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Review

Bacterial Stress Response as an Adaptation to Life in a Soil Environment

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Abstract

The stress response is a metabolic program activated in response to unfavorable environmental factors. Various mechanisms are involved in its activation, depending on the type of stress factor and on the metabolic characteristics of the micro-organism. The stress response mechanisms occurring in bacteria are the general stress response, the stringent response, the oxidative stress response, the TA system, and QS, which is a mechanism of response to population cell density. The end result of the activation of this program, which is resistance to the same stress factor or cross-resistance (i.e. resistance to other types of stress factors), depends on the interaction at various levels between different stress response mechanisms. The phenomenon of resistance is particularly important in the case of soil bacteria, which is often exposed to both natural and anthropogenic stress factors. The stress response determines such diverse microbial functions as survival in periods of starvation, adaptation to the presence of antibiotics, synthesis of antibiotic substances, interactions with a eukaryotic symbiont, and atmospheric oxygen fixation. At the ecosystem level, it helps to maintain climax conditions, i.e. a quantitatively and qualitatively stabilized community of micro-organisms in a given environment, which affects the biological activity of the soil.

Keywords: general stress response, stringent response, nutrient starvation, soil micro-organisms

Introduction

Due to its composition and physical properties, soil is a favourable environment for the development of diverse microflora, the quantity and quality of which depend on soil structure, moisture content, gas phase composition, nutrient content, acidity, temperature, and geographical zone. Soil micro-organisms live on the boundary between two phases, on the surfaces of colloids and in the hydration layer surrounding them. Depending on the specific conditions prevailing in this environment, it is inhabited by specialized populations of micro-organisms forming microbiotopes.

Soil micro-organisms, in contrast with the cells of tissue organisms (which live in conditions of systemic homeostasis), are often exposed to changes in environmental conditions. They have adaptive defence mechanisms or physiological and structural adaptations fixed by evolution, which allow them to survive and function in new, unfavorable conditions. Adaptive mechanisms include a metabolic program known as the stress response. The biological purpose of the stress response is to protect cell components against potentially dangerous environmental factors and to repair damage occurring in stress conditions. The stress response is manifested as a change in the metabolic activity of the cell, resulting from the repression of synthesis of most of the proteins formed in the cell under normal physiological conditions, and induction of the synthesis of a specific group of proteins enabling the cell to function in the new conditions. The biochemical changes are accompanied by physiological changes, such as a temporary slowing or

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stoppage of the division cycle, morphological changes in the cell, or the emergence of resistance to the same stress factor or other types of stress factor.

In certain situations, when an unfavorable stimulus acts with particular intensity or its action is prolonged, components of the cellular structure may be damaged in such a way that activation of defence mechanisms becomes impossible. Such conditions are called severe environmental stress and usually cause the death of the cell, while at the ecological level they lead to elimination of susceptible cells, which entails quantitative and qualitative changes in the composition of the population of a given environment.

Micro-organism populations forming a biotope vary in terms of their genetically conditioned susceptibility to drastic changes in environmental conditions. Some are relatively resistant owing to adaptations that enable them to tolerate a stress factor without the need to activate adaptive mechanisms, while others have mainly adaptive mechanisms, which are more specific but require large energy expenditures, and whose appearance is delayed by the need to synthesize defence molecules.

On the scale of an entire ecosystem, the stress response helps to maintain a state of climax, i.e. a quantitatively and qualitatively stabilized community of micro-organisms in a given environment. This leads, however, to changes in the flow of carbon and energy in the ecosystem as a consequence of the direct effect of stress factors on the metabolism of microbial cells and their effect on the species composition of the biotope [1].

The Stationary Phase of Growth as a Universal Mechanism of Adaptation to Starvation Conditions

Many environments in the biosphere are oligotrophic, with extremely low concentrations of organic carbon compared to a typical rich microbiological medium used for microbial cultures in a laboratory. When nutrient concentration is insufficient for sustaining stable growth, rapid cell division stops and the micro-organisms enter the stationary phase of growth. This process is accompanied by changes at various levels of cellular organization. Since low access to nutrients and the presence of other harmful factors are common conditions in natural environments, it has been suggested that micro-organisms spend most of their lives in the stationary phase [2]. Research by Gray et al. [3] confirms this hypothesis, demonstrating that most of the earth's biomass consists of resting micro-organisms.

In Gram-positive and Gram-negative bacteria, the transition to the stationary phase is principally governed by sigma regulatory proteins. It is commonly accepted that sigma factors are the key to the ability of bacterial cells to adapt and develop in different life conditions. Regulation of gene activity by means of these factors takes place mainly at the transcriptional level, because sigma factors are a subunit of the holoenzyme RNA polymerase, which acquires the ability to recognize the specific regulons of the bound sigma factor.

In optimal life conditions, a housekeeping transcription factor functioning in cells is responsible for the synthesis of proteins typical for these conditions. It is called δ^{70} in *Escherichia coli* and δ^{A} in *Bacillus subtilis* [4]. An alternative to δ^{70}, δ^{32} (encoded by *rpoH*) has been characterized as a component of the heat-shock response in *E. coli* [5]. Environmental cues, such as elevated temperature or other conditions disturbing protein homeostasis, cause the accumulation of misfolded proteins and lead to transcriptional activation of stress genes encoding heat shock proteins (HSPs). HSPs are mainly chaperones and proteases that work in concert to maintain the structural and functional integrity of all living cells [6]. The δ^{S} factor (product of the gene *rpoS*) is a protein in *E. coli* cells which was previously known as RpoS [7] before it was discovered that it performs the functions of an RNA polymerase sigma subunit [8]. It is a mediator of the general stress response. Although the *rpoS* gene is expressed during the logarithmic phase of growth, the amount of the protein encoded by the gene increases sharply at the beginning of the stationary phase and in stress conditions (limited nutrient concentrations, temperature and osmotic shock, and high population density) [9]. The δ^{B} factor controls the general stress regulon of *B. subtilis*. It is activated in response to exposure to physical (e.g., ethanol, heat shock, or osmotic shock) or nutritional cues (e.g., glucose or phosphate starvation or O_2 limitation) [10]. δ^{54}, known as RpoN (product of the gene *rpoN*), differs from other sigma factors in both its structure and its transcription initiation mechanism. It coordinates various processes in the bacterial cell, such as flagellation, utilization of several different carbon and nitrogen sources, or alginate biosynthesis [11].

The effectiveness of alternative δ factors is aided by alarmones of the stringent response, i.e. hyperphosphorylated guanine nucleotides: guanosine tetraphosphate (ppGpp), and guanosine pentaphosphate (pppGpp), abbreviated as (p)ppGpp [12]. (P)ppGpp is an important factor in the regulation of bacterial physiology because it responds to various stresses in such a way that it restricts growth and activates cellular defence and adaptation mechanisms. In *E. coli*, the concentration of (p)ppGpp in the cell depends on the activity of the proteins Rel A and SpoT. Rel A only synthesizes (p)ppGpp (synthetase I activity), while SpoT can both produce (p)ppGpp (synthetase II activity) and degrade it (ppGpp hydrolase activity) [13]. Unlike *E. coli*, a growing number of Gram-positive bacteria possess only a single RelA/SpoT homologue, which displays both (p)ppGpp synthase and hydrolase activities [14].

Several models of the effect of stringent response alarmones on the functions of alternative sigma factors have been proposed. According to Artsimovitch et al. [15], they are capable of interacting directly with the polymerase ß-subunit, changing its specificity and affecting (mainly negatively) the expression of over 80 genes. The indirect mechanism assumes that (p)ppGpp can regulate the activity of genes whose promoters are recognizable by complexes consisting of RNA polymerase and an alternative δ factor, facilitating binding of the sigma factors to core polymerase [16]. In addition, ppGpp has been shown to be necessary not only for the proper functioning of alternative δ factors but also for

their synthesis [17]. Apart from δ factors, other regulators, acting at various levels of gene expression and via various mechanisms, also are involved in the genetic switch that controls entry into the stationary phase of growth.

Stationary-phase bacteria have characteristic structural and functional adaptations to conditions of nutrient deficiency and other harmful environmental factors. The cells become smaller compared to their log-phase counterparts and take on a spherical shape. These changes are the result of two processes: reductive division and dwarfing [18]. Reductive division increases the surface-area-to-volume ratio, producing spherical cells. Dwarfing is a form of self-digestion resulting from the degradation of endogenous cell material, especially from the cytoplasmic and outer membranes. The cell envelope (i.e. the outer membrane, periplasm, peptidoglycan, and inner membrane) undergoes reorganization [19], as a result of which it becomes stiff and more resistant to chemical and physical agents. Changes in the cell envelope take place at the same time as changes in the cytoplasm. The nucleoid undergoes condensation, in which Dps (DNA-binding proteins from starved cells) play a role. These proteins are induced in oxidative stress conditions by OxyR as a result of an expression dependent on housekeeping transcription factor δ^{70}, and in starvation conditions by the RpoS (δ^{s}) transcription factor [20, 21]. Dps proteins protect the DNA from several damaging agents [22]. Some ribosomes are degraded and the remaining ones are transformed into inactive forms as a result of dimerization, which leads to the low level of translation observed in these conditions. Dimerization of ribosomes can be a form of preservation of these structures until the need for them increases.

Changes at the metabolic level involve inhibition of transcription of genes coding for rRNA, tRNA, and ribosomal proteins, i.e. the translation apparatus, leading to a reduction in cellular protein synthesis [7]. Synthesis of lipids and cell wall components is also reduced [23] while protein turnover increases (as much as 5-fold in *E. coli*), which is associated with increased protease and peptidase synthesis in the initial stages of starvation [24]. Mutations that decrease the activity of these enzymes lower the survival rate of stationary-phase cells [25], which confirms the important role of the protein degradation process in survival in starvation conditions. Also observed in these conditions is an increase in the activity of enzymes involved in the utilization of fatty acids, which mainly originate from the cell membrane digestion process [26]. A shift from aerobic metabolism to fermentation also has been observed in these conditions, which may prevent excessive utilization of endogenous reserves during autophagy and is a defence mechanism against the adverse effects of reactive oxygen species induced during aerobic respiration [18]. The physiological manifestation of global changes in metabolism and the architecture of cell structures is inhibition of growth and cell division, as well as the appearance of cross-protection against osmotic stress, oxidative stress induced by hydrogen peroxide [9], and heat stress [27, 28]. Cells at this stage are capable of synthesizing many different secondary metabolites such as antibiotics and toxins.

The stationary phase of growth in bacteria is associated with behavior involving regulation of the number of cells in the population. This phenomenon is probably regulated by a quorum sensing (QS) factor [29, 30] and is manifested as the bacterial death phase. At this stage, 90-99% of cells in the population die and their biomass is a food source for the surviving cells, so that the survival rate of cells in these conditions remains constant for a long period. It has been suggested that bacterial death in these conditions takes place either as the result of a stochastic program or programmed cell death, although the role of each of these two mechanisms in this process is not yet clear.

The phenomenon of programmed cell death, which is well-documented in the case of eukaryotic organisms, consists in the beneficial elimination of cells that are damaged or unnecessary at a given stage of development. Thus cell death in the stationary phase of growth could be an altruistic form of behavior of some of the cells in the population, which die for the sake of the remaining cells. The mechanism of programmed cell death in bacteria may be mediated by the toxin-antitoxin (TA) system, which is a form of response to various stresses, including starvation [31].

Forms of Cellular Organization in the Stationary Phase of Growth

Stationary-phase conditions promote the appearance of various highly specialized lifestyles in micro-organisms. Many Gram-positive bacteria can form resistant spores, while nonsporulating micro-organisms are able to persist in adverse environments as vegetative cells with altered metabolic activity, in a free-living state or forming multicellular aggregates.

Microbial aggregates known as biofilms are formed by micro-organisms – both prokaryotic (eubacteria and archaea) and eukaryotic (unicellular algae and fungi). Biofilms can be single-species, but multi-species biofilms are observed much more frequently. The formation of biofilms in bacteria is induced by suboptimal growth conditions (including a lack of easily assimilable nutrients), harmful stress factors (such as metals or the presence of antibiotics) [32-34], or the presence of specific low-molecular-weight compounds secreted by plants [35, 36].

Micro-organisms living in the form of a biofilm differ morphologically, physiologically, and metabolically from free-living "planktonic" forms. Physiological differences in these cells result from differences in the profile of the genes expressed, which has been proven in comparative functional genomic studies of the two forms [37, 38]. One characteristic trait of cells living in the form of a biofilm, in contrast to their free-living counterparts, is the synthesis of extracellular polysaccharides (EPS), which together with proteins and nucleic acids bound to the cell surface form a biofilm matrix [39, 40]. According to literature data, this structure plays a role in resistance to environmental stresses such as a lack of moisture in the environment, rather than in cell aggregation [40, 41].

Initiation of biofilm formation has been shown to depend on various environmental signals that can modulate the activity of complex regulatory networks including both specific and global regulators. There are few regulators specific only to this structure [43], but the second category includes stringent response alarmones, the RpoS factor (δ^s), and the quorum sensing (QS) factor. It is interesting that QS is regulated, at least in some bacteria, both by RpoS at the beginning of the stationary phase [44] and/or by the level of ppGpp [45].

The significance of these types of stress response in initiating biofilm formation is evidenced by observations that 46% of genes dependent on RpoS are expressed in varying degrees in the immobilized cells. The deletion of the *rpoS* gene negatively affects biofilm formation in *E. coli*, mainly due to lack of production of curli fibres, an adhesion factor strictly dependent on the rpoS gene [46].

Evidence for the role of the stringent response was provided by Taylor et al. [47], and Balzer and McLean [48]. The rel_{Lmo} mutant of *Listeria monocytogenes*, lacking the ability to synthesize (p)ppGpp stringent response alarmones, was found to be incapable of growth on a solid surface, which is the first stage in biofilm formation. Similarly, the double mutant *E. coli relA spoT* had reduced biofilm formation capability in microtitre plates in minimal media, while in chemostat cultures with high cell concentrations and slow replenishment of nutrients it formed a biofilm, but with altered cell density and morphology.

The existence of cells in the form of a biofilm leads to the activation of various stress response mechanisms. One of these is the mechanism of response to cell envelope damage, in which CpxA/CpxR factors act as mediators. This mechanism is usually activated in response to damage to the bacterial cell wall structure in conditions of high temperature or osmotic concentration in the environment. In cells forming a mature biofilm, this program can be initiated by the accumulation in the periplasm of protein precursors of the adhesion factor (e.g. monomers of the subunits CsgA and CsgB, or curli fimbriae involved in aggregation) [49]. Activation of the CpxA/CpxR program inhibits expression of gene encoding factors for cell aggregation and biofilm formation, which may be the first stage of the programmed process of biofilm dispersal. Another explanation for this phenomenon may be the temporary expression of factors stimulating biofilm formation. It has been suggested that adhesion factors such as curli fibres or type IV pili could be needed in the initial stage of biofilm formation, but unnecessary later on.

Cells living in the form of a biofilm exhibit a high level of transcription of the *recA* gene, which is characteristic of conditions promoting DNA damage [37], and the gene coding for the protein Dps, whose functions are associated with protecting DNA against oxidative damage. Overproduction of this protein, which takes place, for example, in the case of the mutant *E. coli* MG1655, leads to resistance to bacteriophage infections and promotes biofilm formation [50]. Indirect evidence for associations between the stress response and biofilm structure is provided by observations indicating the release of prophages from *P. aeruginosa* cells forming mature biofilms [51]. The prophage release process is typical of stress conditions, particularly those inducing DNA damage.

Activation of various stress response mechanisms during biofilm formation and in the mature biofilm is probably the reason for the increase observed in the resistance of these cells to various environmental stress factors. Cells forming biofilms have been found to be more resistant to hydrogen peroxide [52, 53], antibiotics [34], heavy metals [54], and attacks by bacteriophages or amoebae [55] than free-living planktonic cells.

The response to stress induced by nutrient depletion or harmful chemical agents is manifested in many prokaryotic and some eukaryotic micro-organisms in a form referred to as "viable but nonculturable" (VBNC). Although the molecular mechanism governing this process is unknown, many characteristics of this form are similar to those occurring in the stationary phase of growth or dormant form [56, 57].

It has been suggested that in Gram-negative bacteria that lack the ability to form endospores under the influence of harmful environmental factors, VBNC is one of the life forms that allow them to survive unfavorable conditions. Cells in the VBNC state lack the ability to grow on typical non-selective microbiological media they have previously grown on, despite the fact that they are still alive and metabolically active. When the unfavorable factor ceases to be active, such cells quickly exit the VBCN state and can be grown in laboratory conditions [56, 58]. A morphological symptom of cells having entered the VBNC state is a decrease in their size. This phenomenon is accompanied by changes in membrane structure, protein composition, and number of ribosomes. DNA rearrangement probably occurs as well [59].

In the Gram-negative soil bacteria *Myxobacteria*, a structure known as a fruiting body is generated in starvation conditions. This process is regulated by a stringent response and a QS factor [60]. Stringent-response mediators, (p)ppGpp, initiate the formation of the multicellular fruiting body. Involved in this process are signal molecules dependent on the level of (p)ppGpp, of which the most well-researched are A and C. Signal molecule A is formed at the early stage of differentiation of the fruiting body and serves as an indicator of the number of cells, which is a vital factor in the organization of the structure. Signal molecule C directs the cells to create the fruiting body, i.e. to aggregate, and stimulates them to transform into spherical spores that are resistant to environmental stress [61, 62]. The *rel A* mutant of *Myxococcus xanthus*, which is incapable of synthesizing (p)ppGpp, is unable to produce a fruiting body because signal molecule A is not produced [63].

Other resistant forms observed in prolonged stationary-phase conditions are the GASP and SCDI microbial phenotypes. The GASP phenotype is characteristic of both prokaryotic and eukaryotic micro-organisms and appears late in the stationary phase [3, 64, 65]. It involves the transformation of old cells that have been in the stationary phase for a long period into cells with characteristics of young cells. This phenotype is caused by various stable mutations

that restore growth capability in severe conditions. The appearance of cells capable of proliferation in the old population may lead to their domination of the environment due to the complete displacement of old cells [66] or of only some of the cells of the parent wild-type strain [67]. These properties appear, for example, due to mutation of the *rpoS* gene, which reduces the efficiency of transcription of the regulon controlled by RpoS, while cells with no δ^S factor activity at all are completely incapable of transformation into this phenotype [66]. According to Farrell and Finkel [68], the beneficial effects of the mutation which decreases expression of the regulon controlled by rpoS may result from the promotion of binding of core polymerase with δ^D (δ^{70}) and δ^N (δ^{54}) factors, which control the efficiency of glucose, ammonia, and amino acid utilization. Thus far several other mutations leading to the GASP phenotype have been identified, all of which increase the efficiency of the utilization of substances in the environment that originate in dead cells.

SCDI is another phenotype initiated by prolonged starvation [69]. Thus far it has only been observed in the *E. coli* K-12 strain. Cells in this form seem to kill the parent strain or inhibit its growth. The capability of growth inhibition results from a mutation of the *glcC* gene coding for ADP-glucose pyrophosphorylase, a regulator protein in glycogen biosynthesis. Overproduction of glycogen is a necessary condition for transformation of the wild-type strain into the SCDI phenotype. Although the GASP and SCDI phenotypes exhibit certain similarities, it has been suggested that the two phenomena are functionally and genetically distinct [69].

In the case of Gram-positive bacteria, mechanisms activated in response to starvation lead to the development of spores. According to Eymann et al. [70] and Ochi [71], the (p)ppGpp stringent response alarmones mediate the sporulation process, but in both studies their effect was found to be indirect, involving changes in the GTP level. This is evidenced by the fact that (p)ppGpp has been found to require GTP as a substrate and (p)ppGpp may inhibit the activity of inosine monophosphate dehydrogenase, the first enzyme of the GTP synthesis pathway [12].

Resistance to Antibiotics and Synthesis of Antibiotic Agents

Of particular importance in the defence system of free-living micro-organisms and pathogens of humans and farm animals is resistance to antibiotics. Natural antibiotics are secondary metabolites of micro-organisms living in natural environments [72]. Many soil isolates, particularly strains of *Actinomycetes*, are highly efficient producers of various antibiotics [73]. Another source of antibiotics, in such varied environments as surface waters, groundwater, and drinking water (as well as bottom sediments and soils), is residue of antibiotics used on a large scale in animal production [74, 75]. Antibiotic residues in soil originate mainly in contaminated excrement used as fertilizer in agriculture. The quantity of antibiotics in agricultural soil has been estimated at kilograms per hectare, equaling that of pesticides in these environments [76, 77].

Antibiotics in natural environments eliminate susceptible populations of bacteria and promote the growth of bacteria that are resistant to them. Both the ability to produce antibiotics and the ability to eliminate their effects are linked to stress response mechanisms. Antibiotics that inhibit the growth of micro-organisms often lead to resistance to re-exposure to them. This phenomenon has been observed following incubation of bacteria with aminoglycoside antibiotics, which damage ribosomes, or ofloxacin, which inhibits DNA replication [78, 79].

Resistance to antibiotics appears not only after contact with these therapeutic agents, but also, for example, in conditions of restricted access to nutrients or metal ions necessary for growth. A lack of magnesium ions leads to resistance to cationic antibiotics such as polymyxin B in *Psudomonas aeruginosa* [80] and *Salmonella enterica* serovar *Typhimurium* [81]. Limited access to phosphate ions induces resistance to polymyxin B in *P. aeruginosa* [82], while a lack of iron ions leads to resistance to β-lactam antibiotics in *E. coli* [83]. Antibiotic resistance has also been noted following exposure to a factor inducing an oxidative stress response and to weak carboxylic acids. Resistance to fluoroquinolene antibiotics has been noted in *E. coli* cells following application of paraquat and salicylate [84]. This trait was expressed as the capability of these bacteria to survive in the presence of antibiotics, but without the ability to produce new generations.

Literature reports also indicate that the stringent response mechanism plays a role in the process of induced resistance to some antibiotics. Activation of the program has been found to lead to the appearance of resistance to ß-lactam antibiotics. The mechanism of this resistance involves inhibition by stringent response mediators of synthesis of peptidoglycan, which is the main target of the antibiotic, and induction of synthesis of ß-lactamase, an enzyme that hydrolyzes the ß-lactam ring of the antibiotic [85]. Indirect evidence for the role of the stringent response in antibiotic resistance was found in a study by Nguyen et al. [86]. Planktonic and biofilm-forming *Pseudomonas aeruginosa* bacteria incapable of ppGpp synthesis were found to be significantly more susceptible to antibiotics than their isogenic wild-type strain. In addition, the cells of this mutant had an increased level of hydroxyl radicals and were more susceptible to paraquat.

Another mechanism of antibiotic resistance may be increased expression of genes coding for low-molecular-weight shock proteins [87]. These proteins condition resistance to aminoglycoside antibiotics which impair ribosome functions, leading to accumulation of improperly folded proteins and, in consequence, to the death of the cell [88]. Thus resistance to these antibiotics may result from the protective effect of HSP on the structure of cellular proteins, and from their ability to restore the proper structure of denatured proteins.

The ability of bacteria to synthesize antibiotics and other secondary metabolites is also associated with the stringent response. In laboratory conditions, antibiotic syn-

thesis is induced by exposing a microbial culture to starvation conditions, causing it to enter the stationary phase. During the stationary phase, the cells produce numerous secondary metabolites, including antibiotics and toxins. Although their significance for survival in these conditions is not clear, their intensive synthesis suggests that they are important for the producers [89]. Accumulation of stringent response mediators induces synthesis of actinorhodin and undecylprodigiosin in *Streptomyces coelicolor*, and of actinomycin in *Streptomyces antibioticus*. In starvation conditions, strains lacking the ability to synthesize this mediator (relA – relaxed mutants) have been shown not to synthesize enzymes of the biosynthesis pathways for these antibiotics [90, 91]. Synthesis of colicin K in *E. coli* is similarly regulated [89].

Nitrogen Fixation

Current research on the physiological effects of the stress response in bacteria has provided evidence that this process is associated with biological fixation of atmospheric nitrogen. Atmospheric nitrogen fixation, in which nitrogen is reduced to ammonia in the presence of the enzyme nitrogenase, is a phenomenon occurring only in prokaryotes. This process requires a high level of ATP, both in regulation of expression of the nif regulon (which codes for enzymes involved in atmospheric nitrogen fixation) and in the actual reduction of molecular nitrogen [92]. Due to the high energy cost of this process, it is precisely regulated depending on the availability of nitrogen sources in the environment. Nitrogen starvation, in which ammonia or amino acids are lacking in the environment, leads to activation of this process.

Both symbiotic micro-organisms and some species of free-living micro-organisms in the environment are capable of nitrogen fixation. The symbiotic micro-organisms active in this process are the soil root-nodule bacteria *Rhizobium*, which are capable of colonizing the roots of specific legumes in conditions of limited soil nitrogen availability [93]. This symbiotic relationship leads to the formation of organs called nodules on the plant roots. In these nodules the bacteria are transformed into bacteroids capable of fixing atmospheric nitrogen. The bacteroids in the nodules, surrounded by a cell membrane called an envelope membrane, form structures known as symbiosomes [94]. In the symbiosome formation process, free-living *Rhizobia* move from the highly variable soil environment to the more stable conditions prevailing within the plant tissues. The transformation of vegetative forms into bacteroids is accompanied by the loss of ability to continue cell division and by a metabolic shift from ammonia assimilation to reduction of molecular nitrogen. The bacterial stringent response mechanism is involved in the proper progression of the early stages of symbiosis between the symbiotic bacterium *Sinorhizobium meliloti* and its host *Medicago sativa*. Evidence of this is provided by observations that rel_{Sme} mutants, incapable of ppGpp synthesis, are defective for the nodulation process and overproduce succinoglycan, an exopolysaccharide that is important during infection [95].

The stringent response also is involved in the intermediate and late stages of symbiosis between the symbiotic bacterium *Rhizobium etli* and its host *Phaseolus vulgaris*. Accumulation of (p)ppGpp, mediators of the stringent response in these bacteria, is necessary for efficient fixation of atmospheric nitrogen. *R. etli relA* mutants, which lack the ability to synthesize (p)ppGpp under conditions of amino acid starvation, have been shown to be capable of inducing nodules, but the level of activity of nitrogenase (the enzyme involved in reducing N_2 to NH_3) in the nodules was only 25% of the level characteristic of nodules induced by the wild-type strain [96]. Bacterioids of this mutant were hypersensitive to oxidative stress, but were more resistant to heat and osmotic stress [97]. Consistent with these observations, a low level of expression of genes dependent on the δ^N transcription factor was noted in these cells, including the nitrogen fixation genes *rpoN2* and *iscN* and the *prxS* gene coding for peroxiredoxin, an enzyme involved in antioxidant defence [96]. This could indicate that (p)ppGpp alarmones of the stringent response in bacteroids promote expression of genes regulated by the δ^N factor.

Apart from changes in the physiology of bacteroids of the wild-type strain and *relA* mutants, structural and morphological differences have been noted as well. Bacteroids of the *relA* mutant were larger than those of the wild-type strain; moreover, each of them was surrounded by a symbiosome membrane, reducing the amount of space in the symbiosome [96]. *R. etli rsh* mutants (with impaired ability to produce and hydrolyze ppGpp) were also found to be capable of inducing root nodules, but there were fewer of them and they were not involved in atmospheric nitrogen fixation [98]. These results suggest that mediators of the stringent response play an important role in successfully establishing a symbiotic relationship and in the metabolic adaptation of the cells to the conditions prevailing in root nodules.

The bacterial stringent response is also involved in the physiological processes of free-living *R. etli* cells [97]. *R. etli* rel_{Ret} mutants, which are incapable of synthesizing (p)ppGpp, exhibited sensitivity to osmotic stress, heat stress, and oxidative stress generated by H_2O_2, as well as to stationary-culture conditions on a succinate minimal medium. Cells of this mutant were incapable of the adaptive change in shape from cylindrical to oval, which is characteristic of the wild-type strain in the stationary phase of growth. While bacteroids of this strain were characterized by increased sensitivity to H_2O_2, they were more resistant than wild-type strain bacteroids to higher temperatures and osmotic stress.

These data indicate that the stringent response plays a key role in the process of adaptation of free-living forms of *R. etli* to the specific conditions of the soil environment. The hypersensitivity of these cells may result from their inability to form a biofilm, a protective structure typical of free-living microbial cells in the natural environment. The biofilm structure was observed in rhizobia for the first time

by Seneviratne and Jayasinghearachchi [99]. According to the present state of knowledge, it not only protects free-living cells against harmful environmental factors, but also seems to play an important role in initiating infection. It has been suggested that the ability of rhizobial cells to attach to the root surfaces of a sensitive plant and to aggregate facilitates the initial stages of infection. This was confirmed in a study by Fujishige et al. [100], in which both exopolysaccharide deficient mutants and mutants that overproduced it exhibited reduced biofilm formation capability, and an associated reduction in ability to induce root nodules.

The stress response is probably also involved in activating the mechanism of atmospheric nitrogen fixation and the subsequent nitrogen-fixing growth of free-living bacteria, although the general stress response mediated by the sigma factor RpoS (δ^S) seems to play a greater role in this process than the stringent response. A study by Howorth and England [101] demonstrated that free-living atmospheric nitrogen assimilators such as *Azotobacter vinelandii* and *Azomonas agilis*, unlike some symbiotic strains, are incapable of accumulating stringent response alarmones in conditions of amino acid starvation, which is generally believed to determine the metabolic shift in these bacteria from nitrate and amino acid assimilation to atmospheric nitrogen fixation.

The role of the general stress response in the nitrogen fixation process in this group of bacteria was confirmed in a study by Sandercock and Page [102], in which a *rpoS* mutant of *A. vinelandii* incapable of RpoS synthesis had lower viability in a nondesiccating, solid medium and in a liquid medium in conditions of glucose starvation than the wild-type strain. It also exhibited greater sensitivity than the wild-type strain to stress induced by the presence of hydrogen peroxide. Stationary-phase cells of this mutant were particularly susceptible, which was manifested by their quick loss of viability induced by 15 mM of hydrogen peroxide. This hypersensitivity was probably due to a lack of induction of catalase 1, a protein that breaks down hydrogen peroxide, which is regulated by RpoS during the mid-exponential to late-stationary phases. According to this study, *rpoS* expression did not occur exclusively in the stationary phase but was influenced by changes in carbon and nitrogen source availability. A 26- to 28-fold induction of the RpoS protein occurred during acetate-to-glucose and ammonium-to-N_2 diauxic shifts. The reverse metabolic shift to acetate and ammonium led to a decrease in the RpoS protein to its basal level, but did not affect the level of *rpoS* mRNA in the same manner. As the stability of this protein does not change significantly in *A. vinelandii* cells, the most likely explanation for this phenomenon is post-transcriptional regulations taking place in the cells of the bacteria under conditions that restrict growth due to nutrient depletion.

Conclusions

This paper does not cover all of the questions connected with the role of the stress response in the functioning of microbial communities in natural environments. Only some phenomena involving stress response mechanisms of ecological and agricultural significance have been discussed. The conditions in which micro-organisms live in the soil environment are variable and often harmful. Nutrient reserves in the soil may undergo substantial fluctuations, depending on the physical, chemical, and biological conditions prevailing in it. On the other hand, compounds constituting nutrients for micro-organisms influence some physical and chemical characteristics of the natural environment. Moreover, substances associated with human activity may appear in the soil. For this reason, an extremely important aspect of the stress response is the activation of adaptive resistance mechanisms to adverse environmental factors of both natural and anthropogenic origin. The data presented in our paper show that the scope and level of this resistance depend on many factors, including concrete environmental circumstances, the type of micro-organism, and even its strain.

Common to all micro-organisms is a form of adaptation to starvation conditions – known in the literature as nutrient stress – involving the generation of cells capable of functioning in the stationary phase of growth. This cell type appears as the result of a re-programming of the metabolism, mediated by mechanisms of the general stress response, the heat shock response, the stringent response, the TA response, and activity of other regulators, e.g. QS.

Bacteria in the stationary phase are characterized by a number of adaptations that enable them to utilize available sources of energy and carbon sparingly. Most often this is achieved by means of restricted energy expenditures for growth and reproduction, a reduced metabolism rate, and activation of autolysis processes that ensure the supply of new food substrates. The capability of utilizing other food sources present in the environment often appears as well. Cell structures such as the cell envelope, the nucleoid, and the ribosomes become modified in order to increase their stability. As a result of these changes the cells become resistant not only to the starvation conditions characteristic of this phase of growth, but also to other harmful physical and chemical agents.

Specialized forms of life are generated based on the metabolic program activated in the stationary phase of growth. Some of these, such as the fruiting body in *Myxococcus* or endospores in Gram-positive bacteria, are specific stages in the life cycle of these microbes, while others, such as the GASP and SCDI phenotypes, appear to be an irreversible and extreme adaptation to severe conditions. The VBNC state and the biofilm are life forms common to both prokaryotic and eukaryotic micro-organisms, and are often observed in the environment.

The capability of forming a biofilm is probably typical not only of soil micro-organisms but of other types as well, e.g. intestinal bacteria, for which soil is a chance, temporary environment [103]. The biofilm structure is the result of microbial interaction not only with the abiotic soil environment, but also with the biotic component of the soil, which includes plant roots. Rhizosphere micro-organisms are particularly sensitive to signals emitted by plants. Low-molecular-weight plant exudates can stimulate some kinds of bac-

teria to form a biofilm. This phenomenon can be beneficial not only to the bacteria, which become resistant to harmful environmental factors, but to the plants as well. The biofilm structure formed by *B. subtilis* can form a protective layer on the roots of tomato plants, while biofilms produced by rhizobia on the surface of legume roots can facilitate the initiation of infection, which leads to the formation of an active symbiotic relationship. Moreover, rhizobium biofilm formation can be induced by root exudates from the symbiotic partner even when it is not present in the environment, e.g. after harvesting or near the end of the growing season. This indicates that this is a mechanism fixed by evolution with the purpose of creating a niche enabling rhizobia to survive in the soil environment when they cannot enter a symbiotic relationship with a plant partner.

VBNC is another commonly occurring form in which micro-organisms can live in the external environment. Cells in the VBNC state, unlike those organized in a biofilm, are characterized by low metabolic activity, which is probably the main reason for their resistance to environmental factors. The inability of cells to proliferate in laboratory conditions explains the discrepancies often observed by various researchers (particularly microbial ecologists) between the number of micro-organisms grown in soil and water samples and the number determined using direct methods, in which cells are counted under a microscope. This trait is also the reason why knowledge of this form of microbial life is limited.

Atmospheric nitrogen fixation is a form of adaptation of bacteria to nitrogen-limiting conditions. This process is mainly regulated by means of the alternative δ^N factor, although other stress mechanisms also play an important role. The data presented show that the stringent response, depending on the species or strain, determines the life functions of free-living rhizobia, essential stages of infection, and active symbiosis. The general stress response mechanism mediates the adaptation of free-living atmospheric nitrogen assimilators to the changeable conditions of the soil environment.

The stress response also mediates activation of synthesis pathways for antibiotic substances and induced mechanisms of antibiotic resistance. Synthesis of antibiotics is associated with the activation of alternative mechanisms for acquiring carbon sources in stationary-phase conditions. Production of secondary metabolites in the stationary phase is exploited on an industrial scale in production not only of antibiotics, but also of bacteriocins, various enzymes, and polymers useful in industry. The phenomenon of antibiotic resistance appearing as a result of the stress response plays an important role in preserving biodiversity of microbial communities in the natural environment.

The data presented indicate the important role of the stress response program in microbial adaptation to the conditions of the soil environment, the rhizosphere, or the inside of the plant. This determines the life functions of free-living microbial communities in the soil as well as those capable of association with plants. Thus the metabolic program of the environmental stress response is an important factor in shaping the biological activity of the soil.

References

1. SCHIMEL J., BALSER T., WALLENSTEIN M. Microbial stress-response physiology and its implications for ecosystem function. Ecology **88**, (6), 1386, **2007**.
2. KOLTER R., SIEGELE D.A., TORMO A. The stationary phase of the bacterial life cycle. Annu. Rev. Microbiol. **47**, 855, **1993**.
3. GRAY J.V., PETSKO G.A., JOHNSTON G.C., RINGE D., SINGER R.A., WERNER-WASHBURNE M. "Sleeping beauty": quiescence in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. **68**, (2), 187, **2004**.
4. HELMANN J.D., WU M.F.W., KOBEL P.A., GAMO F-J., WILSON M., MORSHEDI M.M., NAVRE M., PADDON C. Global Transcriptional Response of *Bacillus subtilis* to Heat Shock. J. Bacteriol. **183**, (24), 7318, **2001**.
5. ZHAO K., LIU M., BURGESS R.R. The global transcriptional response of *Escherichia coli* to induced σ^{32} protein involves σ^{32} regulon activation followed by inactivation and degradation of σ^{32} *in vivo*. J. Biol. Chem. **280**, (18), 17758, **2005**.
6. BECKMANN R.P., MIZZEN L., WELCH W. Interaction of Hsp70 with newly synthesized proteins: implications for protein folding and assembly. Science **248**, 850, **1990**.
7. REEVE C.A., AMY P.S., MATIN A. Role of protein synthesis in the survival of carbon-starved *Escherichia coli* K-12. J. Bacteriol. **160**, (3), 1041, **1984**.
8. LANGE R., HENGGE-ARONIS R. Identification of a central regulator of stationary-phase gene expression in *Escherichia coli*. Mol. Microbiol. **5**, (1), 49, **1991**.
9. HENGGE-ARONIS R. Back to log phase: sigma S as a global regulator in the osmotic control of gene expression in *Escherichia coli*. Mol. Microbiol. **21**, (5), 887, **1996**.
10. HECKER M., PANÉ-FARRÉ J., VÖLKER U. SigB-dependent general stress response in *Bacillus subtilis* and related gram-positive bacteria. Annu. Rev. Microbiol. **61**, 215, **2007**.
11. CASES I., USSERY D.W., DE LORENZO V. The sigma54 regulon (sigmulon) of *Pseudomonas putida*. Environ Microbiol. **5**, (12), 1281, **2003**.
12. CASHEL M., GENTRY D.M., HERNANDEZ V.J., VINELLA D. The stringent response. In *Escherichia coli* and *Salmonella typhimurium* Cellular and Molecular Biology. (F. C. Neidhardt, Ed. in Chief). ASM Press, Washington D.C. **1**, 1458, **1996**.
13. GENTRY D.R., CASHEL M. Mutational analysis of the *Escherichia coli* spoT gene identifies distinct but overlapping regions involved in ppGpp synthesis and degradation. Mol. Microbiol. **19,** (6), 1373, **1996**.
14. MITTENHUBER G. Comparative genomics and evolution of genes encoding bacterial (p)ppGpp synthetases/hydrolases (the Rel, RelA and SpoT proteins). J. Mol. Microbiol. Biotechnol. **3**, (4), 585, **2001**.
15. ARTSIMOVITCH I., PATLAN V., SEKINE S., VASSYLYEVA M.N., HOSAKA T., OCHI K., YOKOYAMA S., VASSYLYEV D.G. Structural basis for transcription regulation by alarmone ppGpp. Cell **117**, (3), 299, **2004**.
16. JISHAGE M., KVINT K., SHINGLER V., NYSTRÖM T. Regulation of sigma factor competition by the alarmone ppGpp. Genes Dev. **16**, (10), 1260, **2002**.
17. MAGNUSSON L.U., FAREWELL A., NYSTRÖM T. ppGpp: a global regulator in *Escherichia coli*. Trends Microbiol. **13**, (5), 236, **2005**.
18. NYSTRÖM T. Stationary- phase physiology. Annu. Rev. Microbiol. **58**, 161, **2004**.

19. HUISMAN G.W., SIEGELE M., ZAMBRANO M., KOLTER R. Morphological and Physiological Changes during Stationary Phase. American Society for Microbiology Press, Washington, DC **1996**.
20. ALMIRÓN M., LINK A.J., FURLONG D., KOLTER R. A novel DNA-binding protein with regulatory and protective roles in starved *Escherichia coli*. Genes Dev. **6**, (12B), 2646, **1992**.
21. NAIR S., FINKEL S.E. Dps protects cells against multiple stresses during stationary phase. J. Bacteriol. **186**, (13), 4192, **2004**.
22. MARTINEZ A., KOLTER R. Protection of DNA during oxidative stress by the nonspecific DNA-binding protein Dps. J. Bacteriol. **179**, (16), 5188, **1997**.
23. BARKER M.M., GAAL T., JOSAITIS C.A., GOURSE R.L. Mechanism of regulation of transcription initiation by ppGpp. I. Effects of ppGpp on transcription initiation *in vivo* and *in vitro*. J. Mol. Biol. **305**, (4), 673, **2001**.
24. GROAT R.G., SCHULTZ J.E., ZYCHLINSKY E., BOCKMAN A., MATIN A. Starvation proteins in *Escherichia coli*: kinetics of synthesis and role in starvation survival. J. Bacteriol. **168**, (2), 486, **1986**.
25. WEICHART D., QUERFURTH N., DREGER M., HENGGE-ARONIS R. Global role for ClpP-containing proteases in stationary-phase adaptation of *Escherichia coli*. J. Bacteriol. **185**, (1), 115, **2003**.
26. FAREWELL A., DIEZ A.A., DIRUSSO C.C., NYSTRÖM T. Role of the *Escherichia coli* FadR regulator in stasis survival and growth phase-dependent expression of the uspA, fad, and fab genes. J. Bacteriol. **178**, (22), 6443, **1996**.
27. GROSSMAN A.D., TAYLOR W.E., BURTON Z.F., BURGESS R.R., GROSS C.A. Stringent response in *Escherichia coli* induces expression of heat shock proteins. J. Mol. Biol. **186**, (2), 357, **1985**.
28. GIVSKOV M., EBERL L., MØLLER S., POULSEN L.K., MOLIN S. Responses to nutrient starvation in *Pseudomonas putida* KT2442: analysis of general cross-protection, cell shape, and macromolecular content. J. Bacteriol. **176**, (1), 7, **1994**.
29. RIVAS M., SEEGER M., HOLMES D.S., JEDLICKI E. A Lux-like quorum sensing system in the extreme acidophile *Acidithiobacillus ferrooxidans*. Biol. Res. **38**, 283, **2005**.
30. RUIZ L.M., VALENZUELA S., CASTRO M., GONZALEZ A., FREZZA M., SOULÈRE, L., ROHWERDER T., QUENEAU Y., DOUTHEAU A., SAND W., JEREZ C.A., GUILIANI N. AHL communication is a widespread phenomenon in biomining bacteria and seems to be involved in mineral-adhesion efficiency. Hydrometallurgy **94**, 133, **2008**.
31. MUTSCHLER H., GEBHARDT M., SHOEMAN R.L., MEINHART A. A novel mechanism of programmed cell death in bacteria by toxin-antitoxin systems corrupts peptidoglycan synthesis. PLoS Biol. **9**, (3), e1001033, **2011**.
32. CASTONGUAY M.H., VAN DER SCHAAF S., KOESTER W., KROONEMAN J., VAN DER MEER W., HARMSEN H., LANDINI P. Biofilm formation by *Escherichia coli* is stimulated by synergistic interactions and co-adhesion mechanisms with adherence-proficient bacteria. Res. Microbiol. **157**, (5), 471, **2006**.
33. HARRISON J.J., CERI H., TURNER R.J. Multiple metal resistance and tolerance in microbial biofilms. Nat. Rev. Microbiol. **5**, 928, **2007**.
34. HOFFMAN L.R., D'ARGENIO D.A., MACCOSS M.J., ZHANG Z., JONES R.A., MILLER S.I. Aminoglycoside antibiotics induce bacterial biofilm formation. Nature **436**, (7054), 1171, **2005**.
35. CHEN Y., CAO S., CHAI Y., CLARDY J., KOLTER R., GUO J.H., LOSICK R. A *Bacillus subtilis* sensor kinase involved in triggering biofilm formation on the roots of tomato plants. Mol. Microbiol. **85**, (3), 418, **2012**.
36. PÉREZ-GIMÉNEZ J., MONGIARDINI E.J., ALTHABEGOITI M.J., COVELLI J., QUELAS J.I., LÓPEZ-GARCÍA S.L., LODEIRO A.R. Soybean lectin enhances biofilm formation by *Bradyrhizobium japonicum* in the absence of plants. Int. J. Microbiol. **719367**, **2009**.
37. BELOIN C., VALLE J., LATOUR-LAMBERT P., FAURE P., KRZEMINSKI P., BALESTRINO D., HAAGENSEN J.A., MOLIN S., PRENSIER G., Arbeille B., Ghigo J.M. Global impact of mature biofilm lifestyle on *Escherichia coli* K-12 gene expression. Mol. Microbiol. **51**, (3), 659, **2004**.
38. SAUER K., CAMPER A.K. Characterization of phenotypic changes in *Pseudomonas putida* in response to surface-associated growth. J. Bacteriol. **183**, (22), 6579, **2001**.
39. WHITCHURCH C.B., TOLKER-NIELSEN T., RAGAS P.C., MATTICK J.S. Extracellular DNA required for bacterial biofilm formation. Science **295**, (5559), 1487, **2002**.
40. CALDWELL D.E. The calculative nature of microbial biofilms and bioaggregates. Int. Microbiol. **5**, 107, **2002**.
41. WANG X., ROCHON M., LAMPROKOSTOPOULOU A., LÜNSDORF H., NIMTZ M., RÖMLING U. Impact of biofilm matrix components on interaction of commensal *Escherichia coli* with the gastrointestinal cell line HT-29. Cell Mol. Life Sci. **63**, (19-20), 2352, **2006**.
42. WHITE A.P., GIBSON D.L., KIM W., KAY W.W., SURETTE M.G., Thin aggregative fimbriae and cellulose enhance long-term survival and persistence of *Salmonella*. J. Bacteriol. **188**, (9), 3219, **2006**.
43. GHIGO J.M. Are there biofilm-specific physiological pathways beyond a reasonable doubt? Res. Microbiol. **154**, (1), 1, **2003**.
44. SCHUSTER M., HAWKINS A.C., HARWOOD C.S., GREENBERG E.P. The *Pseudomonas aeruginosa* RpoS regulon and its relationship to quorum sensing. Mol. Microbiol. **51**, 973, **2004**.
45. VAN DELDEN C., COMTE R., BALLY A.M. Stringent response activates quorum sensing and modulates cell density-dependent gene expression in *Pseudomonas aeruginosa*. J. Bacteriol. **183**, (18), 5376, **2001**.
46. SCHEMBRI M.A., KJAERGAARD K., KLEMM P. Global gene expression in *Escherichia coli* biofilms. Mol. Microbiol. **48**, (1), 253, **2003**.
47. TAYLOR C.M., BERESFORD M., EPTON H.A., SIGEE D.C., SHAMA G., ANDREW P.W., ROBERTS I.S. *Listeria monocytogenes* relA and hpt mutants are impaired in surface-attached growth and virulence. J. Bacteriol. **184**, (3), 621, **2002**.
48. BALZER G.J., MCLEAN R.J.C. The stringent response genes relA and spoT are important for *Escherichia coli* biofilms under slow-growth conditions. Can. J. Microbiol. **48**, 675, **2002**.
49. PRIGENT-COMBARET C., BROMBACHER E., VIDAL O., AMBERT A., LEJEUNE P., LANDINI P., DOREL C. Complex regulatory network controls initial adhesion and biofilm formation in *Escherichia coli* via regulation of the csgD gene. J. Bacteriol. **183**, (24), 7213, **2001**.
50. LACQUA A., WANNER O., COLANGELO T., MARTINOTTI M.G., AND LANDINI P. Emergence of biofilm-forming subpopulations upon exposure of *Escherichia coli* to environmental bacteriophages. Appl. Environ. Microbiol. **72**, (1), 956, **2006**.

51. WEBB J.S., THOMPSON L.S., JAMES S., CHARLTON T., TOLKER-NIELSEN T., KOCH B., GIVSKOV M., KJELLEBERG S. Cell death in *Pseudomonas aeruginosa* biofilm development. J. Bacteriol. **185**, (15), 4585, **2003**.
52. ELKINS J.G., HASSETT D.J., STEWART P.S., SCHWEIZER H.P., MCDERMOTT T.R. Protective role of catalase in *Pseudomonas aeruginosa* biofilm resistance to hydrogen peroxide. Appl. Environ. Microbiol. **65**, 4594, **1999**.
53. ZHANG X.S., GARCÍA-CONTRERAS R., WOOD T.K. YcfR (BhsA) influences *Escherichia coli* biofilm formation through stress response and surface hydrophobicity. J. Bacteriol. **189**, (8), 3051, **2007**.
54. TEITZEL G.M., PARSEK M.R. Heavy metal resistance of biofilm and planktonic *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. **69**, 2313, **2003**.
55. COSTERTON J.W., STEWART P.S., GREENBERG E.P. Bacterial biofilms: A common cause of persistent infections. Science **284**, (5418), 1318, **1999**.
56. OLIVER J.D. Viable but nonculturable bacteria in food environments. In: P.M. Fratamico and A.K. Bhunia (Eds.), Ford Borne Pathogens: Microbiology and Molecular Biology. Horizon Scientific Press, Norfolk, U.K. **2005**.
57. COUTARD F., POMMEPUY M., LOAEC S., HERVIO-HEATH D. mRNA detection by reverse transcription-PCR for monitoring viability and potential virulence in a pathogenic strain of *Vibrio parahaemolyticus* in viable but non-culturable state. J. Appl. Microbiol. **98**, (4), 951, **2005**.
58. ZHONG L., CHEN J., ZHANG X.H., JIANG Y.A. Entry of *Vibrio cincinnatiensis* into viable but nonculturable state and its resuscitation. Lett. Appl. Microbiol. **48**, (2), 247, **2009**.
59. NILSSON L., OLIVER J.D., KJELLEBERG S. Resuscitation of Vibrio vulnificus from the viable but non-culturable state. J. Bacteriol. **173**, (16), 5054, **1991**.
60. NARIYA H., INOUYE M. MazF, an mRNA interferase, mediates programmed cell death during multicellular *Myxococcus* development. Cell **132**, (1), 55, **2008**.
61. LI S., LEE B.U., SHIMKETS L.J. CsgA expression entrains *Myxococcus xanthus* development. Genes Dev. **6**, (3), 401, **1992**.
62. LOBEDANZ S., SØGAARD-ANDERSEN L. Identification of the C-signal, a contact-dependent morphogen coordinating multiple developmental responses in *Myxococcus xanthus*. Genes Dev. **17**, 2151, **2003**.
63. HARRIS B.Z., KAISER D., SINGER M. The guanosine nucleotide (p)ppGpp initiates development and A-factor production in *myxococcus xanthus*. Genes Dev. **12**, (7), 1022, **1998**.
64. MARTÍNEZ-GARCÍA E., TORMO A., NAVARRO-LLORÉNS J.M. GASP phenotype: presence in enterobacteria and independence of sigmaS in its acquisition. FEMS Microbiol. Lett. **225**, (2), 201, **2003**.
65. FINKEL S.E., ZINSER E., KOLTER R. Long-term survival and evolution in stationary phase. Bacterial Stress Responsem. ASM Press, Washington DC, pp. 231-238, **2000**.
66. ZAMBRANO M.M., SIEGELE D.A., ALMIRÓN M., TORMO A., KOLTER R. Microbial competition: *Escherichia coli* mutants that take over stationary phase cultures. Science **259**, (5102), 1757, **1993**.
67. ROZEN D.E., PHILIPPE N., ARJAN DE VISSER J., LENSKI R.E., SCHNEIDER D. Death and cannibalism in a seasonal environment facilitate bacterial coexistence. Ecol. Lett. **12**, (1), 34, **2009**.
68. FARRELL M.J., FINKEL S.E. The growth advantage in stationary-phase phenotype conferred by rpoS mutations is dependent on the pH and nutrient environment. J. Bacteriol. **185**, (24), 7044, **2003**.
69. LEMONNIER M., LEVIN B.R., ROMEO T., GARNER K., BAQUERO M.-R., MERCANTE J., LEMICHEZ E., BAQUERO F., BLÁZQUEZ J. The evolution of contact-dependent inhibition in non-growing populations of *Escherichia coli*. Proc. Biol. Sci. **275**, (1630), 3, **2008**.
70. EYMANN C., MITTENHUBER G., HECKER M. The stringent response, sigmaH-dependent gene expression and sporulation in *Bacillus subtilis*. Mol. Gen. Genet. **264**, (6), 913, **2001**.
71. OCHI K. Metabolic initiation of differentiation and secondary metabolism by *Streptomyces griseus*: significance of the stringent response (ppGpp) and GTP content in relation to A factor. J. Bacteriol. **169**, (8), 3608, **1987**.
72. GOTTLIEB D. The production and role of antibiotics in soil. J. Antibiot. **29**, (10), 987, **1976**.
73. NANJWADE B.K., CHANDRASHEKHARA S., GOUDANAVAR P.S., SHAMAREZ A.M., MANVI M.V. Production of antibiotics from soil-isolated actinomycetes and evaluation of their antimicrobial activities. Trop. J. Pharmaceut. Res. **9**, 373, **2010**.
74. KÜMMERER K. Drugs In the environment emission of drags, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources – a review. Chemosphere **45**, 957, **2001**.
75. HAMSCHER G., SCZESNY S., HÖPER H., NAU H. Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Anal. Chem. **74**, (7), 1509, **2002**.
76. VAN GOOL S. Possible effect on the environment of antibiotic residues in animal manure. Tijdschr. Diergeneeskd. **118**, (1), 8, **1993**.
77. WINCKLER C., GRAFE A. Stoffeintrag durch Tierarzneimittel und pharmakologisch wirksame Futterzusatzstoffe unter besonderer Berücksichtigung von Tetrazyklinen. UBA-Texte 44/00, Berlin **2000**.
78. MORITA Y., SOBEL M.L., POOLE K. Antibiotic inducibility of the MexXY multidrug efflux system of *Pseudomonas aeruginosa*: involvement of the antibiotic-inducible PA5471 gene product. J. Bacteriol. **188**, (5), 1847, **2006**.
79. DÖRR T., LEWIS K., VULIĆ M. SOS response induces persistence to fluoroquinolones in *Escherichia coli*. PLoS Genet. **5**, (12), e1000760, **2009**.
80. MACFARLANE E.L., KWASNICKA A., OCHS M.M., HANCOCK R.E. PhoP-PhoQ homologues in *Pseudomonas aeruginosa* regulate expression of the outer-membrane protein OprH and polymyxin B resistance. Mol. Microbiol. **34**, 305, **1999**.
81. GROISMAN E.A., KAYSER J., SONCINI F.C. Regulation of polymyxin resistance and adaptation to low-Mg^{2+} environments. J. Bacteriol. **179**, (22), 7040, **1997**.
82. LEWENZA S., FALSAFI R., BAINS M., ROHS P., STUPAK J., SPROTT G.D., HANCOCK R.E. The olsA gene mediates the synthesis of an ornithine lipid in *Pseudomonas aeruginosa* during growth under phosphate-limiting conditions, but is not involved in antimicrobial peptide susceptibility. FEMS Microbiol. Lett. **320**, (2), 95, **2011**.
83. VINELLA D., ALBRECHT C., CASHEL M., D'ARI R. Iron limitation induces SpoT-dependent accumulation of ppGpp in *E. coli*. Mol. Microbiol. **56**, (4), 958, **2005**.

84. ROSNER J.L., SLONCZEWSKI J.L. Dual regulation of inaA by the multiple antibiotic resistance (mar) and superoxide (soxRS) stress response systems of *Escherichia coli*. J. Bacteriol. **176**, (20), 6262, **1994**.
85. O'CONNOR K., ZUSMAN D. Induction of β-lactamase influences the course of development in *Myxococcus xanthus*. J. Bacteriol. **181**, (20), 6319, **1999**.
86. NGUYEN D., JOSHI-DATAR A., LEPINE F., BAUERLE E., OLAKANMI O., BEER K., MCKAY G., SIEHNEL R., SCHAFHAUSER J., WANG Y., BRITIGAN B.E., SINGH P.K. Active starvation responses mediate antibiotic tolerance in biofilms and nutrient-limited bacteria. Science **334**, (6058), 982, **2011**.
87. THOMSEN L.E., OLSEN J.E., FOSTER J.W., INGMER H. ClpP is involved in the stress response and degradation of misfolded proteins in *Salmonella enterica* serovar Typhimurium. Microbiology **148**, 2727, **2002**.
88. VAKULENKO S.B., MOBASHERY S. Versatility of aminoglycosides and prospects for their future. Clin. Microbiol. Rev. **16**, (3), 430, **2003**.
89. KUHAR I., ŽGUR-BERTOK D. Transcription regulation of the colicin K cka gene reveals induction of colicin synthesis by differential responses to environmental signals. J. Bacteriol. **181**, (23), 7373, **1999**.
90. HOYT S., JONES G.H. RelA is required for actinomycin production in *Streptomyces antibioticus*. J. Bacteriol. **181**, (12), 3824, **1999**.
91. KANG S.G., JIN W., BIBB M., LEE K.J. Actinorhodin and undecylprodigiosin production in wild-type and relA mutant strains of *Streptomyces coelicolor* A3(2) grown in continuous culture. FEMS Microbiol. Lett. **168**, (2), 221, **1998**.
92. EYDMANN T., SÖDERBÄCK E., JONES T., HILL S., AUSTIN S., DIXON R. Transcriptional activation of the nitrogenase promoter *in vitro*: adnenosine nucleotides are required for inhibition of NifA activity by NifL. J. Bacteriol. **177**, (5), 1186, **1995**.
93. VAN RHIJN P., VANDERLEYDEN J. The Rhizobium-plant symbiosis. Microbiol. Mol. Biol. Rev. **59**, (1), 124, **1995**.
94. ROTH E., JEON K., STACEY G. Homology in endosymbiotic systems: the term 'symbiosome'. In 'Molecular genetics of plant microbe interactions'. (Eds R Palacios and DPS Verma), 220-225. (American Phytopathology Society Press: St Paul, MN) **1988**.
95. WELLS D.H., LONG S.R. The *Sinorhizobium meliloti* stringent response affects multiple aspects of symbiosis. Mol. Microbiol. **43**, (5), 1115, **2002**.
96. MORIS M., BRAEKEN K., SCHOETERS E., VERRETH C., BEULLENS S., VANDERLEYDEN J., MICHIELS J. Effective symbiosis between *Rhizobium etli* and *Phaseolus vulgaris* requires the alarmone ppGpp. J. Bacteriol. **187**, (15), 5460, **2005**.
97. BRAEKEN K., FAUVART M., VERCRUYSSE M., BEULLENS S., LAMBRICHTS I., MICHIELS J. Pleiotropic effects of a rel mutation on stress survival of *Rhizobium etli* CNPAF512. BMC Microbiol. **10**, (8), 219, **2008**.
98. CALDERÓN-FLORES A., DU PONT G., HUERTA-SAQUERO A., MERCHANT-LARIOS H., SERVÍN-GONZÁLEZ L., DÚRAN S. The stringent response is required for amino acid and nitrate utilization, Nod factor regulation, nodulation and nitrogen fixation in *Rhizobium etli*. J. Bacteriol. **187**, (15), 5075, **2005**.
99. SENEVIRATNE G., JAYASINGHEARACHCHI H.S. Mycelial colonization by bradyrhizobia and azorhizobia. J. Biosci. **28**, (2), 243, **2003**.
100. FUJISHIGE N.A., KAPADIA N.N., DE HOFF P.L., HIRSCH A.M. Investigations of *Rhizobium* biofilm formation. FEMS Microbiol. Ecol. **56**, (2), 195, **2006**.
101. HOWORTH M., ENGLAND R.R. Accumulation of ppGpp in symbiotic and free-living nitrogen-fixing bacteria following amino acid starvation. Arch. Microbiol. **171**, 131, **1999**.
102. SANDERCOCK J.R., PAGE W.J. RpoS expression and the general stress response in *Azotobacter vinelandii* during carbon and nitrogen diauxic shifts. J. Bacteriol. **190**, (3), 946, **2008**.
103. OLSÉN A., JONSSON A., NORMARK S. Fibronectin binding mediated by a novel class of surface organelles on *Escherichia coli*. Nature **338**, (6217), 652, **1989**.