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REVIEWS

Heterogeneity as an Adaptive Trait of Microbial Populations

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Abstract—The review deals with heterogeneity of bacterial populations, a superorganismic characteristic providing for their adaptation to environmental conditions at the population–communication level. This phenomenon attracts increasing attention as an example of collective forms of microbial behavior and the mechanisms of cell survival in communities. Heterogeneity of bacterial populations may be discrete or continuous and may result from both phenotypic and genotypic variations. Heterogeneity of microbial cells results from the interaction of internal and environmental factors, as well as from random fluctuations of the biochemical and physiological characteristics. Cell heterogeneity improves the survival of bacterial populations under heterogeneous or variable environmental conditions, as well as under the effect of stress factors. This phenomenon should be taken into account for the development of strategies for cultivation of the biotechnologically important microorganisms and for the rational therapy of infections.

Keywords: bacterial heterogeneity, variability, population level of organization, nonspecific adaptations

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ONCEPT OF BACTERIAL HETEROGENEITY

Phenotypic heterogeneity is a universal characteristic of living organisms. It implies that the individuals differing in a number of heritable and nonheritable characteristics are present simultaneously within a population. Heterogeneity is not identical to polymorphism, which implies the presence of two or several stable genetic variations which are in stable equilibrium within a population. In the case of heterogeneity, the differences in the characteristics of individual organisms results from the realization of the adaptive possibilities already existing in the genome [1].

Heterogeneity of bacterial populations attracts much attention in the context of investigation of the mechanisms of adaptation to environmental stress factors [2]. This is associated with the specific features of bacterial functioning and development as clonal populations of unicellular organisms with simple cells, small haploid genomes, short life cycle, and a set of specialized systems for the regulation of social behavior. In this case, the variety of genotypes and phenotypes, apart from acting as a material for selection, becomes a means to protect the population from damaging impacts, thus facilitating its survival. Prokaryotes are able to change the level of heterogeneity in their populations, adjusting it according to the environmental conditions. This is manifested, for example, in the hysteresis, when the conditions of preliminary incubation of a microbial population affect its response to external factors [3, 4].

The term "bacterial polymorphism" is usually applied to genetic diversity of the strains within a species, rather than of the cells and isolates within a population [5]. However, the term "polymorphism" is sometimes incorrectly used to describe certain manifestations of bacterial heterogeneity [6]. This results from the problematic status of the borders of bacterial population and from the fact that the term "culture polymorphism" (implying coexistence of morphologically different cells within a population) developed independently of the term "polymorphism" in the theory of evolution of macroorganisms. As a result, a certain convergence of terms exists today. For example, Krasil'nikov [7] in his mid-20 century works treated culture polymorphism as the individual differences between the cells of a given culture, expressed to a different degree depending on its age, species, growth conditions, and the presence or absence of stresses. He stressed that culture polymorphism was not to be identified with species polymorphism, i.e., the presence of phenotypically or genotypically different strains within a microbial species.

Discrimination between these concepts is required to limit the range of issues discussed, to avoid confusion of terms, and to focus the reader's attention on the fact that heterogeneity of a population is a result of realization of the adaptive potential initially inherent to a specific microorganism. Thus, it is an instrument for manifestation of adaptive capacities within a microbial genome.

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INVESTIGATION OF HETEROGENEITY OF BACTERIAL POPULATIONS IN RUSSIA AND WORLDWIDE

The interest of the microbiological research community to the superorganism level of bacterial organization increased in the 1990s due to a number of reasons. Development of the technological base resulted in the possibility of analysis of individual cells using flow cytometers [8]. Moreover, the universal nature of the chemical communication signals was discovered and the concept of quorum sensing was introduced [9]. Finally, the data on the architectonics of bacteria colonies were accumulated and the concept of a biofilm was developed [10, 11].

In Russia, however, investigation of the superorganisms (population–communicative) [12] level of microbial organization have been carried out since mid-20th century. During this period of intense interest in flow cultures, the patterns of development of bacterial cultures were revealed and described, and many important features of the phenotypic and genotypic variability, heterogeneity, and microbial adaptive evolution were noted [12]. The concepts concerning the functioning of bacterial populations were developed using the holistic system approach, which originated from the continued tradition developed of Vernadskii, Timofeev-Resovskii, Winogradsky, and other authors. Unfortunately, these notions did not receive due attention. The major statements of the concept of a bacterial culture as a system similar to a multicellular organism, which have been formulated by Ierusalimskii in the late 1940s–early 1950s, were then rediscovered by Shapiro, Sutherland, and other authors in the 1980s–1990s [12].

The worldwide interest in population heterogeneity emerged in the late 1990s–early 2000s. Traditionally, the cells in a certain growth phase in batch cultures (e.g., in the exponential or stationary growth phase) were considered equivalent [8, 13]. Heterogeneity of cellular characteristics, which was reported in various fields of research, was considered within the framework of the regulation of metabolism and the effect of environmental conditions and random processes. The role of heterogeneity as a universal system feature of every bacterial population was not emphasized. The concept of a homogeneous population results mainly from the experimental procedures, which used the population-based methods to investigate bacterial physiology. These studies yielded immense amounts of data and made it possible to determine the patterns of cell behavior in response to various stimuli or environmental changes [8]. The recently developed methods for analysis of single cells revealed, however, that heterogeneity of bacterial populations in different growth phases should not be ignored [14, 15]. Data are accumulating concerning the role of cellular subtypes in bacterial communities. Four major fields of investigation may be specified: analysis of the phenotypic variability of individual cells in a culture by flow cytometry [8, 13]; investigation of phase variations of bacteria and of different colony morphotypes within a population [16, 17]; description, investigation, and simulation of stochastic heterogeneity [18], which is responsible for the choice of a metabolic pathway in the artificial [19] and natural bistable systems (persister cell formation, functioning of the *lac* operon, etc.) [2, 20]; and investigation of the cells in colonies and biofilms [10, 11]. Moreover, the microheterogeneity of the 16S rRNA genes in bacterial populations came into account recently [21, 22].

In Russia, heterogeneity of microbial populations was studied by several groups of researchers. Investigation of bacterial heteromorphism, i.e., the presence of different cell types within a population (heterocysts, L-form bacteria, mini bacteria, giant cells, dormant forms, etc.) was the first area to develop (1930s–1940s). Subsequently, Ierusalimskii formulated his thesis on the heterogeneity of bacterial populations [12]. Research on the phenotypic [23] and genotypic [24] heterogeneity of the cells in pure continuous cultures developed in the 1960s–1980s during the boom of chemostat cultivation. Since 1960s, Russian microbiologists Rabotnova, Mil'ko, and Vysotskii have studied the dissociation of bacteria. This phenomenon implies the emergence of phase variability in a population, with significant, usually reversible changes in the biochemical and morphological characteristics of the cells and colonies [25]. Such works have recently been associated with investigation of bacterial dormant forms [26]. Works on stochastic heterogeneity, individual differences between the cells of planktonic cultures, colony architectonics, and biofilm structure were minor in Soviet and Russian science.

Although some of these works were the pioneering ones, they were usually a by-product of applied research. The concepts were not formed into a system in order to stress the role of investigation of the organization of bacterial populations.

FORMS OF BACTERIAL HETEROGENEITY

All variants of heterogeneity in bacterial populations fall into two groups: phenotypic and genotypic heterogeneity [2]. The first one manifests itself at the level of gene expression and does not result from mutational or recombinational events, while genotypic heterogeneity results from the changed DNA sequences.

Heterogeneity may be discrete and continuous, depending on the character of manifestation [27]. In the first case, the population consists of two or several homogeneous, phenotypically different groups (subpopulations); in the second case, microorganisms exist in a variety of forms, so that isolation of stable groups with reliably differing characteristics becomes impossible.

Discrete heterogeneity is observed in bacterial populations containing a number of alternative variants, usually genomically programmed ones. While these programmed variants are continuously reproduced under certain conditions, they are not stable in the generation lineage, and may result from phenotypic (sporulating and lysing cells [15]) or genotypic variations (phase variations [16]). Discrete heterogeneity may be observed in the case of formation of colonies with different morphotypes [17] or when mutants emerge with pronounced selective advantages [28]. Saturation of the population with such mutants results in formation of a discrete subpopulation of stress-resistant cell, which coexists with the ancestral form until the complete replacement of the latter.

Continuous heterogeneity of bacterial populations may develop due to variations of the physiological state [29] or due to emergence of various adaptive and nonadaptive mutations [24, 30]. These variants are usually less specialized and are often less reproducible under laboratory conditions than the "discrete" variants. Asymmetric division [31] or development of the morphological of mutational variations providing and insignificant decrease in antibiotic sensitivity [32] may be examples of such heterogeneity.

Phenotypic Heterogeneity of Bacteria

Phenotypic heterogeneity of bacteria is heterogeneity of the cells in a population resulting from nonheritable variations, either random or age-dependent.

Every population contains the organisms differing in their biochemical composition, which varies throughout the cell cycle. Since bacterial resistance to stresses differs depending on the life cycle phase, this diversity increases the resistance of a population as a whole to environmental factors [23]. *Bacillus subtilis* cells were shown to exhibit the lowest catalase activity and thus the highest sensitivity to elevated oxygen levels at the beginning of the cell cycle [33]. Resistance of bacteria bearing plasmids with resistance genes is higher at the phase directly preceding division, when the number of plasmid copies in the cell is higher. Unlike eukaryotic organisms, a new cycle of DNA replication may commence in bacteria before the termination of the previous one. As a result, the genes closest to the initiation site are present in more copies at the later stages of the cell cycle; this may affect the physiology of the cells [13].

Analysis of microbial processes is usually carried out with nonsynchronized populations. The results are therefore averaged along the time interval corresponding to the time of cell division [13]. Techniques of fractionation and synchronization are used to study the activity of microorganisms of a specific age. Fractionation techniques are based on the morphological, physiological, and biochemical differences between the cells. They are usually labor-intensive and have low specificity of isolation. Synchronization techniques are based on selection of the cells belonging to one of the stages of the cycle. Synchronization of cell division is usually successful for two to four generations, which are followed by transition of the cell suspension to asynchronous division [23].

The biochemical composition and physiology of microorganisms may vary not only in the course of cell growth and division, but also as a result of selective activation of specific metabolic processes. The mechanisms of the so-called bistability in bacteria may be an example of such behavior. Bistability is a general feature of genetic networks with negative feedback regulation [19]. For example, lysis caused by prophage activation in *E. coli* cells is launched by the amount and ratios of the repressor cI and regulator Cro, at the moment of the external stimulus; after initiation, it continues to completion [34]. Cell death caused by the activation of a lytic phage plays an important part in formation and development of *Pseudomonas aeruginosa* biofilms [35]. A well-known phenomenon of bistability results in the production of dormant forms [36, 37], such as spores [1, 15] and persister cells [38]. Similar processes result in differentiation of the cells of *B. subtilis, Streptococcus pneumoniae*, and other microorganisms into the lysing and competent ones (ComK being the responsible regulator in *B. subtilis*) [2, 38], emergence of the altruistic, colicin-producing *E. coli* cells (*cka* being the gene regulating production of colicin K) [2, 39], and other variants of discrete phenotypic heterogeneity.

Bistability is the least stable variant of epigenetic variability resulting in development of bacterial heterogeneity. Another known mechanism responsible for cell differentiation in a population is based on DNA methylation. Unlike bistability, it is inherited by the daughter cells and has the frequency of "switching" from 10^{-1} to 10^{-5} per generation [4]. The *pap* operon of *E. coil* (pili associated with pyelonephritis) regulated by DNA methylation is an example of such switches [40].

Genotypic Heterogeneity of Bacteria

Genotypic heterogeneity is formed in bacteria as a result of point mutations or chromosomal rearrangements. Such genetic changes affect the structure or expression level of various proteins and, thus, the emergence of two or more variants within a population.

Discrete genotypic heterogeneity, which is characterized by a limited number of genetically different variants, develops because the frequency of mutations is different in different regions of the genome. For example, early investigation of the repressor of the *E. coli lac* operon revealed significant variation in the frequency of spontaneous mutations in different sites of the gene [41], while investigation of mutations in the "silent" regions of the human genome revealed the "hot" and "cold" spots of mutagenesis hundreds of

thousands base pairs in size [42]. As early as 1988, Cairns et al. [43] observed directedness of mutagenesis with preferential acquisition of the mutations which favor adaptation to the current stress. As for the frequency of mutations resulting in a given phenotype, the target size should be taken into consideration, which depends on the number of proteins required for manifestation of this phenotype, as well as on the structure of the relevant genes. The more genes are involved in realization of a certain process or metabolic pathway, the more probable an impairment of this function is [4].

Concentration of mutations at specific regions of the genome is associated with the character of the DNA matrix and the rate of transcription at this site. The frequencies of point mutations and recombination events were shown to be nonrandomly distributed along the bacterial chromosome. "Hot spots" exist for mutations [44] and recombinations [45]. The first ones are characterized by short tandem repeats, which are responsible for frameshift mutations due to slip-strand mispairing [45, 46]. The second ones are direct or inverted repeats, which facilitate intrachromosomal rearrangements [16], or the sites for specific integration of mobile genetic elements [47]. Mutagenesis and recombination associated with transcription, resulting in the most intense modifications in the actively expressed genes is another phenomenon resulting in directional mutagenesis [48].

Partially directional mutagenesis and recombination, which generate genetic heterogeneity in a bacterial population, seemingly agree with Lamarck's concepts. However, the DNA sequences and the relevant mechanisms regulating the rates of genetic modification were formed in the course of evolution, i.e., they were a subject of natural selection and therefore do not contradict with the original Darwin's notions [4].

The so-called phase variation, a high-frequency mechanism for reversible switching between alternative phenotypes, is an example of discrete genetic heterogeneity. The frequency of phase variations is, as a rule, 10^{-3}–10^{-5}, although sometimes it is as high as 10^{-1} per generation. This mechanism was described for a number of microorganisms, such as *Salmonella typhimurium, Neisseria gonorrhoea, N. meningitidis, Haemophilus influenzae*, and *E. coli* [16]. Phase variations were found to occur by a variety of mechanisms [16], many of which are variations of genetic rearrangements: DNA inversions [49], duplications [50], transpositions [47], homologous recombinations [51, 52], or incorrect coupling due to slip-strand mispairing [53]. Recombination reversing the gene to its initial form results in complete restoration of the original phenotype. Inactivation of the *recA* gene decreases the frequency of phase variations by two to three orders of magnitude [52].

Phase variations were termed phenotypic oscillations or phenotypic switches [53]. Their most characteristic feature is their frequency, which is significantly higher than that of regular point mutations [4].

The above-described mechanisms of epigenetic switching between phenotypes and bacterial bistability are also classified as phase variations. We maintain that clear terminological differentiation is required, since the trigger mechanisms for bistability are fundamentally different from the switches functioning via DNA modification or rearrangement. We are therefore not merging these phenomena into a single group of the mechanisms of phase variation.

Continuous genotypic heterogeneity develops as a result of nondirectional spontaneous mutations. The notion of a microbial population as a community of genetically identical cells is incorrect. Even pure microbial cultures maintained by transfer to fresh media always contain a number of mutant forms, i.e., they are genetically heterogeneous (if the population exceeds 10^6 cells) [24]. Mutations in bacterial cells usually impair the functioning of the cell as a whole. Every model of populations always contains a certain level of mutant subpopulations, which are more or less active and viable. However, changing ambient conditions may favor these forms, making it possible for the population to survive after rearranging its structure [24].

In many cases it is difficult to determine whether the mechanism generating the heterogeneity of the population is a genotypic or a phenotypic one. Phenotypic variations emerging in a population may be inherited by the subsequent generations but are leveled out after several transfers. For example, germination of the dormant forms results in dissociation with formation of a number of colony morphotypes. In the course of subsequent cultivation, most of these reverse to the wild type morphotype [25]. Similar phenomena are associated with easily reversible chromosome rearrangements [17, 55]. However, judging by stability of the morphological variants, only a fraction of them represents the phenotypic phase variations as such, caused by DNA rearrangements. Most of the phenotypic variations disappear during the subsequent transfers.

Variations with intermediate stability may affect not only the visible characteristics of bacteria, but also other physiological features, such as activity of biofilm formation and mutation frequency. For example, our work on the heterogeneity of *P. aeruginosa* populations existing for a long time within a system of swimming pools [56] demonstrated that bacterial subpopulations with a mutator phenotype and decreased activity of biofilm formation, which were persistently present in the biotope, were characterized by easily reversible chromosome malfunctions affecting the genes of the MMR system [57].

SOURCES OF BACTERIAL HETEROGENEITY

Spatial Discontinuity

Spatial discontinuity of the environment is among the major sources of heterogeneity of the cells in a population. This discontinuity may be either an independent external factor or a result of development of bacterial populations. The latter is of special interest, since, according to modern notions, the majority of bacteria exist mostly as biofilms (structured communities of microbial cells).

Phenotypic heterogeneity of bacteria was shown to increase in the course of biofilm and colony formation. In this case, the level of differentiation of bacteria is much higher than in planktonic cultures [10, 58]. Developing three-dimensional structures create gradients of temperature and pH, as well as of the concentrations of minerals and nutrients, oxygen, metabolites, and quorum sensing signals [59]. Local concentrations of these components in every point of space create specific conditions, to which a bacterial cell reacts by changing its physiological state [60]. Complex differentiation develops in mature biofilms and colonies, so that the subpopulations of planktonic, sedentary, lysing, swarming [61], etc. cells develop. Each of these variants, due to peculiarities of its biochemical state, is characterized by a specific spectrum of sensitivities to various environmental factors [49, 62].

The effect of the shape of biofilms and colonies on the genotypic diversity results from localization of the cell clones without mixing, unlike planktonic cultures. Under such conditions, the so-called clonal interference occurs, i.e., an increase in the number of competing variants [63], which, in turn, decreases the efficiency of selection due to the Hill–Robertson effect. This effect implies that selection at one site decreases the efficiency of selection in another one [64]. Conditions are therefore created for the presence of numerous mutant variants within a population, which are not eliminated by natural selection even when they are less competitive than other variants [65]. Thus, due to the structured nature of the environment, isolation, which has been known as a highly important factor in speciation [1], acts even in a microscopic scale [66].

The ecological mechanisms maintaining diversity in a spatially discontinuous environment are incompletely understood. Competition for oxygen, for example, may be an important factor [67]. The phase variations characteristic of a given genotype and highly adapted to specific ecological niches within a biofilm may act as the "source material" for adaptive differentiation [68]. Formation of various colony morphotypes by *P. fluorescens, Serratia marcescens*, and other organisms, which does not occur in planktonic cultures of these bacteria, is a good example of this model [69, 70].

Stress Factors

Effect of stress factors on the phenotypic heterogeneity. Every stress factor affects the functioning of bacterial cells. Switching from favorable conditions to less favorable ones results in unbalanced growth of the population, as changed physicochemical conditions affect the rates of the reactions of biochemical synthesis and, therefore, the ratio of macromolecular components within the cells [71]. Moreover, a complex system containing numerous sensor components of the genetic regulatory networks reacts to environmental signals by activating specific mechanisms of physiological adaptation [72]. These processes result in a situation when numerous cells within a population have to choose their survival strategies. Variety of the solutions in this situation results in the biochemical (and therefore physiological) heterogeneity of the population, which plays a part in improved survival of the stationary-phase cells [73].

Effect of stress factors on the genotypic heterogeneity. Transient (or stress-induced) hypermutagenesis is an important factor in genetic heterogeneity of the cells in the stationary phase and in biofilms [74, 75]. Transient hypermutagenesis is activated in a group of cells within the population experiencing prolonged action of a stress factor. It is caused by a complex of mechanisms and phenomena and involves cross-coupling of several regulons, such as SOS, σ^s and, to some degree, Hfq (the RNA-binding protein) [76, 77]. Several mechanisms of the regulatory increase in mutagenesis intensity, depending on the methods of initiation, cell state at the time of activation of temporary mutagenesis, and the genes involved, are known: SOS mutagenesis [78], adaptive mutagenesis [79], ROSE mutagenesis (dormant cells in a structured environment) [80], MAC mutagenesis (mutagenesis an ageing colonies) [74], etc.

The following are the universal components of every transient mutagenesis:

(1) Suppressed or stopped cell division, so that all accumulated DNA damage should be repaired within a cell, rather than in a row of generations [79].

(2) Suppressed activity of the DNA reparation mechanisms due to decreased rate of transcription (regulatory action of RpoS on MMR reparation systems) [68] and translation (destabilization on the mRNA genes *mutS* and *mutH* by the Hfq protein) [77], and due to titration of the cellular reparation proteins by accumulating DNA damage [81].

(3) Activation of the DNA synthesis mechanisms tolerant to damage (translesion synthesis). In bacterial cells, this function is realized by increased activities of the enzymes PolII, PolIV, and PolV [82, 83]. The two latter proteins belong to the family of Y polymerases termed error-prone polymerases due to the weakened control over the accuracy of the matrix synthesis. Enhanced expression of the relevant genes (*dinB, umuDC*) results in less correct replication and repara-

tive DNA synthesis and therefore to accumulation of mutations [83].

Temporary hypermutagenesis may also result from increased frequency of transpositions occurring at the SOS response activation, decreased activity of the MMR system, and in the case of stress-induced synthesis of heat shock proteins [84].

Involvement of recombination-dependent mechanisms, such as DNA amplification, was also reported for adaptive mutagenesis. In the course of amplification, specific chromosomal regions are duplicated many times, creating a number of copies subjected to mutational changes [85, 86]. This process is RecA-dependent and is probably an alternative to hypermutagenesis caused by Y polymerases [87].

Due to the processes described above, the population enters the stage of temporary mutagenesis, when the number of mutations increases several times, thus increasing the probability of a desired genomic change. Induction of this process in the cells of various microorganisms may result from the action of some antibiotics (fluoroquinolones and/or β-lactams), significantly enhancing the adaptive potential of bacteria [88, 89]. This process is especially important in the case of therapy of infections caused by antibiotic-resistant biofilms.

Thus, bacterial growth during the stationary phase or in a biofilm results in increased genotypic heterogeneity caused by increased rates of mutations.

Stochastic Processes

Stochastic processes play a highly important role in development of heterogeneity in bacterial populations [90]. They may involve regulatory molecules interacting with DNA or act at the level of general physiological processes, such as cell growth or mutagenesis.

Stochasticity in cell processes develops as a result of temperature fluctuations, a low number of the regulatory molecules, or formation of macromolecular aggregates. All these factors may affect the rates of chemical reactions and the local concentrations of the reagents. This, in turn, affects the availability of specific genes for transcription (e.g., by random rearrangements of the chromatin) and the process of gene expression as a whole. In many experiments isogenic populations, which existed originally under the same conditions, produced the cells of different molecular composition. Cell clusters differing in their phenotypes and biological functions were formed [72, 90].

Bacterial cell cycle, for example, is a regular process, but random fluctuations in its duration provide for the constant presence of the cells of all growth phases within a population [23]. Probabilistic distribution of the molecules of regulatory proteins among the cells in the population, with expression suppressed under favorable growth conditions, results in variations of the predetermined states of bacteria, which cause the activation of the programs of individual cell behavior (within the framework of populational adaptation) in response to environmental signals [72]. Numerous instances of bistability in bacteria are among the examples of such behavior [19]. Genotypic heterogeneity is somewhat similar: the relevant mutational and recombinational events in bacterial cells are random [54], although the probability of their manifestation in a specific region of the chromosome and under specific conditions may vary. This heterogeneity may be advantageous under the periodically or stochastically varying conditions, if the rate of the population's response to these variations is low [72]. Results of mathematical simulations confirmed that the growth rate was higher for a heterogeneous population than for a homogeneous culture [91].

Stochastic heterogeneity is under both theoretical (using simulation) and practical investigation. The first mathematical models of heterogeneity in the populations resulting from the variable duration of the cell cycle were developed as early as the 1930s. The list of such models is presently much longer. The group of the so-called cell population balance models has been under development since the late 1960s. However, the models of this group are extremely complex, and the search for more convenient approaches resulted in emergence (in the 1970s) of the models using the Monte Carlo algorithms, which gained popularity in the 1990s–2000s. This approach involves the chemical master equations (CME), stochastic differential equations (SDE), stochastic variable number Monte Carlo model (SVNMC), and other mathematical models. While a fundamentally different method of investigation, the so-called ensemble models, which are based on the individual modeling of each cell, was developed in 2003, its application is as yet limited [18].

As for experimental research on stochastic processes in development of heterogeneity within bacterial populations, the works involving the artificial, specially designed "switches", i.e., elements of the genetic regulatory systems responsible for a certain type of bistability, gained popularity in the last decade. This approach to investigation of stochastic heterogeneity in bacterial populations makes it possible to minimize the effect of the external and internal environment, thus providing for the purity of experiments [18, 19].

Heterogeneity of the Biochemical Composition of the Cells

Heterogeneity of the internal content of the cell is considered by some authors as an additional source of phenotypic heterogeneity [18]. It should be noted, however, that the differences in the biochemical composition of the cells may develop due to random fluctuations of the contents of the structural and regulatory components in the cytoplasm.

Nonuniform (asymmetric) division is a classical mechanism generating the phenotypic heterogeneity of this type [31, 18]. This process implies nonuniform distribution of the cytoplasm between the daughter cells. One of them receives more of the newly synthesized material, while the other becomes the progenitor of an ageing and, finally, dying cell line. Apart from budding eukaryotic microorganisms, the process on nonuniform division was found in bacteria [92].

ROLE OF POPULATION HETEROGENEITY FOR BACTERIA AND HUMANS

Being of small size, bacterial cells are susceptible to all environmental fluctuations, which may be on the scale not compatible with the individual microorganism's capacity for physiological adaptation. Adaptation at the level of populations is therefore especially important for bacteria. As a component of this strategy, heterogeneity is realized at the genetic and epigenetic levels, so that the modulation of both the gene expression and the frequency of their mutations and recombinations becomes possible [2].

Insurance is among the most popular explanations of the role of heterogeneity in survival of bacteria [93]. This principle holds for many ecosystems. Diversity of communities is always a protective mechanism under unstable environmental conditions [94, 95]. Fluctuation of the parameters, both the random ones and those regulated by the social behavior, are responsible for the initial preadaptation of some variants (groups) to specific environmental conditions, enhancing the probability of their survival compared to other variants (groups) [60]. Apart from experimental data, better survival of heterogeneous populations is confirmed by mathematical simulation-based theoretical works [27]. As for the activation of specialized programs differentiating the cells prior to stress action (as a response to preceding factors), this is an instant of altruistic behavior in bacteria. Some cells sacrifice their successful development under current conditions, providing for survival of the population under an unexpected impact of unfavorable factors [60, 96].

Apart from insurance, diversity of the characteristics and states of the cells may also act as a primer, a necessary component and inevitable result of such socially regulated forms of bacterial behavior as, for example, biofilm formation. Development of structured bacterial communities is an important mechanism facilitating bacterial survival [35].

The phenotypic heterogeneity of bacteria resulting from nonuniform division is considered a phenomenon of special adaptive importance. Mathematical models demonstrate that the growth rate and survival of microorganisms with nonuniform division may be higher than in the cultures with symmetrical division [90, 97].

Heterogeneity of the population resulting from accumulation of the mutants becomes the material for natural selection and adaptive evolution [98].

Applied studies of heterogeneity of the populations comprise several aspects. First, this phenomenon should be considered for more precise modeling of the biochemical processes in biotechnology, e.g., in production of nutrients or medications. Moreover, bacterial heterogeneity may result in improved survival of the population, necessitating the clinical and hygienic investigation of this problem. For basic science, research of the heterogeneity of bacterial populations makes it possible to understand some qualities of bacteria characteristic of the population and not following directly from the properties of the individual cells [18].

Application of the term "adaptation" to bacterial heterogeneity meets justifiable objections, since both the random fluctuations at the phenotypic or genotypic level and the random results of the variations induced by stress impacts facilitate survival. Investigation of the heterogeneity of bacterial populations is therefore important from the philosophical point of view, demonstrating that the laws of physics and chemistry, together with probabilistic processes, inevitably result in enhanced adaptation of the organisms to their environment. Thus, some nomogenetic concepts of evolution, which stress that basic laws of evolution are partially predetermined by the physical constants of our universe, have to come into view.

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