Journal of Applied Genetics (2020) 61:123–130
https://doi.org/10.1007/s13353-019-00531-w

MICROBIAL GENETICS • ORIGINAL PAPER

The stringent response of marine bacteria – assessment of (p)ppGpp accumulation upon stress conditions

Klaudia Milewska[1] · Klaudyna Krause[1] · Agnieszka Szalewska-Pałasz[1]

Received: 18 September 2019 / Revised: 31 October 2019 / Accepted: 8 November 2019 / Published online: 26 November 2019

Abstract

Microorganisms are particularly adapted to alterations in their environment. One of the global regulatory mechanisms involved in these adaptations is the stringent response. The unusual nucleotides, guanosine penta and tetraphosphates, (p)ppGpp act as alarmones of this response, heralding nutrient limitation and stressors. Marine bacteria encounter numerous stresses of sparse nutrient supplies and changes in physicochemical conditions. The aim of this work was to assess whether the stress conditions common in marine environment can induce the stringent response and what is a kinetic of this process. The representative bacterial species, *Shewanella baltica*, *Acinetobacter johnsonii*, *Vibrio harveyi,* and *Escherichia coli* were subjected to a variety of stressors. We analyzed the kinetics of (p)ppGpp synthesis by labeling in vivo nucleotides and analysis by thin layer chromatography. The (p)ppGpp accumulation followed the elevated temperature and amino acid starvation for all bacteria tested. The carbon and nitrogen limitation resulted in the response limited to *V. harveyi* and *S. baltica*. The DNA damaging agents induced the (p)ppGpp production in all strains, while osmotic stress did not result in significant alarmone synthesis. The representative marine bacteria species were shown to induce with varying extent the stringent response upon the onset of stress and limitation conditions. Importantly, the in vivo labeling and subsequent separation of the nucleotides by thin layer chromatography serves as a valid method for the analysis of the stringent response and (p)ppGpp accumulation in environmental bacteria.

Keywords Marine bacteria · (p)ppGpp · Stringent response · Environmental stress · Thin-layer chromatography

Introduction

Bacteria can deal with unfavorable conditions in multiple ways, where the time and extent of the reaction is crucial for survival. One of the global and far-reaching mechanisms of adaptation to environmental conditions is the stringent response, where unusual nucleotides (guanosine tetra- and pentaphosphate, ppGpp, and pppGpp, collectively named (p)ppGpp), herald starvation, and various physicochemical stress (Cashel et al. 1996; Potrykus and Cashel 2008 and refs therein). These alarmones are produced promptly after the onset of stress, directly and indirectly affect major cellular processes such as adaptation to adverse conditions, sporulation, biofilm formation, quorum sensing and virulence (Jain et al. 2006; Potrykus and Cashel 2008; Dalebroux et al. 2010). (p)ppGpp has been identified in all free-living eubacteria tested so far (Potrykus and Cashel 2008; Atkinson et al. 2011) and chloroplast-bearing plants (Braeken et al. 2006). The enzymes responsible for its synthesis and degradation could be one dual-function or separate proteins, coded by *rsh* or *rel* (synthesis) and *spo* (hydrolysis) genes, respectively. In model bacterium, *Escherichia coli* and some of β- and γ-proteobacteria, two enzymes are responsible for (p)ppGpp accumulation: the monofunctional synthetase I encoded by *relA* gene and the bifunctional synthetase SpoT which have also (p)ppGpp hydrolase activity. RelA protein, associated with ribosomes, responds to amino acids starvation, while SpoT synthetase activity is induced by limitation of other nutrients or by stresses (Potrykus and Cashel 2008; Dalebroux et al. 2010). In addition, some short enzymes with only synthesis domains were identified in some bacterial species. This

Communicated by: Marek Switonski

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13353-019-00531-w) contains supplementary material, which is available to authorized users.

✉ Agnieszka Szalewska-Pałasz
Agnieszka.Szalewska-Palasz@ug.edu.pl

[1] Department of Bacterial Molecular Genetics, University of Gdańsk, Wita Stwosza 59, Gdańsk, Poland

suggests a possible generalized ppGpp role in all living organisms. (p)ppGpp has a major impact on cellular metabolism and gene expression which are altered in order to survive and to use limited resources in the economical way, e.g., the expression of the growth-related genes is inhibited, while those coding for proteins connected with survival and efficient nutrient utilization are induced (Cashel et al. 1996; Magnusson et al. 2005; Potrykus and Cashel 2008). However, despite almost 50 years of study, many aspects of ppGpp-mediated regulation remain obscure or have been solved only recently e.g., the ppGpp influence on transcription or DNA replication (for review, see Potrykus and Cashel 2008). Most of the knowledge of the mechanisms of bacterial stress response comes from the studies on the model bacteria, *Escherichia coli* that can switch between free-living and intestine environment, soil bacterium *Pseudomonas putida*, or Gram-positive *Bacillus subtilis*.

The marine environments present an exceptionally severe challenge for free-living bacteria because of the varying nutrient supplies and other important factors. Thus, it can be hypothesized that the marine microorganisms are particularly well-adjusted to the altering conditions. 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 and Morita 1983). Understanding the physiological mechanisms of these adjustments is therefore crucial to reveal the molecular mechanisms underlying bacterial stress responses and ultimately leading to survival. However, the information on their stringent response is quite limited with few 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 (Flärdh et al. 1992, 1994; Östling et al. 1996). The presence of (p)ppGpp-metabolizing enzymes and the relevant genes was shown in the phylogenetic analyses of the genomes from microorganisms associated with marine environment (Guzow-Krzemińska et al. 2015). However, the mechanisms and the kinetics of the induction of the stringent response in marine bacteria have not been studied so far. Thus, we employed a series of various stresses to compare the stringent response of representative marine bacteria to the known model of *E. coli*. Importantly, the study on the stringent response in other than model bacteria is often more difficult because of the growth conditions and requirements for guanosine nucleotide labeling (low phosphate medium) and for stress introduction. For the quantification of bacterial alarmones, HPLC method has been employed (Varik et al. 2017); however, this method is relatively complex and has some serious limitations. Thus, it is important to prove that the standard procedure established already 50 years ago (Cashel et al. 1969) can be employed with some culturing modifications to environmental bacteria.

Material and methods

Bacterial strains

Bacterial strains used in this work are listed in Table 1.

Media and growth conditions

Bacteria were cultured in liquid LB broth (Sigma), Marine broth (Difco), MOPS minimal (morpholinepropanesulfonic acid) medium (for *E. coli*, *S. baltica*, *A. johnsonii*) (Mechold et al. 2013), or Tris-minimal medium (*V. harveyi*) (Kaempfer and Magasanik 1967), in shake flasks with agitation (150 rotation per min).

The carbon and nitrogen limitation stress were introduced by pelleting the bacterial cultures and resuspending in relevant minimal (MOPS or Tris) medium depleted of carbon or nitrogen source. The amino acid starvation was achieved by adding serine hydroxamate (SHX) to the cultures at 1.5 mg/ml. The heat stress was introduced by transferring bacterial cultures to water baths with the elevated temperature: 37 °C for *S. baltica* and *A. johnsonii* and 43 °C for *E. coli* and *V. harveyi*. The relevant temperatures were established by increasing culture temperature until the significant growth inhibition was observed (Table 1S). The osmotic stress was achieved by adding LiCl to the growth-inhibiting concentrations, established for each strain − 0.6 M for *E. coli*, 0.2 M for *S. baltica*, and 0.1 M for *A. johnsonii* and *V. harveyi*. The DNA damage stress was introduced by adding EMS (ethyl methanesulfonate) to the final concentration of 28 mM. The inhibition of DNA synthesis was started by adding mitomycin C to the final concentration of 1 mg/ml.

Analysis of (p)ppGpp synthesis

(p)ppGpp levels were measured as previously described, with minor modifications (Mechold et al. 2013). Briefly, bacteria were grown overnight in minimal MOPS (containing 5 mM KH_2PO_4) or Tris medium (containing 1 mM K_2HPO_4) and then washed and resuspended in low-phosphate (0.4 mM KH_2PO_4 or K_2HPO_4, respectively) MOPS or Tris-labeling medium at A_{600} of 0.02. Cultures were grown until they reached A_{600} of 0.2 and diluted again (1:10) in the same medium. [^{32}P] orthophosphoric acid was added to 5,550,000 Bq/

Table 1 Bacterial strains used in this study

Strain	Reference or source
Escherichia coli MG1655	(Jensen 1993)
Shewanella baltica 1 M	Karczewska-Golec et al. (2016)
Acinetobacter johnsonii 17909	ATCC
Vibrio harveyi BB7	(Belas et al. 1982)

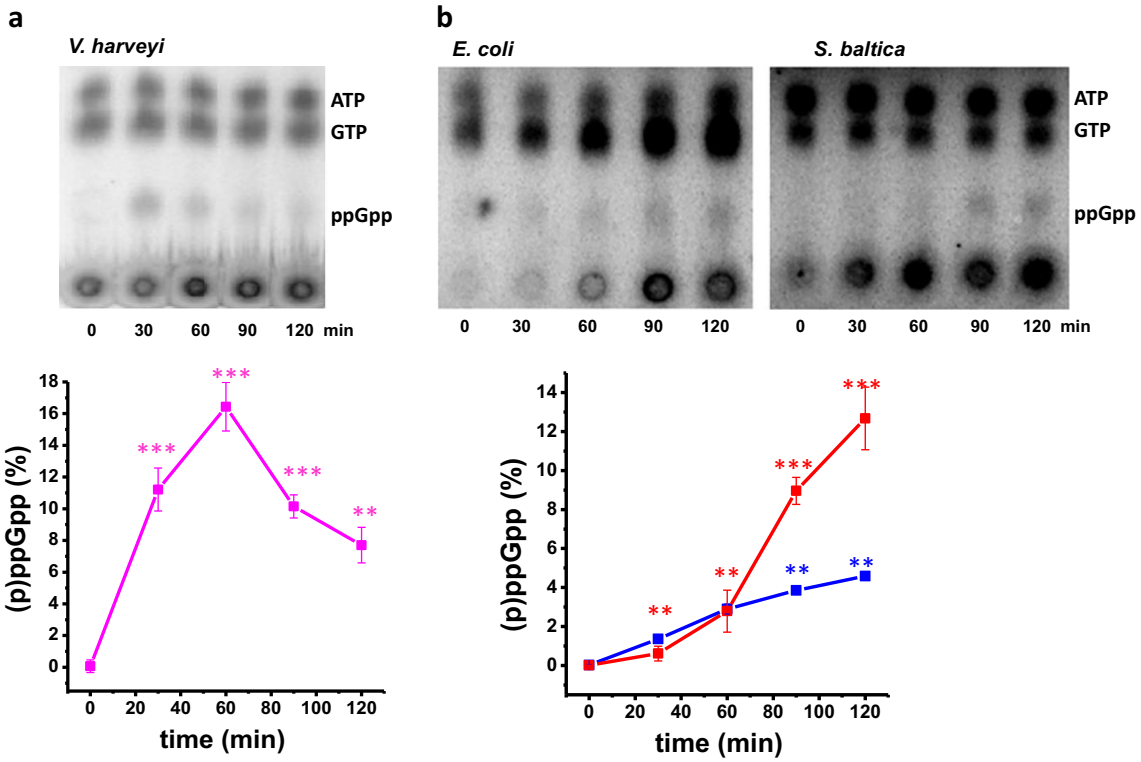


Fig. 1 The stringent response induction by the carbon (A) and nitrogen (B) limitation. The upper panels present an example of the thin-layer chromatogram with ATP, GTP, and ppGpp positions indicated. At the time 0, bacteria were transferred to the media depleted of C and N source. The lower panels show kinetics of relative (p)ppGpp synthesis in *V. harveyi* (magenta squares) (**A**), *E. coli* (blue circles) and *S. baltica* (red triangles) (**B**) based on the densitometrical analysis. The relative (p)ppGpp level was calculated as a ratio of (p)ppGpp amount to the sum of GTP and (p)ppGpp (set as 100 %). The results are from at least three independent experiments. The statistical significance of differences in (p)ppGpp amount compared with time 0 was determined by t-test (*$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$) as indicated above or below plots.

ml, and then bacteria were cultured for at least 2 generations before the first sample was taken. The stress conditions were implemented, and then at indicated time points, samples (50 μl) were taken and then extracted with ice-cold formic acid (13 M) by three cycles of freeze-thaw. Samples were centrifuged (5000 x g, 4 °C, 5 min), and nucleotides present in the supernatant were separated by thin-layer chromatography (TLC) on polyethylenimine (PEI) cellulose TLC plates in 1.5 M potassium phosphate buffer and analyzed in a phosphorimager (Typhoon; GE Healthcare). The spots corresponding to ppGpp and pppGpp were identified according to previously reported characteristics (Mechold and Malke 1997; Mechold et al. 2013).

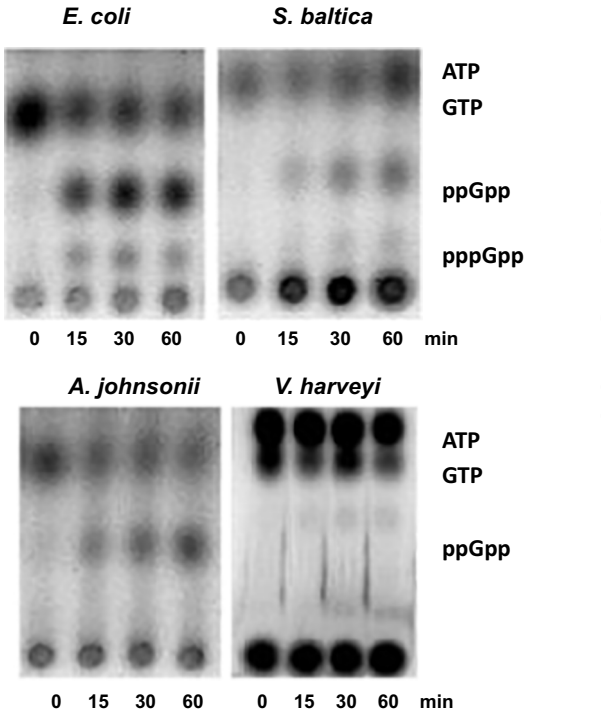
Statistical analysis

Data were analyzed using the GraphPad Prism and PQStat softwares. Student's t-test or one-way ANOVA, followed by Kołmogorow-Smirnow or Shapiro-Wilk tests, respectively, was used to determine statistical significance of the obtained results. The equality of variances was checked using the Fisher-Snedecor and Brown-Forsythe tests. To check the differences between the groups, a post hoc test by Tukey was performed. Difference was considered significant at $p < 0.05$.

Results and discussion

The choice of bacterial strains and stress conditions

To ensure survival, bacteria as unicellular organisms need to react promptly and precisely to the alteration of environmental factors. For bacteria living in the marine habitats, these changes involve both physicochemical and nutrient conditions. To address the question about the adaptive regulation upon stress, we chose to study the reaction to a variety of stresses: carbon and nitrogen source limitation, amino acid depletion by modifying the medium composition, DNA damage, temperature, and osmotic challenge by varying growth parameters. Nutrients are usually quite sparse in most of cold-water marine habitats; however, the availability of them may rapidly increase especially in the enclosed ecosystems such as the Baltic Sea due to floods and river-mediated depositions from agricultural areas. Moreover, the Baltic Sea is exposed to recurrent seasonal variations of salt and anthropogenic pollutions, including various chemical mutagens. Bacterial strains used in this work are listed in Table 1. The choice of bacterial species for this study was based upon several factors: the representation in the natural population of the marine environment, in particular, the Baltic Sea and the possibility to study the selected species under defined laboratory conditions. The



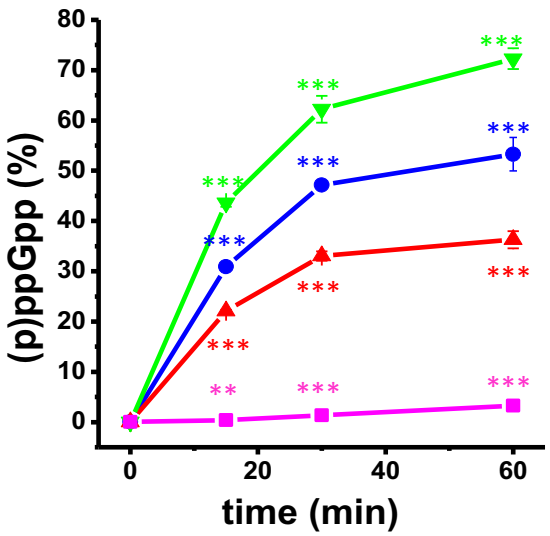


Fig. 2 The stringent response induction by the amino acid starvation. The left panel presents an example of the thin-layer chromatogram with GTP and ppGpp positions indicated. SHX (1.5 mg/ml) was added in the time 0. The right panel shows kinetics of relative (p)ppGpp synthesis in *E. coli* (blue circles) and *S. baltica* (red triangles), *A. johnsonii* (green inverted triangles), and *V. harveyi* (magenta squares) based on the densitometrical analysis. The relative (p)ppGpp level was calculated as a ratio of (p)ppGpp amount to the sum of GTP and (p)ppGpp (set as 100%). The results are from at least three independent experiments. The statistical significance of differences in (p)ppGpp amount compared with time 0 was determined by t-test (*$p \le 0.05$, **$p \le 0.01$, and ***$p \le 0.001$) as indicated above or below plots.

bacterial isolates from the Baltic Sea from the collection of Department of Molecular Biology, University of Gdańsk and results of the analysis by Moskot et al. (2012) supported this choice. As a control, *Escherichia coli* was used due to several reasons: this bacterium serves as a model in molecular biology, and its stringent response was extensively studied; moreover, *E. coli* belongs to proteobacteria as the marine representatives. The strain of *Shewanella baltica* M1 was isolated from the Baltic Sea, and its full genome was sequenced in our lab (Karczewska-Golec et al. 2016). *S. baltica* as other species from *Shewanella* genus plays an important role in the turnover of the organic substances as one of the most important denitrifying strains in the redox interface (Ziemke et al. 1997). *Acinetobacter johnsonii* is a Gram-negative bacterium with broad range of habitats, quite common in the low-salinity water (Guardabassi et al. 1999; Doughari et al. 2011). Its high flexibility in terms of environments suggest the efficient way of dealing with stresses, e.g., by the stringent response. *Vibrio harveyi* is motile marine bacterium, free-living and facultative pathogen that can grow in the wide range of temperatures; however, its efficient growth requires NaCl (Farmer III et al. 2005; Owens and Busico-Salcedo 2006). All three bacterial species have a genetic basis for the stringent response that is homologs of *rsh* genes encoding two paralogue enzymes, similarly to *E. coli*. The comparison of enzymes amino acid sequences of *S. baltica* revealed 60% identity for RelA homolog, Sbal_3146, and 70% for SpoT homolog, Sbal_0353 with *E. coli* counterpart (Figs. 1–2 S). *A. johnsonii* enzymes (RZ95_RS13970 and RZ95_RS01580) revealed quite low level of identity with *E. coli* RelA and SpoT proteins (39 and 34 %, respectively); however, the identity at the nucleotide level was considerably higher (63 and 69%, respectively) (Figs. 3–4 S). *V. harveyi* homologs bear 65% identity to RelA (VIBHAR_03528) and 75% to SpoT (VIBHAR_00627) (Figs. 5–6 S). Numerous species from *Vibrio* genus have additional protein involved in (p)ppGpp synthesis, RelV. Analysis of *V. harveyi* genome sequence revealed presence of *relV*-related gene, VC1224. The protein hypothetically encoded by this gene, VIBHAR_RS09915, shows 71% identity with RelV of *V. cholerae* (Fig. 7 S). All analyses were performed using BLAST. These analyses confirmed that all studied bacteria have an ability to produce (p)ppGpp upon various stresses; thus we proceeded to address the question whether the reaction of marine bacteria to adverse conditions show their specific adaptations to unfavorable environment.

The stringent response induction during nutrient limitation stresses

The typical marine habitat does not provide an abundance of nutrients albeit bacteria employ various methods of

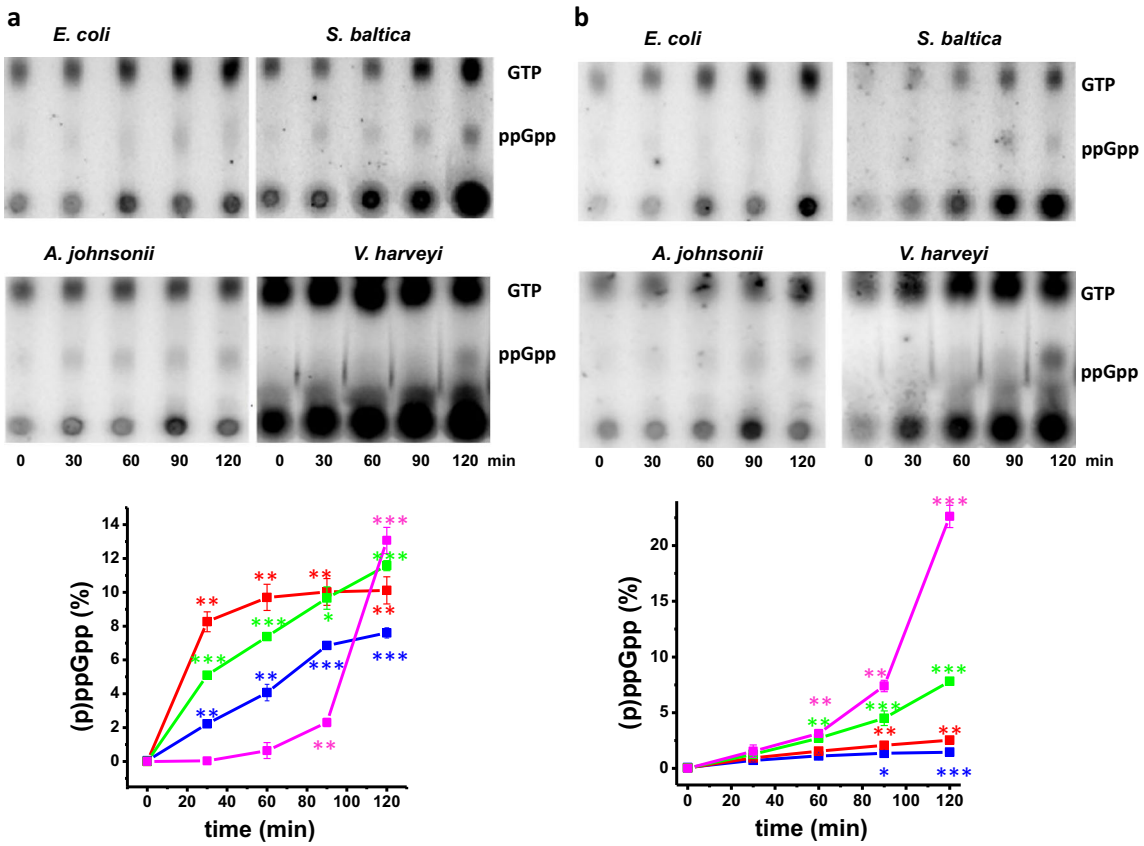


Fig. 3 The stringent response induction by the DNA damaging agents, mitomycin C (A), and EMS (B). The upper panels present an example of the thin-layer chromatogram with GTP and ppGpp positions indicated. The mitomycin C (1 mg/ml) (**A**) or EMS (28 mM) (**B**) was added at the time 0. The lower panels show kinetics of relative (p)ppGpp synthesis in *E. coli* (blue circles), *S. baltica* (red triangles), *A. johnsonii* (green inverted triangles), and *V. harveyi* (magenta squares) based on the densitometrical analysis. The relative (p)ppGpp level was calculated as a ratio of (p)ppGpp amount to the sum of GTP and (p)ppGpp (set as 100%). The results are from at least three independent experiments. The statistical significance of differences in (p)ppGpp amount compared with time 0 was determined by t-test (*$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$) as indicated above or below plots.

utilizing unusual energy and compounds sources. The most common is a deficit of carbon and nitrogen sources. First, we assessed the growth in defined minimal MOPS (morpholinepropanesulfonic acid) medium containing 5 mM KH_2PO_4 (for *E. coli*, *S. baltica*, *A. johnsonii*) (Mechold et al. 2013) or Tris minimal medium containing 1 mM K_2HPO_4, (*V. harveyi*) (Kaempfer and Magasanik 1967), at 30 °C in shake flasks with agitation (150 rotation per min). The generation times, which turned out to be 30 min for *E. coli*, 60 min for *S. baltica* and *A. johnsonii*, and 90 min for *V. harveyi*, have been assessed in the experiments preceding labeling assays. For efficient labeling of nucleotides, bacteria have to be cultured in limiting phosphate medium, containing 0.4 mM KH_2PO_4 or K_2HPO_4, respectively. To check whether these media do not provoke stress in bacteria, we evaluated the (p)ppGpp levels during prolonged cultures up to 210 min. The alarmones synthesis could be observed from 180 min, likely due to the exhausting of available nutrients (data not shown). Thus, the maximal time of experiment was established as 120 min. To provide the limiting growth conditions, we depleted the medium of C and N sources and continued cultures for additional 120 min taking samples every 30 min. Interestingly, only *V. harveyi* accumulated ppGpp during carbon starvation (Fig. 1A), while nitrogen limitation resulted in the induction of the stringent response in *E. coli* and S. *baltica* (Fig. 1B). The amino acid starvation was achieved by adding the toxic amino acid analog serine hydroxamate (SHX) to the cultures at 1.5 mg/ml, and the (p)ppGpp synthesis was observed in all bacterial species (Fig. 2), with the nearly maximum accumulation at 30 min. Notably, the presence of guanosine pentaphosphate was noted only for *E. coli* and in lesser extent in *S. baltica*. Apparently, from tested nutrient deficiencies, the amino acid limitation constitutes the strongest inducing factor for stringent response. The Rel-mediated reaction to the presence of uncharged tRNA bound to ribosomes triggers promptly (p)ppGpp synthesis in order to stop energy-consuming processes such as stable RNA production. The marine bacteria response to amino acid starvation show that their Rel homologs sense this limitation and react similarly to *E. coli* RelA. In contrary, carbon or nitrogen

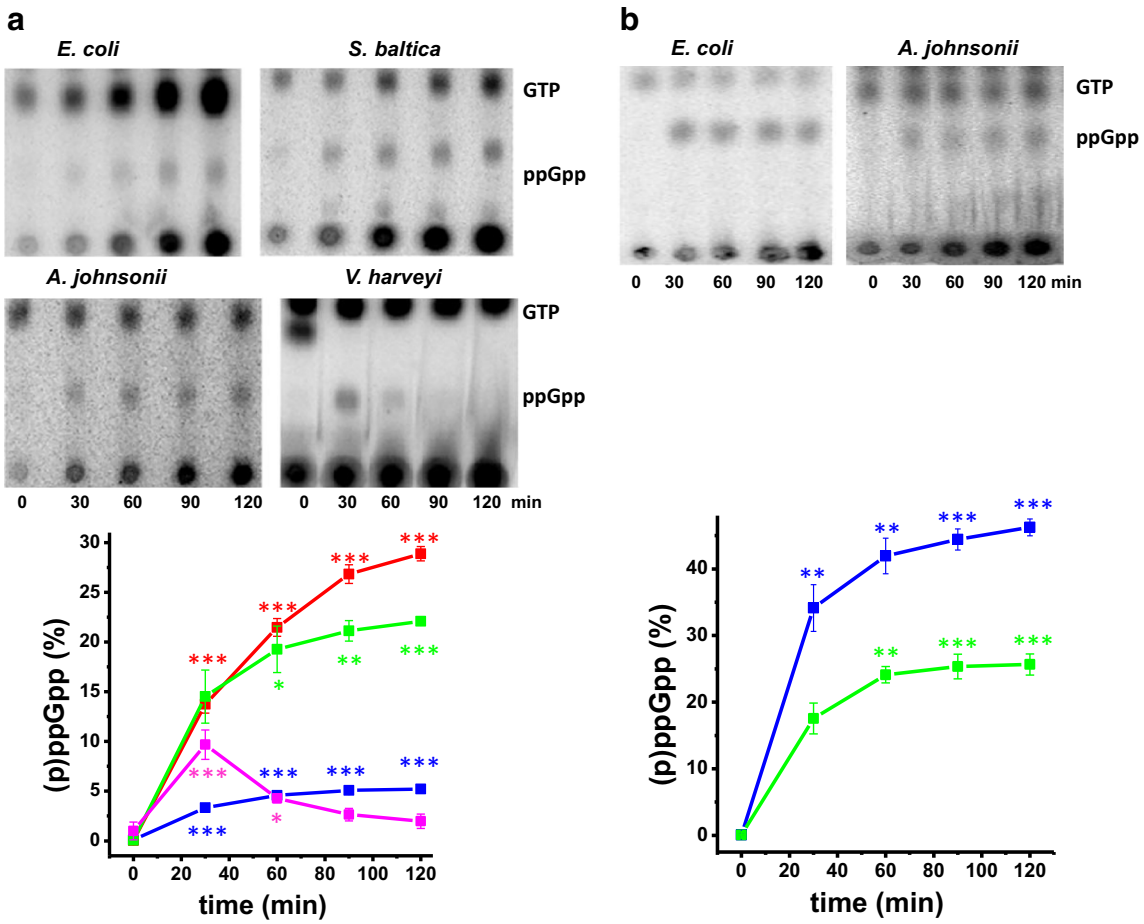


Fig. 4 The stringent response induction by the temperature (A) and osmotic (B) stress. The upper panels present an example of the thin-layer chromatogram with GTP and ppGpp positions indicated. At the time 0, bacteria were transferred to the elevated temperature (43 °C for *E. coli* and *V. harveyi* and 37 °C for *S baltica* and *A. johnsonii*) (A), or LiCl (0.6 M for *E. coli* and 0.1 M for *A. johnsonii*) was added. The lower panels show kinetic of relative (p)ppGpp synthesis in *E. coli* (blue circles) and *S. baltica* (red triangles), *A. johnsonii* (green inverted triangles) and *V. harveyi* (magenta squares) based on the densitometrical analysis. The relative (p)ppGpp level was calculated as a ratio of (p)ppGpp amount to the sum of GTP and (p)ppGpp (set as 100%). The results are from at least three independent experiments. The statistical significance of differences in (p)ppGpp amount compared with time 0 was determined by t-test (*$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$) as indicated above or below plots.

starvation induce only moderate (p)ppGpp accumulation limited to some bacterial strains. This may suggest that marine bacteria are relatively resistant to these limitations which may be a part of their adaptation to the frequent famine periods. Interestingly, it was reported that *Vibrio* sp. responded to the nitrogen limitation with growth inhibition only after 30 h (Nyström et al. 1992). Just in opposite, the carbon limitation was the factor that induced (p)ppGpp accumulation only in *V. harveyi*. These observations point to varying sensitivity of marine bacteria to nutrient deficiency.

The response of marine bacteria to the DNA damaging agents

The free-living bacteria in their environment encounter numerous factors that could either block DNA replication or damage genetic material such as mutagenic pollutants or UV irradiation. The direct effect is usually induction of the SOS response; however, the stringent response could also be involved in overcoming the DNA damage. ppGpp was suggested to facilitate DNA repair by exposing DNA breaks while bound to RNA polymerase paused at the damage site (McGlynn and Lloyd 2000). We employed mitomycin C (in the final concentration of 1 mg/ml) as DNA damaging agent which may subsequently interfere with DNA replication and observed prompt reaction by ppGpp accumulation to this stressor in *S. baltica* and *A. johnsonii*, while *E. coli* and *V. harveyi* exhibited more delayed yet significant response in further time points (Fig. 3A). Another DNA damaging agent, EMS (ethyl methanesulfonate), at the final concentration of 28 mM, provided most pronounced response in *V. harveyi*; nevertheless the ppGpp accumulation was delayed with the highest levels at 120 min after implementation of the stress condition (Fig. 3B). Our results suggest that the stringent response mediates the reaction to DNA damage stress in marine bacteria, facilitating their survival. However, the ppGpp

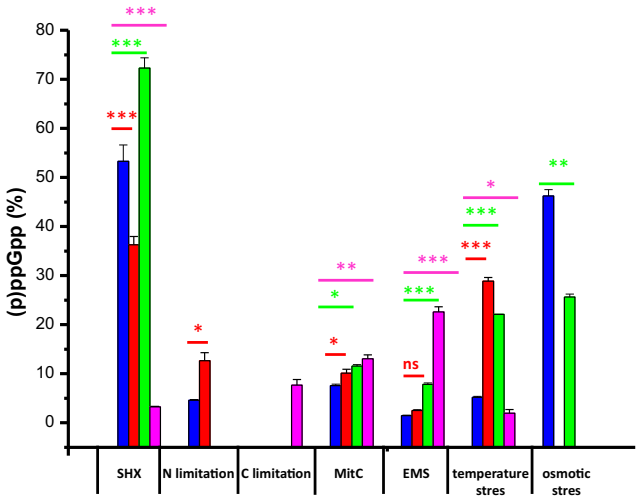


Fig. 5 The summary of (p)ppGpp synthesis upon stringent response induction by various stressors. The stress conditions are indicated below graph, with the data from 60 min time point after onset of stress for SHX and 120 min for other stresses. The (p)pGpp production in *E. coli* (blue) and *S. baltica* (red), *A. johnsonii* (green), and *V. harveyi* (magenta) was calculated as a ratio of (p)ppGpp amount to the sum of GTP and (p)ppGpp (set as 100%). The statistical significance of differences between respective strains compared to *E. coli* as a control was determined by t-test or one-way ANOVA (*$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$; *ns* nonsignificant) as indicated above columns (colors correspond to the column representing relevant strain compared to *E. coli*).

accumulation may not be a first line of defense in the environmental induced DNA damage agents.

The stringent response induction by physicochemical stresses

Aquatic environment is a subject to alteration in basic characteristics such as salinity or temperature. The temperature changes not only seasonally but also depending on the water depth. The marine bacteria could be sensitive to both elevated and reduced water temperature, while high temperature can be more detrimental. The heat stress was introduced by transferring bacterial cultures to water baths with the elevated temperature: 37 °C for *S. baltica* and *A. johnsonii* and 43 °C for *E. coli* and *V. harveyi*. We observed that all analyzed bacteria responded by increased (p)ppGpp synthesis already after 30 min of stress implementation with the highest response of *S. baltica* (Fig. 4A). This observation could be explained by relatively low temperatures of the Baltic Sea which is the most common habitat where this bacterium was identified.

The salinity level in marine environment may fluctuate due to seasonal variations, water inflows, and currents. Bacteria need to adapt to survive these changes. In the laboratory conditions, we implemented increasing LiCl concentrations, established for each strain − 0.6 M for *E. coli*, 0.2 M for *S. baltica*, and 0.1 M for *A. johnsonii* and *V. harveyi* as a concentration causing significant growth inhibition. The choice of this salt was accounted for technical problems with thin-layer chromatography when NaCl was used. LiCl was reported to be employed as osmotic stress factor in the concentrations usually lower than NaCl (Serrano 1996). The introduction of the growth-inhibiting concentrations of LiCl resulted in the significant (p)pGpp synthesis in *E. coli* and moderate in *A. johnsonii* (Fig. 4B) with no response in other strains. The strong response to the osmotic stress in *E. coli* may be attributed to the typical habitat of these bacteria, where the conditions could change from stable habitat of intestines of homeothermic animals to the free-living status (e.g., water, liquid wastes). The marine bacteria can apparently cope with changes in salinity in the way not involving the stringent response.

Conclusions

The studied representative marine bacteria species were shown to be able to induce the stringent response upon the onset of stress and limitation conditions. The adjustment of growth and labeling conditions allowed for application of the standard method of nucleotide visualization to assess the time and level of the (p)ppGpp accumulation upon stress for environmental bacteria. Using this method, it was possible to quantitatively evaluate the accumulation of the stringent response alarmones in comparison to the control bacterium, *E. coli*. We showed that the extent of (p)ppGpp synthesis and time of the response vary depending of the stress conditions (summarized in Fig. 5). The highest (p)pGpp synthesis was stimulated by amino acid starvation for all tested bacteria, while nitrogen and carbon limitation did not efficiently induce the stringent response effector accumulation. From the physicochemical stresses, temperature stress was the most universal in inducing (p)pGpp synthesis, while only *E. coli* and A. *johnsonii* responded to osmotic stress. The DNA damage triggered (p)pGpp synthesis; however, the total alarmone production was moderate. The most sensitive to most of tested stress conditions proved to be *A. johnsonii*, while *S. baltica* did not respond to some conditions. The level of the stringent response may correspond with the ability of bacteria to efficiently confront adverse conditions common in marine environment.

Acknowledgments We thank Drs. Katarzyna Potrykus, Dariusz Nowicki, Monika Maciąg-Dorszyńska, and Grzegorz Cech for discussion, encouragements, and scientific advices.

Funding information This work was funded by National Science Centre, Poland (HARMONIA 2012/06/M/NZ2/00100 to ASP).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

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

Atkinson GC, Tenson T, Hauryliuk V (2011) The RelA/SpoT homolog (RSH) superfamily: distribution and functional of ppGpp synthetases and hydrolases across the tree of life. PLoS ONE 6:e23479. https://doi.org/10.1371/journal.pone.0023479

Belas R, Mileham A, Cohn D et al (1982) Bacterial luminescence: isolation and expression of the luciferase genes from *Vibrio harveyi*. Science 218:791–793. https://doi.org/10.1126/science.10636771

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. https://doi.org/10.1016/j.tim.2005.11.006

Cashel M, Lazzarini RA, Kalbacher B (1969) An improved method for thin-layer chromatography of nucleotide mixtures containing ^{32}P-labeled orthophosphate. J Chromatogr 40:103–109

Cashel M, Gentry DR, Hernandez VJ, Vinella D (1996) The stringent response in *Escherichia coli* and *Salmonella typhimurium* In: Neidhart FC. et al. (eds). *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology. ASM, pp 1458-1496.

Dalebroux ZD, Svensson SL, Gaynor EC, Swanson MS (2010) ppGpp conjures bacterial virulence. Microbiol Mol Biol Rev 74:171–199. https://doi.org/10.1128/mmbr.00046-09

Doughari HJ, Ndakidemi PA, Human IS, Benade S (2011) The ecology, biology and pathogenesis of Acinetobacter spp.: an overview. Microbes Environ 26:101–112. https://doi.org/10.1264/jsme2.me10179

Farmer JJ III, Janda JM, Brenner FW, Cameron DN, Birkhead KM (2005) Genus 1. Vibrio Pacini 1854, 411AL. In Brenner DJ, Krieg NR and Staley JT (eds.). *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2. The Proteobacteria, part B, The Gammaproteobacteria, pp 494–546.

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. https://doi.org/10.1128/jb.174.21.6780-6788.1992

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 relA gene. J Bacteriol 176:5949–5957. https://doi.org/10.1128/jb.176.19.5949-5957.1994

Guardabassi L, Dalsgaard A, Olsen JE (1999) Phenotypic characterization and antibiotic resistance of *Acinetobacter* spp. isolated from aquatic sources. J Appl Microbiol 87:659–667. https://doi.org/10.1046/j.1365-2672.1999.00905.x

Guzow-Krzemińska B, Gąsior T, Szalewska-Pałasz A (2015) Phylogenetic relationship of the stringent response-related genes of marine bacteria. Acta Biochim Pol 62:773–783. https://doi.org/10.18388/abp.2015_1132

Jain V, Kumar M, Chatterji D (2006) ppGpp: stringent response and survival. Microbiology 44:1–10

Jensen KF (1993) The *Escherichia coli* K-12 "wild types" W3110 and MG1655 have an rph frameshift mutation that leads to pyrimidine starvation due to low pyrE expression levels. J Bacteriol 175:3401–3407. https://doi.org/10.1128/jb.175.11.3401-3407.1993

Kaempfer ROR, Magasanik B (1967) Effect of infection with T-even phage on the inducible synthesis of β-galactosidase in *E. coli*. J Mol Biol 27:453–468. https://doi.org/10.1016/0022-2836(67)90051-4

Karczewska-Golec J, Strapagiel D, Sadowska M et al (2016) Draft genome sequence of *Shewanella baltica* M1 isolated from brackish surface water of the Gulf of Gdańsk. Genome Announc 4(3):e00611–e00616. https://doi.org/10.1128/genomeA.00611-16

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

Magnusson LU, Farewell A, Nyström T (2005) ppGpp: a global regulator in *Escherichia coli*. Trends in Microbiology 13:236–242. https://doi.org/10.1016/j.tim.2005.03.008

McGlynn P, Lloyd RG (2000) Modulation of RNA polymerase by (p)ppGpp reveals a RecG-dependent mechanism for replication fork progression. Cell 101:35–45. https://doi.org/10.1016/s0092-8674(00)80621-2

Mechold U, Malke H (1997) Characterization of the stringent and relaxed responses of *Streptococcus equisimilis*. J Bacteriol 179:2658–2667. https://doi.org/10.1128/jb.179.8.2658-2667.1997

Mechold U, Potrykus K, Murphy H et al (2013) Differential regulation by ppGpp versus pppGpp in *Escherichia coli*. Nucleic Acids Res 41:6175–6189. https://doi.org/10.1093/nar/gkt302

Moskot M, Kotlarska E, Jakóbkiewicz-Banecka J et al (2012) Metal and antibiotic resistance of bacteria isolated from the Baltic Sea. Int Microbiol 15:131–139

Nyström T, Olsson RM, Kjelleberg S (1992) Survival, stress resistance, and alterations in protein expression in the marine vibrio sp. strain S14 during starvation for different individual nutrients. Appl Environ Microbiol 58:55–65

Östling 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. https://doi.org/10.1128/jb.178.16.4901-4908.1996

Owens L, Busico-Salcedo N (2006) *Vibrio harveyi*: pretty problems in paradise (Chapter 19). In: Thompson F, Austin B, Swings J (eds) The Biology of Vibrios. ASM Press, pp 266–280. https://doi.org/10.1128/9781555815714.ch19

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

Serrano R (1996) Salt tolerance in plants and microorganisms: toxicity targets and defense responses. Int Rev Cytol 165:1–52. https://doi.org/10.1016/s0074-7696(08)62219-6

Varik V, Oliveira SRA, Hauryliuk V, Tenson T (2017) HPLC-based quantification of bacterial housekeeping nucleotides and alarmone messengers ppGpp and pppGpp. Sci Rep 7:11022. https://doi.org/10.1038/s41598-017-10988-6

Ziemke F, Brettar I, Höfle MG (1997) Stability and diversity of the genetic structure of a *Shewanella putrefaciens* population in the water column of the central Baltic. Aquat Microb Ecol 13:63–74. https://doi.org/10.3354/ame013063