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EXPERIMENTAL ARTICLES

Growth Induction and Stabilization of Population Composition in *Saccharopolyspora erythraea* by Catecholamine Compounds

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Abstract—Dopamine and epinephrine, hormone compounds belonging to the catecholamine group, produced a polymodal effect on the culture of the erythromycin-producing actinobacterium *Saccharopolyspora erythraea*. They stimulated spore germination and stabilized the composition of its population. Plating of monospore suspensions of strains RIA 1387 and RIA 120 in the presence of catecholamines increased the colony number by 2- and 2.3-fold, respectively (with epinephrine), and 3- and 4-fold, respectively (with dopamine). The optimum effect of both catecholamines was attained at a concentration of 10^{-5} M. The influence of exogenous catecholamines resulted in a significant shift in the population structure of the tested strains with an increased share of the colonies of the dominant morphotype, which is the most efficient antibiotic producer. Considerable differences in the biological activity of the catecholamines were revealed after exposure to ultralow temperatures both during medium-term storage (for three months) and after short-term freezing (for 10 min) in liquid nitrogen. An appreciable effect was produced by exogenous dopamine introduced at the resuscitation stage, resulting in a two- to threefold increase in the viability of actinobacterial spores. Almost 100% of the resulting populations were composed of colonies of the dominant morphotype. In contrast to dopamine, epinephrine failed to produce a growth-stimulating effect when added to spore suspensions exposed to ultralow temperatures.

Key words: catecholamines, epinephrine, dopamine, actinobacteria, *Saccharopolyspora erythraea*, growth stimulation, stabilization of the population structure, cryoconservation.

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Catecholamines are evolutionarily conservative compounds belonging to the group of biogenic amines. This group includes neurotransmitters, hormones, histohormones, and pheromones that play important roles in the endocrine systems of humans and animals. Studies of the pathogenesis of a number of human infections demonstrated that bacterial pathogens actively respond to an increase in the organism's cathecholamine level by changing their growth rate and virulence. The growth-inducing effect of exogenous catecholamines was revealed for the first time over 70 years ago, chiefly for gram-negative and some gram-positive pathogenic bacteria [1, 2]. Exogenous catecholamines enhance the virulence of pathogenic microorganisms by increasing the formation of toxins, adhesins, biofilms, and the signal molecules involved in quorum "sensing systems" [1, 2]. It was revealed that catecholamines produce a stimulatory effect on the growth of representatives of the *Aeromonas* group that inhabit ore water and are capable of leaching gold-containing ores [3]. Addition of exogenous catecholamines at submillimolar concentrations to the cultures of microorganisms belonging to diverse taxonomic groups has a stimulatory effect on their growth and development [4]. Importantly, all the above data available in the literature primarily concern actively growing bacterial cells. However, no information is presently available regarding the influence of catecholamines on microbial cells during transition from the inactive to the active state in physiological and metabolic terms. This knowledge is of much importance with respect to the mechanisms of infectious processes, the variability range of their symptoms, and the development of efficient relevant drugs. The continued existence of infection in a latent form may be caused by dormant/persister cells of the pathogen.

Modern methods of conservation of microorganisms use low and ultralow temperatures. The efficiency of conservation of microorganisms in a viable state without changing their original properties depends on the techniques used for their conversion into a profound anabiotic state and for subsequent resuscitation. It may be expedient to use physiologically active compounds of the catecholamine group for restoring the life-sustaining activities of the culture cells after long-term storage. These compounds may be employed in ecological studies on the size and

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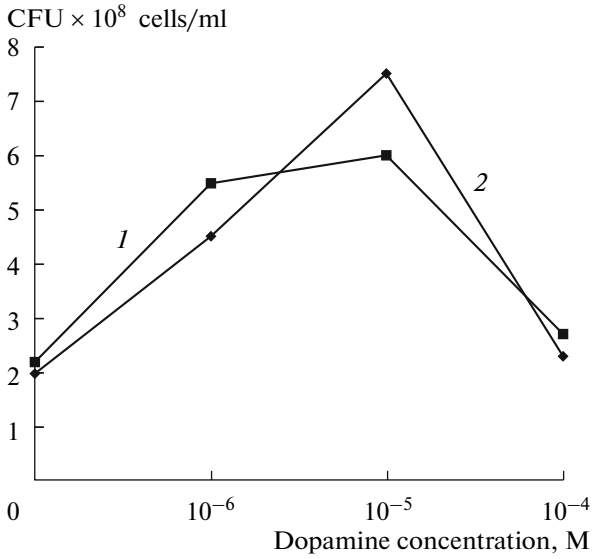

Fig. 1. CFU numbers of *S. erythraea* RIA 1387 and RIA 120 depending on the dopamine concentration in the monospore suspensions: RIA 1387 (*1*) and RIA 120 (*2*).

structure of microbial populations in habitats exposed to permanent or seasonal stressors including extremely low or high temperatures.

The influence of catecholamines on the growth and development of actinobacteria, one of the most advanced and biotechnologically important groups of prokaryotic organisms, is of particular interest. They are characterized by high levels of genetic instability, which may result, under stress, in significant alterations in their taxonomic characteristics and practically relevant properties.

The goal of this work was to investigate the growth-inducing effect of the catecholamines epinephrine and dopamine on the erythromycin producer *Saccharopolyspora erythraea* and their involvement in the formation of the population structure both under optimum conditions for actinobacterial growth and development and after the stress caused by ultralow temperatures.

Table 1. Growth-inducing effect of catecholamines on the cultures of *S. erythraea* RIA 1387 and RIA 120.

Catecholamines (10^{-5} M)	CFU numbers (%) relative to the control value (100%)	
	Cultures of actinobacteria	
	RIA 1387	RIA 120
Epinephrine	200	230
Dopamine	300	400

MATERIALS AND METHODS

The subject of the research were the two *Saccharopolyspora erythraea* strains RIA 1387^{T} [2] and RIA 120 from the Collection of the Laboratory for Classification and Storage of Unique Microorganisms. The cultures were maintained on agar-containing oat medium with 0.25% of yeast extract [5]. Water solution of dimethyl sulfoxide (DMSO) (Reakhim) at a concentration of 5% (vol/vol) was used as a protective medium for the cryoconservation of actinobacteria. Monospore suspensions of actinobacteria in the protective medium were prepared according to [5]. Dopamine (3-hydroxytyramine hydrochloride) (Fluka) and (–) epinephrine ((R)-(–)-3,4-dihydroxy-α-(methylaminomethyl)benzyl alcohol) (Fluka) as freshly prepared aqueous solutions were added to the spore suspensions of actinobacteria immediately before inoculation. The final concentrations of the catecholamines in spore suspensions were 10^{-4} M, 10^{-5} M, and 10^{-6} M. The effects of the catecholamines on the viability and population variability of *S. erythraea* were determined (i) after freezing the cultures for a short time (10 min) by immersing the ampoules with cell suspensions into liquid nitrogen and (ii) during medium-term (three month) storage at low temperatures. The quantitative determination of cell viability was performed by plating *S. erythraea* suspensions on oat agar with 0.25% of yeast extract and counting the colony-forming unit (CFU). The degree of population heterogeneity was estimated from the percentage of colonies of the dominant and the minor morphotypes. The morphotypes were grouped together on the basis of the published data concerning the population composition of *S. erythraea* [6]. The results were processed statistically [7]. A data array was considered homogeneous if the standard deviations did not exceed 11%. The confidence interval level was based on a significance level of 0.05% ($p < 0.05$).

RESULTS

In our preliminary studies, we determined the optimum catecholamine concentration that caused a maximum growth-inducing effect in the tested strains. This concentration was 10^{-5} M. Fig. 1 shows the dependence between the colony number after plating the monospore suspensions and the added dopamine concentration. Inoculating monospore suspensions of strains RIA 1387 and RIA 120 with the addition of epinephrine increased the resulting colony number by 2- and 2.3-fold, respectively, and with the addition of dopamine by 3- and 4-fold, respectively (Table 1). The increase in population density and a qualitative change in the population structure were particularly prominent if dopamine was added to the monospore inoculum of RIA 1387 (Fig. 2). A change in the color of the

[2] The type strain.

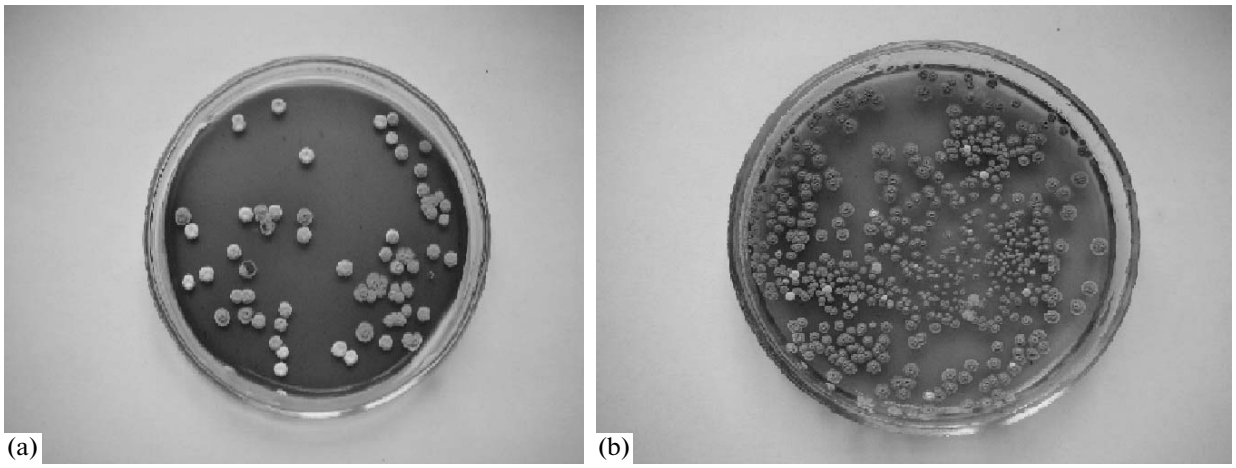

Fig. 2. The influence of the addition of dopamine (10^{-5} M) to the monospore suspension of RIA 1387 on population density: without dopamine (control) (a) and with dopamine added (b).

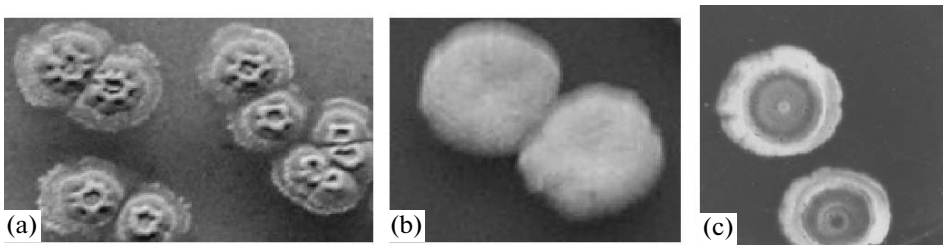

Fig. 3. Intrapopulation variants of *S. erythraea*: main (dominant) (a), white (b), and oligosporic (c).

water-soluble pigment from reddish-brown (Oc5r) in the control (without catecholamines) to fulvous-greenish (Co6r) occurred [8].

Our studies on the populations of both strains enabled us to distinguish between the main (dominant) morphotype and the minor (the white and the oligosporic) morphotypes (Fig. 3). Their basic properties, including antibiotic-producing activity, were studied earlier [6]. After plating the monospore suspensions supplemented with catecholamines, the population composition of the tested cultures underwent significant changes. A drastic change in the population structure occurred, resulting in an increase in the percentage of the colonies of the dominant morphotype characterized by maximum antibiotic productivity (Table 2).

In order to optimize the effect of the catecholamines during storage in liquid nitrogen, we conducted a series of experiments in which we introduced them at various stages of storage of spore suspensions: (i) at the beginning of the storage period; (ii) upon resuscitation; and (iii) twice, both at the beginning of the storage period and upon resuscitation. The effect of a single addition of the catecholamines at the resuscitation stage was studied after freezing the samples for a short time (10 min) in liquid nitrogen. Our results indicate (Table 3) that the viability of both strains after freezing was enhanced by dopamine supplemented to the thawed sample. Adding dopamine twice (before freezing and after thawing) resulted in a significant (two- to threefold) increase in CFU numbers of both strains after three-month storage in liquid nitrogen. The data of our experiments demonstrated that a single addition of dopamine at the resuscitation stage produced practically the same effect as its addition twice (before freezing and upon resuscitation). A certain increase in spore viability occurred when dopamine was added to the protective medium prior to freezing in liquid nitro-

Table 2. Effect of catecholamines on the population structure of strains *S. erythraea* RIA 1387 and RIA 120

Catecholamines (10^{-5} M)	RIA 1387			RIA 120		
	Morphotypes (%)					
	Main	White	Oligosporic	Main	White	Oligosporic
Control (without catecholamines)	58.2	41.6	0.2	84.3	15.5	0.2
Epinephrine	87.8	10.0	2.2	96.0	1.6	2.4
Dopamine	78.5	21.4	0.1	92.9	7.0	0.1

Table 3. Effect of catecholamines on the resuscitation of the spores of *S. erythraea* RIA 1387 and RIA 120 after short-time (10 min) exposure to low temperatures and after medium-term (three month) storage in liquid nitrogen

Addition of catecholamines (10^{-5} M) to the spore suspension	Strain RIA 1387		Strain RIA 120	
	Viability (% of CFU) relative to the control value (100%, without catecholamines)			
	*After freezing (for 10 min)	**After storage (for three months)	***After freezing (for 10 min)	****After storage (for three months)
	Epinephrine			
Before freezing	100	100	100	100
Before freezing and after thawing	100	70	100	54
After thawing	100	ND	100	ND
	Dopamine			
Before freezing	155	146	175	175
Before freezing and after thawing	222.5	312	315	237
After thawing	232	ND	323	ND

Notes: ND stands for not determined.
* $(2.0 \pm 0.2) \times 10^8$ cells/ml = 100%.
** $(2.4 \pm 0.3) \times 10^7$ cells/ml = 100%.
*** $(1.9 \pm 0.1) \times 10^8$ celsl/ml = 100%.
**** $(2.4 \pm 0.1) \times 10^7$ cells/ml = 100%.

Table 4. Changes in the population composition of *S. erythraea* RIA 1387 and RIA 120 in the presence of dopamine (10^{-5} M) during storage (for three months) in liquid nitrogen

Dopamine addition	RIA 1387		RIA 120	
	Morphotypes (% of the total colony number)			
	Main	Minor	Main	Minor
Control (without dopamine)	65.5	34.5	75.0	25.0
At the beginning of storage	91.0	9.0	97.0	3.0
At the beginning of storage and upon resuscitation	98.6	1.4	100	0

gen. The maximum growth-inducing effect of dopamine was attained both after adding it twice and after its one-time addition upon resuscitation.

Importantly, storage resulted in changes in the population composition, so that the content of the dominant morphotype increased (Table 4). In the populations of both strains, the percentage of the minor morphotypes halved after storage in the protective medium with dopamine. As a result of adding dopamine for the second time upon resuscitation, the colonies of the dominant morphotype accounted for almost 100% of the population.

In contrast, the addition of epinephrine to the spores exposed to ultralow temperatures failed to produce growth-inducing and stabilizing effects.

DISCUSSION

Our research revealed that dopamine and epinephrine exerted a stimulatory influence on spore germination in both tested strains of *Saccharopolyspora erythraea* and had a stabilizing effect on their population composition. A significant increase in CFU numbers upon addition of the catecholamines to spore suspensions may be due to a signaling effect of these compounds on initiation of spore germination and further growth. There is evidence in the literature that catecholamines are involved in cytokinesis. It is emphasized that these compounds produce distinct effects at different stages of cell development and they are implicated in cell differentiation [9]. Similar stimulation of spore germination and an effect on cell differentiation were reported for the actinobacteria of the genus *Streptomyces* supplemented with autoregulators of the A factor group [10, 11]. There is evidence that biogenic amines, apart from their growth-stimulating effect, induce cell aggregation with subsequent fruiting body formation in myxobacteria [4]. The activating function of catecholamines is possibly due to their involvement in iron transfer, apart from their cytokinetic effect [2]. Recently, the growth of *S. erythraea* in submerged culture was monitored using microchip

techniques [12]. An important role of iron homeostasis for actinobacterial growth and development was demonstrated. It was established that siderophore production by *S. erythraea* largely depends on the conditions under which the spore inoculum was obtained [13]. In our studies, one of the causes of the growth-stimulating effect of catecholamines could be an increase in siderophore synthesis by actinobacterial cultures after the addition of these compounds to the spore inoculum.

The species-independent growth-stimulating effect of catecholamines on gram-positive and gram-negative bacteria reported by several researchers, suggests that bacteria possess a recognition system for such compounds. Specific dopamine and epinephrine receptors were detected [2]. The biological effects of these compounds and their involvement in metabolic regulation largely depend on the state and activity of the relevant receptors. Obviously, the hormonelike effect of the tested biogenic amines on actinobacteria is due to the operation of appropriate sensory receptors in their cells. This enables the amines to operate as triggering effectors in respect to spore germination and subsequent cell division. Although the issue of the location of respective ligands that perform receptor functions has not been resolved to date, there is evidence of their presence in the cell membranes of gram-negative bacteria [2].

Our studies revealed a significant difference in the biological activity of the tested catecholamines with respect to ultralow temperatures-exposed cultures. Epinephrine produced no biological effect both when added to the protective medium used for three-month storage of actinobacteria in liquid nitrogen and when additionally introduced into the medium at the resuscitation stage. In contrast, the activity of dopamine in the same situation was not only retained, but additionally enhanced, when it was added for the second time upon thawing the spore suspensions. The differences in the biological activity of these catecholamines are likely to be due to the differences in resistance of epinephrine- and dopamine-binding receptor systems to ultralow temperatures. It is known that exposure to low temperatures may change the conformation of receptor molecules, which results in blocking the relevant functional groups that determine their affinity for various catecholamines.

Another important conclusion is that the maximum influence of catecholamines (dopamine) is exerted at the culture resuscitation stage. Studies of the cultures that were frozen in liquid nitrogen for a short time (10 min) revealed a strong effect of the dopamine addition upon thawing the cultures. It was established that a single addition of dopamine upon resuscitation produced the same effect as its two-time addition (Table 3). Apparently, an increase in culture viability with dopamine added before freezing is not related to its cryoprotective effect. Instead, it is probably due to its residual activity still present upon resuscitation.

The analysis of the data obtained demonstrates a polymodal effect of the tested catecholamines on actinobacterial cultures. In our studies, actinobacterial spores were used. They exhibit a more significant genetic heterogeneity than vegetative cells. Potentially, this provides for the generation of a wider spectrum of variants [6, 14]. An important result of these studies is that the population structure of the tested *S. erythraea* strains changed significantly, with the content of the minor morphotypes decreasing in the presence of exogenous catecholamines. The increased homogeneity of the populations was primarily caused by a drastic decrease in colony number of the white nonsporulating morphotype with a concomitant increase in the content of the dominant morphotype. Presumably, population heterogeneity and a loss of the white variant's capacity to form spores did not involve large-scale intragenomic alterations and resulted from disrupting the regulatory mechanisms involved in cell differentiation, as was shown for *S. oligocarbophilus* [15]. In this case, epinephrine and dopamine may operate as signal molecules that trigger intragenomic transitions, apart from initiating spore germination. A similar effect of γ-butyrolactones on weakly differentiated morphotypes was described in streptomycete populations [16].

Interestingly, the addition of dopamine to the spore inoculums of the tested strains of *S. erythraea* resulted in changing in the color of its colonies and of the soluble pigment from reddish–brown to fulvous–greenish. According to the data available in the literature, the color of *S. erythraea* cultures is due to compounds related to flaviolin (2,5,7-trihydroxy-1,4-naphthoquinone), the terminal product of spontaneous oxidation of the precursor compound (1,3,6,8-tetrahydroxynaphthalene) [17]. Presumably, color changes in our studies resulted from incomplete oxidation of flaviolin precursors due to the antioxidant properties of catecholamines [18].

The cyclic structure of the *S. erythraea* [19] genome indicates a significant evolutionary distance between *S. erythraea* and the actinobacteria of the genus *Streptomyces* containing a linear chromosome. Nevertheless, the *S. erythraea* genome possesses a considerable biosynthetic potential. Presumably, secondary metabolism products are a chemical "warehouse" whose exploitation is the basis of the evolutionary strategy enabling mycelial actinobacteria to survive in habitats characterized by intense competition [12]. The catecholamine effect that we observed is comparable to that of hormonelike compounds synthesized by microorganisms. The main function of these compounds is coordinating the primary and secondary metabolism and intensifying the latter under stress [20]. The functions of epinephrine and dopamine manifest themselves in a similar fashion in organisms belonging to different levels of biological evolution.

This facilitates their coexistence in complex ecosystems including micro- and macroorganisms.

Catecholamines can be used at micromolar concentrations in biotechnological processes for the purpose of restoring the viability and morphogenetic stability of actinobacterial cultures producing biologically active substances. In low-temperature microbial banks, they may be used to minimize the destructive effects of ultralow temperatures on microbial cultures.

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