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BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY

New Marker of FAME Profile of *Pseudomonas aurantiaca* Total Lipids

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The fatty acid methyl ester (FAME) profile [1], along with the ribosomal 16S RNA gene sequence [2], is widely used for rapid identification of many microorganisms, including bacterial strains [3]. The FAME profile of bacterial lipids is usually obtained by gas chromatography [4] and/or mass spectrometry [5] (including its variant with in situ thermal hydrolysis and methylation (THM-MS) [6]). For a number of families of bacteria, FAME markers are known. In particular, for *Bacillus subtilis*, these are iso and anteiso 15:0 and 17:0 fatty acids [7, 8]; for *Pseudomonas*, the ratio of 16:0 and 16:1 acids and the presence of 18:1 fatty acids [7]. For the *Pseudomonas* strain *Nakhimovskaya 1948*, the pattern of changes of these biomarkers and their ratio under the influence of various environmental factors remains unknown. *Pseudomonas aurantiaca* strain B-1558, which was used by us in studies of the fatty acid profile of DNA-bound lipids [9, 10], is a Gram-negative bacteria identified as *Pseudomonas chlororaphis* subsp. *aurantiaca Nakhimovskaya 1948* [11], which is used as a producer of phenazine antibiotics [12]. The purpose of this work was to investigate the response of fatty acid biomarkers of adaptation of the *P. aurantiaca* strain VKM B-1558 [9, 10] to an increase in temperature as an environmental factor. In this paper, we analyzed the changes in the content of fatty acid markers of total lipids of the genus *Pseudomonas* and their ratios with temperature in different growth phases. We suggest that another parameter, namely, the 16:0/18:0ω7c ratio, should be used as a biomarker of *P. aurantiaca Nakhimovskaya 1948* and members of the *Pseudomonas* genus. This ratio increases almost twice as the temperature of growth of the bacteria increases by 10°C.

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MATERIALS AND METHODS

Reagents and solvents used in the study were of reagent or analytical grade. The solutions were prepared using milliQ water.

P. aurantiaca strain B-1558 (*Nakhimovskaya 1948*) was obtained from the Collection of Microorganisms (All-Russia Collection of Microorganisms, IBPM Academy of Sciences, Pushchino, Moscow region) [9–11]. Strain type: ATCC 33663 = CIP 106718 = NCIMB 10068 = VKM B-876 (www.strainInfo.net). The total lipid FAME profile was given in [10]; the ribosomal 16S RNA gene sequence was registered in GenBank/EMBL/DDBJ (accession number DQ682655) [11]. This strain was grown and stored according to the protocol described in the study performed with *B. subtilis* OSU-142 [8].

The total lipid FAME profile of *P. aurantiaca* strain B-1558 was analyzed by gas chromatography using TSBA6 or 40 standards, a commercial database, and the MIDI software package (Sherlock Microbial Identiftication System version 4.0, MIDI Inc., Newark, DE) in accordance with the bacterium culture protocols described in it and instrument specifications [4].

The FAME profile was analyzed using a Hewlett-Packard 6890 gas chromatograph equipped with an HP7673A autosampler (a fused-silica capillary column (25m by 0.2 mm) with cross-linked 5% phenylmethyl silicone). During analysis, the instrument parameters were adjusted and controlled automatically by the MIDI computer program [4]. Chromatograms with peak retention times and respective peak areas were produced on the integrator and then transferred to the database for analysis, storage, and delivery of analysis results. Peak assignment and column control were ensured using the Microbial ID 1200-A standard calibration mixture containing nC9–nC20 saturated and 2- and 3-hydroxy fatty acids (for TSBA6 or 40 standards).

Biomarker fatty acids and their ratio (mol %) in the FAME profile of total lipids of the Gram-negative bacterium *P. aurantiaca* B-1558 at different temperatures (°C) and in dependence on the growth phase

Fatty acid	Logarithmic*				Stationary**	
	24	28	34	37	24	28
15:0 iso 2OH/16:1ω7c	35.44	35.92	35.76	45.2	34.97	35.16
16:0	27.43	29.69	35.23	54.8	24.05	28.04
18:1ω7c	17.25	15.81	13.03	–	17.63	15.64
16:0/15:0 iso 2OH/16:1ω7c	0.77	0.83	0.98	1.21	0.69	0.80
16:0/18:1ω7c	1.59	1.88	2.70	–	1.36	1.79

* 24 h of cultivation, ** 36 h of cultivation.

The FAME content in the fatty acid profile was determined using statistical analysis methods and represented as the mean values and standard deviations; each determination was performed 4 to 6 times.

RESULTS AND DISCUSSION

In this paper, we used gas chromatography and the standard set of fatty acids and the MIDI database to investigate the fatty acid (FAME) profile of total lipid extracts of the Gram-negative bacterium *P. aurantiaca*. Particular attention was given to study the effect of temperature and growth phase of bacteria on the biomarker fatty acids. It is known that biomarker FAMEs of the genus *Pseudomonas* are palmitic 16:0 and 16:1 palmitoleic acids in equal proportions as well as 18:1ω7c/ω9t/ω12t, an isomer of oleic acid [7]. Figure 1a shows the results of determination of total lipid FAME profile of *P. aurantiaca* B-1558 (logarithmic phase, 24 h) at different temperatures (24, 28, 34, and 37°C). This figure also shows the content (more than 0.5%) of the following fatty acids: 10:0 3OH, 12:0, 12:0 2OH, 12:1 3OH, 12:0 3OH, 15:0 iso 2OH/16:1ω7c, 16:0, 18:1ω7c, which constitute 100% of the fatty acid composition of total lipids of this bacterium. Five of these acids are hydroxy acids characteristic of surface polysaccharides (glycolipids) of Gram-negative bacteria [13]. As can be seen from the data presented in Fig. 1a, the proportion of C12 acids in the fatty acid profile of bacteria cultured at 24°C is the smallest; this especially applies to 12:0 and 12:1 3OH acids (the content of the latter is less than 2%). The content of the 10:0 3OH acid is significantly higher (4.8%). The major acids in the fatty acid profile of *P. aurantiaca* cultured at 24°C are C16 acids: the sum of 15:0 iso 2OH and 16:1ω7c acids accounts for 35.44%, and the 16:0 acid accounts for 27.43%. The proportion of another biomarker of *Pseudomonas*, 18:1ω7c [4], was 17.25%. Thus, our results are consistent with the data reported in [4], where the fatty acids found by us in the FAME profile of *P. aurantiaca* B-1558 in the highest quantities are considered as biomarkers. With increasing the cultivation temperature of bacteria, only the content of C16 acids in the FAME profile increased (from 30–35% at 24–34°C to 45 and 55% at 37°C). The content of the 16:0 acid at 24°C was less than 30%; however, as the cultivation temperature increased to 34°C, its proportion becomes equal to the sum of proportions of two acids (15:0 iso 2OH and 16:1ω7c) and increased to 55% at 37°C. The level of the remaining acids in the FAME profile of this bacterium decreased with an increase in the cultivation temperature. The proportion of oleic acid isomer 18:1ω7c decreased from 17.25% (at 24°C) to 13% at 34°C. The 12:1 3OH acid disappeared already at 34°C. At 37°C, the fatty acid profile of the bacterium contained only C16 acids.

Figure 1b shows the FAME profile of total lipids of the bacterium cultured at 24 and 28°C for 36 h, which corresponds to the early stationary phase of growth. These data suggest that the FAME profile of total lipids of the bacterium in this growth phase is not changed qualitatively, and the quantitative composition of FAME components remained virtually unchanged, except that the level of the 12:1 3OH acid significantly (from 1.48 to 0.42%) decreased at 28°C.

In the literature, the ratios of the content of fatty acids in the FAME profile of bacteria are used as biomarkers. In view of this, in the table we gave the ratios of the content of fatty acids, which in sum account for as much as 80% of the FAME profile of total lipids of *P. aurantiaca* B-1558. It can be seen that the ratio of the content of 16:0 and the sum of 15:0 iso 2OH and 16:1ω7c acids increased from 0.77 to 1.21 as the temperature increased from 24 to 37°C. This ratio of these biomarkers at different temperatures is also characteristic of other members of this genus, in particular, *P. fluorescens* [14]. In the FAME profile of this bacterium, the ratio of the content of 16:0/16:1ω7c acids in the temperature range from 16 to 26°C remained virtually unchanged in both exponential and stationary phases and increased several times at 36°C in both phases [14]. Such a change in this ratio is not characteristic of other bacteria [2], in particular, of the

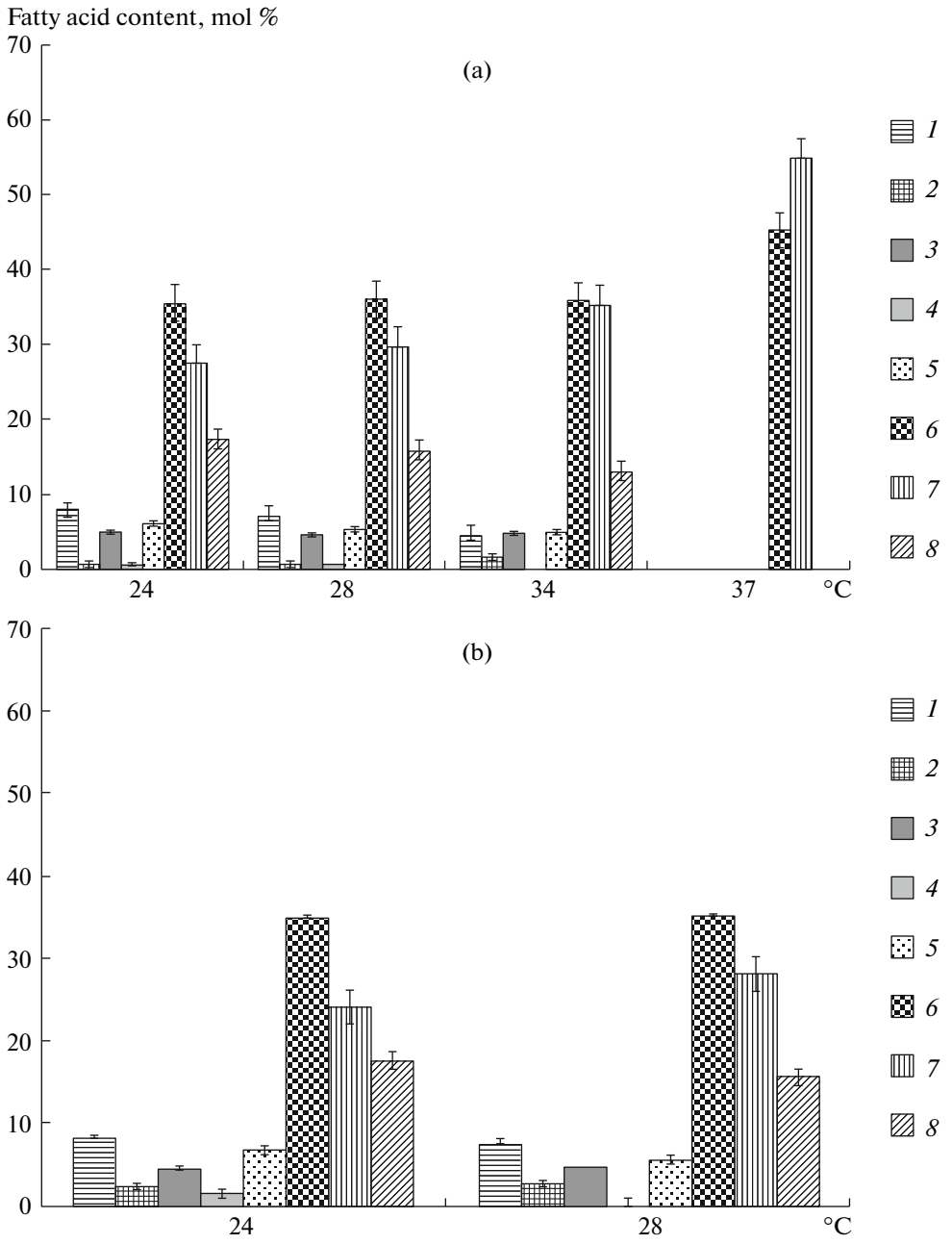

Fig. 1. Changes in the fatty acid profile of total lipids of *P. aurantiaca* B-1558 with increasing temperature in (a) logarithmic growth phase (24 h) and (b) stationary growth phase (36 h). Designations: *1*, 10:0 3OH; *2*, 12:0; *3*, 12:0 2OH; *4*, 12:1 3OH; *5*, 12:0 3OH; *6*, 15:0 iso 2OH/16: 1ω7c; *7*, 16:0; *8*, 18:1ω7c. The confidence intervals are shown.

psychrophilic bacterium *Vibrio* sp. strain 5710 [15]. Since in our experiments the 16:1ω7c acid was represented as the sum with another acid, 15:0 iso 2OH, we suggest that another ratio, 16:0/18:1ω7c, should be used as a biomarker of *P. aurantiaca* strain *Nakhimovskaya 1948* and members of the genus *Pseudomonas*. This ratio also increases with increasing temperature, though only slightly (nearly two times when temperature rises by 10°C). This behavior is not typical of other bacteria, for example, *Vibrio* sp. 5710 [15].

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