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EXPERIMENTAL ARTICLES

Comparative Study of the Fatty Acid Composition of Some Groups of Purple Nonsulfur Bacteria

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Abstract—The fatty acid composition (FAC) of 43 strains of purple nonsulfur bacteria belonging to six genera—*Rubrivivax, Rhodopseudomonas, Rhodoplanes, Blastochloris, Rhodobium*, and *Rhodomicrobium*—was studied by capillary gas chromatography. The cultures were grown on standard medium under standard conditions. Automatic identification of the fatty acid methyl esters and statistical processing of the results were performed by the computerized Microbial Identification System (MIS). Significant differences between the FACs of different genera, species, and, sometimes, strains were revealed. 16S rRNA genes of some of the new isolates, primarily those having a specific FAC, were sequenced. The taxonomic status of a number of the strains in question was determined using the FAC characteristics as one of the criteria. It was shown that the FAC characteristics may be used both for affiliating isolates to known species and for revealing new taxa.

Key words: anoxygenic phototrophic bacteria, purple nonsulfur bacteria, fatty acids, Euclidean distance, taxonomy.

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The fatty acid composition (FAC), along with the polar lipid and quinone compositions, has served as one of the criteria used for the taxonomic description and identification of microorganisms for more than 30 years [1]. This is due to the fact that the FACs of bacteria from different taxonomic groups differ significantly both in the proportions of the main fatty acids (FA) and in the presence of the characteristic minor components. Investigations devoted to studying the FAC of anoxygenic phototrophic bacteria have shown that most of them are characterized by the presence of nonbranched FA with an even number of carbon atoms, saturated or with one double bond, the number of hydroxy acids being low. At the same time, the FACs of taxa of different ranks, including species, may differ significantly. Thus, the comparative study of the family *Ectothiorhodospiraceae* [2] gave an impetus to the revision of its classification. The possibility of identifying new isolates of the family *Rhodobacteraceae* on the basis of their FAC and polar lipid composition has been shown [3]. The comparative analysis of the FACs of a large number of species and strains of purple nonsulfur bacteria performed by Japanese researchers [4, 5] also revealed differences in their FACs at the generic and species levels.

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The FAC of a particular bacterial strain may vary significantly. FA are mainly membrane constituents, forming the hydrophobic part of the lipid bilayer. FAC changes are one of the adaptation mechanisms that developed in the process of evolution; they are aimed at maintaining the functional integrity of membranes under varying environmental conditions.

FAC variations were noted even when one strain of phototrophic bacteria was repeatedly grown under the same cultivation conditions. The most changeable was the C18:1 FA content (up to 5%); the concentrations of other FA varied to a significantly lesser degree [3]. According to the data of Japanese investigators [4, 5], switching from the phototrophic to chemotrophic mode of nutrition caused insignificant changes in the FAC of purple nonsulfur bacteria. However, according to the data of other authors, the content of saturated FAs decreased in several species grown chemotrophically [6]. Significant FAC changes in the process of culture growth and aging were noted [6]. For example, the cyclopropanoic (C19:0cyc) acid content in different *Ectothiorhodospira* strains was 2–3% in the exponential growth phase and 25–33% in the stationary phase. The cultivation temperature exerts a substantial influence on FAC. Thus, it was shown that, in the purple bacteria of the family *Ectothiorhodospiraceae*, an increase in tem-

perature caused the content of C18 and unsaturated FAs to decrease, while the C16 and saturated FA contents increased [7]. The general patterns of the influence of medium salinity on FAC were revealed when anoxygenic phototrophic bacteria belonging to different taxonomic groups and having different FACs were studied [7]. When the salinity was close to the optimum, minimum C16 and saturated FA content and maximum C18 and unsaturated FA content were observed. Changes in FAC occurred when the salinity was either increased or decreased in relation to the optimum.

However, in most cases, the general characteristics of the FAC, despite the changes caused by the cultivation conditions, are retained if the cells are not subjected to significant stress [3]. For example, in the cultures of the type strain of *Rhodovulum adriaticum* grown at 2.5 to 10% salinity, changes in the content of individual FAs reached 10%. Nevertheless, these variations did not prohibit the affiliation of strains to particular species based on their FAC, irrespective of the growth conditions. Naturally, data obtained with cells grown under extreme conditions should not be used for the purpose of comparison. The influence of the abovementioned factors on the FAC may be minimized by growing the cells under standard conditions and harvesting them for analysis in the exponential growth phase.

The extensive experience of studying the FAC of bacteria for taxonomic purposes gives evidence of the fact that the resolving capacity of this criterion varies substantially in different taxa of the same rank, and the effectiveness of its use for identification significantly depends on how profoundly this taxon has been studied. In this connection, of special interest are complex studies of bacteria of different taxonomic groups and the comparison of the specific features of their FACs with other pheno- and genotypic characteristics.

The objectives of our study were as follows: (1) to determine by standard methods the FAC of a large number of strains of purple nonsulfur bacteria (including closely related strains) grown on standard medium and under standard conditions; (2) to reveal statistically significant differences in the FACs of different genera, species, and, possibly, strains of purple nonsulfur bacteria; (3) to carry out the 16S rRNA gene sequencing of some new isolates, above all, of those possessing a specific FAC; and (4) to determine the taxonomic status of a number of the strains studied, using the characteristics of FAC as an additional criterion.

MATERIALS AND METHODS

The FAC of 43 strains of purple nonsulfur bacteria belonging to six genera were studied: 5 strains of *Rubrivivax*, 17 strains of *Rhodopseudomonas*, 3 strains of *Rhodoplanes*, 5 strains of *Blastochloris*, 4 strains of *Rhodobium*, and 9 strains of *Rhodomicrobium.* The strains *Rubrivivax* sp. L-4g, SRg, B-6g, KR-55g, H-4g; *Rhodopseudomonas* sp. KR-31p, KR-32p, KR-91p, KR-103p, KR-109p, KR-111p, ORp, ABp, BORp, MG-3p, UZ-25p; *Rhodoplanes* sp. KR-62p, KR-108p; *Rhodobium* sp. KR-36m; *Blastochloris* sp. KR-70sv, KR-101sv, KR-109sv; *Rhodomicrobium* sp. KR-19mc, KR-86mc, KR-88mc, KR-91mc, KR-97mc, KR-105mc, K1mc, M6mc were isolated by E.I. Kompantseva and are stored at the laboratory of ecology and geochemical activity of microorganisms, Winogradsky Institute of Microbiology, Russian Academy of Sciences (Moscow). The strains *Rhodopseudomonas* sp. 121, 124; *Rhodoplanes* sp. Alt; *Rhodobium* sp. 992, Scotc; and *Blastochloris* sp. 170 were isolated by J.F. Imhoff and are stored at the Marine Microbiology Department, Leibniz Institute of Marine Sciences (Kiel, Germany). Strains from international collections—*Rhodopseudomonas palustris* DSM 123^T, *Rhodopseudomonas* sp. R-1 (ATCC 33872, the former type strain of *Rhodopseudomonas rutila*), *Blastochloris viridis* DSM 133^T, *Rhodobium marinum* DSM 2698^T, and *Rhodomicrobium vannielii* DSM 162^T—were also used in the work.

The FACs were studied at Leibniz Institute of Marine Sciences (Kiel, Germany) in the Marine Microbiology Department headed by Prof. J.F. Imhoff.

All strains were cultivated on the AT medium for purple nonsulfur bacteria [3] (g/l): KH_2PO_4, 1; NH_4Cl, 1; Na_2SO_4, 0.7; $MgCl_2 \cdot 6H_2O$, 0.5; $CaCl_2 \cdot 2H_2O$, 0.1; $NaHCO_3$, 3; NaCl, 1; sodium ascorbate, 0.5; sodium acetate, 1. In addition, 1 ml/l of trace element solution and 1 ml/l vitamin solution, as well as 20 μg/l of vitamin B_{12}, were added to the medium. The pH was adjusted to 7. The saline-water *Rhodobium* strains were grown on the same medium containing additionally 30 g/l of NaCl. $Na_2S \cdot 9H_2O$ (0.5 mM) in the form of a solution neutralized by blowing through CO_2 was additionally introduced into the medium for *Blastochloris.* The media were sterilized by filtration, poured into flasks, and allowed to stand for at least two days before inoculation until oxygen had been completely bound by sodium ascorbate.

Glass flasks (50 ml) with screw caps were used for cultivating the bacteria. The bacteria of all strains were grown under the same conditions in a thermostatically controlled room at 25°C and an intensity of illumination of 2200 lx until the late exponential phase. For comparison, five cultures were grown both under the conditions described above and at an increased temperature (35°C). The cultures were subjected to FAC analysis after four sequential passages under the same conditions. Twelve test cultures of each strain were grown in parallel for the purpose of statistical data processing. The cultivation time was varied from 24 to 48 h for different cultures.

The analysis of FAC was performed according to the technique envisaged by the Microbial Identification System as described by Thiemann and Imhoff [2]. The extracted FA were converted to methyl esters and ana-

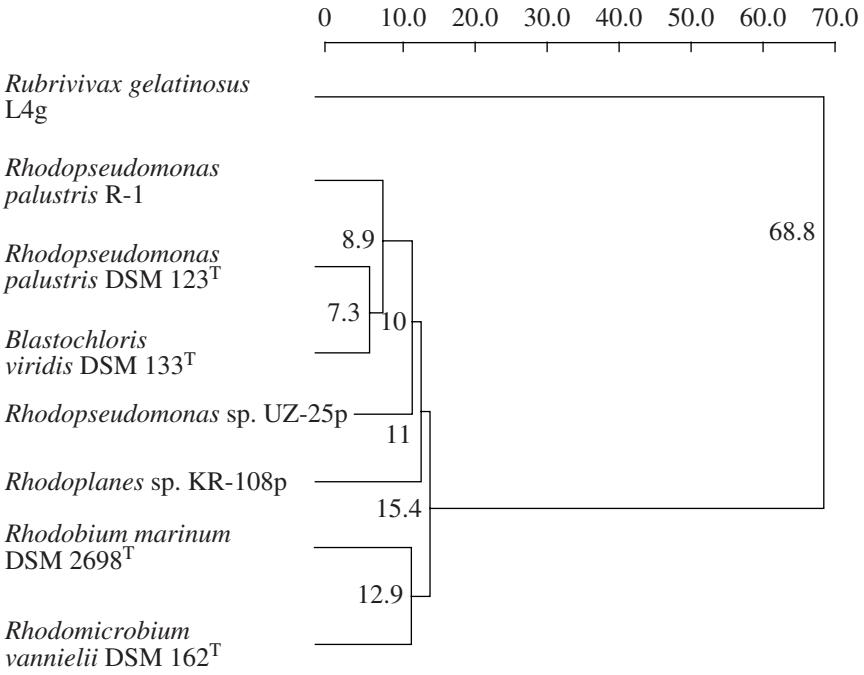

Fig. 1. Euclidean distances between the studied genera and species of purple nonsulfur bacteria.

lyzed using the capillary gas chromatography method on an HP 5890 GC series chromatograph (Hewlett Packard). The automatic identification of the FA methyl esters, the statistical processing of data, and the determination of the degree of FAC similarity, expressed in terms of the Euclidean distance (ED), were carried out using the high-resolution computerized Microbial Identification System (M.I.S.).

DNA was isolated according to the technique described earlier [8]. 16S rRNA gene amplification was carried out with the universal primers 27f (5-AGAGTTTGATCCTGGCTCAG) and 1492r (5-TACGGYTACCTTGTTACGACTT) using a GeneAmp PCR System 2700 device (Applied Biosystems). Sequencing of the amplified 16S rRNA gene fragment was carried out on a GEQ2000XL automatic DNA sequencer (Beckman Coulter) using the Dye Terminator Cycle Sequencing reagent kit (Beckman Coulter) and the protocol attached to the kit. The search for the nucleotide sequences in the GenBank database was carried out with the BLASTn program (http://www.ncbi.nlm.nih.gov/BLAST). The ClustalX program was used for aligning the nucleotide sequences and constructing the similarity matrix for 16S rRNAs [9].

RESULTS AND DISCUSSION

In all of the bacteria studied, saturated and monounsaturated FA containing 16 and 18 carbon atoms predominated: C16:0, C16:1, C18:0, and C18:1 (Table 1). The C14, C15, C19, and C20 acids, as well as FA containing a hydroxyl or cyclic group, occurred in small amounts. The ratios between major FAs, as well as the presence of one or another minor component, varied significantly in different groups of the bacteria studied. Based on the degree of similarity/distinction of the fatty acid spectra expressed in the Euclidean distance (ED), dendrograms were constructed (Figs. 1–6).

By the degree of FAC similarity, the bacteria studied were divided into four groups, with the ED between them being 12.9, 15.4, and 68.8 (Fig. 1). The farthest from the others was the genus *Rubrivivax* (ED 68.8), belonging to the class *Betaproteobacteria*. As distinct from the other bacteria, which belonged to the class *Alphaproteobacteria*, the content of FAs with 16 carbon atoms in a chain (C16) in *Rubrivivax* representatives was several times higher than the content of FAs with

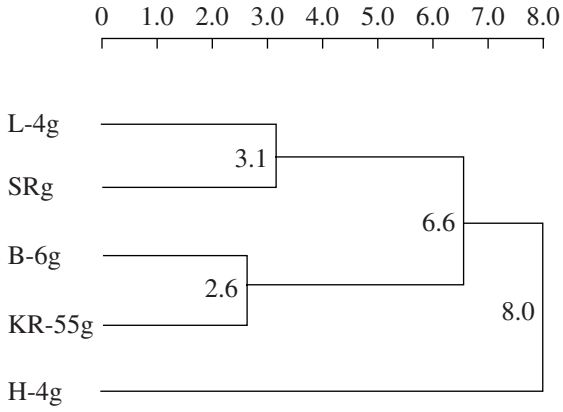

Fig. 2. Euclidean distances between the studied bacterial strains of the genus *Rubrivivax*.

Table 1. Comparative characterization of the fatty acid compositions (%) of the studied strains of purple nonsulfur bacteria

Fatty acids	*Rubrivivax*		*Rhodopseudomonas*				*Rhodoplanes*		*Blastochloris*: 5 strains	*Rhodobium*		*Rhodomicrobium*: 9 strains
	L-4g, SR_g B-6g, KR-55g	H-4g	*Rps. palustris*: 7 strains, including the type strain	KR-91p KR-111p	*Rps. palustris*: 7 strains, including R-1	UZ-25p	KR-62p KR-108p	Alt		3 strains, including the type strain	Scotc	
C16:0	25–28	32	12–15	10–12	9–11	19	8–11	9	9–13	1–2	2	1–2
C16:1cis9	32–38	36	6–12	9–11	2–4	4	3–6	2	5–8	1	1	0–3
C18:0	1	1	3–7	3	8–9	6	2–3	2	1–2	9–10	9	1–3
C18:1cis11	18–23	16	70–75	73–77	75–77	70	78–81	86	71–77	77–79	70	81–88
Sum of C16	64–69	71	19–25	19–23	13–15	23	14–15	11	17–19	2–4	3	4–8
Sum of C18	20–24	17	74–80	76–80	83–86	76	80–85	88	73–78	88–89	79	84–89
C18/C16	0.3–0.4	0.2	3–4	3–4	6–7	3	6	8	4–5	24–47	27	11–24
C16:1 + C18:1	50–58	52	78–85	83–87	77–81	74	82–86	88	77–84	78–80	71	83–90
C16:0 + C18:0	26–29	33	16–21	13–15	17–20	25	11–14	11	11–14	10–12	11	3–5
C16:1 + C18:1/C16:0 + C18:0	2	2	4–6	6–7	4–5	3	6–8	8	6–8	7–8	7	20–30
Characteristic minor fatty acids												
C10:0	<1	<1	0	0	0	0	0	0	0	0	0	0
C10:0-3OH	4–5	5	0	0	0	0	0	0	0	0	0	0
C12:0	3–4	3	0	0	0	0	0	0	0	0	0	0
C14:0	2–4	4	0	0	0	0	0	0	0–<1	0	0	1
C14:0-3OH	0	0	0–<1	<1	<1	<1	0–<1	<1	0	1	<1	0–2
i-C15:0	0	0	0	0	0	0	0	0	0	0	0	1
C16:0-3OH	0	0	0	0	0	0	0	0	0–<1	0	0	2–3
i-C16:0-3OH	0	0	0–<1	<1	0–<1	<1	0–<1	<1	0–<1	0	0	0
C16:1cis7	3–4	2	0	0	0	0	0	0	0	0	0	0–2
C16:1cis11	1	1	0	0	0	0	0	0	0	0	0	0
i-C16:1-3OH	<1	<1	0	0	0	0	0	0	0	0	0	0–1
C18:1cis13	<1	<1	<1–1	<1	<1	<1	0–<1	<1	0–<1	0	0	0
C19:0cyc	0	0	0	0	0	<1	0–2	0	0	0–<1	0	1–2
C19:1cyc	0	0	0	0	0	0	0	0	0–3	2–3	6	0–1
C20:0	0	0	0	0	0	0	0	0	0	1	4	0
C20:1cis13	0	0	0–<1	0	<1	0	0	<1	2–5	3–5	8	1–2

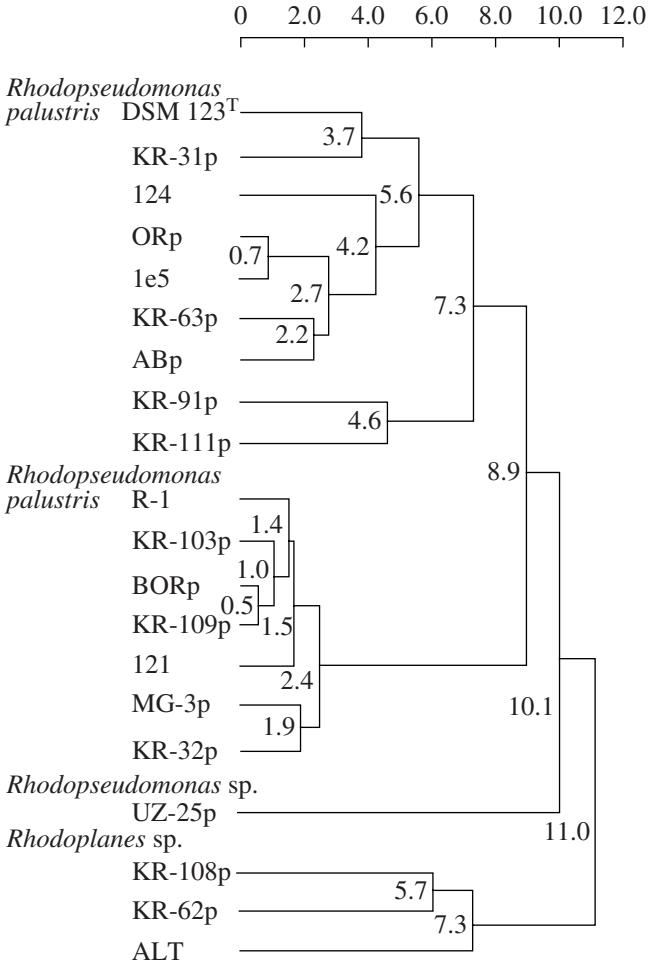

Fig. 3. Euclidean distances between the studied bacterial strains of the genera *Rhodopseudomonas* and *Rhodoplanes*.

18 carbon atoms (C18). The ratio between the sum of C18 FAs and the sum of C16 FAs (C18/C16) was 0.2–0.4 (Table 1), with the C16:0, C16:1cis11, and C18:1cis11 FAs predominating. All the *Rubrivivax* strains studied contained characteristic minor biomarker components: C10:0, C10:0-3OH, C12:0, C14:0, C16:1cis7, C16:1cis11, *i*-C16:1-3OH, and C18:1cis13.

Strains of the genera *Rhodopseudomonas*, *Rhodoplanes*, and *Blastochloris* (Fig. 1) can be combined, according to their FACs, to form one large group separate from other genera (ED 15.4 and 68.8). What these microorganisms had in common was the predominance of the C18:1cis11 FA (70–80%; in one case, 85%) and a significant total C16 FA content (13–25%); the C18/C16 ratio varied between 3 and 8 (Table 1). The characteristic minor components C14:0-3OH, *i*-C16:0-3OH, and C18:1cis13 were present in small amounts (less than 1%) in most of these strains. A significant C20:1cis13 biomarker content (2.5–4.8%) was revealed only in representatives of the genus *Blastochloris*.

In bacteria of the genus *Rhodobium*, the content of the main C18:1cis11 FA was 70–79% (Table 1). However, the C18/C16 ratio in them was much higher (27–46) due to the low C16 FA content, a higher average C18:0 content (compared to *Blastochloris*), and the significant share of the minor components: C14:0-3OH (0.5–1%), C19:1cys (2.2–6%), C20:0 (1–3.5%), and C20:1cis13 (2.8–8%). The presence of the C20:0 FA, which was not revealed in the other groups of the bacteria studied, as well as the absence of the *i*-C16:0-3OH and C18:1cis13 FAs characteristic of the previous group of strains, were the other distinctive features of this cluster.

For the bacteria of the genus *Rhodomicrobium*, a markedly pronounced feature was the predominance of the C18:1cis11 FA, which accounted for 81.2 to 87.7% of the total FA content and was, in fact, the only nonminor component, since the share of each of the remaining FA did not exceed 3% (Table 1). Along with this, the bacteria of this group were distinguished by a large set of minor components, including specific ones. All the strains revealed the C14:0, *i*-C15:0, C16:0-3OH, C19:0cis, and C20:1cis13 FAs; most of the strains, the

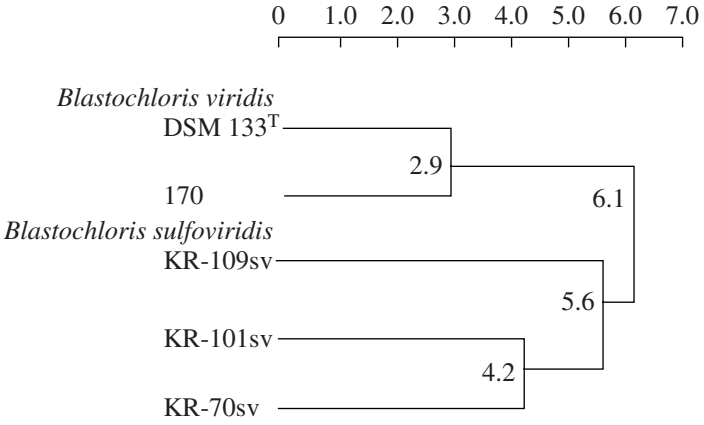

Fig. 4. Euclidean distances between the studied bacterial strains of the genus *Blastochloris*.

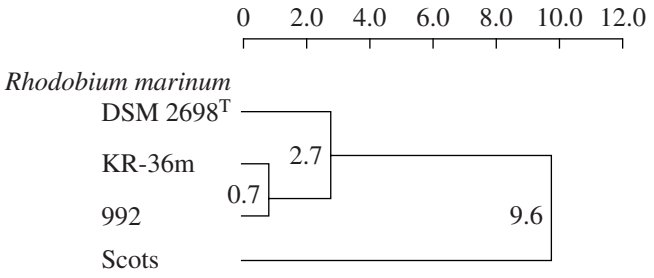

Fig. 5. Euclidean distances between the studied bacterial strains of the genus *Rhodobium*.

C14:0-3OH and C19:1cys FAs; some of the strains, the C15:0-3OH, *i*-C16:0, and *i*-C16:1-3OH FAs. Some of these FAs (*i*-C15:0, C15:0-3OH, and *i*-C16:0) did not occur in the rest of the bacteria studied in this work, and others, apart from *Rhodomicrobium*, were revealed only in individual strains or groups of strains.

The groups of strains described above were, in turn, subdivided into clusters with different degrees of FAC similarity.

The strains of the genus *Rubrivivax* were divided into three clusters with the ED between them being 6.6 and 8 (Fig. 2). The C18/C16 ratio in different clusters constituted 0.2, 0.3, and 0.4 (Tables 1, 2). Unfortunately, we did not include the type strain of *Rubrivivax gelatinosus* in the work for technical reasons. However, partial 16S rRNA gene sequencing (447 nucleotides) was carried out for KR-55g and showed the similarity between this isolate and the *Rubrivivax gelatinosus* type strain to be 100%. Despite the differences in FACs revealed, all the *Rubrivivax* strains under study proved to be close to each other and to *Rvi. gelatinosus* and were assigned to this species.

The representatives of the genera *Rhodopseudomonas, Rhodoplanes*, and *Blastochloris* formed a group of clusters with the ED between them varying between 7.3 and 11. Two clusters sufficiently remote from each other (ED of 8.9) consisted of strains of the species *Rhodopseudomonas palustris* (Fig. 3), one of them including the type strain *Rps. palustris* DSM 123^T and the other including *Rps. palustris* R-1 (ATCC 33872), the former type strain of the species *Rps. rutila*, abolished in 1992 [10]. These clusters differed significantly in the main FA content (Tables 1, 3). The C18/C16 ratio constituted 3–4 for the *Rps. palustris* DSM 123^T cluster and 6–7 for the *Rps. palustris* R-1 cluster. Five strains form these clusters were also grown at 35°C (Table 4), after which FACs exhibited lesser C18 and unsaturated FA contents, which is consistent with the trends of the influence of temperature on FAC revealed earlier for *Ectothiorhodospiraceae* representatives [7]. Despite the substantial changes in FAC due to the temperature increase, the two clusters retained the same distinctions as those noted at 25°C. Although the C18/C16 ratio changed, its value was lower (3) in the representatives of the *Rps. palustris* DSM 123^T cluster and higher (4) in

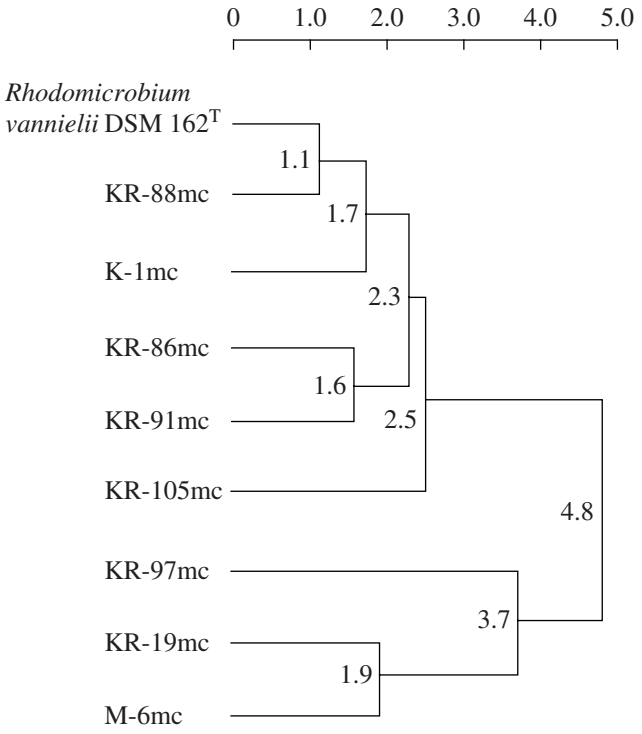

Fig. 6. Euclidean distances between the studied bacterial strains of the genus *Rhodomicrobium.*

the strains of the *Rps. palustris* R-1 cluster. However, the similarity between the partial (415 nucleotides) 16S rRNA sequences of the strains *Rps. palustris* DSM 123^T and *Rps. palustris* R-1 was 100%. No significant morphophysiological differences between the representatives of these clusters were revealed either. Thus, on the one hand, the subdivision of the species *Rps. palustris* into two clusters based on FAC is not accidental, because it is reproduced under different cultivation conditions (25 and 35°C), and, on the other hand, it is not reinforced by other pheno- and genotypic differences.

A separate subcluster (ED 7.3 from the type strain of *Rps. palustris*) was formed by two strains of *Rhodopseudomonas* sp., KR-91p and KR-111p (Fig, 3), which differed in having a lower saturated FA content (Tables 1, 3). No genetic studies of these strains were conducted, and phenotypically they are close to *Rps. palustris*.

An independent cluster remote (ED 10) from the rest of the *Rhodopseudomonas* strains was represented by *Rhodopseudomonas* sp. UZ-25p (Fig. 3). It was characterized by an increased concentration of the C16:0 FA (Tables 1, 3) and the presence of C19:0cyc (0.3%) as a minor component. At present, morphophysiological and genetic studies of strain UZ-25p bacteria are underway with a view to describing them as a new species of the genus *Rhodopseudomonas*.

Three of our isolates with the ED equal to 5.7 and 7.3 between them were in the *Rhodoplanes* sp. cluster (ED from *Rhodopseudomonas* was 11). In terms of FAC, they were closest to representatives of the *Rps. palustris* R-1 cluster, from which they differed in having lower C18:0 content and higher (especially in strain Alt) C18:1cis11 content (Tables 1, 3). As judged from the results of 16S rRNA gene sequencing, strain KR-108p occupied an intermediate position between the two known species of the genus *Rhodoplanes*—*Rpl. roseus* (98.7% similarity) and *Rpl. elegans* (98.9% similarity). The study of these bacteria is currently underway.

Based on the ED (7.3), the bacteria of the genus *Blastochloris* proved to be close to *Rps. palustris* DSM 123^T (Fig. 1). However, they possessed a specific FAC (Tables 1, 5) and could easily be identified owing to the low C18:0 content (0.6–1.8%), as well as the presence of a considerable amount of the normally minor component—the C20:1cis13 FA (2.5–4.3%). The *Blastochloris* strains studied were subdivided into three clusters with the ED between them of 5.6 and 6.1 (Fig. 4). One cluster included two *Blc. viridis* strains, which differed in having a slightly higher C16:1cis9 content, a smaller share of C18:0, as well as in the presence of the minor C16:0-3OH, C17:1cis9, and C18:1cis13 FAs (Table 5). Two other clusters included strains phenotypically close to *Blc. sulfoviridis* but differed in the saturated to unsaturated FA ratio. Unfortunately, the type strain of this species was not included in this work. However, earlier, we conducted a taxonomic study [11] of 11 *Blastochloris* strains (three of them were used in this work), including the type strain *Blc. sulfoviridis* Pl. By the results of DNA–DNA hybridization, the strains phenotypically close to *Blc. sulfoviridis* were subdivided into the same two groups with a 30–40% level of similarity between them. Strain KR-109sv proved to be close (72%) to the type strain *Blc. sulfoviridis* Pl. The strains KR-70sv and KR-101sv formed a separate group with a DNA–DNA hybridization level of 92% between them, a 30–40% hybridization level with the type strain. However, no morphophysiological criteria distinguishing these strains from *Blc. sulfoviridis* were revealed, and they were assigned to this species.

The bacteria of the genus *Rhodobium* formed two clusters (ED 9.6; Fig. 5). One cluster included the type strain of *Rbi. marinum*, as well as strains KR-36m and 992. The affiliation of strain KR-36m isolated from a freshwater sulfur spring with the saline-water species *Rbi. marinum* is confirmed by the DNA–DNA hybridization data and the results of morphophysiological studies [12]. A separate cluster was formed by strain Scotc, which was distinguished by a significantly lower C18:1cis11 content (Table 6). No detailed study of strains 992 and Scotc was conducted.

The studied strains of the genus *Rhodomicrobium* were subdivided by FAC into two closely related clusters (ED 4.8), which differed in the C18/C16 ratio (Fig. 6, Table 6). Morphophysiological studies confirmed the phenotypic similarity between these strains and the type strain of *Rmc. vannielii*. Some distinctions revealed between individual strains did not correlate with the division of the bacteria studied into FAC-based clusters. The partial (604 nucleotides) 16S rRNA sequence of strain KR-19mc, which differed from the other strains in a number of phenotypic features (e.g., in the spectrum of the aromatic compounds used) and FAC, appeared to be similar (99.2–100%) to the 16S rRNA sequences of three nontype strains of *Rmc. vannielii* represented in GenBank (unfortunately, no data on the type strain are available in GenBank). Thus, all the *Rhodomicrobium* strains studied should be assigned to the species *Rmc. vannielii*.

On the whole, the results of our investigation confirmed that FAC is specific to different groups of purple nonsulfur bacteria of the suprageneric, generic, and

Table 2. Fatty acid compositions (%) of the studied strains of the genus *Rubrivivax*

Fatty acids	L-4g	SRg	B-6g	KR-55g	H-4g
C10:0	0.7	0.4	0.6	0.4	0.4
C10:0-3OH	4.6	4.9	5.1	4.4	4.7
C12:0	3.7	3.3	3.9	3.1	3.3
C14:0	1.7	2.6	1.6	3.6	3.7
C16:0	25.9	25.3	28.4	28.2	31.7
C16:1cis7	4.1	3.1	3.5	2.8	2.4
C16:1cis9	37.5	36.3	31.7	32.1	35.7
C16:1cis11	1.0	1.2	0.7	0.8	0.7
i-C16:1-3OH	0.5	0.4	0.6	0.4	0.6
C17:0cyc	0.6	–	–	–	–
C18:0	0.6	1.0	1.1	0.8	0.7
C18:1cis9	0.4	0.4	0.6	–	–
C18:1cis11	18.1	20.5	21.5	22.5	15.6
C18:1cis13	0.4	0.5	0.5	0.4	0.3
Sum of C16	69.0	66.3	64.9	64.3	71.1
Sum of C18	19.5	22.4	23.7	23.7	16.6
C18/C16	0.3	0.3	0.4	0.4	0.2

Table 3. Fatty acid composition (%) of bacteria of the genera *Rhodopseudomonas* and *Rhodoplanes*

Fatty acids	*Rps. palustris* DSM 123^T	KR-31	124	ORp	1 e 5	KR-63p	ABp	KR-91p	KR-111p	UZ-25p	*Rps. palustris* R-1	BORp	KR-103p	KR-109p	121	MG-3p	KR-32p	*Rhodoplanes* sp. KR-62p	KR–108p	Alt
C14:0-3OH	0.4	0.5	0.3	0.0	0.3	0.4	0.0	0.5	0.4	0.3	0.3	0.6	0.5	0.3	0.4	0.4	0.3	0.4	0.0	0.4
C15:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0
C15:0-3OH	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C16:0	15.4	13.6	13.8	13.1	12.9	12.3	14.1	9.9	11.6	18.9	10.4	10.4	11.1	10.5	10.0	9.1	10.1	7.9	11.1	8.5
i-C16:0-3OH	0.6	0.0	0.0	0.7	0.6	0.5	0.4	0.6	0.5	0.6	0.0	0.6	0.6	0.6	0.8	0.5	0.7	0.6	0.5	0.5
C16:1cis9	8.7	11.5	6.2	6.0	5.7	7.1	7.2	8.7	10.7	3.7	3.9	3.3	3.4	3.0	3.6	3.6	2.2	6.2	2.5	2.4
C17:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0
C18:0	4.2	2.8	2.6	6.5	7.0	4.9	4.5	2.6	2.5	5.5	8.3	8.5	8.3	8.8	9.4	8.1	8.3	3.3	2.1	1.7
C18:1cis11	70.0	70.5	74.6	72.0	72.3	37.3	72.0	77.3	73.4	69.5	74.8	75.4	75.0	75.5	74.5	76.6	76.7	80.7	78.0	85.6
C18:1cis13	0.5	0.4	1.0	0.7	0.6	0.4	0.7	0.4	0.4	0.6	0.7	0.7	0.6	0.6	0.7	0.6	0.5	0.6	0.0	0.3
C19:0cyc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C19:1cyc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.0
C20:1cis13	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.3	0.4	0.3	0.4	0.5	0.7	0.6	0.0	0.0	0.4
Sum of C16	24.7	25.1	20.0	19.8	19.2	19.9	21.7	19.2	22.8	23.2	14.3	14.3	15.1	14.1	14.4	13.2	13.0	14.7	14.1	11.4
Sum of C18	74.7	73.7	78.2	79.2	79.9	78.6	77.2	80.3	76.3	75.6	83.8	84.6	83.9	84.9	84.6	85.3	85.5	84.6	80.1	87.6
C18/C16	3.0	2.9	3.9	4.0	4.2	3.9	3.6	4.2	3.3	3.3	5.9	5.9	5.6	6.0	5.9	6.5	6.6	5.8	5.7	7.7

Table 4. Fatty acid compositions (%) of five strains of the genus *Rhodopseudomonas* grown at 35°C

Fatty acids	*Rps. palustris* DSM 123^T			*Rps. palustris* R-1 cluster	
	ORp	1e5	ABp	R-1	MG-3p
C16:1cis9	3.3	3.4	4.0	2.7	2.3
C16:0	20.6	22.7	21.6	18.2	16.2
C18:0	15.3	15.4	13.0	15.0	15.9
C18:1cis11	56.9	55.4	58.9	61.4	62.6
Sum of C16	23.9	26.1	25.6	20.9	18.5
Sum of C18	72.2	70.8	71.9	76.4	78.5
C18/C16	3.0	2.7	2.8	3.7	4.2
C16:1 + C18:1	60.2	58.8	62.9	64.1	64.9
C16:0 + C18:0	35.9	38.1	34.6	33.2	32.1
C16:1 + C18:1/C16:0 + C18:0	1.7	1.5	1.8	1.9	2.0

Table 5. Fatty acid composition (%) of bacteria of the genus *Blastochloris*

Fatty acids	*Blc. viridis*		*Blc. sulfoviridis*		
	DSM 133^T	170	KR-109sv	KR-70sv	KR-101sv
C14:0	0.4	0.3	–	–	–
C16:0	9.7	9.3	9.6	12.6	11.8
C16:0-3OH	0.6	0.5	–	–	–
i-C16:0-3OH	–	0.6	1.4	0.8	–
C16:1cis9	8.4	7.7	6.3	5.9	5.2
C17:1cis9	0.3	0.5	–	–	–
C18:0	0.6	0.6	0.9	1.8	1.8
C18:1cis11	76	76.1	77.2	71.2	75.2
C18:1cis13	0.3	0.4	–	–	–
C19:1cyc	–	–	1.0	3.1	–
C20:1cis13	2.5	3.8	3.3	4.5	4.8
Sum of C16	18.7	18.1	17.3	19.3	17.0
Sum of C18	76.9	77.1	78.1	73.0	77.0
C18/C16	4.1	4.3	4.5	3.8	4.5

Table 6. Fatty acid compositions (%) of bacteria of the genera *Rhodobium* and *Rhodomicrobium*

Fatty acids	*Rhodobium*				*Rhodomicrobium*								
	DSM 2698^T	KR-36m	992	Scotc	DSM 162^T	KR-88mc	K-1mc	KR-91mc	KR-86mc	KR-105mc	KR-19mc	M-6mc	KR-97mc
C14:0	0.0	0.0	0.0	0.0	1.2	0.9	1.3	1.0	1.3	0.8	0.9	1.5	1.3
14:0-3OH	1.0	0.8	0.7	0.5	0.5	0.6	0.0	0.0	0.5	1.0	2.5	1.0	0.8
i-C15:0	0.0	0.0	0.0	0.0	0.7	0.9	0.7	0.9	0.6	1.0	0.7	0.6	0.8
C15:0-3OH	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.0	0.6	0.0	0.0	0.0
C16:0	2.2	1.5	1.4	2.0	1.3	1.8	1.1	2.1	1.3	1.3	1.8	1.6	2.0
i-C16:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.6	0.0
C16:0-3OH	0.0	0.0	0.0	0.0	1.7	1.5	2.4	1.6	2.4	1.8	1.5	1.8	2.6
C16:1cis7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.0
C16:1cis9	0.9	0.7	0.5	0.9	0.7	1.3	0.6	0.0	0.0	1.8	0.8	0.8	3.2
i-C16:1-3OH	0.0	0.0	0.0	0.0	0.0	0.0	1.2	1.1	0.7	0.0	0.0	0.0	0.0
C18:0	0.0	0.0	0.0	0.0	1.4	1.8	1.0	2.2	3.1	1.8	2.1	2.6	2.9
i-C18:0	9.2	9.2	9.7	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0
C18:1cis9	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C18:1cis11	77.3	79.3	78.9	70.0	87.2	87.1	87.7	86.6	86.3	85.7	83.6	83.1	81.2
C19:0cyc	0.3	0.0	0.0	0.0	1.5	1.2	1.8	1.2	1.6	1.5	1.3	0.9	1.7
C19:1cyc	2.6	2.2	2.7	6.0	0.5	0.5	0.5	1.3	0.0	0.0	0.9	0.0	0.0
C20:0	1.0	1.0	1.1	3.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C20:1cis13	2.8	4.6	4.7	8.0	1.4	1.5	0.8	2.0	2.1	1.6	2.2	1.8	2.1
Sum of C16	3.1	2.2	1.9	2.9	3.7	4.6	5.3	4.8	4.4	4.9	5.2	6.8	7.8
Sum of C18	87.7	88.5	88.6	79.0	88.6	88.9	88.7	88.8	89.4	87.5	85.7	86.6	84.1
C18/C16	28.3	40.2	46.6	27.2	24.0	19.3	16.7	18.5	20.3	17.9	16.5	12.7	10.8

species levels and can be used for the purpose of their taxonomy.

It should be pointed out that the results of FAC determinations by different authors [1, 3–6] for the same bacterial strains may differ significantly. These distinctions are predominantly caused by the cultivation conditions rather than the use of different analytical methods [6]. Our data also differ from the data obtained by other authors [1, 4–6]. This is primarily connected with the use of media of different composition, as well as with differences in the cultivation temperature (25°C in our work; 30°C and higher in most investigations performed by other authors).

The differences in FAC between closely related strains revealed by us did not always correlate with their geno- and phenotypic differences. Thus, based on FAC, the strains of the species *Rps. palustris* and *Rmc. vannielii* were subdivided into clusters that did not differ from one another in the results of 16S rRNA gene sequencing and in morphophysiological characteristics. Strains of *Blc. sulfoviridis* fell into two clusters both on FAC and on the level of DNA–DNA hybridization; however, no significant phenotypic differences between them were revealed. Along with this, some of the studied isolates, which differed substantially in FAC from the related strains, are likely to be independent taxa. Currently, we are completing the description of strain UZ-25p as a new species; this strain is remote from other strains of the genus *Rhodopseudomonas* by FAC, and the results of 16S rRNA gene sequencing. Other strains with specific FAC (*Rhodoplanes* sp. KR-108p, *Rhodobium* sp. Scots, *Rubrivivax* sp. H-4) were revealed as well, and their further study is of taxonomic interest.

Thus, the FAC characteristics of the studied groups of purple nonsulfur bacteria can be used both for affiliating isolates to known genera and species and for revealing new taxa of the species and generic levels.

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