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Emergence of dissociants in colonies of *Rhodococcus erythropolis* - producer of carotenoids

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ABSTRACT

The frequency of dissociative transitions of the studied strain has been determined. It is discovered that at prolonged cultivation the quantity of emerged dissociants in colonies increases. If a colony is in such conditions of growth, when the emerged variants have selective advantages over the initial variant, then dissociation ability will be found quickly and yield of the target product may change.

Keywords: *Rhodococcus erythropolis*, *colony*, *dissociation*

At present heterogeneity of population of microorganisms draws close attention of researchers in connection with development of biotechnology, genetic and cellular engineering, general and medical microbiology, environmental problems and soil microbiology. One of the important processes forming heterogeneity is dissociation, i.e. splitting of population of bacteria on variants differing by genetic, physiological-biochemical and morphological properties, including ability to synthesis of practically valuable substances and growth rate. The S-R dissociation in many cases complicates bacteriological diagnostics of a number of infectious diseases, for example, Sonne dysentery, escherichiosis, caused by *E.coli*, etc. The R-form of bacteria is usual for mycobacterium of tuberculosis, bacilli of anthrax and the causative agent of plague. Reversible dissociative transitions occur with high frequency about 10^2-10^4 per one cell division. Differences of dissociants are of permanent nature (3, 7).

The issue of emergence of dissociants in colonies is not paid sufficient attention. However it is important in the selective breeding of productive variant.

The present study is aimed at measurement of quantity of emerged dissociants in colonies of different ages on the example of R-, S - and M- variants of *Rhodococcus erythropolis* - strain 44 *(Mycobacterium rubrum)* - producer of carotenoids (1-3).

The morphology of colonies and cells of these three dissociants of *rhodococcus* has been described earlier (1). The frequency of dissociative transitions of the studied strain has been determined by the Luria -Delbruck method (3).

$$R \underset{3.10^{-5}}{\overset{5.10^{-5}}{\rightleftharpoons}} S \underset{10.10^{-5}}{\overset{1.10^{-5}}{\rightleftharpoons}} M$$

Table 1: Number of cells in colonies of different age of R-, S - and M- dissociants *R. erythropolis*

Age of colonies, days	Dissociant	Number of diffused colonies	Quantity of cells in 1 colony, x10^7	Diameter of colonies, mm
5-6	R	6	10	2,0-2,5
	S	6	8	2,0
	M	6	8	2,0-2,5
10-12	R	10	65	5,0-6,0
	S	11	55	2,5-3,0
	M	9	66	6,0-7,0
20-22	R	8	50	6,0-7,0
	S	8	60	3,5-4,0
	M	8	74	8,0-9,0

Individual colonies of *rhodococcus* were obtained by screening on agarized medium meat-and-peptone agar + mash (1:1). For determination of total number of cells and a ratio of dissociants in one colony it was cut out by scalpel together with agar and transferred to a sterile test tube with two balls and 2 ml of physiological solution, vigorously stirred up during 5-8 min and then a screening on Petri dishes has been made after a series of tenfold dilutions in physiological solution. 5-10 dishes were taken on last two dilutions. Slowly growing *rhodococci* were incubated at 30^oC during 5-22 days. In each variant of experiment 2000-10 000 colonies were counted. For one dissociant a number of cells in three or four typical colonies of identical diameter, distant from other colonies on a dish not less than on 2 cm were determined simultaneously. Experiment was repeated 2-3 times. Average data are presented in the Tables 1 and 2.

The colonies of three dissociants 5-6-days old slightly differ by diameter and total number of cells (Table 1). By the 10-th-12-th day of growth the diameter of the S-colonies changes slightly, and that of the R-and M-colonies increases 2-3 times. However the quantity of cells in colonies of all dissociants remains identical and makes 5.10^8 - 7.10^8 cells. By the 20-th-22-nd day the diameter of R-, S-and M-colonies and quantity of cells in them changes a little. The increase in the size of colonies at M-variant in comparison with the S - variant is connected with the increased synthesis of carotenoids (2). It is possible to assume that the large size of R-colonies is connected with more friable arrangement of cells in them in comparison with the S-variant. The population of R-cells is the most heterogeneous from the dissociants by electric surface properties (3-6). This assumption requires further study.

Table 2: Ratio of dissociants in multiple-aged colonies of R-, S-and M-dissociants of *R. erythropolis*

Age of colonies, days	Dissociant	Ratio of dissociants in colonies, %		
		R	S	M
5-6	R	99,89	0,11	0
	S	0,06	99,90	0,04
	M	0	0,12	99,88
10-12	R	99,9	0,1	0
	S	0,1	99,8	0,1
	M	0	0,3	99,7
20-22	R	99,8	99,3	0,6
	S	0,1	0,3	99,7
	M	0	0,2	0

The Table 2 shows that the R- and M-colonies of *rhodococcus* of all studied ages contain dissociants, making in a population from 100-th to the tenth of percent. At long cultivation the quantity of the emerged dissociants in colonies increases. When the S-dissociants are aged on dishes there are morphologically atypical colonies. At some colonies the radius of one sector is longer, than that of the rest of colony; the surface of the colony remains brilliant. At screening of such colonies 5-7 % of M-dissociant are revealed in the population. There are the S-colonies with secondary growth; the surface of a colony becomes hilly, especially in the center. At screening of such colonies about 7.5 % of R-cells are found in the population.

Thus, at selective breeding of active variants of a producer it is necessary to consider existence of dissociants in individual colonies. Emergence of dissociants in a colony could be assumed in advance, proceeding from frequency of their emergence. If the colony gets to such conditions of growth where the emerged variant will have selective

advantages over the initial variant, the dissociation ability of a producer will be found quickly and the yield of the target product may change.

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