

Regular paper

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

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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 B- 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

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### 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 Grampositive 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  $\beta$ - and  $\gamma$ -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

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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-Łyżen, Paweł Olszewski, Marta Moskot, Magdalena Balut, 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 CIP-RES 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  $\geq$ 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 ReIA 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  $\beta$  and  $\gamma$ -proteobacteria form highly supported clades with bootstrap supports of 100. These organisms are Gramnegative 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  $\beta$  and  $\gamma$ -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  $\beta$  and  $\gamma$ -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  $\gamma$ -*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  $\gamma$ -*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  $\alpha$ -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).

	10	20	30	40	50	60	)	70	80	90	100	110	120
RelSeq	EEVVALAAKYMNETDA	. Afv <b>kk</b> al <b>d</b> ya	TAAHFYOV	 RKSGEPYIVHP	IQVAGI	LA-DLHLDAV	 /TVA <mark>C</mark> GF	LHDVVED	I	.    EFDFGKDVRDIV	 DGVTKLGKVE	YKSHEE-QL	 AENHRK
A.coralici	YELVEKVAAYKPDLDE	ALLNRAYVYA	MQKHGAQK	RASGDPYFSHP	LEVAAI	LT-DMHLDE	TVAVAL	LHDTIED	T-DATRKEII	OQHFGPKIGQLVI	EGLTKLKRLD	LVSKKA-AQ	AENLRK
1.salexige M.maris	DELIARIRRYFPKVDA	DFVRRAYDFA	EEAHRPQE	RASGDPIFSHP RQSGEPYFAHV	AQVAMI	LA-DLRMDVA	ATVCTGL	LHDVIED	T-PATLEDL	IDAF SEEVASLVI	NGVIKLIRIE	LRSKRT-KQ	AENFRK AENLQK
H.baltica	YELVERVLAYOPDADE	DALNRAYVFA	MVRHGAQT	RHSGDPYYAHP	VSVAGI	LT-DLKLDYS	STIIAGI	LHDTVED	T-DVTLEEI	ELFSKDIAEIVI	DGVTKLTQLE	SSSRAA-KQ	AENFQK
E.vulgaris	YELVERVLEYDPDADE	AMLNRAYVYI	VQKHGTQT	RASGDPYFSHP	VEVAGL	MT-DLKLDQI	TIATAL	LHDTVED	T-LATIDDI	EKNFGGEVARLVI	DGVIKLDKLE	QMPENE-RA	AENLRK
J.aquimari Paracoccus	QDLVALVRAYNPRTDA EDLLALVRNYNPRSNC	DLIERAYAFG	AEMHSGQT	RRSGEPYFTHP PHSGEPYFTHP	VEVACL	LT-EQRLDD	TIACAL	LHDVIED	T-RASESDVI	ERFGRDVAELVI	DGVTKLTKIQ	LSSSET-KQ	AENFRK
P.ubique	NELINKVKGYNKFLNP	ERLDKAYNFA	VKAHQNQK	RASGDPYSVHP	IEVANI	LT-DLKLDS	TITTGI	LHDTIED	T-FATYDTI	KTEFGDEVAELVI	DGVTKLINDE	NTANAN-SK	VENFRK
C.lytica E agariphi	KELLRVSYLTLSDEDK	KLIRSAFEIA	VDAHKDQF	RKSGEAYIFHP RKSGEAYIFHP	IAVARI IAVARI	VASEIGLDAV	SIASAL	LHDVVED	T-EYTLADII	ERLEGETVAKIVI RELEGETVALIVI	DGLTKIAHLK	KDMNVS-QQ	AENFRK
Flavobacte	KELLRISYQTLTDEDK	KLIRKAFDVA	VDAHKEQF	RKSGEAYIFHP	IAVAKI	VASEIGLGAT	<b>TSIAAA</b> I	MHDVVED	T-DITVDDI	KMFNPKIAKIV	EGLTKIAKVK	TDQDVS-VQ	AENFRK
O.hongkong P.bellarii	RALLRAMQDRADNDDR DDLLKSFNRPVTDEAK	KLIRKAFKLA ALILKAFNFA	QDAHADVE NKAHMGVE	RKSGEPYIFHP RKSGEPYILHP	LEVAQI LAVAKI	VAKEIGLGPV VTSEIGLGAE	/SVAAAI (SATAAI	LHDVVED	S-DYTLEDII T-DYSLODII	DCLFGEEIARII ENMFGKKVANLVI	DGLTKISGVF DGLTKLSGTF	DSKOAV	AENFRK NFRK
G.tropica	KQLVEVCQEHIENVDE	EAISKAFKLC	YLSHQDMK	RASGEPYYYHP	VEVAKI	VASEINIDD	/SVIASI	LHDTVED	T-DVNLDDI	RYWFGEEVAVII	DGVTKITGVF	KSRDSKQ	AEAFMK
	130	140	150	160 	170	180	) 	190 	200	210	220 	230	240
RelSeq	MLMAMSKDIRVILVKL	ALRLHNMRTL	KHLR-KEK	ERISRETMEI	YAPLAH	RLGISRIKW	ELEDLAF	RYLNETE	FYKISHMMN	KRREREALVDD	IVTKIKSYTT	EQGLFGDVY	GRPKHI
A.coralici T.salexige	LVLAMSQDIRVLLVKL	ALRLHNMRTL ALRVHNMRTL	GHMA-PEK HFIKNPEK	RARISQEIMDI RRRIAAEIMDI	YAPLAG	RIGINEIKD	SLEDLAF ELEDLAF	FAEINPDA	RTSIKARLD	LNQRHASRIAS. (LRSQGGDMVNS)	IETDLTDRLR IIAELHEKLA	DAGVIADIS	GRMKTP
M.maris	LVVAISDDVRVLIVKL	CERLHNMRTL	DAISRAEK	RERIALETLEI	YAPLAR	RIGINRVCV	ELEDLAF	EHVNASA	HESINTRLK	RLRDAHAEEVSV	VSAAMTDSLA	KAGIEGRIF	GREKRP
H.atlantic	FILATISDIRVLLVKL	ALRLHNMRTI ALRLHNMRTI	HFRKKASS	RERTARETMDI	YGPLAR	RIGLSIFAG	EMEDLAF	FQELNPEA	RRAILYRQEI	ELALENAGDLER	IREALQELME	ESGIACRIK	GRKKQP
E.vulgaris	FLLAMSEDIRVLLVKL	GERLHNMRTL	HFIKKPEK	RORIARETMDI	YAPLAE	RVGMYEYMR	MQLLAF	FEQIEPEA	YTTITNRLQ	DIREODGGOVDA	IALDMKHALA	EAGLSVEVS	GREKHP
Paracoccus	LFMAMSRDBRVILVKL	ACRLHNMRTI	RSMR-PEK	QVKKARETMDI	YAPLAG	RMGMQWMRE	ELEDLAF	KVINPEA	RSSIIRREV	SLQRDSGDIIGQ:	ITADIRTEME	KEGIEADVF	GRAKKP
P.ubique	LILATSKDIRVLLVKI	ALRLHNMRTI	KAIPKEEK	RKRIAQETMEI	YAPLAD	RMGMHRIRD	ELEDLSE	FEILNNDA	RKLIKIRLD	EIKLDKKDIFEE	LSFELSEILN	DNHINAEIY	GREKTP
F.agariphi	MLLTLNDDVRVIIIKI	AERLHNMQTM	IDSMR-PEK	DIKIASETLYI	YAPLAH	RIGLYNIKN	ELEDLSI	KYTEPDV	YFDILNKIKI	OSKEGQDEYIRE	FNELIKKSLD	REGLHYTIK	GRPKSI
Flavobacte O.hongkong	MLLTLNDDVRVILIKI MLLTISDDIRVIIIKL	ACRLHNMQTM ACRLHNMRTM	IDSMA-EYK IESMP-AHK	DAKIASETLYI OVKIASETLYL	YAPLAH YAPLAH	RLGLYNIKT, RLGLYNIKT	DLEDLGI ELEDLSI	KYTEPEI RYTEPEV	FKEIISKIKI YRDIVLKLKS	SKADEIKYLKR	ISSVLS <b>E</b> SLN FTTKIREELK	EEGIEYSIK KENFNFTIK	GRPKSI ERTKSI
P.bellarii	MLLTLSDDVRVILIKL	ALRLHNMRTI	DSMP-RNK	QLKIAGETLYV	FAPLAH	RLGLYSIKT	ELEDLSI	RYKHPEA	YQQIDLQLH	QEERINYLVHV	FAKPIQKKLY	EEHFDFTIS	GRPKSI
G.tropica	LLLTMAEDIRVVLIKF	AURLHNMRTI	онрк-век	PIQIASETMDL	YAPLAH	RFGLFRIKN	SLEDLCE	<u>KT</u> IDPTS	YKFVARKLRI	SKKEDREEFIQE	FMDPIKNELG	RMNFKFEIN	GRPKH
	-												
	250	260	270	280	290	300	) 	310	320	330	340 	350	360
RelSeq	YSIYRKMRDKKKRFDQ	IFDLIAIRCV	METQS	DVYAMVGY	IHELWR	PMPGREKDY	IAAP <b>K</b> A	4GYQSIHT	TVYGPKG-P	EIQIRTKEMHQ	VA <mark>E</mark> YGVAAHW	AYKKGVRGK	VNQ
A.coralici T.salexige	YSIWQKMQRKEVGIEQ	LSDIFGFRVI LSDIMAFRLV	VEIEE VDDIG	GCYQCLGA	VHRSWA MHGSYP	WVPGRFKDY VVPGRFKDY	ISTPKON ISTPKPN	GYRSIHI GYRSLHT	GVIGPHRQR	ELQIRIRRMHE	VAEIGVAAHI IAELGVAAHW	NYKQRDG	DQR-VH
M.maris	YSIWRKLERKGLTFEE	IADIYAFRLI	VDTPD	DCYRALGV	IHQSWR	CVPERFRDF	SLPKP	NYRSLHT	TVMGPKNVR	ELQIRTEEMES	VAESGVAAHW	RYKNSSYTY	DADAAQ
H.atlantic	YSLWRKLEKKSISFRD	VADLFAFRVI	VSSVE	DCYRVLGK	VHALWA	CIPDRERDY	I SVPKPN I SVPKPN	IGYASLHI	TVRASGNRR	ELQIRTEEMDR	TAEFGVAAHW	GYKNHSYGF:	DVD-SA
E.vulgaris	YSIWRKMAERHVSFEQ	VTDIMAFRVI USDIVCERU	CDDVA	DCYRAMGV	LHTTWQ	FLPGKEKDY	SOPKSN	GYRSLHT	SLIYGKSMR	EVQIRTRDMHR	TNEFGLAAHW	AYKQAER	PD
Paracoccus	FSVWRKMQEKQLAFSR	LSDIYGFRII	TRTEM	DCYRALGV	IHHRWR	AVPGREKDY	I SQPKSN I SQPKSN	GYRSIHI	TVSGRDGKR	EVQIRTROMHE	VAERGVAAHW VAEAGVAAHW	AYRDGVR	TR
P.ubique	FSIWRKVQKKRVSLEQ	VTDIIGFRII	LKNID	DCYKTLGI	FHKKWN VTDNFT	CIPGKEKDY	SSPKIN	IGYKSIHT	AVIGSNKKP	EIQIRTNEMHE	FAERGVASHW	QYKSSEK	F
F.agariphi	FSIRRKMMKQGVSFDE	VYDKFAVRI I	YKSDT-AN	EKFLAWKIYSI	VTDHFR	PNPIRLRDW	I SSPKSI	<b>IGYEALHI</b>	TVMGPKGRW	EVQIRSERMNE	IA <b>EKG</b> YAAHY	KYKQ	D
Flavobacte O.hongkong	YSIRRKMKNQGVTFDE YSIRKKMINOGISFDE	VYDKFALRI I TYDKFATRI I	YKSNP-HD LDSPP-ET	EKFLAWKIYSV EKADAWRVYSV	VTDHYR VTDFYR	PSPSRIRDW	ISSPKSI ISAPKSN	IGYEALHI IGYESLHI	TVMGPKGRWV	EIQVRSERMDE:	IA <b>EK</b> GYAAHY VA <b>EK</b> GYAAHW	KYKN	G
P.bellarii	YSIWNKMQNKKISFSE	IYDLLAIRIV	FKPKPGLS	EKRQCFDILSL	ITDIYK	PKPDRIRDW	TIPKAN	IGYEALHV	TVMGPEGQW	EVQIRTERMDE	IAERGFAAHY	KYKG	D
G.tropica	FSIYRKMQRQQKPFEE	IYDLFAIR	LENPH	TKEDCWRVYSI	ITDWYT	PIPERFRDF	ISVPKAN		TVITNKGRKV	EVQIRTRRMDD	IAEKGLAAHW	KYKEG	A
	370	380	390	400	410	420	) 	430	440	450	460	470	480
RelSeq	AEQKVGMNWIKEL	VELQDASNGE	-AVDFVDS	VKEDIFSERIY	VFTPTG	AVQELPKDS	PIDFAY	AIHTQVG	EKAIGAKVNO	GRMVPLTAKLKT	GDVVEIVTNP	NSFGPSRDW	IKLVKT
A.coralici T.salexige	-INEGKQYRWIREL	LDILEQASGF	EEFLEH	TKLELFQDQVF	CFTPKG	DLIALPRGA	/PIDFAY	(AVHIDVG (AVHS <b>E</b> VG	DSCVGARIDO	GRIMPVVTELSNO	GDEVEIIRAK GDQVEIVTSK	GAT-PPQAW. TST-PSPNW	EQIVVT
M.maris	AAGG-DPLERLRPF	VEILNQGGDF	EEFLEH	AKLEMFADQVY	CFTPKG	DLISLPVGA	PLDFAY	AVHTELG	HTTVAAKIN	GRERPLETELION	GDVVAIVKGG	VRQ-PPAGW	ENLAVT
H.atlantic	RAAGLDPEDSLLSF RAAGLDPAANLEAF	AELIQDGGDF	eefleh sefmeh	AKMEMP RD1 VF AKLEMYREHVF	AF TPRG AFTPKG	KLIILPAGAN	IPLDFA1	AVHSAVG	DECVGVRING	SEIKPLRRPLKN(	GDITEIIRGP GDVVEVIRGK	APQ-AIHGW	EALAIT
E.vulgaris	GQVGWLRDL	IEIVDASHDA	EELLEH	TRMAIYQDRIF	AFTPKG	ALHQLPKGS	TAVDFAF	AVHTELG	TQTVGAKIN	RHMPLRTQLNN	GDVVEILKGK	NAE-POMSW	LGFVIT
Paracoccus	NPFAVDPAEWLRQM	TDRFD-TEDH	DEFLEA	VKLEMISDQVF	CFTPKG	DVIKLPKGAI	(PIDFA)	AIHTRIG	NSCVGAKID	GIRVPLWIRLKN	GQSVEIVIAE	GQR-PQATW	LDIVVT
P.ubique	NSLSWKEYDWLKDL	VEIIEKNENF	EHSYEY	TKLQMFQENVF	CFTPKG	SVIKLPKDAT	PIDFAY	AVHTKIG	NTAIGCEIN	SNKSELQDILRNO	GDRVNIITSK	NQS-PSLHW	IPTTKT
F.agariphi	TEKEDSLDSWVAKL	QEALESNETN	-AVDEVEE	FKLNLYSKEIY	VF TPQG VFTPKG	DLKSLPKGAT	I DE DE AF	NIHTEVG	MRTRGAKVN	SKLVPLSYKLHS	GDQVDILTSD	SAK-PNQSW	LDYATT
Flavobacte	NSEEHGLEVWLNQL	KEALESQAAN REMLENNDGS	-AVDEVED	FKLNLYSKEIY	IFTPKG	DIKSLPKGAT	TLDFAF	SINTDIG	VKTRGTRVN	KLVPLNHVLNS	GDQVEIITSV	NQK-PSVQW	LDYVTT
P.bellarii	NTAESEIDRWLEKI	RELLQNPESC	-ALDFLDE	FKLNLYSQEII	IFTPKG	DIKTIPAGA	CVLDFAY	DIHTELG	NKCIGAKVNI	IQLVPMSYVLSS	GDQVEILTSD	KQN-PKPSW	LEIAVT
G.tropica	QQGSDTLDKFVNWV	RDVLDNPRPD	AATDFVKD	FQLNLYKDEIY	VFTPDG	ELRTLPRNAT	[PIDFAF	FEIHSEIG	ERAMAAKVN	GKMVPLRQKLHN	GDQVEIITGN	KIN-LNP <b>D</b> W	IDDVVT
		500	510								500		
	490   .	.	510	520	530	54( ••••	, 	550 	560	570 	580 	590 	600 
RelSeq	NKARNKIRQFFKNQDK	ELSVNKGRDM	LVSYF-QE	QGYVANKYLDK	KRIEAI	LPKVSVKSER	SLYAAV	GFGDISP	VSVFNKLTE	KERREEE	R	AKAKAEAEE	LVN
A.COFALICI T.salexige	GKAKARIRRFVRLKRR	QQFSDLGQQI	LQKAF		KQIEPL	LGRFHAEAVE	EDLYAGI	GEGLESA	LEVVHAVHPI	PVVEPK	vTR	KEENV	VP
M.maris	GRARAAIRRLIRESER	DEFHRIGKIM	AEHAF	RREGRTLIE	DDLKDA	LSRLEVKDV	EMYETI	GRGRISS	VDMLNAAFP-	-GRLDE		RPD-	
H.atlantic	GRARSAMRKLVRDKET	TEFRRLGQGL	INMAL	RRAGIDPID	VKMNHT.	AVQSGMATTE ARLAGFENLE	SEDDEEL SEMAEAL	GRGE IDL	NDVIVAAFP	GYRPER		EDD-	
E.vulgaris	GRARAAIRRAVRLKER	GEVAEIGQKL	YDEIV	TRVPAKIGK	KALREA	LKRLEMEEPH	DLFYAI	GAAKITD	RAVMEALMP-	-GSTEG		MAD-	
	CONTRACTOR OF STREET	D D D D D D D D D D D D D D D D D D D		THE PRIME I D		A NOVOLD ST		00000000	The second s				-

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.bellari G.tropica	610GGEI AAGGGEI AAG	620 KHENKDVLKVRS GRSKRSKKG- GRSKRSKKG- LIQD-SKARL- HAIDDETES- VRMDSEHTPL- DEIDG- IDKESITAKYD- IDKESITAKYD- IDKNEISKKYD- KAVQKEKIEKK 	630 II.I. ENGVIIQASGI ALPIRGLDGI -LLSCEKLPG' -VVRGRDLSPG' -SLSIRGLTGG -ARAVVGLDPK. -RRPFAGLBAD -DLVFCKEEEK -LLVFCKEEEK -LLVFCKEEEK -LLVFCKEEEK -LLVFCKEEEK -LLVFCKEEK -LLVFCKEEK -LLVFCKEEK	640 	650 I PVPGDPIEG AVPGDPIVG PLPGDRIVG PLPGDRIVG PVPGPRIVG PVPGPRIVG PVPGPRIVG PIPGDVFG PIPGDVFG PIPGDVVG PIPGDVVG PIPGDVVG	660 IIITKGRGIAIH ILEPGKGITI) IVTTGKGVTI JQIAGKGVDV JQIAGKGVDV JEVGGGUVVI ITNRGGVSI ITNRGGVSI ITNRGGVVI ITNRGIKVI ITINBGIKVI ITINBGIKVI ITINBGIKVI ITINBGIKVI ITINBGIKVI	670 IRADCINITSCO IRADCINITSCO ITIDCETLEGRO ITIDCETLEGRO ITIDCETLEGRO ITIDCETLEGRO ITIDCETLEGRO ITIDCETLEGRO INVASCHMAT INVASCO IRKUCPNAISLQ IRKUCPNAISLQ IRKUCPNAISLQ IRKTCPEAIKLM IRAMCNNAQHLL	680 	690 ENDIDESAME ANDDDAS- ANDADAS- SURPIASTDFI RWTELARTGA SURPIASTDFI RWTELARTGA SUKCRSRG QWAPGHHA-A QWAPGHHA-A QWAPGHHA-A KWIDSSQEE- KWIDSSQEE- KWIDSSQEE- KWIDSSQEE- KWIDSSQEE- KWIDSSQEE- KWIDSSQEE- KWIDSAGA	700 JJ. YQAEIDIYQ SYMARIQAVH YAVSRILIAI TAIGRIXIM AVGRLYVI FAVSRILIAI TSDVIEMTI AYSTVLNLTI FTANIKLTS FKAMLNIT YTTVLVIRG -YLSHIYIRG -FLGAIKVI	710 LINERGSLAE: ANEPGSLGSI ANEPGSLGSI HINEPGALAE: SNQRGSLAT SNRRGVLAK SNDRGVLGR: RHDAGVLGR: IDNIGLSD IDNIGLIVNQ MDTGLTNEI IDTVGLVNKQ GDRVGMIND	720 VLQIL IAETV LSTVI IAKTV VCKTI ICTLI ICTLI ISSLI LTSII LTKVI VTQII ITNVI ITNVI
RelSeq A.coralici T.salexige M.maris H.baltica H.taltantic E.vulgaris J.aquimari Paracoccus P.ubique C.lytica F.agariphi Flavobacte O.hongkong P.bellarii G.tropica	730 S-NSTKSISTVN A-ANDANIHNLT G-KNGGNIMNLK S-ENRGNIAAVT S-ENRGNITSIH A-QAGANISNLS G-AQGANISNLS G-SHKLNISNVE SQMHVNMRSUN SNMHVNIQNIT SNDLNVNIRSIN SKSLETNMKSIN	740 AQPTRDMFANI W-NTAPDFTKM W-NTAPDFTKM IV-NRSIDFFEM TR-NRADFFDM IK-NRNEDFFDI UT-DENEDFTDI FV-DENEDFTDI FV-DENEDFFRL FS-TDGGTFSG LS-GEAGIFNG LA-GEAGIFNG LA-GDEGVFEG VS-SDSGMFEG	750 II.H HYSFGIPNITHI EFDVEVFDIRHI EFDVEVFDIRHI LIDVEVFDIRHI LEDVEVFDIRHI EVEVELRHEHI KITVIVKINISHI KITVIVKINISHI KITVIVVKNISHI LITVVVKDIHI LITVVVKDIHI LITLYVGISHI	760 II TITVVENIKA TQTIRQLRA TIQTLRQLRA TIQILAALRA ANILAAIRM TQILAALRS TRILSALRA HATMMALET HNLLTALEA JATMMALET HNLLTALEA JKLLYANLKK JKLUANLKK JKLUANLKK JKLUANLKK JKLUSLKKE NKLWANLKK	770 VPDVVSVKR KPCVSEVVR TVVINSVDR CETVVSADR LSAVESVDR LSAVESVDR LSAVESVDR SDAVQAER ESDVAQVE ESDVAQVE LSAVESVDR LIGUKVSR LEGVSSVDR LEGVSVDR LEGVSVVLR							

Figure 7. Consensus alignment of Rsh homologs from selected marine bacteria.

The Streptococcus equisimilis RelSeq 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.  $\alpha$ -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. equi*similis* 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  $\alpha$ -,  $\beta$ - and  $\gamma$ -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 ReIA, SpoT<br/>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		NP289338.1	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 putida Pseudomonas syringae	KF278708.1 KJ830937.1	WP_003252420.1 YP_236765.1	WP_003253381.1 YP_233320.1	-	γ–proteobacteria γ–proteobacteria	soil plant pathogen
Pseudomonas putida Pseudomonas syringae Psychrobacter arcticus	KF278708.1 KJ830937.1 NR_075054.1	WP_003252420.1 YP_236765.1 WP_011279650.1	WP_003253381.1 YP_233320.1 WP_011280986.1	- - -	γ–proteobacteria γ–proteobacteria γ–proteobacteria	soil plant pathogen soil
Pseudomonas putida Pseudomonas syringae Psychrobacter arcticus Psychrobacter sp. JCM	KF278708.1 KJ830937.1 NR_075054.1 -	WP_003252420.1 YP_236765.1 WP_011279650.1 GAF52056.1	WP_003253381.1 YP_233320.1 WP_011280986.1 GAF52608.1	- - -	γ-proteobacteria γ-proteobacteria γ-proteobacteria γ-proteobacteria	soil plant pathogen soil marine
Pseudomonas putida Pseudomonas syringae Psychrobacter arcticus Psychrobacter sp. JCM Rheinheimera nanhaiensis	KF278708.1 KJ830937.1 NR_075054.1 - FJ169968.1	WP_003252420.1 YP_236765.1 WP_011279650.1 GAF52056.1 GAB60100.1	WP_003253381.1 YP_233320.1 WP_011280986.1 GAF52608.1 GAB58932.1	- - - -	<ul> <li>γ-proteobacteria</li> <li>γ-proteobacteria</li> <li>γ-proteobacteria</li> <li>γ-proteobacteria</li> <li>γ-proteobacteria</li> </ul>	soil plant pathogen soil marine marine

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

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.

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