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CONFERENCE PROCEEDINGS

Regulatory Functions of Bacterial Exometabolites

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Abstract—This review deals with the issue of growth autoregulation and survival in bacterial cultures under starvation conditions. Based on our results and on published data, the conclusion has been drawn that low-molecular products of metabolism (carboxylic acids, amino acids, and other metabolites) perform regulatory functions. The same compounds also control the ecological relationship between microorganisms at the interspecific level, and affect their antagonistic activity. It is suggested that complexes of bacterial metabolites can be used for controlling the composition of various microbiocenosis, including those of humans.

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Recently, considerable evidence has been accumulated in the literature suggesting that all the compounds (or most of them) excreted by cells perform certain communicative functions at the population level, in interspecific relations, and in microbial–host interactions. The inhibitory effect of bacterial metabolites, an effect which induces bacteriostasis in high-density cultures has been studied in the most detail. This work is primarily concerned with two other aspects of culture growth: autostimulation of cell growth, and autoregulation of cell survival under starvation conditions.

Autoregulation of cell survival. The death rates of *Escherichia coli* M-17 cells suspended in distilled water, physiological solution, or solutions of other salts were the highest at low (2×10^6 CFU/ml and lower) and extreme (more than 20×10^9 CFU/ml) concentrations. A certain intermediate (critical) concentration range ($0.6–2.0 \times 10^9$ CFU/ml) proved to be optimal for their survival [1–3]. Similar patterns were observed in experiments performed on other bacteria, although the concentrations optimal for their survival varied, e.g., reaching as low as $0.5–1 \times 10^8$ CFU/ml for *Salmonella enteritidis* [3, 4].

The partial removal of low-molecular exometabolites by dialysis or sorption allowed us to increase bacterial survival in high-density suspensions. If these compounds were removed almost completely by high-flux dialysis, the rate of cell death increased again. In the cultures with a density below critical, metabolite removal resulted in a decrease in cell survival [2, 3].

The low-molecular metabolites from high and low-density cultures (HD and LD metabolites) differed in their biological effect. The HD metabolites added to the cultures with a density of 10^{10} CFU/ml or higher accelerated bacterial death, while the LD metabolites had no noticeable effect on bacterial survival. This has allowed us to assume that high-density cultures produce specific compounds accelerating cell death factors (ACD factors). The ACD activity reached its peak when CFU decreased by approximately 50%, then it began to decrease, and disappeared completely at a concentration of 10^9 CFU/ml. In the low-density cultures ($5 \times 10^6–5 \times 10^7$ CFU/ml), the ACD factors were active only when the test culture liquids were diluted $10^3–10^7$-fold, since at lower dilutions, the products of cell autolysis present in these culture liquids served as growth substrates for the test culture. The minimum working concentration of ACD factors was not higher than tens of picograms per milliliter [2, 3].

The use of low-density cultures allowed us to monitor the ACD factor dynamics during cultivation of bacteria, because, during the experiment, the medium was diluted to the extent that all the compounds, except the ACD factors, did not influence the test culture survivability. During the lag phase, the ACD factor activity was comparatively low, but rapidly increased afterwards. Its peak occurred during the first half of the exponential phase; afterwards, the concentration of ACD factors decreased and then increased again at the beginning of the stationary phase [3, 5]. On the basis of the dynamics of ACD factors, one can assume that their presence in the culture liquid may cause the decrease in cell survival during the lag phase, which is a phenomenon well-known but poorly understood. Our present understanding of autoregulation allows us to think, with minor reservations, of the ACD compounds as fac-

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tors that induce apoptosis in bacterial cells. The factor d_2 [6] (a "killing factor", [7]), as well as the compounds produced in the presence of a death-accelerating substrate, may perform an analogous function [8].

Autostimulants of microbial growth. A comparatively small number of studies have investigated the characteristics of autostimulants that induce bacterial growth. In addition to the previously known results [9, 10], we studied the dynamics of autostimulation of *E. coli* M-17 growth under starvation conditions during growth in flasks and in a fermentor; we have developed methods to isolate growth stimulators (ARK-1 and ARK-2) and studied their influence on the growth and viability of bacteria.

The concentration of autostimulants reached its peak during the stationary phase of bacterial growth. As the cell density increased, it caused an increase in the autostimulant concentration. However, the stimulatory effect of the culture liquid in the stationary growth phase was observed only after its dilution, whereas the undiluted culture liquid inhibited bacterial growth. This means that various concentrations of the same metabolites may produce either a stimulatory or inhibitory effect. A study of the dynamics of cell numbers in batch cultures was performed. The result confirmed that the growth started and ended with synchronous cell division [11]. Immediately before the division of cells, the stimulator concentration increased markedly, then it dropped during the division process, and after the process termination it was restored practically to the initial level. Investigation into the autostimulation dynamics during the step growth of bacteria yielded analogous results [9]. The growth autostimulants also accumulated during the storage of high-density and ultrahigh-density cultures, their activity being noticeably higher than during bacterial growth.

Starvation-induced autooscillations. During the storage of cultures with certain cell densities or during the incubation of the stationary phase cultures, changes in bacterial numbers (CFU) occurred not monotonously, but exhibited pronounced oscillatory behavior. Oscillations were observed if the density of the stored suspension was slightly higher ($3–11 \times 10^9$ CFU/ml) or lower ($0.4–6 \times 10^8$ CFU/ml) than the critical cell concentration; these oscillations were accompanied by periodic changes in the concentrations of growth stimulators and ACD factors. Each increase in cell concentration was preceded by an increase in the concentration of growth stimulators, and each decrease was preceded by an increase in the ACD factor concentration [5]. In order to explain the generation mechanism of these oscillations, a mathematical model has been developed [12], which describes, in addition to the oscillations in bacterial cell numbers, the relationships between the lag phase duration and inoculum density, acceleration of cell death induced by high population density, substrate-induced stimulation of cell death [8], as well as certain other phenomena of cell development.

Biological properties and chemical nature of *E. coli* M-17 regulatory exometabolites. Low molecular weight fractions of *E. coli* M-17 exometabolites obtained by dialysis or ultrafiltration of the culture liquid of the stationary phase culture (ARK-1 preparation), or of the culture liquid of a starving high-density culture (ARK-2 preparation), exhibited the ability to stimulate cell growth to or accelerate the death of bacteria. Both preparations contained growth autostimulants; the latter also contained ACD factors. The preparations stimulated the growth of *E. coli* M-17 and some other microorganisms, and showed certain specificity. The maximum stimulatory effect was experimentally observed on *E. coli* M-17; this effect was somewhat less pronounced on *E. coli* K-12, and still less in experiments with the representatives of other species (*Salmonella enteritidiss* and *Serratia marcescens*) [13]. This fact corresponded well with earlier results obtained by other authors [9]. Furthermore, it was shown that *E. coli* M-17 metabolites enhanced the growth of the normal human symbionts *Lactobacillus acidophilus* (strains D-75 and D-76) and *Bifidobacterium adolescentis* MC-42, but not of *Lactobacillus bulgaricus* ATCC 21815, which is not a normal human symbiont. These facts agree well with the ability of *E. coli* M-17 (as the colibacterin preparation) to restore the natural balance of human microflora; we assume that this restoration mechanism is related to the stimulatory effect of the metabolites produced by this strain [14].

The addition of ARK (ARK-1 or ARK-2) to the mixed cultures of *E. coli* M-17 with *E. coli* K-17 or with *S. enteritidis*) made *E. coli* M-17 grow faster (approximately 2.6-fold) in comparison to the other component [14]. The addition of ARK into the mixed culture during starvation enhanced the viability of *E. coli* M-17 and decreased that of the competitive strain [15]. Thus, *E. coli* M-17 exometabolites enhanced the antagonistic activity of the strain, although they had no bactericidal effect (see above) [13]. The ARK preparations enhanced the antagonistic activity of *L. acidophilus* D-75 and D-76: the addition of these preparations to the mixed culture of *L. acidophilus* and *S. enteritidis* resulted in an almost 100-fold decrease in *S. enteritidis* content. When solid media were supplemented with ARK-1, the zone of *S. enteritidis* growth inhibition by lactobacilli increased 1.75-fold [13, 14].

Our further task was to isolate and identify the growth stimulators of *E. coli* M-17. Our preliminary experiments have shown that, during separation on TSK gel HW-40 (pH 7.4), growth stimulators and inhibitors were detected in the neighboring fractions, whereas the ACD factors were represented by at least two compounds—ACD_1 and ACD_2; the ACD_2 factor might contain an amino group. Using a three-stage purification process, we also obtained two fractions of

growth stimulators; the less active one contained organic amines [1, 3]. By frontal anion-exchange chromatography, appropriate procedures for sorption and removal of metabolites were chosen; it has been shown that the initial culture liquids may contain up to five growth stimulators acting individually or in combination with other compounds. The metabolites that exhibited the highest sorption affinity were the most potent growth stimulators. One could suggest that these compounds had individual, as well as group, activity. We intended to identify these compounds in subsequent experiments, and then investigate other fractions and their influence on the activity of the compounds that had already been isolated.

The sorption of low molecular weight exometabolites onto an anion-exchange resin with the subsequent elution of growth stimulators at pH 3.1 was the first stage of the scheme (after ultrafiltration). At the second stage (HPLC), two groups of active fractions were revealed in these stimulators. Glutamic acid was the sole active component of the first group. After additional separation, it was found that the second group contained succinic acid as an active component [16, 17]. The success of these investigations was due to the concept of the multicomponent structure of growth stimulators and, therefore, of the application of diverse methods to monitor the changing activity of the fractions as the separation progressed. It was possibly the lack of such concepts and control methods that did not allow other authors to achieve similar results [18].

The fact that "ordinary" metabolites were found to be the major components of growth stimulators, instead of some specific compounds (for instance, such as AI-1 or AI-2), allowed us to assume that other products of *E. coli* metabolism may display stimulatory activity [4, 19]. After investigating the composition of low-molecular metabolites from *E. coli* M-17 culture liquid, a composition was made of all the identified compounds. The activity of this composition was no lower than that of the prototype. Other growth stimulators (methionine, lysine, and acetate) and inhibitors (alanine, aspartate, histidine, formate, cysteine, indole) were revealed in this composition. Other compounds did not show any individual activity. It was confirmed that succinate was the major component of the stimulatory complex, since it showed the highest activity; it was present in the medium in higher concentrations than the other stimulators (except for acetate). The second important stimulator was glutamic acid [4, 19].

To study the combined effect of the metabolites, aside from their full composition (Comp-1), several mixtures were composed which contained only growth stimulators (Σ_{st}), growth inhibitors (Σ_i), neutral compounds (Σ_n), and some others. The stimulatory activity of these mixtures decreased according to the following series: Comp-1 > [$\Sigma_{st} + \Sigma_n > \Sigma_{st}$ > individual stimulators. Hence, the synergetic effect of growth stimulators was confirmed, and the capacity of neutral compounds to enhance their activity revealed. These results agreed well with the conclusions based on the results of frontal anion-exchange chromatography.

Likewise, Σ_i inhibited growth more effectively than individual compounds. The presence of acetate intensified the effect of other inhibitors, but only at high enough concentrations. On the contrary, low acetate concentrations decreased this effect to the extent of its neutralization, or even resulted in a slight stimulatory effect. Thus, acetate was found capable of modulating the stimulatory activity of the extracellular medium: at low concentrations, it intensified the stimulatory effect and diminished the inhibitory effect, whereas it showed its own inhibitory activity at high concentrations and augmented the inhibitory effect of other compounds. These properties of acetate suggest that it is a density-dependent factor affecting the changes of the phases of culture development [4, 19].

The hypothesis that metabolites may have regulatory functions was confirmed by recent molecular biological studies. For instance, it was shown that on addition of acetate to *E. coli* medium, the expression of 37 proteins increased and the expression of 60 proteins decreased. Formate inhibited the synthesis of 10 proteins out of the total of 37 acetate-induced proteins [20, 21]. There is evidence of the regulatory effect of pH and propionate [22], as well as of the synergetic effect of short-chain amino acids.

The study of the dynamics of already identified compounds revealed that an exchange of metabolites between cells and the adjacent medium occurred immediately after inoculation. Unless the culture was overly diluted, growth stimulators were released to the medium; otherwise the cells consumed these stimulators. The extension of the lag phase resulted from this phenomenon. During the cell growth cycle, the overall stimulatory activity of the medium changed with the changes in the exometabolite (stimulator) concentrations: the activity intensified by the second hour with increasing acetate concentration, as well as in the final phase, simultaneously with the increasing concentrations of acetate and succinate. Both increases preceded the synchronous division of the culture [23].

The metabolite compositions possessed the same properties as the prototypes investigated (ARK): they intensified the antagonistic activity of *E. coli* M-17 during its growth and starvation in mixed cultures with *S. enteritidis*, stimulated the of growth *L. acidophilus* D-75 and D-76, and increased their antagonistic activity against a wide spectrum of pathogenic bacteria.

The composition similar to the exometabolite composition of the starvation medium of *E. coli* M-17 (Comp-2) showed the capacity to accelerate the death of high-density (25×10^9 cells/ml) test cultures. The presence of acetate (and probably of some other metabolites) caused fluctuations in the number of *E. coli* M-17 cells. As the cell number increased, acetate was consumed by the cells and returned in lesser amounts to

the medium after their death. Out of all the compounds present in the medium, glycine was shown to be the most active cell-death stimulator of *E. coli* M-17. Its addition to the composition accelerated the cell death, and its removal would enhance survivability. However, the identity of glycine as an ACD_2 factor requires further examination.

The specificity of the ARK functions, metabolite compositions, and their components was confirmed during experiments on solid nutrient media. We studied the effect of different concentrations of these components in agarized media; the concentration of 50 μg/ml was used in order to compare their activity. Succinate had a much weaker stimulatory effect on the growth of *E. coli* BL colonies than on those of *E. coli* M-17 and inhibited the growth of *S. enteritidis*. Lactate stimulated only *E. coli* M-17 growth and inhibited the growth of other strains. It was confirmed (see [24]) that aspartic acid inhibited the growth of *E. coli* M-17, while it stimulated the growth of other strains of *E. coli*. An increase in heterogeneity or the bimodality of the size distribution of the colonies under the influence of metabolites indicated that all (or most) of them influenced not the whole population, but only its segments, subpopulations. One can assume that subpopulations stimulate the growth of each other as they exchange appropriate metabolites and form the total pool of exometabolites within their population. The list of these metabolites must include various gaseous compounds influencing bacterial growth, such as CO_2, H_2S, H_2, etc.

The results of our experiments on solid and liquid media correlated well with each other. However, there were some differences. Aspartate, alanine, and lactate, inhibiting the growth of a particular strain on solid media, could induce its growth in a liquid medium, since in the latter case they operated at the population level, rather than at the subpopulation level. Therefore, it was sufficient to induce the growth of a single cell for the appearance of the stimulatory effect.

On the basis of the data obtained, a new concept was proposed concerning the possibility of the application of low-molecular exometabolites for medical purposes. In pursuing these aims, the synthetic composition "Actoflor-C" was developed; this composition surpasses the prototype preparations (ARK) in its ability to stimulate growth and to intensify the antagonistic activity of the normal microflora. In our experiments on laboratory animals, the efficiency of this composition for the correction of dysbiotic states and the treatment of intestinal ulcers was demonstrated [25, 26].

According to the results of our experiments on the growth-stimulating complex "bios" [27] and some other investigations [28], the metabolic autoregulation system of yeasts resembles in many respects the bacterial one, but differs from it in utilizing vitamins and other similar compounds (more specialized than amino acids and fermentation products) as regulators. Although the autoregulation process of multicellular organisms is based on the effect of still more specific compounds, it still uses *L*- and *D*-amino acids (glycine, glutamate, aspartate, *D*-serine, etc.) and even simpler compounds (CO_2, CO, H_2S, NO, H_2). Information transfer in the nervous system is based on a variety of simple chemical signals. Each presynaptic ending is able to generate several mediators, a combination of which may differ even in synapses from one neuron [29].

The data presented agree well with the concept of metabolic regulation [30] that works at all levels of life and is thought to be the lowest and most ancient level in the hierarchy of regulatory processes. Its evolution can be traced back to chemical regulation based on two opposite phenomena: inhibition of the reaction by the resulting product, and autocatalysis. Further investigations into the relationship between the system of metabolic regulation and other systems, including the system of production and activation of resting forms of microorganisms [6], or of production and consumption of inductors AI-2 by *E. coli* cells [31], are of particular interest.

REFERENCES

1. Vakhitov, T.Ya., Kulikov, S.V., Yashina, O.Yu., Morozova, E.N., and Ososkova, L.I., The Olly Principle in Microbial Populations, in *Otechestvennyi proizvodstvennyi opyt. Mikrobiologicheskaya promyshlennost'* (Russian Industrial Experience. Microbiological Industry), Moscow: VNII SENTI, 1988, issue 4, pp. 9–12.
2. Vakhitov, T.Ya. and Petrov, L.N., Survival of *Escherishia coli* Cells during Storage in Suspensions of Different Concentration, *Mikrobiologiya*, 1992, vol. 61, no. 6, pp. 1087–1095.
3. Vakhitov, T.Ya., Autoregulation of Survival of *Escherichia coli* M-17 Cultures During Starvation, *Cand. Sci. (Biol.) Dissertation*, St. Petersburg: SPbGU, 1993.
4. Momot, E.N., Metabolic Regulation in the Course of Growth and Starvation of *Escherichia coli* M-17 Culture *Cand. Sci. (Biol.) Dissertation*, St. Petersburg: SPbGU, 2002.
5. Vakhitov, T.Ya., Oscillations in Bacterial Population During Starvation, *Biofizika*, 1999, vol. 44, no. 3, pp. 503–504 [*Biophysics* (Engl. Transl.), vol. 44, no. 3, pp. 495–496.
6. El'-Registan, G.I., Rest as a Form of Microbial Adaptation, in *Bukharin O.V., Gintsburg A.L., Romanova Yu.M., El'-Registan G.I. Mekhanizmy vyzhivaniya bakterii* (Mechanisms of Bacterial Survival), Moscow: Meditsina, 2005.
7. Barlati, S. and Majerfeld, J., Partial Characterization of the Factor Responsible for Tryptophanless Death in *Bacillus subtilis*, *J. Bacteriol.*, 1970, vol. 101, no. 2, pp. 355–360.
8. Strange, R.E. and Dark, F.A., "Substrate Accelerated Death" of *Aerobacter Aerogenes, J. Gen. Microbiol.*, 1965, vol. 39, no. 2, pp. 215–228.
9. Pshenichnov, R.A., Kolotov, V.M., Barikhin, S.Ya., Tkachenko, A.G., Sokolova, N.A., and Ivakina, A.M., On the Existence of the System of Specific Autometab-

olite Regulation of Development of Microbial Populations. Initial Observations on Revealing the Autofactor Stimulating Culture Development, *Ekologiya*, 1975, no. 3, pp. 42–50.

10. Pshenichnov, R.A., Kolotov, V.M., Barikhin, S.Ya., and Tkachenko, A.G., Realization of the Feedback Principle in the System of Autometabolic Growth Regulation of the populations of *Escherichia coil* clones, in *Mechanizmy regulyatsii razvitiya bacterial'nykh populyatsii* (Mechanisms of the Regulation of Development of Bacterial Populations), Sverdlovsk: UNTs AN SSSR, 1977, pp. 3–9.
11. Culter, R.G. and Evans, J.E., Synchronization of Bacteria by a Stationary-Phase Method, *J. Bacteriol.*, 1966, vol. 91, no. 2, pp. 469–476.
12. Vakhitov, T.Ya. and Yashina, O.Yu., Description of Growth Kinetics and Survival of a Microbial Population Considering the Autoregulative Factors of its Development, in *Peredovoi proizvodstvennyi opyt v meditsinskoi promyshlennosti, rekomenduemyi dlya vnedreniya* (Progressive Experience in Medical Industry Recommended for Application), Moscow: VNII SENTI, 1990, issue 2, pp. 24–26.
13. Vakhitov, T.Ya., Yashina, O.Yu., Petrov, L.N., and Korolyuk, A.M., Study of the Effect of the M-17 *Escherichia coli* M-17 Growth Autostimulator Preparation on the Growth of Pure and Mixed Bacterial Cultures, *Zh. Microbiol. Epidemiol. Immunobiol.*, 2003, no. 3, pp. 20–24.
14. Vakhitov, T.Ya., Dobrolezh, O.V., Petrov, L.N., Verbitskaya, N.B., and Zadaura, E.Yu., Comparative Study of the Effect of *Escherichia coli* M-17 Growth Autostimulating Preparations and Fructooligosaccharides on Growth and Antagonistic Activity of Lactobacilli, *Zh. Microbiol. Epidemiol. Immunobiol.*, 2001, no. 3, pp. 80–83.
15. Vakhitov, T.Ya., Dobrolezh, O.V., and Petrov, L.N., Effect of the Aktoflor Preparation on Survival of *Escherichia coli* M-17 and *Salmonella enteritidis* Under Starvation in Mixed Cultures, *Zh. Microbiol. Epidemiol. Immunobiol.*, 2000, no. 6, pp. 67–68.
16. Vakhitov, T.Ya., Visnol'd, N.V., Protasov, E.A., and Petrov, L.N., Isolation and Identification of *Escherichia coli* Growth Autostimulators, *Russian Journal HIV/AIDS and Related Problems*, 1999, vol. 3, no. 1, pp. 116–117.
17. Vakhitov, T.Ya., Protasov, E.A., Visnol'd, N.V., Tolparov, Yu.N., and Petrov, L.N., Isolation and Identification of *Escherichia coli* Growth Autostimulators, *Zhurn. Mikrobiol. Epidemiol. Immunobiol*, 2003, no. 2, pp. 7–12.
18. Weichart, D.H. and Kell, D.B., Characterization of an Autostimulatory Substance Produced by *Escherichia coli*, *Microbiology (UK)*, 2001, vol. 147, pp. 1875–1885.
19. Vakhitov, T.Ya., Momot, E.N., Shalaeva, O.N., and Petrov, L.N., Composition and Biological Activity of *Escherichia coli* M-17 Exometabolites, *Zhurn. Mikrobiol. Epidemiol. Immunobiol*, 2003, no. 6, pp. 20–25.
20. Arnold, C.N., McElhanon, J., Lee, A., Leonhart, R., and Siegele, D.A., Global Analysis of *Escherichia coli* Gene Expression During the Acetate-Induced Acid Tolerance Response, *J. Bacteriol.*, 2001, vol. 183, no. 7, pp. 2178–2186.
21. Kirkpatrick, C., Maurer, L.M., Oyelakin, N.E., Yoncheva, Y.N., Maurer, R., and Slonczewski, J.L., Acetate and Formate Stress: Opposite Responses in the Proteome of *Escherichia coli, J. Bacteriol.*, 2001, vol. 183, no. 21, pp. 6466–6477.
22. Stancik, L.M., Stancik, D.M., Schmidt, B., Barnhart, D.M., Yoncheva, Y.N., and Slonczewski, J.L., pH-Dependent Expression of Periplasmic Proteins and Amino Acid Catabolism in Escherichia Coli, *J. Bacteriol.*, 2002, vol. 184, no. 15, pp. 4246–4258.
23. Vakhitov, T.Ya., Momot, E.N., and Tolparov, Yu.N., Dynamics and Functions of Exametabolites in the Course of Growth of Batch Culture of *Escherichia coli* M-17, *Zhurn. Mikrobiol. Epidemiol. Immunobiol,* 2005, no. 1, pp. 16–21.
24. Oleskin, A.V., Botvinko, I.V., and Tsavkelova, E.A., Colonial Organization and Intercellular Communication in Microorganisms, *Mikrobiologiya*, 2000, vol. 69, no. 3, pp. 309–327 [*Microbiology* (Russ. Transl., 2000, vol. 69, no. 3, pp. 249–265.
25. Vakhitov, T.Ya., Petrov, L.N., and Bondarenko, V.M., The Concept of a Probiotic Preparation Containing Original Microbial Metabolites, *Zhurn. Mikrobiol. Epidemiol. Immunobiol*, 2005, no. 5, pp. 108–114.
26. Petrov, L.N., Bondarenko, V.M., Vakhitov, T.Ya., and Vorob'ev, A.A., QS-Systems in Bacteria and the Prospects of Development of new Metabolic Probiotic Preparations, *Vestnik RAMN*, 2006, no. 1, pp. 38–45.
27. Meisel', M.N., *Funktsional'naya morfologiya drozhzhevykh organizmov* (Functional Morphology of Yeasts), Moscow: Akad. Nauk SSSR, 1950.
28. Chen, H., Fujita, M., Feng, Q., Clardy, J., and Fink, G.R., Tyrosol Is a Quorum-Sensing Molecule in *Candida Albicans, Proc. Natl. Acad. Sci. USA*, 2004, vol. 101, no. 14, pp. 5048–5052.
29. Zefirov, A.L., Mediators, an Evolution of Concepts, *Vestnik RAMN*, 2005, no. 1, pp. 49–52.
30. Shvarts, S.S., Metabolic Regulation of Animal Growth and Development on the Population and Organismic Levels, *Izv. Akad. Nauk SSSR, Ser. Biologicheskaya*, 1972, no. 6, pp. 822–835.
31. Xavier, K.B. and Bassler, B.L., Interference with AI-2-Mediated Bacterial Cell-Cell Communication, *Nature*, 2005, vol. 437, pp. 750–753.