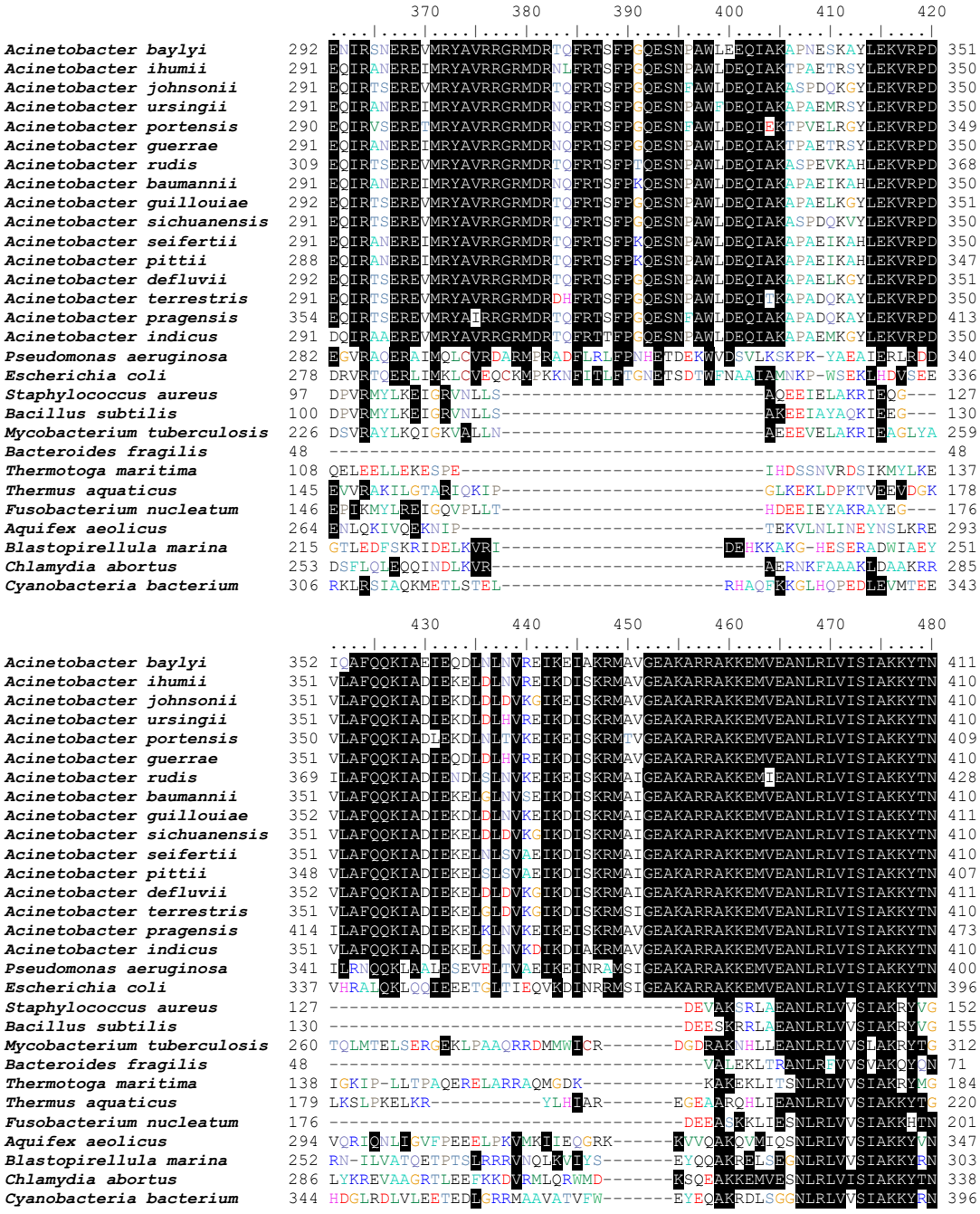
[1] Protein sequences were selected from the NCBI database (https://www.ncbi.nlm.nih.gov/protein) and aligned using CLUSTALW implemented within BioEdit using default parameters [115]. Black background denotes 55% identity, which highlights the high sequence identity within the *Acinetobacter* species.

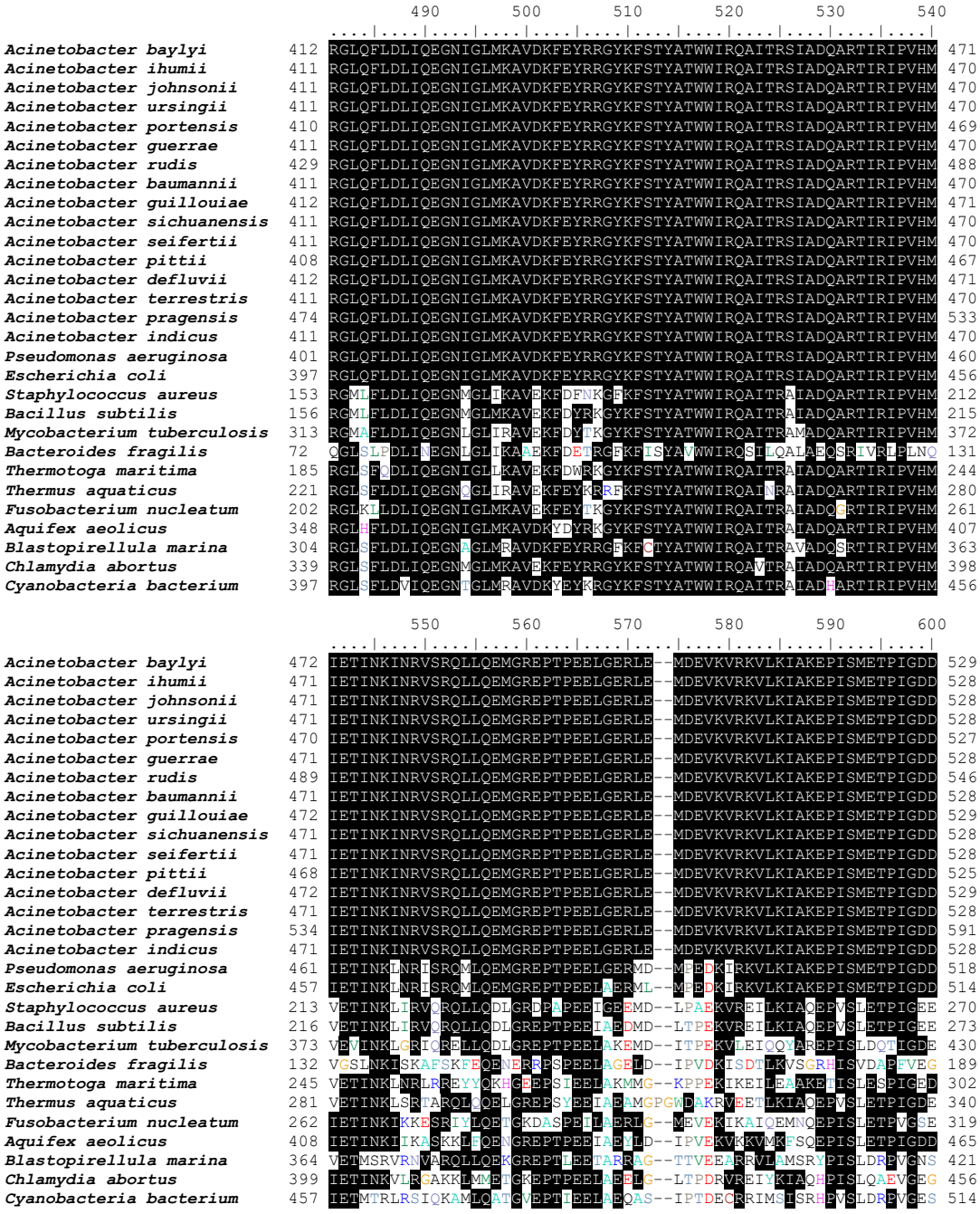
Figure A2.6. Sequence alignment of Sigma70 subunit (RpoD)[1].

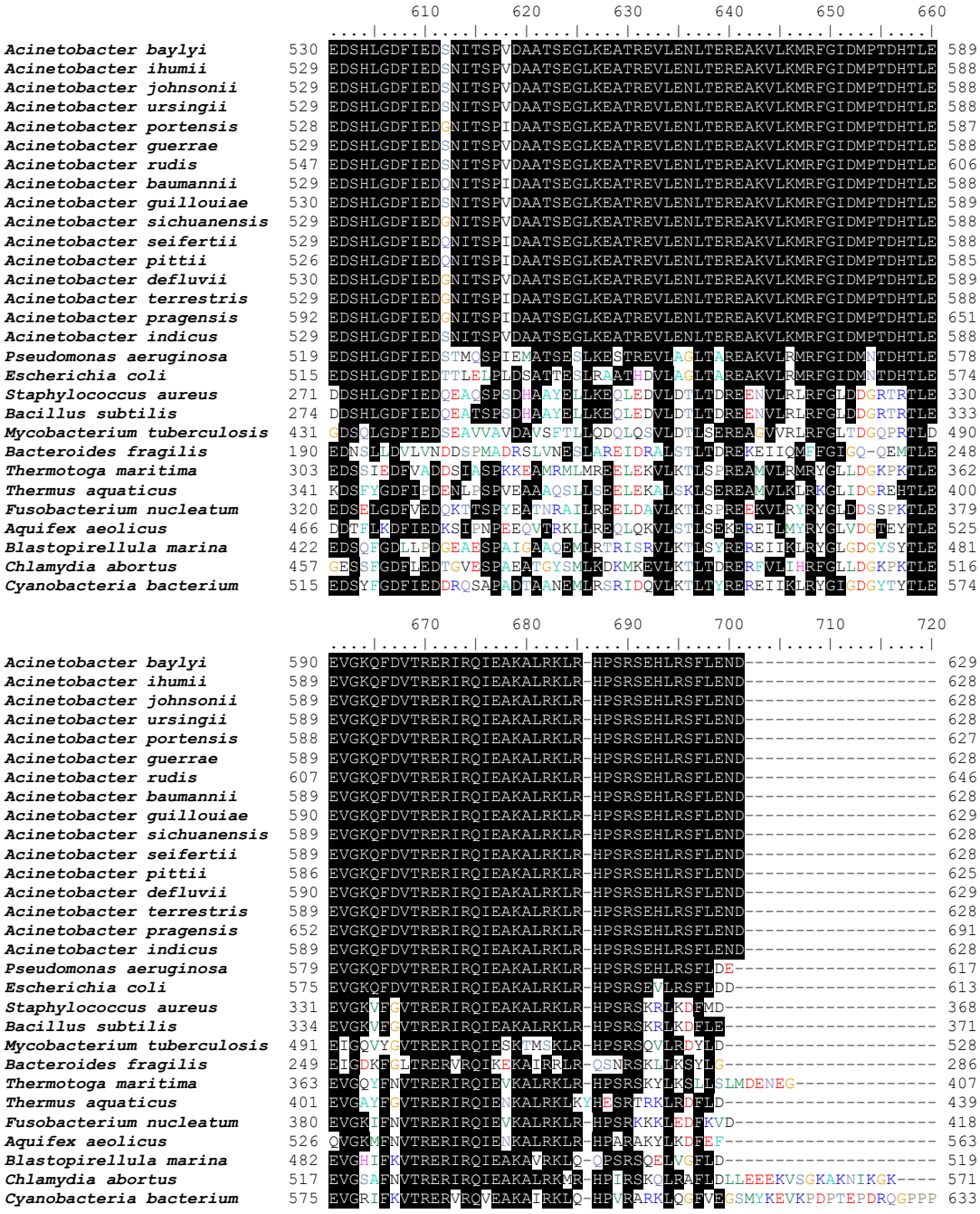



130 140 150 160 170 180
Acinetobacter baylyi 58 HERAPESDDAMFGDS-TEA-DEVAEEEAAAVLASVENEPGRTTDPVRMYMREMGTVELLT 115
Acinetobacter ihumii 58 HERAPESDDAMFGES-TEAADEVAEEEAAAVLASVESEPGRTTDPVRMYMREMGTVELLT 116
Acinetobacter johnsonii 58 HERAPESDDAMFEGG-NEAADEVAEEEAAAVLASVENEPGRTTDPVRMYMREMGTVELLT 116
Acinetobacter ursingii 58 HERAPESDDAMFGES-TEAADEVAEEEAAAVLASVESEPGRTTDPVRMYMREMGTVELLT 116
Acinetobacter portensis 58 HERAPESDDAMFEGN-NEATDEVAEEEAAAVLASVENEPGRTTDPVRMYMREMGTVELLT 116
Acinetobacter guerrae 58 HERAPESDDAMFGDS-TEAADEVAEEEAAAVLASVESEPGRTTDPVRMYMREMGTVELLT 116
Acinetobacter rudis 77 HERAPESDDAMFEGN-TEGTDEVAEEEAAAVLASVENEPGRTTDPVRMYMREMGTVELLT 135
Acinetobacter baumannii 58 HERAPESDDAMFGES-ADATDEVAEEEAAAVLASVENEPGRTTDPVRMYMREMGTVELLT 116
Acinetobacter guillouiae 58 HERAPETDDAMFEGN-SEAGDEVAEEEAAAVLASVENEPGRTTDPVRMYMREMGTVELLT 116
Acinetobacter sichuanensis 58 HERAPETDDAMFEGN-SEAADEVAEEEAAAVLASVENEPGRTTDPVRMYMREMGTVELLT 116
Acinetobacter seifertii 58 HERAPESDDAMFGES-TDTADEVAEEEAAAVLASVENEPGRTTDPVRMYMREMGTVELLT 116
Acinetobacter pittii 55 HERAPESDDAMFGES-ADATDEVAEEEAAAVLASVENEPGRTTDPVRMYMREMGTVELLT 113
Acinetobacter defluvii 58 HERAPESDDAMFEGN-SEATDEVAEEEAAAVLASVENEPGRTTDPVRMYMREMGTVELLT 116
Acinetobacter terrestris 58 HERAPESDDAMFDGNNTEATDEVAEEEAAAVLASVESEPGRTTDPVRMYMREMGTVELLT 117
Acinetobacter pragensis 121 HDRAPESDDAMFEGG-NEAADEVAEEEAAAVLASVENEPGRTTDPVRMYMREMGTVELLT 179
Acinetobacter indicus 58 HERAPESDDAMFEDT-TEAADEVAEEEAAAVLASVENEPGRTTDPVRMYMREMGTVELLT 116
Pseudomonas aeruginosa 58 FETAPDADALLLAEA---DTDEAAEEAAAALAAVESDIGRTTDPVRMYMREMGTVELLT 114
Escherichia coli 56 MEEAPDADDIMLAEN---TADEDAEEAAAEVLSSVESEIGRTTDPVRMYMREMGTVELLT 112
Staphylococcus aureus 1 ----------MSDNTVKIKKQTIDPTLTLEDVKKQL 26
Bacillus subtilis 1 ----------MAD------KQTHETELTFDQVKEQL 20
Mycobacterium tuberculosis 34 KRTAAKSASGSPPAK--------RATKPAARSVKPASAPQDTTTSTIPKRKTRAAAKSAA 85
Bacteroides fragilis 1 ------MRQLKITKSITN----------RESASLDKYLQEIGREDLLT 32
Thermotoga maritima 21 -------EQTQEQTQ---------EQKE------TLP----------PQIE 40
Thermus aquaticus 19 KEVEVKEPEPLPELE--------AAED-------LQDLPEPDPELLASEPELEDLADPLD 63
Fusobacterium nucleatum 25 YEEINEELG----------DDFPAENIEQLINEMLEQG-IKIVD 57
Aquifex aeolicus 42 VESKEEYEEESEETQ---------EEEEEVVVSGSLTSSSRDGDPVKLYTREMGKIPLLK 92
Blastopirellula marina 31 DGDSDDTPLVDVDSDNDNSVDDSS------DEFLGDDG-ETWSDDPVRMYLTQMGEIPLLT 84
Chlamydia abortus 70 LNQADVERQKERKKE----------AKELEGLAKRTEGPPDDPVRMYLKEMGTVPLLT 117
Cyanobacteria bacterium 117 NAEHHGMPQAERDVAEAAALDDETFQRDIDEAVQEDGPVRRIDDPVRMYLTQMGSIPLLT 176
190 200 210 220 230 240
Acinetobacter baylyi 116 REGEISIAKRIEEGIRDVLHSIAYWPNAVEVVLQEYNDYVTGERRLADILSGYLDPETDD 175
Acinetobacter ihumii 117 REGEISIAKRIEEGIRDVLHSIAYWPNAVEVVLQEYNDFLTGERRLADILSGYLDPETDE 176
Acinetobacter johnsonii 117 REGEISIAKRIEEGIRDVLHSIAYWPNAVEVVLQEYKDYEAGERRLADILSGYLDPETDE 176
Acinetobacter ursingii 117 REGEISIAKRIEEGIRDVLHSIAYWPNAVEVVLQEYNDFLTGERRLADILSGYLDPETDE 176
Acinetobacter portensis 117 REGEISIAKRIEEGIRDVLHSIAYWPNAVEVVLQEYKDYETGERRLADILSGYLDPESDE 176
Acinetobacter guerrae 117 REGEISIAKRIEEGIRDVLHSIAYWPNAVEVVLQEYNDFLTGERRLADILSGYLDPETDE 176
Acinetobacter rudis 136 REGEISIAKRIEEGIRDVLHSIAYWPNAVEVVLTEYKEYQNGERRLADILSGYLDPETNE 195
Acinetobacter baumannii 117 REGEISIAKRIEEGIRDVLNSIAYWPNAVEVVLKEYNDFLTGERRLADILSGYLDPETDE 176
Acinetobacter guillouiae 117 REGEISIAKRIEEGIRDVLHSIAYWPNAVEVVLQEYKDYETGERRLADILSGYLDPESDE 176
Acinetobacter sichuanensis 117 REGEISIAKRIEEGIRDVLHSIAYWPNAVEVVLQEYKDYENGERRLADILSGYLDPETDE 176
Acinetobacter seifertii 117 REGEISIAKRIEEGIRDVLNSIAYWPNAVEVVLKEYNDFLTGERRLADILSGYLDPETDE 176
Acinetobacter pittii 114 REGEISIAKRIEEGIRDVLNSIAYWPNAVEVVLKEYNDFLTGERRLADILSGYLDPETDE 173
Acinetobacter defluvii 117 REGEISIAKRIEEGIRDVLHSIAYWPNAVEVVLQEYKDYEAGERRLADILSGYLDPETDE 176
Acinetobacter terrestris 118 REGEISIAKRIEEGIRDVLHSIAYWPNAVEVVLKEYSDVAEGERRLADILSGYLDPESNE 177
Acinetobacter pragensis 180 REGEISIAKRIEEGIRDVLHSIAYWPNAVEVVLQEYKDYEAGERRLADILSGYLDPESDE 239
Acinetobacter indicus 117 REGEISIAKRIEEGIRDVLHSIAYWPNAVEVVLQEYKDYENGERRLADLLSGYLDPETDE 176
Pseudomonas aeruginosa 115 REGEIEIAKRIEEGIREVMSAIAQEPGTVDSILADYNRIVAEGGRLSDVLSGYIDPDDGS 174
Escherichia coli 113 REGEIDIAKRIEDGINQVQSVAEYPEAITYLLEQYDRVEAEEARLSDLITGFVDPN--- 169
Staphylococcus aureus 27 IEKGKKEGHLSHEEIAEKL----------QNFDIDS--DQMDDFFDQLND----- 64
Bacillus subtilis 21 TESGKKRGVLTYEEIAERM----------SSFEIES--DQMDEYYEFLGE----- 58
Mycobacterium tuberculosis 86 AKAPSARGHATKPRAPKDA----------QHEAATDPEDALDSVEELDAEEDLDV 130
Bacteroides fragilis 33 VEEEVELAQRIRKG----------DR---------- 48
Thermotoga maritima 41 RR----IKKIISLGKKKG----------YITYEDIDK 63
Thermus aquaticus 64 LEGPLEADLLPEEGLLEEE----------EEELSLPKVSTSDPVRQYLHEIG--- 105
Fusobacterium nucleatum 58 EE---QLDELGEDEIREKDLG----------DDYVDSEEHDDILEDDTDDKLDDTDD- 101
Aquifex aeolicus 93 REEEIYYARQIEIGRKIMRRGLMRTSFLVEKILDDWAKLCNGKLKIQEVMDLVEYEN-DD 151
Blastopirellula marina 85 RQEEIFLARKIELTRAKFRGMLLECDYVIQDAIKVLRRVQDGELPFDRTVQVSVTDRLEK 144
Chlamydia abortus 118 REEEVEISKRIEKAQVQIERIILRFRYSSKEAISIAQYLINGKERFDKTISEKEVEDKAH 177
Cyanobacteria bacterium 177 RDEEIRLAKKIETTRMIFRRRCLESDYIVAEAVKILHLVHSGDLPFDRTMRISTAESNHK 236


250 260 270 280 290 300
Acinetobacter baylyi 176 DIPEVLEEEAE-LEEDDESTSKTTKDVKLDDDEEEESEGDDDS---EGDSGPDPELA 231
Acinetobacter ihumii 177 DIPEVLEEEAE-LDEDDESSSK-TKEVKLDDEDEDEE-SEGDDDS---EGESGPDPEVA 230
Acinetobacter johnsonii 177 AIPEVLEEESE-IDEEDDSSTK-STKDVKLDDDEEEEE-SESDDDS---EGESGPDPELA 230
Acinetobacter ursingii 177 DIPEVLEEEAE-LEEDEESSSK-TTKDVKLDDDEEEE-SEGDDDS---EGESGPDPEVA 230
Acinetobacter portensis 177 EIPEVIE-EAE-IEVEDDSATK-SAKNVKLDDEEEEEE-SDSDDDS---EGESGPDPELA 229
Acinetobacter guerrae 177 DIPEVLEEEAE-LEEDEEASSK-TTKDVKLDDDEEEE-SESDDDS---EGESGPDPEVA 230
Acinetobacter rudis 196 EIPEVIVEEEE-IDEDDRSTASK-PTKDVKLDDDEEEEE--ESDSED---EAESGPDPELA 248
Acinetobacter baumannii 177 DIPEVLEDVEE-LEEEDESSTK-STKEVKLDDDEEEE-SEGDDDS---EGDSGPDPEVA 230
Acinetobacter guillouiae 177 VIPEVLEDEAEVIVEEEDTSAK-PTKEVKLDDEDEEEE-SESDDDS---EGESGPDPELA 231
Acinetobacter sichuanensis 177 EIPEVLEEEAD-LDEEEESSSK-PTKDVKLDDDDEEEE-AEGEDDS---EGESGPDPELA 230
Acinetobacter seifertii 177 DIPEVLEDVEE-LEEQDESSSK-STKEVKLDDDEEEE-SEGDDDS---EGDSGPDPEVA 230
Acinetobacter pittii 174 DIPEVLEEVEE-LEEEDESSSK-STKEVKLDDDEEEE-SEGDDDS---EGDSGPDPEVA 227
Acinetobacter defluvii 177 VIPEVLEEEAEEIDEEEEASKK-PTKDVKLDDDEEEE-AESEDES---EGESGPDPELA 231
Acinetobacter terrestris 178 EIPEVIEEAEI-VVEKPEAATK-TTKDVKLDDDEDEEA---EESDDDS---EVESGPDPELA 230
Acinetobacter pragensis 240 EIPEVLEDEAE-IAEEDDTSGK-SAKDVKLDDDEEEE-SESDDDS---EGESGPDPELA 293
Acinetobacter indicus 177 EIPEVLEDEAV-LDDEEESSSK-STKDVKLDDDEEEE-SESDDDS---EADSGPDPELA 230
Pseudomonas aeruginosa 175 LPAEEVEPVNL-----KDDSAD--SKE--KDDEEEESD-DSSDSDD---EGDGPDPEEA 221
Escherichia coli 169 -AEEDLAPTAT-----HVGSEL--SQEDLDDDEDEDEE-DGDDDSA---DDDNSIDPELA 217
Staphylococcus aureus 64 ---NDISLVNE----------KDSSD----------TDEKL---NPSD------- 86
Bacillus subtilis 58 ---QGVELISE----------NEETEDPNIQQL-AKAEEEF---DLND------- 89
Mycobacterium tuberculosis 131 EPGEDLDLDAA----------DLNLDDLEDDVA-PDADDDL---DSGDDEDHEDL 171
Bacteroides fragilis 48 ---------- 48
Thermotoga maritima 64 AFPPDFEGFDTNLIER----------IHEELEKHG---------- 88
Thermus aquaticus 105 ----QVPLLTL----------EEEID-----LA-RKVEEGM---EAIK------- 130
Fusobacterium nucleatum 101 ----------ENDDIEESFTEFDDEF---NPEYIEDVSED 128
Aquifex aeolicus 152 DFEDRFDEFKRFFIEKGYELAKAYRNLLDVREEYIKNPSEESRKKYLYAHARMNKIIKEM 211
Blastopirellula marina 145 EQILGRLPHNL----------KELDTLLKRNYRDYRIATSKSRSESQRAEAWVRLGRR 192
Chlamydia abortus 178 FLKLLPKLISLLKEE----------DAYLESLLLALKQNNLSKEE---TAKLNEDLEKC 223
Cyanobacteria bacterium 237 DKLSQRIPANL----------PTIEKLLARNREDWATLKT-CTNASKRRQLLSGLASR 283
310 320 330 340 350 360
Acinetobacter baylyi 232 KVRFEELEAAWKNTKTVIEKHGRNSEEANAALESLATVFMMFKFTPRLFDIISEMIRGTH 291
Acinetobacter ihumii 231 KVRFEELEAAWKNTKTVIEQHGRSSEQAKQALESLATVFMMFKFTPRLFDIISEMIRGTH 290
Acinetobacter johnsonii 231 KARFEELTAAWKNTRTIIEKHGRSSKEAEDALESLATVFMMFKFTPRLFDIISEMIRGTH 290
Acinetobacter ursingii 231 KVRFEELEAAWNNTKAVIEQHGRSSEQANEALESLASVFMMFKFTPRLFDIISEMIRGTH 290
Acinetobacter portensis 230 KARFEELANAWQTTQAVIAKHGRNSIEGAAALEALASVFMMFKFTPRLFEIISEMIRGTH 289
Acinetobacter guerrae 231 KVRFEELDAAWKNTKAVIEQHGRSSEQAKDALESLATVFMMFKFTPRLFDIISEMIRGTH 290
Acinetobacter rudis 249 RVRFEELENAWNQAKQVIAQHGRNSDVANEAMQSLATIFMMFKFTPRLFDIISEMIRGTH 308
Acinetobacter baumannii 231 KVRFEELEAAWAQTKAVIEKHGRNSPEANEALESLATVFMMFKFTPRLFDIISEMIRGTH 290
Acinetobacter guillouiae 232 KARFEELEAVWNSAKVAIAQHGRNSIQGTEAMEALATVFMMFKFTPRLFDIISEMIRGTH 291
Acinetobacter sichuanensis 231 KIRFEELENAWNEAKATLAKSGRNSADSKAAMEALATVFMMFKFTPRLFDIISEMIRGTH 290
Acinetobacter seifertii 231 KVRFEELEAAWTQTKAIIEKHGRNSREANEALESLATVFMMFKFTPRLFDIISEMIRGTH 290
Acinetobacter pittii 228 KVRFEELEAAWAKTKAVVEKHGRNSPEAEEALEALATVFMMFKFTPRLFDIISEMIRGTH 287
Acinetobacter defluvii 232 KARFEELEAAWHTAKESIAKSGRNSPESKEAMEALASVFMMFKFTPRLFDIISEMIRGTH 291
Acinetobacter terrestris 231 RIRFEELEDAWKLTKIAIEKYGRSSKQAEEAQQALATVFMMFKFTPRLFDIISEMIRGTH 290
Acinetobacter pragensis 294 KARFEELENAWKNTRTAIEKHGRSSKEGEDALEALASVFMMFKFTPRLFEIISEIIRGTH 353
Acinetobacter indicus 231 KVRFEELTEAWKNTKDVIAKHGRNSKEAEAALQALASVFMMFKFTPRLFDIISEMIRGTH 290
Pseudomonas aeruginosa 222 RLRFTAVSEQLDKAKKALKKHGRSSKQATAELTGLAELFMPIKLVPKQFDALVARVRSAL 281
Escherichia coli 218 REKFEELRAQYVVTRDTIKAKGRSHAAAQEEILKLSEVFKQFRLVPKQFDYLVNSMRVMM 277
Staphylococcus aureus 86 ---LSAPPGVKIN---------- 96
Bacillus subtilis 89 ---LSVPPGVKIN---------- 99
Mycobacterium tuberculosis 172 EAEAAVAPGQTADDDEEIAEPTEKDKASGDFVWDEDESEALRQARK------DAELTASA 225
Bacteroides fragilis 48 ---------- 48
Thermotoga maritima 88 -INIVENEPEEEEISASSDE---------- 107
Thermus aquaticus 130 --KLSEATGLDQELIR---------- 144
Fusobacterium nucleatum 129 ELSNEKLLNLGNSAKVD---------- 145
Aquifex aeolicus 212 KIKYAKFERVTEEIIKIYKEYARKRKDLNARIRKIKALYKDPDYFAQNF--------LID 263
Blastopirellula marina 193 RRRSVKLVEELGLRTQRIES----------KI 214
Chlamydia abortus 224 RIRTQAYLRCFHCRHNVTEDFG----------EVVFKAY 252
Cyanobacteria bacterium 284 RRRMATLTEELCLRTGRIIP----------LM 305


370 380 390 400 410 420
Acinetobacter baylyi 292 ENIRSNEREVMRYAVRRGRMDRTQFRTSFPGQESNPAWLEEQIAKAPNESKAYLEKVRPD 351
Acinetobacter ihumii 291 EQIRANEREIMRYAVRRGRMDRNLFRTSFPGQESNPAWLDEQIAKTPAETRSYLEKVRPD 350
Acinetobacter johnsonii 291 EQIRTSEREVMRYAVRRGRMDRTQFRTSFPGQESNEAWLDEQIAKASPDQKGYLEKVRPD 350
Acinetobacter ursingii 291 EQIRANEREIMRYAVRRGRMDRNQFRTSFPGQESNPAWSDEQIAKAPAEMRSYLEKVRPD 350
Acinetobacter portensis 290 EQIRVSEREIMRYAVRRGRMDRNQFRTSFPGQESNEAWLDEQIEKTPVELRGYLEKVRPD 349
Acinetobacter guerrae 291 EQIRANEREIMRYAVRRGRMDRNQFRTSFPGQESNPAWLDEQIAKTPAETRSYLEKVRPD 350
Acinetobacter rudis 309 EQIRTSEREVMRYAVRRGRMDRTQFRTSFPTQESNPAWLDEQIAKASPEVKAHLEKVRPD 368
Acinetobacter baumannii 291 EQIRANEREIMRYAVRRGRMDRNQFRTSFPKQESNPAWLDEQIAKAPAEIKAHLEKVRPD 350
Acinetobacter guillouiae 292 EQIRTSEREVMRYAVRRGRMDRTQFRTSFPGQESNPAWLDEQIAKAPAELKGYLEKVRPD 351
Acinetobacter sichuanensis 291 EQIRTSEREVMRYAVRRGRMDRTQFRTSFPGQESNPAWLDEQIAKASPDQKVYLEKVRPD 350
Acinetobacter seifertii 291 EQIRANEREIMRYAVRRGRMDRTQFRTSFPKQESNPAWLDEQIAKAPAEIKAHLEKVRPD 350
Acinetobacter pittii 288 EQIRANEREIMRYAVRRGRMDRTQFRTSFPKQESNPAWLDEQIAKAPAEIKAHLEKVRPD 347
Acinetobacter defluvii 292 EQIRTSEREVMRYAVRRGRMDRTQFRTSFPGQESNPAWLDEQIAKAPAELKGYLEKVRPD 351
Acinetobacter terrestris 291 EQIRTSEREVMRYAVRRGRMDRDHFRTSFPGQESNPAWLDEQIKAPADQKAYLEKVRPD 350
Acinetobacter pragensis 354 EQIRTSEREVMRYAIRRGRMDRTQFRTSFPGQESNEAWLDEQIAKAPADQKAYLEKVRPD 413
Acinetobacter indicus 291 DQIRAAEREVMRYAVRRGRMDRTQFRTTFPGQESNPAWLDEQIAKAPAEMKGYLEKVRPD 350
Pseudomonas aeruginosa 282 EGVRAQERAIMQLCVRDARMPRADFLRLFPNHETDEKWVDSVLKSKPK-YAEAIERLRDD 340
Escherichia coli 278 DRVRTQERLIMKLCVEQCKMPKKNFITLFTGNETSDTWFNAAIAMNKP-WSEKLHDVSEE 336
Staphylococcus aureus 97 DPVRMYLKEIGRVNLLS----------AQEEIELAKRIEQG--- 127
Bacillus subtilis 100 DPVRMYLKEIGRVNLLS----------AKEEIAYAQKIEEG--- 130
Mycobacterium tuberculosis 226 DSVRAYLKQIGKVALLN----------AEEEVELAKRIEAGLYA 259
Bacteroides fragilis 48 ---------- 48
Thermotoga maritima 108 QELEELLEKESPE----------IHDSSNVRDSIKMYLKE 137
Thermus aquaticus 145 EVVRAKILGTARIQKIP----------GLKEKLDPKTVEEVDGK 178
Fusobacterium nucleatum 146 EPIKMYLREIGQVPLLT----------HDEEIEYAKRAYEG--- 176
Aquifex aeolicus 264 ENLQKIVQEKNIP----------TEKVLNLINEYNSLKRE 293
Blastopirellula marina 215 GTLEDFSKRIDELKVRI----------DEHKKAKG-HESERADWIAEY 251
Chlamydia abortus 253 DSFLQLEQQINDLKVR----------AERNKFAAAKLDAAKRR 285
Cyanobacteria bacterium 306 RKLRSIAQKMETLSTEL----------RHAQEKKGLHQPEDLEVMTEE 343
430 440 450 460 470 480
Acinetobacter baylyi 352 IQAFQQKIADIEQDLNLNVREIKEIAKRMAVGEAKARRAKKEMVEANLRLVISIAKKYTN 411
Acinetobacter ihumii 351 VLAFQQKIADIEKELDLNVREIKDISKRMAVGEAKARRAKKEMVEANLRLVISIAKKYTN 410
Acinetobacter johnsonii 351 VLAFQQKIADIEKDLDLDVKGIKEISKRMAVGEAKARRAKKEMVEANLRLVISIAKKYTN 410
Acinetobacter ursingii 351 VLAFQQKIADIEKDLDLHVREIKDISKRMAVGEAKARRAKKEMVEANLRLVISIAKKYTN 410
Acinetobacter portensis 350 VLAFQQKIADLEKDLNLTVKEIKEISKRMTVGEAKARRAKKEMVEANLRLVISIAKKYTN 409
Acinetobacter guerrae 351 VLAFQQKIADIEQDLDLHVREIKDISKRMAVGEAKARRAKKEMVEANLRLVISIAKKYTN 410
Acinetobacter rudis 369 ILAFQQKIADIENDLSLNVKEIKEISKRMAIGEAKARRAKKEMIEANLRLVISIAKKYTN 428
Acinetobacter baumannii 351 VLAFQQKIADIEKELGLNVSEIKDISKRMAIGEAKARRAKKEMVEANLRLVISIAKKYTN 410
Acinetobacter guillouiae 352 VLAFQQKIADIEKDLDLNVKEIKDISKRMAIGEAKARRAKKEMVEANLRLVISIAKKYTN 411
Acinetobacter sichuanensis 351 VLAFQQKIADIEKELDLDVKGIKDISKRMAIGEAKARRAKKEMVEANLRLVISIAKKYTN 410
Acinetobacter seifertii 351 VLAFQQKIADIEKELNLSVAEIKDISKRMAIGEAKARRAKKEMVEANLRLVISIAKKYTN 410
Acinetobacter pittii 348 VLAFQQKIADIEKELSLSVAEIKDISKRMAIGEAKARRAKKEMVEANLRLVISIAKKYTN 407
Acinetobacter defluvii 352 VLAFQQKIADIEKELDLDVKGIKDISKRMAIGEAKARRAKKEMVEANLRLVISIAKKYTN 411
Acinetobacter terrestris 351 VLAFQQKIADIEKELGLDVKGIKDISKRMSIGEAKARRAKKEMVEANLRLVISIAKKYTN 410
Acinetobacter pragensis 414 ILAFQQKIADIEKELKLNVKEIKEISKRMAVGEAKARRAKKEMVEANLRLVISIAKKYTN 473
Acinetobacter indicus 351 VLAFQQKIADIEKELGLNVKDIKDIAKRMAVGEAKARRAKKEMVEANLRLVISIAKKYTN 410
Pseudomonas aeruginosa 341 ILRNQQKLAALESEVELTVAEIKEINRAMSIGEAKARRAKKEMVEANLRLVISIAKKYTN 400
Escherichia coli 337 VHRAIQKLQQIEEETGLTIEQVKDINRRMSIGEAKARRAKKEMVEANLRLVISIAKKYTN 396
Staphylococcus aureus 127 ----------DEVAKSRLAEANLRLVVSIAKRYVG 152
Bacillus subtilis 130 ----------DEESKRRLAEANLRLVVSIAKRYVG 155
Mycobacterium tuberculosis 260 TQLMTELSERGEKLPAAQRRDMMWICR-------DGDRAKNHLLEANLRLVVSLAKRYTG 312
Bacteroides fragilis 48 ----------VALEKLTRANLRFVVSVAKQYQN 71
Thermotoga maritima 138 IGKIP-LLTPAQERELARRAQMGDK----------KAKEKLITSNLRLVVSIAKRYMG 184
Thermus aquaticus 179 LKSLPKELKR----------YLHIAR-------EGEAARQHLIEANLRLVVSIAKKYTG 220
Fusobacterium nucleatum 176 ----------DEEASKKLIESNLRLVVSIAKKHTN 201
Aquifex aeolicus 294 VQRIQNLIGVFPEEELPKVMKIIEQGRK------KVVQAKQVMIQSNLRLVVSIAKKYVN 347
Blastopirellula marina 252 RN-ILVATQETPTSLRRRVNQLKVIYS-------EYQQAKRELSEGNLRLVVSIAKKYRN 303
Chlamydia abortus 286 LYKREVAAGRTLEEFKKDVRMLQRWMD-------KSQEAKKEMVESNLRLVISIAKKYTN 338
Cyanobacteria bacterium 344 HDGLRDLVLEETEDLGRRMAAVATVFW-------EYEQAKRDLSGGNLRLVVSIAKKYRN 396

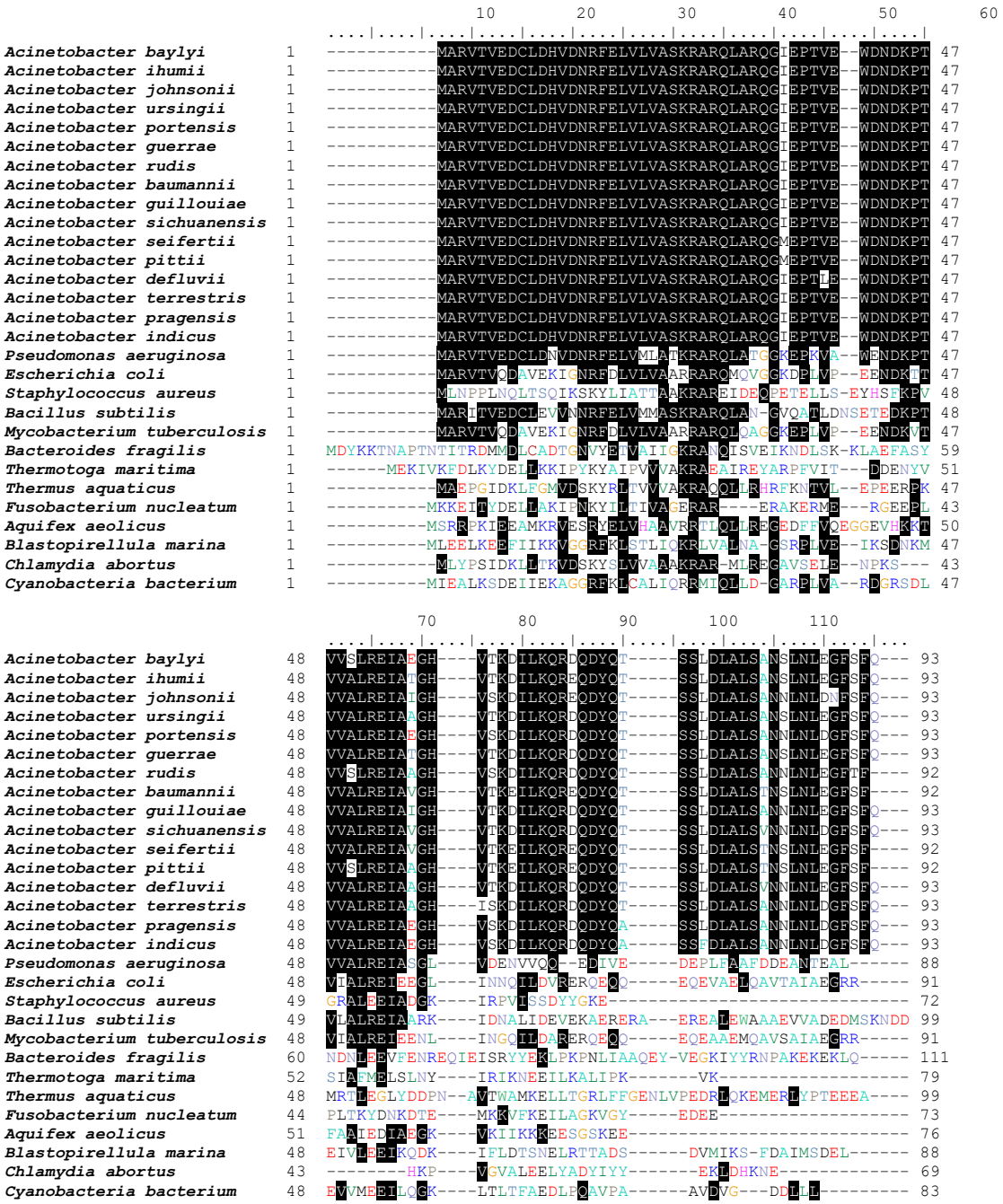

490 500 510 520 530 540
Acinetobacter baylyi 412 RGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWWIRQAITRSIADQARTIRIPVHM 471
Acinetobacter ihumii 411 RGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWWIRQAITRSIADQARTIRIPVHM 470
Acinetobacter johnsonii 411 RGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWWIRQAITRSIADQARTIRIPVHM 470
Acinetobacter ursingii 411 RGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWWIRQAITRSIADQARTIRIPVHM 470
Acinetobacter portensis 410 RGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWWIRQAITRSIADQARTIRIPVHM 469
Acinetobacter guerrae 411 RGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWWIRQAITRSIADQARTIRIPVHM 470
Acinetobacter rudis 429 RGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWWIRQAITRSIADQARTIRIPVHM 488
Acinetobacter baumannii 411 RGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWWIRQAITRSIADQARTIRIPVHM 470
Acinetobacter guillouiae 412 RGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWWIRQAITRSIADQARTIRIPVHM 471
Acinetobacter sichuanensis 411 RGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWWIRQAITRSIADQARTIRIPVHM 470
Acinetobacter seifertii 411 RGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWWIRQAITRSIADQARTIRIPVHM 470
Acinetobacter pittii 408 RGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWWIRQAITRSIADQARTIRIPVHM 467
Acinetobacter defluvii 412 RGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWWIRQAITRSIADQARTIRIPVHM 471
Acinetobacter terrestris 411 RGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWWIRQAITRSIADQARTIRIPVHM 470
Acinetobacter pragensis 474 RGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWWIRQAITRSIADQARTIRIPVHM 533
Acinetobacter indicus 411 RGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWWIRQAITRSIADQARTIRIPVHM 470
Pseudomonas aeruginosa 401 RGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWWIRQAITRSIADQARTIRIPVHM 460
Escherichia coli 397 RGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWWIRQAITRSIADQARTIRIPVHM 456
Staphylococcus aureus 153 RGMLFLDLIQEGNMGLIKAVEKFDFNKGFKFSTYATWWIRQAITRAIADQARTIRIPVHM 212
Bacillus subtilis 156 RGMLFLDLIQEGNMGLMKAVEKFDYRKGYKFSTYATWWIRQAITRAIADQARTIRIPVHM 215
Mycobacterium tuberculosis 313 RGMAFLDLIQEGNLGLIRAVEKFDYTKGYKFSTYATWWIRQAITRAMADQARTIRIPVHM 372
Bacteroides fragilis 72 QGLSLPDLIQEGNLGLIKAAEKFDETRGFKFLSYAVWWIRQSILQALAEQSRIVRLPLNQ 131
Thermotoga maritima 185 RGLSFQDLIQEGNIGLLKAVEKFDWRKGYKFSTYATWWIRQAITRAIADQARTIRIPVHM 244
Thermus aquaticus 221 RGLSFLDLIQEGNQGLIRAVEKFEYKRRFKFSTYATWWIRQAINRAIADQARTIRIPVHM 280
Fusobacterium nucleatum 202 RGLKILDLIQEGNIGLMKAVEKFEYTKGYKFSTYATWWIRQAITRAIADQERTIRIPVHM 261
Aquifex aeolicus 348 RGLHFLDLIQEGNIGLMKAVDKYDYRKGYKFSTYATWWIRQAITRAIADQARTIRIPVHM 407
Blastopirellula marina 304 RGLSFLDLIQEGNAGLMRAVDKFEYRRGFKFCTYATWWIRQAITRAVADQSRTIRIPVHM 363
Chlamydia abortus 339 RGLSFLDLIQEGNMGLMKAVEKFEYRRGYKFSTYATWWIRQAVTRAIADQARTIRIPVHM 398
Cyanobacteria bacterium 397 RGLSFLDVIQEGNIGLMRAVDKYEYKRGYKFSTYATWWIRQAITRAIADEARTIRIPVHM 456
550 560 570 580 590 600
Acinetobacter baylyi 472 IETINKINRVSRQLLQEMGREPTPEELGERLE--MDEVKVRKVLKIAKEPISMETPIGDD 529
Acinetobacter ihumii 471 IETINKINRVSRQLLQEMGREPTPEELGERLE--MDEVKVRKVLKIAKEPISMETPIGDD 528
Acinetobacter johnsonii 471 IETINKINRVSRQLLQEMGREPTPEELGERLE--MDEVKVRKVLKIAKEPISMETPIGDD 528
Acinetobacter ursingii 471 IETINKINRVSRQLLQEMGREPTPEELGERLE--MDEVKVRKVLKIAKEPISMETPIGDD 528
Acinetobacter portensis 470 IETINKINRVSRQLLQEMGREPTPEELGERLE--MDEVKVRKVLKIAKEPISMETPIGDD 527
Acinetobacter guerrae 471 IETINKINRVSRQLLQEMGREPTPEELGERLE--MDEVKVRKVLKIAKEPISMETPIGDD 528
Acinetobacter rudis 489 IETINKINRVSRQLLQEMGREPTPEELGERLE--MDEVKVRKVLKIAKEPISMETPIGDD 546
Acinetobacter baumannii 471 IETINKINRVSRQLLQEMGREPTPEELGERLE--MDEVKVRKVLKIAKEPISMETPIGDD 528
Acinetobacter guillouiae 472 IETINKINRVSRQLLQEMGREPTPEELGERLE--MDEVKVRKVLKIAKEPISMETPIGDD 529
Acinetobacter sichuanensis 471 IETINKINRVSRQLLQEMGREPTPEELGERLE--MDEVKVRKVLKIAKEPISMETPIGDD 528
Acinetobacter seifertii 471 IETINKINRVSRQLLQEMGREPTPEELGERLE--MDEVKVRKVLKIAKEPISMETPIGDD 528
Acinetobacter pittii 468 IETINKINRVSRQLLQEMGREPTPEELGERLE--MDEVKVRKVLKIAKEPISMETPIGDD 525
Acinetobacter defluvii 472 IETINKINRVSRQLLQEMGREPTPEELGERLE--MDEVKVRKVLKIAKEPISMETPIGDD 529
Acinetobacter terrestris 471 IETINKINRVSRQLLQEMGREPTPEELGERLE--MDEVKVRKVLKIAKEPISMETPIGDD 528
Acinetobacter pragensis 534 IETINKINRVSRQLLQEMGREPTPEELGERLE--MDEVKVRKVLKIAKEPISMETPIGDD 591
Acinetobacter indicus 471 IETINKINRVSRQLLQEMGREPTPEELGERLE--MDEVKVRKVLKIAKEPISMETPIGDD 528
Pseudomonas aeruginosa 461 IETINKLNRISRQMLQEMGREPTPEELGERMD--MPEDKIRKVLKIAKEPISMETPIGDD 518
Escherichia coli 457 IETINKLNRISRQMLQEMGREPTPEELAERML--MPEDKIRKVLKIAKEPISMETPIGDD 514
Staphylococcus aureus 213 VETINKLIRVQRQLLQDLGRDPAPEEIGEEMD--LPAEKVREILKIAQEPVSLETPIGEE 270
Bacillus subtilis 216 VETINKLIRVQRQLLQDLGREPTPEEIAEDMD--LTPEKVREILKIAQEPVSLETPIGEE 273
Mycobacterium tuberculosis 373 VEVINKLGRIQRELLQDLGREPTPEELAKEMD--ITPEKVLEIQQYAREPISLDQTIGDE 430
Bacteroides fragilis 132 VGSLNKISKAFSKFEQENERRPSPEELAGELD--IPVDKISDTLKVSGRHISVDAPEVEG 189
Thermotoga maritima 245 VETINKLNRLREEYYQKHGREPSIEELAKMMG--KPPEKIKEILEAAKEPISLESPIGED 302
Thermus aquaticus 281 VETINKLSRTAROLQELGREPSYEEIAEAMGPGWDAKRVEETLKIAQEPVSLETPIGDE 340
Fusobacterium nucleatum 262 IETINKIKKESRIYLQETGKDASPEILAERLG--MEVEKIKAIQEMNQEPISLETPVGSE 319
Aquifex aeolicus 408 IETINKIIKASKKLLQENGREPTPEEIAEELD--IPVEKVKKVMKFSQEPISLETPIGDD 465
Blastopirellula marina 364 VETMSRVRLVAROLLQEKGREPTLEETARRAG--TTVEEARRVLAMSRYPISLDRPVGNS 421
Chlamydia abortus 399 IETINKVLRGAKKLMMETGKEPTPEELAEELG--LTPDRVREIYKIAQHPISLQAEVGEG 456
Cyanobacteria bacterium 457 IETMTRLRSIQKAMLQATGVEPTIEELAEQAS--IPTDEGRRIMSISRHPVSLDRPVGES 514


610 620 630 640 650 660
Acinetobacter baylyi 530 EDSHLGDFIEDSNITSPVDAATSEGLKEATREVLENLTEREAKVLKMRFGIDMPTDHTLE 589
Acinetobacter ihumii 529 EDSHLGDFIEDSNITSPVDAATSEGLKEATREVLENLTEREAKVLKMRFGIDMPTDHTLE 588
Acinetobacter johnsonii 529 EDSHLGDFIEDSNITSPVDAATSEGLKEATREVLENLTEREAKVLKMRFGIDMPTDHTLE 588
Acinetobacter ursingii 529 EDSHLGDFIEDSNITSPVDAATSEGLKEATREVLENLTEREAKVLKMRFGIDMPTDHTLE 588
Acinetobacter portensis 528 EDSHLGDFIEDGNITSPIDAATSEGLKEATREVLENLTEREAKVLKMRFGIDMPTDHTLE 587
Acinetobacter guerrae 529 EDSHLGDFIEDSNITSPVDAATSEGLKEATREVLENLTEREAKVLKMRFGIDMPTDHTLE 588
Acinetobacter rudis 547 EDSHLGDFIEDSNITSPVDAATSEGLKEATREVLENLTEREAKVLKMRFGIDMPTDHTLE 606
Acinetobacter baumannii 529 EDSHLGDFIEDQNITSPIDAATSEGLKEATREVLENLTEREAKVLKMRFGIDMPTDHTLE 588
Acinetobacter guillouiae 530 EDSHLGDFIEDSNITSPVDAATSEGLKEATREVLENLTEREAKVLKMRFGIDMPTDHTLE 589
Acinetobacter sichuanensis 529 EDSHLGDFIEDGNITSPVDAATSEGLKEATREVLENLTEREAKVLKMRFGIDMPTDHTLE 588
Acinetobacter seifertii 529 EDSHLGDFIEDQNITSPIDAATSEGLKEATREVLENLTEREAKVLKMRFGIDMPTDHTLE 588
Acinetobacter pittii 526 EDSHLGDFIEDQNITSPIDAATSEGLKEATREVLENLTEREAKVLKMRFGIDMPTDHTLE 585
Acinetobacter defluvii 530 EDSHLGDFIEDGNITSPVDAATSEGLKEATREVLENLTEREAKVLKMRFGIDMPTDHTLE 589
Acinetobacter terrestris 529 EDSHLGDFIEDGNITSPIDAATSEGLKEATREVLENLTEREAKVLKMRFGIDMPTDHTLE 588
Acinetobacter pragensis 592 EDSHLGDFIEDGNITSPIDAATSEGLKEATREVLENLTEREAKVLKMRFGIDMPTDHTLE 651
Acinetobacter indicus 529 EDSHLGDFIEDSNITSPVDAATSEGLKEATREVLENLTEREAKVLKMRFGIDMPTDHTLE 588
Pseudomonas aeruginosa 519 EDSHLGDFIEDSTMQSPIEMATSESLKESTREVLAGLTAREAKVLRMRFGIDMNTDHTLE 578
Escherichia coli 515 EDSHLGDFIEDTTLELPLDSATTESLRAATHDVLAGLTAREAKVLRMRFGIDMYTDHTLE 574
Staphylococcus aureus 271 DDSHLGDFIEDQEAQSPSDSAAYELLKEQLEDVLDTLTDREENVLRLRFGLDDGRTRTLE 330
Bacillus subtilis 274 DDSHLGDFIEDQEATSPSDSAAYELLKEQLEDVLDTLTDREENVLRLRFGLDDGRTRTLE 333
Mycobacterium tuberculosis 431 GDSQLGDFIEDSEAVVAVDAVSFTLLQDQLQSVLDTLSEREAGVVRLRFGLTDGQPRTLD 490
Bacteroides fragilis 190 EDNSLLDVLVNDDSPMADRSLVNESLAREIDRALSTLTDREKEIIQMRFGIGQ-QEMTLE 248
Thermotoga maritima 303 EDSSIEDFVADDSLPSPKKEAMRMLMREELEKVLKTLSPREAMVLRMRYGLLDGKPKTLE 362
Thermus aquaticus 341 KDSFYGDFIEDENLESPVEAAAQSILSEELEKALSKLSEREAMVLKLRKGLIDGREHTLE 400
Fusobacterium nucleatum 320 EDSELGDFVEDQKTSPYEAINRAILREELDAVLKTLSPREEKVLRYRYGLDDSSPKTLE 379
Aquifex aeolicus 466 DDTFLKDFIEDKSLPNPEEQVTRKLLREQLQKVLSTLSEKEREIIMYRYGLVDGTEYTLE 525
Blastopirellula marina 422 EDSQFGDLLEDGEAESPAIGAAQEMLRTRISRVLKTLSYREREIIKLRYGLGDGYSYTLE 481
Chlamydia abortus 457 GESSFGDFLEDTGVESPAEAIGYSMLKDKMKEVLKTLTDREREVLIHRFGLLDGKPKTLE 516
Cyanobacteria bacterium 515 EDSYFGDFIEDDRQSAPADTAANEMLRSRIDQVLKTLTYREREIIKLRYGIGDGYTYTLE 574
670 680 690 700 710 720
Acinetobacter baylyi 590 EVGKQFDVTRERIRQIEAKALRKLR-HPSRSEHLRSFLEND---------- 629
Acinetobacter ihumii 589 EVGKQFDVTRERIRQIEAKALRKLR-HPSRSEHLRSFLEND---------- 628
Acinetobacter johnsonii 589 EVGKQFDVTRERIRQIEAKALRKLR-HPSRSEHLRSFLEND---------- 628
Acinetobacter ursingii 589 EVGKQFDVTRERIRQIEAKALRKLR-HPSRSEHLRSFLEND---------- 628
Acinetobacter portensis 588 EVGKQFDVTRERIRQIEAKALRKLR-HPSRSEHLRSFLEND---------- 627
Acinetobacter guerrae 589 EVGKQFDVTRERIRQIEAKALRKLR-HPSRSEHLRSFLEND---------- 628
Acinetobacter rudis 607 EVGKQFDVTRERIRQIEAKALRKLR-HPSRSEHLRSFLEND---------- 646
Acinetobacter baumannii 589 EVGKQFDVTRERIRQIEAKALRKLR-HPSRSEHLRSFLEND---------- 628
Acinetobacter guillouiae 590 EVGKQFDVTRERIRQIEAKALRKLR-HPSRSEHLRSFLEND---------- 629
Acinetobacter sichuanensis 589 EVGKQFDVTRERIRQIEAKALRKLR-HPSRSEHLRSFLEND---------- 628
Acinetobacter seifertii 589 EVGKQFDVTRERIRQIEAKALRKLR-HPSRSEHLRSFLEND---------- 628
Acinetobacter pittii 586 EVGKQFDVTRERIRQIEAKALRKLR-HPSRSEHLRSFLEND---------- 625
Acinetobacter defluvii 590 EVGKQFDVTRERIRQIEAKALRKLR-HPSRSEHLRSFLEND---------- 629
Acinetobacter terrestris 589 EVGKQFDVTRERIRQIEAKALRKLR-HPSRSEHLRSFLEND---------- 628
Acinetobacter pragensis 652 EVGKQFDVTRERIRQIEAKALRKLR-HPSRSEHLRSFLEND---------- 691
Acinetobacter indicus 589 EVGKQFDVTRERIRQIEAKALRKLR-HPSRSEHLRSFLEND---------- 628
Pseudomonas aeruginosa 579 EVGKQFDVTRERIRQIEAKALRKLR-HPSRSEHLRSFLDE---------- 617
Escherichia coli 575 EVGKQFDVTRERIRQIEAKALRKLR-HPSRSEVLRSFLDD---------- 613
Staphylococcus aureus 331 EVGKVFGVTRERIRQIEAKALRKLR-HPSRSKRLKDFMD---------- 368
Bacillus subtilis 334 EVGKVFGVTRERIRQIEAKALRKLR-HPSRSKRLKDFLE---------- 371
Mycobacterium tuberculosis 491 EIGQVYGVTRERIRQIESKTMSKLR-HPSRSQVLRDYLD---------- 528
Bacteroides fragilis 249 EIGDKFGLTRERVRQIKEKAIRRLR-QSNRSKLLKSYLG---------- 286
Thermotoga maritima 363 EVGQYFNVTRERIRQIEVKALRKLR-HPSRSKYLKSLLSLMDENEG---------- 407
Thermus aquaticus 401 EVGAYFGVTRERIRQIENKALRKLKYHESRTRKLRDFLD---------- 439
Fusobacterium nucleatum 380 EVGKIFNVTRERIRQIEVKALRKLR-HPSRKKKLEDFKVD---------- 418
Aquifex aeolicus 526 QVGKVFNVTRERIRQIENKALRKLR-HPARAKYLKDFEF---------- 563
Blastopirellula marina 482 EVGHIFKVTRERIRQIEAKAVRKLQ-QPSRSQELVGFLD---------- 519
Chlamydia abortus 517 EVGSAFNVTRERIRQIEAKALRKMR-HPIRSKQLRAFLDLLEEEKVSGKAKNIKGK---- 571
Cyanobacteria bacterium 575 EVGRIFKVTRERVRQVEAKAIRKLQ-HPVRARKLQGFVEGSMYKEVKPDPTEPDRQGPPP 633

```
                                .
Acinetobacter baylyi          629 - 629
Acinetobacter ihumii          628 - 628
Acinetobacter johnsonii       628 - 628
Acinetobacter ursingii        628 - 628
Acinetobacter portensis       627 - 627
Acinetobacter guerrae         628 - 628
Acinetobacter rudis           646 - 646
Acinetobacter baumannii       628 - 628
Acinetobacter guillouiae      629 - 629
Acinetobacter sichuanensis    628 - 628
Acinetobacter seifertii       628 - 628
Acinetobacter pittii          625 - 625
Acinetobacter defluvii        629 - 629
Acinetobacter terrestris      628 - 628
Acinetobacter pragensis       691 - 691
Acinetobacter indicus         628 - 628
Pseudomonas aeruginosa        617 - 617
Escherichia coli              613 - 613
Staphylococcus aureus         368 - 368
Bacillus subtilis             371 - 371
Mycobacterium tuberculosis    528 - 528
Bacteroides fragilis          286 - 286
Thermotoga maritima           407 - 407
Thermus aquaticus             439 - 439
Fusobacterium nucleatum       418 - 418
Aquifex aeolicus              563 - 563
Blastopirellula marina        519 - 519
Chlamydia abortus             571 - 571
Cyanobacteria bacterium       634 T 634
```

[1] Protein sequences were selected from the NCBI database (https://www.ncbi.nlm.nih.gov/protein) and aligned using CLUSTALW implemented within BioEdit using default parameters [115]. Black background denotes 55% identity, which highlights the high sequence identity within the *Acinetobacter* species.

Figure A2.7. Sequence alignment of Omega subunit (RpoZ)[1].



[1] Protein sequences were selected from the NCBI database (https://www.ncbi.nlm.nih.gov/protein) and aligned using CLUSTALW implemented within BioEdit using default parameters [115]. Black background denotes 55% identity, which highlights the high sequence identity within the *Acinetobacter* species.

Appendix B (Chapter 3)

Table A3.1. Crystallographic statistics.

	ACIAD0746 Native	YeiE-EBD Native	ACIAD0746 SeMet
Data collection			
Space group	$P2_12_12_1$	$P4_32_12$	$P2_12_12_1$
Cell dimensions (Å)	*a*=107.334, *b*=110.694, *c*=111.481[a]	*a*=*b*=71.098, *c*=196.336	*a*=106.597, *b*=109.69, *c*=110.268[a]
Wavelength, (Å)	1.0	1.0	0.97932 (Se peak)
Resolution, (Å)	26.23-2.294 (2.376-2.294)	31.8-1.929 (1.998-1.929)	100-2.7 (2.7-2.75)
R_{merge}, %[b]	4.7 (40.3)	9.1[c]	6.5 (44.4)
Average Redundancy	6.6 (4.9)	6.1 (3.3)	3.2 (3.2)
$\langle I/\sigma(I) \rangle$	45.9 (4.2)	29.3 (0.9)	16.7 (2.1)
Completeness (%)	99.1 (98.0)	99.5 (99.9)	95.8 (92.9)
Refinement			
Resolution (Å)	26.23-2.294	31.8-1.929	
No. reflections	57744	38749	
R_{work} / R_{free}	0.2077 / 0.2446	0.1564 / 0.1819	
No. atoms			
Macromolecules	9238	3233	
Ligand/ion	4	68	
Water	461	3233	
B factors (Å^2)			
Macromolecules	67.64	35.14	
Ligand/ion	62.67	50.79	
Water	54.06	43.46	
R.M.S deviations			
Bond lengths (Å)	0.003	0.004	
Bond angles (°)	0.54	0.68	
Ramachandran			
Preferred/allowed/ disallowed (%)	97 / 2.5 / 0.25	97.8 / 2.2 / 0	

[a]The b and c axes are not the same between the ACIAD0746 Native and SeMet crystals

[b]$R_{merge} = |I_{obs} - \langle I \rangle| / I_{obs}$, where I_{obs} is the intensity measurement and $\langle I \rangle$ is the mean intensity for multiply recorded reflections.

[c]No symmetry-related reflections are observed in the highest resolution bin. The next highest resolution bin had R_{merge} 70%

[d]R_{work} and $R_{free} = |F_{obs} - F_{calc}| / |F_{obs}|$ for reflections in the working and test sets, respectively. R_{free} is the cross-validation R factor for the test set with 5% of the reflections omitted in the model refinement.

Table A3.2. Representative orthologs in different bacterial phyla.

Homolog / Phylum	Rep. Organism	e-value	Identity (%)	DBD e-value[1]	DBD identity (%)	EBD e-value	EBD identity (%)	GenBank Protein ID	Genome Links
FinR (fpr)									
Pseudomonadota	*Acinetobacter bayly*	0	100	0	100	0	100	WP_004922487.1	SEED, NCBI
Pseudomonadota	*Pseudomonas aeruginosa PAO1*	3e-69	40.55	4e-23	51.22	3e-69	40.55	WP_003114438.1	SEED, NCBI
Elusimicrobiota	*Elusimicrobia bacterium*	6e-80	46.74	2e-23	54.32	6e-80	46.74	TXH26191.1	NCBI
YeiE (yeiH)									
Acidobacteriota	*Holophaga foetida*	1e-31	32.85	9e-07	37.10	1e-31	32.85	WP_005035719.1	SEED, NCBI
Actinomycetota	*Rhodococcus erythropolis*	3e-36	31.46	6e-05	30.00	3e-36	31.46	WP_020905889.1	SEED NCBI
Armatimonadota	*Capsulimonas corticalis*	6e-46	31.74	3e-14	43.75	6e-46	31.74	BDI28264.1	NCBI
Bacillota	*Bacillus cereus*	7e-41	26.94	3e-10	29.27	7e-41	26.94	AAP12040.1	SEED, NCBI
Bacillota[3]	*Geobacillus kaustophilus*	4e-37	28.12	2e-11	39.71	4e-37	28.12	WP_011230392.1	SEED, NCBI
Bacteroidota	*Flavobacterium johnsonia johnsoniae*	1e-31	29.20	1e-08	35.29	1e-31	29.20	WP_012024944.1	SEED NCBI
Bdellovibrionota	*Bdellovibrio sp. NC01*	2e-31	31.20	6e-06	31.82	2e-31	31.20	WP_142697313.1	NCBI
Campylobacterota	*Nitratifractor salsuginis*	5e-61	36.61	1e-18	47.37	5e-61	36.61	WP_013552986.1	SEED, NCBI
Chloroflexota	*Ktedonobacter racemifer*	1e-39	31.16	9e-11	33.33	1e-39	31.16	WP_007919445.1	SEED NCBI
Deferribacterota	*Deferribacter desulfuricans*	7e-65	33.67	5e-19	43.21	7e-65	33.67	WP_013008140.1	SEED, NCBI
Deinococcota	*Allomeiothermus silvanus*	1e-39	32.67	1e-11	38.03	1e-39	32.67	WP_013159155.1	SEED, NCBI
Fibrobacterota	*Hallerella succinigenes*	2e-27	27.07	6e-06	30.14	2e-27	27.07	WP_100424460.1	NCBI
Fusobacteriota	*Sebaldella termitidis*	3e-30	24.40	0.001	28.57	3e-30	24.40	WP_012859543.1	SEED, NCBI

Gemmatimonadota	*Gemmatirosa kalamazoonesis*	4e-47	35.12	8e-11	39.19	4e-47	35.12	AHG88493.1	NCBI
Ignavibacteriota	*Ignavibacteriales bacterium*	5e-36	29.35	2e-09	38.33	5e-36	29.35	PJA96986.1	NCBI
Lentisphaerota	*Victivallis vadensis*	5e-23	28.36	6e-04	29.31	5e-23	28.36	WP_116882856.1	SEED, NCBI
Myxococcota	*Myxococcus stipitatus*	1e-33	33.09	5e-08	36.23	1e-33	33.09	WP_015347476.1	SEED, NCBI
Pseudomonadota	*Cronobacter sakazakii*	3e-74	40.21	3e-23	52.70	4e-49	38.19	AKE95361.1	NCBI NCBI
Pseudomonadota	*Escherichia coli (Enterobacteriaceae)*	2e-73	41.24	1e-22	52.44	2e-73	41.24	WP_000548294.1	SEED, NCBI
Rhodothermota	*Rubricoccus marinus*	7e-61	41.98	7e-17	56.06	7e-61	41.98	WP_094552207.1	NCBI
Spirochaetota	*Gracilinema caldarium*	8e-30	29.80	5e-08	35.38	8e-30	29.80	WP_013969574.1	SEED, NCBI
Synergistota	*Dethiosulfovibrio peptidovorans*	3e-61	36.64	1e-20	46.99	3e-61	36.64	WP_005660474.1	SEED NCBI
Verrucomicrobiota	*Opitutaceae bacterium*	9e-23	30.89	3e-09	40.00	9e-23	30.89	EIP99677.1	SEED, NCBI
***cysI/cysJ* & sulfite reductases**									
Bacillota	*Caldicellulosiruptor hydrothermalis*	1e-43	28.09	8e-12	34.72	1e-43	28.09	WP_013403423.1	SEED, NCBI
Bacillota	*Geobacillus kaustophilus*	1e-44	30.22	6e-09	27.50	1e-44	30.22	WP_044731581.1	SEED, NCBI
Planctomycetota	*Blastopirellula marina DSM 3645*	1e-53	35.66	2e-13	40.28	1e-53	35.66	WP_002651497.1	SEED , NCBI
Chromate Transporter									
Chlamydiota	*Chlamydia abortus*	6e-36	30.28	1e-12	44.87	6e-36	30.28	SHE14101.1	NCBI
Isolated, unknown function of neighbors, or no genome									
Archaea Euryarchaeota	*Methanococcoides sp. SA1*	1e-60	35.93	2e-26	53.85	2e-31	31.75	WP_172364814.1	NCBI
Aquificota	*Sulfurihydrogenibium*	7e-32	24.44	2e-07	30.88	7e-32	24.44	WP_007547258.1	SEED NCBI

	yellowstonense								
Atribacterota	*Candidatus Atribacteria bacterium*	2e-33	28.12	4e-12	37.50	2e-33	28.12	NLL61784.1	None
Bacillota	*Bacillus subtilis (Bacillus)*	1e-38	28.89	1e-06	26.87	1e-38	28.89	WP_003243173.1	SEED, NCBI
Balneolota	*Balneola vulgaris*	9e-31	27.00	1e-07	37.70	9e-31	27.00	WP_018127304.1	NCBI
Chlorobiota	*Chloroherpeton thalassium*	1e-32	28.28	1e-07	34.43	1e-32	28.28	WP_012500379.1	SEED, NCBI
Mycoplasmatota	*Acholeplasmatales bacterium*	3e-33	23.91	6e-16	36.59	3e-33	23.91	MCI5938531.1	None
Nitrospinota	*Nitrospina bacterium*	1e-50	31.51	1e-11	35.48	1e-50	31.51	MBI4667023.1	None
Nitrospirota	*Leptospirillum ferriphilum*	1e-35	31.89	2e-14	43.06	1e-35	31.89	KGA94897.1	SEED NCBI
Thermodesulfobacteriota	*Thermosulfurimonas dismutans*	5e-84	43.88	5e-26	56.79	5e-84	43.88	OAQ21728.1	NCBI
Thermotogota	*Athalassotoga saccharophila*	2e-36	26.53	7e-09	31.58	2e-36	26.53	WP_161848395.1	NCBI

[1] DBD comparisions computed for resides 1 to 85; word length 2.

[2] EBD comparisons computed for residues 89 to 293

[3] Examples of at least two paralogs present

Table A3.3. Phyla in which orthologs are absent.

Phylum	**Organism and metabolic pathways in KEGG database**	**Number of LTTRs[1]**	**Uniprot search phylum (number proteins in database)**
Chrysiogenetota	*Desulfurispirillum indicum* No sulfate metabolism	5 Uniprot	Chrysiogenetes (9533)
Caldisericota	*Caldisericum exile* No sulfate metabolism	0 Uniprot; 6 NCBI	Caldisericum (5731)

Calditrichota	*Caldithrix abyssi* Sulfate to PAPs No sulfite reductase	7 Uniprot; 25 NCBI	Caldithrix (30,343)
Coprothermobacterota	*Coprothermobacter proteolyticus* No sulfate metabolism	0	Coprothermobacter (2746)
Dictyoglomota	*Dictyoglomus thermophilum, Dictyoglomus turgidum* No sulfate metabolism	0	
Kiritimatiellaeota	*Kiritimatiella glycovorans* Sulfate to APS No sulfite reductase	14 Uniprot	Kiritimatiellaeota (33,142)
Nitrososphaerota	*Candidatus Nitrososphaera gargensis* *Nitrososphaera viennensis* Assimilatory sulfate reduction pathway Alkanesulfonate transport	1 Uniprot	Nitrososphaera (8,452)
Thermomicrobiota	*Thermomicrobium roseum* No sulfate metabolism	6 Uniprot	Thermomicrobium (6,971)
Thermoproteota	*Thermoproteus tenax* *Thermoproteus uzoniensis* Assimilatory and dissimilatory sulfate reduction Sulfate to APS to sulfite	19 uniprot	Thermoproteus (16,998)

Table A3.4. Measurement of ACIAD0746- and YeiE sulfite affinity through Isothermal titration calorimetry (ITC).

Protein (full-length)	Ligand	K_D Site 1 (M)	ΔG Site 1 (kJ/mol)	ΔH Site 1 (kJ/mol)	-TΔS Site 1 (kJ/mol)	K_D Site 2 (M)	ΔG Site 2 (kJ/mol)	ΔH Site 2 (kJ/mol)	-TΔS Site 2 (kJ/mol)
ACIAD 0746	Sulfite	566E-9 ± 82.9E-9	-35.7	-3.35 ± 3.05	-32.3	13.7 E-6 ± 0.645 E-6	-27.8	-24.7 ± 0.209	-3.09
YeiE	Sulfite	801E-9 ± 11.1E-9	-34.8	4.25 ± 0.875	-39.1	1.57 E-6 ± 0.025 9E-6	-33.2	-215 ± 12.2	182

The values are the average of triplicates with errors shown.

Table A3.5. ACIAD0746 transcriptional regulation at different promoters associated with sulfur metabolism.

Promoter	Sulfite (1mM)	Sulfate (1mM)	Cysteine (1mM)
cysP	No regulation	No regulation	No regulation
sbp	No regulation	No regulation	No regulation
ssu	No regulation	No regulation	No regulation
cysDN	Activation	No regulation	No regulation
cysH	No regulation	No regulation	No regulation
cysI	Activation	No regulation	No regulation
fpr	No regulation	No regulation	No regulation
fpr2	Activation	No regulation	No regulation
cysK	No regulation	No regulation	No regulation
cysM	No regulation	No regulation	No regulation

All experiments were performed in triplicate.

Table A3.6. Affinity of ACIAD0746 for promoters associated with sulfur metabolism.

Promoter	ACIAD0746 Affinity with No Sulfite (nM)	ACIAD0746 Affinity with 1 mM Sulfite (nM)
cysP	4.9 ± 2.8	69.6 ± 35.9
sbp	9.3 ± 10.0	57.8 ± 14.8
ssuA	2.7 ± 1.5	26.5 ± 1.6
cysDN	8.6 ± 5.7	48.6 ± 2.2
cysH	64.0 ± 15.6	64.8 ± 33.9
cysI	3.2 ± 0.99	5.2 ± 2.9
cysK	56.6 ± 20.8	10.8 ± 2.8
cysM	82.9 ± 12.2	80.4 ± 11.2
fpr	155.4 ± 42.6	121.3 ± 8.8

The values are the average of triplicates ± SEM.

Table A3.7. Key resources table.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
Escherichia coli XL-1 Blue	Stratagene	Cat#200249
Escherichia coli BL21 (DE3) RIPL	Stratagene	Cat#230280
Escherichia coli B834 (DE3)	Stratagene	Cat#69041-3
Pseudomonas aeruginosa PAO1	Laboratory of Balazs Rada	N/A
Acinetobacter baylyi ADP1	Laboratory of Ellen Neidle	N/A
Chemicals, peptides, and recombinant proteins		
Fast-link ligase buffer	Epicenter	Cat#LK0750H
ZraI	New England Biolabs	Cat#R0659S
T4 DNA ligase	New England Biolabs	Cat#M0202S
BspQI	New England Biolabs	Cat#R0712S
XbaI	New England Biolabs	Cat#R0145S
XhoI	New England Biolabs	Cat#R0146S
SspI-HF	New England Biolabs	Cat#R3132S
StuI	New England Biolabs	Cat#R0187S
EcoRI	New England Biolabs	Cat#R0101S
L-methionine	Sigma-Aldrich	Cat#64319-100G-F
L-alanine	Sigma-Aldrich	Cat#A7627
L-serine	Sigma-Aldrich	Cat#S4500
L-cysteine	Fisher Scientific	Cat#ICN10144480
Paraffin oil	J.T. Baker	Cat#S894-07
Sodium sulfite	J.T. Baker	Cat#3922-01
Sodium sulfate	J.T. Baker	Cat#8028-06
Adenosine 5’-phosphosulfate	Sigma-Aldrich	Cat#A5508-5MG
Iron (III) chloride hexahydrate	Sigma-Aldrich	Cat#236489
NAD^+	Sigma-Aldrich	Cat#N7004-5G
NADH	Sigma-Aldrich	Cat#N8129-5G
$NADP^+$	Sigma-Aldrich	Cat#N5755-1G
NADPH	Sigma-Aldrich	Cat#10107824001
O-acetyl-L-serine hydrochloride	Sigma-Aldrich	Cat#A6262
Sodium sulfide	Sigma-Aldrich	Cat#208043-100G
Sodium thiosulfate	J.T. Baker	Cat#3953-01
RNase inhibitor, murine	New England Biolabs	Cat#M0314S
Bovine serum albumin (BSA)	Fisher Scientific	Cat#23209

Ribonucleotide solution mix (rNTPs)	New England Biolabs	Cat#N0466S
DNase I	New England Biolabs	Cat#M0303S
SYBR™ Gold Nucleic Acid Gel Stain	ThermoFisher Scientific	Cat#S11494
SYBR™ Green I Nucleic Acid Gel Stain	ThermoFisher Scientific	Cat#S7563
Protogel 30%	National Diagnostics	Cat#EC-890
ACIAD0746	This paper	N/A
YeiE	This paper	N/A
FinR	This paper	N/A
A. baylyi ADP1 RNA polymerase holoenzyme	Provided by Juliana Brondani	[193]
P. aeruginosa PAO1 RNA polymerase holoenzyme	Provided by Derrick Afful	[107]
Critical commercial assays		
Crystal Screen	Hampton Research	Cat#HR2-110
Index	Hampton Research	Cat#HR2-144
Oligonucleotides		
See **Table A3.8** for oligonucleotides	Integrated DNA Technologies	N/A
Recombinant DNA		
pET28b(+).SapKO-CH.ACIAD0746	This paper	N/A
pET28b(+).SapKO-CH.YeiE	This paper	N/A
pET28b(+).SapKO-CH.YeiE_EBD	This paper	N/A
pUC18.cysP-P	This paper	To be send to Addgene
pUC18.cysK-P	This paper	To be send to Addgene
pUC18.cysDN-P	This paper	To be send to Addgene
pUC18.cysH-P	This paper	To be send to Addgene
pUC18.cysI-P	This paper	To be send to Addgene
pJB3.pUC18.fpr-P	This paper	To be send to Addgene
pDAP16.pET28b.PAO1.finR.CHx5	This paper	N/A
pDAP20.pUC18.PAO1.finR-fpR	This paper	To be send to Addgene
Software and algorithms		
HKL2000	Minor et al., 2006	http://www.hkl-xray.com

CCP4i	Winn et al., 2011	http://www.ccp4.ac.uk/ccp4i_main.php
Phenix	Adams et al., 2010	https://www.phenix-online.org/
Coot	Emsley et al., 2010	https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/
Pymol	The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.	https://pymol.org/2/
ScanProsite	De Castro et al., 2006	https://prosite.expasy.org/scanprosite/
Mega6	Tamura et al., 2013	http://www.megasoftware.net/
Jalview	Waterhouse et al., 2009	http://www.jalview.org/
GelQuantNET	BiochemLab Solutions	http://biochemlabsolutions.com/GelQuantNET.html
Prism 7	GraphPad Software	https://www.graphpad.com/scientific-software/prism/
Other		
Microbatch Plates	Fisher Scientific	Cat#50-035-5
MicroCal PEAQ-ITC	Malvern Instruments	N/A
NanoDrop Lite Spectrophotomer	ThermoFisher Scientific	N/A
GE Storm 865 Scanner	GE Healthcare Life Sciences	N/A
Genewiz Sequencing	Genewiz	https://www.genewiz.com/en
Plasmidsaurus Sequencing	Plasmidsaurus	https://www.plasmidsaurus.com/

Table A3.8. Oligonucleotides.

Oligonucleotide	Sequence	Use
ACIAD0746-F	GGGCTCTTCGATGCGCATGACATTACGTC	To clone full length ACIAD0746
ACIAD0746-R	CAGCTCTTCAGTGATTCACTCGATTTTCACAGAAG	To clone full length ACIAD0746
YeiE FL-F	CCGCTCTTCAATGCACATCACCCTCCGGC	To clone full length YeiE
YeiE FL-R	GGGCTCTTCAGTGACGCGGTACATTCGCGGG	To clone full length YeiE
YeiE_EBD-F	GAGCTCTTCTATGATTCGTATCTATGCCAGTAGTACC	To clone EBD YeiE
YeiE-EBD-R	GGGCTCTTCAGTGACGCGGTACATTCGCGGG	To clone EBD YeiE
FPM1	ACGCTCTTCCATGAATTCACCCTCCGCC	To clone full length FinR
FPM2	TGCTCTTCTGTGGGGAATCAGCGGACGCACC	To clone full length FinR
CysP-P-FOR	TGCTAAATGACGATGCAT	To clone *cysP* promoter
CysP-P-REV	CTGTTTCCAGTATGCACC	To clone and amplify *cysP* promoter
CysK-P-FOR	TTAACAACTAAGAAATATGAACTTGCC	To clone and amplify *cysK* promoter
CysK-P-REV	ACCTAAAACCGTTGCACC	To clone and amplify *cysK* promoter
CysDN-P-FOR	GACATTTTGTGCTTTAAACGG	To clone and amplify *cysD* promoter
CysDN-P-REV	CGAATAATATGAATACTCTCAGCT	To clone and amplify *cysD* promoter
CysH-P-FOR	CTTGTCTGCATACTCTGTATTAAG	To clone and amplify *cysH* promoter
CysH-P-REV	CGTAATCTGGAATATCACGTG	To clone and amplify *cysH* promoter
cysI_New_P-F	AAACGCAATGGACGGTATTC	To clone *cysI* promoter
cysI_New_P-R	AGCCACACCGATGACCTATG	To clone and amplify *cysI* promoter
finR-thru-fpr-1-For	GGCACGAACCTATAAACTGGG	To clone *aciad0746_fpr* promoter
finR-thru-fpr-Rev	TTACCAATAGTTTTCTACTGCGATATTGC	To clone *aciad0746_fpr* promoter

FPM3	CAGATGAAGTAGAACTGCCG	To clone *finR_fprA* promoter
FPM4	CATGCAACGTCATACCCTC	To clone *finR_fprA* promoter
CysP-P-FOR_NewJ	GGCTCGGTCTTGAATAGAG	To amplify *cysP* promoter
cysI_P-F_NewJ	GGTTTGATCACGGAACTGAGC	To amplify *cysI* promoter
F-cysM_J	ATGGCGTCTTTCCAACATAA	To amplify *cysM* promoter
R-cysM_J	CGGTATGACGTTGTTGAGAT	To amplify *cysM* promoter
F-ssu_J	GAGAAAGCACGTCAAGAAGG	To amplify *ssu* promoter
R-ssu_J	GCACAGCCACTCAGCATCAT	To amplify *ssu* promoter
F-sbp_J	AAAAATCTTTCGCTGCGTTG	To amplify *sbp* promoter
R-sbp_J	ATGAGATCGTTGTCTTCACC	To amplify *sbp* promoter
TA_Template1_F	GATCAAATAAAGGTGCTCCAAG	To amplify *aciad0746_fpr* promoter
TA_Template1_R	GTAGAAAAATCTTGCAGCATGC	To amplify *aciad0746_fpr* promoter
F-fpr2	TAAACAGAGTGTCATTCCAGT	To amplify *fpr2* promoter
R-fpr2	CTGGACGATACATGCTTCTG	To amplify *fpr2* promoter
FPM5	CCAGGCTTCCTCGTCTAGAGC	To amplify *finR_fprA* promoter
FPM6	CAAAGACTCCTAGGAAAACGC	To amplify *finR_fprA* promoter
FPM7	CCATATCCATATTCTGGATAAGCATTATCCAGACAATTCATTTTG	To amplify MP1
FPM8	CAAAATGAATTGTCTGGATAATGCTTATCCAGAATATGGATATGG	To amplify MP1
FPM9	CCATATCCATATTCTGGATAAGCATTATCCAGACAATTCATTTTGCGGATATTT	To amplify MP2
FPM10	AAATATCCGCAAAATGAATTGTCTGGATAATGCTTATCCAGAATATGGATATGG	To amplify MP2

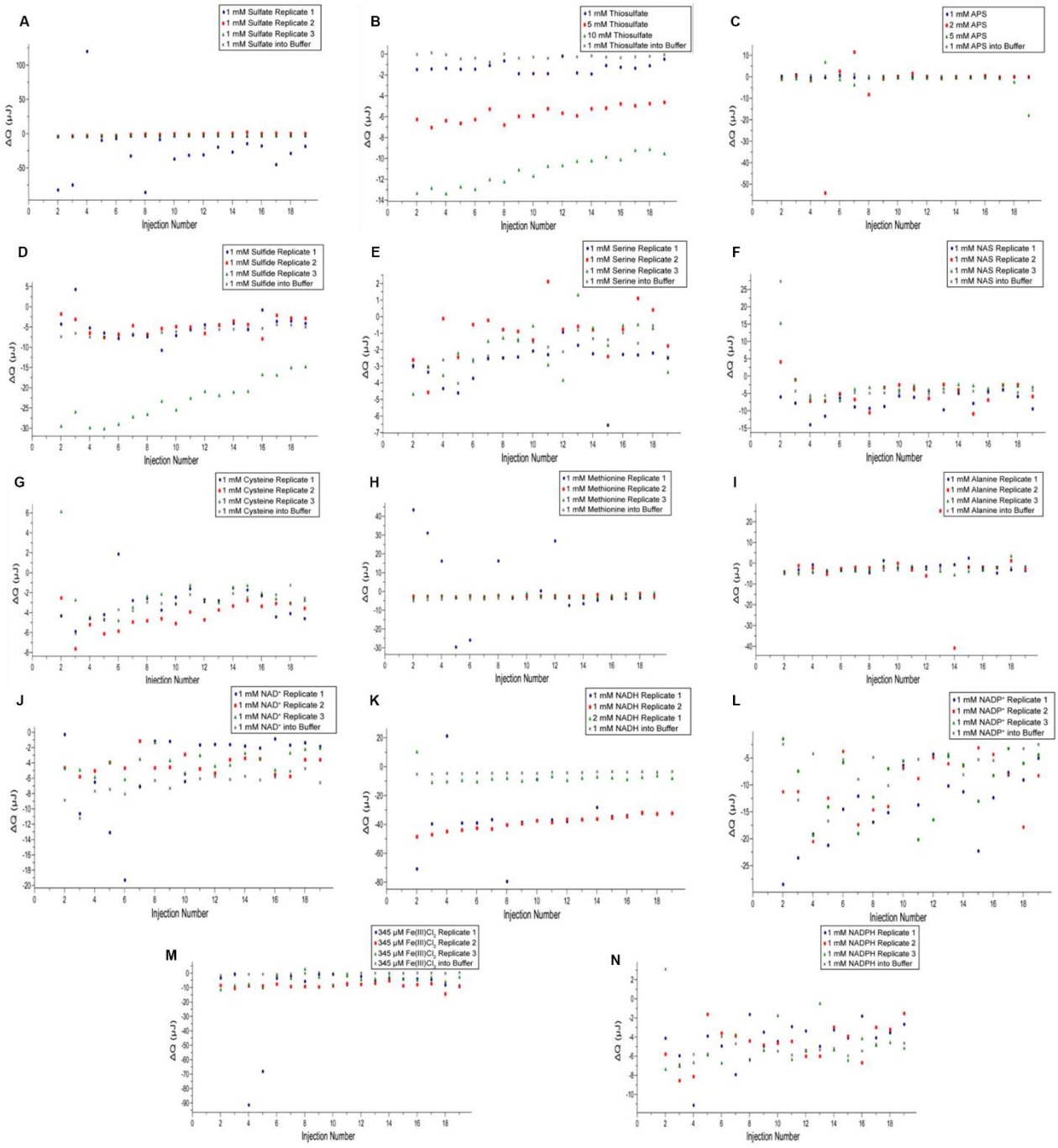


Figure A3.1. ITC analysis of ACIAD0746 with several intermediates in the sulfur assimilation pathway. No binding was detected when ACIAD0746 was titrated with (A) sulfate, (B) thiosulfate, (C) APS, (D) sulfide, (E) serine, (F) NAS, (G) cysteine, (H) methionine, (I) alanine, (J) NAD^+, (K) NADH, (L) $NADP^+$, (M) $FeCl_3$, (N) NADPH.