ISOPRENOID QUINONE COMPOSITION IN THE CLASSIFICATION OF *RHODOSPIRILLACEAE*¹

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Fifty five strains of 13 species of *Rhodospirillaceae* and one strain of *Chromatium vinosum* were examined for isoprenoid quinone composition. These bacteria were divided into the following five categories on the basis of their predominant quinone patterns: Q-10—*Rhodospirillum rubrum*, *Rhodopseudomonas palustris*, *Rhodopseudomonas capsulata*, *Rhodopseudomonas sphaeroides*, and *Rhodopseudomonas sulfidophila*; Q-10+MK-10—*Rhodopseudomonas acidophila*; Q-9+MK-9—*Rhodospirillum fulvum*, *Rhodospirillum molischianum*, *Rhodopseudomonas viridis*, and *Rhodopseudomonas globiformis*; Q-8—*Rhodospirillum photometricum* and atypical strains of *Rhodopseudomonas gelatinosa*; Q-8+MK-8—*Rhodospirillum tenue*, *R. gelatinosa*, and *C. vinosum*. The significance of the quinone system in *Rhodospirillaceae* taxonomy is discussed in comparison with available information on other taxonomic properties such as morphology and photosynthetic membrane systems and on some phylogenetic characteristics.

Respiratory isoprenoid quinones are essential components of membrane-bound electron transport systems in bacteria. In addition to interest in their functions in electron transport mechanisms, a growing concern with the significance of the quinone system in bacterial systematics has led to intensive study. At present, the diagnostic value of isoprenoid quinones in bacterial taxonomy is well realized (1), and an analysis of isoprenoid quinone composition of bacterial strains is one of the most prevalent chemotaxonomic methods (2).

There are some preliminary reports in the literature on predominant quinone patterns in phototrophic purple nonsulfur bacteria, *Rhodospirillaceae* (3–5). The available information on this subject has also been summarized in recent re-

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Table 1. Bacterial strains studied.

Species and strain designation ^a	Other designations	Source ^b	Reference
<i>Rhodospirillum rubrum</i>			
*ATCC 11170	DSM 467	ATCC	
IFO 3986		IFO	
SW20		HIRAISHI & KITAMURA	(8)
<i>Rhodospirillum tenue</i>			
*2761	ATCC 25093, DSM 109	N. PFENNIG	(30)
SW18		HIRAISHI & KITAMURA	(8)
<i>Rhodospirillum fulvum</i>			
WP1		HIRAISHI & KITAMURA	(8)
WF2		HIRAISHI & KITAMURA	(8)
WF4		HIRAISHI & KITAMURA	(8)
WF6		HIRAISHI & KITAMURA	(8)
<i>Rhodospirillum molischianum</i>			
*N131	ATCC 14031, DSM 120	N. PFENNIG	
<i>Rhodospirillum photometricum</i>			
IL256		our own collection	
WP4		HIRAISHI & KITAMURA	(8)
WP5		HIRAISHI & KITAMURA	(8)
WP6		HIRAISHI & KITAMURA	(8)
<i>Rhodopseudomonas palustris</i>			
*ATCC 17001	DSM 126	A. YOSHIKAWA	
A		H. TAKAHASHI	(31)
IL137		our own collection	
RS22		HIRAISHI & KITAMURA	(8)
SA37		HIRAISHI & KITAMURA	(8)
SA42		HIRAISHI & KITAMURA	(8)
<i>Rhodopseudomonas viridis</i>			
*F	ATCC 19567, DSM 133	N. PFENNIG	
NR7		our own collection	
<i>Rhodopseudomonas acidophila</i>			
*ATCC 25092	DSM 137	ATCC	(9)
PT1		our own collection	
PT6		our own collection	
NR9		our own collection	
<i>Rhodopseudomonas globiformis</i>			
*DSM 161		DSM	(11)
<i>Rhodopseudomonas gelatinosa</i>			
*ATCC 17011		ATCC	
A3		H. TAKAHASHI	(32)
IL144		our own collection	
KA49		HIRAISHI & KITAMURA	(8)
KE2		our own collection	

Table 1. (Continued)

Species and strain designation ^a	Other designations	Source ^b	Reference
FR2		HIRAISHI & KITAMURA	(8)
FR3		HIRAISHI & KITAMURA	(8)
SA34		HIRAISHI & KITAMURA	(8)
<i>Rhodopseudomonas capsulata</i>			
*ATCC 11166		A. YOSHIKAWA	
IL155		our own collection	
HP8		HIRAISHI & KITAMURA	(8)
C2		HIRAISHI & KITAMURA	(8)
MC9R		our own collection	
SA47		HIRAISHI & Kitamura	(8)
<i>Rhodopseudomonas sphaeroides</i>			
*ATCC 17023		ATCC	
IFO 12203		IFO	
B1		H. TAKAHASHI	(32)
B3		H. TAKAHASHI	(32)
IL102		our own collection	
RS1		HIRAISHI & KITAMURA	(8)
RS2		HIRAISHI & KITAMURA	(8)
TWN		HIRAISHI & KITAMURA	(8)
C7		HIRAISHI & KITAMURA	(8)
KA38		HIRAISHI & KITAMURA	(8)
KA63		HIRAISHI & KITAMURA	(8)
NR3		HIRAISHI & KITAMURA	(8)
<i>Rhodopseudomonas sulfidophila</i>			
*W4	DSM 1374	T. A. HANSEN	(10)
TW13		our own collection	
<i>Chromatium vinosum</i>			
*D	ATCC 17899, DSM 180	K. SHIMADA	

^a Type or neotype strains are asterisked.

^b ATCC, American Type Culture Collection, Rockville, U.S.A.; IFO, Institute for Fermentation, Osaka, Japan; DSM, Deutsche Sammlung von Mikroorganismen, Göttingen, Federal Republic of Germany; N. Pfennig, Universität Konstanz, Konstanz, Federal Republic of Germany; A. Yoshikawa, Mitsubishi Gas Chemical Co., Niigata, Japan; H. Takahashi, Tohoku University, Sendai, Japan; T. A. Hansen, University of Groningen, Haren, The Netherlands; K. Shimada, Tokyo Metropolitan University, Tokyo, Japan.

views (1, 6). These reports have shown that ubiquinones (Q-8, Q-9, or Q-10) represent the major quinones within representative species of *Rhodospirillaceae*, and some species of these bacteria also contain menaquinones (MK-8 or MK-9) with polyprenyl side chains of the same length as the present ubiquinones. However, it is not yet possible to establish the taxonomic significance of isoprenoid quinones in *Rhodospirillaceae* because no detailed information regarding the quinone system is available for several species (6) such as *Rhodospirillum photo-*

metricum, *Rhodopseudomonas acidophila*, *Rhodopseudomonas globiformis*, and *Rhodopseudomonas sulfidophila* and also because no attempt has been made to relate this system to other well-defined taxonomic properties.

This article deals with the quinone system in 13 species of *Rhodospirillaceae* and, for comparison, one species of *Chromatiaceae*. The significance of isoprenoid quinones in *Rhodospirillaceae* taxonomy is discussed in comparison with available information on other taxonomic properties such as morphology and intracytoplasmic membrane systems and on some phylogenetic characteristics.

MATERIALS AND METHODS

Strains and cultivation. Details of the bacterial strains tested and their sources are listed in Table 1. The species and strain names are used according to their designations with which we received them from culture collections and individuals. All names of the bacterial species referred to here are described in the Approved Lists of Bacterial Names (7).

Unless otherwise indicated, mineral base RM2 (pH 7.0) (8) was used for all growth media. DL-Sodium malate (3.6 g/l) and yeast extract (0.5 g/l) were the usual carbon source and growth factors, respectively. The medium for *Rhodospirillum fulvum*, *Rhodospirillum molischianum*, and *R. photometricum* was supplemented with filter-sterilized sodium ascorbate (0.5 g/l). The pH of the medium for *R. acidophila* was adjusted to 5.8 (9). The medium for *R. sulfidophila* contained a high concentration of NaCl (25 g/l) (10). *R. globiformis* was cultured according to PFENNIG (11). The organisms were grown in completely filled screw-capped bottles (280 ml) at 30° under incandescent illumination (ca. 5,000 lx). Cultures at the early stationary phase of growth were harvested by centrifugation, washed with distilled water, and stored at 0–4° until assayed, not more than 3 hr.

Extraction and purification of quinones. Preliminary experiments established that higher concentrations of isoprenoid quinones were often obtained from fresh wet cells than from freeze-dried cells of the same strains. In this study, therefore, quinones were extracted directly from fresh stationary cells in all cases. The quinone extraction was made with chloroform–methanol (2: 1, v/v) according to COLLINS et al. (12). The total quinone extracts were purified by thin-layer chromatography (TLC) using Whatman PK6F silica gel plates and a developing solvent of petroleum benzine–diethyl ether (9: 1, v/v) (2); as markers, available Q-10 and vitamin K₁ were co-chromatographed. The ubiquinone and menaquinone samples obtained were further purified by TLC on the same plates developed in 100% benzene and hexane–benzene–chloroform (5: 2: 1, v/v), respectively (2).

Spectrophotometric analysis. Ultraviolet absorption spectra of quinone samples in ethanolic solution were recorded with a Hitachi 200-20 spectrophotometer. Quinone concentration was determined by the difference spectrum of the reduced minus the oxidized state, using the following millimolar extinction coef-

ficients: ubiquinone, $\Delta\epsilon_{275\text{nm}}=12.9\text{ mM}^{-1}\text{ cm}^{-1}$; menaquinone, $\Delta\epsilon_{245\text{nm}}=28.7\text{ mM}^{-1}\text{ cm}^{-1}$ (13).

Reversed-phase TLC. Components of quinone samples were separated by reversed-phase TLC using Whatman LKC18F plates with acetone–water (95: 5, v/v, for ubiquinone; 98: 2, v/v, for menaquinone) as the solvent system. The separated quinone homologues were detected as yellow-colored bands in visual light and as fluorescence-quenched regions in 254 nm ultraviolet light, and then identified by comparison with known quinones co-chromatographed. Scanning patterns of the quinone chromatograms were obtained with a Helena Quick Scan R&D densitometer and their relative percentage to the total peak area was calculated.

High-performance liquid chromatography (HPLC). The number of isoprene units and the relative percentage of components of quinone samples were also determined by HPLC which was performed on a Shimadzu Liquid Chromatograph LC-3A equipped with a Zobax ODS pre-packed column, a Shimadzu Spectrophotometric Detector SPD-2A, and a microcomputer, Shimadzu Chromatopac C-RIA; solvent, methanol–isopropyl ether (3: 1, v/v); solvent flow, 1 ml/min. This system was identical with that of TAMAOKA et al. (14).

Standard quinones. Vitamin K₁ and ubiquinone standards (Q-6 through Q-10) were obtained from Sigma Chemical Co., except Q-8 which was prepared from *Flavobacterium lutescens* IFO 12997 (15). Q-10 (H₂) was kindly supplied by Dr. H. Kuraishi. Reference menaquinones were prepared from *Cytophaga aquatilis* IAM 12365 (MK-6) (15), *Cytophaga heparina* IFO 12017 (MK-7) (15, 16), *Curto-bacterium testaceum* IAM 1537 (MK-8 through MK-11) (14), and *Arthrobacter globiformis* IFO 12137 (MK-9(H₂)) (14, 17).

RESULTS

Chromatographic and spectrophotometric behavior of quinones

In the TLC system with the solvent of petroleum benzine–diethyl ether (9: 1,

Table 2. *R_f* values (on reversed-phase TLC) and HPLC retention times of standard quinone homologues.

Ubiquinone	<i>R_f</i>	HPLC retention time (min)	Menaquinone	<i>R_f</i>	HPLC retention time (min)
Q-6	0.55	6.2	MK-6	0.61	9.5
Q-7	0.49	7.7	MK-7	0.56	12.2
Q-8	0.42	9.7	MK-8	0.50	15.8
Q-9	0.36	12.5	MK-9	0.45	20.5
Q-10	0.29	16.1	MK-9 (H ₂)	NT ^a	23.1
Q-10 (H ₂)	NT	17.6	MK-10	0.40	27.2
			MK-11	0.35	36.6

^a Not tested.

Table 3. Quinone composition of *Rhodospirillaceae*

Organism	Total ubiquinone content ($\mu\text{mol/g}$ dry wt)	Percentage of total ubiquinones ^a						
		Q-6	Q-7	Q-8	Q-9	Q-10	Q-10 (H ₂)	Others ^b
<i>Rhodospirillum rubrum</i>								
ATCC 11170	2.02				(5)	(93)		(2)
IFO 3986	5.27			t ^c	5	92		3
SW20	5.17			t	6	92		1
<i>Rhodospirillum tenue</i>								
2761	2.99	t	3	94	1			1
SW18	2.87	t	3	93	1			2
<i>Rhodospirillum fulvum</i>								
WP1	4.00		t	4	89	2		4
WF2	3.38		t	5	90	2		3
WF4	4.33		t	4	89	2		5
WF6	5.12		t	3	92	2		3
<i>Rhodospirillum molischianum</i>								
N131	3.50		t	3	89	2		6
<i>Rhodospirillum photometricum</i>								
IL256	ND ^d		7	89	4			
WP4	3.78		8	90	2			
WP5	3.24		6	87	2			5
WP6	4.28		5	91	4			
<i>Rhodopseudomonas palustris</i>								
ATCC 17001	2.59				2	98		
A	2.97				3	96		1
IL137	2.98				3	97		t
RS22	2.17			t	3	96		t
SA37	ND				2	98		
SA42	ND				2	97		t
<i>Rhodopseudomonas viridis</i>								
F	2.53			(2)	(94)	(4)		
NR7	2.26			5	93	2		
<i>Rhodopseudomonas acidophila</i>								
ATCC 25092	4.88				3	97		t
PT1	3.36				4	94	1	t
PT6	2.87				4	94	1	t
NR9	2.43				8	92		t
<i>Rhodopseudomonas globiformis</i>								
DSM 161	ND			(5)	(93)	(2)		
<i>Rhodopseudomonas gelatinosa</i>								
ATCC 17011	2.38		t	91	1	t		7
A3	4.05		t	97	1			1
IL144	2.44		t	97	2			t
KA49	2.39		t	98	1			t
KE2	2.36		4	90	2			4

species and *Chromatium vinosum*.

Total menaquinone content (μ mol/g dry wt)	Percentage of total menaquinones ^a							
	MK-6	MK-7	MK-8	MK-9	MK-9 (H ₂)	MK-10	MK-11	Others ^b
absent								
absent								
absent								
0.91	2	2	89	t				6
0.88	1	1	88	t				9
0.68	t		6	89	t	t		4
0.75	1		6	84	t	t		8
0.61	t		6	90				4
1.11	t		4	91		t		5
1.22	t		5	87	2	1		4
absent								
absent								
absent								
absent								
absent								
absent								
0.98		(1)	(6)	(93)				
0.64		t	5	93	t			
0.26		t	3	4		91	1	t
0.37	t		2	6		85	3	3
0.33			t	5		93	t	2
0.26			t	7		86	t	6
ND			(3)	(95)		(2)		
0.74	t	t	97	t				2
0.92	t	t	97	t				1
0.70	t	t	97	1				1
0.61	t	t	97	1				1
absent								

Table 3.

Organism	Total ubiquinone content ($\mu\text{mol/g}$ dry wt)	Percentage of total ubiquinones ^a						
		Q-6	Q-7	Q-8	Q-9	Q-10	Q-10 (H ₂)	Others ^b
FR2	2.06		1	92	5	1		1
FR3	2.22		2	94	4			t
SA34	2.35		(3)	(93)	(4)			
<i>Rhodopseudomonas capsulata</i>								
ATCC 11166	5.10				1	99		
IL155	4.58				3	95	2	
HP8	3.96				4	94	2	
C2	4.78				(4)	(96)		
MC9R	ND				(3)	(97)		
SA47	ND				3	95	2	
<i>Rhodopseudomonas sphaeroides</i>								
ATCC 17023	5.14			t	t	99		
IFO 12203	5.49			t	t	99		
B1	4.72					100		
B3	ND					100		
IL102	ND				t	99		
RS1	5.48				t	99		
RS2	5.08				t	99		
TWN	5.68			t	t	99		
C7	ND			t	t	99		
KA38	5.82			t	t	99		
KA63	4.81			t	t	99		
NR3	4.98					100		
<i>Rhodopseudomonas sulfidophila</i>								
W4	2.70			t	t	98		t
TW13	3.08				(1)	(99)		
<i>Chromatium vinosum</i>								
D	4.25		10	80	1	4		5

^a Calculated from the peak area on HPLC or on reversed-phase TLC with densitometry (TLC data given in parentheses).

^b Unidentified homologues.

v/v), the lipids extracts from all 56 test strains showed a fluorescence-quenching band of the same *R_f* value (0.2–0.3) as the reference ubiquinone. Each of the quinone samples of this fraction had an ultraviolet difference spectrum identical with that of typical ubiquinone (13) with a maximum decrease in absorption at 272–275 nm. Another band that exhibited the same mobility (*R_f*, 0.5–0.6) as vitamin K₁ was also found in the lipid samples from about one-third of the test strains. The difference spectrum of the quinone samples of this fraction was characterized by a maximum increase in absorption at 245 nm and a maximum decrease at 263–270 nm, both of which are characteristic of menaquinone (13).

The data for the ubiquinone and menaquinone composition of the test strains

are summarized in Table 3.

Ubiquinones were found in all test strains as mentioned above, with their amounts varying between 2.02 and 5.82 $\mu\text{mol/g}$ dry weight. The length of the poly-prenyl side chain of the predominant ubiquinones differed from species to species. Q-10 predominated in all test strains of *Rhodospirillum rubrum*, *Rhodopseudomonas palustris*, *R. acidophila*, *Rhodopseudomonas capsulata*, *Rhodopseudomonas sphaeroides*, and *R. sulfidophila*. Q-9 predominated in *R. molischianum* N131, *R. globiformis* DSM 161, and all test strains of *R. fulvum* and *Rhodopseudomonas viridis*. Q-8 predominated in all test strains of *Rhodospirillum tenue*, *R. photometricum*, and *Rhodopseudomonas gelatinosa*. *Chromatium vinosum* D contained Q-8 as the major ubiquinone. In most strains tested, the predominant ubiquinones accounted for 90% and more of the total ubiquinone content. Although one to three minor ubiquinones were also found in these organisms, the amount of each minor component was less than 10% of the total ubiquinone content.

Menaquinones were recovered from *R. molischianum* N131, *R. globiformis* DSM 161, *R. gelatinosa* strains ATCC 17011, A3, IL144, and KA49, and all test strains of *R. tenue*, *R. fulvum*, *R. acidophila*, and *R. viridis*. *C. vinosum* D also contained menaquinones. The total menaquinone content of these strains ranged from 0.26 to 2.11 $\mu\text{mol/g}$ dry weight, corresponding with 5 to 50% of the total ubiquinone content on the dry weight basis of the same strains. The number of isoprene units of the predominant menaquinones was the same as that of the side chain of the major ubiquinones concurrently present. Namely, MK-10 predominated in the *R. acidophila* strains, MK-9 predominated in the *R. fulvum*, *R. molischianum*, *R. viridis*, and *R. globiformis* strains, and MK-8 predominated in the *R. tenue* and *R. gelatinosa* strains. *C. vinosum* D had MK-8 as the major menaquinone and a considerable amount (40% of the total menaquinones) of an unidentified homologue that showed a HPLC peak with a retention time of 7.7 min. With the exception of the major component of *C. vinosum* D, the predominant menaquinone constituted 84 to 97% of the total menaquinone content of each strain.

None of the test strains of *R. rubrum*, *R. photometricum*, *R. palustris*, *R. capsulata*, *R. sphaeroides*, or *R. sulfidophila* produced menaquinones. It is of particular interest that the remaining *R. gelatinosa* strains KE2, FR2, FR3, and SA34 also lacked menaquinones. Concurrent studies have shown that the *R. gelatinosa* strains devoid of menaquinones differ from typical strains of this species in certain phenotypic characteristics (8).

DISCUSSION

In view of the present results, the *Rhodospirillaceae* species studied are divided into the following five categories on the basis of the isoprenoid quinone system: Q-10 — *R. rubrum*, *R. palustris*, *R. capsulata*, *R. sphaeroides*, and *R. sulfidophila*; Q-10+MK-10 — *R. acidophila*; Q-9+MK-9 — *R. fulvum*, *R. molischianum*, *R.*

viridis, and *R. globiformis*; Q-8 — *R. photometricum* and *R. gelatinosa* (atypical strains); Q-8+MK-8 — *R. tenue* and *R. gelatinosa*. There has been a general view concerning the quinone system in the *Rhodospirillaceae* that all species which contain Q-10 lack menaquinones and all species which contain ubiquinones other than Q-10 also possess menaquinones with the corresponding isoprenoid side chain as the second component (1, 5). The results of this study extend the earlier results in this area (1, 3–6) and provide further evidence that there are some *Rhodospirillaceae* species that do not fit the above general concept.

Reports on the quinone system in certain species of the phototrophic purple bacteria have been somewhat confusing. Some workers found Q-9 as the major ubiquinone in *R. rubrum* (18), *R. sphaeroides* (4), and *R. gelatinosa* (19), whereas other investigators found Q-10 in the former two species and Q-8 in the latter (3, 5). Our results appear to clarify this situation completely. The available information on the quinone composition of *C. vinosum* is also lacking in uniformity. The predominant quinones in this organism reported so far are as follows: Q-*n* (20), Q-7+MK-*n* (18), Q-7+MK-7 (4), Q-8+MK-8 (21), and Q-*n*+MK-8 (22). Our data clearly indicate that Q-8 and MK-8 are present as the major quinone components in *C. vinosum* D, which also contains a considerable amount of the unknown menaquinone. At any rate, further studies on additional strains of this species are necessary to clear up this confusion.

As described here, both genera *Rhodospirillum* and *Rhodopseudomonas* are considerably heterogeneous groups with respect to the quinone system. This suggests that a taxonomic inconsistency exists in the *Rhodospirillaceae*. The phenotypic heterogeneity and taxonomic questions which have long existed in these groups of phototrophic bacteria have already been discussed (23, 24). Also, recent studies on their phylogenetic traits, e.g., amino acid sequence of cytochromes *c* (25) and oligonucleotide signature of 16S ribosomal RNAs (26, 27), have shown that there is no correlation between the phylogenesis and the standard taxonomy of the *Rhodospirillaceae*. This is not surprising because the genera now defined for the phototrophic purple bacteria are meant to be determinative categories for the identification of newly isolated strains, irrespective of the genetic or evolutionary relatedness thereof (23, 24). However, we take the ground that, if information is available, a classification system for bacterial species must be based on a combination of phenotypic, chemotaxonomic, and phylogenetic properties, rather than a single one.

To evaluate the significance of the quinone system in *Rhodospirillaceae* systematics, we attempted to compare the data presented here with those on some phylogenetic and phenotypic properties of these bacteria available in the literature and to re-classify them based on a combination of these characteristics. For this purpose, the available information was arranged in the following manner. First, according to DICKERSON (25), the *Rhodospirillaceae* species were divided into three groups that have different structural types of *c*-type cytochromes, i.e., "long," "medium,"

Table 4. Classification of *Rhodospirillaceae* species based on a combination of cytochrome *c* structural classes, intracytoplasmic membrane systems, morphology, and isoprenoid quinone patterns.

Photosynthetic membrane system ^a	Cell form	Long cytochromes ^b		Medium cytochromes ^b		Short cytochromes ^b	
		Species	Quinone system	Species	Quinone system	Species	Quinone system
Vesicles	ovoid/sphere	<i>R. capsulata</i> <i>R. sphaeroides</i>	Q-10 Q-10	<i>R. globiformis</i>	Q-9 + MK-9		
Lamellae	rod (budding)	<i>R. palustris</i>	Q-10	<i>R. acidophila</i> <i>R. viridis</i>	Q-10 + MK-10 Q-9 + MK-9		
Tubes	rod/spiral					<i>R. gelatinosa</i> <i>R. tenue</i>	Q-8 + MK-8 Q-8 + MK-8
Vesicles	spiral	<i>R. rubrum</i>	Q-10				
Stacks	spiral	<i>R. photometricum</i>	Q-8	<i>R. fulvum</i> <i>R. molischianum</i>	Q-9 + MK-9 Q-9 + MK-9		

^a Based on information from PENNIG and TRÜPER (23, 28).

^b Based on information from DICKERSON (25).

and "short" cytochromes *c*. Second, they were also separated into five groups based on their ultrastructural and morphological characteristics (23, 28) — that is, those species that have spherical to ovoid cell forms and vesicular intracytoplasmic membrane systems, those species that have rod-shaped cells multiplying by budding and lamellar membrane systems, those species that have slender rod-shaped to spirilloid cells and membrane systems with tubular intrusions, those species that have spiral cell forms and vesicular membrane systems, and those species that have spiral cell forms and several stacks of lamellar membranes. Finally, the *Rhodospirillaceae* species re-classified on the basis of the above criteria were tabulated with their quinone patterns (Table 4).

The information contained in Table 4 indicates that the *Rhodospirillaceae* species can be divided into at least eight groups (surrounded by broken lines) based on the new classification system. Interestingly, each of the eight groups is quite uniform in the quinone system, with the exception of the *R. acidophila*–*R. viridis* group. Since YAMADA et al. (17) suggested that the quinone system might be of value in grouping bacteria at the genus level, it seems logical to conclude that the eight groups of the phototrophic bacteria can be defined as the new genus-groups. *R. sulfidophila* is not listed in Table 4 due to lack of information on its cytochrome *c* size. However, one would predict that this species is a member of the *R. capsulata*–*R. sphaeroides* group because of its cell form of the ovoid type, its membrane system of the vesicular type, and its quinone system of Q-10. Also, the atypical *R. gelatinosa* strains that have the quinone system of Q-8 are not tabulated, because they apparently belong to a species different from *R. gelatinosa*. The amino acid sequence analyses of *c*-type cytochromes revealed close affinities between *R. capsulata* and *R. sphaeroides*, between the budding species, between *R. gelatinosa* and *R. tenue*, between *R. rubrum* and *R. photometricum*, and between *R. fulvum* and *R. molischianum* (25). Similar results were obtained with oligonucleotide sequence studies of 16S ribosomal RNAs (26, 27). The results of these phylogenetic studies with the *Rhodospirillaceae* encourage our conclusion.

It is evident from the present results that the isoprenoid quinone composition, as well as the conventionally-recognized, phenotypic characteristics of the *Rhodospirillaceae* yields valuable taxonomic information. Also, whether or not the quinone system in these bacteria is of phylogenetic significance, it seems that in their classification the cytochrome *c* structural classes and the quinone structural types are correlated. Namely, those species with medium or short cytochromes *c* generally contain both ubiquinone and menaquinone, whereas those species with long cytochromes *c* lack menaquinone (Table 4).

In this paper, we did not deal with rhodoquinone, which was initially discovered in *R. rubrum* (29). This type of isoprenoid quinone is also present in certain *Rhodospirillaceae* species other than *R. rubrum*, such as *R. photometricum*, *R. acidophila*, and the atypical *R. gelatinosa* strains (HIRAISHI and HOSHINO, in preparation). Further study in this area should provide considerable insight into our un-

derstanding of the significance of rhodoquinone in *Rhodospirillaceae* taxonomy.

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