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REVIEW

Persistence and Adaptive Mutagenesis in Biofilms

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Abstract—The mechanisms of formation of persisting cells in planktonic and structured microbial populations are considered. The relations between persistence and adaptive mutagenesis are discussed. Persisting cells are suggested to be among the major objects of adaptive mutagenesis and act as an instrument of the microevolution processes in microbial biofilms.

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The processes of biofilm formation and the major characteristics of biofilms have been considered in reviews recently published in this journal [1, 2]. The present review concentrates, therefore, on issues insufficiently covered by them.

The biofilms formed by eukaryotic microorganisms are not considered in this review, since due to the specific patterns of their formation this problem deserves special consideration.

MICROBIAL METABOLISM IN BIOFILMS. THE "BIOFILM" PHENOTYPE

The resistance of biofilms to external factors is characterized as persistence. To a significant degree, persistence is determined by the physiological and biochemical characteristics of the biofilm-forming microorganisms; the latter are phenotypically different from the same microorganisms growing as planktonic populations.

The traits of the "biofilm" phenotype were originally specified by Costerton et al., who discussed the differences between planktonic cultures, microbial colonies, and biofilms [3, 4]. The parameters of this phenotype have been recently successfully investigated by physiological and biochemical methods and by molecular techniques. These results are described in recent reviews and monographs, e.g., [5, 6]. We provide information directly related to the topic reviewed.

The biofilm phenotype develops mainly due to the action of quorum sensing (QS) factors on a microbial culture (see below) and to the mutual interaction of the biofilm-forming microorganisms.

Some mechanisms of microbial interaction in biofilms have been considered in our previous review [1].

We want, however, to present some new data of utmost interest. Since microbial biofilms are crucial in the resistance of microbial infections to chemotherapy, most of these works are focused on biofilm resistance to antibiotics.

Spatial organization and physiological heterogeneity (in growth phase and synthesis of metabolic products) should be a priori expected in microbial populations of biofilms. However, while structural stratification may be detected by direct optical and biochemical techniques, e.g., FISH or determination of growth of anaerobic microorganisms under aerobic conditions in biofilms containing aerobic satellites [7], metabolic heterogeneity, which in many cases may be responsible for persistence of biofilms, is more difficult to observe.

In one of the most successful works with *Staphylococcus epidermidis* biofilms [8], immunofluorescence determination of pulse-labeled DNA and green fluorescent protein (GFP) induced by low tetracycline concentrations were used to detect the physiological and biochemical heterogeneity of the population. In stationary colony-like biofilms, the zone of active metabolism was up to 38 μm deep, i.e., it was limited by oxygen penetration as determined by the microelectrode technique. Since the content of dead cells did not exceed 10%, a significant fraction of cells were viable but metabolically inactive. In general, microbial cells in the biofilm occurred in four different states: growing aerobically, growing fermentatively, dead, and dormant. We suggest that the latter type comprises persistent cells, the role of which in biofilm stability will be discussed in the following sections of this review. The authors concluded that such physiological and biochemical heterogeneity possibly played some part in biofilm resistance to antibiotics. Indeed, cells in different physiological states may launch the mechanism of adaptive mutagenesis, supplemented by exchange of metabolic products and horizontal gene transfer, thus activating the microevolutionary processes (see below), including the mechanisms responsible for antibiotic resistance [9, 10].

Multispecies biofilms have been described as more stable than the biofilms formed by a single microbial species. This results from the synergistic relationships

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often formed between the biofilm components. Such research is usually carried out on binary biofilms, since the data obtained from analysis of these models are more easily interpreted [11].

Important results were obtained in [12], where nonpathogenic *Veillonella parvula*, when growing in binary biofilms with the pathogenic *Streptococcus mutans*, changed its metabolism, so that *S. mutans* became significantly more resistant to various antibacterial agents. Although the mechanism of this phenomenon remains unknown, the authors concluded that the chemotherapeutical treatment required activity against the microbial community including a pathogen, rather than against the pathogen alone.

The effect of microbial satellites (dissipotrophs) on microbial "producers" under starvation conditions is of special interest. This interaction may occur in the ecotopes where the original carbon and energy sources are available only to a limited number of the components of the microbiocenosis ("producers").

This is the case, for example, in well bottom zones of flooded oil fields where petroleum components are the only source of carbon and energy. An enhanced rate and completeness of hexadecane utilization was observed in a binary biofilm containing oil-oxidizing bacteria and their satellites unable to utilize paraffins. The satellite thus prevents starvation stress by stimulating formation of the products of hydrocarbon decomposition [13, 14]. Another possible means by which an oil-oxidizing producer is stimulated is under elevated salinity of stratal water. In this case, formation of a binary biofilm containing an oil-oxidizing microorganism and its halophilic satellite, incapable of oil utilization, results in enhanced expression of the metabolic pathway of ectoine production in the cells of the satellite. Ectoine, an osmoprotective agent, which is excreted from the cell and absorbed by the oil-degrading microorganism, protects the latter from osmotic shock [15].

Formation of persistent cells is still another mechanism of biofilm resistance.

RESISTANCE OF BIOFILMS TO STRESS FACTORS AND ANTIMICROBIAL AGENTS. PERSISTENT CELLS

The existence of persistent cells, which are resistant both to aggressive environmental factors and antimicrobial agents, is a striking characteristic of biofilms.

Persistence, a noninherited (phenotypic) ability of a small part of a bacterial population to survive even after treatment with lethal doses of penicillin, was discovered early in the "antibiotic era" [16] and has been confirmed by many researchers [17, 18]. We have demonstrated one of its manifestations—namely, that, under "bacteriostatic" concentrations of antibiotics inhibiting protein synthesis, synthesis of some proteins, especially the components of the cell membrane, still occurs, albeit at a decreased rate [19, 20]. The optical density of the population increases slowly due to growth of some of the cells [21].

Persistence should be differentiated from drug indifference, another genetically uninherited phenomenon occurring in all microbial populations and resulting from the usually decreased sensitivity of slowly growing or dormant cells to antibacterial agents. Insensitivity of dormant bacterial cells to penicillin and other inhibitors of cell wall biosynthesis is a classical example [22, 23].

Numerous publications (hundreds of experimental articles and tens of reviews) deal with the mechanism of persistence, since this phenomenon plays an important role in recurring (chronic) microbial infections. In such cases, antibiotic treatment eliminates most free-living (planktonic) cells of the pathogen and a significant part of the sensitive cells of the pathogen in biofilms. The immune system deals with the remaining planktonic persistent cells. Persistent cells in biofilms are, however, inaccessible to the immune system; after termination of antibiotic treatment, they resume growth, causing another ictus of infection.

According to the Centers for Disease Control and Prevention (CDC, United States), approximately 65% of all infections result from formation of biofilms in macroorganisms [24]. Moreover, biofilms are formed on almost all medical implants (catheters, prostheses, etc.) Persistence is therefore an important medical problem. Since biofilms cause biofouling and biodegradation of various materials, this issue is significant for nonmedical activities as well [25].

In spite of efforts to overcome the resistance of biofilms to various treatments, the mechanisms of this resistance, especially the mechanisms responsible for formation of persistent cells, remain debatable. We will consider briefly the major opinions on these processes existing in the modern scientific literature; for detail, reviews [26–29] are recommended.

MECHANISMS OF FORMATION OF PERSISTING CELLS

Several alternative and complementary opinions exist concerning the mechanisms responsible for formation of persisting cells.

Assessment of the hypotheses of persistent cell formation requires consideration of the following traits of persisting cells. First, cultures grown from these cells have the same sensitivity to antibiotics as do the initial parent cells. Second, persistent cells constitute a minor part of the total population (10^{-6} or less), although their number increases during the stationary growth phase. Thus, the original concept relating persistence to dormancy remains valid.

Under changing environmental conditions, microbial populations probably face a choice: whether to continue growth, spreading in space and competing for nutrients, or to stop growth, concentrating their

resources on protection from possible stress impacts. In reality, both strategies are used: while the main part of the population is growing, a small part of it acts as a "safeguard" and prepares mechanisms for protection from stress. The ratio of such cells depends on the microbial species and may vary in the course of evolution.

Investigation of the metabolism of persistent cells is hampered by the need to obtain them in quantities sufficient for experiments. In the case of *Escherichia coli*, this problem was partially solved, since the *hip* mutant, which has an increased content of persistent cells, was isolated [30]. The *hip* locus includes two genes, *hipA* and *hipB*, forming the *hipBA* operon. In *hipA7*, the best-studied mutant, persistent cells may constitute up to 1% of the population, i.e., 10^4 times more than in the wild-type strain. Although another locus, *hipQ*, responsible for increased content of persistent cells, was found in *E. coli* [31], these mutants are as yet insufficiently studied.

Two methods have been developed for the isolation of persistent cells of the *hip* mutants. The first includes treatment of the exponential phase *E. coli* cells with ampicillin. After lysis of most of the population, the surviving persistent cells are collected by centrifugation [32]. This method is certainly suitable only for obtaining small amounts of cells.

The second method is based on the fact that persistent cells are in a dormant state and their gene expression is therefore inhibited. Special sorting devices (MoFlo cell-sorters) may be used to isolate the major, strongly fluorescent fraction (ofloxacin-sensitive cells) and a small fraction of weakly fluorescent cells (ofloxacin-resistant persistent cells) in cell populations containing the GFP reporter gene [33]. Although the weakly fluorescent fraction was 20 times more resistant to the antibiotic than the strongly fluorescent population, this method is too labor-consuming for large-scale application.

Modern microfluidic chambers make it possible to observe directly the behavior of individual growing cells of the *hipA7* and *hipQ* mutants and compare them to the wild-type cells [34]. This approach revealed two types of cells in *hipA7* and *hipQ* cultures—rapidly growing, ampicillin-sensitive cells (normal cells) and slowly growing, antibiotic-resistant cells (persistent cells). Both cell types were present in the population prior to antibiotic treatment. Since conversion of persistent cells to rapidly growing, antibiotic-sensitive cells was demonstrated, persistence did not result from a genetic mutation.

Two types of persistence were shown to exist. Persistent cells of the first type are a subpopulation of nongrowing cells, which develops during the stationary phase of the previous growth cycle. Their number therefore depends on the inoculum volume. Prior to growth, such cells experience a prolonged lag phase (up to 14 h), unlike regular cells with 40-min lag phase duration. The second type of persistent cells develops during the growth of a population. Their ratio depends on the number of cells in the culture and does not depend on inoculum volume. A wild-type population contains, apart from the normal, antibiotic-sensitive cells, both types of persistent cells [34].

Unlike the *hipA* and *hipQ* mutations, which increase the number of persisting cells, mutations in the genes *glpD* (glycerol-3-phosphate dehydrogenase) and *plsB* (glycerol-3-phosphate acyltransferase) result in decreased numbers of persistent cells in stationary-phase cultures [35]. Mutation in the *phoU* gene, encoding the product participating in orthophosphate transport and regulation of the synthesis of alkaline phosphatase, has a similar effect [36]. The mechanism of this phenomenon is unknown, although it is most probably related to maintenance of persistence, rather than to its development [26].

Analysis of gene expression in persistent cells obtained from *hipA7* cultures demonstrated that the picture of expression agrees with the notion of persistence as a dormant state [32]. Expression of approximately 300 genes in such cells was found to be enhanced. These genes include *rmf*, encoding the translation inhibitor; components of the SOS system (*recA, umuDC, uvrAB, sulA*, etc.); and the genes responsible for response reactions to heat and cold shock (*htrA, htpX, cspH, clpB, cbpAB*, etc.). Importantly, the genes of the toxin–antitoxin (TA) system (*dinJ/yafQ, yefM, relBE, mazEF*, etc.) also belong to this group. In the slowly growing cells isolated by Shah et al. [33] by sorting (see above), the profile of transcribed genes was rather similar, although not coinciding completely with that for *hipA7* persistent cells.

The genes with decreased expression in persistent cells are those with the products involved in energy metabolism or in less vitally important functions, such as flagella formation.

Due to the abundance of persistence-related genes, a library search for the transposon inserts resulting in enhanced bacterial survival in the presence of antibiotics was unsuccessful [35].

What are the mechanisms of switching to the protective mode of metabolism?

The hypothesis of the role the toxin–antitoxin system plays in this process is presently the most popular.

TOXIN–ANTITOXIN (TA) SYSTEMS AND FORMATION OF PERSISTING CELLS

The role of TA systems in plasmid maintenance in prokaryotic cells is presently well studied [37], while their other functions are less understood. The typical TA system includes two genes, usually combined in an operon, i.e., sharing a common promoter. One of these genes encodes the toxin; another, the antitoxin [38]. The toxin is a stable compound inhibiting one of the important cell functions. The antitoxin is highly labile and is rapidly decomposed when its formation

stops. It neutralizes the effect of the toxin and acts (in a complex with the toxin) as a regulator of its own synthesis. Over ten TA modules are known in *E. coli* [39]. The characteristics of some toxins and their targets are presented in the table [40].

While the toxin is always a protein, the nature of the antitoxin may vary. For example, it may be a small RNA complementary to the mRNA of the toxin and inhibiting translation. In other cases, the antitoxin is a protein neutralizing the toxin by formation of a protein–protein complex [41].

A popular model of persistent cell formation involving the TA system implies that the toxins (for example, HipA or RelE proteins) inactivate the antibiotic-sensitive target in the cell, such as ribosome functioning (for antibiotics inhibiting protein synthesis), the cell-wall synthesis system (for penicillins and other functionally similar antibiotics), or the apparatus of DNA replication (for fluoroquinolones and similar agents). Thus, the target becomes insensitive to antibacterial agents and does not respond to metabolic failures by accumulation of toxic products (proteins with mistakenly included amino acids, etc.) [26, 27, 32]. Blocking of the target by the toxin transfers the cell to a dormant state with suppressed synthesis of the antitoxin, resulting in persistence. After removal of the antibacterial agent, antitoxin synthesis is restored and it deactivates the toxin, causing renewed bacterial growth. The scheme of the action of the toxin is presented on Fig. 1.

The disadvantage of the hypotheses based on the role of TA systems in persistence formation is that, since persisting cells acquire resistance to a number of antibacterial factors, including extreme physicochemical stresses, either a single toxin should block a number of targets or a number of TA systems should participate in development of persistence. Moreover, data are accumulating on the role of other genes, not involved in TA systems, in formation of persistent cells. For example, apart from TA modules (*hipA* and *mazF*), enhanced expression of such genes as *dnaJ* in *E. coli* (chaperon synthesis) or *pmrC* in *Salmonella typhimurium* (associated with phospholipid synthesis) resulted in elevated numbers of persistent cells resistant to penicillin and ciprofloxacin [42].

Alternative models of persistence formation not involving TA systems directly were therefore developed. One example is the suggestion of the role of an "allarmone," guanosine polyphosphate [43]. The authors demonstrated that deletion of the *relA* and *spoT* genes responsible for (p)ppGpp synthesis suppresses almost completely the capacity of *hipA7* mutants for increased formation of persistent cells. This defect may be compensated for by the *relA* gene in a *trans* position. This gene encodes a shortened RelA protein, which catalyzes (p)ppGpp synthesis not requiring ribosomes. This model suggests that an increase in guanosine polyphosphate intracellular level, resulting in a dormant state and persistence, is the primary event in formation of persistent cells. The dormant state, however, is not a necessary requirement for persistence, since enhanced *hipA7* expression, while not inhibiting growth, results in a sharp increase in persistence [44].

Major families of *E. coli* toxins

Toxin	Target	Activity	Suppressed process
CcdB	DNA gyrase	Double-stranded cuts	Replication
RelE	Translating ribosomes	mRNA cleavage	Translation
MazF	RNA	Endoribonuclease	Translation
ParE	DNA gyrase	Double-stranded cuts	Replication
Doc	Translating ribosomes	mRNA cleavage	Translation
VapC	RNA	Endoribonuclease	Unknown
ξ-Toxin	Unknown	Phosphotransferase	Unknown
HipA	*EF-TU*	Protein kinase	Translation
HigB	Translating ribosomes	mRNA cleavage	Translation

Recently, an improved search procedure demonstrated the presence of numerous genes responsible for decreased or suppressed formation of persisting cells in a library of *E. coli* knockout mutations [45]. Mutations in the genes *dnaJ, dnaK* (chaperon production), *apaH* (diadenosine tetraphosphatase gene), *surA* (peptidylprolyl *cis-trans* isomerase gene), *fis* and *hns* (global regulation genes), *hnr* (regulator of the *rpoS* gene), *dksA* (regulator of rRNA transcription), *ygfA* (5-formyltetrahydrofolate cycloligase gene), and *yigB* (flavin mononucleotide phosphatase gene) resulted in tenfold decreased formation of persistent cells, while not affecting the growth rate or the minimal inhibiting concentration of ofloxacin.

Importantly, only a few (~2%) of about 300 genes exhibiting enhanced expression in persistent cells are involved in the process of persistence formation. These are TA modules, SOS response genes, genes encoding hot and cold shock proteins, and some others. The products of other genes are probably necessary for some other vital functions of persisting cells. Predominance of the genes involved in global metabolic regulation certainly indicates the multiplicity of the mechanisms of persistence formation.

PREVENTION OF BIOFILM AND PERSISTING CELL FORMATION

The formation of biofilms in an infected macroorganism and emergence of persistent cells in such biofilms are major problems for chemotherapy of infec-

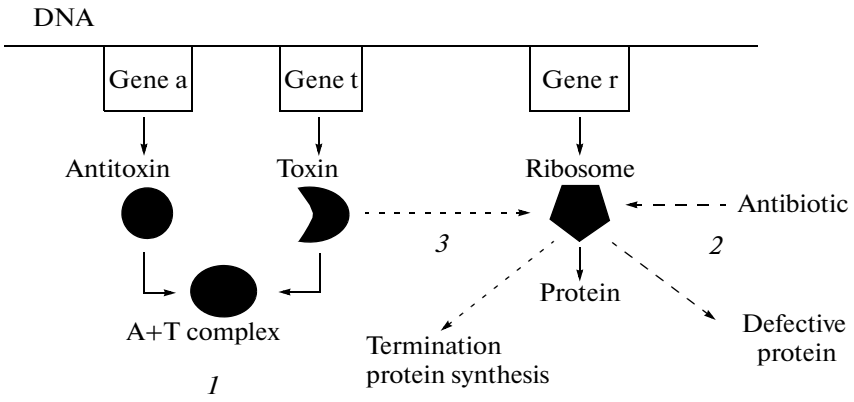

Fig. 1. Schematic representation of persistence formation: the toxin and antitoxin form an inactive complex and protein synthesis on the ribosomes occurs normally (*1*); antibiotic treatment results in formation of defective proteins suppressing microbial growth (dashed arrows) (*2*); the toxin blocks the ribosome and prevents synthesis of the defective protein (dotted arrows), resulting in formation of a persistent cell (*3*).

tions. Moreover, biofilms forming on technological equipment and in pipelines cause them to corrode [25]. Numerous attempts to prevent biofilm formation or functioning have therefore been made. Although significant progress is still in the future, several approaches to these problems were developed.

For example, a method was suggested for treating chronic infections caused by antibiotic-resistant persisting cells in the biofilms of a microbial pathogen. The method involves pulse-dosing of the antibiotic. After elimination of the sensitive cells of the pathogen, antibiotic treatment is interrupted for a period sufficient for the activation of persisting cells and their transition from the dormant state. Antibiotic treatment is then resumed. A correct choice of the interruption intervals for antibiotic treatment makes it possible to cure a chronic disease [27]. The author suggests that such discontinuous treatment does not occur in nature and persisters have no protection against it. Paradoxically, he suggests that the patients' absent-mindedness in the course of prolonged medication was often the reason for their recovery. Noncompliance of the dosage regimen results in varying doses of antibiotics, and this pulse-dosing may result in eradication of persisting cells. The optimal regimen of pulse-dosing may act as a "cure" for persistence.

Another approach relies on a so-called "prodrug" with the preparations that are inactive outside the cell and are converted by the relevant enzymes into active antimicrobial agents when inside a persisting cell. The molecules of such antimicrobial preparation should bind covalently to a number of targets, resulting in cell death. Irreversible binding to a target should prevent removal of the preparation by excretion systems, such as pumps or systems of multiple drug resistance (MDR) [27].

However, in general, treating the formed biofilms is a very difficult task. Attempts have been made to affect the metabolic processes responsible for the initial stages of biofilm formation.

In the case of biocorrosion, special coatings preventing adhesion of biofilm-forming microorganisms are seemingly a good solution. Such coatings, however, proved to be either inefficient or prohibitively expensive. In the case of medical devices, this coating should also be nontoxic, a requirement not easily combined with antibacterial activity.

Attachment of an antimicrobial agent nontoxic to the macroorganism as a long-chain polymer covalently bound to the protected surface is one of the approaches to partial solution of this problem. Poly[2-(dimethylamino)ethylmethacrylate] and *n*-alkyl polyethyleneimine were used as such immobilized polymers. Contact with such surfaces resulted in death of all *Staphylococcus aureus* and *E. coli* cells, including persistent ones [46, 47].

Suppression of microbial adhesion to phase boundaries is a theoretically attractive possibility. Natural "antiadhesins" produced by competing microorganisms are most promising in this respect.

For example, such antiadhesins already described include volatile compounds [48], *n*-alkane mixture [49], or specific hydrolases [50]. Biosurfactants produced by some bacilli also possess antiadhesive properties [51, 52].

Moreover, the cells of one of the strains of marine gliding *Cytophaga* sp. prevent penetration of other strains of this microorganism into the biofilm by production of an antiadhesin of glycoprotein nature [53].

Recently, nanoparticles of superparamagnetic iron oxide (SPION) were used to inhibit growth and biofilm formation by *S. epidermidis*. The exact mechanism of this phenomenon remains unknown [54].

Attempts to use antagonists of the QS factors as inhibitors of biofilm formation are presently the most attractive field of research.

QS factors are known to participate in the regulation of various aspects of bacterial behavior, including bioluminescence, production and secretion of virulence factors, motility, conjugation, spore formation, and, last but not least, biofilm formation [55, 56].

Tens of QS factors and signal components have been recently discovered, although for some of them the mechanism of action has not been studied and the proof of their role in global metabolic regulation (including the existence of relevant receptors) has not been presented. At least six metabolic pathways involved in realization of the functions of the QS factors are presently identified.

However, those QS factors for which the proof of their regulatory role is convincing and the search for inhibitors is promising will be listed below. Among these classical QS factors are acyl derivatives of homoserine lactone (AHL) found in gram-negative bacteria [57]. At least three other types of QS factors exist in gram-negative bacteria: 4-quinolone derivatives, including 2-heptyl-3-hydroxy-4(1H)-quinolone [58]; long-chain hydroxyketones, such as (S)-3-hydroxytridecane-4-one [59]; and, finally, a set of universal autoinducers, 4,5-dihydroxy-2,3-pentandione (DPD) derivatives, which are active in both gram-positive and gram-negative bacteria [60]. The fact that organic derivatives of boric acid, so-called boronic acids with alkyl or aryl substituents, are inhibitors of this QS autoinducer is highly unusual [61, 62].

Specific oligopeptide autoinducers (AIPs) are among the few signal metabolites characteristic only of gram-positive bacteria. They contain 5–17 amino acids, usually undergoing posttranslational modification with inclusion of lactone, thiolactone, isoprenoid, and other structures [63].

Antagonists or inhibitors were found for all these factors, which prevent biofilm formation and are potentially promising as agents facilitating chemotherapy of infections [56, 64].

THE RELATION BETWEEN PERSISTENCE AND ADAPTIVE MUTAGENESIS

"Adaptive mutations" is the term for mutations that originate in a slowly growing or dormant microbial population under continuous stress and counteract the factors causing this stress. Paradoxically, adaptive mutations, which are spontaneous, do not look random because they result in the phenotypic changes promoting survival of the population under stress conditions [65]. Supermutagenesis begins as a consequence of growth arrest, resulting in some small part of the population gaining the ability to grow in the presence of a certain stress factor.

Molecular mechanisms of adaptive mutagenesis are insufficiently studied. However, the existence of certain processes that may potentially result in "directed" mutations has been established [66–69].

These processes are based on several mechanisms. Generally, the most probable one is decreased precision of DNA replication due to mutations in the genes responsible for prevention of replication errors and removal of their consequences via reparation. The SOS regulation system is believed to play a special role in adaptive mutagenesis. Recombination may occur in dormant cells, resulting in "unprotected" DNA synthesis, which increases the probability of erroneous replication. While most of these errors should certainly result in cell death, in some cases the mutations may be beneficial for survival under stress conditions.

Moreover, both under exposure to stress factors and in the stationary growth phase [66], the frequency of transposition of mobile genetic elements increases, this also promoting supermutagenesis [70].

Possible involvement of the RpoS sigma factor one of the RNA polymerase components in adaptive mutagenesis attracts most attention. Seven sigma factors are known for *E. coli* RNA polymerase: RpoD, RpoN, RpoS, RpoH, RpoF, RpoE, and FecI. However, only RpoS is induced upon transition from the exponential to the stationary growth phase and under stress conditions. The remaining sigma factors regulate transcription under specific environmental conditions [68].

The major pathways and mechanisms of participation of the RpoS sigma factor in metabolic regulation are presented on Fig. 2.

During the stationary growth phase, the role of RpoS is to prevent DNA from damage caused by stress factors via enhanced expression of the Dps protein, which forms a nucleoprotein complex with DNA. However, if damage has already occurred, RpoS launches mechanisms, one of which involves a special DinB DNA polymerase that carries out replication bypassing the damage without correction [71]; this increases the mutation rate.

Surprisingly, a number of factors responsible for the ability to form biofilms and persistent cells coincide with those initiating adaptive mutagenesis. For example, RpoS is important for biofilm formation (a process involving up to 30 genes dependent on RpoS), and its mutations therefore suppress this process [72]. RpoS also positively regulates formation of the extracellular polymer matrix [73]. On the other hand, as was already mentioned, the SOS response genes play a key role both in formation of persistent cells and in adaptive mutagenesis. In both processes, some other chaperon proteins also participate [74, 75].

That it is sometimes impossible to differentiate between genetic variability resulting from adaptive mutagenesis and caused by increased frequency of horizontal gene transfer hampers interpretation of the results on increased capacity of biofilm microbial communities for mutagenesis. In fact, the works of many authors demonstrate that the frequency of

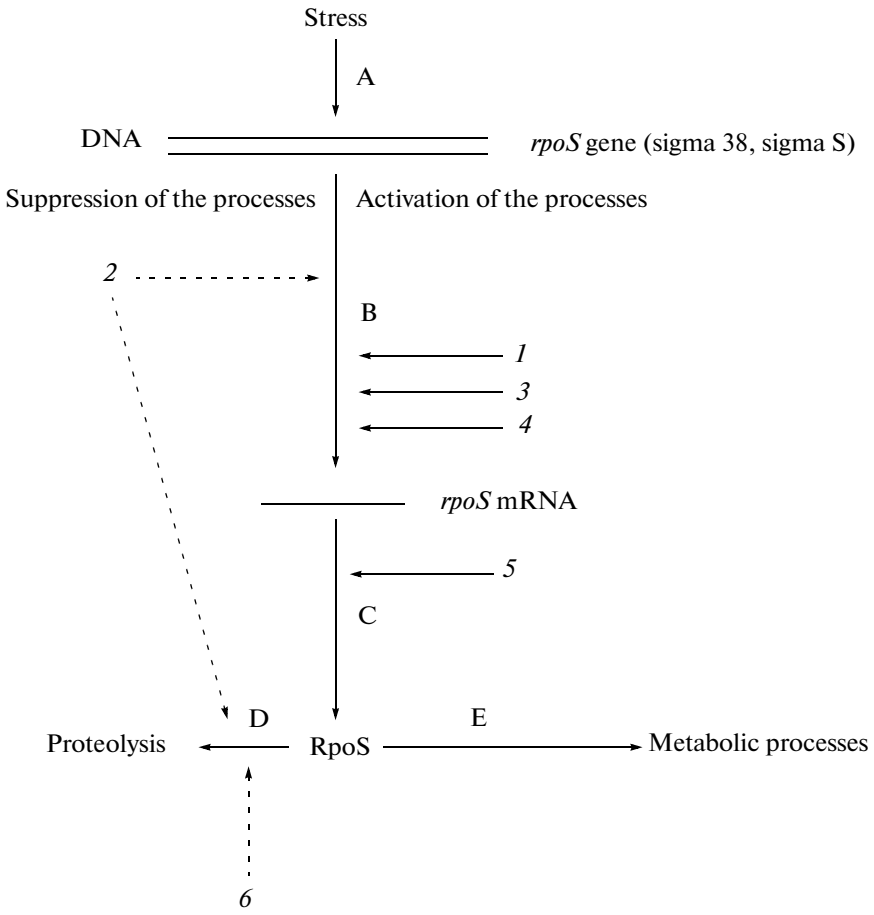

Fig. 2. Schematic representation of the RpoS sigma factor formation and role in the regulation of metabolic processes (based on [68, 82–84] with modifications). (A) induction of *rpoS* under the influence of stress factors, which results in decreased growth rate (limitation of carbon, nitrogen, and phosphorus sources; heat, hyperosmotic, or acid shock, etc.), RpoS proteolysis; (B) additional pathways of regulation of *rpoS* transcription (stimulation, solid lines to the right and inhibition, dashed lines to the left): transcription stimulation by two-component systems BarA/UvrY9 (*E. coli*) and GacA/GacS (*Pseudomonas*) involved in regulatory phosphorylation and preventing RpoS proteolysis (*1*); ArcB/ArcA, another two-component switch of regulatory phosphorylation, stimulating RpoS proteolysis and suppressing its transcription (*2*); activation of *rpoS* transcription by the complex of cAMP + CRP protein (in *E. coli*) or the TetR protein factor (in *Pseudomonas*) (*3*); growth phase-independent stimulation of rpoS transcription by guanosine polyphosphates and global signal molecules, acetate and acetyl phosphate (they probably act via other intermediates) (*4*); (C) regulation of mRNA translation by RpoS: increased translation rate due to the changes of mRNA secondary structure caused by the Hfg RNA-binding protein in a complex with small nontranslated RNAs (DsrA, RprA) (*5*); binding of the Hfg protein to another small nontranslated RNA, OxyS, suppresses *rpoS* translation; (D) regulation of RpoS proteolysis by phosphorylation, which activates RssB/ArcA, resulting in coordination between RpoS proteolysis and transcription of the *rpoS* gene and energy metabolism of the cell (*6*); (E) regulation of RpoS-dependent genes (about 10% of the *E. coli* genome).

genetic exchange in biofilms is tens of times higher than in planktonic cultures [76–78].

In a recent work [79], one of the most ingenious attempts to deal with this issue was made. The authors compared the frequency of deletion mutants (*gtfBC*) in planktonic cultures and biofilms upon recombination of two highly homologous genes, *gtfB* and *gtfC*, encoding glycosyl transferases that catalyze formation of glucans, compounds involved in cell adhesion and biofilm formation. In the biofilm, the frequency of *gtfBC* recombinants was 1.5–2 times higher than in the planktonic culture. The authors explained this effect by reference to selection of recombinants under conditions of impaired biofilm structure resulting from mutation of the glycosyl transferase gene. However, in conclusion, the authors noted that they did not hope to terminate the discussion concerning the role of adaptive mutations in variability of microbial components of the biofilms. They simply stated that selection was an overwhelming factor under the conditions of their experiment.

On the other hand, *P. aeruginosa* isolates obtained from patients with cystic fibrosis often exhibit hypermutability [80], this probably resulting from mutations in the genes (e.g., *mutS*) involved in the system of DNA reparation. Since these isolates form biofilms in

infected macroorganisms, the authors suggested that existence as biofilms favors development of hypermutability due to the damage in DNA reparation systems. Sensitivity to antibiotics—rifampicin and ciprofloxacin—was used as a marker. Transcriptional profiles were determined using DNA microarrays by comparison of planktonic cultures and model biofilms. The cultures grown as biofilms were shown to exhibit ~100 times higher mutability than planktonic cultures. This results primarily from a sharply (by several times) decreased expression of a number of genes (*ahpC*, *katA, sodB,* etc.) encoding the enzymes responsible for DNA protection against oxidative damage.

In work [81], a very important observation was made. The authors proved convincingly that the rate of mutations in *P. aeruginosa* microcolonies within a biofilm was 100 times higher than in planktonic cultures. For in situ investigation of mutations, a *P. aeruginosa* transformant was used that contained a GFP gene with a mutation causing a reading frame shift. Fluorescence-recovering mutations were observed only in the microcolonies of this transformant in the biofilm, but not in the remaining part of the biofilm and not in planktonic cultures of this transformant. The authors suggested that biofilm microcolonies acted as the centers of microevolutionary processes due to adaptive mutagenesis favoring the growth of the microcolonial populations.

Although these works did not investigate the relation between formation of persistent cells and increased mutability in the population, the coincidence of conditions required for adaptive mutagenesis and formation of persistence is sufficient for a substantiated, although as yet speculative, suggestion that persistent cells may be among the important tools for the microevolutionary processes in structured microbial communities, which result in increased variability of microbial populations and their adaptation to changing environmental conditions. Moreover, an increased rate of genetic information exchange in biofilms certainly plays an important part in the processes of microevolution.

REFERENCES

1. Nikolaev, Yu.A. and Plakunov, V.K., Biofilm—"City of Microbes" or an Analogue of Multicellular Organisms?, *Mikrobiologiya*, 2007, vol. 76, no. 2, pp. 149–163 [*Microbiology* (Engl. Transl.), vol. 76, no. 2, pp. 125–138].
2. Smirnova, T.A., Didenko, L.V., Azizbekyan, R.R., and Romanova, Yu.M., Structural and Functional Characteristics of Bacterial Biofilms, *Mikrobiologiya*, 2010, vol. 79, no. 4, pp. 432–446 [*Microbiology* (Engl. Transl.), vol. 79, no. 4, pp. 413–423].
3. Costerton, J.W and Lappin-Scott, H.M, Introduction to Microbial Biofilms, in *Microbial Bioflms*, Lappin-Scott, H.M. and Costerton, J.W., Eds., Cambridge, UK: Cambridge Univ. Press, 1995, pp. 1–11.
4. Donlan, R.M. and Costerton, J.W., Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms, *Clin. Microbiol. Rev.*, 2002, vol. 15, pp. 167–193.
5. Costerton, J.W., The Biofilm Primer, in *Springer Series on Biofilms*, Eckey, C., Ed., Berlin: Springer, 2007.
6. Palmer, R.J. and Stoodley, P., Biofilms 2007: Broadened Horizons and New Emphases, *J. Bacteriol.*, 2007, vol. 189, pp. 7948–7960.
7. Okabe, S., Ito, T., and Saton, H., Sulfate-Reducing Bacterial Community Structure and Their Contribution to Carbon Mineralization in a Wastewater Biofilm Growing Under Microaerophilic Conditions, *Appl. Microbiol. Biotechnol.*, 2003, vol. 63, pp. 322–334.
8. Rani, S.A., Pitts, B., Beyenal, H., Veluchamy, R.A., Lewandowski, Z., Davison, W.M., Buckingham-Meyer, K., and Stewart, P.S., Spatial Patterns of DNA Replication, Protein Synthesis, and Oxygen Concentration within Bacterial Biofilms Reveal Diverse Physiological States, *J. Bacteriol.*, 2007, vol. 189, pp. 4223–4233.
9. Krasovec, R. and Jerman, I., Bacterial Multicellularity as a Possible Source of Antibiotic Resistance, *Med. Hypotheses*, 2003, vol. 60, pp. 484–488.
10. Woo, P.C., To, A.P., Lau, S.K., and Yuen, K.Y., Facilitation of Horizontal Transfer of Antimicrobial Resistance by Transformation of Antibiotic-Induced Cell-Wall-Deficient Bacteria, *Med. Hypotheses*, 2003, vol. 61, pp. 503–508.
11. Burmølle, M., Webb, J.S., Rao, D., Hansen, L.H., Sørensen, S.J., and Kjelleberg, S., Enhanced Biofilm Formation and Increased Resistance to Antimicrobial Agents and Bacterial Invasion Are Caused by Synergistic Interactions in Multispecies Biofilms, *Appl. Environ. Microbiol.*, 2006, vol. 72, pp. 3916–3923.
12. Luppens, S.B., Kara, D., Bandounas, L., Jonker, M.J., Wittink, F.R., Bruning, O., Breit, T.M., Ten, Cate, J.M., and Crielaard, W., Effect of *Veillonella parvula* on the Antimicrobial Resistance and Gene Expression of *Streptococcus mutans* Grown in a Dual-Species Biofilm, *Oral Microbiol. Immunol.*, 2008, vol. 23, pp. 183–189.
13. Zhurina, M.V., Strelkova, E.A., Plakunov, V.K., and Belyaev, S.S., Effect of Reconstituted Biofilm Composition on Bacterial Hydrocarbon-Oxidizing Activity, *Mikrobiologiya*, 2008, vol. 77, no. 5, pp. 701–703 [*Microbiology* (Engl. Transl.), vol. 77, no. 5, pp. 625–627].
14. Strelkova, E.A., Zhurina, M.V., and Plakunov, V.K., Application of Reconstituted Biofilms for Accelerated Degradation of Petroleum Hydrocarbons, *Vestnik Biotekhnol. Fiz.-Khim. Biol. im. Yu.A. Ovchinnikova,* 2008, vol. 3, no. 4, pp. 28–30.
15. Plakunov, V.K., Zhurina, M.V., and Belyaev, S.S., Resistance of the Oil-Oxidizing Microorganism *Dietzia* sp. to Hyperosmotic Shock in Reconstituted Biofilms, *Mikrobiologiya*, 2008, vol. 77, no. 5, pp. 581–589 [*Microbiology* (Engl. Transl.), vol. 77, no. 5, pp. 515–522].
16. Bigger, J.W., Treatment of Staphylococcal Infections with Penicillin, *Lancet*, 1944, pp. 497–500.
17. McCune, R.M., Jr., and Tompsett, R., Fate of *Mycobacterium tuberculosis* in Mouse Tissues as Determined

by the Microbial Enumeration Technique. I. Persistence of Drug Susceptible Bacilli in the Tissues Despite Prolonged Antimicrobial Therapy, *J. Exp. Med.*, 1956, vol. 104, pp. 737–763.

18. McDermott, W., Microbial Persistence, *Yale J. Biol. Med.,* 1958, vol. 30, pp. 257–291.
19. Plakunov, V.K., Differential Sensitivity of Bacterial Protein Synthesis to Antibiotics, *Antibiotiki*, 1971, no. 6, p. 766.
20. Plakunov, V.K. and Kozyreva, L.F., Differences in vivo Antibiotic Sensitivity of Bacterial Protein Synthesis, *Mikrobiologiya*, 1971, vol. 40, no. 6, pp. 981–987.
21. Plakunov, V.K. and Kozyreva, L.F., "Residual Growth of *Staphylococcus aureus* in the Presence of "Bacteriostatic" Concentrations of Antibiotics Inhibiting Protein Synthesis, *Mikrobiologiya*, 1971, vol. 40, no. 2, pp. 311–316.
22. Aridesi, J.N., Zahller, E., Roe, F., and Stewart, P.S., Role of Nutrient Limitation and Stationary Phase Existence in *Klebsiella pneumonia* Biofilm Resistance to Ampicillin and Ciprofloxacin, *Antimicrob. Agents Chemother.*, 2003, vol. 47, pp. 1251–1256.
23. Levin, B.R. and Rozen, D.E., Noninherited Antibiotic Resistance, *Nat. Rev. Microbiol.,* 2006, vol. 4, pp. 556–562.
24. Hall-Stoodley, L., Costerton, J.W., and Stoodley, P., Bacterial Biofilms: from the Natural Environment to Infectious Diseases, *Nat. Rev. Microbiol.,* 2004, vol. 2, pp. 95–108.
25. Sanders, P.F and Sturman, P.J, Biofouling in the Oil Industry, in *Petroleum Microbiology*, Ollivier, B. and Magot, M., Eds., Washington, DC: ASM Press, 2005, pp. 171–198.
26. Lewis, K., Persister Cells, Dormancy and Infectious Disease, *Nat. Rev. Microbiol.,* 2007, vol. 5, pp. 48–56.
27. Lewis, K, Multidrug Tolerance of Biofilms and Persister Cells, in *Bacterial Biofilms. Current Topics in Microbiology and Immunology*, Romeo, T., Ed., Berlin: Springer, 2008.
28. Jayaraman, R., Bacterial Persistence: Some New Insights Into an Old Phenomenon, *J. Biosci.*, 2008, vol. 33, pp. 795–805.
29. Sharma, A.M. and Yadav, S., Biofilms: Microbes and Disease, *Brazilian J. Infectious Diseases*, 2008, vol. 12, pp. 526–530.
30. Black, D.S., Kelly, A.S., Madris, M., and Moyed, H.S., Structure and Organization of *hip*, an Operon That Affects Lethality Due to Inhibition of Peptidoglycan Synthesis, *J. Bacteriol.*, 1991, vol. 173, pp. 5732–5739.
31. Wolfson, J.S., Hooper, D.C., McHugh, G.L., Bozza, M.A., and Swartz, M.N., Mutants of *Escherichia coli* K12 Exhibiting Reduced Killing by Both Quinolone and Beta-Lactam Antimicrobial Agents, *Antimicrob. Agents Chemother.,* 1990, vol. 34, pp. 1938–1943.
32. Keren, I., Shah, D., Spoering, A., Kaldalu, N., and Lewis, K., Specialized Persister Cells and Mechanism of Multidrug Tolerance in *E. coli, J. Bacteriol.*, 2004, vol. 186, pp. 8172–8180.
33. Shah, D., Zhang, Z., Kodursky, A., Kaldalu, N., Kurg, K., and Lewis, K., Persisters: a Distinct Physiological State of *E. coli, BMC Microbiology,* 2006, vol. 6, pp. 53–57.
34. Balaban, N.Q., Merrin, J., Chait, R., Kowalik, L., and Leibler, S., Bacterial Persistence as a Phenotypic Switch, *Science*, 2004, vol. 305, pp. 1622–1625.
35. Spoering, A.L., Vulic, M., and Lewis, K., *GlpD* and *PlsB* Participate in Persister Cell Formation in *Escherichia coli, J. Bacteriol.*, 2006, vol. 188, pp. 3494–3497.
36. Li, Y. and Zhang, Y., *Pho U* Is a Persister Switch Involved in Persister Formation and Tolerance to Multiple Antibiotics and Stresses in *Escherichia coli, Antimicrob. Agents Chemother.*, 2007, vol. 51, pp. 2092–2099.
37. Hayes, F., Toxins-Antitoxins: Plasmid Maintenance, Programmed Cell Death and Cell Cycle Arrest, *Science*, 2003, vol. 301, pp. 1496–1499.
38. Gerdes, K., Christensen, S.K., and Lobner-Olessen, A., Prokaryotic Toxin-Antitoxin Stress Response Loci, *Nature Rev. Microbiol.,* 2005, vol. 3, pp. 371–382.
39. Pedersen, K., Christensen, S.K., and Gerdes, K., Rapid Induction and Reversal of a Bacteriostatic Condition Controlled by the Expression of Toxins and Antitoxins, *Mol. Microbiol.*, 2002, vol. 45, pp. 501–510.
40. Van Melderen, L. and De Bast, M.S., Bacterial Toxin-Antitoxin Systems: More Then Selfish Entities?, *PLoS Genetics*, 2009, vol. 5, no. 3, e1000437 (www.plosgenetics.org).
41. Fozo, E.M., Hemm, M.R., and Storz, G., Small Toxic Proteins and the Antisense RNAs That Repress Them, *Microbiol. Mol. Biol. Rev.,* 2008, vol. 72, pp. 579–589.
42. Vazquez-Laslop, N., Lee, H., and Neyfakh, A.A., Increased Persistence in *Escherichia coli* Caused by Controlled Expression of Toxins or Other Unrelated Proteins, *J. Bacteriol.*, 2006, vol. 188, pp. 3494–3497.
43. Korch, S.B., Henderson, T., and Hill, T.M., Characterization of the hipA7 Allele of *Escherichia coli* and Evidence That High Persistence Is Governed by (p) ppGpp Synthesis, *Mol. Microbiol.*, 2003, vol. 50, pp. 1199–1213.
44. Korch, S.B. and Hill, T.M., Ectopic Over Expression of Wild Type and Mutant *hipA* Genes in *Escherichia coli.* Effects of Macromolecular Synthesis and Persister Formation, *J. Bacteriol.*, 2006, vol. 188, pp. 3826–3836.
45. Hansen, S., Lewis, K., and Vulić, M., The Role of Global Regulators and Nucleotide Metabolism in Antibiotic Tolerance in *E. coli, Antimicrob. Agents Chemother.*, 2008, vol. 52, pp. 2718–2726.
46. Lee, S.B., Koepsel, R.R., Morley, S.W., Matyjaszewski, K., Sun, Y., and Russell, A.J., Permanent, Nonleaching Antibacterial Surfaces. 1. Synthesis by Atom Transfer Radical Polymerization, *Biomacromolecules*, 2004, vol. 5, pp. 877–882.
47. Milovic, N.M., Wang, J., Lewis, K., and Klibanov, A.M., Immobilized N-Alkylated Polyethylenimine Avidly Kills Bacteria by Rupturing Cell Membranes with No Resistance Developed, *Biotechnol. Bioeng.*, 2005, vol. 90, pp. 715–722.
48. Nikolaev, Yu.A., Prosser, Dzh.I., and Vittli, R.I., Regulation of the Adhesion of *Pseudomonas fluorescens* Cells to Glass by Extracellular Volatile Compounds, *Mikrobiologiya*, 2000, vol. 69, no. 3, pp. 352–355 [*Microbiology* (Engl. Transl.), vol. 69, no. 3, pp. 287–290].

49. Nikolaev, Yu.A., Panikov, N.S., Lukin, S.M., and Osipov, G.A., Saturated C_{21}–C_{33} Hydrocarbons Are Involved in the Self-Regulation of *Pseudomonas fluorescens* Adhesion to a Glass Surface, *Mikrobiologiya*, 2001, vol. 70, no. 2, pp. 174–181 [*Microbiology* (Engl. Transl.), vol. 70, no. 2, pp. 138–144].

50. Kaplan, J.B., Ragunath, C., Ramasubbu, N., and Fine, D.H., Detachment of *Actinobacillus actinomycetemcomitans* Biofilm Cells by an Endogenous Beta-Hexosamidase Activity, *J. Bacteriol.*, 2003, vol. 185, pp. 4693–4698.

51. Mireles, J.R., Toguchi, A., and Harshey, R.M., *Salmonella enterica* Serovar *typhimurium* Swarming Mutants with Altered Biofilm-Forming Abilities: Surfactin Inhibits Biofilm Formation, *J. Bacteriol.*, 2001, vol. 183, pp. 5848–5854.

52. Batrakov, S.G., Rodionova, T.A., Esipov, S.E., Polyakov, N.B., Sheichenko, V.I., Shekhovtsova, N.V., Lukin, S.M., Panikov, N.S., and Nikolaev, Yu.A., A Novel Lipopeptide, an Inhibitor of Bacterial Adhesion, from the Thermophilic and Halotolerant Subsurface *Bacillus licheniformis* Strain 603, *Biochim. Biophys. Acta*, 2003, vol. 1634, pp. 107–115.

53. Burshard, R.P. and Sorongon, M.L., A Gliding Bacterium Strain Inhibits Adhesion and Motility of Another Gliding Bacterium Strain in Marine Biofilm, *Appl. Environm. Microbiol.*, 1998, vol. 64, pp. 4079–4083.

54. Taylor, E.N. and Webster, T.J., The Use of Superparamagnetic Nanoparticles for Prosthetic Biofilm Prevention, *Int. J. Nanomedicine*, 2009, vol. 4, pp. 145–152.

55. Khmel', I.A., Quorum-Sensing Regulation of Gene Expression: Fundamental and Applied Aspects and the Role in Bacterial Communication, *Mikrobiologiya*, 2006, vol. 75, no. 4, pp. 457–464 [*Microbiology* (Engl. Transl.), vol. 75, no. 4, pp. 390–397].

56. Ni, N., Li, M., Wang, J., and Wang, B., Inhibitors and Antagonists of Bacterial Quorum Sensing, *Medicinal Res. Rev.*, 2009, vol. 29, pp. 65–124.

57. Ravn, L., Christensen, A.B., Molin, S., Givskov, M., and Gram, L., Methods for Detecting Acylated Homoserine Lactones Produced by Gram-Negative Bacteria and Their Application in Studies of AHL-Production Kinetics, *J. Microbiol. Methods*, 2001, vol. 44, pp. 239–251.

58. Pesci, E.C., Milbank, J.B., Pearson, J.P., McKnight, S., Kende, A.S., Greenberg, E.P., and Iglewski, B.H., Quinolone Signaling in the Cell-to-Cell Communication System of *Pseudomonas aeruginosa, Proc. Natl. Acad. Sci. USA,* 1999, vol. 96, pp. 11229–11234.

59. Higgins, D.A., Pomianek, M.E., Kraml, C.M., Taylor, R.K., Semmelhack, M.F., and Bassler, B.L., The Major *Vibrio cholerae* Autoinducer and Its Role in Virulence Factor Production, *Nature*, 2007, vol. 450, pp. 883–886.

60. Winzer, K., Hardie, K.R., and Williams, P., LuxS and Autoinducer-2: Their Contribution to Quorum Sensing and Metabolism in Bacteria, *Adv. Appl. Microbiol.*, 2003, vol. 53, pp. 291–396.

61. Chen, X., Schauder, S., Potier, N., Van Dorsselaer, A., Pelczer, I., Bassler, B.L., and Hughson, F.M., Structural Identification of a Bacterial Quorum-Sensing Signal Containing Boron, *Nature*, 2002, vol. 415, pp. 545–549.

62. Ni, N., Choudhary, G., Peng, H., Li, M., Chou, H.T., Lu, C.D., Gilbert, E.S., and Wang, B., Inhibition of Quorum Sensing in *Vibrio harveyi* by Boronic Acids, *Chem. Biol. Drug Des*, 2009, vol. 74, pp. 51–56.

63. McDowell, P., Affas, Z., Reynolds, C., Holden, M.T.G., Wood, S.J., Saint, S., Cockayne, A., Hill, P.J., Dodd, C.E.R., Bycroft, B.W., Chan, W.C., and Williams, P., Structure, Activity and Evolution of the Group I Thiolactone Peptide Quorum-Sensing System of *Staphylococcus aureus, Mol. Microbiol.*, 2001, vol. 41, pp. 503–512.

64. Wood, N.K., Insights on *Escherichia coli* Biofilm Formation and Inhibition from Whole-Transcriptome Profiling, *Environ. Microbiol.,* 2009, vol. 11, pp. 1–15.

65. Hall, B.G., Adaptive Mutagenesis: a Process That Generates Almost Exclusively Beneficial Mutations, *Genetics*, 1998, vol. 102/103, pp. 109–125.

66. Velkov, V.V., New Insights into the Molecular Mechanisms of Evolution: Stress Increases Genetic Diversity, *Mol. Biol.*, 2002, vol. 36, no. 2, pp. 277–285 [*Molecular Biology* (Engl. Transl.), vol. 36, no. 2, pp. 209–215].

67. Sung, H.-M. and Yasbin, R.E., Adaptive, or Stationary-Phase, Mutagenesis, a Component of Bacterial Differentiation in *Bacillus subtilis, J. Bacteriol.*, 2002, vol. 184, pp. 5641–5653.

68. Dong, T., Joyce, C., and Schellhorn, H.E., The Role of RpoS in Bacterial Adaptation, in *Bacterial Physiology: A Molecular Approach*, El-Sharoud, W., Ed., Berlin: Springer, 2008.

69. Dutova, T.A., Mordkovich, N.N., and Tsygankov, Yu.D., Using Adaptive Mutagenesis System for Identification of Early Genes Encoding de novo Purine Biosynthesis in Methylotrophic Yeast *Pichia methanolica* MH4, *Genetika*, 2009, vol. 45, no. 10, pp. 1361–1368 *Russ. J. Genetics* (Engl. Transl.), vol. 45, no. 10, pp. 1200–1207].

70. Eishenbaum Z., Livneh Z. UV Light Induces *IS10* Transposition in *Escherishia coli, Genetis*, 1998, vol. 149, pp. 1173–1181.

71. Layton, J.C. and Foster, P.L., Error-Prone DNA Polymerase IV Is Controlled by the Stress-Response Sigma Factor, RpoS, in *Escherichia coli, Mol. Microbiol.*, 2003, vol. 50, pp. 549–561.

72. Schembri, M.A., Kjaergaard, K., and Klemm, P., Global Gene Expression in *Escherichia coli* Biofilms, *Mol. Microbiol.*, 2003, vol. 48, pp. 253–267.

73. Weber, H., Pesavento, C., Possling, A., Tischendorf, G., and Hengge, R., Cyclic-di-GMP-Mediated Signalling within the Sigma Network of *Escherichia coli, Mol. Microbiol.*, 2006, vol. 62, pp. 1014–1034.

74. Velkov, V.V., Stress-Induced Evolution and the Biosafety of Genetically Modified Microorganisms Released into the Environment, *J. Biosci.*, 2001, vol. 26, pp. 667–683.

75. Chow, K.C. and Tung, W.L., Magnetic Field Exposure Stimulates Transposition through the Induction of *DnaK/J* Synthesis, *Biochem. Biophys. Res. Commun.*, 2000, vol. 270, pp. 745–748.

76. Stoodley, P., Sauer, K., Davies, D.G., and Costerton, J.W., Biofilms as Complex Differentiated Communities, *Annu. Rev. Microbiol.*, 2002, vol. 56, pp. 187–209.

77. Van Elsas, J.D. and Bailey, M.J., The Ecology of Transfer of Mobile Genetic Elements, *FEMS Microbiol. Ecol.*, 2002, vol. 42, pp. 183–197.

78. Jefferson, K.K., What Drives Bacteria to Produce a Biofilm?, *FEMS Microbiol. Letts.*, 2004, vol. 236, pp. 163–173.

79. Banas, J.A., Miller, J.D., and Fuschino, M.E., Evidence That Accumulation of Mutants in a Biofilm Reflects Natural Selection Rather Than Stress-Induced Adaptive Mutation, *Appl. Environ. Microbiol.*, 2007, vol. 73, pp. 357–361.

80. Driffield, K., Miller, K., Bostock, J.M., O'Neill, A.J., and Chopra, I., Increased Mutability of *Pseudomonas aeruginosa* in Biofilms, *J. Antimicrob. Chemother.*, 2008, vol. 61, pp. 1053–1056.

81. Conibear, T.C.R., Collins, S.L., and Webb, J.S., Role of Mutation in *Pseudomonas aeruginosa* Biofilm Development, *PLoS ONE*, 2009, vol. 4, no. 7, e6289 (www.plosone.org).

82. Coleman, C., Baker, C., and Nickerson, A., The Role of Sigma Factors in Regulation Bacterial Stress Responses and Pathogenesis, in *Molecular Paradigms of Infectious Disease. A Bacterial Perspective*, Nickerson, C.A. and Schurr, M., Eds., Springer Science+Business Media, 2006, pp. 438–482.

83. Burtnick, M.N., Downey, J.S., Brett, P.J., Boylan, J.A., Frye, J.G., Hoover, T.R., and Gherardini, F.C., Insights into the Complex Regulation of *rpoS* in *Borrelia burgdorferi, Mol. Microbiol.*, 2007, vol. 65, pp. 277–293.

84. Hengge, R, The Two-Component Network and the General Stress Sigma Factor RpoS (σ^s) in *Escherichia coli*, in *Bacterial Signal Transduction: Networks and Drug Targets*, Utsumi, R., Ed., Landes Bioscience and Springer Science+Business Media, 2008, pp. 40–53.