

Isoprenoid quinone composition of some marine *Alteromonas*, *Marinomonas*, *Deleya*, *Pseudomonas* and *Shewanella* species

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The isoprenoid quinone composition of 51 Gram-negative, aerobic, marine bacteria representing the genera *Alteromonas*, *Marinomonas*, *Deleya*, *Pseudomonas* and *Shewanella* was examined using high-performance liquid chromatography. *Alteromonas* and *Marinomonas* strains contained ubiquinone with eight isoprene units as their most abundant component, whilst ubiquinones with nine isoprene units predominated in *Deleya* and marine *Pseudomonas* strains. Members of the genus *Shewanella* contained both ubiquinones and menaquinones. The presence of two types of isoprenoid quinones in *Shewanella* is thought to be unique amongst Gram-negative, aerobic bacteria. In addition, methylmenaquinones were also found in *Shewanella putrefaciens* strains. The results demonstrate that the analysis of isoprenoid quinones offers a rapid and effective method of differentiating between some marine bacteria.

Introduction

Gram-negative, aerobic bacteria are commonly isolated from marine environments. The ecological and economic significance of these organisms has been reported (Yaphe & Morgan, 1959; Yazawa *et al.*, 1988; Akagawa-Matsushita *et al.*, 1991; Hayashida *et al.*, 1991). However, identification of these organisms remains problematic due to limited taxonomic information. Problems with their identification are further compounded by the fact that aerobic bacteria inhabiting marine environments share essentially similar physiological and biochemical characters.

Amongst aerobic marine bacteria possessing polar flagella, members of the genera *Alteromonas*, *Marinomonas* and *Shewanella* have often been confused with each other and with members of the genus *Pseudomonas*

because of their phenotypic similarity. The genus *Alteromonas* was created to encompass marine 'Pseudomonas-like' organisms which had a G+C content of 38–50 mol% (Baumann *et al.*, 1972) and as such were very different from members of the genus *Pseudomonas* (58–70 mol%). Though this division resolved the conflict that one genus accommodates organisms with so wide a range of G+C contents, it was still arbitrary as it was entirely based on G+C content and each genus might remain heterogeneous. Subsequent to this, rRNA–DNA hybridization studies revealed that the genus *Alteromonas* was highly heterogeneous (Van Landschoot & De Ley, 1983). Two species, *A. communis* and *A. vagum* were found to constitute a distinct rRNA branch and were transferred to the newly named genus *Marinomonas* (Van Landschoot & De Ley, 1983). The genera *Alteromonas* and *Marinomonas* share many phenotypic similarities and can only be separated on the basis of a few characteristics.

Two further species of *Alteromonas* – *A. putrefaciens*, which had been transferred from *Pseudomonas*, and *A. hanedai* – were reassigned to the genus *Shewanella*, within the family *Vibrionaceae*, as a result of 5S rRNA sequencing (MacDonell & Colwell, 1985). Further, *A. colwelliana* was subsequently assigned to this genus on the basis of studies utilizing similar methodologies (Coyne *et al.*, 1989).

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Abbreviations: Q, ubiquinone; MK, menaquinone; MMK, methylmenaquinone.

Table 1. Strains used

Names in quotation marks have not been validated. Abbreviations: T, type strain; AJ, Central Research Laboratories, Ajinomoto Co., Kawasaki, Japan; ATCC, American Type Culture Collection, Rockville, MD, USA; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; IAM, Institute of Applied Microbiology, The University of Tokyo, Bunkyo-ku, Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Japan; NCDO, National Collection of Dairy Organisms, Reading, UK; NCIMB, National Collection of Industrial and Marine Bacteria, Torry Research Station, Aberdeen, UK; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, UK.

Name	Strain no.	Source	Other designations	Previous assignment	DNA homology group*	Reference(s)†
<i>Ateromonas aurantia</i>	IAM 12912	NCIMB 2049	ATCC 33041, Gauthier 57			15
<i>Ateromonas citrea</i>	IAM 12644 [†]	NCIMB 1889	ATCC 29719, Gauthier 10			13, 22
<i>Ateromonas citrea</i>	IAM 12913	ATCC 29720				13
<i>Ateromonas espejana</i>	IAM 12640 [†]	ATCC 29659	NCIMB 2127, Baumann 261	' <i>Vibrio haloplanktis</i> '		7, 22
<i>Ateromonas haloplanktis</i>	IAM 12915 [†]	ATCC 14393	NCIMB 2084, ZoBell & Upham 545, Baumann <i>et al.</i> 215			21, 22
<i>Ateromonas haloplanktis</i>	IAM 12916	ATCC 19648	Johnson ASU-1	' <i>Vibrio marinopraescens</i> '		22
<i>Ateromonas haloplanktis</i>	IAM 12917	ATCC 23821	NCIMB 1545, Colwell 651	' <i>Pseudomonas enalia</i> '		21, 22
<i>Ateromonas haloplanktis</i>	IAM 12918	ATCC 27127	NCIMB 1964, Baumann 121	' <i>Ateromonas marinopraescens</i> '		21, 22
<i>Ateromonas haloplanktis</i>	IAM 12919	NCIMB 19	ATCC 19855, MacLeod B-16	<i>Pseudomonas</i> sp.		11, 14, 22
<i>Ateromonas lateoviolacea</i>	IAM 12924 [†]	NCIMB 1893	ATCC 33492, Gauthier CERBON CH130			11, 14, 22
<i>Ateromonas lateoviolacea</i>	IAM 12925	NCIMB 2035	ATCC 33493, Gauthier CERBON CH101			3, 8, 22
<i>Ateromonas macleodii</i>	IAM 12920 [†]	ATCC 27126	NCIMB 1963, Baumann 107			12, 22
<i>Ateromonas rubra</i>	IAM 12643 [†]	NCIMB 1890	ATCC 29570, Gauthier CERBON 18			7, 22
<i>Ateromonas undina</i>	IAM 12922 [†]	ATCC 29660	NCIMB 2128, Baumann 272	' <i>Pseudomonas nigrifaciens</i> '		5, 6, 8, 22
<i>Ateromonas nigrifaciens</i>	IAM 13019 [†]	ATCC 19375	NCIMB 8614, NCTC 10691, NCDO 755			5, 10, 22
<i>Ateromonas nigrifaciens</i>	IAM 13161	ATCC 25013				3, 6, 22
<i>Marinomonas communis</i>	IAM 12914 [†]	ATCC 27118	NCIMB 1961, Baumann 8	<i>Ateromonas communis</i>		3, 6, 22
<i>Marinomonas vaga</i>	IAM 12923 [†]	ATCC 27119	NCIMB 1967, ATCC 27132	<i>Ateromonas vaga</i>		10, 12
<i>Pseudomonas nautica</i>	IAM 12929 [†]	DSM 50418	NCIMB 568, ZoBell 632	<i>Pseudomonas perfectomarina</i>		9, 10
<i>Pseudomonas stutzeri</i>	IAM 12931	NCIMB 301	ATCC 14405			9, 22, 23
' <i>Pseudomonas atlantica</i> '	IAM 12927	NCIMB 301	ATCC 14405			10, 20
' <i>Pseudomonas bathycetes</i> '	IAM 13162	ATCC 23597	ATCC 19262, IAM 12376			20
' <i>Pseudomonas bathycetes</i> '	IAM 13163	ATCC 23599				10, 22
' <i>Pseudomonas carrageenonora</i> '	IAM 12662	IFO 12985	ATCC 43555, NCIMB 302			
' <i>Pseudomonas halosaccharolytica</i> '	IAM 12662	ATCC 29423				
' <i>Pseudomonas piscicida</i> '	IAM 12642	NCIMB 1142	ATCC 15057, NCIMB 1142, IAM 12932			
' <i>Pseudomonas piscicida</i> '	IAM 12933	ATCC 15802	NCIMB 854, Weeks 14			1, 10, 24
<i>Deleya aquamarina</i>	IAM 12550 [†]	ATCC 14400	NCIMB 557, DSM 30161, ZoBell 558	<i>Alcaligenes aquamarinus</i>		3, 4
<i>Deleya aquamarina</i>	IAM 12551	ATCC 27128	NCIMB 1980, Baumann 134	<i>Alcaligenes aestus</i>		1, 2
<i>Deleya aquamarina</i>	IAM 12645	ATCC 33127	NCIMB 2116, Austin <i>et al.</i> L1	<i>Alcaligenes faecalis</i> subsp. <i>homari</i>		3, 4
<i>Deleya cupida</i>	IAM 12552 [†]	ATCC 27124	NCIMB 1978, Baumann 79	<i>Alcaligenes cupidus</i>		3, 4
<i>Deleya halophila</i>	IAM 13009 [†]	CCM 3662	Quesada <i>et al.</i> F5-7			19
<i>Deleya marina</i>	IAM 14107 [†]	ATCC 25374		<i>Pseudomonas marina</i>		4, 10
<i>Deleya marina</i>	IAM 14108	ATCC 35142	Baumann 143			4
<i>Deleya marina</i>	IAM 12928	ATCC 27129	DSM 50416, Baumann 140	<i>Pseudomonas marina</i>		4, 10
<i>Deleya pacifica</i>	IAM 12553 [†]	ATCC 27122	NCIMB 1972, Baumann 62	<i>Alcaligenes pacificus</i>		3, 4
<i>Deleya venusta</i>	IAM 12554 [†]	ATCC 27125	NCIMB 1979, Baumann 86	<i>Alcaligenes venustus</i>		3, 4
<i>Deleya venusta</i>	IAM 14110	ATCC 35137	Baumann 84			4
<i>Deleya venusta</i>	IAM 14111	ATCC 35138	Baumann 89			4
<i>Deleya venusta</i>	IAM 14112	ATCC 35139	Baumann 94			4
<i>Shewanella hanedai</i>	IAM 12641 [†]	ATCC 33224	NCIMB 2157, Baumann 281	<i>Ateromonas hanedai</i>		16, 17, 22
<i>Shewanella putrefaciens</i>	IAM 12079 [†]	IFO 3908	ATCC 8071, NCIMB 10471			16, 17, 18, 22
<i>Shewanella putrefaciens</i>	IAM 1510	ATCC 12099	NCTC 10695	' <i>Pseudomonas rubescens</i> '	I	18
<i>Shewanella putrefaciens</i>	IAM 13595	AJ 1592	NCTC 10737		II	18
<i>Shewanella putrefaciens</i>	IAM 13596	AJ 2258	NCTC 10735, IAM 1477		III	18
<i>Shewanella putrefaciens</i>	IAM 13614	NCTC 10738	NCIMB 11157		IV	18, 22
<i>Shewanella putrefaciens</i>	IAM 13615	NCTC 10762			IV	18
<i>Shewanella putrefaciens</i>	IAM 13616	NCTC 10763			IV	18, 22
<i>Shewanella putrefaciens</i>	IAM 13617	NCTC CL 256/73			III	18, 22
<i>Shewanella putrefaciens</i>	IAM 13618	NCTC CL 549/73			III	18
<i>Shewanella putrefaciens</i>	IAM 13619	NCTC CL 106/76			III	18

* According to Owen *et al.* (1978).

† 1, Akagawa & Yamamoto (1989); 2, Austin *et al.* (1981); 3, Baumann *et al.* (1972); 4, Baumann *et al.* (1983); 5, Baumann *et al.* (1984); 6, Bowditch *et al.* (1984); 7, Chan *et al.* (1978); 8, Coyne *et al.* (1989); 9, Döhler *et al.* (1987); 10, De Vos *et al.* (1989); 11, Gauthier (1976a); 12, Gauthier (1976b); 13, Gauthier (1982); 14, Gauthier (1982); 15, Gauthier & Breittmayer (1979); 16, Jensen *et al.* (1980); 17, MacDonnell & Colwell (1985); 18, Owen *et al.* (1978); 19, Quesada *et al.* (1984); 20, Quimley & Colwell (1968); 21, Reichelt & Baumann (1973); 22, Van Landschoot & De Ley (1983); 23, Yaphe (1957); 24, ZoBell & Upham (1944).

In a previous study (Baumann *et al.*, 1972), four Gram-negative, aerobic species which possessed many features in common with marine *Pseudomonas* were assigned to the genus *Alcaligenes*, largely on the basis of peritrichous flagellation. Subsequent studies involving molecular techniques (Kerstens & De Ley, 1980), and comparative biochemical studies of the regulatory mechanism of aspartokinase (Baumann & Baumann, 1974) and the catabolic pathway for D-fructose and D-glucose (Sawyer *et al.*, 1977) revealed these organisms to be quite different from the genus *Alcaligenes*, and as such they were reassigned to the newly created genus *Deleya* (Baumann *et al.*, 1983). The genus also contains a species originally assigned to the genus *Pseudomonas*, *Deleya marina*, which unlike the other species in this genus has polar flagella and was included largely on the basis of the phylogenetic implications of aspartokinase regulation (Baumann *et al.*, 1983).

The genera discussed above have been created on the basis of advanced molecular and biochemical information; although there are phenotypic differences between these organisms, they are few in number. Therefore, there is a pressing need to uncover new phenotypic characteristics.

Chemotaxonomic analyses have proved of value in the characterization of a wide variety of prokaryotes (Goodfellow & Minnikin, 1985). These characteristics are of increasing importance in the delineation of prokaryotic taxa (Murray *et al.*, 1990); for example, they are employed in the descriptions of *Aminobacter* (Urakami *et al.*, 1992), *Sphingobacterium antarcticus* (Shivaji *et al.*, 1992) and *Brachybacterium nesterenkovi* (Gvozdyak *et al.*, 1992). The value of isoprenoid quinone analyses has been discussed by Collins & Jones (1981). In this study, the isoprenoid quinone composition of 51 strains of Gram-negative, aerobic, marine bacteria was analysed, and the results presented and discussed.

Methods

Organisms and growth condition. Strains, listed in Table 1, were grown in modified marine broth: peptone, 5.0 g (Kyokuto Pharmaceutical Industrial Co., Tokyo, Japan), yeast extract, 1.0 g (Oriental Yeast Co., Tokyo, Japan), 750 ml of natural seawater and 250 ml of distilled water. The final pH was adjusted to pH 7.4. Cultures were incubated for up to 15 h at 25 °C with shaking at 120 r.p.m. Biomass was harvested and lyophilized at early stationary growth phase as described previously (Akagawa & Yamasato, 1989).

Respiratory quinone analysis. Quinones were extracted with chloroform/methanol (2:1, v/v) from lyophilized cells and purified by preparative thin-layer chromatography on Merck Kieselgel 60F₂₅₄ (20 × 20 cm, 0.25 mm thickness) using benzene as the developing solvent. Quinone spots were detected by UV irradiation. Spots were scraped from the TLC plates and recovered in acetone. High-

performance liquid chromatography was used to determine the isoprenoid quinone composition. The apparatus comprised a Shimadzu liquid chromatograph LC-6A and a spectrophotometric detector SPD-2AM (Shimadzu) fitted with a reverse-phase type Cosmosil 5C₁₈ column (4.6 mm i.d. × 150 mm, Nacalai Tesque Inc., Kyoto, Japan). The mobile phase used was a mixture of methanol and isopropyl ether (3:1, v/v), as described by Tamaoka *et al.* (1983). Ubiquinones and menaquinones were detected by monitoring at 275 nm and 270 nm respectively. Isoprenoid quinones were identified by comparison with known standards: phyloquinone, which was purchased from Wako Pure Chemical Industries (Osaka, Japan), and Q-6, Q-7, Q-8, Q-9, Q-10, MK-6, MK-7, MK-8, MK-9 and MK-10, which were a gift from S. Yamada (Tokyo Res. Lab., Eisai Co., Tokyo, Japan). The relative molar ratios of quinone homologues were determined by reference to the standard mixtures containing equimolar amounts of phyloquinone and Q-10.

Chemicals. All organic solvents were of analytical grade, with the exception of methanol which was of chromatographic grade (Nacalai Tesque Inc.).

Results and Discussion

The quinone compositions as determined by HPLC are shown in Table 2. Ubiquinones were the only type of isoprenoid quinones found in all strains, with the exception of members of the genus *Shewanella*.

Sixteen *Alteromonas* strains and two *Marinomonas* strains contained Q-8 as the most abundant component (84–98%) accompanied by small amounts of Q-6, Q-7, Q-9 and Q-10. The genera *Alteromonas* and *Marinomonas*, which are not phylogenetically related, are almost indistinguishable using phenotypic characteristics (Van Landschoot & De Ley, 1983). In this study, members of the genera could not be separated by quinone analysis (Table 2). Ubiquinone-8 has been found in species of *Xanthomonas*, *Hydrogenophaga*, *Alcaligenes*, *Bordetella*, *Comamonas*, *Variovorax*, *Frateuria* and *Moraxella* (Yamada *et al.*, 1982; Fletcher *et al.*, 1987; Yamada *et al.*, 1969; Nishimura *et al.*, 1986), and as such is widely distributed in the rRNA superfamilies I, II and III that correspond to the β and γ subdivisions in the class *Proteobacteria*. The lack of variation between these genera is therefore not surprising. However, *Alteromonas* and *Marinomonas*, which have often been confused with *Pseudomonas*, were clearly distinguished by isoprenoid quinone analysis. As reported previously (Akagawa & Yamasato, 1989), all strains of the genus *Deleya* contained Q-9 as the most abundant quinone (90–98%), accompanied by small amounts of Q-6, Q-7, Q-8 and Q-10. *Deleya*, which is the only taxon available for Gram-negative, aerobic, marine, peritrichous species, includes one polarly flagellated species, *Deleya marina* (Baumann *et al.*, 1983). This species was concluded to be a member of the genus *Deleya* from a comparative study of the metabolic pathways of D-fructose (Sawyer *et al.*, 1977) and immunological comparisons of certain

Table 2. Isoprenoid quinone composition of the tested strains

Isoprenoid quinones were abbreviated as follows: Q-8, ubiquinone with eight isoprene units; MK-6, Menaquinone with six isoprene units; MMK-7, methylmenaquinone with seven isoprene units. Quinone compositions are presented as percentages. Where two types of quinone have been detected, values appearing outside parentheses are the percentages of ubiquinones or menaquinones alone, and values appearing within parentheses are the percentages of the total quinone profile.

Organism* MMK-8	Quinone composition (%)								
	Q-6	Q-7	Q-8	Q-9	Q-10	MK-6	MK-7	MK-8	MMK-7
<i>Alteromonas aurantia</i> IAM 12912	9	1	87	1					
<i>Alteromonas citrea</i> IAM 12644 ^T	5	1	88	4					
<i>Alteromonas citrea</i> IAM 12913	1	1	96	2					
<i>Alteromonas espejiana</i> IAM 12640 ^T		1	98	1					
<i>Alteromonas haloplanktis</i> IAM 12915 ^T		2	97	1	1				
<i>Alteromonas haloplanktis</i> IAM 12916		2	98						
<i>Alteromonas haloplanktis</i> IAM 12917		1	84	15					
<i>Alteromonas haloplanktis</i> IAM 12918	2	1	95	2					
<i>Alteromonas haloplanktis</i> IAM 12919		1	98	1					
<i>Alteromonas luteoviolacea</i> IAM 12924 ^T	2	1	96	1					
<i>Alteromonas luteoviolacea</i> IAM 12925	2	5	88	5					
<i>Alteromonas macleodii</i> IAM 12920 ^T			97	2	1				
<i>Alteromonas rubra</i> IAM 12643 ^T	2	1	96						
<i>Alteromonas undina</i> IAM 12922 ^T		6	94						
<i>Alteromonas nigrifaciens</i> IAM 13019 ^T		1	97	2					
<i>Alteromonas nigrifaciens</i> IAM 13161			97	2					
<i>Marinomonas communis</i> IAM 12914 ^T		4	95	1					
<i>Marinomonas vaga</i> IAM 12923 ^T		8	92						
' <i>Pseudomonas piscicida</i> ' IAM 12933	1	1	97	1					
' <i>Pseudomonas piscicida</i> ' IAM 12934		t	100						
' <i>Pseudomonas atlantica</i> ' IAM 12927 ^T		1	96	2					
' <i>Pseudomonas carrageenovora</i> ' IAM 12662		1	97	1	1				
<i>Pseudomonas nautica</i> IAM 12929 ^T			3	93					
<i>Pseudomonas stutzeri</i> IAM 12931			3	95	2				
' <i>Pseudomonas bathycetes</i> ' IAM 13162			16	84					
' <i>Pseudomonas bathycetes</i> ' IAM 13163			2	98					
' <i>Pseudomonas halosaccharolytica</i> ' IAM 12642			1	98					
<i>Deleya aquamarina</i> IAM 12550 ^T			10	90					
<i>Deleya aquamarina</i> IAM 12551			8	92					
<i>Deleya aquamarina</i> IAM 12645			4	95					
<i>Deleya cupida</i> IAM 12552 ^T			2	97					
<i>Deleya halophila</i> IAM 13009 ^T			2	97	1				
<i>Deleya marina</i> IAM 14107 ^T			9	90					
<i>Deleya marina</i> IAM 14108			8	91					
<i>Deleya marina</i> IAM 12928			9	91					
<i>Deleya pacifica</i> IAM 12553 ^T			4	95					
<i>Deleya venusta</i> IAM 12554 ^T			2	97					
<i>Deleya venusta</i> IAM 14110			3	96					
<i>Deleya venusta</i> IAM 14111			2	98					
<i>Deleya venusta</i> IAM 14112			2	98					
<i>Shewanella hanedai</i> IAM 12641 ^T	t(t)	37(35)	61(58)	1(1)			100(6)		
<i>Shewanella putrefaciens</i> IAM 12079 ^T	2(1)	24(11)	74(34)				95(44)	2(2)	2(6)
<i>Shewanella putrefaciens</i> IAM 1510	1(t)	34(15)	64(28)				96(52)	2(1)	2(1)
<i>Shewanella putrefaciens</i> IAM 13595	1(1)	23(16)	76(54)				77(22)		21(6)
<i>Shewanella putrefaciens</i> IAM 13596		46	52	2					
<i>Shewanella putrefaciens</i> IAM 13614	3(2)	48(31)	49(32)				89(31)	3(1)	9(3)
<i>Shewanella putrefaciens</i> IAM 13615	3(2)	43(28)	53(35)				88(31)		11(4)
<i>Shewanella putrefaciens</i> IAM 13616	2(1)	37(21)	61(35)				85(36)	5(2)	9(4)
<i>Shewanella putrefaciens</i> IAM 13617	1(1)	55(51)	43(40)				83(5)		17(1)
<i>Shewanella putrefaciens</i> IAM 13618	3(2)	58(47)	39(32)			1	94(18)		5(1)
<i>Shewanella putrefaciens</i> IAM 13619	1(1)	55(40)	43(31)				91(25)		7(2)

* t, Type strain.

enzymes (DeLong *et al.*, 1984). The ubiquinone profile of *Deleya marina* shown in Table 2 is the same as those of other *Deleya* species that are peritrichously flagellated. The result is not contrary to the assignment of this species to genus *Deleya*. The quinone profile of *Deleya* species is similar to that of strains from the genus *Halomonas* (Franzmann & Tindall, 1990). One of the most important taxonomic problems in the *Halomonadaceae* is the differentiation of *Deleya* and *Halomonas* (Franzmann & Tindall, 1990). The results presented here highlight the phenotypic similarity of these two genera. Further differential chemotaxonomic characterization and systematic studies on *Deleya*, marine *Pseudomonas* and *Halomonas* species, including a larger number of isolates, are necessary.

Two marine *Pseudomonas* strains, *Pseudomonas nautica* IAM 12929^T and *Pseudomonas stutzeri* IAM 12931 (formerly *Pseudomonas perfectomarina*) contained Q-9 as the most abundant quinone (93% and 95%, respectively) with small amounts of Q-8 and Q-10; as such they show similarities to some species of the *Halomonadaceae* and members of the genus *Pseudomonas sensu stricto*, i.e. rRNA homology group I (Franzmann & Tindall, 1990; Yamada *et al.*, 1982; Oyaizu & Komagata, 1983). Marine *Pseudomonas* species are heterogeneous and none of them belong to *Pseudomonas sensu stricto*. *Pseudomonas nautica* has been assigned to the rRNA superfamily II by rRNA-DNA hybridization (De Vos *et al.*, 1989), with no close relatives. However, the quinone profile for this strain is the same as those of authentic *Pseudomonas* (Yamada *et al.*, 1982; Oyaizu & Komagata, 1983). *Pseudomonas stutzeri* IAM 12931 was previously *P. perfectomarina* and was reassigned to this species on the basis of DNA-DNA hybridization studies (Döhler *et al.*, 1987). Recently, Rossello *et al.* (1991) reported that *Pseudomonas stutzeri* contains several genomic groups and clearly, further investigations of this group are required. One terrestrial strain of *Pseudomonas stutzeri* IAM 1054 was reported to contain Q-9 (Yamada *et al.*, 1982), in agreement with results for the marine strain studied here. The genomic heterogeneity uncovered by Rossello *et al.* (1991) was not confirmed here by isoprenoid quinone analysis.

Four misnamed *Pseudomonas* strains, '*Pseudomonas piscicida*' IAM 12933 and IAM 12934, '*Pseudomonas atlantica*' IAM 12927 and '*Pseudomonas carrageenovora*' IAM 12662 all contained Q-8 as the most abundant component and showed the same ubiquinone profiles as those of *Alteromonas* and *Marinomonas*. These four strains should be reassigned to the genera *Alteromonas* or *Marinomonas*, in agreement with the findings of De Vos *et al.* (1989). The latter reported that '*Pseudomonas atlantica*' IAM 12927 and '*Pseudomonas carrageenovora*' IAM 12662 are not *Pseudomonas* and belong to the

Alteromonas haloplanktis rRNA branch on the basis of rRNA-DNA hybridization studies; the results presented here agree with their findings. Two strains of '*Pseudomonas bathycetes*' and '*Pseudomonas halosaccharolytica*' IAM 12462 were found to contain Q-9 as the major ubiquinone. These results demonstrate that '*Pseudomonas bathycetes*' exhibits a profile similar to those of *Deleya* strains and supports the finding that '*Pseudomonas bathycetes*' should be assigned to the genus *Deleya* or its relatives, as presented by De Vos *et al.* (1989). The quinone profile of '*Pseudomonas halosaccharolytica*' IAM 12462 agreed with those of authentic *Pseudomonas* species (Yamada *et al.*, 1982; Oyaizu & Komagata, 1983); however, without further study it is difficult to determine the true identity of this strain.

As shown in Table 2, *Shewanella* strains contained both ubiquinones and menaquinones; in addition, methylmenaquinones were detected in most *Shewanella putrefaciens* strains with the exception of strain IAM 13596. The presence of both ubiquinones and menaquinones is a common feature of members of the family *Enterobacteriaceae* but has not been observed in any aerobic Gram-negative rod with the exception of *Shewanella putrefaciens* (Collins & Jones, 1981; Moule & Wilkinson, 1987). This may be an important differential characteristic among aerobic, motile marine bacteria. Further investigation of the quinone profiles of other *Shewanella* species, i.e. *S. colwelliana*, *S. benthica* and *S. alga*, is required. Amongst the strains of *S. putrefaciens* studied here, the quinones Q-7 and Q-8 were predominant components, followed by MK-7 and, with the exception of IAM 13596, MMK-7 was generally the fourth largest component. The presence of MMK in some strains of *Shewanella putrefaciens* has already been reported by Itoh *et al.* (1985) and Moule & Wilkinson (1987); however, its taxonomic importance has not yet been recognized. *Shewanella* is heterogeneous with respect to DNA homology (Owen *et al.*, 1978) and has been assigned to four homology groups. In the present study, we studied *Shewanella putrefaciens* strains from all four DNA-DNA homology groups presented by Owen *et al.* (1978), as indicated in Table 1. Owen's homology group I strains, IAM 12079^T and IAM 1510, contained a larger amount of MK-7, 44–52% of the total quinones, as compared to the other strains, in which MK-7 did not exceed 36% of the total isoprenoid quinone content. In the homology group II strains, IAM 13595 and IAM 13596, the most abundant quinone was Q-8 followed by Q-7; in the homology group III strains, IAM 13617, IAM 13618 and IAM 13619, the most abundant quinone was Q-7 followed by Q-8. The homology group IV strains, IAM 13614, IAM 13615 and IAM 13616, contained almost equal amounts of Q-7 and Q-8. These differences in quinone profile between the four homology groups

may indicate a subspecific structure in agreement with the work of Owen *et al.* (1978). The presence of MMKs in *Shewanella putrefaciens* may be an important characteristic in delineating this species; further studies are necessary.

In conclusion, determination of isoprenoid quinone profiles offers simple and clear criteria to distinguish the following three groups: (1) *Alteromonas* and *Marinomonas*; (2) *Shewanella*; and (3) marine *Pseudomonas*, *Deleya* and *Halomonas*. Its usefulness in primary grouping of natural isolates of aerobic, Gram-negative, motile, marine bacteria has been proved.

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References

- AKAGAWA, M. & YAMASATO, K. (1989). Synonymy of *Alcaligenes aquamarinus*, *Alcaligenes faecalis* subsp. *homari*, and *Deleya aesta*: *Deleya aquamarina* comb. nov. as the type species of the genus *Deleya*. *International Journal of Systematic Bacteriology* **39**, 462–466.
- AKAGAWA-MATSUSHITA, M., YAMADA, Y. & YAMASATO, K. (1991). Characterization and identification of a restriction enzyme producing marine bacterium *Deleya marina* IAM 14114. *Bulletin of the Japan Federation for Culture Collections* **7**, 28–32.
- AUSTIN, B., RODGERS, C. R., FORNS, J. M. & COLWELL, R. R. (1981). *Alcaligenes faecalis* subsp. *homari* subsp. nov., a new group of bacteria isolated from moribund lobsters. *International Journal of Systematic Bacteriology* **31**, 72–76.
- BAUMANN, L. & BAUMANN, P. (1974). Regulation of aspartokinase activity in non-fermentative, marine eubacteria. *Archives of Microbiology* **95**, 1–18.
- BAUMANN, L., BAUMANN, P., MANDEL, M. & ALLEN, R. D. (1972). Taxonomy of aerobic marine eubacteria. *Journal of Bacteriology* **110**, 402–429.
- BAUMANN, L., BOWDITCH, R. D. & BAUMANN, P. (1983). Description of *Deleya* gen. nov. created to accommodate the marine species *Alcaligenes aestus*, *A. pacificus*, *A. cupidus*, *A. venustus*, and *Pseudomonas marina*. *International Journal of Systematic Bacteriology* **33**, 793–802.
- BAUMANN, P., BAUMANN, L., BOWDITCH, R. D. & BEAMAN, B. (1984). Taxonomy of *Alteromonas*: *A. nigrifaciens* sp. nov., nom. rev.; *A. macleodii*; and *A. haloplanktis*. *International Journal of Systematic Bacteriology* **34**, 145–149.
- BOWDITCH, R. D., BAUMANN, L. & BAUMANN, P. (1984). Description of *Oceanospirillum kriegii* sp. nov. and *O. jannaschii* sp. nov. and assignment of two species of *Alteromonas* to this genus as *O. commune* comb. nov. and *O. vagum* comb. nov. *Current Microbiology* **10**, 221–230.
- CHAN, K. Y., BAUMANN, L., GARZA, M. M. & BAUMANN, P. (1978). Two new species of *Alteromonas*: *Alteromonas espejiana* and *Alteromonas undina*. *International Journal of Systematic Bacteriology* **28**, 217–222.
- COLLINS, M. D. & JONES, D. (1981). Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. *Microbiological Reviews* **45**, 316–354.
- COYNE, V. E., PILLIDGE, C. J., SLEDJESKI, D. D., HORI, H., ORTIZ-CONDE, B. A., MUIR, D. G., WEINER, R. M. & COLWELL, R. R. (1989). Reclassification of *Alteromonas colwelliana* to the genus *Shewanella* by DNA–DNA hybridization, serology and 5S ribosomal RNA sequence data. *Systematic and Applied Microbiology* **12**, 275–279.
- DELONG, E. F., BAUMANN, L., BOWDITCH, R. D. & BAUMANN, P. (1984). Evolutionary relationships of superoxide dismutases and glutamine synthetases from marine species of *Alteromonas*, *Oceanospirillum*, *Pseudomonas* and *Deleya*. *Archives of Microbiology* **138**, 170–178.
- DE VOS, P., VAN LANDSCHOOT, A., SEGERS, P., TYTGAT, R., GILLIS, M., BAUWENS, M., ROSSAU, R., POT, B., KERSTERS, K., LIZZARAGA, P. & DE LEY, J. (1989). Genotypic relationships and taxonomic localization of unclassified *Pseudomonas*-like strains by deoxyribonucleic acid: ribosomal ribonucleic acid hybridizations. *International Journal of Systematic Bacteriology* **39**, 35–49.
- DÖHLER, K., HUSS, V. A. R. & ZUMFT, W. G. (1987). Transfer of *Pseudomonas perfectomarina* Baumann, Bowditch, Baumann, and Beaman 1983 to *Pseudomonas stutzeri* (Lehmann and Neumann 1896) Sijderius 1946. *International Journal of Systematic Bacteriology* **37**, 1–3.
- FLETCHER, M. T., BLACKALL, P. J. & DOHENY, C. M. (1987). A note on the isoprenoid quinone content of *Bordetella avium* and related species. *Journal of Applied Bacteriology* **62**, 275–278.
- FRANZMANN, P. D. & TINDALL, B. J. (1990). A chemotaxonomic study of members of the family *Halomonadaceae*. *Systematic and Applied Microbiology* **13**, 142–147.
- GAUTHIER, M. J. (1976a). Morphological, physiological, and biochemical characteristics of some violet-pigmented bacteria isolated from seawater. *Canadian Journal of Microbiology* **22**, 138–149.
- GAUTHIER, M. J. (1976b). *Alteromonas rubra* sp. nov., a new marine antibiotic-producing bacterium. *International Journal of Systematic Bacteriology* **26**, 459–466.
- GAUTHIER, M. J. (1977). *Alteromonas citrea*, a new Gram-negative, yellow-pigmented species from seawater. *International Journal of Systematic Bacteriology* **27**, 349–354.
- GAUTHIER, M. J. (1982). Validation of the name *Alteromonas luteoviolacea*. *International Journal of Systematic Bacteriology* **32**, 82–86.
- GAUTHIER, M. J. & BREITMAYER, V. A. (1979). A new antibiotic-producing bacterium from seawater: *Alteromonas aurantia* sp. nov. *International Journal of Systematic Bacteriology* **29**, 366–372.
- GOODFELLOW, M. & MINNIKIN, D. E. (1985). Introduction to chemosystematics. In *Chemical Methods in Bacterial Systematics*, pp. 1–16. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- GVOZDYAK, O. R., NOGINA, T. M. & SCHUMANN, P. (1992). Taxonomic study of the genus *Brachybacterium*: *Brachybacterium nesterenkovi* sp. nov. *International Journal of Systematic Bacteriology* **42**, 74–78.
- HAYASHIDA, S., TANAKA, S., TERAMOTO, Y., NANRI, N., YOSHINO, S. & FURUKAWA, K. (1991). Isolation of anti-algal *Pseudomonas stutzeri* strains and their lethal activity for *Chattonella antiqua*. *Agricultural and Biological Chemistry* **55**, 787–790.
- ITOH, T., FUNABASHI, H., KATAYAMA-FUJIMURA, Y., IWASAKI, S. & KURAISHI, H. (1985). Structure of methylmenaquinone-7 isolated from *Alteromonas putrefaciens* IAM 12079. *Biochimica et Biophysica Acta* **840**, 51–55.
- JENSEN, M. J., TEBO, B. M., BAUMANN, P., MANDEL, M. & NEALSON, K. H. (1980). Characterization of *Alteromonas hanedai* (sp. nov.), a nonfermentative luminous species of marine origin. *Current Microbiology* **3**, 311–315.
- KERSTERS, K. & DE LEY, J. (1980). Classification and identification of bacteria by electrophoresis of their proteins. In *Microbiological Classification and Identification*, pp. 273–297. Edited by M. Goodfellow & A. G. Board. London: Academic Press.
- MACDONELL, M. T. & COLWELL, A. R. (1985). Phylogeny of the *Vibrionaceae*, and recommendation for two new genera, *Listonella* and *Shewanella*. *Systematic and Applied Microbiology* **6**, 171–182.
- MOULE, A. L. & WILKINSON, S. G. (1987). Polar lipids, fatty acids, and isoprenoid quinones of *Alteromonas putrefaciens* (*Shewanella putrefaciens*). *Systematic and Applied Microbiology* **9**, 192–198.
- MURRAY, R. G. E., BRENNER, D. J., COLWELL, R. R., DE VOS, P., GOODFELLOW, M., GRIMONT, P. A. D., PFENNIG, N., STACKEBRANDT, E. & ZAVARZIN, G. A. (1990). Report of the Ad Hoc committee on approaches to taxonomy within the *Proteobacteria*. *International Journal of Systematic Bacteriology* **40**, 213–215.
- NISHIMURA, Y., KANBE, K. & IZUKA, H. (1986). Taxonomic studies of aerobic coccobacilli from seawater. *Journal of General and Applied Microbiology* **32**, 1–11.

- OWEN, R. J., LEGROS, R. M. & LAPAGE, S. P. (1978). Base composition, size and sequence similarities of genome deoxyribonucleic acids from clinical isolates of *Pseudomonas putrefaciens*. *Journal of General Microbiology* **104**, 127–138.
- OYAZU, H. & KOMAGATA, K. (1983). Grouping of *Pseudomonas* species on the basis of cellular fatty acid composition and the quinone system with special reference to the existence of 3-hydroxy fatty acids. *Journal of General and Applied Microbiology* **29**, 17–40.
- QUESADA, E., VENTOSA, A., RUIZ-BERRAQUERO, F. & RAMOS-CORMENZANA, A. (1984). *Deleya halophila*, a new species of moderately halophilic bacteria. *International Journal of Systematic Bacteriology* **34**, 287–292.
- QUINGLEY, M. M. & COLWELL, R. R. (1968). Proposal of a new species *Pseudomonas bathycetes*. *International Journal of Systematic Bacteriology* **18**, 241–252.
- REICHEL, J. L. & BAUMANN, P. (1973). Change of the name *Alteromonas marinopraescens* (ZoBell and Upham) Baumann *et al.* to *Alteromonas haloplanktis* (ZoBell and Upham) comb. nov. and assignment of strain ATCC 23821 (*Pseudomonas enalia*) and strain c-A1 of De Voe and Oginsky to this species. *International Journal of Systematic Bacteriology* **23**, 438–441.
- ROSSELLO, R., GARCIA-VALDES, E., LALUCAT, J. & URSING, J. (1991). Genotypic and phenotypic diversity of *Pseudomonas stutzeri*. *Systematic and Applied Microbiology* **14**, 150–157.
- SAWYER, M. H., BAUMANN, P. & BAUMANN, L. (1977). Pathways of D-fructose and D-glucose catabolism in marine species of *Alcaligenes*, *Pseudomonas marina*, and *Alteromonas communis*. *Archives of Microbiology* **112**, 169–172.
- SHIVAJI, S., RAY, M. K., RAO, N. S., SAISREE, L., JAGANNADHAM, M. V., KUMER, G. S., REDDY, G. S. N. & BHARGAVA, P. M. (1992). *Sphingobacterium antarcticus* sp. nov., a psychrotrophic bacterium from the soils of Schirmacher Oasis, Antarctica. *International Journal of Systematic Bacteriology* **42**, 102–106.
- TAMAOKA, J., KATAYAMA-FUJIMURA, Y. & KURAISHI, H. (1983). Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. *Journal of Applied Bacteriology* **54**, 31–36.
- URAKAMI, T., ARAKI, H., OYANAGI, H., SUZUKI, K. & KOMAGATA, K. (1992). Transfer of *Pseudomonas aminovorans* (den Dooren de Jong 1926) to *Aminobacter* gen. nov. as *Aminobacter aminovorans* comb. nov. and description of *Aminobacter aganoensis* sp. nov. and *Aminobacter niigataensis* sp. nov. *International Journal of Systematic Bacteriology* **42**, 84–92.
- VAN LANDSCHOOT, A. & DE LEY, J. (1983). Intra- and intergeneric similarities of the rRNA cistrons of *Alteromonas*, *Marinomonas* (gen. nov.) and some other Gram-negative bacteria. *Journal of General Microbiology* **129**, 3057–3074.
- YAMADA, Y., AIDA, K. & UEMURA, T. (1969). Enzymatic studies on the oxidation of sugar and sugar alcohol. V. Ubiquinone of acetic acid bacteria and its relation to classification of genera *Gluconobacter* and *Acetobacter*, especially of the so-called intermediate strains. *Journal of General and Applied Microbiology* **15**, 181–196.
- YAMADA, Y., TAKINAMI-NAKAMURA, H., TAHARA, Y., OYAZU, H. & KOMAGATA, K. (1982). The ubiquinone systems in the strains of *Pseudomonas* species. *Journal of General and Applied Microbiology* **28**, 7–12.
- YAPHE, W. (1957). The use of agarase from *Pseudomonas atlantica* in the identification of agar in marine algae (*Rhodophyceae*). *Canadian Journal of Microbiology* **3**, 987–993.
- YAPHE, W. & MORGAN, K. (1959). Enzymic hydrolysis of fucoidin by *Pseudomonas atlantica* and *Pseudomonas carrageenovora*. *Nature, London* **183**, 761–762.
- YAZAWA, K., ARAKI, K., OKAZAKI, N., WATANABE, K., ISHIKAWA, C., INOUE, A. & KONDO, K. (1988). Production of eicosapentaenoic acid by marine bacteria. *Journal of Biochemistry* **103**, 5–7.
- ZOBELL, C. E. & UPHAM, H. C. (1944). A list of marine bacteria including descriptions of sixty new species. *Bulletin of the Scripps Institution of Oceanography of the University of California* **5**, 239–292.