CHEMICAL COMPOSITION OF THE LOW-MOLECULAR-MASS FRACTION OF YERSINIA PSEUDOTUBERCULOSIS AS A FUNCTION OF THE TEMPERATURE OF CULTIVATION

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The dependence of the chemical composition of the low-molecular-mass fraction of the microbial cells of Yersinia pseudotuberculosis (str. 2781) on the temperature of cultivation (6 and 37°C) has been investigated.

The surrounding medium exerts a substantial influence on the morphological and biological properties of bacteria, the temperature factor being one of the most important and determining the nature of the metabolism of the bacteria and the choice by them of alternative routes of biochemical adaptation to changing conditions. Particular interest is presented by the relationship with the temperature of pathogenic bacteria – facultative parasites possessing a dual (saprophytic and parasitic) nature, i.e., capable of multiplying (living) both in the organisms of warm-blooded animals with their relatively high and constant temperature and also in materials of the environment with low and continuously varying temperature.

Such microorganisms include the pseudotuberculosis microbe which causes endemic pseudotuberculosis and is capable of multiplying over a wide range of temperatures (from 0 to 40°C) [1]. It possesses psychrophilic properties [2] and, moreover, a low temperature of cultivation favors the activation of certain pathogenicity factors of this microorganism and, consequently, a rise in the virulence of the population. Raising the temperature leads to a fall and, after a number of successive passages, even to the complete loss of virulence [3].

The study of the chemical composition of the pseudotuberculosis microbe at various temperatures of cultivation is necessary for the elucidation of the mechanisms of its high metabolic plasticity. Up to the present time, the chemical composition of the main components of its outer membrane have been studied as a function of the temperature of cultivation and so have the polypeptide, carbohydrate, and fatty-acid compositions of the bacterial cells [4, 5] and the activity of the enzymes at various temperatures (from 0 to 42°C) [6, 7].

We have studied for the first time the chemical composition of the low-molecular mass fraction (LMF) of the microbial cells of Y. pseudotuberculosis (strain 2781) as a function of the temperature of cultivation (6 and 37°C). As is known, this fraction is a substrate for the biosynthesis of the main high-molecular-mass components of the cell (proteins, carbohydrates, glycoproteins, etc.).

The microorganism was cultivated on meat-peptone broth containing 0.1% of glucose, at 6 and 37°C. For investigation we took the microbial mass in the stationary growth phase. A suspension of the microbes was separated by centrifugation at 4000 rpm and was washed with physiological solution three times. The LMF was obtained by alcoholic extraction from the microbial mass of the bacteria [8].

The analytical results (Table 1) showed a predominance of carbohydrates and nucleic acids and also of phosphorus, free and bound, in the "hot" variant, which indicates the existence of more intensive metabolism in the process of cultivating this microorganism at 37°C as compared with low-temperature (6°C) cultivation.

No free monosaccharides were detected in a study of the carbohydrate composition by the PC method in solvent system 1 (see Experimental) in LMFs from the "hot" and "cold" variants.

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TABLE 1. Analytical Characteristics of the LMF of Yersinia tuberculosis, Strain 2781, as a Function of the Temperature

Conditions of cultivation, °C	Composition, %						
	carbohydrate	protein	nucleic acids	heptoses	organic phosphates	inorganic phosphates	succinic acid
6	12,7	13,6	27,1	0,64	3,1	0,78	8,35
37	18,8	17,3	30,1	0,40	3,8	1,10	3,72

TABLE 2. Characteristics of the LMF of Y. pseudotuberculosis Strain 2781, as a Function of the Temperature Cultivation ($\times 10^{-2}$)

Conditions of cultivation, °C	Monosaccharide				Phosphoric esters				Free amino acids, % on dry substance															
	$GlcNH_2$	Glc	Man	Rib	Glc	Man	Rib	глицерин	Asp	Thr	Ser	Glu	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg	Pro	Cys
6	+	+	+	+	—	—	—	+	Trace amounts												—		—		
37	+	+	Tr.	+	+	+	+	+	1,2	1,5	5	2	1	3,5	3	1,5	2	4,5	3	3,0	3,5	0,5	1,5	0,4	—

Spots were revealed at the level of oligosaccharides. Acid hydrolysis and the PC of the hydrolysates in the same solvent system showed the presence of Glc, Rib, and $GlcNH_2$ in the fractions investigated. In the preparations of the "cold" variant, Man was detected, in addition. In the "hot" variant, only traces of this monosaccharide were found (Table 2). The chromatographic investigation of the preparations in solvent system 4 showed the presence of phosphorylated sugars in them. The PC of these preparations in system 1 before and after their treatment with acid phosphatase revealed phosphoric esters of Glc, Man, Rib, and glycerol in the "hot" variant, the phosphorylated Man being detected in the predominating amount, but only phosphorylated Rib and phosphorylated glycerol in the "cold" variant (see Table 2). It is obvious from the results obtained that a considerable part of the Man and Glc passes into the phosphorylated state during the "hot" cultivation of the microorganism under investigation.

These results permit the assumption that on the "hot" cultivation of the pseudotuberculosis microbe the energy metabolism is intensified, which is probably connected with adaptation to the high temperature. At the same time, this is evidence in favor of the assumption that "cold" cultivation is more natural for this microorganism, since it is a facultative psychrophile [5, 7].

Investigations of the preparation by the PC method in system 3 and on an amino acid analyzer showed the presence of free amino acids in the LMF of the "hot" variant but only trace amounts of them on "cold" cultivation (Table 2). This confirmed the hypothesis put forward previously that with a lowering of the temperature of cultivation the demand for amino acids by the pseudotuberculosis microbe decreases [9].

Of organic acids, in the LMF of the microbial cells we detected succinic acid by the PC method in solvent system 2 while twice as much of it was found in the "cold" as in the "hot" variant. Consequently, on "cold" cultivation the metabolic processes with the participation of organic acids and amino acids take place more economically.

Thus, on the basis of the results that we have obtained it may be assumed that the choice of routes for the metabolism of organic acids and amino acids in the microorganism investigated depends on the temperature of cultivation and, in its turn, affects the biosynthesis of high-molecular-mass compounds (proteins, carbohydrates, lipids, etc.).

EXPERIMENTAL

Descending PC was conducted on Filtrak FN-15 and FN-12 papers. The following solvent systems were used: 1) butan-1-ol–pyridine–water (6:4:3); 2) diethyl ether–85% formic acid–

water (70:1:9); 3) butan-1-ol–acetic acid–water (40:15:5); and 4) n-propanol–ammonia–water (6:3:1). Monosaccharides and sugar alcohols were detected with a saturated solution of silver nitrate in acetone, phosphoric esters by means of the Isherwood reagent, amino acids with a 0.5% solution of ninhydrin in acetone, and organic acids with a 0.5% solution of Bromphenol Blue in ethanol [10].

The quantitative analysis of the free amino acids was performed on a Hitachi-835 high-speed amino acid analyzer using a column (2.6 × 250 mm) containing the 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 [11].

The total amount of monosaccharides was determined by the phenol/sulfuric acid method [12], proteins by Lowry's method [13], nucleic acids according to Spirin [14], heptoses by a modification of Similova's method [15], and phosphorus by Chen's method [16].

The organic acids were determined quantitatively by elution from the chromatogram with 70% ethanol followed by redox [sic] titration with 0.1 N NaOH.

Isolation of the Low-Molecular-Mass Fraction. The moist microbial cells were extracted with boiling 80% ethanol to stop enzymatic processes and were centrifuged at 12,000 rpm for 30 min. The deposit was treated successively with hot 40% and 20% ethanols and with hot distilled water. The supernatants were combined, evaporated to the minimum volume, and lyophilized. The preparation obtained was used for the investigations.

Acid Hydrolysis. The preparation (10 mg) was subjected to hydrolysis with 2 N HCl at 100°C for 3 h and was neutralized by repeated evaporation with double-distilled water. The hydrolysate was used for the chromatographic investigation.

Enzymatic Dephosphorylation. The preparation (5 mg) was dissolved in 1 ml of acetate buffer (pH 5), and 0.5 ml of acid phosphatase with a concentration of 1 mg/ml in the same buffer was added. The mixture was kept overnight in a thermostat at 37°C and was then treated rapidly with ion-exchange resins (anion- and cation-exchangers) and filtered and the filtrate was evaporated in a vacuum evaporator to dryness [17]. The dry residue investigated chromatographically in solvent system 1.

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NATURAL EFFECTORS OF β-1,3-GLUCANASES

ACTIVATOR OF THE β-1,3-GLUCANASES OF MARINE MOLLUSKS

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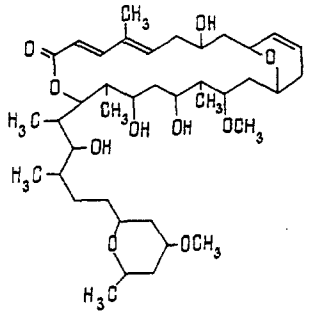
The capacity of certain extracts of marine invertebrates of the Indian and Pacific Oceans for activating β-1,3-glucanases has been detected. It has been shown that an individual compound – swinholide A from the sponge Theonella swinhoei – specifically activates β-1,3-glucanases of bivalve mollusks. In the presence of swinholide A both the hydrolysis of laminarin by β-1,3-glucanase L-IV from Spisula sachalinensis and the transglycosylation reaction are accelerated.

The search for effectors of β-1,3-glucanases in extracts of marine invertebrates of the Indian and Pacific Oceans has shown that, together with an inhibiting action [1], some metabolites bring about an activation of the enzymes. More than 300 extracts of invertebrates belonging to the most important systematic groups have been tested. It has been established that the most promising sources of activators are soft corals, sponges, and ascidians. Thus, 12 extracts out of 100 samples of soft corals, one out of 125 samples of sponges, and five out of 44 samples of ascidians activated β-1,3-glucanase L-IV from Spisula sachalinensis.

An individual compound of natural origin accelerating the enzymatic reaction of β-1,3-glucanases has been found for the first time. The substance, isolated from the sponge Theonella swinhoei has been identified as a representative of the macrolides – swinholide A [2, 3]. Macrolides from sponges form a new group of physiologically active compounds that have no structural analogues among related substances of microbial origin. It is known that swinholide A possesses antifungal activity [3].



Swinholide A

We have found that swinholide A is an activator of two isoenzymes: endo-β-1,3-glucanases L-III and L-IV from the crystalline style of the bivalve mollusk S. sachalinensis. Activation by swinholide A is expressed to a smaller degree in relation to the endo-β-1,3-glucanase L-O from the marine mollusk Chlamys albidus. Under the same condition, no changes

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