ACTA ABP BIOCHIMICA POLONICA

Vol. 62, No 4/2015
773–783
http://dx.doi.org/10.18388/abp.2015_1132

Regular paper

Phylogenetic relationship of the stringent response-related genes of marine bacteria

Beata Guzow-Krzemińska✉, Tomasz Gąsior and Agnieszka Szalewska-Pałasz

Department of Molecular Biology, University of Gdansk, Gdańsk, Poland

Bacteria living in marine environment encounter various challenges and limitations, thus in order to survive, they need to employ efficient stress-response mechanisms. One of these mechanisms is the stringent response, where unusual nucleotides, guanosine tetra- and pentaphosphates, herald starvation and physico-chemical stresses. All so far sequenced free-living bacteria contain the gene(s) responsible for (p)ppGpp synthesis — *rsh* (named after *Escherichia coli* genes, *relA* and *spoT*). Two similar genes were identified mostly in β- and γ-*proteobacteria* while other bacteria have only one gene coding the dual function of (p)ppGpp synthesis and degradation. Although the presence of (p)ppGpp-mediated response to the stress conditions has been shown for a few, and predicted for some other marine microorganisms, the (p)ppGpp effects may vary among different organisms. Thus, in this work we asked whether marine bacteria could have evolved a genetic adaptation specifically suited to adapt to environment with limited resources. The phylogenetic analyses of SpoT, RelA and RSH proteins from organisms associated with marine environment showed, however, that the evolutionary correlations obtained for these proteins are congruent with those constructed for 16S rRNA sequences and reflect taxonomical relationships of these organisms. Likewise, the similarity of specific amino acid residues indispensable for catalytic activity of these enzymes is very high, and any observed changes parallel with the taxonomical and evolutionary relationships. However, potential homologs of Mesh1 enzyme (metazoan SpoT homologs) that occur in both eukaryotic and prokaryotic organisms and contain the hydrolytic domain orthologous to SpoT were identified in *Cellulophaga, Erythrobacter* and *Flavobacterium* genera for the first time, as well as in soil bacterium *Cytophaga hutchinsonii* and freshwater *Rhodothermus marinus*.

Key words: ppGpp, stringent response, marine bacteria

Received: 30 July, 2015; revised: 18 September, 2015; accepted: 05 October, 2015; available on-line: 07 December, 2015

INTRODUCTION

Marine environment is one of the most challenging habitats, because of recurrent changes in salinity, nutrient availability, temperature and many other factors such as pollution and UV radiation. Marine microorganisms, one of the most abundant groups in this habitat, are responsible for most of biomass turnover and food and energy cycles (Sogin *et al.,* 2006). Unicellular organisms, including bacteria, are particularly sensitive to environmental alterations and challenges. Thus, a key role in their survival plays a prompt and effective response to these changes at the biochemical and metabolic level. In fact, marine bacteria are particularly well-adapted to an environment with limited resources; it has been documented that they can stop and resume their biological activities faster than bacteria that thrive in less restrictive environments (Amy *et al.,* 1983; Kurath & Morita, 1983). However, the knowledge about the specific adaptation mechanisms in marine environment is limited. For example, one of the global regulatory mechanisms ensuring the survival under the stress condition, the stringent response, is studied mostly in Gram-negative models of *Escherichia coli,* soil bacterium *Pseudomonas putida* or Gram-positive model bacterium *Bacillus subtilis*.

During the stringent response, unusual nucleotides, guanosine tetra- and pentaphosphate, ppGpp and pppGpp, referred to as (p)ppGpp, are synthesized promptly after starvation and physico-chemical stress, directly and indirectly affecting all major cellular processes such as sporulation, biofilm formation, quorum sensing, adaptation to adverse conditions, bacterial virulence (Potrykus & Cashel, 2008 and refs therein, Dalebroux *et al.,* 2010). However, the effects vary among different organisms and may depend on the type of stress, (p)ppGpp levels, the mechanism of (p)ppGpp action and the inducing conditions. (p)ppGpp has been identified in all free living eubacteria tested (Potrykus & Cashel, 2008) and chloroplast bearing plants (Braeken *et al.,* 2006) but the enzymes responsible for its metabolism differ.

Escherichia coli and some of β- and γ-*proteobacteria* have two similar 74 kDa RSH (Rel Spo homolog) proteins: synthetase I, encoded by the *relA* gene, responsible for ribosome-dependent production of ppGpp upon amino acid starvation, and bifunctional synthetase/hydrolase, product of the *spoT* gene. SpoT-mediated production of ppGpp is induced by limitation of other nutrients (carbon, iron, nitrogen, phosphate, fatty acids) or by stresses (membrane, osmotic). Both enzymes bear high similarity to each other, however the strong hydrolase activity, localized in the N-terminal part of the protein (HD domain), is present only in SpoT. The synthesis activity is dependent on a neighboring domain that is similar in both proteins. The C-terminal domain is responsible for regulation of the enzyme's activity, and, for RelA, interaction with ribosomes.

A functional and structural study was performed on the RelSeq protein from *Streptococcus equisimilis*, including the crystal structure and mutational analysis of domains

✉e-mail: beata.guzow@biol.ug.edu.pl
*The results were presented at the 6th International Weigl Conference on Microbiology, Gdańsk, Poland (8–10 July, 2015).

and importance of particular amino acid residues (Hogg *et al.*, 2004). This protein, named RelSeq is an example of a single RSH enzyme with bifunctional synthesis and hydrolytic activities, present in many bacterial groups. The variety of (p)ppGpp metabolism-related enzymes has been evolutionarily classified by Mittenhuber (2001). Later, the thorough analysis including the class of short enzymes with only synthesis domains (for e.g. present in Gram-positive bacteria) was presented by Atkinson and collaborators (2011). An ortholog of the functional ppGpp hydrolase domain was also discovered in animal cells (Sun *et al.,* 2010). This suggests a possible general role for ppGpp in all living organisms, not just bacteria and plants.

The presence of (p)ppGpp-mediated regulation in marine bacteria is expected from several lines of evidence: i) evolutionary benefits for their survival under conditions of nutrient and stress challenges, ii) impaired survival of strains with defective (p)ppGpp synthetase genes (Ostling *et al.,* 1995; 1996), iii) vast majority of bacteria analyzed to date have genes coding for (p)ppGpp-synthetizing enzymes. However, the information on the stringent response in marine microorganisms is very limited with only a handful of publications describing the stringent response of a single species, *Vibrio* sp. S14 identified later as *V. angustum* which can synthesize ppGpp during amino acid and carbon starvation (Flardh *et al.,* 1992; 1994; Ostling *et al.,* 1996).

It was also hypothesized that the stringent response in marine bacteria may differ from the *E. coli* model: marine microorganisms retain a considerably higher residual rate of ribosomal synthesis during starvation (Flardh *et al.,* 1992) and cell division occurs at a notably lower critical cell mass (Amy *et al.,* 1983). Thus, we asked in this work whether marine bacteria could have evolved a specific genetic adaptation mechanism in terms of the stringent response to ensure optimal survival and efficient usage of the limited resources in this environment.

MATERIALS AND METHODS

All sequences used in this study were obtained from GenBank (http://www.ncbi.nlm.nih.gov/) or UniProt (http://www.uniprot.org/) databases and are presented in Table 1, except of *Flavobacterium* sp. and *Paracoccus* sp. that were generated in our lab (Joanna Karczewska-Golec, Maja Kochanowska-Łyżeń, Paweł Olszewski, Marta Moskot, Magdalena Bałut, Arkadiusz Piotrowski, Piotr Golec and Agnieszka Szalewska-Palasz, to be published elsewhere) and deposited as a Whole Genome Shotgun project at DDBJ/EMBL/GenBank under the accession number JYGZ00000000 for *Flavobacterium* sp. and JYGY00000000 for *Paracoccus* sp.

We selected marine bacteria for which SpoT, RelA or RSH homolog protein sequences were available. Moreover, 16S rRNA sequences from the same taxa were downloaded. Sequences of *Anabaena cylindrica* and *A. variabilis* were also used in further analysis. The similarity searches for sequences were carried out by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and alignments were done using MAFFT (http://www.ebi.ac.uk/Tools/msa/mafft/). Next, the alignments were adjusted manually using MEGA5 (Tamura *et al.*, 2011).

The trees were calculated using RaxML v.8 on CIPRES Science Gateway V 3.3 (https://www.phylo.org/portal2/home.action) (Miller *et al.*, 2010). For SpoT, RelA or Rsh trees PROTGAMMA model was employed and 100 bootstrap replicates were performed. For 16S rRNA tree, a GTR model was used and 100 bootstrap replicates were performed. The 16S rRNA tree was visualized using FigTree v 1.4.2. The branches representing multiple species belonging to the same genus are shown as collapsed. Other trees were visualized using TreeView (Page, 1996). Bootstrap supports ≥70 are shown above branches.

RESULTS AND DISCUSSION

We performed independent phylogenetic analyses of SpoT, RelA and RSH proteins from organisms associated with marine environment (Figs. 1, 2 and 3, respectively). We selected bacteria that are reportedly present in the Baltic Sea or other marine environment and whose SpoT, RelA or RSH sequences were available (Table 1). The selection was based on information from publication records (Mudryk & Podgórska, 2005; Cabaj *et al.,* 2006; Riemann *et al.,* 2008; Stolle *et al.,* 2011; Sjöstedt *et al.,* 2012) and we also added strains that were isolated from the Baltic Sea in our laboratory. However, only some of them are strictly marine bacteria, while others are more flexible with respect to their habitats as they may occur in soil, rivers, as pathogens of different organisms etc. The information on their habitats is provided in Table 1.

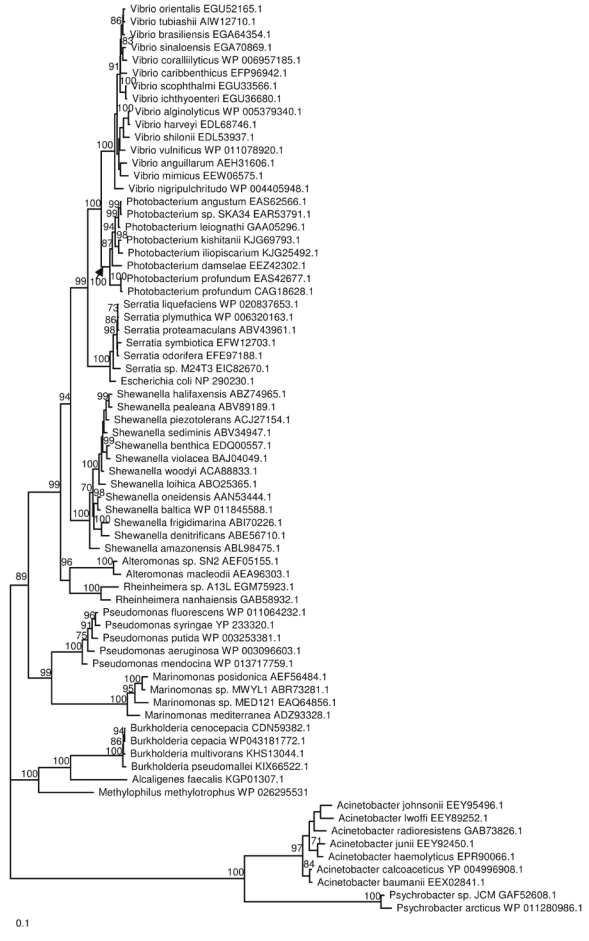


Figure 1. Maximum likelihood phylogeny of SpoT homolog proteins from selected bacteria associated with marine environments.
The tree was generated using RaxML and bootstrap supports are provided above branches. Names of species are followed with GenBank accession numbers of their SpoT amino acid sequences.

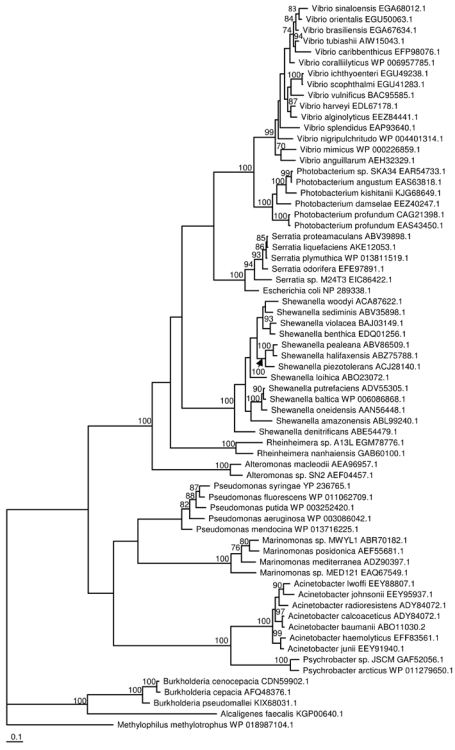


Figure 2. Maximum likelihood phylogeny of RelA homolog proteins from selected bacteria associated with marine environments.

The tree was generated using RaxML and bootstrap supports are provided above branches. Names of species are followed with GenBank accession numbers of their RelA amino acid sequences.

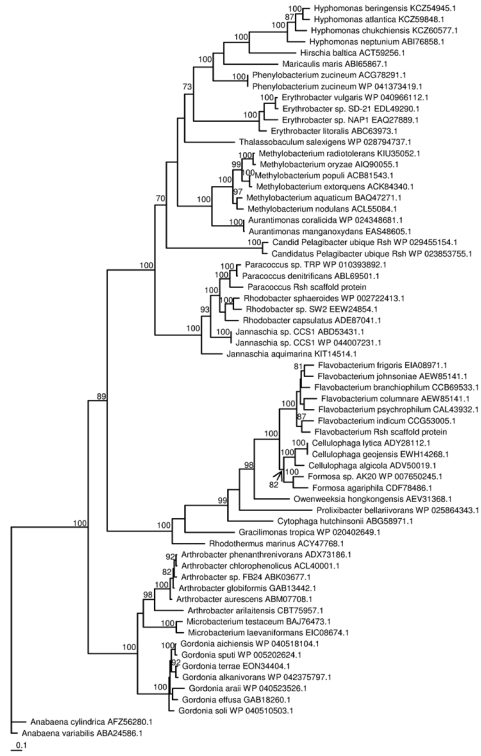


Figure 3. Maximum likelihood phylogeny of RSH homolog proteins from selected bacteria associated with marine environments.

The tree was generated using RaxML and bootstrap supports are provided above branches. Names of species are followed with GenBank accession numbers of their RSH amino acid sequences.

Figure 4. Maximum likelihood phylogeny based on 16S rRNA gene sequences from selected bacteria associated with marine environments.

The tree was generated using RaxML and bootstrap supports are provided above branches. Names of species are followed with GenBank accession numbers of their 16S rRNA nucleotide sequences. Some branches are presented as collapsed for multiple species of the same genus and only names of genera are provided.

In case of SpoT homologs, we used 71 sequences from different taxa and the final alignment had 734 positions. For RelA homolog analysis, we downloaded 67 sequences and the final alignment had 800 positions. In case of RSH we included 66 sequences and the alignment had 795 positions including *Anabaena* spp. that was used as an outgroup.

Simultaneously, we used the 16S rRNA gene to construct a phylogenetic tree (Fig. 4) for all organisms that were used in our analyses of proteins. In total, 128 taxa were selected and the final alignment of 16S rRNA used for phylogenetic analysis had 1437 positions. *Anabaena cylindrica* and *A. variabilis* were used as an outgroup. To simplify the tree, some branches representing species belonging to the same genus were collapsed. In all trees (Figs. 1, 2, 3, and 4) numbers above branches indicate bootstrap supports based on 100 replicates.

In the 16S rRNA tree (Fig. 4) bacteria that belong to β and γ-*proteobacteria* form highly supported clades with bootstrap supports of 100. These organisms are Gram-negative bacteria and occur in different environments. *Alteromonas, Marinomonas, Photobacterium, Rheinheimera, Shewanella* and *Vibrio* represent aquatic species (mainly marine bacteria) while others such as *Acinetobacter, Pseudomonas* or *Serratia* are not strictly associated with aquatic habitats and are often causative agents of diseases. Bacteria belonging to β and γ-proteobacteria encode two

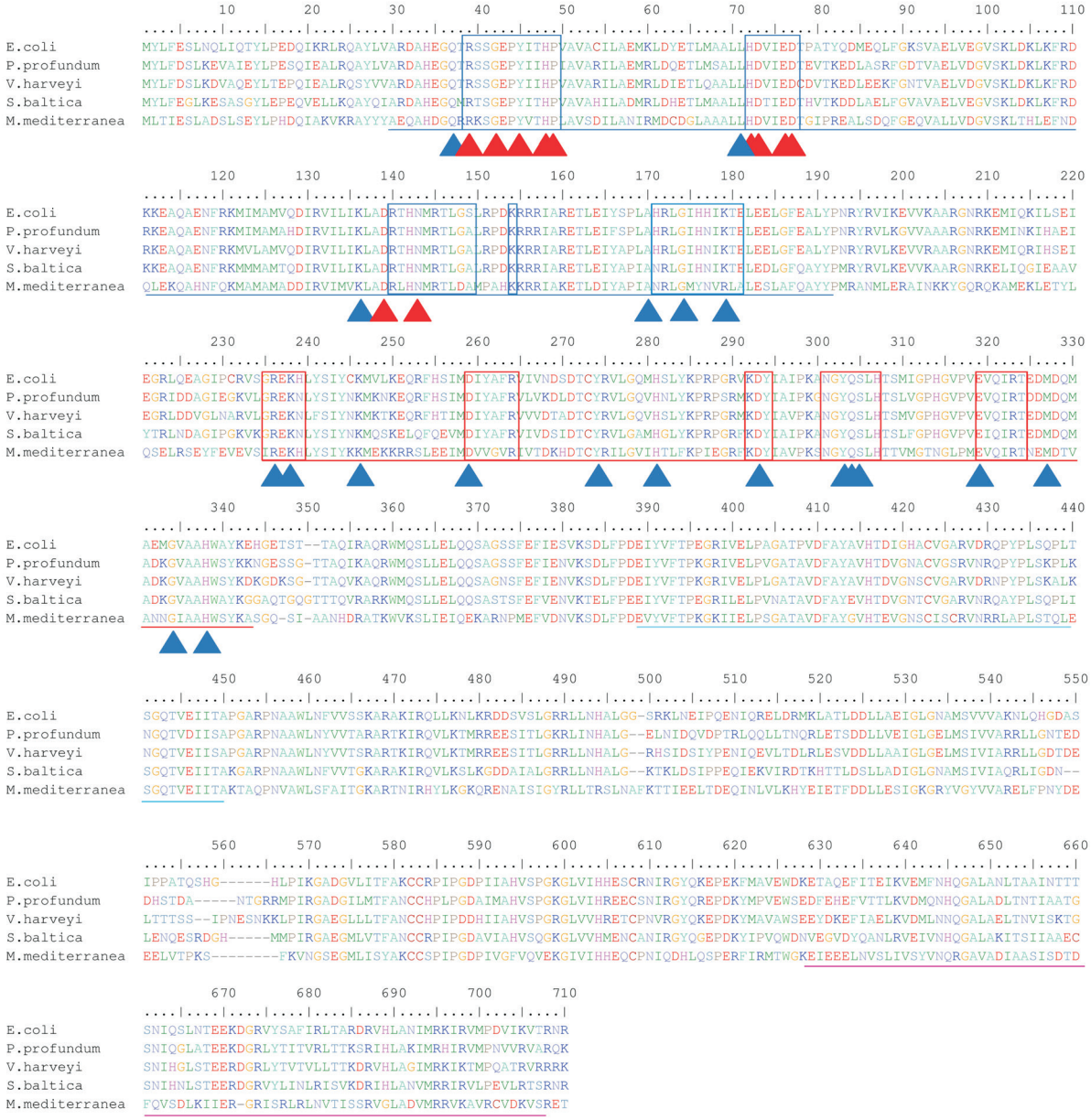


Figure 5. Consensus alignment of SpoT homologs from selected marine bacteria.
The *E. coli* SpoT was added for comparison. Positions that are indispensable for catalytic activity are indicated with triangles (blue — amino acid residues conserved in SpoT, RelA and RSH, red — amino acid residues conserved only in SpoT and bifunctional RSH enzymes) (based on Hogg *et al.*, 2004). Blue lines underneath the sequences indicate the hydrolytic domain, red — synthesis domain, light blue — TGS domain, magenta — ACT domain. The nucleotide binding pocket is indicated by blue and red boxes, for hydrolytic and synthesis domains, respectively (based on Atkinson *et al.*, 2011).

paralogue enzymes in a single genome. SpoT and RelA homologs probably evolved after gene duplication or gene transfer, thus β and γ-*proteobacteria* gained an additional protein involved in the (p)ppGpp metabolism.

Atkinson *et al.* (2011) proposed a hypothetical evolutionary history of RSH, RelA and SpoT and their functions in different lineages of bacteria suggesting gene duplication and then loss of the synthetase function of SpoT in *Moraxellaceae*. In our study, SpoT homologs from *Acinetobacter* and *Psychrobacter* spp. are also very divergent from those in other γ-*proteobacteria*. They form a highly supported clade in the SpoT tree, but with particularly long branches (Fig. 1) that reflects their individuality and perhaps a separate evolutionary history. In contrast, RelA proteins from *Acinetobacter* and *Psychrobacter* spp. do not differ significantly from homologs of other γ-*proteobacteria* (Fig. 2) and the RelA phylogenetic tree is congruent with the 16S rRNA tree (Fig. 4).

Other organisms have only a single RSH protein that is considered as an ancestral state. In 16S rRNA tree (Fig. 4) subclades representing each group of bacteria belong to *Actinobacteria*, *Bacterioidetes* and α-*proteobacteria*, and are highly supported with bootstrap values of 100. Among them *Hyphomonas, Hirschia, Maricaulis, Erythrobacter, Thalassobaculum, Aurantimonas, Pelagibacter, Jannaschia, Flavobacterium, Cellulophaga, Formosa, Owenweeksia, Prolixibacter, Rhodothermus* and *Gracilimonas* are associated with aquatic habitats. Others represent various lifestyles (Ta-

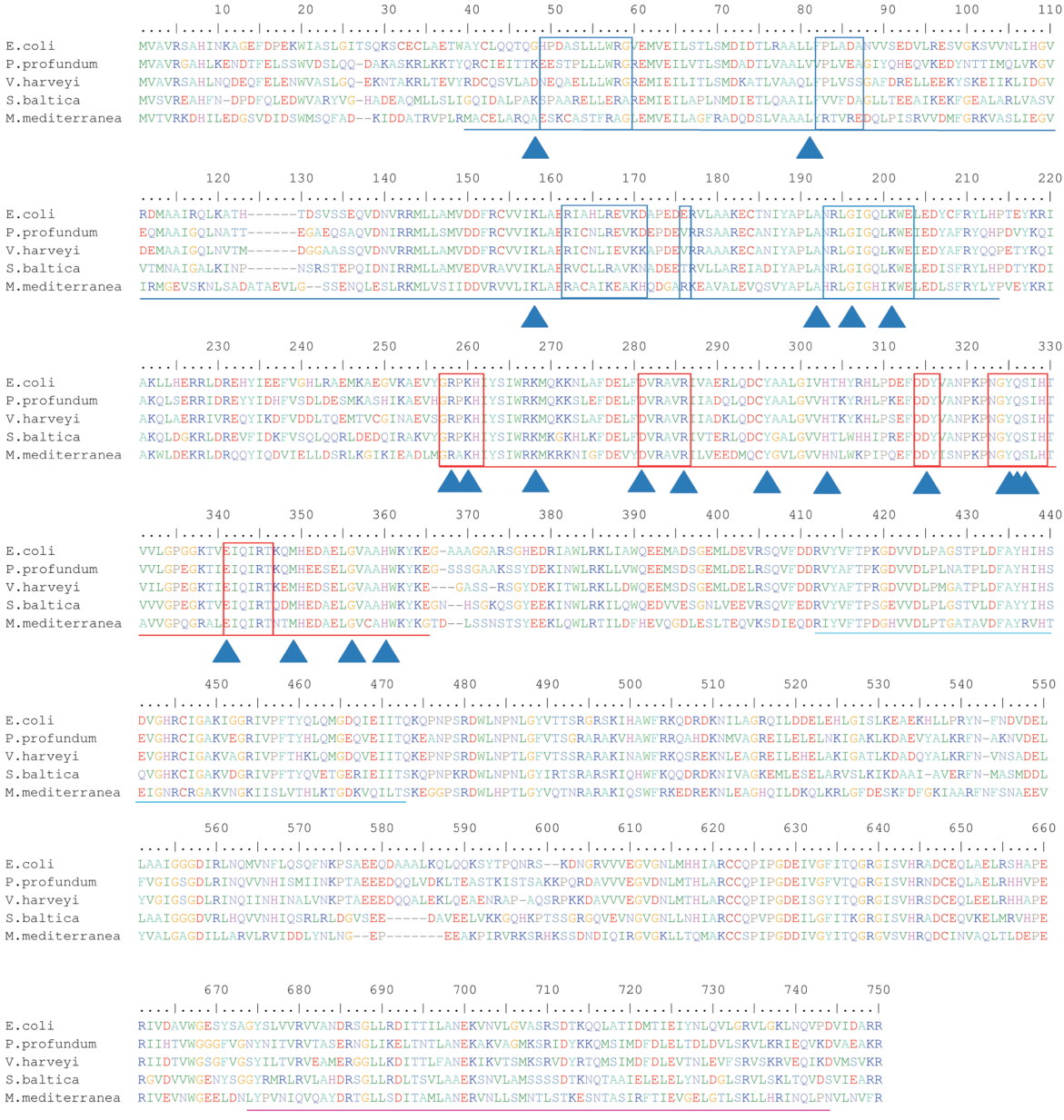
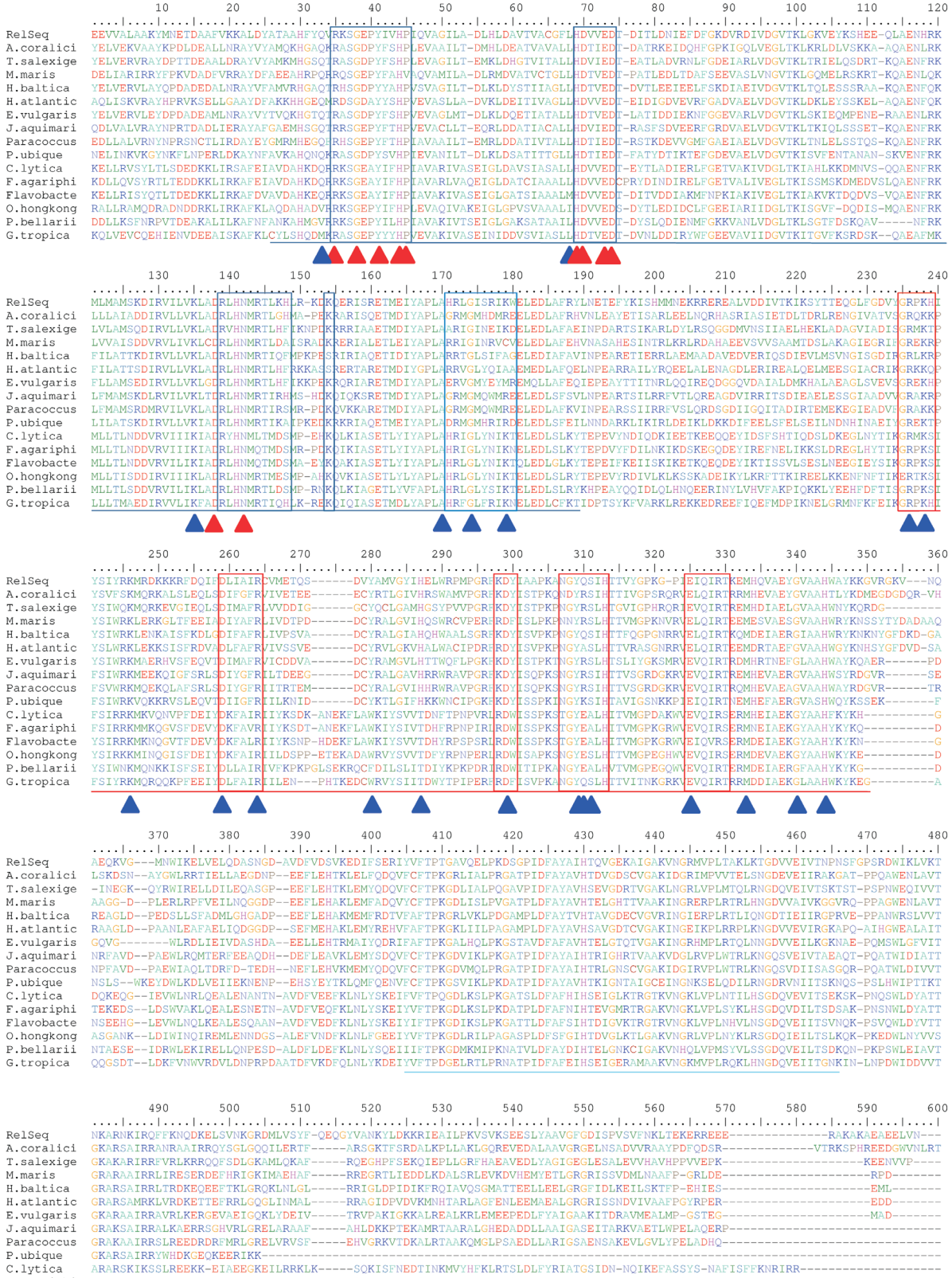


Figure 6. Consensus alignment of RelA homologs from selected marine bacteria.
The *E. coli* RelA was added for comparison. Positions that are indispensable for catalytic activity are indicated with blue triangles as amino acid residues conserved in SpoT, RelA and RSH (based on Hogg *et al.*, 2004). Blue lines underneath the sequences indicate the hydrolytic domain, red — synthesis domain, light blue — TGS domain, magenta — ACT domain. The nucleotide binding pocket is indicated by blue and red boxes, for hydrolytic and synthesis domains, respectively (based on Atkinson *et al.*, 2011).


RelSeq
A.coralici
T.salexige
M.maris
H.baltica
H.atlantic
E.vulgaris
J.aquimari
Paracoccus
P.ubique
C.lytica
F.agariphi
Flavobacte
O.hongkong
P.bellarii
G.tropica

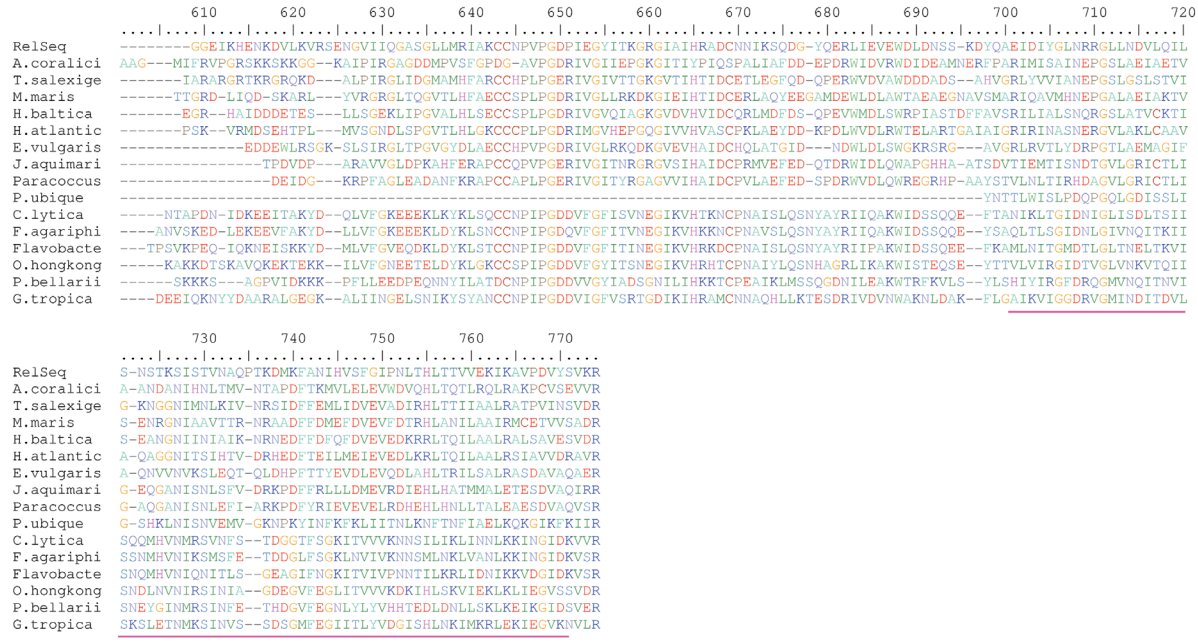


Figure 7. Consensus alignment of Rsh homologs from selected marine bacteria.
The *Streptococcus equisimilis* Rel*Seq* was added for comparison. Positions that are indispensable for catalytic activity are indicated with triangles (blue — amino acid residues conserved in SpoT, RelA and RSH, red — amino acid residues conserved only in SpoT and bifunctional RSH enzymes) (based on Hogg *et al.*, 2004). Blue lines underneath the sequences indicate the hydrolytic domain, red — synthesis domain, light blue — TGS domain, magenta — ACT domain. The nucleotide binding pocket is indicated by blue and red boxes, for hydrolytic and synthesis domains, respectively (based on Atkinson *et al.*, 2011).

ble 1). Comparison of 16S rRNA tree (Fig. 4) and RSH tree (Fig. 3) shows that each subgroup i.e. α-*proteobacteria*, *Bacterioidetes* and *Actinobacteria* form a highly supported clade with bootstrap supports of 100 in each tree. The evolutionary relationships obtained for the RSH protein are congruent with those determined on the basis of 16S rRNA data and reflect taxonomical resolution of these organisms.

We also generated and presented consensus sequence alignments of SpoT, RelA and RSH homologs from selected marine bacteria analysed in this study (Figs. 5, 6 and 7 respectively). *Escherichia coli* homologs were used as reference for SpoT and RelA alignment (Fig. 5 and 6), while RelSeq from *Streptococcus dysgalactiae* subsp. *equisimilis* served as reference for bifunctional RSH proteins (Fig. 7). According to Hogg and collaborators (2004), we indicated sites that are indispensable for catalytic activities in RSH proteins with triangles. Analysis of consensus sequence alignments showed that all important positions in hydrolytic and synthesis domains are conserved in all SpoT homologs (Fig. 5). In case of RelA homologs from marine bacteria, the hydrolytic domain is highly mutated and that leads to loss of its activity as was also reported for other bacterial species (e.g. Atkinson *et al.*, 2011), but the synthesis domain of RelA is conserved (Fig. 6). Bifunctional RSH proteins present as a single enzyme in these organisms exhibit high similarity in amino acid residues responsible for hydrolytic and synthesis activity of proteins (Fig. 7). Carboxyterminal region of RelA, SpoT and RSH also contains two domains: TGS and ACT. The conserved TGS region in SpoT and RSH plays a role in the regulation of catalytic activity of the enzyme, e.g. sensing the fatty acid starvation by binding the acyl carrier protein (Battesti &Bouveret, 2006; Potrykus & Cashel, 2008). This region is also conserved in analysed marine bacteria. The presence of ACT domain in CTD region was reported for typical RSH, RelA and SpoT enzymes, and it is also present in the sequences of marine bacteria chosen for these studies. The level of conservation of this domain is higher for RelA than for RSH. The ACT domain was suggested to play a role in modulating the intramolecular interactions and regulation of the enzyme activity. Thus, the differences in the amino acid sequences of these domains may indicate specific adaptations to environmental stresses.

The presence of an enzyme containing ppGpp hydrolysis domain (Mesh1) has been reported for metazoa (Sun *et al.*, 2010). Some of bacterial genera also harbor Mesh1 homologs (Atkinson *et al.*, 2011), thus we performed the search for Mesh1 in the collection of microorganisms analyzed in this study. In the analyses based on *Drosophila melanogaster* and bacterial (*Methylobacterium extorquens DM4*) Mesh1 sequences (Atkinson *et al.*, 2011) we found Mesh1 homologs for e.g. in *Burkholderia* spp., *Cellulophaga* spp., *Cytophaga* spp., *Erythrobacter* spp., *Flavobacterium* spp., *Methylobacterium aquaticum, Methylobacterium populi, Pelagibacter ubique, Pseudomonas* spp., *Rhodobacter sphaeroides* and *Rhodothermus marinus*. These bacterial species belong to α-, β- and γ-*proteobacteria*. The presence of Mesh1 in these classes of bacteria has been reported by Atkinson *et al.* (2011), including genera such as: *Pseudomonas, Methylobacterium, Burkholderia* and *Rhodobacter*. In some genera, such as *Cellulophaga, Cytophaga, Erythrobacter* or *Flavobacterium, Pelagibacter, Rhodothermus*, the presence of Mesh1 has not been reported previously. Although the role of Mesh1 in bacteria is unknown, the presence of Mesh1 homologs in the genomes of marine bacteria confirms that their genetic background regarding (p)ppGpp metabolism follows the pattern described for other microorganisms.

Table 1. List of organisms analysed in this study and GenBank Accession Numbers of their 16S rRNA gene sequences and RelA, SpoT or RSH protein sequences.
Organism from which amino acid sequences were used for consensus alignment are indicated in bold. Information on habitat of the organisms is also provided. *"widespread" indicates that bacteria can inhabit various environments: soil, freshwater, marine water etc. **Accession number for whole genome sequencing, 16S DNA coordinates are 700320–701869.

Species	16S RNA	RelA	SpoT	Rsh	Taxonomical subdivision	Enviroment
Acinetobacter baumannii	U10874.1	ABO11030.2	EEX02841.1	–	γ–proteobacteria	widespread*
Acinetobacter calcoaceticus	AY346313.2	ADY84072.1	YP_004996908.1	–	γ–proteobacteria	widespread
Acinetobacter johnsonii	DQ864703.1	EEY95937.1	EEY95496.1	–	γ–proteobacteria	widespread
Acinetobacter junii	AB777646.1	EEY91940.1	EEY92450.1	–	γ–proteobacteria	pathogen
Acinetobacter haemolyticus	NR_117622	EFF83561.1	EPR90066.1	–	γ–proteobacteria	pathogen
Acinetobacter lwoffii	DQ371237.1	EEY88807.1	EEY89252.1	–	γ–proteobacteria	widespread
Acinetobacter radioresistens	NR_026210	ADY84072.1	GAB73826.1	–	γ–proteobacteria	widespread
Alcaligenes faecalis	KF500593.1	KGP00640.1	KGP01307.1	–	β–proteobacteria	widespread
Alteromonas macleodii	Y18231.1	AEA96957.1	AEA96303.1	–	γ–proteobacteria	marine
Alteromonas sp. SN2	GU166736.2	AEF04457.1	AEF05155.1	–	γ–proteobacteria	marine
Arthrobacter aurescens	AB741459.1	–	–	ABM07708.1	Actinobacteria	soil
Arthrobacter arilaitensis	KP284570.1	–	–	CBT75957.1	Actinobacteria	cheeses
Arthrobacter chlorophenolicus	NR_074518.1	–	–	ACL40001.1	Actinobacteria	soil
Arthrobacter globiformis	NR_026187.1	–	–	GAB13442.1	Actinobacteria	soil
Arthrobacter phenanthrenivorans	KP980596.1	–	–	ADX73186.1	Actinobacteria	soil
Arthrobacter sp. FB24	NR_074590.1	–	–	ABK03677.1	Actinobacteria	soil
Aurantimonas coralicida	LC020223.1	–	–	WP_024348681.1	α–proteobacteria	marine
Aurantimonas manganoxydans	NR_118836.1	–	–	EAS48605.1	α–proteobacteria	marine
Burkholderia cenocepacia	KJ605842.1	CDN59902.1	CDN59382.1	–	β–proteobacteria	widespread
Burkholderia cepacia	AY741362.1	AFQ48376.1	WP043181772.1	–	β–proteobacteria	widespread
Burkholderia pseudomallei	AJ131790.1	KIX68031.1	KIX66522.1	–	β–proteobacteria	widespread
Candidatus Pelagibacter ubique	NR_074224.1	–	–	WP_029455154.1	α–proteobacteria	marine
Candidatus Pelagibacter ubique		–	–	WP_023853755.1	α–proteobacteria	marine
Cellulophaga algicola	NR_074452.1	–	–	ADV50019.1	Bacteroidetes	marine
Cellulophaga geojensis	NR_118002.1	–	–	EWH14268.1	Bacteroidetes	marine
Cellulophaga lytica	NR_074464.1	–	–	ADY28112.1	Bacteroidetes	marine
Cytophaga hutchinsonii	NR_112977.1	–	–	ABG58971.1	Bacteroidetes	soil
Erythrobacter litoralis	NR_112040.1	–	–	ABC63973.1	α–proteobacteria	marine
Erythrobacter sp. NAP1	AY326259.1	–	–	EAQ27889.1	α–proteobacteria	marine
Erythrobacter sp. SD–21	AF325445.1	–	–	EDL49290.1	α–proteobacteria	marine
Erythrobacter vulgaris	KM387388.1	–	–	WP_040966112.1	α–proteobacteria	marine
Escherichia coli		NP._289338.1	NT_290230.1		γ–proteobacteria	intestinal
Flavobacterium branchiophilum	NR_104713.1	–	–	CCB69533.1	Bacteroidetes	marine, fish pathogen
Flavobacterium columnare	AY842901.1	–	–	AEW85141.1	Bacteroidetes	marine, fish pathogen
Flavobacterium indicum	KJ635872.1	–	–	CCG53005.1	Bacteroidetes	freshwater
Flavobacterium frigoris	AJ557887.1	–	–	EIA08971.1	Bacteroidetes	freshwater
Flavobacterium johnsoniae	NR_074455.1	–	–	AEW85141.1	Bacteroidetes	widespread
Flavobacterium psychrophilum	AF090991.1	–	–	CAL43932.1	Bacteroidetes	marine, fish pathogen
Flavobacterium sp.	JYGZ01000000	–	–	WP_008254028.1	Bacteroidetes	marine
Formosa agariphila	NR_042770.1	–	–	CDF78486.1	Bacteroidetes	marine
Formosa sp. AK20	HE653972.1	–	–	WP_007650245.1	Bacteroidetes	marine
Gordonia aichiensis	NR_037030.1	–	–	WP_040518104.1	Actinobacteria	pathogen
Gordonia alkanivorans	NR_026488.1	–	–	WP_042375797.1	Actinobacteria	soil
Gordonia araii	EF164924.1	–	–	WP_040523526.1	Actinobacteria	pathogen

Gordonia effusa	NR_041008.1	–	–	GAB18260.1	Actinobacteria	pathogen
Gordonia soli	NR_043331.1	–	–	WP_040510503.1	Actinobacteria	soil
Gordonia sputi	NR_037031.1	–	–	WP_005202624.1	Actinobacteria	pathogen
Gordonia terrae	AY771333.1	–	–	EON34404.1	Actinobacteria	soil
Gracilimonas tropica	EF988655.2	–	–	WP_020402649.1	Bacteroidetes	marine
Hirschia baltica	NR_074121.1	–	–	ACT59256.1	α–proteobacteria	marine
Hyphomonas atlantica	KF863142.1	–	–	KCZ59848.1	α–proteobacteria	marine
Hyphomonas beringensis	KF863136.1	–	–	KCZ54945.1	α–proteobacteria	marine
Hyphomonas chukchiensis	KF863137.1	–	–	KCZ60577.1	α–proteobacteria	marine
Hyphomonas neptunium	NR_074092.1	–	–	ABI76858.1	α–proteobacteria	marine
Jannaschia aquimarina	NR_109177.1	–	–	KIT14514.1	α–proteobacteria	marine
Jannaschia sp. CCS1	NR_074163.1	–	–	ABD53431.1	α–proteobacteria	marine
Marinomonas mediterranea	NR_114181.1	ADZ90397.1	ADZ93328.1	–	γ–proteobacteria	marine
Marinomonas posidonica	NR_074719.1	AEF55681.1	AEF56484.1	–	γ–proteobacteria	marine
Marinomonas sp. MED121	–	EAQ67549.1	EAQ64856.1	–	γ–proteobacteria	marine
Marinomonas sp. MWYL1	NR_074778.1	ABR70182.1	ABR73281.1	–	γ–proteobacteria	marine
Maricaulis maris	NR_041967.1	–	–	ABI65867.1	α–proteobacteria	marine
Methylobacterium aquaticum	LC026011.1	–	–	BAQ47271.1	α–proteobacteria	plants
Methylobacterium extorquens	KP676602.1	–	–	ACK84340.1	α–proteobacteria	soil
Methylobacterium nodulans	JN685307.1	–	–	ACL55084.1	α–proteobacteria	plants
Methylobacterium oryzae	GU294332.1	–	–	AIQ90055.1	α–proteobacteria	plants
Methylobacterium populi	AB698694.1	–	–	ACB81543.1	α–proteobacteria	plants
Methylobacterium radiotolerans	GU294333.1	–	–	KIU35052.1	α–proteobacteria	plant
Methylophilus methylotrophus	NR_041257.1	WP_018987104.1	WP_026295531	–	β–proteobacteria	sewage
Microbacterium laevaniformans	EU879962.1	–	–	EIC08674.1	Actinobacteria	freshwater
Microbacterium testaceum	HE716908.1	–	–	BAJ76473.1	Actinobacteria	plants
Owenweeksia hongkongensis	NR_074100.1	–	–	AEV31368.1	Bacteroidetes	marine
Paracoccus denitrificans	Y17512.1	–	–	ABL69501.1	α–proteobacteria	soil
Paracoccus sp. TRP	EF070124.1	–	–	WP_010393892.1	α–proteobacteria	sewage
Paracoccus sp.	YGY01000000	–	–	WP_011747719.1	α–proteobacteria	marine
Phenylobacterium zucineum	NR_074119.1	–	–	WP_041373419.1	α–proteobacteria	facultative intracellular
Phenylobacterium zucineum		–	–	ACG78291.1	α–proteobacteria	facultative intracellular
Prolixibacter bellariivorans	NR_113041.1	–	–	WP_025864343.1	Bacteroidetes	marine
Photobacterium angustum	NR_119046.1	EAS63818.1	EAS62566.1	–	γ–proteobacteria	marine
Photobacterium damselae	Y18496.1	EEZ40247.1	EEZ42302.1	–	γ–proteobacteria	fish pathogen
Photobacterium iliopiscarium	NR_111990.1	–	KJG25492.1	–	γ–proteobacteria	marine
Photobacterium kishitanii	NR_042852.1	KJG68649.1	KJG69793.1	–	γ–proteobacteria	marine
Photobacterium leiognathi	KC617878.1	–	GAA05296.1	–	γ–proteobacteria	marine
Photobacterium profundum	NR_036943.1	CAG21398.1	CAG18628.1	–	γ–proteobacteria	marine
Photobacterium profundum		EAS43450.1	EAS42677.1	–	γ–proteobacteria	marine
Photobacterium sp. SKA34	–	EAR54733.1	EAR53791.1	–	γ–proteobacteria	marine
Pseudomonas aeruginosa	CP007224.1**	WP_003086042.1	WP_003096603.1	–	γ–proteobacteria	widespread
Pseudomonas fluorescens	AY538263.1	WP_011062709.1	WP_011064232.1	–	γ–proteobacteria	widespread
Pseudomonas mendocina	KJ150296.1	WP_013716225.1	WP_013717759.1	–	γ–proteobacteria	widespread
Pseudomonas putida	KF278708.1	WP_003252420.1	WP_003253381.1	–	γ–proteobacteria	soil
Pseudomonas syringae	KJ830937.1	YP_236765.1	YP_233320.1	–	γ–proteobacteria	plant pathogen
Psychrobacter arcticus	NR_075054.1	WP_011279650.1	WP_011280986.1	–	γ–proteobacteria	soil
Psychrobacter sp. JCM	–	GAF52056.1	GAF52608.1	–	γ–proteobacteria	marine
Rheinheimera nanhaiensis	FJ169968.1	GAB60100.1	GAB58932.1	–	γ–proteobacteria	marine
Rheinheimera sp. A13L	JF951744.1	EGM78776.1	EGM75923.1	–	γ–proteobacteria	freshwater

Rhodobacter capsulatus	HM370064.1	–	–	ADE87041.1	α–proteobacteria	widespread
Rhodobacter sphaeroides	NR_029215.1	–	–	WP_002722413.1	α–proteobacteria	widespread
Rhodobacter sp. SW2	–	–	–	EEW24854.1	α–proteobacteria	widespread
Rhodothermus marinus	NR_029282.1	–	–	ACY47768.1	Bacteroidetes	freshwater
Serratia liquefaciens	NR_122057.1	AKE12053.1	WP_020837653.1	–	γ–proteobacteria	plants
Serratia odorifera	NR_114157.1	EFE97891.1	EFE97188.1	–	γ–proteobacteria	pathogen
Serratia plymuthica	KJ729609.1	WP_013811519.1	WP_006320163.1	–	γ–proteobacteria	soil
Serratia proteamaculans	AB334771.1	ABV39898.1	ABV43961.1	–	γ–proteobacteria	plants
Serratia sp. M24T3	HQ538811.2	EIC86422.1	EIC82670.1	–	γ–proteobacteria	plant pathogen
Serratia symbiotica	NR_117512.1	–	EFW12703.1	–	γ–proteobacteria	insect symbiont
Shewanella amazonensis	NR_074842.1	ABL99240.1	ABL98475.1	–	γ–proteobacteria	freshwater and marine
Shewanella baltica	AJ000214.1	WP_006086868.1	WP_011845588.1	–	γ–proteobacteria	marine
Shewanella benthica	AB008796.1	EDQ01256.1	EDQ00557.1	–	γ–proteobacteria	marine
Shewanella denitrificans	NR_074813.1	ABE54479.1	ABE56710.1	–	γ–proteobacteria	marine
Shewanella frigidimarina	NR_026057.1	–	ABI70226.1	–	γ–proteobacteria	soil
Shewanella loihica	NR_074815.1	ABO23072.1	ABO25365.1	–	γ–proteobacteria	marine
Shewanella halifaxensis	NR_074822.1	ABZ75788.1	ABZ74965.1	–	γ–proteobacteria	marine
Shewanella oneidensis	NR_074798.1	AAN56448.1	AAN53444.1	–	γ–proteobacteria	marine
Shewanella pealeana	NR_114421.1	ABV86509.1	ABV89189.1	–	γ–proteobacteria	marine
Shewanella pealeana		–	ABV89189.1	–	γ–proteobacteria	marine
Shewanella piezotolerans	NR_074738.1	ACJ28140.1	ACJ27154.1	–	γ–proteobacteria	marine
Shewanella putrefaciens	DQ307731.1	ADV55305.1	–	–	γ–proteobacteria	marine
Shewanella sediminis	NR_074819.1	ABV35898.1	ABV34947.1	–	γ–proteobacteria	marine
Shewanella violacea	NR_074924.1	BAJ03149.1	BAJ04049.1	–	γ–proteobacteria	marine
Shewanella woodyi	NR_074846.1	ACA87622.1	ACA88833.1	–	γ–proteobacteria	marine
Streptococcus dysgalactiae subsp. *equisimilis*	–	–	–	Q54089		pathogen
Thalassobaculum salexigens	NR_116122.1	–	–	WP_028794737.1	α–proteobacteria	marine
Vibrio alginolyticus	DQ173157.1	EEZ84441.1	WP_005379340.1	–	γ–proteobacteria	marine
Vibrio anguillarum	X16895.1	AEH32329.1	AEH31606.1	–	γ–proteobacteria	marine
Vibrio brasiliensis	KC508793.1	EGA67634.1	EGA64354.1	–	γ–proteobacteria	marine
Vibrio caribbenthicus	–	EFP98076.1	EFP96942.1	–	γ–proteobacteria	marine
Vibrio coralliilyticus	HM771346.1	WP_006957785.1	WP_006957185.1	–	γ–proteobacteria	marine
Vibrio harveyi	JN990076.1	EDL67178.1	EDL68746.1	–	γ–proteobacteria	marine
Vibrio ichthyoenteri	HG931122.1	EGU49238.1	EGU36680.1	–	γ–proteobacteria	marine
Vibrio mimicus	KJ604709.1	WP_000226859.1	EEW06575.1	–	γ–proteobacteria	marine
Vibrio nigripulchritudo	NR_121769.1	WP_004401314.1	WP_004405948.1	–	γ–proteobacteria	marine
Vibrio orientalis	NR_113788.1	EGU50063.1	EGU52165.1	–	γ–proteobacteria	marine
Vibrio scophthalmi	NR_025992.1	EGU41283.1	EGU33566.1	–	γ–proteobacteria	marine
Vibrio shilonii	NR_114417.1	EDL51111.1	EDL53937.1	–	γ–proteobacteria	marine
Vibrio sinaloensis	DQ451210.1	EGA68012.1	EGA70869.1	–	γ–proteobacteria	marine
Vibrio splendidus	EU091337.1	EAP93640.1	–	–	γ–proteobacteria	marine
Vibrio tubiashii	KP329558.1	AIW15043.1	AIW12710.1	–	γ–proteobacteria	marine
Vibrio vulnificus	HM996972.1	BAC95585.1	`	–	γ–proteobacteria	marine

Marine microorganisms need to cope with changes in their environment and rely on signalling molecules such as (p)ppGpp to adapt to challenging conditions. Their lifestyles might be the reason for the evolution of two genes belonging to the RelA/SpoT family. However, we did not find any specific adaptation of marine bacteria in these terms as there are no obvious correlations with the presence of single RSH enzyme or both RelA and SpoT proteins and the bacterial lifestyles. Moreover, the similarity of amino acid sequences, and in particularly, specific amino acid residues indispensable for catalytic activity of enzymes is very high, and any observed changes are parallel with the taxonomical and evolutionary correlations.

Acknowledgements

We thank Dr. Katarzyna Potrykus for helpful comments, discussion and critical reading of the manuscript.

Acknowledgment of financial support

This work was supported by the grant from the National Science Center (Poland) (grant number N N301 550540) to A.S-P.

REFERENCES

Amy PS, Pauling C, Morita RY (1983) Recovery from nutrient starvation by a marine *Vibrio* sp. *Appl Environ Microbiol* **45**: 1685–1690. PMID: 6191662.

Atkinson GC, Tenson T, Hauryliuk V (2011) The RelA/SpoT Homolog (RSH) Superfamily: Distribution and Functional Evolution of ppGpp Synthetases and Hydrolases across the Tree of Life. Stiller JW, ed. *PLoS ONE* **6**: e23479. DOI: 10.1371/journal.pone.0023479.

Battesti A, Bouveret E (2006) Acyl carrier protein/Spot interaction, the switch linking SpoT dependent stress response to fatty acid metabolism. *Mol Microbiol* **62**: 1048–1063. DOI: 10.1111/j.1365-2958.2006.05442.x.

Braeken K, Moris M, Daniels R, Vanderleyden J, Michiels J (2006) New horizons for (p)ppGpp in bacterial and plant physiology. *Trends Microbiol.* **14**: 45–54. DOI: 10.1016/j.tim.2005.11.006.

Cabaj A, Palińska K, Kosakowska A, Kurlenda J (2006) Heterotrophic bacteria from brackish water of the southern Baltic Sea: biochemical and molecular identification and characterization. *Oceanologia* **48**: 525–543.

Dalebroux ZD, Svensson SL, Gaynor EC, Swanson MS (2010) ppGpp conjures bacterial virulence. *Microbiol Mol Biol Rev* **74**: 171–99. DOI: 10.1128/MMBR.00046-09.

Flärdh K, Cohen PS, Kjelleberg S (1992) Ribosomes exist in large excess over the apparent demand for protein synthesis during carbon starvation in marine *Vibrio* sp. strain CCUG 15956. *J Bacteriol* **174**: 6780–6788. PMID: 1383195.

Flärdh K, Axberg T, Albertson NH, Kjelleberg S (1994) Stringent control during carbon starvation of marine *Vibrio* sp. strain S14: molecular cloning, nucleotide sequence, and deletion of the *rel*A gene. *J Bacteriol* **176**: 5949–5957. PMID: 7928955.

Hogg T, Mechold U, Malke H, Cashel M, Hilgenfeld R (2004) Conformational antagonism between opposing active sites in a bifunctional RelA/SpoT homolog modulates (p)ppGpp metabolism during the stringent response. *Cell* **117**: 57–68. DOI: http://dx.doi.org/10.1016/S0092-8674(04)00260-0.

Kurath G, Morita RY (1983) Starvation-survival physiological studies of a marine *Pseudomonas* sp. *Appl Environ Microbiol.* **45**: 1206–1211. PMID: 16346265.

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees in *Proceedings of the Gateway Computing Environments Workshop (GCE),* New Orleans, LA pp 1–8. DOI: 10.1109/GCE.2010.5676129.

Mudryk ZJ, Podgórska B (2005) Generic composition and respiratory activity of heterotrophic bacteria of marine sandy beach (Baltic Sea). *Pol J Ecol* **53**: 97–103.

Ostling J, Flärdh K, Kjelleberg S (1995) Isolation of a carbon starvation regulatory mutant in a marine *Vibrio* strain. *J Bacteriol* **23**: 6978–6982. PMID: 7592494.

Ostling J, Holmquist L, Kjelleberg S (1996) Global analysis of the carbon starvation response of a marine *Vibrio* species with disruptions in genes homologous to relA and spoT. *J Bacteriol* **178**: 4901–4908. PMID 8759854

Page RD (1996) TreeView: an application to display phylogenetic trees on personal computers. *Computer applications in the biosciences: CABIOS* **12**: 357–358. DOI: 10.1093/bioinformatics/12.4.357.

Potrykus K, Cashel M (2008) (p)ppGpp: still magical? *Annu Rev Microbiol* **62**: 35–51. DOI: 10.1146/annurev.micro.62.081307.162903.

Riemann L, Leitet C, Pommier T, Simu K, Holmfeldt K, Larsson U, Hagström A (2008) The native bacterioplankton community in the central Baltic sea is influenced by freshwater bacterial species. *Appl Environ Microbiol* **74**: 503–515. DOI: 10.1128/AEM.01983-07.

Sjöstedt J, Koch-Schmidt P, Pontarp M, Canbäck B, Tunlid A, Lundberg P, Hagström A, Riemann L (2012) Recruitment of members from the rare biosphere of marine bacterioplankton communities after an environmental disturbance. *Appl Environ Microbiol* **78**: 1361–1369. DOI: 10.1128/AEM.05542-11.

Sogin ML, Morrison HG, Huber JA, Welch MD., Huse SM, Neal PR, Arrieta JM, Herndl GJ (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". *Proc Natl Acad Sci USA* **103**: 12115–12120. DOI: 10.1073/pnas.0605127103.

Stolle C, Labrenz M, Meeske C, Jürgens K (2011) Bacterioneuston community structure in the southern Baltic sea and its dependence on meteorological conditions. *Appl Environ Microbiol* **77**: 3726–3733. DOI: 10.1128/AEM.00042-11.

Sun D, Lee G, Lee JH, Kim HY, Rhee HW, Park SY, Kim KJ, Kim Y, Kim BY, Hong JI, Park C, Choy HE, Kim JH, Jeon YH, Chung J (2010) A metazoan ortholog of SpoT hydrolyzes ppGpp and functions in starvation responses. *Nat Struct Mol Biol* **17**: 1188–1194. DOI: 10.1038/nsmb.1906 .

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, and Kumar S (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**: 2731–2739. DOI: 10.1093/molbev/msr121.