*Biochemistry (Moscow), Vol. 66, No. 4, 2001, pp. 415-421. Translated from Biokhimiya, Vol. 66, No. 4, 2001, pp. 511-519.*


# Effects of Culture Method and Growth Phase on Free Lipid Composition of *Yersinia pseudotuberculosis*

**S. I. Bakholdina[1]*, I. N. Krasikova[1], L. S. Buzoleva[2], F. N. Shubin[2], and T. F. Solov'eva[1]**

[1]*Pacific Institute of Bioorganic Chemistry, Far East Branch of the Russian Academy of Sciences, pr.100-letiya Vladivostoka 159, Vladivostok, 690022 Russia; fax: (4232) 31-4050; E-mail: piboc@stl.ru*
[2]*Institute of Epidemiology and Microbiology, Siberian Branch of the Russian Academy of Medical Sciences, ul. Sel'skaya 1, Vladivostok, 690087 Russia*

Received June 28, 2000

**Abstract**—The influence of culture method (free-floating cells in liquid nutrient broth or bacteria attached to agar surface on solid agarized medium of the same formulation) and bacterial age on the composition of free lipids in *Yersinia pseudotuberculosis* (O:Ib serovar, strain KS 3058) grown in the cold (5°C) has been investigated. The specific growth rate of the bacteria on solid medium was about threefold less than that in liquid medium. The qualitative composition of phospholipids and fatty acids only slightly depended on the bacterial culture method. At the same time, the colonially growing cultures contained somewhat more total lipids, they synthesized more phospholipids, in the linear growth phase they contained more lysophosphatides, and they had higher fatty acid unsaturation index and higher pathogenic potential than their "planktonic" counterparts grown in otherwise identical conditions. The bacterial growth phase influenced the amount of 3-hydroxytetradecanoic acid and, indirectly, that of lipopolysaccharide. The dynamics of changes in the amount of this acid with bacterial age was opposite in the surface and broth cultures.

*Key words*: growth curves, culture method, lipids, fatty acids, phospholipids, lipopolysaccharides, pathogenicity, *Yersinia pseudotuberculosis*

*Yersinia pseudotuberculosis* is a human pathogen, the causative agent of a pseudotuberculosis infection. Pseudotuberculosis is widespread in Russia, in the Far East region in particular, and a significant fraction (about 30-50%) occurs in outbreaks [1]. Resistance to various environments and ability to grow at low temperatures are among the most important epidemic properties of the pseudotuberculosis microbe. It is adapted to colonization of solid substrates including foods stored in home refrigerators. Soil is the main reservoir of *Y. pseudotuberculosis* [1].

In nature, bacteria frequently grow on solid surfaces as aggregates, colonies, and biofilms [2]. Such nonhomogeneous microbial growth is incomparably more diverse and more complicated than homogeneous growth characteristic of liquid cultures [3]. A peculiarity of colonial growth on solid media is that organisms are in conditions of physiological "narrowness", so that each bacterium should compete with others for available limited amounts of nutritious substances and oxygen [4]. Immobilization of cells is also known to decelerate the rate of their growth [5].

The study of the immobilized microorganisms is now of increased interest to researchers. This is motivated by the great practical significance of this problem. According to some published data, 65% of human bacterial infections are related to organisms growing as biofilms [6]. Unlike free-floating cells, immobilized bacteria are much more insensitive to antibiotics and are well protected from attack by the immune system [6].

The structural characteristics of biofilms and the ability of colonially growing bacteria for cell differentiation [7, 8] suggest the presence of significant modifications in the structure of their cell walls. In fact, the composition of cell envelope proteins of bacteria growing as biofilms was shown to be greatly different from that of

*Abbreviations*: CFU) colony-forming units; NA) nutrient agar; NB) nutrient broth; LP) logarithmic phase; LGP) linear growth phase; ESP) early stationary phase; SP) stationary phase; LSP) late stationary phase; FA) fatty acids; NL) neutral lipids; PL) phospholipids; PE) phosphatidylethanolamine; PG) phosphatidylglycerol; DPG) diphosphatidylglycerol; MePE and diMePE) mono- and dimethyl ethers of phosphatidylethanolamine; LPE) lysophosphatidylethanolamine; LPS) lipopolysaccharide.

* To whom correspondence should be addressed.

0006-2979/01/6604-0415$25.00 ©2001 MAIK "Nauka/Interperiodica"

free-floating cells [6]. Cultures grown on a liquid medium and on agar were also established to differ in their fatty acid composition [9]. It should be expected that the culture method will influence the structure and composition of other cell envelope components, especially lipids, which are involved in control over the inner medium of the organism and its interaction with the environment and rapidly respond to changes in cultivation conditions.

The lipid composition of pseudotuberculosis bacteria has been rather well investigated [9-14]. However, the data in the literature were mainly obtained for batch liquid cultures in a particular growth phase. The purpose of the present work was to comparatively study the lipid composition of free-floating (liquid nutrient medium) and immobilized (solid agarized medium) cells of *Y. pseudotuberculosis* (O:Ib serovar, strain KS 3058) in different stages of development. *Y. pseudotuberculosis* exhibits high biological activity including pathogenic properties at rather low growth temperature [15]; therefore, this investigation was concerned with cultures which were grown in the cold.

## MATERIALS AND METHODS

**Bacterial strains, cultivation of bacteria, and construction of growth curves.** The strain KS 3058 of *Y. pseudotuberculosis* O:Ib serovar obtained as described in work [16] was used in this study. The strain was typical in its morphological, cultural, biochemical, and antigenic properties and did not contain plasmids. For construction of growth curves, the bacteria were grown on a nutrient broth (NB) (Makhachkala, Russia) in flasks (1 liter) with intensive aeration and on nutrient agar (NA) (Makhachkala, Russia) in 3 ml tubes on standardized slants of 5 cm length. To do this, initial suspension of 1.02 absorbance at wavelength 600 nm and 1 cm quartz cell was prepared. Eight milliliters of this bacterial suspension was inoculated into 300 ml of a broth to adjust the absorbance to 0.02. To inoculate the slants, two drops of the initial bacterial suspension were uniformly placed on the top of the agar. The absorption of bacterial cells washed off the agar with 2 ml of saline was 0.02. Growth of cells was monitored by the absorption of bacterial suspensions at 600 nm ($A_{600}$) each 2.5 h. Three aliquots were taken from each flask. From slants, the bacteria were washed off with 2 ml of saline. The measurements were performed for 3 tubes in parallel. With $A_{600}$ values higher than 0.5, dilutions were made. Experiments were carried out for 21 days on the liquid medium and for 30 days on the solid medium. After plotting the time dependence of $\log A_{600}$ for both media, the time of generation and specific growth rate were calculated [5]. Biomass yields were determined by weight after drying aliquots of stationary phase bacterial suspension at 108°C for 48 h.

For the definition of colony-forming units (CFU), liquid culture (0.1 ml) was inoculated on plates with solid medium at the required dilutions [17].

**Isolation and characterization of lipids and fatty acids.** For lipid analysis, cells were collected according to curves constructed for both media at various phases of growth. On the liquid medium cells were studied after 24 h (logarithmic phase, LP), 2 days (linear growth phase, LGP), 6 days (early stationary phase, ESP), 9 days (stationary phase, SP), and 21 days (late stationary phase, LSP). On the solid medium cells were studied after 2 days (LP), 6 days (LGP), 9 days (ESP), 17 days (SP), and 30 days (LSP), respectively. The cells were killed by a 1% phenol solution for 20 min and separated from the culture liquid by centrifugation at 4,000 rpm for 20 min. Then the cells were treated with chloroform–methanol mixture (2 : 1) at 5-8°C as described [18]. Lipid extracts were separated by two-dimensional thin-layer chromatography (TLC) in systems described in [19]. Phospholipids (PLs) were identified by comparison with authentic standards using specific spray reagents [20]. The total PL content in cells (in % of dry bacterial weight) and amounts of individual PLs (in % of total PLs) were determined by the phosphorus content in total lipid extracts by the method described in [21].

The fatty acids (FA) were obtained hydrolyzing biomass with 6 M NaOH (100°C, 4 h) and were analyzed as methyl ethers by gas–liquid chromatography as described in [22]. Eicosanoic acid was used as internal standard for quantitative analysis of fatty acids. All data presented here are means of three or more independent experiments; the range of experimental errors did not exceed 5%.

## RESULTS AND DISCUSSION

**Curves of growth**. In this work, total lipid extracts from *Y. pseudotuberculosis* cultivated on liquid and solid nutrient media were investigated. The contents of lipids in Gram-negative bacteria are known to depend on the nutrient medium composition and growth phase of cells [14, 23, 24]. Comparative analysis of bacterial lipid profiles is possible only for experiments in which cells of the same growth phase and grown on a medium of the same composition are used. In this regard, the top priority problem was to study the influence of culture method (nutrient broth and agarized nutrient broth) on the kinetics of *Y. pseudotuberculosis* growth (Fig. 1).

Comparison of the growth curves revealed a significant difference between them. Thus, the generation time for the given strain on the liquid medium was 5 h, while on the solid medium it was 15 h; the specific growth rate on NB (0.06 $h^{-1}$) was in three times higher than that on NA (0.018 $h^{-1}$). On the liquid medium, the logarithmic phase lasted 1 day, the linear growth phase about 3 days, and then the stationary phase began. On the solid nutrient medium, due to lengthening of the generation time,

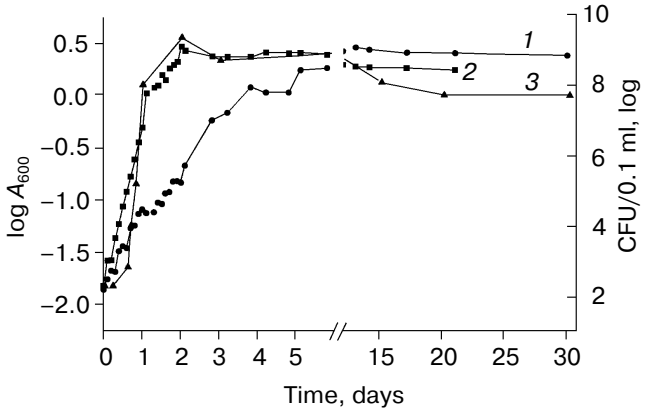


**Fig. 1.** Growth curves of *Y. pseudotuberculosis* on nutrient agar (*1*) and nutrient broth (*2*) based on bacterial suspension absorbance and curve of growth on nutrient agar (*3*) based on number of colony-forming units.

the logarithmic phase lasted about 3 days, the linear growth phase about 7 days, and the stationary phase began only on the 8th day.

With identical inoculation doses, the yield of bacteria attached to agar was slightly less than that of free-floating cells (210 and 236 mg from 300 ml of a medium). Taking into account that the concept of "best growth" includes shortening generation time and greater yield of cells at the end of cultivation [25], it can be concluded that the liquid nutrient medium is more favorable for the growth of the pseudotuberculosis microbe.

The high viability of the pseudotuberculosis bacterium cultivated in the cold should be noted. Thus, the readings of CFU/0.1 ml show (Fig. 1, curve *3*) that the period of preservation of M-concentration of the bacteria (maximum quantity of cells) was about 2 weeks in our experiment. Then a drop in the number of viable cells to 84% was observed, after which their level remained constant for 2.5-3 months (the period of observation).

**Characterization of total lipid extracts**. The free lipid content in *Y. pseudotuberculosis* varied with the cultivation method and the age of the culture (Fig. 2a). On both media, exponentially growing cells were characterized by a maximum content of lipids (7 and 19% for NA and NB, respectively), which sharply decreased as the culture entered the linear growth phase. Further deceleration of the bacterial reproduction rate on entering the stationary phase of growth was accompanied by rather small changes in lipid contents, and the dynamics of these changes depended on the cultivation method: on the liquid medium some decrease in the level of lipids was observed, while the lipid content increased on the solid medium. As a result, colonially growing cells contained somewhat more lipids in the stationary phase of growth than free-floating cells did.

Total lipid extracts contain phospholipids (PLs) and neutral lipids (NLs); their relative contents depended on age of cells, cultivation method (Fig. 2b), and were apparently determined by the growth rate of the bacterium [26]. On both media, the level of NLs exceeded the level of PLs throughout the life cycle of the bacterium. Exceptions were the immobilized cells in LGP and the free-floating cells in LSP, in which the proportions of PLs achieved a maximum value, 50% of the total extracted lipids. A high content of NLs (up to 80%, mostly as free FAs) in lipid extracts of the pseudotuberculosis microbe is unusual for Gram-negative bacteria. It is interesting that

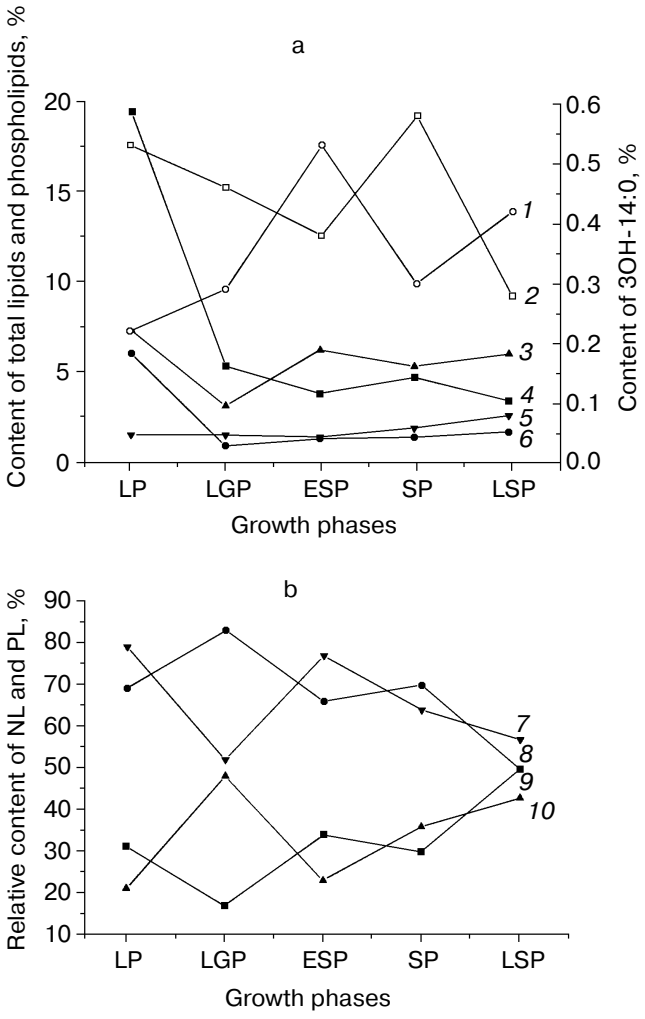


**Fig. 2.** Content of 3-hydroxytetradecanoic acid (*1*, *2*), total lipids (*3*, *4*), and phospholipids (PL; *5*, *6*) in cells (% of dry bacterial mass) (a) and relative contents (% of total lipids) of neutral lipids (NL; *7*, *8*) and PL (*9*, *10*) in lipid extracts (b) of *Y. pseudotuberculosis* grown on nutrient broth (*1*, *4*, *6*, *8*, *9*) and nutrient agar (*2*, *3*, *5*, *7*, *10*) in various growth phases: LP, logarithmic; LGP, linear growth; ESP, early stationary; SP, stationary; LSP, late stationary.

a similar substantial increase in the neutral lipid fraction was observed in the marine psychrophylic *Vibrio* sp. under starvation [27].

**Analysis of phospholipid fractions**. The yield of phospholipids from the *Y. pseudotuberculosis* cells was rather low on both media (Fig. 2a). The character of the changes in the content of PLs with culture age depended on the cultivation method and had opposite tendencies: decreasing of PL was observed on NB and its content increased on NA. The maximum PL for broth cultures was noted in LP, and for colonially growing cultures in SP. At the end of the life cycle, the immobilized bacteria had a noticeably higher PL level than the free-floating bacteria (2.6 and 1.7%, respectively).

Comparison of the lipid extracts showed that the broth and surface cultures had identical qualitative PL compositions, as was established for pseudotuberculosis bacteria earlier [13]. The major components were phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), mono- and dimethyl ethers of PE (MePE and diMePE), and lysophosphatidylethanolamine (LPE) (Table 1). As minor components, from one to three PLs of unknown structure were present in the lipid extracts. One of them (PL-X) was noted earlier in some lipid extracts of pseudotuberculosis bacteria and represented a phospholipid that did not contain choline or amino or monosaccharide residues [13]. The two other unidentified PLs contained 3-hydroxytetradecanoic acid (data are not shown) and are apparently biosynthetic precursors of lipid A [28].

Phosphatidylethanolamine was the dominant phospholipid throughout cell cycle only on the solid nutrient medium, while DPG become dominant in the middle of the stationary phase on the liquid medium. On both media, the proportion PG in the total lipid fraction decreased with aging of the cells. Simultaneously, the content of DPG increased. An exception were the immobilized cells in the linear growth phase, whose level of DPG decreased twofold versus that in the logarithmic phase cells (Table 1).

We noted a low content of PE at all stages of growth (25-44 and 26-51% for NB and NA, respectively, Table 1). This was partially compensated by a high level of PE derivatives (MePE, diMePE, and LPE) for which PE is a precursor. The maximum level of methylated derivatives was found in the logarithmic phase of growth, and then their content gradually decreased to a minimum value in the stationary phase. In free-floating cells, their content was 2-4 times greater than in the colonial cultures. The content of LPE decreased with deceleration of culture

**Table 1.** Relative content of individual *Y. pseudotuberculosis* phospholipids depending on culture method and growth phase (% of total PL)

| Growth phase* | LP | | LGP | | ESP | | SP | | LSP | |
|---|---|---|---|---|---|---|---|---|---|---|
| Medium** | NB | NA | NB | NA | NB | NA | NB | NA | NB | NA |
| Growth time (days) | 1 | 2 | 2 | 6 | 6 | 9 | 9 | 17 | 21 | 30 |
| | Phospholipids | | | | | | | | | |
| PE | 25.6 | 30.3 | 30.5 | 33.6 | 44.1 | 26.0 | 36.5 | 51.6 | 33.9 | 50.8 |
| PG | 23.0 | 14.3 | 19.3 | 8.5 | 7.4 | 9.3 | 6.5 | 4.8 | 5.2 | 4.9 |
| DPG | 13.9 | 20.1 | 18.1 | 9.7 | 26.7 | 25.7 | 37.1 | 33.1 | 39.5 | 36.0 |
| LPE | 7.2 | 8.4 | 6.1 | 35.9 | 5.5 | 18.2 | 4.5 | 6.5 | 4.6 | 3.2 |
| PL-X | — | 5.5 | — | 1.1 | 2.6 | 5.2 | 3.7 | 1.4 | 2.0 | 1.6 |
| $PL\text{-}X_1 + PL\text{-}X_2$ | — | 6.2 | 5.2 | 1.9 | — | 11.1 | — | — | — | — |
| MePE | 18.5 | 10.3 | 16.3 | 7.0 | 10.0 | 4.3 | 6.4 | 2.6 | 12.7 | 3.5 |
| diMePE | 11.75 | 4.9 | 4.6 | 2.3 | 3.6 | — | — | — | 2.3 | — |
| ΣPE*** | 63.1 | 53.9 | 57.9 | 78.8 | 63.2 | 48.5 | 47.4 | 60.7 | 53.5 | 57.5 |
| PE + PG + DPG | 62.5 | 64.7 | 67.9 | 51.8 | 78.2 | 61.0 | 80.1 | 89.5 | 78.6 | 91.7 |
| ΣPE***/PG + DPG | 1.7 | 1.6 | 1.5 | 4.3 | 1.9 | 1.4 | 1.1 | 1.6 | 1.2 | 1.4 |

* Phases of growth: LP, logarithmic; LGP, linear growth; ESP, early stationary; SP, stationary; LSP, late stationary.
** NB, nutrient broth; NA, nutrient agar.
*** ΣPE = PE + LPE + MePE + diMePE.

growth and with entry into the SP. This tendency did not occur for the *Y. pseudotuberculosis* grown on the solid medium: an abnormally high level of LPE was observed in the linear growth phase and in early stationary phase (35.9 and 18.2%, respectively).

The presence of LPE in lipid extracts apparently arises from the action of endogenous phospholipases whose activity, as shown for *Escherichia coli* [23], depends on age of culture and is mostly exhibited in the last stages of the exponential phase and on entering the stationary phase. The fact that LPE is mainly formed in the immobilized cells is probably connected to peculiarities of colonial growth of pseudotuberculosis bacteria, for example, to their higher enzymatic activity (in comparison with free-floating cells [29]) and is directed to increasing cell membrane permeability toward macromolecules in a phase of intensive cell division [30]. It is of interest that similar significant amounts of lysophosphatides (up to 37%) were detected earlier in lipid extracts of marine psychrophilic bacteria of the genus *Vibrio* [31]. Their level in these microorganisms also depended on cultivation temperature and age, reaching a maximum in the logarithmic phase of growth on cultivation in the cold.

Comparison of the lipid composition of pseudotuberculosis bacteria grown as broth cultures with that earlier published [13] showed a significant difference between them. We suppose that this is due to the lack of glucose in the growth medium; this may inhibit phosphatidylglycerol synthetase, a key enzyme in biosynthesis of PE [32]. The analysis of the lipid composition in free-floating pseudotuberculosis bacteria grown on glucose-containing NB (0.5%) provides support for this (data not shown).

**Fatty acid composition of immobilized and free-floating *Y. pseudotuberculosis*.** Hexadecanoic (16:0), hexadecenoic (16:1c9), octadecenoic (*cis*-vaccenic, 18:1c11), methylencyclohexanoic (cyclopropanoic, 17:0cy), and 3-hydroxytetradecanoic (3OH-14:0) acids were the dominant FAs (Table 2). On both media and on all stages of

**Table 2.** Dependence of *Y. pseudotuberculosis* fatty acid content on culture method and bacterial growth phase (% of total fatty acids)

| Growth phase* | LP | | LGP | | ESP | | SP | | LSP | |
|---|---|---|---|---|---|---|---|---|---|---|
| Medium** | NB | NA | NB | NA | NB | NA | NB | NA | NB | NA |
| Growth time (days) | 1 | 2 | 2 | 6 | 6 | 9 | 9 | 17 | 21 | 30 |
| | | | | | Fatty acids | | | | | |
| 12 : 0 | 3.4 | 2.7 | 2.6 | 2.7 | 2.6 | 2.2 | 3.4 | 2.4 | 3.7 | 3.8 |
| 13 : 0 | 0.9 | 0.3 | 0.1 | 0.1 | 0.1 | 0.3 | 0.2 | 0.4 | 0.2 | 0.5 |
| 14 : 0 | 4.6 | 2.8 | 2.6 | 0.8 | 2.8 | 0.8 | 0.7 | 0.6 | 0.6 | 1.2 |
| 15 : 0 | 4.5 | 2.4 | 1.4 | 2.4 | 1.8 | 5.0 | 1.7 | 6.8 | 2.7 | 6.7 |
| $\Delta^2$-14 : 1 | 1.6 | 1.4 | 1.2 | 1.3 | 1.6 | 1.1 | 1.4 | 1.0 | 1.8 | 1.2 |
| 3OH-14 : 0 | 1.5 | 8.0 | 4.5 | 6.3 | 6.1 | 4.7 | 3.3 | 6.3 | 7.2 | 6.4 |
| Σ3OH-14 : 0*** | 3.1 | 9.4 | 5.7 | 7.6 | 7.7 | 5.8 | 4.7 | 7.3 | 9.0 | 7.6 |
| 16 : 0 | 37.0 | 24.6 | 19.5 | 16.0 | 18.2 | 14.4 | 20.6 | 13.9 | 19.9 | 15.2 |
| 16 : 1c9 | 15.0 | 33.7 | 40.8 | 43.0 | 28.1 | 36.4 | 30.0 | 30.8 | 24.2 | 28.8 |
| 17 : 0 | 2.3 | 0.9 | 1.3 | 1.6 | 1.6 | 3.5 | 1.7 | 4.3 | 2.0 | 4.2 |
| 17 : 0cy | 2.4 | 2.1 | 3.4 | 4.5 | 14.7 | 10.9 | 15.7 | 16.3 | 17.9 | 15.3 |
| 18 : 0 | 11.5 | 4.1 | 0.9 | 1.0 | 0.8 | 0.9 | 1.0 | 0.9 | 1.1 | 1.7 |
| 18 : 1c9 | 4.8 | 3.1 | 0.5 | 0.5 | 0.4 | 0.6 | 0.4 | 0.5 | — | 1.3 |
| 18 : 1c11 | 6.0 | 12.8 | 20.7 | 19.2 | 19.4 | 18.7 | 19.0 | 15.1 | 17.4 | 13.2 |
| 19 : 0 | 0.4 | 0.5 | 0.1 | 0.3 | 0.2 | 0.2 | 0.3 | 0.4 | 0.2 | 0.4 |
| Other fatty acids | 4.1 | 0.6 | 0.4 | 0.3 | 1.6 | 0.3 | 0.6 | 0.3 | 1.1 | 0.1 |
| U/S + C**** | 0.3 | 0.9 | 1.6 | 1.7 | 1.0 | 1.3 | 1.0 | 0.9 | 0.7 | 0.8 |

*, ** Same as in Table 1.

*** In this row, the total amount of 3OH-14:0 includes $\Delta^2$-14:1 which is artificially obtained by dehydration 3OH-14:0 during alkaline hydrolysis [22].

**** U/S + C, unsaturation indices determined as the ratio of unsaturated FAs to the sum of saturated and cyclopropanoic FAs.

growth, the total content of unsaturated FAs was noticeably above that of saturated FAs. This is due to the low cultivation temperature and maintains the required membrane fluidity for the normal function of the bacterial cell at low temperature.

The dynamics of the changes in the FA levels with the *Y. pseudotuberculosis* growth phase was typical of Gram-negative bacteria [14] and was due to the type of fatty acids investigated. However, the dependence of unsaturated FAs on growth phase in the *Y. pseudotuberculosis* was unusual; their content increased in rapidly growing cells and gradually decreased on entry into the stationary phase, while the FA unsaturation index increased over the whole life cycle in other Gram-negative bacteria [14]. On both media, the cells contained the maximum of unsaturated FAs and the minimum of saturated FAs in the linear growth phase. As a result, such cells apparently had the most fluid membrane. On the contrary, the cells had the lowest unsaturation index and the lowest membrane fluidity in the logarithmic phase. Thus, the state of the membrane sharply changed as the cells passed from one stage of growth to another in fast growing cultures. No significant changes in unsaturation index and membrane fluidity were observed in the cells in the later stages of growth (ESP, SP, LSP).

Changing from the liquid to the solid culture of *Y. pseudotuberculosis* decreased the saturated FA content and increased the content of unsaturated FA and of saturated FAs with an odd number of carbon atoms. The amount of cyclopropanoic acid had little dependence on culture method. As a result, the immobilized cells had a higher FA unsaturation index than free-floating cells at all growth stages.

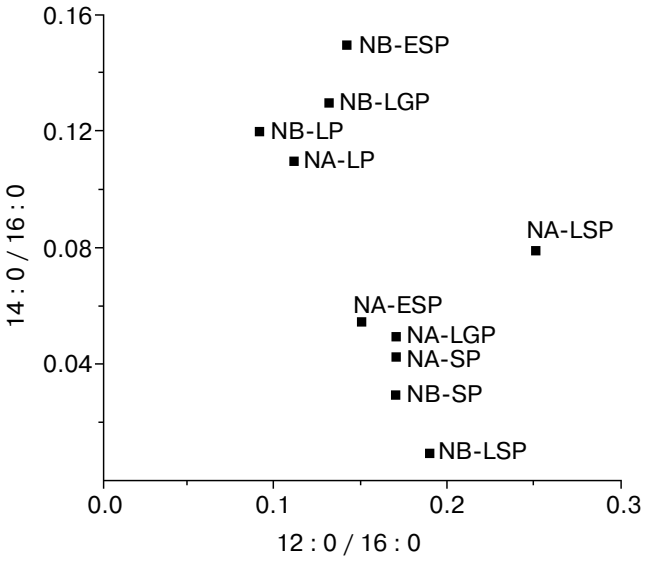


**Fig. 3.** *Y. pseudotuberculosis* pathogenicity as a ratio of 12:0/16:0 and 14:0/16:0 fatty acids in relation to cultivation method (NB, nutrient broth; NA, nutrient agar) and phase of growth: LP, logarithmic; LGP, linear growth; ESP, early stationary; SP, stationary; LSP, late stationary.

Among the fatty acids present in all investigated samples, of special interest is 3OH-14:0. Unlike other FAs, which can be present in free or bound lipids, it is found only in lipopolysaccharides (LPS), the major glycophospholipid of the outer membrane of Gram-negative bacteria [34]. The amount of 3OH-14:0 in hydrolyzates is determined by the amount of LPS in cells. In turn, data on the content and phase dynamics of 3OH-14:0 can be used for characterization of modifications in the amount of LPS. As follows from Table 2, the content of 3OH-14:0 in hydrolyzates depended on the growth phase, and the dynamics of changes in the amount of this acid with aging of cells in surface and broth cultures was opposite (Fig. 2a). However, a correlation between the total lipid content and the PE content in lipid extracts, on the one hand, and the 3OH-14:0 content in the total FA fraction, on the other, is observed on both media: when more free lipids were present in the cells, the amount of 3OH-14:0 and thus LPS was lower. This suggests that the *Y. pseudotuberculosis*, like other Gram-negative bacteria [35], has a mechanism for controlling the synthesis of lipid-containing compounds which operates differently in solid and liquid cultures. The growth rate of the bacteria probably plays a key role in the regulation of free lipid and LPS synthesis.

The content of 3OH-14:0 and, indirectly, of LPS (an important factor of Gram-negative bacterial pathogenicity [34]) depends markedly on the culture method and growth phase. Thus, it seems that *Y. pseudotuberculosis* grown on different nutrient media and in different stages of growth can differ in pathogenicity. To evaluate the pathogenic potential of bacteria of the genus *Yersinia*, some authors use cell fatty acid quantitative analysis, namely, 12:0/16:0 and 14:0/16:0 ratios [36]. As follows from the data shown in Fig. 3, bacteria grown on NB have a high pathogenic potential only in the stationary phase of growth. The highest pathogenic potential, close to that of wild *Y. pseudotuberculosis* strains [36], is seen on cultivation on NA in the linear growth phase and the early stationary and stationary phases. Therefore, immobilization of cells during the growth of the bacterium on the solid nutrient media is a factor enhancing the pathogenic potential of *Y. pseudotuberculosis*. A lack of correlation between 3OH-14:0 content and pathogenicity degree at all stages of growth is worth noting.

Thus, the lipid composition of *Y. pseudotuberculosis* grown on solid substrates has some peculiarities in comparison with that of free-floating cells. The immobilized cultures contained somewhat more total lipids and synthesized more PLs, in the linear growth phase their lysophosphatide fraction was higher, and they had higher

unsaturation index and higher pathogenic potential than free-floating cells grown in otherwise identical conditions. For both culture methods used, a clearly expressed influence of growth phase on *Y. pseudotuberculosis* lipid composition was also observed.

## REFERENCES


1. Somov, G. P. (1997) *Zh. Mikrobiol. Immunol.*, **5**, 7-11.
2. Costerton, J. W., Lewandowski, Z., Caldweil, D. E., Korber, D. R., and Lappin-Scot, H. M. (1995) *Annu. Rev. Microbiol.*, **49**, 711-745.
3. Panikov, N. S. (1991) *Kinetics of Growth of Microorganisms* (Zvyagintsev, D. G., ed.) [in Russian], Nauka, Moscow, pp. 211-221.
4. Rose, A. H. (1971) in *Chemical Microbiology* (Egorov, N. S., ed.) [Russian translation], Mir, Moscow, p. 263.
5. Varfolomeev, S. D., and Gurevich, K. G. (1999) *Biokinetics: Practical Course* [in Russian], Fair-Press, Moscow, p. 635.
6. Potera, C. (1999) *Science*, **283**, 1837-1839.
7. Werthen, M., Hermansson, M., and Elwing, H. (1998) *Eighth Int. Symp. on Microbial Ecology (ISME-8), Microbiol Biosystems. New Frontiers: Program and Abstract*, Halifax, Nova Scotia, Canada, p. 346.
8. Shapiro, J. A. (1998) *Annu. Rev. Microbiol.*, **52**, 81-104.
9. Vasyurenko, Z. P., Ruban, N. M., and Samygin, V. M. (1997) *Mikrobiol. Zh.*, **59**, 24-30.
10. Vasyurenko, Z. P., and Znamenskii, V. A. (1980) *Mikrobiol. Zh.*, **42**, 462-469.
11. Solov'eva, T. F., Yermak, I. M., Moroz, S. I., Krasikova, I. N., Novikova, O. D., Khomenko, V. A., Frolova, G. M., Ivanova, E. P., Timchenko, N. F., and Ovodov, Yu. S. (1988) *Biol. Membr.* (*Moscow*), **5,** 492-500.
12. Krasikova, I. N., Khotimchenko, S. V., Solov'eva, T. F., and Ovodov, Yu. S. (1991) *Khim. Prirod. Soedin.*, **4**, 579-580.
13. Krasikova, I. N., Khotimchenko, S. V., Solov`eva, T. F., and Ovodov, Yu. S. (1995) *Biochim. Biophys. Acta*, **1257**, 118-124.
14. Vasyurenko, Z. P., Frolov, A. F., Smirnov, V. V., and Ruban, N. M. (1992) in *Fatty Acid Profiles of Bacteria Pathogenic for the Human* (Zakharov, I. Ya., ed.) [in Russian], Naukova Dumka, Kiev, pp. 95-114.
15. Smirnov, G. B. (1990) *Biomed. Sci.*, **1**, 223-225.
16. Shovadaeva, G. A., Shubin, F. N., Shaginyan, I. A., Markov, A. P., Gintsburg, A. L., and Smirnov, G. B. (1991) *Mol. Genet. Microbiol. Virusol.*, **11**, 23-27.
17. Labinskaya, A. S. (1978) *Microbiology with Engineering Microbiological Researches* [in Russian], Meditsina, Moscow, p. 394.
18. Rothfield, L., and Perlman, M. (1966) *J. Biol. Chem.*, **241**, 1386-1392.
19. Vaskovsky, V. E., and Khotimchenko, S. V. (1982) *High. Resol. Chrom.*, **5**, 635-636.
20. Vaskovsky, V. E., and Terekhova, T. A. (1979) *High. Resol. Chrom.*, **2**, 671-672.
21. Vaskovsky, V. E., Kostetsky, E. Y., and Vasendin, I. M. (1975) *J. Chromatogr.*, **114**, 129-141.
22. Krasikova, I. N., Bakholdina, S. I., Khotimchenko, S. V., and Solov'eva, T. F. (1999) *Biochemistry (Moscow)*, **64**, 338-344.
23. Ruban, E. L. (1977) *Microbial Lipids and Lipases* [in Russian], Nauka, Moscow, p. 216.
24. Rose, A. H. (1989) in *Microbial Lipids* (Ratledge, C., and Wilkinson, C. G., eds.) Vol. 2, Academic Press, London, pp. 255-278.
25. Markov, K. I. (1965) in *Experimental Microbiology* (Byrdarov, S. V., ed.) Meditsina i Fizkul'tura, Sofia, p. 289.
26. Gilbert, P., and Brown, R. W. (1978) *J. Bacteriol.*, **133**, 1066-1072.
27. Oliver, J. D., and Stringer, W. F. (1984) *Appl. Environ. Microbiol.*, **47**, 461-466.
28. Nishijima, M., and Raetz, C. R. H. (1979) *J. Biol. Chem.*, **16**, 7837-7844.
29. Hilge, B., and Relm, H. J. (1989) *Forum. Microbiol.*, **12**, 58.
30. Scandella, C. Y., and Kornberg, A. (1971) *Biochemistry*, **10**, 4447-4456.
31. Oliver, J. D., and Colwell, R. R. (1973) *J. Bacteriol.*, **114**, 897-908.
32. Raetz, C. R. H. (1978) *Microbiol. Rev.*, **42**, 614-659.
33. Wollenweber, H.-W., and Rietschel, E. Th. (1990) *J. Microbiol. Meth.*, **11**, 195-211.
34. Rietschel, E. Th., Kirikae, T., Schade, F. U., Mamat, U., Schmidt, G., Loppnow, H., Ulmer, A. J., Zahringer, U., Seudel, U., di Padova, F., Schreier, M. D., and Brade, H. (1994) *FASEB J.*, **80**, 217-224.
35. Raetz, C. R. H., and Dowhan, W. (1990) *J. Biol. Chem.*, **265**, 1235-1238.
36. Leclercq, A., Wauters, G., Decallonne, J., El Lioui, M., and Vivegnis, J. (1996) *Med. Microbiol. Lett.*, **5**, 182-194.