BIOCHEMICAL CHARACTERIZATION OF THE VITAL ACTIVITY OF *Yersinia pseudotuberculosis* IN A CULTURE MEDIUM AS A FUNCTION OF THE TEMPERATURE OF CULTIVATION

T. I. Burtseva, S. A. Cherkasova, and G. P. Somov

UDC 577.122.32+577.121.4+
579.222+543.544.42

A biochemical evaluation of the accumulation of amino acids and organic acids in the culture medium during the cultivation of Yersinia pseudotuberculosis str. 2781 as a function of the temperature has been made by methods of quantitative chromatographic analysis. A considerable decrease in the biosynthesis of the products of vital activity under investigation at 37°C as compared with the cultivation of the same microorganism at 6°C has been found. This permits the assumption that the temperature of cultivation of Y. pseudotuberculosis is a factor regulating the biosynthesis of the amino acids and metabolites of the Krebs cycle necessary for its vital activity.

The amounts of individual components (carbohydrates, organic acids, and amino acids) in microbial cells and in their growth m dium reflects changes in the energy metabolism of the cells and their metabolic processes according to the conditions of the ex ernal medium. A determination of the products of the vital activity of microorganisms may be a basis for the subsequent study of the influence of external factors on their biosynthesis and for the discovery of the mechanisms of the homeostatic regulation of the biological systems under investigation.

The object of our investigation was *Y. pseudotuberculosis,* which causes epidemic pseudotuberculosis. It is capable of multiplying within a wide range of temperatures and retains its biological and pathogenic properties at a low temperature [1].

In a recent communication [2] we showed the chemical composition of the low-molecular mass fractions of the microbial cells of *Y. pseudotuberculosis* str. 2781 as a function of the temperature of cultivation. In the present communication we estimate the accumulation in the culture medium of the products of the vital activity of this microorganism; in the present case, organic acids and amino acids, and their subsequent participation in metabolic processes of the microbe under investigation as a function of the temperature of cultivation.

The culture liquid obtained after the cultivation of *Y. pseudotuberculosis* at 6°C and 37°C for predetermined intervals of time was subjected to analysis for its content of organic acids and free amino acids. The investigation was performed over 30 days of continuous cultivation of the microorganism. As a result, at 37°C citric and succinic acids were found in the culture liquid, the most intense accumulation of these products taking place 6-9 h from the beginning of cultivation (Table 1), which corresponds to the phase of exponential growth of this microorganism under these conditions (Fig. 1).

On subsequent cultivation, the dynamics of the accumulation of these products on the culture liquid leveled out and then after about 7 days, began to fall rapidly, reaching its minimum value after 30 days (Table 1) — a time coinciding with the death of the microorganisms (Fig. 1). No other acids of the Krebs cycle could be detected. After 5-6 days of cultivation, pyruvic acid could also be detected in the culture liquid as a precursor of the Krebs cycle and a product of the deamination of serine and cysteine in the metabolic processes of the vital activity of the microorganisms [6].

As a result of cultivation at 6°C, only traces of pyruvic acid were detected in the culture liquid. This can probably be explained by the assumption that under these conditions practically all the pyruvic acid is converted during biosynthesis into citric acid and then the formation of the other organic acids of the Krebs cycle takes place. In favor of this hypothesis are experiments as the result of which, on "cold" cultivation, not only citric and succinic acids but also malic, α-ketoglutaric, and

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Branch, Russian Academy of Sciences, Vladivostok. Scientific-Research Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences, Vladivostok.
Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 439-442 and 446-447, May-June, 1993. Original article submitted August 13, 1992.

0009-3130/93/2903-0376$12.50

TABLE 1. Metabolism of Organic Acids in the Culture Medium as a Result of the Vital Activity of *Y. pseudotuberculosis* str. 2781 as a Function of the Temperature of Cultivation*

Organic acid	Content (mg/ml) at the following time of cultivation, h															
	6	12	72	168	240	360	480	720	6	12	24	48	72	168	240	720
	6°C								37°C							
Citric	—	4	46,6	25,3	30,7	29,8	25,1	15,7	25,3	79,1	85,3	70,3	70,1	50,7	31,6	2,3
Malic	—	—	21,3	37,8	30,1	35,7	22,1	13,3	—	—	—	—	—	—	—	—
Succinate	—	34,6	129,3	42,6	30,2	33,1	22,6	18,3	70,6	98,6	95,3	92,1	90,3	63,7	39,6	5,8
α-Ketoglutaric	—	—	8,5	12,0	30,6	30,8	19,3	7,1	—	—	—	—	—	—	—	—
Fumaric	—	—	46,8	50,1	48,7	42,5	12,3	—	—	—	—	—	—	—	—	—
Pyruvic	—	—	Traces						5,3	9,2	20,3	37,2	32,2	13,8	4,6	—

*All the values given in the Table must be multiplied by 10^{-4}.

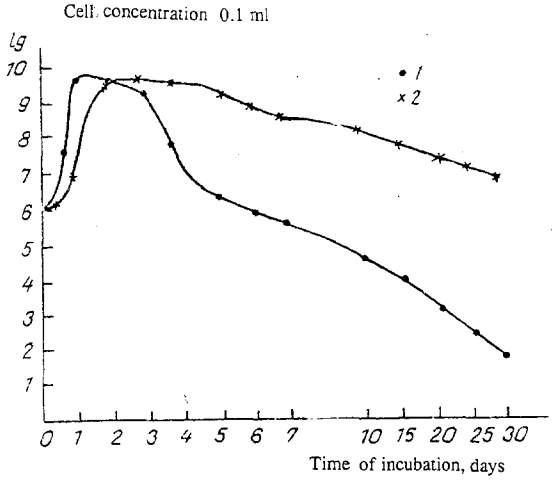

Fig. 1. Curves of the growth of *Y. pseudotuberculosis* str. 2781 at 37°C (1) and 6°C (2).

fumaric acids were detected in the culture liquid in appreciable amounts (Table 1). It must be mentioned that all the organic acids of the Krebs cycle were completely absent from the nutrient broth; i.e., the acids that we had detected in the culture liquid were the products of the vital activity of the microorganism under investigation.

In the "cold" cultivation, all the organic acids shown in Table 1 were detected to a greater or smaller degree over the whole of the experiment beginning from the phase of exponential growth of the microbe. After 17-18 days from the beginning of cultivation, the concentration of organic acids began gradually to fall and then, at the end of the experiment (25-30 days) reached its minimum value. This probably coincides with the beginning of the death of the microorganism (Fig. 1).

Peculiarities of the metabolism of amino acids under the given conditions for cultivating *Y. pseudotuberculosis* str. 2781 consisted in the fact that at 37°C the cultivation of the microorganism took place mainly with intense absorption of free amino acids from the nutrient broth, while at 6°C an almost total decrease in the consumption of free amino acids from the broth was observed except for a slight consumption of aspartic acid and serine. Then the concentration of these amino acids stabilized, with a subsequent gradual decrease in it up to the time of the death of the microorganism (Table 2). Our results agree with those of I. V. Domaradskii showing that on the 'cold' cultivation of *Y. pseudotuberculosis* the consumption of amino acids by it falls [4].

The results that we obtained require further more concrete investigation but, nevertheless, the experiments performed indicate that the cultivation of *Y. pseudotuberculosis* at 37°C independently of the composition of the nutrient medium is accompanied by a definite decrease in the biosynthesis of the metabolites of the Krebs cycle and of the free amino acids necessary for the subsequent biosynthesis of proteins from them. Thus, possessing various enzyme systems for the metabolism of organic amino acids, this microorganism performs the synthesis and decomposition of these substances to a considerable degree at a low temperature. At an elevated temperature the normal mechanism of biosynthesis is disturbed or, for some compounds it ceases completely. The microorganism therefore switches on its metabolic pathways to the consumption of substances to a greater degree from outside.

It is obvious that in the human and animal organism a rise in the temperature may exert a similar influence on the organism under investigation, inhibiting its biological activity in the biosynthesis of product necessary for vital activity. But in the macroorganism the causative agent of pseudotuberculosis uses available metabolites for growth and multiplication, including amino acids and organic acids. All this creates the condition for the saprophytic nutrition of *Y. pseudotuberculosis* and the possibility of its existence outside the macroorganism — for example, in the soil, in vegetable crops, dairy products, etc.

Thus, the investigation that we have performed also provides evidence in favor of the assumption that the vital activity of *Y. pseudotuberculosis* at a low temperature is natural for it.

TABLE 2. Amino Acid Metabolism in the Culture Medium as the Result of Vital Activity of *Y. pseudotuberculosis* str. 2781 as a Function of the Temperature of Cultivation*

Amino acids	Nutrient broth	Amount (mg/ml) at the following times of cultivation, h															
		6	12	72	168	240	360	480	720	6	12	24	48	72	168	240	720
		6°C								37°C							
Aspartic acid	11,5	10,3	9,2	8,9	9,6	16,7	16,7	15,4	12,8	4,9	3,2	1,6	4,7	5,9	6,1	3,7	1,3
Threonine	13,0	13,2	14,2	20,2	18,9	20,5	22,7	18,9	15,4	12,7	9,3	7,4	10,3	11,3	13,7	12,1	9,4
Serine	11,9	10,3	9,3	11,9	15,4	21,7	20,3	19,8	18,7	1,7	1,2	0,9	7,8	8,5	7,3	7,8	6,9
Glutamic acid	17,1	17,8	17,6	32,0	32,2	37,5	32,8	33,5	31,8	8,3	6,5	12,0	18,3	18,5	19,1	15,4	14,1
Glycine	7,6	7,3	7,1	13,0	16,9	18,5	20,7	21,2	19,8	8,9	7,3	5,4	7,6	7,3	8,5	7,9	7,5
Alanine	26,2	26,4	27,3	51,3	68,0	65,5	63,7	60,8	61,1	15,3	10,2	17,9	27,3	28,3	27,9	26,2	24,7
Valine	22,2	23,7	24,3	42,4	62,0	65,7	63,8	57,9	55,2	19,7	15,3	10,0	13,4	17,3	19,4	20,5	22,4
Methionine	14,6	14,7	14,5	24,6	28,3	25,7	27,2	25,4	23,1	8,6	7,2	6,9	12,7	14,7	15,8	12,1	10,1
Isoleucine	15,8	18,7	21,3	35,7	49,3	43,5	47,6	40,2	38,7	21,1	17,4	13,0	17,3	15,7	17,9	18,2	18,6
Leucine	36,2	38,2	45,9	77,3	102,2	97,5	98,2	85,6	70,3	25,3	23,7	24,9	31,3	37,8	38,1	37,9	39,1
Tyrosine	24,2	24,9	27,8	31,8	44,1	48,5	47,6	32,7	31,8	18,2	15,3	10,9	15,7	17,3	17,8	17,9	17,4
Phenylalanine	22,2	27,8	32,4	49,3	62,5	60,8	63,5	67,8	57,5	18,2	23,7	18,0	17,4	19,4	19,8	16,4	15,5
Lysine	26,0	29,7	32,4	62,1	79,8	80,5	79,8	78,4	61,5	23,2	22,5	22,3	24,1	25,6	27,8	28,1	30,5
Histidine	8,3	8,2	7,8	18,2	21,1	25,4	23,7	24,9	21,3	10,3	8,7	6,1	9,2	12,3	10,1	9,7	8,0
Arginine	12,3	13,8	17,7	34,3	33,7	38,5	30,1	28,5	22,8	8,3	5,6	12,5	13,1	17,4	16,5	16,1	14,6
Proline	5,1	5,2	6,1	9,5	7,7	8,9	9,2	7,3	6,2	8,7	7,3	6,7	7,5	8,7	8,1	8,7	8,1

*All the values given in the Table must be multiplied by 10^{-4}.

EXPERIMENTAL

The microorganism *Y. pseudotuberculosis* was cultivated in meat-peptone broth containing 0.1% of glucose at 6°C and 37°C for 30 days. After predetermined intervals of time, 5-ml samples of the culture medium were taken. Each aliquot was used for seeding on Petri dishes containing nutrient medium, and after a day the number of colonies of the microorganism in the dishes per 1 ml of culture medium were calculated. The results obtained were used to plot a curve of the growth of the microorganism.

The microbes were isolated from the remainder of each aliquot by centrifugation at 6000 rpm. The culture liquid obtained was used for the subsequent biochemical investigations.

Descending paper chromatography was performed on Filtrak FN-15 and FN-12 papers. For the chromatography of the organic acids and amino acids we used the following solvent systems, respectively: 1) diethyl ether—85% formic acid—water (70:1:9); and 2) *n*-butanol—acetic acid—water (40:15:5).

Organic acids were detected with a 0.05% solution Bromophenol Blue in ethanol, and amino acids with a solution of ninhydrin in acetone [5].

The quantitative analysis of the free amino acids was conducted in a Hitachi-835 high-speed amino acid analyzer on a 2.6 × 1250 mm column with resin No. 2619. Before the performance of the quantitative analysis of the amino acids, the preparation was freed from proteins, carbohydrates, organic acids, and other impurities as described in [6].

The quantitative content of organic acids was determined by elution from the chromatogram with 70% ethanol followed by redox titration with a 0.01 N solution of sodium hydroxide [sic].

REFERENCES

1. G. P. Somov, *Far Eastern Hemorrhagic Fever. Human Pseudotuberculosis* [in Russian], Leningrad (1978), pp. 3-13.
2. S. A. Cherkasova, T. I. Burtseva, and G. P. Somov, *Khim. Prir. Soedin.*, No. 3-4, 312 (1992).
3. L. S. Olenicheva and G. T. Atarova, in: *The Direct Deamination of Amino Acids by the Causative Agent of Pseudotuberculosis. Physiology and Genetics of the Microorganisms.* Proceedings of a Conference of Microbiologists of the Northern Caucasus [in Russian], Rostov-na-Donu (1969), pp. 70-73.
4. I. V. Domaradskii, *The Causative Agents of Pasteurelloses and Closely Related Diseases* [in Russian], Meditsina, Moscow (1978), pp. 113-146.
5. I. M. Hais and K. Macek, *Paper Chromatography*, Academic Press, New York, 3rd edn. (1963).
6. E. N. Andreyuk, E. V. Valagurova, and N. N. Mal'tseva, *Instrumental Methods in Soil Microbiology* [in Russian], Kiev (1982), pp. 83-87.