ISSN 0026-2617, Microbiology, 2009, Vol. 78, No. 1, pp. 48–55. © Pleiades Publishing, Ltd., 2009.
Original Russian Text © N.B. Strazhevskaya, A.L. Mulyukin, A.S. Shmyrina, A. Kraus, V. Lorentz, R.I. Zhdanov, G.I. El'-Registan, 2009, published in Mikrobiologiya, 2009, Vol. 78, No. 1, pp. 59–67.

EXPERIMENTAL ARTICLES

Characteristics of *Pseudomonas aurantiaca* DNA Supramolecular Complexes at Various Developmental Stages

N. B. Strazhevskaya[a], A. L. Mulyukin[b,1], A. S. Shmyrina[a], A. Kraus[c], V. Lorentz[c], R. I. Zhdanov[a], and G. I. El'-Registan[b]

[a] *Research Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow, Russia*
[b] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia*
[c] *Martin Luther University, Halle-Wittenberg, Germany*

Received October 25, 2007

Abstract—Differences in viscoelasticity (η) and molecular mass (M) values, as well as in the fatty acid profile of lipids in DNA supramolecular complexes (SC), isolated from *Pseudomonas aurantiaca* cultures at the exponential and stationary growth phases, were established for the first time. Typical characteristics of DNA SC from actively growing cells were the following: $\eta = 315 \pm 15$ dl/g, $M_{DNA} = 39 \times 10^6$ Da, $C_{16:0} > C_{18:0} > C_{18:1}$ present as basic fatty acids (FA) in a pool of loosely DNA-bound lipids; the tightly DNA-bound lipid fraction consisted of only two acids $C_{18:0} > C_{16:0}$. Significantly higher values of viscoelasticity $\eta = 779 \pm 8$ dl/g and $M_{DNA} = 198 \times 10^6$ Da were observed for DNA SC of the stationary phase cells; one more FA, $C_{14:0}$, was detected in the loosely bound lipid fraction, while lipids tightly bound to DNA contained mainly $C_{16:0} > C_{18:1} > C_{18:0} > C_{14:0}$ FA. The content of saturated FA in the DNA-bound lipids in the stationary phase cells was twice as high than in the exponential phase cells. The fraction of tightly bound lipids from the stationary phase cells contained nine times more unsaturated fatty acids than the fraction from proliferating cells. These differences in FA composition of DNA-bound lipids demonstrate the importance of lipids for the structural organization and functioning of genomic DNA during bacterial culture development.

Key words: DNA supramolecular complexes, DNA viscoelasticity and molecular mass, DNA-bound lipids, DNA fatty acid profile, bacterial physiological age.

DOI: 10.1134/S002626170901007X

Recently, lipids, in addition to being essential components of cellular membranes, were discovered to play a significant part in the organization of chromosomes, DNA–membrane complex, and nuclear material [1–5]. Lipids were demonstrated to be capable of DNA regulation through both direct interaction with the DNA structure and the influence upon the activity of nucleic acid metabolism enzymes [1–3, 5]. Mild phenol extraction [6] allowed isolation from mammalian, avian, piscine, and bacterial cells, as well as T2 phage high-molecular (3×10^8–3×10^9 Da) DNA preparations in the form of supramolecular complexes (SC) containing minor amounts of RNA (1–3%), acid nonhistone proteins, and specific lipids [1, 4, 5]. The isolated DNA SC preparations contained two lipid fractions which were loosely and tightly bound to DNA (fractions 1 and 2, respectively). The composition of eukaryotic DNA-bound lipid fractions was shown to change in the course of DNA conformational transitions (superspiralization–relaxation), malignancies, and treatment with antitumor drugs [1, 5].

Less is known about the composition and functions of DNA-bound lipids in prokaryotes. Mild phenol extraction of DNA was used to demonstrate the presence of DNA-bound lipids in *Escherichia coli* B, *Shigella sonnei*, and *Salmonella typhimurium* [4, 7, 8]. In *E. coli* B, DNA-bound lipids were shown to be represented by neutral lipids (NL) and phospholipids (PL) in amounts of 0.78 and 0.11%, respectively [1, 4, 5]; heat-labile and heat-resistant strains of *Shigella sonnei* contained 0.92 and 0.75% of NL, respectively, and practically equal amounts of PL (0.1–0.14%) [7]. Cardiolipin (CL) and phosphatidylethanolamine (PE), along with free fatty acids (FA) and their esters, were detected in the DNA SC of *E. coli* and T2 phage [1, 4, 5]. In the case of DNA preparations isolated from *S. typhimurium* under the more hard conditions of detergent–phenol extraction (treatment with 1% SDS, chloroform–isoamyl alcohol, 24 : 1, and phenol), a lower molecular mass DNA ($M = 19.7 \times 10^6$ Da) and less lipids (0.17%) were obtained, than with the mild phenol extraction procedure, where DNA of 180×10^6 Da and more lipids (0.89%) were isolated. However, both procedures

[1] Corresponding author; e-mail: andlm@mail.ru

resulted in the domination of NL over PL in the fractions of loosely and tightly DNA-bound lipids [4, 8].

Previously we have shown the DNA SC from *Pseudomonas aurantiaca* cells in the exponential and stationary phases and of anabiotic cells to be different in viscoelasticity, indicating the superspiralization degree of DNA loops, and in the sensitivity towards alkylhydroxybenzene derivatives [9], the autoregulators synthesized by bacteria of this genus [10, 11] and involved in the mechanisms controlling phenotype variability processes stipulated by intragenomic rearrangements and more pronounced at the stationary phase [9]. Study of the FA profile of DNA-bound lipids in the stationary phase cells showed that DNA-bound lipids were enriched with butyric acid ($C_{4:0}$), saturated palmitic and stearic acids ($C_{16:0}$ and $C_{18:0}$), and unsaturated oleic acid $C_{18:1}$ [12].

The goal of the present work was a comparative study of DNA SC, isolated by a number of various procedures from *P. aurantiaca* bacterial cells at different culture growth stages and FA profile analysis of DNA-bound lipids.

MATERIALS AND METHODS

Gram-negative strain *Pseudomonas aurantiaca* V-1558 (VKM) was an object of the study.

Growth media and conditions. *P. aurantiaca* cells were grown in a synthetic media [9], containing the following (g/l): glucose, 2.0; $KH_2PO_4 \cdot 3H_2O$, 0.1; $(NH_4)_2SO_4$, 0.5; K_2HPO_4, 1; $CaCl_2$, 0.2; $MgSO_4 \cdot 7H_2O$, 0.1; yeast extract (Difco) 0.05; and the following microelements (mg/l): $FeSO_4 \cdot 7H_2O$, 20; $MnCl_2 \cdot 4H_2O$, 20; $ZnSO_4 \cdot 7H_2O$, 0.4; $B(OH)_3$, 0.5; $CuSO_4 \cdot 5H_2O$, 0.05; and $Na_2MoO_4 \cdot 2H_2O$, 0.2; pH 7.25 (after sterilization). Bacteria were cultured at 28°C in 2-l flasks (500 ml of the medium) on a shaker (140–160 rpm) to the exponential and stationary growth phase (24 and 48 h, respectively). The stationary phase culture grown in the same medium was used as inoculum; the initial OD_{540} was 0.1 (l = 10 mm, Specord, Carl Zeiss, Jena, Germany). Dry cell weight (DCW) was determined after drying at 105°C until a constant weight.

Biochemical procedures. Double-distilled water purified with a milliQ system was used to prepare all solutions. Reagents and salts were of reagent grade or analytically pure. Organic solvents were distilled immediately before use.

Mild phenol procedure [6], along with two different detergent–phenol methods (I and II) [13, 14], were used to isolate bacterial DNA SC. Mild extraction was performed by mixing equal volumes of cell suspensions in 0.14 M NaCl (pH 7.0) and a 66% solution of phenol in water, pH 8.5, stirring carefully on a circular shaker (60–70 rpm) for 1 h, and centrifuging for 20 min at 5000 *g*. The upper DNA-containing phase was collected with a special pipette, mixed with an equal volume of 66% aqueous phenol (pH 8.5), stirred for 20 min, and centrifuged for 1 h at the conditions described above. The DNA solution was then dialyzed against 0.14 M NaCl (pH 7.0) at 2–4°C for 3 days. Both detergent–phenol extraction procedures included cell resuspending in 0.01 M Tris–HCl solution in 1 M NaCl (pH 7.6) and addition of an equal volume of the solution containing 0.02 M Tris–HCl, 1 M NaCl, and 50 mM EDTA (pH 7.6). Then, an equal volume was added of either the solution containing 1% SDS, 1 M NaCl, Tris–HCl, and 0.01 M EDTA (pH 9.6), or the solution containing 1% Brij-35, 0.4% desoxycholate, 1 M NaCl, and 10 mM EDTA for method I [13] and method II [14], respectively. The mixtures were centrifuged at 5000 *g* for 1 h, the DNA precipitate was dissolved in 50 ml of cold 0.14 M NaCl solution (pH 7.0), and treated twice with a 66% phenol solution (pH 8.5) according to the technique described in [6]. Spectrophotometric analysis was used to determine the DNA content [15].

Viscoelasticity of the DNA SC solutions (20 μg per 1 ml of 0.14 M NaCl, pH 7.0) was measured using Struchkov and Strazhevskaya high-gradient capillary viscoelastometer [16] at 25°C and expressed in specific viscosity units η_{sp}/C (dl/g). Molecular mass of DNA in the preparations was calculated from characteristic viscosity values (0.002% DNA), measured using a low-gradient three-bead viscosimeter at 25°C in 0.14 M NaCl gradients of 26, 35, and 50 s^{-1}, calibrated against a Zimm rotary viscoelastometer [5, 6].

Isolation of the DNA-bound lipids. DNA-bound lipids were isolated using the methods described in [1, 5]. At the first stage, DNA preparations were treated with 35% ethanol for 24 h at 37°C, then two volumes of cold 96% ethanol were added for DNA precipitation. The isolated DNA was treated twice with 70% ethanol. Ethanol extracts were combined into one and evaporated in a vacuum at 40°C and the remainder was dissolved in chloroform–methanol, 1 : 2 vol/vol, following Bligh–Dyer technique [17] to obtain the fraction of loosely bound lipids (fraction 1). After the DNA precipitate was incubated with lipid-free DNase I (Sigma, United States) in 5 ml of 0.01 M $MgCl_2$ for 2 h at 37°C, the fraction of tightly bound lipids (fraction 2) was then extracted using the Bligh–Dyer procedure.

Fatty acids methyl esters (FAME) analysis. Samples of fractions 1 and 2 were treated with the methylating agent, trimethylsulfonium hydroxide (Macherey & Nagel, Germany) for 1 h. FAME analysis was performed using a Hewlett-Packard 6890 gas chromatograph/mass spectrometer equipped with a capillary HP-5 MS column (30 m, 0.25 mm thin layer, J&W Scientific) and an HP 5973 detector (Agilent Technologies, Palo Alto, United States) at 45–650 m/z range [12]. Helium was used as a carrier gas at a flow rate of 0.8 ml/min. To quantify each component's content in the mixture, calibration against FAME external standards (SUPELCO, United States) was performed.

Table 1. Comparison of *P. aurantiaca* DNA SC extraction techniques in terms of DNA yield and characteristics and of FA content in DNA SC

DNA SC isolation technique	FA content in DNA SC, μg FA/mg DNA		DNA content, mg/g of DCW		Viscoelasticity, dl/g		Molecular mass, Da	
Detergent–phenol method I*	11.19		1.72		320 ± 8		40×10^6	
Detergent–phenol method II*	31.18		0.88		310 ± 6		38×10^6	
Mild phenol procedure	Exp.	Stat.	Exp.	Stat.	Exp.	Stat.	Exp.	Stat.
	9.2	20.56	8.26	10.71	315 ± 15	779 ± 8	39×10^6	198×10^6

* Stationary phase cells.

Total cellular lipids analysis. Cells were dried out under helium flow (DCW 3 mg) and then treated with a 0.4 ml 1N HCl solution in methanol at 80°C for 3 h. Resulting FAME and other lipids were extracted with hexane. Hexane was evaporated and the remainder was derivatized with 20 μl of bis-(trimethylsilyl)-trifluoracetamide for 15 min at 80°C and diluted with hexane to 100 μl [18]. Volatile FA derivatives were analyzed as described above.

Statistic processing. Viscoelasticity (η) measurements are represented as average values ± standard deviation and subjected to statistics analysis using Student's *t* test. The value $P < 0.05$ was critical to consider the differences significant. FAME analysis was performed in three experiments of six ($n = 6$) repeats. The FA profile tables present only the average values without their standard deviations.

RESULTS

Since DNA-bound lipids represent a specific pool of lipids, their quantity and composition depend on the DNA SC isolation technique being used. The first series of experiments was carried out to compare several *P. aurantiaca* DNA SC isolation techniques, namely the mild phenol technique, and two variations of detergent–phenol techniques, including the stage of cellular membrane destruction with either SDS or Brij-35. Comparative values of efficiency for DNA SC isolation from the stationary phase pseudomonas, viscoelasticity and molecular mass of the preparations, and FA composition of DNA-bound lipids for these procedures are presented in Table 1. As follows from comparison of FA specific content in the DNA, detergent–phenol method II was more efficient in isolating DNA-bound lipids (Table 1). FA profile analysis (Table 2) revealed differences in FA quantity in the DNA preparations obtained by various methods. In both fractions of DNA SC isolated with method II, $C_{4:0}$ acid was the predominant one (25.7 and 37.5%, respectively), while in the fractions of DNA-bound lipids obtained by phenol and detergent–phenol method I, $C_{16:0}$ acid was the most abundant (32–43%).

No significant differences were revealed in the FA composition of both loosely and tightly DNA-bound lipid fractions isolated from the stationary-phase cultures of *P. aurantiaca* cells depending on the DNA SC isolation technique, except for the presence of minor eicosanoic acid $C_{20:0}$, detected only in the case of the phenol procedure. The differences concerned quantitative relations and the amount of the major FA (≥10% abundance), and the percentage of minor FA. Thus, four major FA were revealed in the lipids isolated from DNA SC by the phenol procedure, that was $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, and $C_{14:0}$. The lipid fractions of DNA SC isolated by phenol–detergent method I contained three major FA, i.e. $C_{16:0} > C_{18:0} > C_{18:1}$, while in the preparations, isolated by the phenol–detergent method II, FA $C_{4:0} > C_{16:0} > C_{18:0} > C_{18:1}$ were characteristic (see Table 2). Therefore, the similarity between FA composition of fractions 1 and 2 implies that the presence of DNA-bound lipids in *P. aurantiaca* is not an artifact of the isolation procedure.

Additional, more convincing evidence was obtained in the course of comparative study of the FA profiles of total and DNA-bound lipids. The 2- and 3-hydroxy FA typical of *Pseudomonas* [19] were found among *P. aurantiaca* total lipids, but not in fractions 1 or 2 of the DNA SC preparations isolated either by the phenol procedure (Table 3), or by any of the phenol–detergent ones (data not shown). Besides, short-chain FA $C_{4:0}$, $C_{6:0}$, $C_{8:0}$, or $C_{10:0}$ (butyric, caproic, caprylic, or capric acids, respectively) were not found in total lipid fractions of the stationary phase cells, yet they were detected in the fractions of DNA-bound lipids. Among the total lipids of pseudomonads at both the stationary and exponential growth phases, $C_{16:1}$ (palmitoleic), $C_{16:0}$, and $C_{18:1}$ FA were dominating (Table 3). In contrast with total lipids,

Table 2. FA composition (% of the total amount) of DNA-bound lipids in DNA SC isolated from *P. aurantiaca* stationary phase cell cultures by various techniques

FA	Loosely bound lipids (fraction 1)			Tightly bound lipids (fraction 2)		
	Phenol	Detergent/phenol		Phenol	Detergent/phenol	
		Method I	Method II		Method I	Method II
$C_{4:0}$	0.9	2.0	25.7	0.4	7.2	37.5
$C_{6:0}$	1.5	2.0	4.3	0.6	2.8	3.0
$C_{8:0}$	2.1	2.0	1.7	1.8	7.0	1.5
$C_{10:0}$	1.5	4.1	1.7	0.7	2.8	1.5
$C_{12:0}$	3.9	8.1	2.1	1.8	7.0	1.5
$C_{14:0}$	10.8	8.1	4.3	11	7.0	0.8
$C_{14:1}$	0.2	0.4	0.2	1	0.3	0.8
$C_{16:0}$	43.0	40.7	25.7	32.1	35.1	22.6
$C_{16:1}$, sum of isomers	8.4	8.2	8.5	8.2	7.0	7.5
$C_{18:0}$	18.1	12.2	12.9	11	14.0	15.0
$C_{18:1}$, sum of isomers	8.4	12.2	12.9	30.2	9.8	8.3
$C_{20:0}$	1.2	–	–	1.2	–	–
Saturated FA	83	79.2	78.4	60.6	83.1	83.4
Unsaturated FA	17	20.8	21.6	39.4	16.9	16.6

both fractions of DNA-bound lipids, isolated by any of the three methods, were poor in $C_{16:1}$ but enriched with $C_{18:0}$, and together contained more of the saturated FA than of the unsaturated ones (Tables 2 and 3).

The next goal of the study was to reveal the possible differences in FA composition of pseudomonad DNA-bound lipids between the cultures at various growth phases. Comparative study of the FA composition of both loosely and tightly DNA-bound lipids in *P. aurantiaca* was performed using the mild phenol procedure of DNA SC isolation. The following data favored the choice. Little difference was observed between the mild phenol procedure and detergent–phenol method II in terms of FA specific content, while the maximum yield of DNA isolated from pseudomonad cultures of the stationary and exponential growth phases (10.71 and 8.26 mg/g DCW, respectively) was achieved by using the phenol procedure (Table 1). The DNA SC preparations isolated by the phenol procedure displayed higher values of viscoelasticity and molecular mass, which indicated better preservation of the native structure and DNA polymerization degree, compared to the DNA SC isolated by the detergent–phenol methods (Table 1). On the other hand, the phenol procedure allowed us to reveal the difference between the DNA SC preparations isolated from pseudomonad cultures at different growth stages in terms of viscoelasticity and molecular mass values, which were $\eta = 315 \pm 15$ dl/g and $M = 39 \times 10^6$ Da in case of exponentially growing cells, and $\eta = 779 \pm 8$ dl/g and $M = 198 \times 10^6$ Da in case of stationary phase cells (Table 1).

Therefore, since the mild phenol procedure resulted in the best preservation of native DNA macromolecule structure, it was chosen for the comparative study of FA composition in loosely and tightly DNA-bound lipids of *P. aurantiaca* cultures at the stationary and exponential growth phases.

The first difference was that FA content in the whole pool of DNA-bound lipids was 2.2 times higher in the stationary phase cells, than in those proliferating. However, the overall (for two fractions) ratio between saturated FA (SFA) and unsaturated FA (UFA) in the cultures of different stages was close (Table 4). Moreover, while SFA prevailed over UFA in the DNA lipid fractions from both the stationary and exponential growth phases, their proportion varied. The differences in the

Table 3. FA composition (% of the total amount) of total lipids and fractions of DNA-bound lipids of *P. aurantiaca* cells in the exponential and stationary phases (phenol procedure of DNA SC isolation)

FA	Exponential growth phase			Stationary growth phase		
	Fraction 1	Fraction 2	Total lipids	Fraction 1	Fraction 2	Total lipids
$C_{4:0}$	–	–	–	0.9	0.4	–
$C_{6:0}$	–	–	–	1.5	0.6	–
$C_{8:0}$	–	–	–	2.1	1.8	–
$C_{10:0}$	–	–	–	1.5	0.7	–
$C_{12:0}$	0.2	–	0.6	3.9	1.8	0.9
$C_{3OH\text{-}10:0}$	–	–	0.3	–	–	1.1
$C_{3OH\text{-}12:0}$	–	–	0.3	–	–	0.4
$C_{2OH\text{-}12:0}$	–	–	1.5	–	–	3.6
Dodecanediol	–	–	–	–	–	1.2
$C_{14:0}$	1.1	1.5	0.7	10.8	11.0	1.5
$C_{14:1}$	–	–	–	0.2	1	–
$C_{15:0}$	0.6	0.6	–	–	–	–
$C_{16:0}$	41.6	31.1	35.2	43.0	32.1	33.0
$C_{16:1}$, sum of isomers	1.7	1.1	41.6	8.4	8.2	36.0
$C_{17:0}$	1.0	1.0	0.1	–	–	1.1
$C_{18:0}$	32.3	54.3	2.5	18.1	11.0	3.0
$C_{18:1}$, sum of isomers	14.1	7.4	17.2	8.4	30.2	18.2
$C_{18:2}$, unidentified isomers	5.1	–	–	–	–	–
$C_{18:2}$, linoleic acid	0.6	1.1	–	–	–	–
$C_{19:0}$	0.4	–	–	–	–	–
$C_{20:0}$	0.6	1.9	–	1.2	1.2	–
$C_{OH\text{-}20:0}$	0.4	–	–	–	–	–
$C_{22:0}$	0.3	–	–	–	–	–
Saturated FA	78.5	90.4	41.2	83	60.6	45.8
Unsaturated FA	21.5	9.6	58.8	17	39.4	54.2

SFA/UFA ratio were specific for the fraction of DNA lipids and also depended on the growth phase of pseudomonad culture. Thus, fraction 1 (loosely DNA-bound lipids) was more enriched with SFA in the stationary phase cells than in the exponentially growing cells; the corresponding values of SFA/UFA ratio for the pool were 4.9 and 3.7. A different tendency was observed in the case of tightly bound lipids (fraction 2). Here, in the stationary phase the amount of DNA-bound UFA increased by an order of magnitude and the SFA, by a factor of 1.5, compared to the exponential phase. The SFA/UFA ratio for the lipids of the fraction (1.5 and 9.4) was therefore higher in proliferating cells (Table 4). It should be noted that the SFA/UFA ratio in the pool of total lipids was practically the same for the exponential and stationary growth phases (0.7 and 0.8, respectively) and was lower than in any of the fractions of DNA-bound lipids.

The differences in the FA composition of DNA-bound lipids in *P. aurantiaca* cells depending on the growth phase were also determined (Table 3). In the case of the exponential growth phase, $C_{16:0} > C_{18:0} > C_{18:1}$ were the major FA of fraction 1, and $C_{18:0} > C_{16:0}$ were dominating in fraction 2. In fraction 1 of the stationary phase cells, $C_{16:0} > C_{18:0} > C_{14:0}$ were the dominating FA, while in fraction 2 they were $C_{16:0} > C_{18:1} > C_{18:0} > C_{14:0}$. $C_{14:0}$ acid, while being revealed in trace amounts in the total lipid fraction of proliferating cells was among the major acids in both fractions of DNA-bound lipids in the stationary phase cells. Interestingly, in fraction 2 of tightly DNA-bound lipids, $C_{18:1}$ was among the major FA, and $C_{14:0}$ content was considerably higher than in the corresponding fraction of the exponential phase cells (Table 3). Additional balance calculations showed that the total pool of UFA in tightly DNA-bound lipids of the stationary phase cells increased due to the elevated relative content of $C_{18:1}$ FA with simultaneous decrease of $C_{16:1}$ FA specific content.

We would like to specifically emphasize a number of contrasting differences between the FA profiles of DNA-bound and total cellular lipids. For example,

Table 4. FA content and ratio between saturated and unsaturated FA in fractions of DNA-bound lipids (phenol procedure of DNA isolation)

Parameters	Cells at the exponential growth phase			Cells at the stationary growth phase		
	Fraction 1	Fraction 2	DNA-bound lipids	Fraction 1	Fraction 2	DNA-bound lipids
Sum of FA, μg/mg of DNA	6.9	2.3	9.2	15.50	5.06	20.56
SFA, μg/mg of DNA	5.42	2.08	7.5	12.87	3.06	15.93
UFA, μg/mg of DNA	1.48	0.22	1.7	2.63	2.00	4.63
SFA/UFA	3.7	9.43	4.35	4.89	1.53	3.44
UFA/SFA	0.27	0.11	0.23	0.21	0.65	0.29

$C_{18:0}$, being the major FA in DNA-bound lipids, was a minor one in the total lipid pool of the cultures at both growth phases. The isomers of $C_{16:1}$, which dominated in total cellular lipid fractions, were detected only in trace amounts among DNA-bound lipids. The presence of $C_{18:2}$ (linoleic) acid only in fraction 1 of the exponential phase cells was strikingly surprising. Therefore, *P. aurantiaca* DNA SC characteristics and FA profile of DNA-bound lipids varied significantly, depending on the culture growth phase.

DISCUSSION

As already demonstrated for *E. coli, Sh. sonnei*, and *S. thyphimurium*, as well as for some eukaryotic organisms [4, 5, 7, 8], the presence of a special pool of lipids in *P. aurantiaca* DNA SC was established in the present work. However, the very existence of DNA-bound lipids still requires evidence. The presence of DNA-bound lipids in *P. aurantiaca* is supported by the following data: i) DNA-bound lipids were detected independently of the isolation procedure used; ii) FA of loosely and tightly DNA-bound lipids varied in the composition and relative quantity; and iii) FA composition of DNA-bound and total cellular lipids differed significantly (Tables 2 and 3). Above all, 2- and 3-hydroxy FA, typical representatives of *Pseudomonas* total cellular lipids pseudomonads [19], were not detected in the DNA-bound lipids pool. Earlier, in experiments with the introduction of labeled (by ^{14}C-acetate) and unlabeled lipids into the cell homogenate prior to DNA isolation these FA were proved to be DNA SC components rather than isolation procedure artifacts [5].

FA profile analysis of DNA-bound lipids isolated from DNA SC of *P. aurantiaca* cells by various procedures demonstrated their fundamental similarity, but also revealed some differences, depending on the nucleic acid isolation technique. The major differences concerned DNA SC yield and FA amount (Table 1); less notable variations were observed in the FA composition of lipid fractions 1 and 2 (Table 2). Variations in the amount of lipids in DNA SC preparations depending on the isolation procedure have been shown by different studies [4, 5, 7, 8]. For example, total amount of NL and PL in the DNA preparations isolated from *S. thyphimurium* by mild phenol extraction was higher than under more severe conditions (treatment with 1% SDS, chloroform–isoamyl alcohol, 24 : 1, and phenol); yet, NL to PL ratio in both cases was similar [5, 8]. Obviously, high DNA yield in its isolation from *P. aurantiaca* cells by the phenol procedure allowed us to detect the minor $C_{20:0}$ FA, in contrast to DNA SC lipid fractions obtained by either of the detergent–phenol procedures (Table 2). The possibility to isolate a wider range of FA of the nucleic complex favored the choice of the phenol method for a comparative study of DNA-bound lipids in pseudomonad cells at various growth phases.

Another reason for this choice was better preservation of the native DNA structure by the mild phenol procedure, indicated by the values of viscoelasticity and molecular mass of the isolated DNA SC (Table 1). These characteristics varied significantly depending on the *P. aurantiaca* growth phase. Viscoelasticity and molecular mass of the DNA SC preparations isolated from the stationary phase cells were 2.5 and 5 times higher, respectively, than the values obtained for the cultures in the exponential growth phase; this finding confirms our earlier results [9]. Lower viscoelasticity and molecular mass values of DNA isolated from exponentially growing cells are probably due to DNA replication in the phase, which provides for partially relaxed and unwound DNA strings less resistant towards isolation reagents. Apart from DNA superspiralization and nucleoid compactization [9, 20], DNA binding with proteins [21] and lipids [5] in the stationary phase cells also contributes to higher values of DNA viscoelasticity and molecular mass. The importance of lipids was dem-

onstrated by the fact that mild delipidization of high molecular DNA (3×10^8 Da) by 35% ethanol or lipolytic enzymes led to DNA fragmentation into subunits of $(50 \pm 10) \times 10^6$ Da and a tenfold decrease in viscoelasticity [5]. The above data implied a prominent role for lipids in DNA structure stabilization and thus served the ground for our investigation of the FA pool of DNA-bound lipids at various *P. aurantiaca* culture development stages.

In the present work, a positive correlation between FA content of the DNA-bond lipids (per DNA mass unit) and values of viscoelasticity and molecular mass of DNA isolated from the stationary phase cells was observed in comparison to exponentially growing cells (Table 1), which proved the importance of lipids in DNA SC reorganization. On the other hand, pools of FA from DNA-bound lipids were shown to be more abundant in saturated than in unsaturated FA (Table 3). The DNA–FA binding energy depends upon the FA saturation degree, for example, saturated $C_{18:0}$ acid binds DNA better than $C_{18:1}$, $C_{18:2}$, or $C_{18:3}$ FA [22]. These data suggests an explanation for $C_{14:0}$ appearance and high $C_{18:0}$ content among the major FA of both fractions of DNA-bound lipids. We can assume that these two acids bind specifically with DNA, as their high content in DNA SC contrasts with their low amounts in the pool of pseudomonad total cellular lipids (Table 3). Comparative enrichment of tightly bound lipids with $C_{18:0}$ and $C_{18:1}$ FA may be related to the differences in localization of the lipids bearing these FA on the DNA molecule. $C_{18:0}$ and $C_{18:1}$ are assumed to preferably bind to the DNA minor groove where they are more tightly packed [22]. For this reason, an additional stage of DNA destruction is necessary for the isolation of lipids of this localization, which was taken into account in our experiments on isolation of the tightly DNA-bound lipid fraction.

Interestingly, the fraction 2 of tightly DNA-bound lipids was enriched with $C_{18:1}$ and $C_{16:1}$ FA in *P. aurantiaca* stationary phase cells in contrast to the FA pool in proliferating cells marked by the dominance of saturated FA (Table 3). So far, there is no evidence of the difference associated with the transition from active replication to growth arrest in the pseudomonads under study. However, the role of lipids the regulation of replication has been already shown for another gram-negative bacterium, *E. coli* [23–25]. For example, cardiolipin (CL) and phosphatidylglycerol suppress binding of the DnaA regulatory protein to both ATP and *oriC*, DNA segment where replication starts; moreover, CL, bearing $C_{18:1}$ FA, exhibits a stronger inhibitory effect than CL with $C_{18:0}$ FA [25]. In this connection, it should be mentioned that it is CL with which DNA-bound phospholipids of bacteria (*E. coli* and *Sh. sonnei*) are enriched (80%) [1, 4, 7]. We demonstrated that during active DNA replication (in the exponential growth phase) both fractions of DNA-bound lipids were enriched with $C_{18:0}$ FA (32.3 and 54.3%, respectively), but depleted in $C_{18:1}$ FA (14.1 and 7.4%, respectively). On the contrary, during the stationary phase, that is in the absence of DNA replication, both lipid fractions were depleted in $C_{18:0}$ FA (18.1 and 11%, respectively), yet fraction 2 was enriched with $C_{18:1}$ FA (30.2%).

Therefore, modifications of the FA profile of DNA-bound lipids at various growth phases correspond to variations in the physicochemical characteristics of DNA preparations, which implies an important role of lipids in the structural and functional organization of *P. aurantiaca* DNA.

ACKNOWLEDGMENTS

The work was supported by the Russian Foundation for Basic Research, project no. 07-04-01011.

REFERENCES

1. Struchkov, V.A., Strazhevskaya, N.B., and Zhdanov, R.I., DNA-Bound Lipids of Normal and Tumor Cells: Retrospective and Outlooks for Functional Genomics, *Bioelectrochemistry,* 2002, vol. 58, pp. 23–30.
2. Albi, E. and Viola Magni, M.P., The Role of Intranuclear Lipids, *Biol. Cell*, 2004, vol. 96, pp. 657–667.
3. Manzoli, F.A., Capitani, S., Mazzotti, G., Barnabei, O., and Maraldi, N.M., Role of Chromatin Phospholipids on Template Availability and Ultrastructure of Isolated Nuclei, *Adv. Enz. Res.,* 1981, vol. 20, no. 2, pp. 247–262.
4. Struchkov, V.A. and Strazhevskaya, N.B., DNA-Bound Lipids of Eukaryotes (Loach Sperm, pigeon Erythrocytes) and Prokaryotes (*E. coli* B, Phage T2), *Biokhimiya*, 1990, vol. 55, no. 7, pp. 1266–1275.
5. Struchkov, V.A. and Strazhevskaya, N.B., DNA-Bound Lipids: Composition and Possible Functions, *Biokhimiya*, 1993, vol. 58, no. 8, pp. 1154–1175.
6. Struchkov, V.A., On the Nature of "Superpolymeric" Deoxyribonucleic Acid, *Biofizika*, 1962, vol. 7, no. 3, pp. 538–550.
7. Shepelev, A.P., Shestopalov, A.V., Ryndich, A.A., Staviskii, I.M., and Yagovkina, V.V., DNA-Bound Lipids of Heat-Resistant and Heat-Labile Strains of *Shigella sonnei, Byull. Eksperim. Biol. i Med*, 1995, vol. 120, no. 2, pp. 332–333.
8. Sinyak, K.M. and Danilenko, I.I., Lipid Content in DNA Preparations of Different Viscosity and Characterization of These Lipids, *Dokl. AN USSR, Seriya V*, 1976, no. 12, pp. 1119–1122.
9. Mulyukin, A.L., Vakhrushev, M.A., Strazhevskaya, N.B., Shmyrina, A.S., Zhdanov, R.I., Suzina, N.E., Duda, V.I., Kozlova, A.N., and El'-Registan, G.I., Effect of Alkylhydroxybenzenes, Microbial Anabiosis Inducers, on the Structural Organization of *Pseudomonas aurantiaca* DNA and on the Induction of Phenotypic Dissociation, *Mikrobiologiya*, 2005, vol. 74, no. 2, pp. 157–165 [*Microbiology* (Engl. Transl.), vol. 74, no. 2, pp. 128–135].
10. Osipov, G.A., El'-Registan, G.I., Svetlichnyi, V.A., Kozlova, A.N., Duda, V.I., Kaprel'yants, A.S., and Pomazanov, V.V., Chemical Nature of the *Pseudomonas carboxydoflava* Autoregulatory factor d, *Mikrobiologiya*, 1985, vol. 54, no. 2, pp. 186–190.

11. Kozubek, A., Pietr, S., and Czerwonka, A., Alkylresorcinols Are Abundant Lipid Components in Different Strains of *Azotobacter chroococcum* and *Pseudomonas* spp., *J. Bacteriol.*, 1996, vol. 178, no. 14, pp. 4027–4031.
12. Zhdanov, R., Shmyrina, A., Zarubina, T., Mulyukin, A., El-Registan, G., Haupt, N., Kraus, A., and Lorenz, W., Nature of DNA-Bound Fatty Acids in *Pseudomonas aurantiaca, FEMS Microbiol. Letts.*, 2006, vol. 265, no. 1, pp. 151–158.
13. Stonington, O.G. and Pettijohn, D.E., The Folded Genome of *Escherichia coli* Isolated in a Protein-DNA-RNA Complex, *Proc. Natl. Acad. Sci. USA*, 1971, vol. 68, no. 1, pp. 6–9.
14. Sueoka, N. and Hammers, J.M., Isolation of DNA-Membrane Complex in *Bacillus subtilis, Proc. Natl. Acad. Sci. USA*, 1974, vol. 71, no. 12, pp. 4787–4791.
15. Spirin, A.S., Spectrophotometric Determination of Nucleic Aids Content, *Biokhimiya*, 1958, vol. 23, no. 5, pp. 656–662.
16. Struchkov, V.A. and Strazhevskaya, N.B., Capillary Elastoviscosimeter, USSR Inventor's Certificate no. 3842226/24/26, *Bull. Izobret.*, 1986, no. 40, p. 152.
17. Bligh, E.G. and Dyer, W.J., A Rapid Method of Total Lipid Extraction and Purification, *Can, J. Phys.*, 1959, vol. 37, pp. 911–917.
18. Pikuta, E., Lysenko, A., Suzina, N., Osipov, G., Kuznetsov, B., Tourova, T., Akimenko, V., and Laurinavichius, K., *Desulfotomaculum alkaliphilum* sp. nov., a New Alkaliphilic, Moderately Thermophilic, Sulfate-Reducing Bacterium, *Int. J. Syst. Evol. Microbiol.*, 2000, vol. 50, pp. 25–33.
19. Mukwaya, G.M. and Welch, D.F., Subgrouping of *Pseudomonas cepacia* by Cellular Fatty Acid Composition, *J. Clin. Microbiol.,* 1989, vol. 27, no. 12, pp. 2640–2646.
20. Frenkiel-Krispin, D., Levin-Zaidman, S., Shimoni, E., Wolf, S.A., Wachtel, E.T., Arad, T., Finkel, S.E., Kolter, R., and Minsky, A., Regulated Phase Transitions of Bacterial Chromatin: a Non-Enzymatic Pathway for Generic DNA Protection, *The EMBO J.,* 2001, vol. 20, pp. 1184–1191.
21. Azam, T.A., Iwata, A., Nishimura, A., Ueda, S., and Ishihama, A., Growth Phase-Dependent Variation in Protein Composition of the *Escherichia coli* Nucleoid, *J. Bacteriol.*, 1999, vol. 181, no. 20, pp. 6361–6370.
22. Zhdanov, R.I., D'yachkov, E.P., Struchkov, V.A., Strazhevskaya, N.B., and D'yachkov, P.N., DNA-Bound Lipids: Modeling DNA Interaction with Stearic and Unsaturated Fatty Acids, *Izv. Akad. Nauk, Ser. Khim.*, 2003, vol. 52, no. 9, pp. 1794–1800.
23. Yung, B.Y. and Kornberg, A., Membrane Attachment Activates dnaA Protein, the Initiation Protein of Chromosome Replication in *Escherichia coli, Proc. Natl. Acad. Sci. USA*, 1988, vol. 85, pp. 7202–7205.
24. Crooke, E., Castuma, C.T., and Kornberg, A., The Chromosome Origin of *Escherichia coli* Stabilizes dnaA Protein During Rejuvenation by Phospholipids, *J. Biol. Chem.*, 1992, vol. 267, no. 22, pp. 16779–16782.
25. Makise, M., Mima, S., Katsu, T., Tsuchiya, T., and Mizushima, T., Acidic Phospholipids Inhibit the DNA-Binding Activity of DnaA Protein, the Initiator of Chromosomal DNA Replication in *Escherichia coli, Mol. Microbiol.*, 2002, vol. 46, no. 1, pp. 245–256.