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EXPERIMENTAL ARTICLES

Effect of Cell Lipid Composition on the Formation of Nonspecific Antibiotic Resistance in Alkanotrophic Rhodococci

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Abstract—The antibiotic resistance and lipid composition of rhodococci grown in rich organic media with gaseous or liquid *n*-alkanes were studied. Hydrocarbon-grown rhodococci exhibited an increased resistance to a wide range of antibiotics (aminoglycosides, linkosamides, macrolides, β-lactams, and aromatic compounds). The enhanced antibiotic resistance of rhodococci grown on *n*-alkanes correlated with an increased content of total cell lipids (up to 14–28%) and saturated straight-chain fatty acids ($C_{16:0}$, $C_{18:0}$, $C_{21:0}$) and was accompanied by the appearance of cardiolipin and phosphatidylglycerol in cells. These lipid compounds are supposed to promote the formation of nonspecific antibiotic resistance in rhodococci by decreasing the permeability of their cell envelope to antibiotics.

Key words: Rhodococcus, n-alkanes, antibiotic resistance, total cell lipids, phospholipids, fatty acid composition.

The ability of rhodococci to utilize gaseous (propane, *n*-butane) and liquid *n*-alkanes largely determines their structural and metabolic organization. Rhodococci grown on *n*-alkanes are distinguished by their specific physiological properties. Thus, rhodococci grown in the presence of propane or *n*-butane are characterized by an enhanced resistance to antibiotics [1, 2].

The cell envelope of rhodococci involves a hydrophobic lipid layer containing covalently bound mycolic acids. In its barrier properties, this layer, which is known to determine the resistance of rhodococci to many antimicrobial agents [3], is very similar to the peptidoglycan layer of gram-negative bacteria. The lipid composition of rhodococci depends on the type of the substrate used as the carbon source [4–6]. Some lipid components of the cell envelope presumably promote the formation of antibiotic resistance in alkanotrophic rhodococci by decreasing the transport of antibiotics into cells.

Data available in the literature on changes in the lipid composition of the cell envelope of microorganisms during the development of their resistance to antibiotics are scarce and often contradictory [7, 8]. In this connection, the present work was undertaken to study the effect of the cell lipid composition of rhodococci grown on *n*-alkanes on the formation of their broad-spectrum resistance to antibiotics.

MATERIALS AND METHODS

Seventeen propane-oxidizing strains of *Rhodococcus ruber* used in this study (IEGM 71, 75, 76, 77, 78, 81, 84, 85, 86, 90, 91, 94, 242, 333, 337, 371, and 378) were obtained from the specialized collection of alkanotrophic microorganisms of the Institute of Ecology and Genetics of Microorganisms [9].

Cultures were grown at 28°C for 3–7 days on nutrient agar (Oxoid, Unipath Ltd., United Kingdom) or mineral agar medium. In the latter case, either *n*-hexadecane (Sigma, St. Louis, United States) or an air–gas mixture containing 20 vol % of propane and *n*-butane served as the source of carbon and energy. The mineral agar medium, based on K medium [10], contained (g/l) KNO_3, 1.0; K_2HPO_4, 1.0; KH_2PO_4, 1.0; NaCl, 1.0; $MgSO_4$, 0.2; $CaCl_2$, 0.02; $FeCl_3$, 0.001; agar, 15.0; and 1 ml/l of a trace element solution. Agar was purchased from Difco Laboratories, Detroit, United States.

Susceptibility to antibiotics was assayed using standard test disks purchased from Oxoid or Mosmedpreparaty (Moscow, Russia). The disks were impregnated with 23 antibiotics differing in their mechanism of action. The diameter of the growth inhibition zones was measured in mm after 3–7 days of incubation. Tests were carried out in triplicate. For a more detailed study of antibiotic resistance, rhodococci were grown in nutrient broth (Oxoid) or liquid mineral medium with propane or *n*-hexadecane (2.0 vol %) in the presence of one of the following antibiotics taken in two concentrations (μg/ml): gentamicin, 0.1 and 1.0; kanamycin, 1.0 and 10.0; lincomycin, 10.0 and 90.0; oxacillin, 5.0 and 20.0;

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Table 1. Effect of growth substrate on the susceptibility of rhodococci to antibiotics expressed in the diameter of growth inhibition zones measured in mm

Antibiotic	Nutrient agar	Mineral medium with	
		propane	*n*-hexadecane
Aminoglycosides			
Gentamicin	36.2 ± 4.8	17.4 ± 2.4	29.1 ± 4.2
Kanamycin	32.7 ± 5.4	22.4 ± 5.2	30.2 ± 7.2
Monomycin	33.6 ± 4.0	17.5 ± 3.6	ND
Neomycin	29.3 ± 6.2	15.4 ± 5.6	25.7 ± 4.0
Streptomycin	33.1 ± 2.8	25.0 ± 3.8	ND
Macrolides			
Oleandomycin	33.2 ± 8.8	9.2 ± 5.4	16.3 ± 7.2
Erythromycin	40.1 ± 6.8	14.1 ± 7.7	29.5 ± 4.8
Lincosamides			
Lincomycin	25.1 ± 8.0	9.4 ± 5.6	11.3 ± 3.4
Clindamycin	21.2 ± 8.4	15.2 ± 7.2	12.6 ± 6.8
Penicillins			
Ampicillin	36.6 ± 6.0	33.3 ± 6.2	39.7 ± 8.8
Benzylpenicillin	38.1 ± 4.4	35.5 ± 3.4	33.4 ± 8.4
Carbenicillin	44.2 ± 6.4	32.3 ± 3.6	51.8 ± 5.8
Methicillin	8.2 ± 4.4	6.0 ± 0.8	6.0 ± 1.4
Oxacillin	20.2 ± 4.2	9.3 ± 6.2	12.3 ± 5.7
Tetracyclines			
Doxycycline	25.1 ± 6.4	37.5 ± 5.0	35.6 ± 9.4
Tetracycline	31.2 ± 6.0	33.3 ± 5.2	41.7 ± 8.0
Nalidixic acid	6.3 ± 1.0	6.1 ± 0.8	6.0 ± 1.0
Polymyxin	9.1 ± 4.2	6.0 ± 1.6	9.2 ± 3.2
Ristomycin	26.2 ± 7.2	27.7 ± 8.0	34.2 ± 6.8
Rifampicin	41.0 ± 8.0	23.6 ± 10.4	45.0 ± 14.7
Fusidic acid	50.1 ± 3.4	46.2 ± 8.4	54.3 ± 4.0
Chloramphenicol	30.1 ± 5.4	15.0 ± 7.6	13.3 ± 6.9
Cephalexin	14.5 ± 6.3	9.8 ± 6.7	14.4 ± 5.8

Note: ND stands for "no data."

oleandomycin, 1.0 and 5.0; chloramphenicol, 2.0 and 20.0; and erythromycin, 0.5 and 5.0. Antibiotics were purchased from Sigma and were 95–99% pure.

Total cell lipids were extracted as described in the handbook [11]. Lipid extracts were subjected to mild alkaline hydrolysis and their fatty acid composition was determined after esterification with absolute methanol in the presence of benzene. Methylated fatty acids were analyzed on a Chrom-5 gas chromatograph (Laboratorni Pristroje, Praha, Czech Republic) equipped with a flame–ionization detector and column (3.7 m × 4.0 mm ID), with the stationary phase representing 10% PEGA on an N-AWDMCS Inerton. The carrier gas was helium at a flow rate of 24 ml/min; the evaporator, column, and detector were kept at 310, 180, and 210°C, respectively; the volume of samples was 10 μl. Authentic samples of methylated fatty acids purchased from Sigma were used as reference standards.

Phospho- and glycolipids in lipid extracts were quantified by the thin-layer chromatography of their acetone precipitates on 20 × 20 cm TLC plates (Larne Kalur, Haapsalu, Estonia) with KSKG silica gel (5 × 20 μm particle size; 130 × 25 μm layer thickness). Plates were developed in one direction in a chloroform–methanol–water (65 : 25 : 4) mixture at 30°C. The volume of samples was 10 μl. Phospholipids were detected by spraying the developed plates with the phosphomolybdic acid reagent [12], and glycolipids were visualized with a solution of anthrone [13]. Individual phospholipids on developed plates were detected by staining them

Table 2. Effect of antibiotics on the total lipid content (% of dry cell weight) of *R. ruber* IEGM 333 cells grown on different substrates

Antibiotic	Nutrient agar	Mineral medium with	
		propane	*n*-hexadecane
Control (without antibiotics)	14.6 ± 4.2	11.8 ± 2.5	22.3 ± 8.2
Gentamicin	15.3 ± 5.1	11.9 ± 0.6	23.1 ± 0.4
	($P > 0.1$)	($P > 0.1$)	($P > 0.1$)
Kanamycin	15.0 ± 3.5	11.3 ± 8.4	25.4 ± 2.2
	($P > 0.1$)	($P > 0.1$)	($P > 0.1$)
Lincomycin	14.9 ± 6.3	14.2 ± 4.4	27.6 ± 3.5
	($P > 0.1$)	($P < 0.1$)	($P < 0.05$)
Oxacillin	15.1 ± 7.3	10.4 ± 3.0	22.9 ± 1.1
	($P > 0.1$)	($P > 0.1$)	($P > 0.1$)
Chloramphenicol	13.5 ± 3.0	17.9 ± 5.6	26.2 ± 7.8
	($P > 0.1$)	($P < 0.05$)	($P < 0.1$)
Erythromycin	16.3 ± 7.4	18.3 ± 6.3	26.1 ± 7.3
	($P > 0.1$)	($P < 0.05$)	($P < 0.1$)

Note: Given are the mean data of triplicate experiments. Parenthesized are the significance of the difference between the control and the experiment at the significance levels $P < 0.1$ and $P < 0.05$.

with ninhydrin and Dragendorff reagent [14]. Authentic samples of phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, cardiolipin, and trehalose dibehenate purchased from Sigma were used as reference standards.

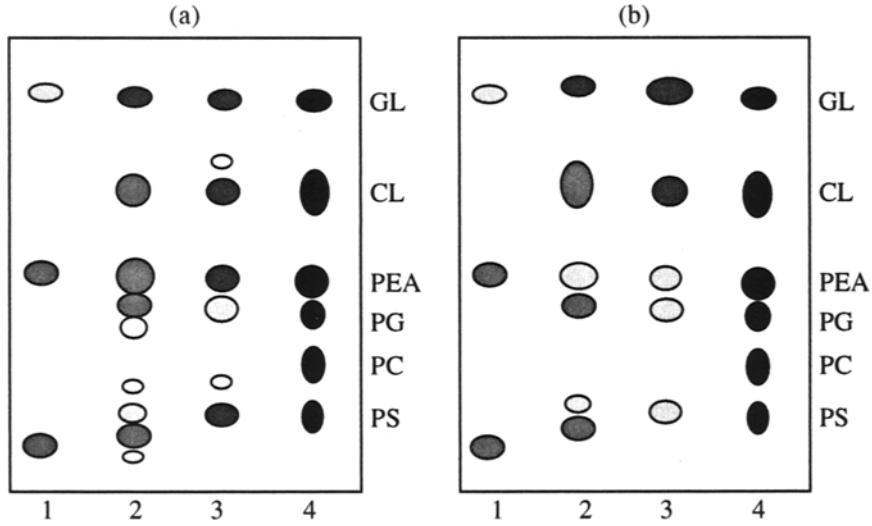
The results were statistically processed as described in the handbook [15].

RESULTS AND DISCUSSION

Rhodococci grown on nutrient agar, propane + *n*-butane, and *n*-hexadecane considerably differed in their sensitivity to antibiotics (Table 1). In particular, rhodococci grown on gaseous hydrocarbons exhibited a high resistance to oleandomycin, lincomycin, oxacillin, and cephalexin (the mean diameter of growth inhibition zones was 9–10 mm), as well as to neomycin, erythromycin, clindamycin, and chloramphenicol (the mean diameter of growth inhibition zones was 14–15 mm). Rhodococci grown on liquid hydrocarbons also exhibited an enhanced resistance to a wide range of antibiotics (aminoglycosides, linkosamides, macrolides, β-lactams, and aromatic compounds), although not so pronounced as in the case of their growth on gaseous hydrocarbons (Table 1). It should be noted that hydrocarbon-grown rhodococci showed a high resistance to antibiotics whose mechanism of action was related to the inhibition of protein synthesis or, as in the case of oxacillin, to the suppression of the synthesis of the cell envelope components. This implied that the targets of these antibiotics are intracellular, and, therefore, the high antibiotic resistance of hydrocarbon-grown rhodococci may be due to the impaired permeability of their cell envelope by virtue of some changes in its lipid composition.

The comparative study of the antibiotic resistance and cell lipid composition of rhodococci grown on different substrates showed the existence of a good correlation between the amount of total lipids in hydrocarbon-grown cells and their resistance to hydrophobic antibiotics, such as lincomycin, chloramphenicol, and erythromycin. For illustration, Table 2 presents the results of the determination of the total cell lipids in *R. ruber* IEGM 333 cells grown in nutrient broth and in mineral medium with propane or *n*-hexadecane in the presence of subinhibitory concentrations of various antibiotics. As is evident from this table, *n*-hexadecane-grown cells had more lipids (22.3%) than cells grown on propane or in nutrient broth (11.8 and 14.6%, respectively). These results agree with data of other authors obtained in experiments with other species of rhodococci, *R. erythropolis* and *R. rhodochrous* [16, 17].

Different antibiotics added to the cultivation medium differently affected the cell lipid content of *R. ruber* IEGM 333 cells grown on *n*-hexadecane. Thus, growth in the presence of the hydrophobic antibiotics lincomycin, erythromycin, and chloramphenicol was accompanied by a significant rise in the content of total cell lipids (by 5.3, 3.8, and 3.9%, respectively). At the same time, hydrophilic antibiotics (aminoglycosides and oxacillin) virtually did not cause any changes in the content of total cell lipids in rhodococci grown on



Thin-layer chromatography of phospholipids extracted from *R. ruber* IEGM 333 cells grown (a) without and (b) with chloramphenicol. Chloroform–methanol–water (65 : 25 : 4) system. Lanes: 1, extract of nutrient broth–grown cells; 2, extract of propane-grown cells; 3, extract of *n*-hexadecane-grown cells; 4, mixture of authentic phospholipids. PS is phosphatidylserine, PC is phosphatidylcholine, PG is phosphatidylglycerol, PEA is phosphatidylethanolamine, CL is cardiolipin, GL is glycolipid (*D*-(+)-trehalose 6,6'-dibehenate).

n-hexadecane. In the case of propane-grown rhodococci, chloramphenicol, erythromycin, and lincomycin raised the content of total cell lipids by 6.1, 6.5, and 2.4%, respectively. It should be emphasized that antibiotics did not cause an adaptive increase in the cell lipid content of rhodococci grown in nutrient broth.

The increase in the total lipid content of *R. ruber* cells grown on hydrocarbons in the presence of hydrophobic antibiotics can be considered to be a manifestation of the adaptive response of cells, which lies in a thickening of the bacterial cell envelope and, hence, in the enhancement of its barrier function. This assumption is corroborated by the fact that rhodococci grown on agar media with propane or *n*-hexadecane in the presence of lincomycin, rifampicin, or chloramphenicol mainly produce R-forms, which are known to differ from S-forms in that they are more resistant to antibiotics and have a more rigid and thicker cell envelope with twice as many lipids [18].

Phospholipids play a significant role in the regulation of the transport of various compounds, including antibiotics, through the outer cell membrane. As can be seen from Fig. 1a, rhodococci grown on propane or *n*-hexadecane contained a wider range of phospholipids than rhodococci grown in nutrient broth. Thus, nutrient broth–grown cells contained only phosphatidylethanolamine, whereas propane-grown cells contained, in addition, phosphatidylserine, phosphatidylglycerol, diphosphatidylglycerol (cardiolipin), glycolipid, unidentified phospholipid with $R_f = 0.14$, and three minor phospholipids that failed to be stained with reagents for amino and choline groups. Cells grown on *n*-hexadecane contained small amounts of phosphatidylserine, phosphatidylethanolamine, cardiolipin, glycolipid, phosphatidylglycerol, and two minor unidentified phospholipids lacking amino and choline groups.

Chloramphenicol at a concentration of 5 μg/ml did not affect the phospholipid composition of nutrient broth–grown rhodococci (Fig. 1b). At the same time, the addition of this antibiotic to the cultivation medium with propane and *n*-hexadecane led to the disappearance of minor phospholipids in cells. Furthermore, cells grown on propane in the presence of chloramphenicol exhibited a lowered content of the acidic phospholipid phosphatidylethanolamine and an elevated content of the neutral phospholipid cardiolipin. In cells grown on *n*-hexadecane in the presence of this antibiotic, the content of the acidic phospholipids phosphatidylserine and phosphatidylethanolamine also decreased, whereas the content of glycolipid increased.

Thus, hydrocarbon-grown rhodococci are characterized by the presence of the neutral phospholipid cardiolipin and its precursor phosphatidylglycerol. These components of the cell membrane probably diminish the permeability of the cell envelope to antibiotics, thereby promoting the resistance of rhodococci to antibiotics. This assumption agrees with the reported ability of cardiolipin to enhance the rigidity of the cell envelope of staphylococci and thus to reduce their susceptibility to bacitracin and enramycin [19].

R. ruber IEGM 333 cells grown in nutrient broth were characterized by a high content of unsaturated and branched fatty acids (52.7%), which did not change in the presence of antibiotics in the cultivation medium (Table 3). The dominant fatty acids were palmitic ($C_{16:0}$), heptadecenic ($C_{17:1}$), and oleic ($C_{18:1}$) acids. Tuberculostearic acid ($C_{10Me18:0}$) was present in an amount of only 3.6%, which is typical of this rhodococ-

Table 3. Effect of antibiotics on the fatty acid composition of *R. ruber* IEGM 333 cells grown on different substrates (data are expressed in % of total fatty acids)

Fatty acid	Control			Gentamicin		Kanamycin			Oxacillin			Oleandomycin		Chloramphenicol			Erythromycin	
	1	2	3	1	3	1	2	3	1	2	3	1	2	1	2	3	1	3
$C_{9:0}$	–	5.5	0.2	–	–	–	–	–	–	–	–	–	–	–	2.2	–	–	–
$C_{10:0}$	1.1	0.3	0.4	0.1	–	1.2	0.7	–	–	4.9	–	1.9	2.0	0.4	0.4	0.2	0.1	–
$C_{11:0}$	–	1.4	0.5	0.6	0.5	1.9	0.7	0.1	0.6	7.0	–	1.8	5.9	0.7	0.4	–	0.3	–
$C_{12:0}$	1.1	1.2	0.6	0.2	1.6	1.2	1.2	1.6	0.6	2.3	1.5	1.8	1.5	0.6	0.6	0.9	0.5	0.1
$C_{12:1}$	–	0.9	0.2	–	–	–	–	–	–	–	–	–	–	–	1.3	–	–	–
$C_{13:0}$	0.6	0.3	0.2	0.2	0.2	0.9	0.6	–	0.3	1.5	–	1.1	1.1	0.5	0.3	–	0.2	–
$C_{13:1}$	–	1.7	0.7	–	1.1	–	–	1.5	1.6	–	1.4	–	–	–	0.8	–	–	1.2
$C_{14:0}$	8.9	7.0	2.3	4.6	6.3	4.3	6.8	6.5	15.4	4.4	6.4	3.3	6.0	2.4	6.3	9.3	2.0	–
$C_{14:1}$	–	1.8	0.2	–	0.4	–	0.1	0.3	0.3	–	0.6	1.0	–	–	0.9	0.3	–	–
$C_{15:0}$	3.2	2.5	0.8	3.9	0.5	6.9	4.6	0.3	3.7	2.6	0.6	4.3	3.3	5.7	3.3	11.1	4.2	3.1
$C_{15:1}$	3.9	2.2	0.2	–	0.9	–	–	1.0	–	–	0.2	–	–	–	1.5	–	–	–
$C_{10Mel5:0}$	–	–	–	–	–	1.8	–	–	–	–	–	–	–	–	–	–	–	–
$C_{16:0}$	24.5	38.0	47.2	33.8	42.2	24.4	44.7	54.4	25.0	27.1	54.0	24.5	35.4	27.6	47.3	44.7	29.6	64.4
$C_{16:1}$	18.7	11.9	6.6	26.5	11.1	13.1	–	8.2	10.4	3.2	14.4	12.4	–	13.2	8.3	5.9	15.2	–
$C_{10Mel6:0}$	3.0	3.9	3.1	1.3	0.3	2.7	0.9	–	–	–	–	1.1	1.3	0.2	–	–	1.0	–
$C_{17:0}$	1.0	0.3	0.2	2.3	–	5.2	3.2	–	3.7	–	–	2.8	2.1	3.8	0.7	0.1	7.3	0.7
$C_{17:1}$	13.6	4.5	1.9	3.8	3.1	2.7	–	5.4	5.6	–	2.7	2.3	1.3	3.1	3.8	1.2	2.4	0.8
$C_{10Mel7:0}$	3.4	0.4	–	1.2	–	3.4	1.4	–	–	–	–	–	1.7	3.6	–	–	1.7	–
$C_{18:0}$	7.4	0.8	–	2.4	1.3	4.2	10.5	1.8	2.9	13.9	1.9	6.1	12.2	2.0	0.9	1.5	3.1	–
$C_{18:1}$	12.5	12.0	13.8	16.6	11.8	11.8	14.7	4.3	13.1	10.1	4.3	9.0	11.7	9.3	10.6	3.1	15.0	11.2
$C_{18:2}$	–	–	3.3	–	1.0	–	–	1.6	–	–	1.5	–	–	–	–	13.6	–	–
$C_{10Mel8:0}$	3.6	0.8	0.6	2.7	10.6	17.7	9.8	11.2	13.2	9.7	10.1	23.2	9.1	27.4	10.3	7.3	17.3	18.6
$C_{19:0}$	–	3.4	0.8	–	–	–	–	–	1.9	13.1	–	1.3	4.4	–	1.0	–	–	–
$C_{19:1}$	–	–	1.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
$C_{20:1}$	–	–	0.2	–	6.6	–	–	1.4	–	–	–	–	–	–	–	0.6	–	–
$C_{21:0}$	–	–	14.3	–	–	–	–	0.4	–	–	–	–	–	–	–	–	–	–
Saturated fatty acids	47.8	60.7	67.5	48.1	52.6	50.2	73.0	65.1	52.2	77.0	64.4	47.6	73.9	43.7	63.4	67.8	47.3	68.3
Unsaturated fatty acids	52.7	40.1	32.4	52.1	46.3	49.8	26.9	34.9	46.1	23.0	35.2	53.3	25.1	56.8	37.5	31.4	52.6	31.8
Branched fatty acids																		
C_9–C_{15}	18.8	24.8	6.3	9.6	11.5	16.4	14.7	11.3	22.5	22.7	10.7	14.2	19.8	10.3	18.0	21.8	7.3	4.4
C_{16}–C_{21}	81.7	76.0	93.6	90.6	87.4	83.6	85.2	88.7	75.8	77.1	88.9	82.6	79.2	90.2	82.9	77.4	92.6	95.7

Note: 1, nutrient broth–grown cells; 2, propane-grown cells; 3, *n*-hexadecane-grown cells. Symbol "–" stands for "not detected."

cal species. The fatty acid composition of nutrient broth–grown rhodococci did not considerably change in response to the addition of antibiotics to the cultivation medium. The only changes involved the appearance of undecanoic acid ($C_{11:0}$) in an amount of 0.3–1.9% of total fatty acids and the disappearance of pentadecenoic acid ($C_{15:1}$). Most of the antibiotics tested reduced the cellular content of heptadecenoic by 2–5 times and augmented the relative content of oleic and tuberculostearic acids.

Earlier, we reported the tendency of the majority of rhodococcal strains to increase the content of unsaturated fatty acids when grown on propane [20]. Conversely, propane-grown cells of *R. ruber* IEGM 333 exhibited an elevated content of saturated straight-chain fatty acids, reaching 60.7% of the total fatty acids (Table 3). The amount of palmitic acid in propane-grown cells was 13.5% greater than in cells grown in nutrient broth. Moreover, propane-grown cells contained C_{11} and C_{19} saturated fatty acids, lacking in cells grown in the broth. In general, antibiotics considerably diminished the content or even caused a complete disappearance of unsaturated C_{12}–C_{17} fatty acids from cells of *R. ruber* IEGM 333 and increased the content of stearic ($C_{18:0}$) and tuberculostearic acids about tenfold.

R. ruber IEGM 333 cells grown in the mineral medium with *n*-hexadecane (Table 3) predominantly synthesized saturated straight-chain fatty acids that have the same number of carbon atoms as *n*-hexadecane, particularly palmitic acid (47.2%). These cells also contained long-chain $C_{20:1}$ and $C_{21:0}$ fatty acids, lacking in cells grown on propane or in nutrient broth. In this case, the addition of antibiotics to the cultivation medium caused no considerable changes in the fatty acid composition of the cells, except for a diminution or complete disappearance of some short-chain C_9–C_{13} fatty acids and a 10-fold increase in the content of tuberculostearic acid.

To summarize, rhodococci grown on hydrocarbons exhibited a higher content of saturated straight-chain fatty acids, palmitic acid ($C_{18:0}$) in particular, and a lower content of unsaturated hexadecenoic ($C_{16:1}$) and heptadecenoic ($C_{17:1}$) acids than rhodococci grown in nutrient broth. The content of branched fatty acids in hydrocarbon-grown cells was also lower than in cells grown in nutrient broth (5.1 and 3.7% of total fatty acids in, respectively, propane-grown and *n*-hexadecane-grown cells as compared to 10% in nutrient broth–grown cells). The presence of short-chain branched and unsaturated fatty acids in the cell envelope of rhodococci grown in nutrient broth probably loosens the lipid bilayer of the cell membrane, thereby increasing its permeability to various chemical substances, antibiotics in particular [21]. Conversely, the predominance of saturated straight long-chain fatty acids in propane-grown and *n*-hexadecane-grown rhodococci must decrease the permeability of the cell envelope to antibiotics and reduce their penetration to cellular targets, thereby increasing the resistance of hydrocarbon-grown cells to antibiotics.

Thus, the assimilation of gaseous and liquid *n*-alkanes by rhodococci is accompanied by some changes in the lipid composition of their cell envelope (an increase in the amount of total lipids and changes in the relative content of particular fatty acids and phospholipids), which play a significant role in the development of the adaptive nonspecific resistance of alkanotrophic rhodococci to antibiotics.

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