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EXPERIMENTAL ARTICLES

Isolation and Comparative Analysis of Glycolipid Fractions in Bifidobacteria

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Abstract—A comparative TLC analysis of lipid extracts from *Bifidobacterium longum* B 379 M, *B. bifidum* 791, and *B. adolescentis* 94 BIM has been performed. It is demonstrated that carbohydrate-containing lipid components were present in the bacteria, which differed in their chromatographic mobility (R_f) from similar compounds isolated from actinomycetes *Stomatococcus mucilaginosus* PCM 2415^T, *Nocardiopsis dassonvillei* PCM 2492, *Propionibacterium propionicum* PCM 2431, *Saccharopolyspora hirsuta* PCM 2279 (= ATCC 27875^T), *Rhodococcus equi* PCM^T 559 (= ATCC 3969), and *Gordonia bronchialis* PCM 2167. Polar lipids of bifidobacteria exhibited the closest similarity to their counterparts from propionic acid bacteria. Preparative chromatography (silica gel column I; elution with chloroform, acetone, and methanol) of the lipid extract of *B. adolescentis* 94 BIM made it possible to isolate fractions containing nonpolar lipids, glycolipids, and phospholipids. Further purification of the glycolipid fraction (column II; eluant, methanol gradient in chloroform) produced preparations of glycolipids and phospholipids. The preparations were studied by two-dimensional TLC using solvent systems chloroform–methanol–H_2O MiLi Q (65 : 25 : 4, v/v/v) and *n*-butanol–acetic acid–H_2O MiLi Q (60 : 20 : 20, v/v/v) for directions I and II, respectively. Two major glycolipids were revealed (G_1 and G_2), in addition to compounds characteristic of the polar lipid group and minor glycolipids (g), the latter being present in considerably lesser amounts.

Key words: actinomycetes, bifidobacteria, glycolipids, chemotaxonomic markers.

The majority of microorganisms representing the order Actinomycetales are soil inhabitants. Pathogens are rare among Actinomycetales, the best known examples include *Mycobacterium tuberculosis*, *M. leprae*, *Nocardia asteroids*, and *Actinomyces israeli*. Bifidobacteria, which are representatives of normal microflora of humans and animals, produce biologically active substances exhibiting immunomodulating, radioprotective, and antineoplastic activities. The biological activity of bifidobacteria resides in cell wall components, such as peptidoglycan, extracellular polysaccharides, glycoproteins, phospho- and glycolipids, and complexes of lipoteichoic acids with proteins [1–5].

The high resistance of Actinomycetales to various chemicals (including antibiotics), which exceeds considerably that of other microorganisms, prompted researchers to focus on the structure of cell walls of actinomycetes. The presence of diverse lipids and glycoconjugates is a major feature of the cell wall in actinomycetes. The cell surface in actinomycetes is characterized by the presence of amphiphilic lipids, which are in direct contact with the habitat of the microorganisms. These compounds differ in polarity, ranging from nonpolar waxes (belonging to the phthiocerol group) to polar lipooligosaccharides and glycopeptidolipids. The presence of such components as peptidoglycan (used in taxonomy), muramyl dipeptide (known as an adjuvant), glycolipids, and phospholipids distinguishes representatives of the order Actinomycetales from other microorganisms. Glycolipids and mycolic acids of actinomycetes are used as chemotaxonomic markers. These compounds are specific for particular groups of actinomycetes. For example, the length of the acyl chain in mycolic acids is important for chemodiagnosing genera belonging to the family Nocardiaceae. Representatives of the genus *Bifidobacterium* are characterized by the presence of glycolipids, phospholipids, and other lipid derivatives of unknown chemical structure [6–10].

There is ample evidence that glycolipids of Actinomycetales are highly immunoreactive. The presence of genus-specific glycolipids was demonstrated for the taxa *Rothia*, *Curtobacterium*, *Nocardiopsis*, *Propionibacterium*, *Saccharopolyspora*, *Rhodococcus*, etc. Major cellular glycolipids of actinomycetes appear as a class of compounds the identification of which allows judgments to be made as to the hetero- or homogeneity of microbial taxa [1, 2, 6, 7, 11, 12].

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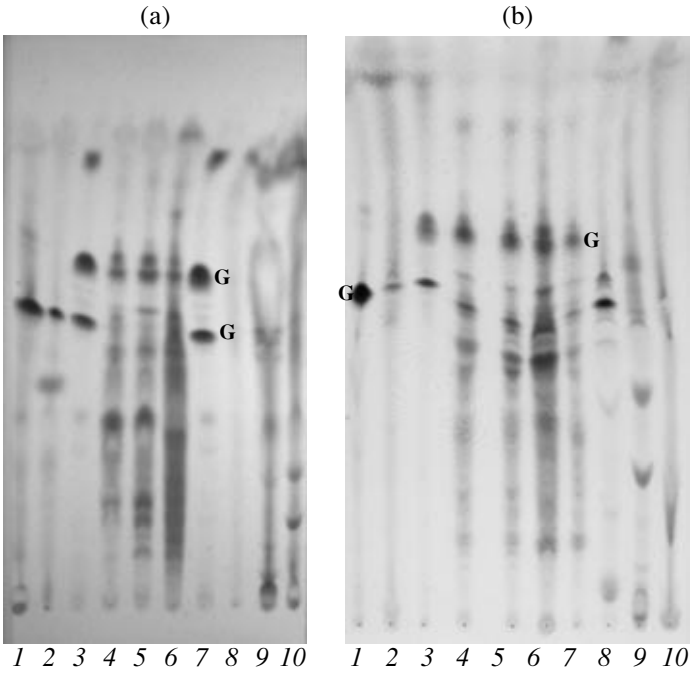

Fig. 1. Location of carbohydrate-containing lipid components (G) on thin-layer chromatograms of lipid extracts of bifidobacteria and representatives of actinomycetes.

a: *1*, *St. mucilaginosus* PCM 2415^T; *2*, *S. hirsuta* PCM 2279 (= ATCC 27875^T); *3*, *P. propionicum* PCM 2431^T; *4–6*, *B. adolescentis* 94 BIM; *7*, *P. propionicum* PCM 2431^T; *8* and *9*, *N. dassonvillei* PCM 2492; and *10*, *R. equi* PCM^T 559 (= ATCC 3969).

b: *1*, *St. mucilaginosus* PCM 2415^T; *2*, *S. hirsuta* PCM 2279 (= ATCC 27875^T); *3*, *P. propionicum* PCM 2431^T; *4* and *5*, *B. longum* B 379 M; *6*, *B. adolescentis* 94 BIM; *7*, *B. bifidum* 791; *8*, *N. dassonvillei* PCM 2492; *9*, *R. equi* PCM^T 559 (= ATCC 3969); *10*, *G. bronchialis* PCM 2167.

Solvent system: chloroform–methanol–H_2O MiLi Q (65 : 25 : 4, v/v/v); detection, treatment with 0.5% solution of orcinol in ethanol supplemented with 3% H_2SO_4.

In this work, we sought to isolate glycolipid (G) fractions from bifidobacteria and subject them to comparative analysis.

MATERIALS AND METHODS

The objects our studies were bifidobacterium strains *Bifidobacterium longum* B 379 M, *B. bifidum* 791 (courtesy of researchers at the Gabrichevsky Research Institute of Epidemiology and Microbiology, Moscow), and *B. adolescentis* 94 BIM (obtained successively from *B. adolescentis* MC-42 and *B. adolescentis* 91-BIM by autoselection for a particular spectrum of antibiotic resistance and a certain level of proteolytic activity; the ultimate strain was deposited—as *Bifidobacterium adolescentis* BIM B-87—with the Scientific Collection of Type and Industrially Important Nonpathogenic Microorganisms of the Institute of Microbiology, National Academy of Sciences of Belarus—known as the Belarussian Collection of Nonpathogenic Microorganisms).

The microorganisms were cultured in a modified synthetic Eagle's medium for tissue culture, supplemented with 0.5% lactose and 0.05% ascorbic acid, and TSB medium [1]. Our modification of Eagle's medium makes it possible to obtain lipid components containing no undesirable admixtures. A physiologically active culture of bifidobacteria (generation III), grown on TSB medium, served as an inoculum. Bifidobacteria were grown under microaerophilic conditions at 37°C (thermostated chamber) for 24–48 h (steady-state growth phase). To control sterility, aliquots of the culture liquid were taken at intervals and seeded on blood agar and beef peptone agar; in addition, preparations of the bacteria for microscopic examination (made according to standard techniques) were viewed in a Karl Zeiss microscope (Germany) at 1000×. Used in the experiments were morphologically homogeneous cultures of rodlike bifidobacteria. The cells were harvested by centrifugation (36000 *g*) of the culture liquid on a Heraeus Sepatech Biofuge 28 RS (Germany). The cells were killed by treatment with 1% formalin (v/v) and washed to eliminate the growth medium. The amount of the biomass was determined by drying the washed cells to a constant weight at 105°C (weight method), as well as nephelometrically (590 nm), using a KFK-2 colorimeter (Russia). Thereafter, the biomass of bifidobacteria was washed three times by phosphate-buffered saline (PBS; 0.01 M PO_4^{-3}, 0.15 M NaCl, pH 7.3) at 8000 *g*

Quantitative ratios of fractions isolated by preparative column chromatography of the extract of lipids of *B. adolescentis* 94 BIM

Column I, fraction	Content		Column II, fraction	Content	
	mg	%		mg	%
Lipid extract	586	100	I-Ac	122.5	100
I-Chl	89.1	15.2	I-Chl2	8.5	6.94
II-Chl	4.3	0.73	II-Chl2	3.8	3.1
III-Chl	2.2	0.37	I-5% Met	8.7	7.1
IV-Chl	9.8	1.67	II-5% Met	20.0	16.33
I-Ac	122.5	20.9	III-5% Met	5.9	4.82
II-Ac	26.9	4.59	IV-5% Met	10.3	8.41
III-Ac	0.8	0.15	I-10% Met	12.6	10.29
IV-Ac	2.8	0.49	II-10% Met	4.0	3.26
V-Ac	2.3	0.39	III-10% Met	3.2	2.61
I-Met	175	29.86	IV-10% Met	5.6	4.57
II-Met	26.6	4.55	I-15% Met	3.4	2.79
III-Met	9.1	1.55	II-15% Met	1.5	1.22
IV-Met	5.7	0.97	III-15% Met	2.1	1.71
			IV-15% Met	4.0	3.26
			I-20% Met	2.1	1.71
			II-20% Met	2.2	1.8
			III-20% Met	8.0	6.53
			I-30% Met	3.3	2.69
			II-30% Met	3.0	2.45
			I-50% Met	1.1	0.9
			II-50% Met	0.2	0.16
			I-100% Met	0	0
			II-100% Met	0	0
Total	477.1	81.42		113.5	92.65

(20 min), until all components of the medium were eliminated. The lipid component was extracted from the PBS-washed biomass using a 2 : 1 mixture of chloroform and methanol (v/v). The extractive solvent was added to the biomass (15 to 30 ml per 1 g), and the mixture was incubated at 37°C and continuous stirring for 18–24 h. Each portion of the biomass was extracted three times; lipid extracts were pooled and concentrated at 40–45°C in a vacuum rotary evaporator (Büchi-Rotavapor R).

The lipid component thus obtained (586 mg) was dissolved in 5.86 ml chloroform and separated by preparative column chromatography. Column I (45 × 1.8 cm) was packed with preactivated (at 120°C for 2 h) Silica gel 60 (70–230 mesh; Merck). Lipids were eluted in sequence by chloroform (400 ml), acetone (500 ml), and methanol (400 ml), at a rate of 3 ml/min, and fractions of 100 ml (*V*) were collected. Each fraction was concentrated at 40–45°C using the same vacuum rotary evaporator. Lipid fractions were designated with account for the eluant type and volume: I-Chl–IV-Chl (chloroform), I-Ac–V-Ac (acetone), and I-Met–IV-Met (methanol). The fraction I-Ac (122.5 mg), containing glycolipids, was further separated by preparative column chromatography. Column II (35 × 1.2 cm) was packed with Silica gel 60 (200–300 mesh; Merck) and eluted with a gradient of methanol in chloroform (i.e., 0, 5, 10, 15, 20, 30, 50, and 100% solutions of methanol in chloroform were passed through the column, and fractions of 25–50 ml were collected). The fractions were evaporated under nitrogen. The following designations were used: I-Chl2 and II-Chl2 (chloroform); I-5% Met–IV-5% Met (5% methanol); I-10% Met–IV-10% Met (10% methanol); I-15% Met–IV-15% Met (15% methanol); I-20% Met–III-20% Met (20% methanol); I-30% Met and II-30% Met (30% methanol); I-50% Met and II-50% Met (50% methanol); and I-100% Met and II-100% Met (100% methanol). Control of preparative lipid separation and analysis of glycolipids were performed by TLC. Aliquots of 5 μl, each containing 50 mg lipid per 1 ml) were applied onto 10 × 10 cm and 20 × 20 cm Silica gel 60 plates (Merck). The solvent system used was chloroform–methanol–H_2O MiLi Q (65 : 25 : 4, v/v/v). The chromatograms were treated using the following reagents: (1) 0.5% solution of vanillin (Sigma) in ethanol supplemented with 3% H_2SO_4 or iodine vapors (detection of lipids); (2) 0.5% solution of orcinol (Sigma) in ethanol supplemented with 3% H_2SO_4 (detection of carbohydrate-containing compounds); (3) 0.5% solution of ninhydrin (Sigma) in water-saturated butanol (detection of free amino groups); and (4) molybdenum reagent (detection of phospholipids). Following drying, chromatograms treated with vanillin or orcinol were heated (incubation at 120°C in a drying oven) for 10–15 min. Ninhydrin-treated chromatographs were warmed in a flow of warm air.

A comparative analysis of lipid extracts isolated from the biomass of bifidobacteria and other representatives of actinomycetes (*Stomatococcus mucilaginosus* PCM 2415^T, *Nocardiopsis dassonvillei* PCM 2492, *Propionibacterium propionicum* PCM 2431, *Saccharopolyspora hirsuta* PCM 2279 (= ATCC 27875^T), *Rhodococcus equi* PCM^T 559 (= ATCC 3969), and *Gordonia bronchialis* PCM 2167) was performed by studying the chromatographic (TLC) behavior of samples (50 mg/ml); the solvent system used was chloroform–methanol–H_2O MiLi Q (65 : 25 : 4, v/v/v). The samples of lipid extracts of actinomycetes were the courtesy of researches of the Ludwik Hirschfeld Institute of Immunology and Experimental Therapeutics. Lipid extracts were obtained from the biomass of strains deposited with the collection of this institute of the Polish Collection of Microorganisms (PCM). The mobility (R_f) and the extent of purification of lipids were determined by two-dimensional TLC on Silica gel 60 (Merck) plates, using distinct solvent systems.

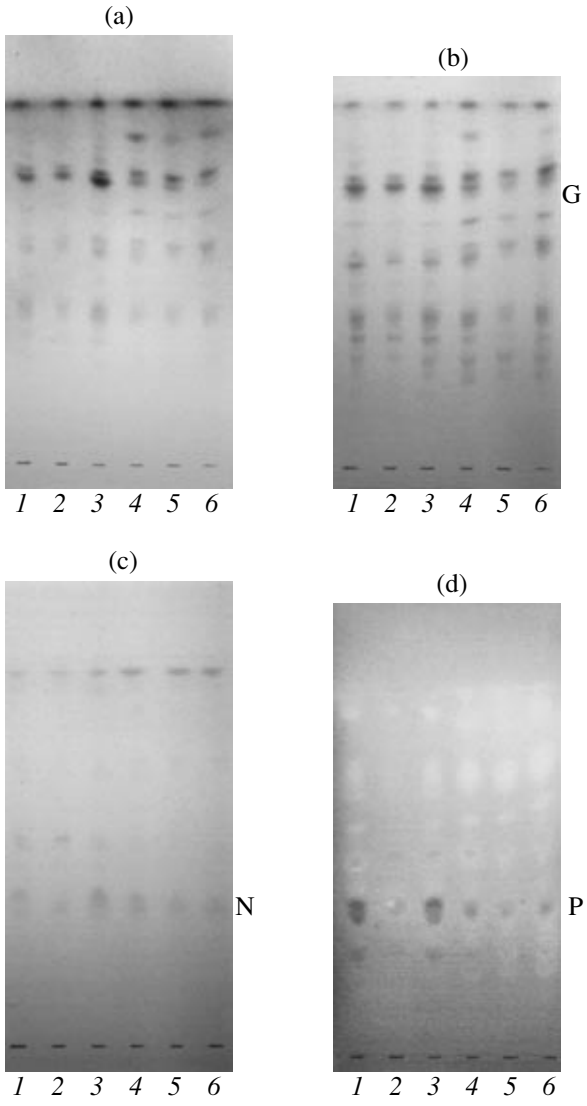

Fig. 2. Ratio of lipid components separated by TLC of lipids extracted from bifidobacteria grown in Eagle's media (*1–3*) or TSB (*4–6*): *1* and *4*, *B. bifidum* 791; *2* and *5*, *B. adolescentis* 94 BIM; *3* and *6*, *B. longum* B 379 M; G, glycolipids; N, lipids containing free amino groups; P, phospholipids.

Solvent system: chloroform–methanol–H_2O MiLi Q (65 : 25 : 4, v/v/v); detection: (a) treatment with 0.5% solution of vanillin in ethanol supplemented with 3% H_2SO_4; (b) treatment with 0.5% solution of orcinol in ethanol supplemented with 3% H_2SO_4; (c) treatment with 0.5% solution of ninhydrin in butanol; (d) treatment with molybdenum reagent.

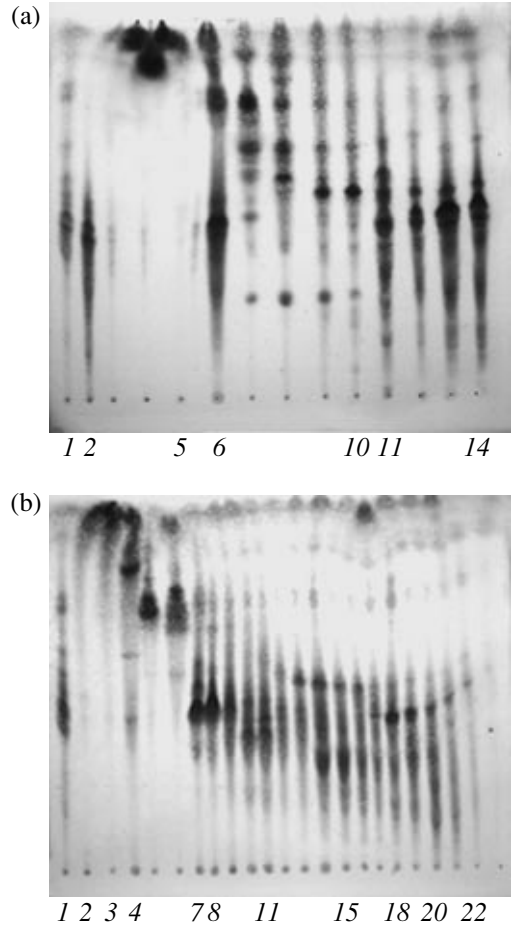

Fig. 3. Thin-layer chromatogram of fractions obtained by preparative chromatography of the extract of lipids of *B. adolescentis* 94 BIM on column I (A) and column II (B).

a: *1*, lipid extract; *2–5*, chloroform fractions (I-Chl–IV-Chl); *6–10*, acetone fractions (I-Ac–V-Ac); *11–14*, methanol fractions (I-Met–IV-Met).

b: *1*, lipid extract; *2* and *3*, chloroform fractions (I-Chl2 and II-Chl2); and methanol fractions *4–7*, I-5% Met–IV-5% Met; *8–11*, I-10% Met–IV-10% Met; *12–15*, I-15% Met–IV-15% Met; *16–18*, I-20% Met–III-20% Met; *19* and *20*, I-30% Met and II-30% Met; *21* and *22*, I-50% Met and II-50% Met; and *23*, I-100% Met and II-100% Met.

Solvent system: chloroform–methanol–H_2O MiLi Q (65 : 25 : 4, v/v/v); detection: treatment with 0.5% solution of vanillin in ethanol supplemented with 3% H_2SO_4.

RESULTS AND DISCUSSION

The biomass of bifidobacteria, obtained as a result of 48-h submerged cultivation on synthetic Eagle's medium and TSB medium, was used as a material for chloroform–methanol (2 : 1, v/v) extraction of lipids. A comparative TLC analysis of the lipid extracts revealed the presence of carbohydrate-containing lipid components in the bifidobacteria under study. These components differed from similar compounds isolated from other representatives of the order Actinomycetales (including closely related and phylogenetically remote) in their R_f values (Fig. 1). Polar lipids of bifidobacteria (*B. longum* B 379 M, *B. bifidum* 791, and *B. adolescentis* 94 BIM) exhibited closest similarity to their counterparts from propionic acid bacteria (*P. propionicum* PCM 2431). Analysis of lipid component ratios in extracts of *B. longum* B 379 M, *B. bifidum* 791, and *B. adolescentis* 94 BIM, cultured in diverse nutrient media, demonstrated that nonpolar lipids and glycolipids were the predominant species (Fig. 2).

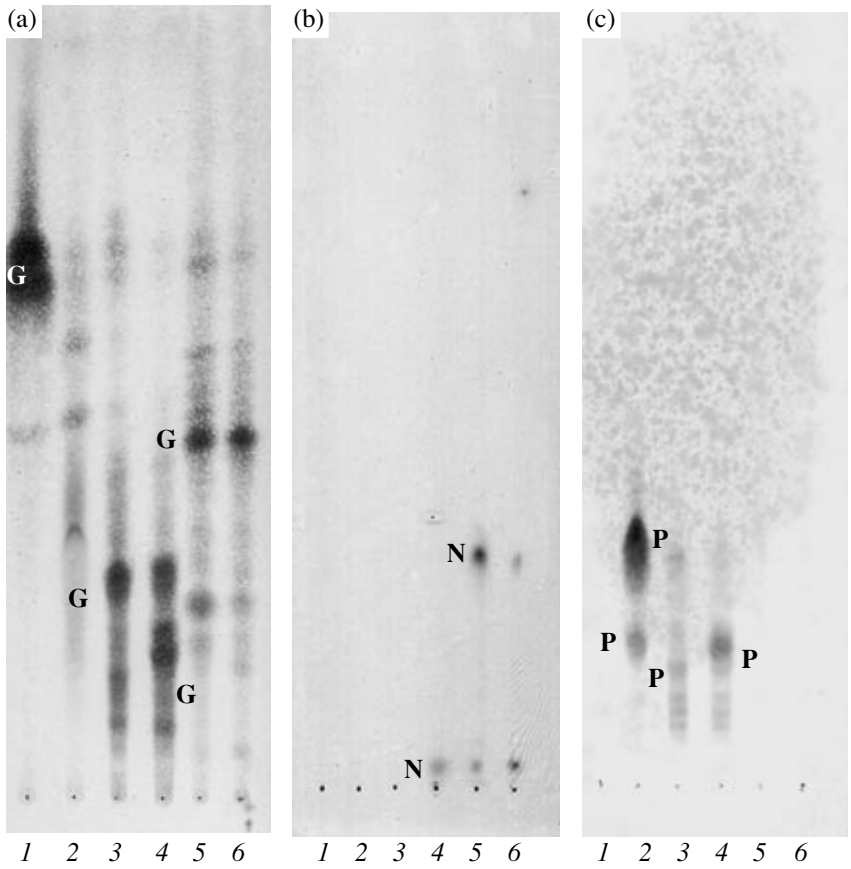

Fig. 4. Analysis of the composition of the fractions obtained by preparative column chromatography of the extract of lipids of *B. adolescentis* 94 BIM on column I (*1–4*) and column II (*5–6*): *1*, II-5% Met; *2*, I-10% Met; *3*, III-20% Met; *4*, I-30% Met; *5*, IV-Ac; *6*, V-Ac; G, glycolipids; N, lipids containing free amino groups; P, phospholipids.
Solvent system: chloroform–methanol–H_2O MiLi Q (65 : 25 : 4, v/v/v); detection: (a) treatment with 0.5% solution of vanillin in ethanol supplemented with 3% H_2SO_4; (b) treatment with 0.5% solution of orcinol in ethanol supplemented with 3% H_2SO_4; (c) treatment with 0.5% solution of ninhydrin in butanol; (c) treatment with molybdenum reagent.

Preparative chromatography (column I packed with Silica gel 60, 70–230 mesh; Merck) of the extract of lipids of *B. adolescentis* 94 BIM (sequential elution with chloroform, acetone, and methanol) allowed the following fractions to be obtained: chloroform, I-Chl–IV-Chl (nonpolar lipids), acetone, I-Ac–V-Ac (glycolipids), and methanol, I-Met–IV-Met (phospholipids). The fraction I-Ac (122.5 mg), containing glycolipids, was further separated by preparative column chromatography (column II packed with Silica gel 60, 200–300 mesh; Merck; the granules of silica were less in size, but more homogeneous). The column was eluted with a gradient of methanol in chloroform (0, 5, 10, 15, 20, 30, 50, and 100% solutions). Efficient elution of glycolipids takes place starting from 5% methanol; as the content of methanol in the eluant increases, the eluted fractions become enriched in phospholipids (quantitative predominance). This second chromatography made it possible to obtain major glycolipid and phospholipid fractions (Fig. 3b). Most of the major glycolipids of *B. adolescentis* 94 BIM were present in the fraction II-5% Met.

At the next stage, we determined the composition of the fractions obtained by preparative column chromatography of the extract of lipids of *B. adolescentis* 94 BIM. Analysis of thin-layer chromatograms (treated with vanillin, orcinol, ninhydrin, and molybdenum reagent) revealed fractions with quantitative predominance of glycolipids, phospholipids, or lipids containing free amino groups (Fig. 4). Thus, glycolipids were detected in fractions IV-Ac, V-Ac, and II-5% Met. The fraction I-10% Met contained predominantly phospholipids. Small amounts of phospholipids were also found in fractions III-20% Met and I-30% Met; the latter contained lipids with free amino groups.

Further characterization of the fractions obtained by preparative column chromatography was carried out in experiments involving two-dimensional TLC (using various solvent systems). As a result (Fig. 5), two major glycolipids were identified (G_1 and G_2), as well as several polar lipids and minor glycolipids (g). When studied by two-dimensional TLC with the solvent systems chloroform–methanol–H_2O MiLi Q (65 : 25 : 4, v/v/v;

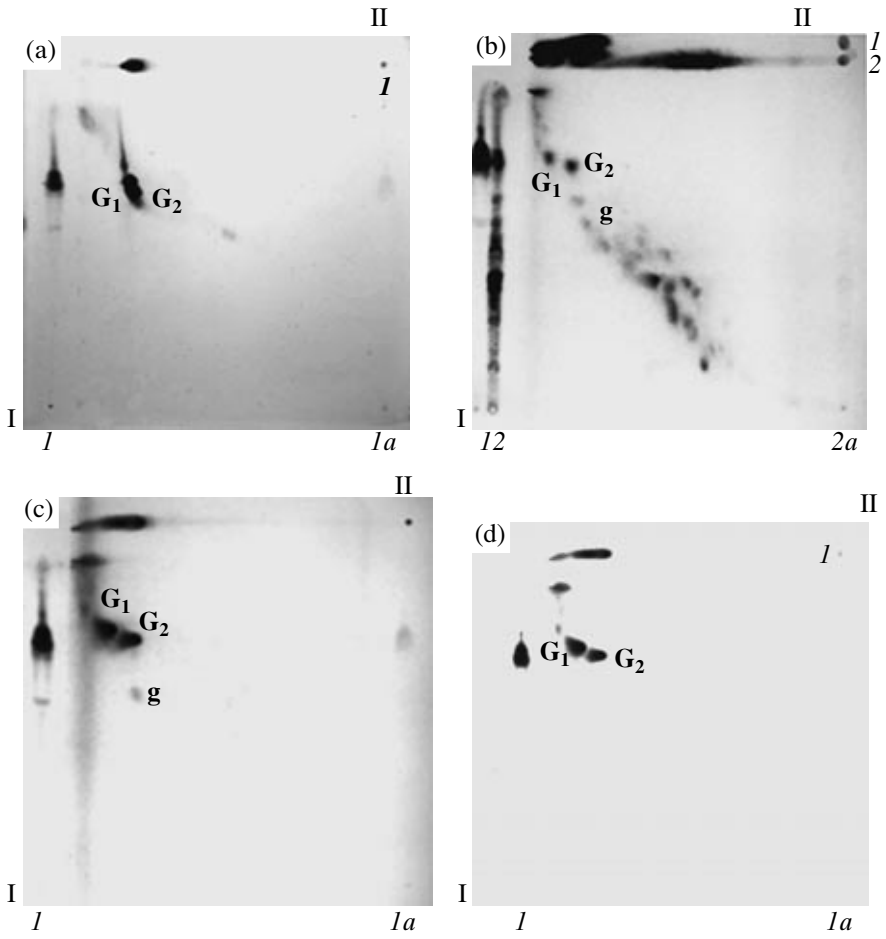

Fig. 5. Two-dimensional TLC of glycolipids of *B. adolescentis* 94 BIM (50 mg/ml; *1* and *2*, 12 μl; *1a* and *2a*, 24 μl): *1* and *1a*, fraction II-5% Met (column II); *2* and *2a*, lipid extract; G_1 and G_2, major glycolipids; g, minor glycolipids.

(a) chloroform–methanol–H_2O MiLi Q (65 : 25 : 4, v/v/v; direction I) and chloroform–acetic acid–methanol–H_2O MiLi Q (80 : 15 : 12 : 4, v/v/v/v; direction II); (b)–(d) chloroform–methanol–H_2O MiLi Q (65 : 25 : 4, v/v/v; direction I) and *n*-butanol–acetic acid–H_2O MiLi Q (60 : 20 : 20, v/v/v; direction II); detection: treatment with 0.5% solution of orcinol in ethanol supplemented with 3% H_2SO_4.

direction I) and chloroform–acetic acid–methanol–H_2O MiLi Q (80 : 15 : 12: 4, v/v/v/v; direction II), the two major glycolipids exhibited similar chromatographic mobility. The use of the systems chloroform–methanol–H_2O MiLi Q (65 : 25 : 4, v/v/v; direction I) and *n*-butanol–acetic acid–H_2O MiLi Q (60 : 20 : 20, v/v/v; direction II) made it possible to separate G_1 and G_2.

Thus, using extraction, preparative column chromatography, and TLC, we succeeded in isolating glycolipid fractions of bifidobacteria and performed a comparative analysis of these substances.

Methods of molecular biology and various types of chromatography are widely used as tools in numerical taxonomy, chemotaxonomy, and molecular systematics. In this respect, studying cell wall glycoconjugates (including major glycolipids of actinomycetes) as chemotaxonomic markers is of considerable theoretical and practical importance [11–14]. For example, cells of three thermophilic taxa of actinomycetes, belonging to the genera *Micropolyspora*, *Faenia*, and *Saccharopolyspora*, were shown to share a common glycolipid marker, dimannosylglycerol. The genus specificity of this marker confirmed reclassification of actinomycetes and inclusion of *Micropolyspora* and *Faenia* species into the genus *Saccharopolyspora*. The phylogenetic relationship of the actinomycetes in question was further proved by sequencing their 16S ribosomal RNAs. A similar example is provided by the cells of the genus *Nocardiopsis*, which contain polar lipids, i.e., phosphatidylcholine (PC) and two major glycolipids with distinct structures—diacylated trehalose (DAT) and dimonomannosyl diglyceride (DMDG) [6, 7, 11]. When studied by TLC, these compounds form a specific glycolipid profile characteristic of the taxon *Nocardiopsis*, which allows the species of this genus to be distinguished from representatives of similar genera, *Acinomadura* and *Nocardia* (in the past, *Nocardiopsis* species were classified with *Acinomadura* and *Nocardia*). Characteristic profiles of carbohydrate-containing

lipid components were described for the microaerophilic bacterium *Propionibacterium propionicum* and the opportunistic microorganism *Rothia dentocariosa*, which inhabit the mouth cavity [2]. Nocardia-like bacteria are characterized by the presence of phenolic glycolipids, sulfolipids, and glycopeptidolipids. In addition to serving as chemotaxonomic markers, glycolipids of actinomycetes may be used in laboratory diagnostics (identification of etiological factors of actinomycoses [1, 2, 6, 7, 11, 12].

According to the data reported by Veerkamp *et al.* [8–10], glycolipids of *Bifidobacterium bifidum* var. *pennsylvanicus* are represented by galactolipids—mono-, di-, and trigalactosyl diglycerides; mono- and diacyl derivatives of monogalactosyl diglycerides; and monoacyl derivatives of digalactosyl diglycerides (monogalactosyl and digalactosyl monoglycerides are present in minor amounts). In this work, we demonstrated, by comparative TLC of lipid extracts from distinct species of bifidobacteria, that their carbohydrate-containing lipid components differ in chromatographic mobility (R_f) from similar compounds of other actinomycetes (*Stomatococcus mucilaginosus*, *Nocardiopsis dassonvillei*, *Propionibacterium propionicum*, *Saccharopolyspora hirsuta*, *Rhodococcus equi*, and *Gordonia bronchialis*. Polar lipids of the bifidobacteria under study exhibited closest similarity to their counterparts from the propionic acid bacteria. The three representatives of the genus *Bifidobacterium—B. longum* B 379 M, *B. bifidum* 791, and *B. adolescentis* 94 BIM—are characterized by the presence of major and minor glycolipids, which form a specific TLC profile. We were able to demonstrate that these compounds are chemotaxonomic markers of genus-specificity, appropriate for use in rapid tests for identification of microorganisms of the order Actinomycetales.

Introduction into practice of rapid identification of industrial strains of microorganisms (based on detection of chemotaxonomic markers) is a topical problem in biotechnology (production of bacterial preparations of new-generation and biologically active additives). Monitoring of chemotaxonomic markers may be used, in addition to molecular-biological methods of DNA analysis (RFPL, pulsed electrophoresis, ribotyping, and PCR-fingerprinting), for certifying industrial microorganisms, in which case it would warrant the identity of an industrial strain to its prototype, developed by researchers and deposited with microorganism collections. Yet another important aspect of certification is that it allows the developers' rights to be protected if unauthorized use of the strain takes place. Determining chemotaxonomic markers has certain advantages over the conventional system of identification (based on morphological, cultural, physiological, and biochemical properties of microorganisms), which include rapid availability of results, good resolution, obviousness and reproducibility of data, and low cost [15–17].

Further studies of the lipid components (including the chemical structure and biological role of the compounds) is expedient, because, in addition to their taxonomic importance, the lipids in question may be involved in specific cellular reactions that underlie the probiotic effects of bifidobacteria. Isolation from bifidobacteria of new biologically active substances (polysaccharides and enzymes, glycolipids and phospholipids, etc.) and their development, aided by high technologies, into efficient medicines for prevention and treatment of diseases constitutes a separate field of research activity [18–20].

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