

INFLUENCE OF THE TEMPERATURE OF CULTIVATION ON THE LIPID
COMPOSITION OF THE BACTERIUM *Yersinia pseudotuberculosis*

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The temperature of cultivation exerts a considerable influence on the composition and biological properties of the components of the outer membranes of Gram-negative bacteria [1, 2]. *Yersinia pseudotuberculosis* (the causative agent of Far Eastern scarlatina-like fever) belongs among the psychrophiles and demonstrates an anomalous, in comparison with other Gram-negative bacteria, behavior with a rise in the growth temperature that consists in an increase in the number of molecules of lipopolysaccharide (LPS) with a shortened O-specific carbohydrate chain when it is grown at 37°C [3].

The biosynthesis of the LPS takes place with the participation of phospholipids (PLs) [4], the composition and physical state of which also depend very strongly on the surrounding medium [5]. The nonstandard behavior of the LPS of *Y. pseudotuberculosis* with a change in the conditions of growth has impelled us to study the phospholipid composition of this bacterium at various temperatures of cultivation.

The lipids were extracted with chloroform-methanol (2:1, vol./vol.) as described in [4]. The PLs were separated from the neutral fraction of the lipids by precipitation with acetone, and they were separated by two-dimensional TLC in the systems of [6, 7] and were identified by comparison with authentic samples using specific reagents [6]. The amount of PLs was determined from the phosphorus content [8]. The fatty-acid composition after hydrolysis with 6 N NaOH was determined with the aid of GLC and chromatomass spectrometry [9] (Table 1).

It was established that the amount of PLs in extracts of PLs in extracts of the microbial mass of *Y. pseudotuberculosis* depends on the temperature of growth of the bacterium and reaches a maximum when it is grown in the cold. Of the total lipids of this bacterium, 90% consists of PLs. The microbial cells grown at 37°C contain a considerably smaller amount of PLs (~30% of the total lipid content), which agrees with results obtained on the bacterium *Escherichia coli* [5].

As has been shown previously [10, 11] the main components of the phospholipid fraction of lipid extracts of Gram-negative bacteria are cardiolipin (CL), phosphatidylglycerol (PG), and phosphatidylethanolamine (PE). In actual fact, the proportion of these PLs in *Y. pseudotuberculosis* at all the temperatures studied ranged from 91 to 97%. With a variation in the temperature of cultivation the PE content underwent the smallest changes and reached a maximum on growth in the cold. The CL and PG were more sensitive to a variation in the temperature, which is apparently connected with features of the biosynthesis of these PLs [11], but they are also synthesized in larger amount at low temperatures.

An important element of the structure of PLs, regulating the synthesis of the LPS, is formed by fatty acids [4]. A study of the qualitative fatty-acid composition of the pseudotuberculosis bacterium showed that it was similar to that of other Gram-negative bacteria, did not depend on the temperature, and included the following fatty acids: palmitic [m/z 270 (M⁺); 239 (M - 31); 227 (M - 43)]; palmitoleic [m/z 268 (M⁺); 236 (M - 32); 194 (M - 74)]; cycloheptadecanoic [m/z 282 (M⁺); 250 (M - 32); 208 (M - 74)]; and oleic [m/z 296 (M⁺); 264 (M - 32); 222 (M - 74)]. They were present both in the free form and as components of the PLs.

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TABLE 1. Phospholipid and Fatty-Acid Compositions of *Y. pseudotuberculosis*

T. °C	Yield, %			Amount, %												C _{16:1} / C _{16:0}	C _{18:1} / C _{18:0}	
				phospho- lipids			Fatty acids											
	C _{16:0}		C _{16:1}				C _{17:0}		C _{18:1}		C _{16:1} / C _{16:0}		C _{18:1} / C _{18:0}					
	ΣLs	PLs	FAs	Cl	PG	PE	PLs	FAs	PLs	FAs	PLs	FAs	PLs	FAs	FLs	PLs	PLs	FAs
4	5.4	4.8	0.5	6.71	1.94	9.35	20.3	31.4	52.1	19.1	9.5	1.5	17.7	27.5	2.58	1.94	0.87	1.87
21	4.0	1.3	1.7	7.43	4.4	64.90	23.5	44.1	28.7	24.8	86.2	11.4	12.3	13.7	1.19	0.5	0.52	0.45
37	4.0	1.2	2.8	14.67	7.37	78.84	27.9	52.0	17.7	20.4	44.8	9.2	9.6	9.8	1.61	0.39	0.34	0.19

With a rise in the growth temperature the degree of unsaturation of the fatty acids of *Y. pseudotuberculosis*, like those of other Gram-negative bacteria, decreased by a factor of 3-4 (see Table 1). Thus, in spite of the special position of the pseudotuberculosis microbe due to its psychrophilicity the variations in the compositions of the phospholipids and fatty acids in it respond to a change in the conditions of growth are similar to those that are observed in other enterobacteria and are apparently not connected with the anomalous behavior of the LPS of *Y. pseudotuberculosis* with a change in the temperature of cultivation.

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