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Glucose as a Growth Medium Factor Regulating Lipid Composition of *Yersinia pseudotuberculosis*

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Abstract—Effects of glucose and growth temperature on *Yersinia pseudotuberculosis* O:1b serovar lipid composition have been studied. These growth parameters were shown to have drastic effects on biosynthetic processes in the pseudotuberculosis bacteria. The temperature effect is the most universal, extending to cell growth and to free lipid and lipopolysaccharide content and composition; it is most conspicuous in the bacteria cultivated on glucose-containing nutrient broth. The effect of glucose is selective, affecting only free lipids and depending on temperature (glucose favors phospholipid (PL) synthesis in the cold and inhibits it at 37°C); the effect of glucose is more evident in the cold. Determination of the contents of individual PL in percent dry bacterial weight indicates that the most obvious effect of glucose and/or growth temperature is on phosphatidylethanolamine (PE) content: on both media and at both temperatures an overall decrease in PL content stems from the inhibition of PE synthesis and is attended by decreasing ratio of neutral to acidic lipids.

Key words: glucose, growth temperature, phospholipids, neutral lipids, lipopolysaccharide, *Yersinia pseudotuberculosis*

Bacterial lipids are biologically important components of bacterial cell membranes. They are important in various metabolic and structural tasks and they play an essential role in defining the permeability barrier of the bacterial membrane. The functional significance of lipids is largely determined by their localization in the cell envelope, their structural heterogeneity, the ability for rapid adaptation with changes in environmental conditions, and the presence of polar groups in their molecules [1].

Phospholipids and lipopolysaccharides are the basic polar lipids of Gram-negative bacteria [2, 3]. Though their biosynthetic pathways are rather well known [4, 5], the mechanisms regulating these processes are not sufficiently investigated. Studies on *Escherichia coli* and related bacteria have revealed that the absolute lipid content and composition depends rather little on culture conditions and appears to be regulated within the cells [1, 4, 6-8]. Changes in structural lipids required for adaptation of bacteria to changing growth conditions involve mainly the hydrophobic part of the lipid molecules, leaving constant the polar groups and, especially, the ratio of uncharged zwitterionic phosphatidylethanolamine to the sum of acidic phosphatidylglycerol and diphosphatidylglycerol having negative charge [4]. Marked changes in polar lipid composition occur when cells experience extreme environmental factors [9] or when the level of specific enzymes is decreased due to mutation [10, 11].

In this communication, we show that the presence of glucose in the medium causes significant changes in the polar lipid composition of *Yersinia pseudotuberculosis*, an enteric organism with psychrophilic properties [12]. Glucose affects only free cell lipids (phospholipids and neutral lipids), and the nature of its influence is affected by growth temperature.

MATERIALS AND METHODS

Bacterial strains and their cultivation. Strain KS 3058 of *Y. pseudotuberculosis* O:1b serovar, which is typical in morphological, cultural, biochemical, and antigenic properties, was used in this study. This plasmid-free strain was chosen to eliminate the possible influence of plasmids on lipid synthesis; it was obtained as described in [13]. Experiments were performed at 5°C (the "cold" variant) and at 37°C (the "warm" variant) since growth temperature is one of the prime concerns in the ubiquity of *Y. pseudotuberculosis* in nature [12] and was shown to have a

Abbreviations: DPG) diphosphatidylglycerol; LPS) lipopolysaccharide; NL) neutral lipids; NB) nutrient broth; PE) phosphatidylethanolamine; PG) phosphatidylglycerol; PL) phospholipids.

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significant effect on the composition of the bacterial cells [14, 15]. The bacteria were grown on nutrient broth (NB, Makhachkala, Russia) or on NB in the presence of 0.5% glucose (NB + Glc) in vessels (1 liter) with intensive aeration for 6 days at 5°C or 36 h at 37°C (in both cases the times correspond to stationary growth phase [16]). The media used were always from the same batch and were prepared identically for each experiment. When the bacteria had reached the desired phase, the cells were killed by adding 1% phenol and incubating for 20 min. Then the cells were separated from the culture liquid by centrifugation at 4,000 rpm for 20 min.

Isolation and characterization of lipids. For free lipid isolation, bacterial cells were treated with a mixture of $CHCl_3$–CH_3OH (2 : 1 v/v) at 5-8°C [14]. Lipid extracts were separated by two-dimensional thin-layer chromatography in systems $CHCl_3$–CH_3OH–C_6H_6–28% NH_4OH (65 : 30 : 10 : 6 v/v) (1st direction) and $CHCl_3$–CH_3OH–AcOH–CH_3COCH_3–C_6H_6–H_2O (70 : 30 : 4 : 5 : 10 : 1 v/v) (2nd direction) [17, 18]. The PL were identified by comparison with authentic standards using specific spray reagents [18]. The total content of PL in cells (in percent dry bacterial weight) and of individual PL (in percent of total PL) were determined based on the phosphorous content in lipid extracts by the method described in work [19].

Isolation and characterization of lipopolysaccharides. The total content of LPSs in the cells was determined from the amount of 3-hydroxytetradecanoic acid, a constitutive component of the *Y. pseudotuberculosis* LPS lipid moiety [20], in alkaline hydrolyzates of dry bacteria (6 M NaOH, 100°C, 4 h) assuming that the molar ratio of this fatty acid in the LPS is genetically controlled and remains unchanged under all growth conditions [21] and using eicosanic acid as an internal standard. For isolation of LPS, defatted cells were extracted with 45% hot aqueous phenol by the Westphal method [22] as described in [23]. The polymerization degree of the O-specific polysaccharides in the LPS was determined by calculating the molar ratio of mannose (the *Y. pseudotuberculosis* O:1b serovar LPS O-specific side chain monosaccharide [24]) to the sum of L-glycero-D-manno- and D-glycero-D-manno-heptoses (the core oligosaccharide [25]). Monosaccharides, with D-xylose as an internal standard, were derivatized to their alditol acetates after hydrolysis (1 M trifluoroacetic acid, 100°C, 2 h) and analyzed by GLC.

All the data presented here are means of three or more independent experiments. The range of experimental errors did not exceed 5%.

RESULTS AND DISCUSSION

To estimate the roles of glucose and cultivation temperature in *Y. pseudotuberculosis* polar lipid synthesis, we compared the free lipid and LPS compositions of bacteria grown either on pure NB or on NB containing 0.5% glucose (NB + Glc) at different temperatures. As seen from the data of Table 1, glucose had no effect on the growth of *Y. pseudotuberculosis* at low temperature. Both "cold" variants of these bacteria grew uniformly well giving high biomass yields and almost equal amounts of free lipids.

PL and NL were the main components of lipid extracts. However, their relative contents changed on changing the composition of the medium. A greater quantity of PL (4.6%) resembling that earlier found in *Y. pseudotuberculosis* cells grown on peptone in the presence of glucose with PL pattern typical for this organism (PE, PG, and DPG [26, 27], Table 2, column 1) was synthesized in the bacteria cultivated on glucose-containing NB (Table 1, column 1; Table 2, column 2). The main PL was PE, which constituted 78.9% of the total PL fraction.

Elimination of glucose from the growth medium repressed of PL synthesis. Consequently, the amount of PL extracted from these cells did not exceed 1.7% (Table 1, column 2). The low content of PL was compensated by increasing NL fraction, which dominated under these conditions. In addition to PE, PG, and DPG, mono- and dimethyl ethers of PE, lyso-PE, and also PL-X of unknown structure were detected in lipid extracts of the bacterium (Table 2, column 3). The contribution of PE to the total PL was reduced to 44%, the level of PG was unchanged, and that of DPG was increased. As a result, the ratio of uncharged zwitterionic PL to the sum of negatively charged PL (PE/(PG + DPG)) was decreased more than threefold.

A quite distinct change in lipid composition caused by the presence of glucose in the growth medium was observed in *Y. pseudotuberculosis* grown at 37°C. Although at this temperature growth of the bacterium was greatly impaired on both media (biomass yields from the "warm" variants were 5 or 10 times less than they were from the "cold" cells, Table 1), the bacterium grew slightly better on glucose-containing NB (the yield of biomass was twofold greater) than they did on glucose-free NB. The degree of restriction of aerobic bacterial growth somewhat depends on incubation temperature. Because the solubility of oxygen in water increases with decreasing temperature, the growth of microorganism at lower temperatures is less limited by the availability of oxygen. This may explain why the total biomass yield of *Y. pseudotuberculosis* grown in the cold was greater than that at 37°C. It was also shown that despite fast cell division at physiological temperature, only 34-46% of the inoculated pseudotuberculosis cells grew, while in the cold practically all (96-100%) cells grew [28]. However, the clear tendency to increasing biomass yield in a case of the "warm" variant growing on glucose-containing NB at 37°C shows that glucose is a preferred substrate for *Y. pseudotuberculosis*, at least at 37°C.

PL synthesis in the "warm" variant was inhibited on the addition of glucose to the growth medium. Under

Table 1. Influence of glucose and growth temperature on *Y. pseudotuberculosis* biomass, PL, and LPS yields

Lipids	Yield (% of dry bacterial weight)			
	5°C		37°C	
	NB + Glc	NB	NB + Glc	NB
	1	2	3	4
Biomass*	0.7	0.8	0.15	0.08
Total lipids	7.1	6.2	9.1	4.5
Neutral lipids	2.5	4.6	6.9	0.5
Phospholipids	4.6	1.7	2.2	4.0
3OH14 : 0**	0.55	0.53	0.72	0.69
LPS	1.2	1.5	n.d.***	n.d.***
Polymerization degree****	4.0	5.3	0.5	0.5

* Biomass yields were calculated in g/liter of nutrient broth.
** 3OH14:0 (3-hydroxytetradecanoic acid) content was determined in alkaline hydrolyzates of bacteria with eicosanic acid as standard as described earlier [16].
*** n.d., not determined.
**** Polymerization degrees of the O-specific side chains in LPS were determined as described in [23].

Table 2. Glucose and growth temperature effects on the *Y. pseudotuberculosis* relative phospholipid contents

Phospholipids	Content (% of total phospholipids)				
	5°C			37°C	
	(NB + Glc)*	NB + Glc	NB	NB + Glc	NB
	1	2	3	4	5
PG	9.1	7.8	7.4	8.9	2.4
DPG	12.3	10.7	26.7	25.9	24.3
PE	76.0	78.9	44.1	52.8	72.4
Lyso-PE	2.3	2.6	5.5	5.1	0.8
MePE			10.0		
DiMePE			3.6		
ΣPE	78.3	81.5	63.2	57.9	73.2
PL-X			2.6	7.3	
PE + PG + DPG	94.5	97.4	78.2	87.6	99.1
PE/(PG + DPG)	3.5	4.3	1.3	1.5	2.7

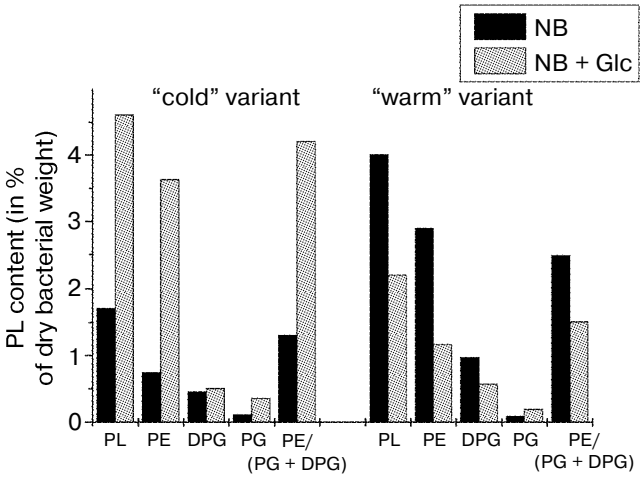
* These data are taken from [14].

these conditions, the content of PL in the bacteria was only 2.2% (Table 1, column 3). An amount of PL isolated from the "warm" cells grown on NB with glucose was almost twofold greater (Table 1, column 3 and 4, respectively) but at expense of NL accumulation, the total amount of extracted lipids remaining high. The qualitative and quantitative composition of PL in the "warm" variant was also significantly changed (Table 2, columns 3 and 4). Lysol-PE and PL-X were detected in the bacteria grown on glucose-containing NB in addition to PE, PG, and DPG present in the cells cultivated on NB. Lipid extracts obtained from them contained consider-

ably less PE, the amount of DPG remained constant, and the content of PG increased from 2.4 (NB) to 8.9% (NB + Glc). As a result, the ratio of neutral to acidic lipids decreased to the value characteristic of the "cold" variants of *Y. pseudotuberculosis* growing on the glucose-free medium.

Thus, glucose affects the bacterium oppositely at the two growth temperatures, i.e., it favors PE and PL synthesis in the cold and inhibits their formation at 37°C. Thus, it should be noted that the nutrient media contained protein hydrolyzate in addition to glucose. When two substrates are present in the medium, the substrate providing faster growth of the bacteria is usually preferred. In our experiment, *Y. pseudotuberculosis* grew more intensively, at least at 37°C, on the glucose-containing NB (Table 1). The fact that PL synthesis was inhibited under these conditions (Table 1, column 3; Table 2, column 4) suggests possible catabolite repression of this process with glucose. In the cold, catabolite repression does not occur, and this may be due to the more "economical" feasibility, less energy-requiring synthesis of PL from glucose directly rather than from amino acids (the pathway of PL biosynthesis from amino acids includes more steps and occur with great energy consumption than that from glucose). It is also known that nitrogen-deficient media can partially suppress glucose-governed catabolite repression.

On the other hand, synthesis of some lipid enzymes is known to be temperature-sensitive [7, 10]. This may account for the temperature dependence of the *Y. pseudotuberculosis* lipid composition shown in the Tables 1 and 2. It is also conceivable that the lipid enzyme patterns in this organism can be changed by changes of the medium composition on adding glucose to a form that is functional in the cold but nonfunctional at 37°C, and *vice versa*.



Individual phospholipid composition based on dry bacterial biomass (NB is nutrient broth)

Enzymes of PE biosynthesis appear to be likely candidates because the changes in PE content were the most pronounced.

Unlike free lipids, the LPS composition of *Y. pseudotuberculosis* was unaffected by glucose in the growth medium. When grown at the same temperature on the two media, the bacteria synthesized equal amounts of LPS that were extracted with identical yields, had the same monosaccharide compositions (identical to that earlier established for *Y. pseudotuberculosis* O1:b serovar LPS [20, 25, 26]), and were similar in polymerization degrees of their O-polysaccharides (Table 1). At the same time, cultivation temperature produced a marked effect on LPS synthesis: both "warm" variants of the bacterium synthesized slightly more LPS molecules which had shorter O-polysaccharide side chains than did the bacterium grown in the cold. These data are in good agreement with previously reported characteristics of LPS from the "warm" variants of pseudotuberculosis bacteria [15].

Thus, both glucose and growth temperature exert drastic control over biosynthetic processes in *Y. pseudotuberculosis*. The temperature effect has a universal character, extending to growth of the cells as well as to free lipid and LPS content and composition, and it is most conspicuous in the bacteria growing on the glucose-containing NB. The effect of glucose is more selective, affecting only free lipids, is temperature dependent, and is more evident in the cold. Determination of individual PL amounts in percent dry bacteria weight (see figure) shows that the most obvious effect of glucose and/or growth temperature is on PE content: on both media the overall decrease in PL content arises from inhibition of PE synthesis and is attended with decreasing PE/(PG + DPG) ratio.

These results differ from data of studies on *E. coli* and many other bacteria, whose lipid compositions were not dramatically altered by changes in growth conditions [4, 6-8, 29]. Changes in the composition of polar head groups, if any, manifested themselves in modulation of O-specific polysaccharide length in the LPS of many bacteria [30], including bacteria of the genus *Yersinia* [31], and in inhibition of PL synthesis at elevated temperature. However, unlike pseudotuberculosis (Tables 1 and 2), it was no more 50% and left the PE/(PG + DPG) ratio unchanged [32].

The changes of PL patterns in *Y. pseudotuberculosis* caused by glucose and/or growth temperature are thus reminiscent of the situation in some *E. coli* mutants defective in the formation of PE (due to the deletion of the *pss* gene coding phosphatidylserine synthetase, a biosynthetic precursor of PE) and accumulating negatively charged PL [4, 11, 33]. However, in contrast to the mutant *E. coli* strains, which were rendered conditionally lethal [11] or had lowered growth rate [33] when there was too little of PE, *Y. pseudotuberculosis* retained viability and continued to grow and divide. The fact that the bacteria with quite abnormal PL compositions were uni-

formly viable indicates that most of the membrane functions of this organism are relatively unaffected by perturbing its polar head group composition and its PE content.

PE is the only PL capable of forming non-bilayer structures under natural conditions [1, 7]. In mutant strains of *E. coli* deprived of PE [33] or containing it only in small amounts [11], DPG in concert with divalent cations (Mg^{2+}) forms non-bilayer structures necessary for normal function of the bacterial cells [7, 33]. The fact that the amount of DPG was increased more than twofold (the "cold" variant) or at least did not change (the "warm" one) with change in carbon source and with significantly decreasing amount of PE synthesized by pseudotuberculosis bacteria indirectly suggests that a similar mechanism may operate in *Y. pseudotuberculosis* if for some reason full synthesis of PE cannot occur.

Such atypical for Gram-negative bacteria reaction of *Y. pseudotuberculosis* lipids in response to changes of cultivation conditions may be related to the facultative psychrophilic nature of this microorganism, which is able to grow and divide as at 37°C in warm-blooded animals in which they are parasites, and also in the environment at relatively low temperature [12]. The wide range of temperature and similarly wide range of nutrient substrates led to the development of unique adaptation mechanisms in pseudotuberculosis bacteria, and the observed large changes in lipid composition on changing temperature or carbon source may be examples of this.

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