LIPIDS AND LIPOPOLYSACCHARIDES OF THE GENUS *Flavobacterium*

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The fatty acid compositions of the free lipids of three strains and the monosaccharide compositions of the lipopolysaccharides of four strains of new species of microorganisms of the genus *Flavobacterium* have been studied. The acidic nature of the polysaccharides of the LPSs of the microorganisms studied has been shown.

Earlier [1], four strains of bacteria of the epiphytic microflora of macrophytic algae were isolated from the thalli of brown algae from the littorals of the Barents Sea and the Sea of Japan, and these were assigned to four new species of microorganisms of the genus *Flavobacterium*: *F. laminarium* (strain 322B), *F. fucusianum* (strain 416B); *F. japonicum* (strain 85C) and *F. desmarestia* (strain 85). The present work is devoted to an investigation of the free lipids and lipopolysaccharides (LPSs), which determine the type specificities of the microorganisms under study.

The free lipids were obtained by chloroform extraction of the lyophilized biomass of the microorganisms. The lipid fractions were hydrolyzed [2] and were analyzed by chromatomass spectrometry. The lipid fraction from *F. fucusianum* was not analyzed. It follows from the results given in Table 1 that the main acid components of the free acids of the cell walls of the strains studied are residues of hexadecanoic, octadecanoic, and octadecenoic acids, their quantitative amounts in the lipid fractions of the three strains being almost identical. This fact may possibly serve as a chemotaxonomic characteristic of the genus as a whole.

The results of a chemical analysis of the biomass after the elimination of the lipid fraction are given in Table 2. The high ash content – from 8 to 29% – must be mentioned.

The lipopolysaccharides (LPSs), isolated by aqueous phenolic extraction using Westphal's method [3] exhibited serological activity. On Ouchterlony double diffusion in agar [4] the LPSs reacted specifically with the homologous antisera, forming a single precipitation band and did not react with sera to the other strains.

The analytical results in Table 3 show considerable differences in the LPSs isolated with result to their amounts of monosaccharides, protein, and nucleic acids and, consequently, in spite of the use of a standard isolation procedure, the degrees of purity of the LPS preparations were different.

The IR spectra of the LPSs each has an absorption band in the 1100 cm^{-1} region that is characteristic for the polysaccharide component and for nucleic acids [5]. An absorption band at 1625 cm^{-1} shows the presence of N-acylated hexosamine residues [6], and absorption in the 1665 cm^{-1} region is due to the vibrations of the C=O groups of protein molecules [5] and that in the 2850 cm^{-1} region to the presence of long-chain fatty acids in lipid A of the LPSs [6].

To determine the monosaccharide compositions, the LPSs were subjected to acid hydrolysis. The hydrolysates were investigated by paper and gas-liquid chromatography in the form of the corresponding polyol acetates [7] and aldononitrile acetates [8] using authentic samples of the monosaccharides. The results are given in Table 4. Common monosaccharide residues of the LPSs investigated were galactosamine, glucosamine, glucose, and galactose. The LPS from *F. japonicum* was an exception, lacking galactosamine. At the same time, the LPSs from

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TABLE 1. Fatty Acid Compositions of the Free Lipids*

Fatty acid	Composition, %		
	F. laminarium	F. japonicum	F. desmarestia
14:0	0,3	0,6	—
15:0	—	0,8	—
16:0	23,0	23,0	14,8
16:1	—	2,2	3,6
17:0	1,4	9,1	8,2
18:0	15,9	14,4	14,9
18:1	33,7	40,0	33,0
18:2	—	2,6	—
20:0	1,2	-	3,4
22:0	3,0	—	9,9
24:0	—	—	2,6

*The amounts of the minor components are not given.

**The fatty acids were identified in the form of their methyl esters by the GLC-MS method.

TABLE 2. Main Components of the Biomass of the Microorganisms

Species of microorganism	Composition, %					
	free lipids	mono-saccharides	proteins	nucleic acids	phosphorus	ash
F. laminarium	4,2	6,8	29,4	9,8	2,5	24,6
F. fucusianum	3,6	23,4	29,2	19,4	2,5	15,2
F. japonicum	6,5	17,7	25,9	9,0	1,3	8,1
F. desmarestia	10,0	5,7	34,8	10,2	6,5	29,1

TABLE 3. Analytical Results for the LPSs

Species of microorganism	Composition, %								
	yield LPSs*	mono-saccharides	proteins	nucleic acids	heptoses	KDO	acyl groups	uronic acids	phosphorus
F. laminarium	0,3	17,5	10,4	13,0	—	6,9	1,3	6,3	2,4
F. fucusianum	0,4	46,4	18,1	3,5	—	—	1,1	2,4	1,7
F. japonicum	0,1	23,8	38,6	3,9	—	—	0,9	2,5	2,7
F. desmarestia	0,3	48,4	4,8	2,7	13,9	2,1	2,5	7,9	3,5

*On the weight of the dry bacterial cells.

TABLE 4. Monosaccharide Compositions of the LPSs (mole %)*

Species of microorganisms	Gal	Glc	Fuc	Rha	L-D Hep	$GalNH_2$**	$GlcNH_2$**
F. laminarium	11,0	4,4	2,1	—	—	7,6	8,2
F. fucusianum	3,5	42,9	—	—	—	2,8	3,8
F. japonicum	10,2	10,2	3,4	—	—	—	3,0
F. desmarestia	2,5	12,0	—	31,6	2,4	1,6	6,8

*The molar percentages were calculated on the amounts of monosaccharides in the LPSs determined by the phenol/sulfuric acid method.

**The amounts of hexosamines were determined with the aid of an amino acid analyzer.

F. japonicum and F. laminarium contained, in addition, fucose residues, and the LPS from F. desmarestia rhamnose and L-glycero-D-mannoheptose residues. Attention is attracted by the fact that the LPS molecules of the three strains had no heptose region. Furthermore, the PLSs of two strains lacked 2-keto-3-deoxyoctonic acid (KDO) – the linking unit between lipid A and the polysaccharide, which is a fairly rare occurrence for the LPSs of Gram-negative bacteria.

The presence of uronic acid residues in the LPSs of all the microorganisms studied was established by analytical methods, and this shows the acidic nature of the polysaccharides present in them.

Thus, the LPSs of three out of the four new species of the genus Flavobacterium differ with respect to their monosaccharide composition from the majority of LPSs of Gram-negative bacteria that have been studied [9].

EXPERIMENTAL

Descending paper chromatography was performed on Filtrak FN-3 and FN-12 papers in the butan-1-ol–pyridine–water (6:4:3) solvent system. Monosaccharides were revealed with the following reagents: reducing sugars with an alkaline solution of $AgNO_3$; amino sugars with 0.2% ninhydrin in acetone; and KDO with 2-thiobarbituric acid.

The GLC of the monosaccharides in the form of the corresponding polyol acetates and aldononitrile acetates was performed on a Pye Unicam 104 chromatograph with a flame-ionization detector in a glass column (0.4 × 150 cm) packed with 3% of QF-1 on Gas Chrom Q (100-120 mesh) in the temperature interval of 175-225°C with programming of the temperature at the rate of 5°C/min. The GLC of the fatty acid methyl esters was performed on a Tsvet-100 chromatograph with a flame-ionization detector in a glass column (0.4 × 300 cm) packed with 6% of DEGS on Chromaton N (100-120 mesh) at 175°C. The chromato-mass spectrometric analysis of the fatty acid methyl esters was carried out on a LKB 9000 S spectrometer using the same column.

The total monosaccharide content was determined by the phenol/sulfuric acid method [10], KDO with the aid of the modified TBA reaction [11], protein according to Lowry [12], phosphorus as in [13], uronic acids as in [16], acryl groups as in [15], ash gravimetrically, and heptoses by a modification of Sinilova's method [14].

The IR spectra of the LPSe (5 mg) were recorded on a Carl Zeiss UR-20 spectrometer in KBr tablets. The quantitative analysis of the amino sugars in the hydrolysates of the LPSs was performed on a LKB Biocal amino acid analyzer using columns (0.9 × 45 cm) packed with Jeol LC-R2 resin.

Microorganisms. The growth of the biomass of the microorganisms was carried out in the semiindustrial fermenters of the Institute of Microbiology of the USSR Academy of Sciences on meat-peptone broth diluted in a ratio of 1:5 with water containing 3% of sea salt, at pH 7.2 for 72 h. After the suspension of microbes had been centrifuged, they were lyophilized. The yield of the biomass of the microorganisms was 1-2 g per 1 liter of medium.

Isolation of the Free Lipids. The lyophilizate of the biomass was extracted three times with chloroform and the extract was dried. The yield of chloroform extracts was 3-10% of the weight of the dry microbial mass.

Hydrolysis of the Free Lipids. Free-lipid fractions (100 mg each) were boiled with 0.5 N KOH in methanol, and 15 ml of a solution obtained by boiling 2 g of NH_4Cl in 60 ml of dry methanol with 3 ml of H_2SO_4 for 15 min was added. The fatty acids were extracted from the mixture with hexane (3 × 25 ml) and, after evaporation, they were methylated with diazomethane in diethyl ether [17] and were analyzed by the GLC-MS method. The chromatograms were read quantitatively by the internal area normalization method.

The isolation of the LPSs was carried out from the chloroform-washed dry powder of the bacteria by extraction with 45% aqueous phenol, and they were freed from nucleic acids by three ultracentrifugations at 105,000 g and were lyophilized.

Hydrolysis of the LPSs. The LPSs (5-10 mg) were hydrolyzed in sealed tubes with 1 ml of 0.5 N H_2SO_4 at 100°C for 10 h. The hydrolysates were neutralized with $NaCO_3$, treated with KU-2 cation-exchange resin (H^+ form), concentrated in vacuum at 40°C, and analyzed by paper and gas-liquid chromatography in the form of the corresponding polyol acetates and aldononitrile acetates. For the quantitative determination of the amino sugars, the LPSs (5 mg) were hydrolyzed with 0.5 ml of 4 N HCl at 100°C for 4 h, and the hydrolysate was evaporated several times with methanol and was analyzed on an automatic amino acid analyzer.

Preparation of Antisera. Antisera were obtained by immunizing rabbits intravenously by the sevenfold injection of a suspension of $1 \cdot 10^9$ live bacterial cells of the appropriate strain. The animals were injected with 12 ml of the cell suspension every days. Blood was taken 7 days after the last injection from an incision in the auricular vein of the rabbit. Erythrocytes were precipiated by centrifugation at 500 rpm, and the sera so obtained were lyophilized.

SUMMARY

The fatty acid compositions of the free lipids of the cell walls of three of the four strains studied of new species of the genus Flavobacterium have been determined by the GLC-MS method. The monosaccharide compositions of the LPSs of all the stains investigated have been determined by paper and gas-liquid chromatography. The chemical and serological heterogeneity of the LPSs of the microorganisms studied has been shown. The acidic nature of the polysaccharidic component of the LPSs has been revealed by analytical methods.

LITERATURE CITED

1. T. P. Stupakova, I. E. Mishustina, and T. G. Kameneva, Mikrobiologiya, 52, 967 (1983).
2. L. Hartman and C. A. Lago Regino, Lab. Pract, 22, 475 (1979).
3. O. Westphal, O. Luderitz, and F. Bister, Z. Naturforsch., 76, 146 (1952).
4. O. Ouchterlony, Acta Path. Microbiol. Scand., 32, 231 (1953).
5. S. Levine, H. Stevenson, and L. Chambers, J. Bacteriol., 65, 101 (1953).
6. B. Adhakari, D. Chakravarti, and P. C. Adhakari, J. Inst. Chem., 50, 18 (1978).
7. J. S. Sawardeker, J. H. Sloneker, and A. R. Jeanes, Anal. Chem., 37, 1602 (1965).
8. V. M. Easterwood and B. J. L. Huff, Svensk. Paperstidn., 72, No. 37, 1602 (1965).
9. F. M. Unger, Adv. Carbohydr. Chem. Biochem., 38, 323 (1981).
10. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, Anal. Chem., 28, 350 (1956).
11. T. I. Burtseva, L. I. Glebko, and Yu. S. Ovodov, Anal. Biochem., 64, 1 (1975).
12. O. H. Lowry, N. F. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193, 265 (1951).
13. L. Hortman, M. Cardoso Elias, and W. Esteves, Analyst, 105, 173 (1980).
14. T. I. Burtseva and Yu. S. Obodov, Khim. Prir. Soedin., 159 (1982).
15. H. Trutnovsky, A. B. Sakla, and A. A. Taleb, Microchem. J., 20, 415 (1975).
16. L. P. Kosheleva and L. I. Glebko, Khim. Prir. Soedin., 500 (1982).
17. E. Th. Rietshel, H. Gottert, O. Luderitz, and O. Westphal, Eur. J. Biochem., 28, 166 (1972).

STRUCTURE OF ERGOLIDE

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The epigeal part of Erigeron khorassanicus Boiss. has yielded a new sesquiterpene lactone of the pseudoguaiane type – ergolide $C_{17}H_{22}O_5$, mp 179-180°C (ethanol) $[\alpha]_D^{20}$ + 123° (c 4.88; ethanol). On the basis of chemical transformations and spectral characteristics, its structure and configuration have been established as 6-acetoxy-4-oxo-1,7αH,6,8,10βH-pseudoguai-11(13)-en-8,12-olide.

The isolation from Erigeron khorassanicus Boiss., family Asteraceae of the known lactone britanin [1, 2] and the new compounds erigerolide, $C_{19}H_{26}O_7$, mp 152-153°C (ethanol), $[\alpha]_D^{20}$ + 2° (c 6.36; ethanol) and ergolide (I), $C_{17}H_{22}O_5$ (corrected composition), mp 179-180°C (ethanol), $[\alpha]_D^{20}$ + 123° (c 4.88; ethanol) has been reported previously [2].

In the present paper we give information to establish the structure and stereochemistry of ergolide (I).

In the IR spectrum of (I), bands showing its α,β-unsaturated lactone nature are located at 1770 and 1670 cm^{-1}. At 1740 cm^{-1} the absorption bands of the carbonyl of a five-membered ring and the band of an ester carbonyl coincide.

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