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EXPERIMENTAL ARTICLES

Dynamics of the Growth and Population Composition of Mixed Cultures of R, S, and M Dissociants of *Pseudomonas aeruginosa*

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Abstract—The population composition of polycultures of *Pseudomonas aeruginosa* dissociants (R + M and R + S + M) developing on media with various contents and ratios of nitrogen and phosphorus has been studied. Irrespective of its proportion (10 to 90%) in the inoculum, the R variant accounted for 65 to 84% of the whole population of linear-phase and stationary-phase binary cultures of R and M dissociants, which differ in terms of energy metabolism and nutritional requirements. After prolonged cultivation, the population in the binary culture contained only R cells (100%), which are characterized by minimum requirements with respect to the main biogenic elements. These data agree with the predictive data of model studies and can be attributed to regulation of the population composition of bacterial cultures by trophic factors. It was established that the proportion of M cells, which are distinguished by maximum nutrient requirements and enhanced stability, increased during two developmental stages of the *Ps. aeruginosa* polycultures (R + M and R + S + M): the lag phase and the decay stage. This result cannot be due to the influence of trophic factors and presumably results from changes in the levels of autoregulatory factors (anabiosis autoinducers) involved in stress resistance and plausibly in the adaptive interconversion of dissociants upon transfer to a new medium (during the lag phase) and under starvation conditions (at the onset of the decay phase).

Key words: dissociation, dissociant polycultures, biogenic elements, population composition, autoregulatory factors.

Transitions between dissociant forms significantly contribute to the heterogeneity of bacterial populations. The transitions are due to intragenomic alterations that occur at a high rate (exceeding that of random mutations by several orders of magnitude) and produce pleiotropic effects. This phenomenon results in frequent reversible changes in a large number of morphological, physiological, and biochemical properties of cells, including the production of extracellular hydrolases and the efficiency of biopolymer degradation, the capacity for synthesizing biologically active compounds and substances of practical value, and resistance to external factors. In the case of actual and potential pathogens, intragenomic alterations also influence the activity of virulence factors and the properties that enable cells to persist.

Dissociant ratios in bacterial populations vary depending on selective environmental factors. Dissociation can present difficulties for researchers and decrease the efficiency of bacteria in industrial microbiological processes because active variants may be replaced by inactive variants. In natural habitats, dissociation provides for the adaptation of microbial populations to environmental factors and extends the range of environmental conditions within which microbial species can survive [1].

We have previously investigated the energy metabolism of three *Pseudomonas aeruginosa* K-2 dissociants (R, S, and M) during growth of their cultures on a mineral medium with glucose. We demonstrated that the oxidative pathway of glucose utilization prevails in the R dissociant. The M dissociant is predominantly characterized by fermentation accompanied by formic acid formation and medium acidification to a pH value of 3.3. The S dissociant alternately uses both pathways of glucose utilization. Accordingly, the glucose-6-phosphate dehydrogenase activity of the M dissociant was found to be two orders of magnitude lower than that of the

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R and S dissociants [2, 3]. The physiological and biochemical features of the dissociants that we revealed correlate with their different requirements with respect to the main biogenic elements (particularly carbon). The M and the R dissociants are characterized by maximum and minimum C, N, and P requirements, respectively [4]. The higher the nutrient requirements of a dissociant, the lower the percentage of its cells in a mixed culture at the stationary growth stage [5]. However, the dynamics of the population composition during the development of bacterial polycultures, including the decay stage, has not yet been studied.

The goal of this work was to elucidate the dynamics of the growth and population composition of polycultures of *Ps. aeruginosa*, paying special attention to cell behavior during the lag phase and the decay stage.

MATERIALS AND METHODS

This study was conducted with the R, S, and M dissociants of a *Pseudomonas aeruginosa* strain isolated from the formation waters of a Siberian oil field and stored in the culture collection of the Microbiology Department at Moscow State University. The pseudomonads were grown on a mineral media with glucose. The contents of $NaNO_3$, NaH_2PO_4, and glucose were varied (0.03–0.5, 0.004–0.03, and 0.22–3.8%, respectively), whereas the contents of KCl (0.06%) and $MgSO_4$ (0.02%) were kept constant. The bacteria were cultivated in 50-ml tubes containing 10 ml of medium on a shaker (180 rpm) at 30°C. The cell suspensions in a physiological solution to be used as inoculum were obtained from 1-day-old cultures of pseudomonad dissociants grown on an agarized mixture of nutrient broth and wort (1 : 1). The density of the inoculum was nephelometrically adjusted to a cell concentration of 10^9 cells/ml. The inoculum dose was 3%. The growth of the bacteria was estimated from the optical density (OD) of the culture. For convenience, readings taken from an FEK 56M nephelometer were multiplied by 100. The dissociant percentage in a population (the population composition) was determined from the morphology of the colonies obtained by plating decimal dilutions of the culture onto a solid nutrient broth–wort medium.

The pH value of the medium was measured with a Checker micropotentiometer (HANNA Instruments). The glucose concentration in the medium was estimated by a semiquantitative method, using the indicator strips (Diagluc) employed for determining glucose levels in blood. The productivity of the dissociants with respect to extracellular proteases (proteolytic capacity) was determined using a modified Anson method. A proteolytic activity unit corresponds to the enzyme quantity that, within 1 min, converts the amount of sodium caseinate containing 1 μmol (0.181 mg) of tyrosine to hydrolysis products that are not precipitable with trichloroacetic acid.

The productivity of the dissociants in terms of synthesis of autoregulatory factors (represented in *Ps. aeruginosa* by alkylhydroxybenzenes (AHBs) of an alkylresorcinol type) was determined using a highly specific colorimetric test with a diazotized derivative of 3,3'-dimethoxybenzidine (Fast Blue B Salt diazotized, FBB, Sigma). The AHB quantity was expressed in μg/g of dry cell mass (DCM). The working reagent solution was obtained by dissolving 5 mg of FBB in 10 ml of 5% acetic acid followed by its dilution with a fivefold amount of *n*-propanol. 5-*n*-pentadecylresorcinol, 4-*n*-hexylresorcinol, *n*-decylresorcinol, 2,5-dibutylresorcinol, and 2-nonyl-5-decylresorcinol were used as the standards. Samples of pseudomonad extracts or standard alkylresorcinol solutions were evaporated until dry and supplemented with 2 ml of the working reagent solution; then, the reaction mixture was incubated in the dark for 1 h. The extinction value and the absorption spectrum of the colored product were determined in 0.2-cm cuvettes with a Specord M-400 spectrophotometer (Jena, Germany) operating in an automatic two-beam mode using the working reagent solution as a control.

The tables and plots contain the mean values of the results of two to three experiments, each of which was performed in triplicate. The mean values were calculated using Microsoft Excel 2000.

RESULTS AND DISCUSSION

Our earlier studies concerning the energy metabolism of *Ps. aeruginosa* dissociants revealed that the R and M variants display the most significant differences in their glucose metabolism patterns: the R variant oxidizes glucose, and the M variant ferments it to formic acid [2, 3]. Therefore, we used binary (R + M) bacterial cultures in the first part of our investigation in order to elucidate the behavioral patterns of the dissociants in mixed cultures. A polyculture obtained by mixing these two dissociants was grown for 10 days in a medium containing (%) glucose, 2; nitrates, 1.1; and phosphates, 0.055. The percent ratio of the R and M dissociants in the inoculum was 40 : 60. Monocultures of R and M dissociants grown on the same medium served as control systems. We monitored the culture growth (OD) and population composition (dissociant percentages, Fig. 1).

The lag phase of the binary culture was 10 h long. The maximum biomass yield (OD = 250) was obtained by the 70th hour of cultivation. After that, the decay stage began. All the glucose was completely consumed by the 70th hour. The cell density hardly changed between 160 and 250 h of cultivation (OD = 60). At the end of the lag phase, the medium became slightly acidified, which was followed by a monotonic increase in pH up to a value of 9.5.

The pattern of changes in the M dissociant percentage in the developing culture was nonlinear. The number of cells yielding M colonies reliably increased at

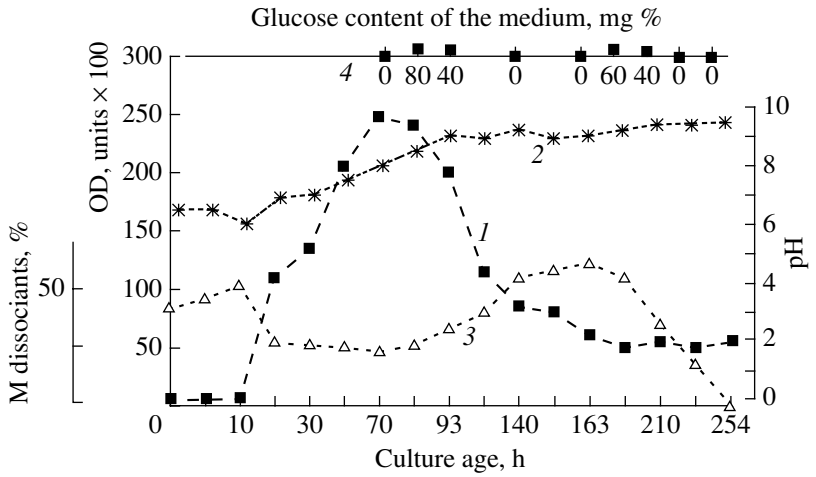


Fig. 1. Growth dynamics and population composition of a binary R + M culture of *Ps. aeruginosa*: (*1*) optical density (OD × 100) of the culture; (*2*) pH of the medium; (*3*) M dissociants, % of the CFU number; (*4*) residual glucose content, mg %.

two points: during the lag phase (from 40 to 50–60%) and during the decay phase (from 25 to 60%). During the growth period, the binary culture was increasingly dominated by the R variant, which is characterized by minimum requirements with respect to the main biogenic elements [4]. The R dissociant percentage was 76% during the stationary phase, which is in accordance with the results of calculations in model systems [6]. As for the decay stage of the binary culture, the R dissociant percentage dropped to 40% by the 163th hour. There was an inverse relationship between the R dissociant percentage and the optical density of the culture. The R dissociant percentage increased for the second time in an autolysing decaying culture with a stabilized optical density value (OD = 60), amounting to 100% by the 250th hour. Inoculation of this culture onto solid medium resulted in retarded growth of the colonies. This effect is characteristic of the germination of dormant bacterial cells and suggests their presence in the 250-h culture. The formation of dormant bacterial cells in autolysing cell suspensions has been demonstrated for a number of non-spore-forming bacteria [7–9].

A comparative study of the development of the binary *Ps. aeruginosa* culture (R + M) and of monocultures revealed that the R culture (grown on the medium described above) displayed the same properties as the binary culture and reached the stationary phase by the 70th hour (OD = 270 and pH 8.8). The population composition of the R monoculture did not change throughout the whole developmental cycle. The growth of the M monoculture ceased at a much earlier point, specifically, by the 22nd hour; the optical density attained was significantly lower (OD = 65), and the growth medium was acidified to a pH value of 3.5. If the bacterial culture was grown further, the M population was 100% replaced by an R population.

Thus, M cells were replaced by R cells during the development of both the M monocultures and the R + M binary cultures of *Ps. aeruginosa.* An increase in the percentage of M cells occurred at two developmental stages: during the lag phase and during the decay phase. These changes in the population composition are unlikely to be due to trophic environmental factors, since the medium contained a complete set of nutrients during the lag phase and was glucose-limited during the decay phase.

The question to be raised is whether the above pattern of changes in the population composition is also characteristic of the lag phase of other types of mixed cultures of *Ps. aeruginosa* dissociants and of polycultures grown on other media. The following experiments investigated the behavior of bacterial populations in R + S + M polycultures. We used a mixture containing equal percentages of R, S, and M dissociants to act as inoculum. The polycultures were grown for 24 h (logarithmic phase) on four media with equal glucose concentrations (3.8%) and different nitrate and phosphate contents. Medium 1 contained 0.05% nitrate and 0.005% phosphate; medium, 2 0.5 and 0.005%; medium 3, 0.05 and 0.03; and medium 4, 0.5 and 0.03%. The nitrate–phosphate ratio was, therefore, 10, 100, 1.7, and 7 in media 1, 2, 3, and 4, respectively.

The R + S + M polycultures were characterized by the same growth dynamics on all these media (Table 1), but the patterns of population composition (dissociant percentage) were different (Fig. 2). The R dissociant was eliminated at the beginning of the lag phase (before the 6th hour) on all the media, and the M (medium 1, 2, or 4) or the S dissociant (medium 3) dominated the population at this stage. The M cell number decreased in all the cultures upon the onset of active growth and the transition to the logarithmic phase. The R variant dominated the culture grown on the richest medium (medium 4) at the end of the lag phase and the beginning of the logarithmic phase. The M cell number decreased at the beginning of the logarithmic phase in

the culture grown on the medium 3, and the S dissociant became dominant. Importantly, the M dissociant prevailed on the still poorer medium (medium 1), even though it exhibits maximum requirements with respect to the main biogenic components and consumes glucose with the resulting formation of significant amounts of formic acid.

A comparison of the population composition of cultures growing at different contents of nitrogen (Figs. 2a and 2b correspond to medium 1 and 2; Figs. 2c and 2d, to medium 3 and 4) and phosphorus (Figs. 2b and 2c, medium 2 and 3) showed significant differences in the ratios between the R and the S cells. At the same time, the cultures grown on medium 2 and medium 3 had the same population composition and R and S percentages, although these media were characterized by the most significant differences between their N/P ratios (100 and 1.7, respectively).

Among the trophic factors involved, it is, therefore, the absolute nitrogen and phosphorus contents, not their ratio, that exert the main influence on the population composition of the R + S + M polycultures of *Ps. aeruginosa* during the lag phase and at the beginning of the logarithmic phase. This conclusion is supported by the fact that the M dissociant (Fig. 2a) prevailed at the early stages of development of the culture grown on medium 1 (with minimum nitrogen and phosphorus contents) whereas the R variant (Fig. 2d) overwhelmingly dominated the culture on medium 4, which was similar to medium 1 in terms of the N/P ratio but contained maximum amounts of these elements. The results obtained are consistent with the data presented in [3].

The inocula used in the above studies with R + S + M polycultures or R + M binary cultures of *Ps. aeruginosa* were characterized by equal R, M, and S (in the polycultures) percentages. The following studies investigated the influence of the R/M ratio in the inoculum on the population composition of binary cultures grown on a standard medium containing 0.19% glucose, 0.1% nitrates, and 0.01% phosphates. We used five different mixtures of R and M cells as inocula (Table 2). The differences in maximum biomass yields were negligible, but the population composition of these cultures was significantly different.

The composition of the stationary phase populations was the same (78 and 22% R and M cells, respectively), with all the cultures being characterized by R/M ratios between 3 : 7 and 7 : 3 in the inoculum. This population composition was dictated by the requirements of the R and M dissociants with respect to the main biogenic elements, and it was consistent with results obtained with growth variation models for mixed populations [6]. However, if the share of a dissociant in the inoculum was brought down to 10%, its percentage at the end of the active growth stage of the mixed binary culture (Table 2) was below the value predicted in model studies [6].

Of special interest in this context is the following result, established by us as we prepared mixtures of

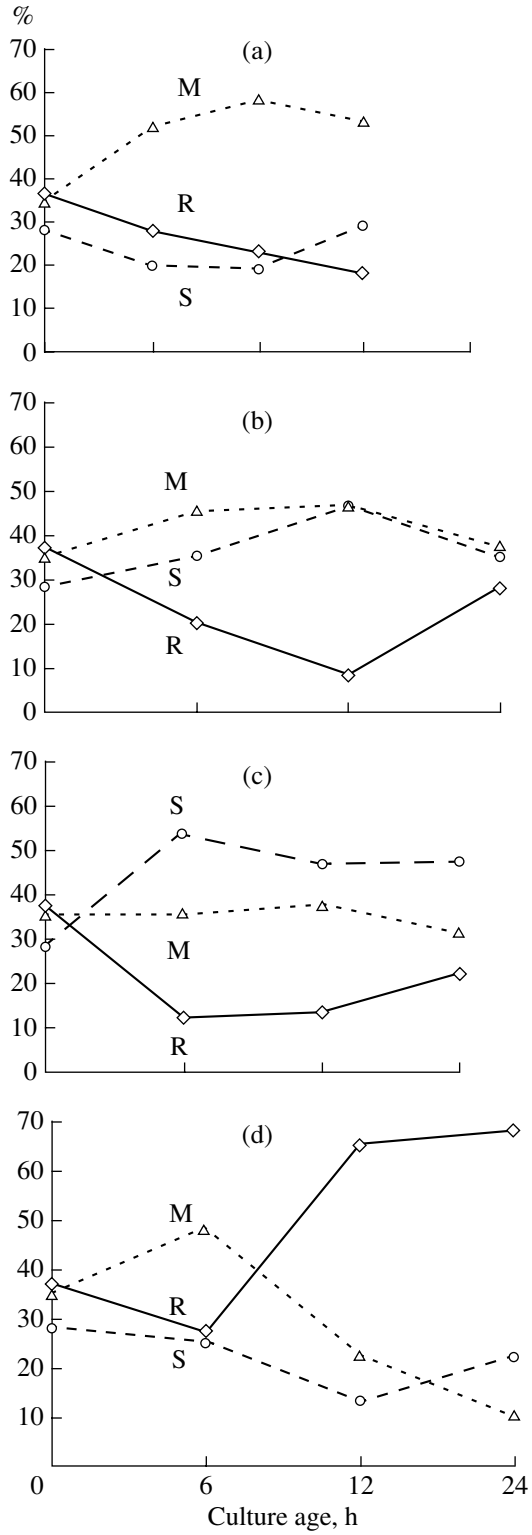
Table 1. Growth dynamics of R + S + M polycultures of *Ps. aeruginosa* on various media

Culture age, h	Optical density (×100)			
	Medium 1	Medium 2	Medium 3	Medium 4
0	5	5	5	5
6	5	5	5	5
11	10	12	10	10
24	90	88	94	92

Table 2. Influence of the dissociant ratio in the inoculum on the growth yield and population composition of *Ps. aeruginosa* polycultures in the stationary phase

Inoculum		Stationary phase		
Dissociant ratio, %		Culture density, OD × 100	Dissociant ratio, %	
R	M		R	M
10	90	64	65	35
30	70	66	78	22
50	50	57	78	22
70	30	60	78	22
90	10	60	84	16

Ps. aeruginosa dissociant cells for polyculture inocula: If equal amounts of R, S, and M cells (at a ratio of 1 : 1 : 1) were mixed in a physiological solution to obtain a cell density of 10^9 cells per ml, incubated for 3 h without being transferred to a nutrient medium, and thereupon plated onto an agarized medium, the percentage of M colonies increased to 50–60% in the resulting population. What was the reason for this change in the population composition of the inoculum? If we assume that the R and S cells were predominantly lysed, then we should expect a change in the proteolytic activity of the cultures. However, the extracellular protease activities of R, S, and M dissociants grown as monocultures in nutrient broth were 85, 99, and 104 units/l, i.e., the differences were insignificant. Alternatively, we can suggest that the M dissociant is selected in a system in which it grows at the expense of a partially autolysed inoculum (including R, S, and M cells). However, the requirements of the R and S cells with respect to the main biogenic elements are more limited than those of the M cells. Therefore, the cell density in R and S monocultures exceeds that in an M monoculture as early as at the logarithmic stage (at 16 h), as was shown in [3]. Below is an explanation based on the assumption of the enhanced stress resistance of M cells. The low growth rate of the M dissociant compared to the R and S dissociants does not militate against this assumption. Apart from postulating that more resistant cells are selected under stressful conditions resulting from transferring the culture to a new medium (during the lag



Medium 1

Concentration		N/P ratio
N	P	
0.05	0.005	10

Medium 2

Concentration		N/P ratio
N	P	
0.5	0.005	100

Medium 3

Concentration		N/P ratio
N	P	
0.05	0.03	1.7

Medium 4

Concentration		N/P ratio
N	P	
0.5	0.03	17

Fig. 2. Ratios of the R, S, and M dissociants of *Ps. aeruginosa* (expressed in %) in mixed cultures grown on media with various nitrogen and phosphorus contents and ratios: (a) medium 1, (b) medium 2, (c) medium 3, and (d) medium 4.

phase) or starvation (caused by incubating the inoculum cell suspension in physiological solution), we also suggest that dissociation processes are induced by the SOS system and other stress responses [10]. Based on these ideas and the results given in Fig. 2, it seems likely that the changes in dissociant percentages and the

dominance of the M dissociant during the first 6 h of polyculture growth occur in response to "new medium stress."

While the nutrient requirements of dissociants influence the growth of *Ps. aeruginosa* polycultures and the dissociant percentages in stationary-phase populations [4], other population-level control factors manifest themselves during the lag phase and the decay phase. We should reemphasize that the M percentage in polyculture populations increases both during the lag phase, when a complete set of nutrients is available, and during the decay stage, characterized by limited nutrient amounts. Therefore, the M percentage does not depend on the concentrations of biogenic elements in the medium. The fact that the increase in the M percentage in lag phase polycultures occurs on media with various contents and ratios of nitrates and phosphates does not militate against this conclusion.

The data obtained suggest that, apart from trophic regulators of the population composition, the dynamics of dissociant percentages in mixed bacterial cultures (polycultures) is controlled by autoregulatory factors d_1 (anabiosis autoinducers) and d_2 (autolysis autoinducers). Their synthesis has been demonstrated in various taxonomic groups of pro- and eukaryotic microorganisms, including representatives of the genus *Pseudomonas* [11]. In *Pseudomonas* species, the d_2 factors are alkylhydroxybenzenes (AHBs) of an alkylresorcinol type [12], whose quantitative determination is based on a highly sensitive colorimetric reaction with the diazotized derivative of 3,3'-dimethoxybenzidine. This method was earlier used to determine AHBs of plant and microbial origins [13, 14]. In our studies, we determined the AHB amount in the culture liquid (CL) at the stage when their maximum yield was attained [11, 14], i.e., at the onset of the stationary phase in the R, S, and M monocultures of *Ps. aeruginosa* grown on synthetic medium with glucose (Table 3). The M and S dissociant were characterized by the maximum and minimum production of extracellular AHBs (expressed hereinafter in μg/g of dry cell mass). The R cells contained the maximum intracellular AHB amounts. Hence, the dissociants displayed significant differences in their capacity to synthesize AHBs, the autoregulators of bacterial growth and development.

Below is an analysis of the results of our studies in terms of these concepts. The AHB level dynamics in developing microbial cultures has two maxima, occurring in the lag phase and the stationary phase [11, 14]. This is consistent with changes in the population composition of the *Ps. aeruginosa* polycultures. The maximum AHB yield in the cells of the R dissociants suggests that they possess advantages over the other dissociants when an old (250-h) culture assumes the hypometabolic and anabiotic states. This hypothesis seems to account for the fact that the R dissociants replace the other variants (e.g., the M dissociants in our experiments) and prevail in older cultures (Fig. 1).

Table 3. Total quantity of extracellular AHBs synthesized by the R, S, and M dissociants of *Ps. aeruginosa* on a synthetic medium with glucose

Dissociant	Wet biomass, g/l	Dry cell weight, g/l	CL AHBs, μg/g of DCM	Intracellular AHBs, μg/g of DCM
S	2.89	0.67	253.7	41.2
M	2.03	0.47	319.1	58.5
R	2.86	0.66	303	87.8

The population composition changes in the *Ps. aeruginosa* cultures during the lag phase (Fig. 2) and decay phase (Fig. 1) may be due either to dissociation processes or to selection pressures favoring the stress-resistant M dissociant. The involvement of AHBs in dissociation processes has been demonstrated for *Bacillus cereus* [15], *Staphylococcus aureus* [16], and *Salmonella typhimurium* [17]. It may be linked to their capacity to induce SOS responses [10] and function as endogenous mutagens, as we have previously shown for auxotrophic *S. typhimurium* mutants reverting to a prototrophic condition (the Ames test) [17]. The induction of the M dissociant and its selective development during the lag phase in the R + S + M polycultures and the R + S binary cultures with unbalanced N and P contents could be due to its enhanced stress resistance and to its AHB level (Table 3).

This suggestion is supported by data on the resistance of the R, S, and M dissociants of *Ps. aeruginosa* to immobilization in polyacrylamide gel (PAAG) [18]. When embedded in PAAG, the M variant demonstrated the highest viability level among dissociants grown on a nitrogen-limited medium, which attests to its comparatively high resistance to PAAG.

Importantly, the regulation of the dissociant spectrum of *Ps. aeruginosa* polycultures by trophic factors, including the contents and ratios of biogenic elements, does not rule out the influence of autoregulatory factors (AHBs) on the population composition, particularly under stress, i.e., during the lag phase and the decay phase. These two types of factors are interrelated, since the AHB yield varies depending on the growth medium [11, 19], while culture growth and transitions between developmental stages are subject to regulation by extracellular AHB concentrations [11, 14].

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REFERENCES

1. Mil'ko, E.S. and Egorov, N.S., *Geterogennost' populyatsii bakterii i protsess dissotsiatsii* (Heterogeneity of

Bacterial Populations and the Dissociation Processes), Moscow: Mosk. Gos. Univ., 1991.

2. Mil'ko, E.S. and Krasil'nikova, E.N., Peculiarities of Carbohydrate Metabolism of R, S, and M Dissociants of *Pseudomonas aeruginosa, Mikrobiologiya,* 1999, vol. 68, no. 2, pp. 211–214.
3. Mil'ko, E. and Il'inykh, I.A., The Effect of Major Nutrient Elements on the Growth and Population Homogeneity of the R, S, and M Dissociants of *Pseudomonas aeruginosa* and the Glucose Oxidation and Fermentation Pathways, *Mikrobiologiya*, 2004, vol. 73, no. 1, pp. 45–50.
4. Fursova, P.V., Mil'ko, E.S., Il'inykh, I.A., Maksimov, V.N., and Levich, A.P., The Requirements of *Pseudomonas aeruginosa* Dissociants for Carbon, Nitrogen, and Phosphorus, *Mikrobiologiya*, 2004, vol. 73, no. 1, pp. 45–50.
5. Maksimov, V.N., The Main Concepts of General Ecology, *Ekologiya mikroorganizmov* (Microbial Ecology), Moscow: ACADEMA, 2004, pp. 12–28.
6. Fursova, P.V., Mil'ko, E.S., Il'inykh, I.A., and Levich, A.P., Approaches to the Control of the Community Composition of *Pseudomonas aeruginosa* Dissociants: Experimental Data and Model Calculations, *Biotekhnologiya*, 2005, no. 1, pp. 73–82.
7. Mulyukin, A.L., Lusta, K.A., Gryaznova, M.N., Babusenko, E.S., Kozlova, A.N., Duzha, M.V., Mityushina, L.A., Duda, V.I., and El'-Registan, G.I., Formation of Resting Cells in Microbial Suspensions Undergoing Autolysis, *Mikrobiologiya*, 1997, vol. 66, no. 1, pp. 42–49.
8. Demkina, E.V., Soina, V.S., and El'-Registan, G.I., Formation of Resting Forms of *Arthrobacter globiformis* in Autolyzing Cell Suspensions, *Mikrobiologiya*, 2000, vol. 69, no. 3, pp. 383–388.
9. Loiko, N.G., Soina, V.S., Sorokin, D.Yu., Mityushina, L.A., and El'-Registan, G.I., Production of Resting Forms by the Gram-Negative Chemolithoautotrophic Bacteria *Thioalkalivibrio versutus* and *Thioalkalimicrobium aerophilum, Mikrobiologiya*, 2003, vol. 72, no. 3, pp. 328–337.
10. Margulis, A.B., Il'inskaya, O.N., Kolpakov, A.I., and El'-Registan, G.I., Induction of SOS Response of Cells by Microbial Autoregulatory Factors, *Genetika*, 2003, vol. 39, no. 9, pp. 1180–1184.
11. Svetlichnyi, V.A., Savel'eva, I.D., Nekrasova, V.K., El'-Registan, G.I., and Romanova, A.K., Organotrophic Growth of *Pseudomonas carboxydoflava* and Synthesis of an Autoregulatory Factor, *Mikrobiologiya*, 1982, vol. 51, no. 4, pp. 606–610.
12. Osipov, G.A., El'-Registan, G.I., Svetlichnyi, V.A., Kozlova, A.N., Duda, V.I., Kaprel'yants, A.S., and Pomazanov, V.V., On the Chemical Nature of the Autoregulatory Factor d_1 of *Pseudomonas carboxydoflava, Mikrobiologiya*, 1985, vol. 54, no. 2, pp. 186–190.
13. Tluscik, F., Kozubek, A., and Mejbaum-Kaizenellenbogen, W., *Alkylresorcinols* in Rye (*Secale cereale* L.) Grains, *Act. Soc. Bot. Pol.*, 1981, vol. 54, no. 7, pp. 645–651.
14. Mulyukin, A.L., Kozlova, A.N., Kaprel'yants, A.S., and El'-Registan, G.I., The d_1 Autoregulatory Factor in *Micrococcus luteus* Cells and Culture Liquid: Detection and Accumulation Dynamics, *Mikrobiologiya*, 1996, vol. 65, no. 1, pp. 20–25.
15. Doroshenko, E.V., Loiko, N.G., Il'inskaya, O.N., Kolpakov, A.I., Gornova, I.B., Klimanova, E.V., and El'-Registan, G.I., Characterization of *Bacillus cereus* Dissociants, *Mikrobiologiya*, 2001, vol. 70, no. 6, pp. 811–819.
16. Il'inskaya, O.N., Kolpakov, A.I., Shmidt, M.A., Doroshenko, E.V., Mulyukin, A.L., and El'-Registan, G.I., The Role of Bacterial Growth Autoregulators (Alkyl Hydroxybenzenes) in the Response of Staphylococci to Stresses, *Mikrobiologiya*, 2002, vol. 71, no. 1, pp. 23–29.
17. Il'inskaya, O.N., Kolpakov, A.I., Zelenikhin, P.V., Kruglova, Z.F., Choidash, B., Doroshenko, E.V., Mulyukin, A.L., and El'-Registan, G.I., The Effect of Anabiosis Autoinducers on the Bacterial Genome, *Mikrobiologiya*, 2002, vol. 71, no. 2, pp. 194–199.
18. Khabibullin, S.S., Fesenko, D.O., Nasedkina, T.V., Golod, N.A., and Zaitseva, A.A., The Use of Stabilized Bacterial Cells for Development of Biochips, *Materialy II Mezhdunarodnogo kongressa "Biotekhnologiya: sostoyanie i perspektivy razvitiya," 10–14 noyabrya 2003 g.*, (Proc. II Int. Congr. "Biotechnology: Current State and Development Prospects," Nov. 10–14, 2003), Moscow, vol. 2, pp. 223–224.
19. El'-Registan, G.I., Duda, V.I., and Svetlichnyi, V.A., Dynamics of the d Autoregulatory Factors in Batch Cultures of *Pseudomonas carboxydoflava* and *Bacillus cereus, Mikrobiologiya*, 1983, vol. 52, no. 2, pp. 238–243.