EFFECT OF A SUPRAOPTIMAL TEMPERATURE ON DISSOCIATION OF CERTAIN SPECIES OF MICROORGANISMS

Yu. V. Kulyash

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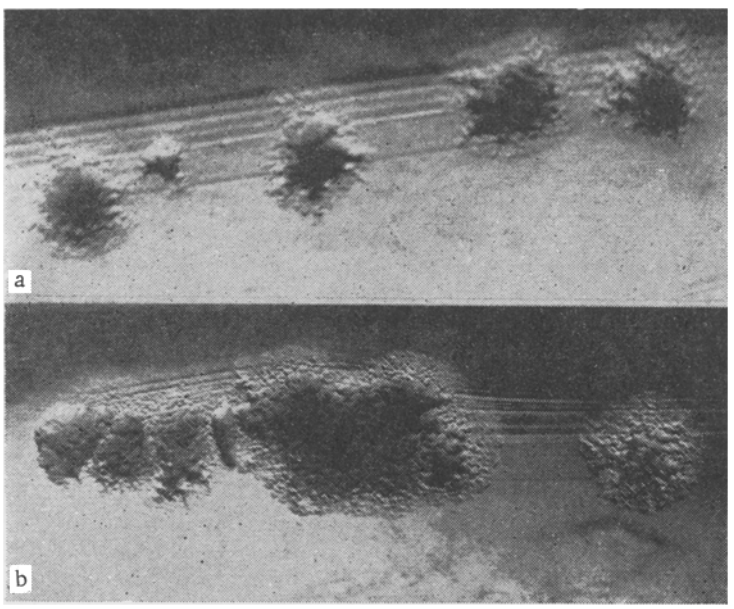
Several investigations [3, 5, 8, 10, 13] have shown that with an increase of temperature the metabolism of microorganisms may change considerably, and sometimes dissociation of the strain takes place [1, 11, 18].

In the present investigation the influence of a supraoptimal temperature was studied on the process of dissociation of Escherichia coli and Staphylococcus aureus. The activity of the acetylation enzyme and the content of citric and lactic acids were determined in S- and R-variants of these strains. The effect of malonic acid on growth of the dissociants was also studied.

EXPERIMENTAL METHOD

The test strains consisted of E. coli and S. aureus in the S-form, cultivated at 37° and 45° on 5% nutrient agar (pH 7.5) supplied by the Daghestan Research Institute of Nutrient Media. Seedings were made on the 3rd day of cultivation. The activity of coenzyme A and the content of citric and lactic acids were determined in the original S-forms of E. coli and S. aureus, grown at 37°, and in the R-variants obtained at 45°. The S-forms of these strains grown at 45° were also investigated at the second passage, before the onset of dissociation. A 24-hour culture, washed twice with 0.85% NaCl solution, was used. The activity of coenzyme A was determined by the method of Handschumacher and co-workers [12], based on the acetylation of p-aminoazobenzene. Citric acid was determined by A. P. Safronov's method [7], and lactic acid by the color reaction with p-hydroxydiphenyl [9]. Malonic acid was used as succinate dehydrogenase inhibitor. The nutrient agar was prepared in $2 \cdot 10^{-2}$, $1 \cdot 10^{-2}$, and $1 \cdot 10^{-3}$ M solutions of malonic acid.



R-forms of E. coli (a) and S. aureus (b) obtained at a temperature of 45°. Magnification 16.

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TABLE 1. Effect of a Supraoptimal Temperature on Process of Acetylation in S- and R-Forms of E. coli and S. aureus (mean data of 10 determinations)

Strain and form	Temp. of cultivation	Acetylated p-aminobenzene (in μg/1000 billion bacterial cells
S-form	37°	
E. coli		35,49±0,98
St. aureus		18,83±2,16
R-form	45°	
E. coli		17,98±1,53
St. aureus		12,30±2,48
R-form	45°	
E. coli		42,49±1,25
St. aureus		41,43±2,97

TABLE 2. Effect of a Supraoptimal Temperature on Content of Citric and Lactic Acids in S- and R-Forms of E. coli and S. aureus

Strain and form	Temp. of cultivation	Citric acid (in μg/25 billion bacterial cells)	Lactic acid (in μg/100 mg crude biomass)
S-form	37°		
E. coli		14,05±1,0	7,15±0,67
St. aureus		16,85±0,53	9,45±0,73
S-form	45°		
E. coli		19,90±0,61	33,45±1,49
St. aureus		10,05±0,72	33,3±1,6
R-form	45°		
E. coli		28,85±0,72	17,15±0,97
St. aureus		35,0±0,64	20,5±1,17

TABLE 3. Effect of Malonic Acid on Growth of S- and R-Forms of E. coli and S. aureus at Different Temperatures

Strain and form	Temp. of cultivation	Concentration of inhibitor (in moles)		
		$2\cdot10^{-2}$	$1\cdot10^{-2}$	$1\cdot10^{-3}$
S-form	37°			
E. coli		++	++	++
St. aureus		++	++	++
S-form	45°			
E. coli		+—	+—	++
St. aureus		+—	++	++
R-form	45°			
E. coli		—	+	++
St. aureus		—	+	++

Note: —) no growth; +—) single colonies; +) scanty growth; ++) moderate growth.

EXPERIMENTAL RESULTS

Cultivation of the S-forms of E. coli (see figure, a) and S. aureus (see figure, b) at 45° showed that with each passage, growth became less luxuriant and the culture either died at the 5th-10th passage or dissociated (in this case growth again became luxuriant). Hence, under the influence of supraoptimal temperature, stable R-variants were obtained, which were kept at 45° for 1 year. The R-forms of E. coli and S. aureus thus obtained did not undergo reversion when grown at 37° during 10 passages.

The results of the determinations of the activity of the acetylation enzyme showed that under the influence of a temperature of 45° the process of acetylation was depressed in the S-forms of these strains, but during subsequent dissociation the indices of acetylation increased, and actually exceeded the original values (Table 1).

According to reports in the literature [13, 17], the pantothenic acid consumption of certain microorganisms increases with a rise of temperature. It may be postulated that the depression of coenzyme A activity during the action of a supraoptimal temperature on S- and R-forms is connected with a deficiency of pantothenic acid, which is probably synthesized more intensively in R-variants as a result of adaptation of their metabolism.

With a rise of temperature to 45° the amount of citric acid in the S-form of E. coli rose by 35%, and in S. aureus it fell by 40%. The amount of lactic acid rose by almost 4 times in both E. coli and S. aureus. In this case it may be concluded that glycolysis was sharply predominant in the S-forms under the influence of a supraoptimal temperature.

In the R-variants grown at 45° an increase was observed in the content of both citric and lactic acid, which was approximately doubled (Table 2).

Malonic acid showed maximal inhibitory action on growth of the R-forms of E. coli and S. aureus, thus suggesting that the intensity of the aerobic processes was higher in them (Table 3). The results given in Table 3 are in agreement with data published in the literature [3, 4, 6].

SUMMARY

Stable R-variants of E. coli and S. aureus were produced at a temperature of 45°.

A study was carried out on the effect of a temperature of 45° on the process of acetylation and on the content of citric and lactic acids in S- and R-forms of given strains. In addition, the effect of malonic acid on the growth of microbes was also studied.

It was established as a result of the experiments that under the action of supraoptimal temperature the process of acetylation is depressed and glycolysis is markedly intensified in the S-forms of E. coli. The R-variants of these strains have shown a certain increase in acetylation, as well as a simultaneous increase in the content of citric and lactic acids. Malonic acid produced the greatest inhibitory effect on the growth of R-forms.

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