Saccharospirillum impatiens gen. nov., sp. nov., a novel γ-*Proteobacterium* isolated from hypersaline Ekho Lake (East Antarctica)

Matthias Labrenz,[1]† Paul A. Lawson,[2] Brian J. Tindall,[3] Matthew D. Collins[2] and Peter Hirsch[1]

[1]Institut für Allgemeine Mikrobiologie, Christian-Albrechts-Universität, Kiel, Germany

[2]School of Food Biosciences, University of Reading, UK

[3]DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany

Correspondence

Matthias Labrenz

mla@gbf.de

Five Gram-negative, motile, aerobic to microaerophilic spirilla were isolated from various depths of the hypersaline, heliothermal and meromictic Ekho Lake (East Antarctica). The strains are oxidase- and catalase-positive, metabolize a variety of sugars and carboxylic acids and have an absolute requirement for sodium ions. The predominant fatty acids of the organisms are $C_{16:1}\omega7c$, $C_{16:0}$ and $C_{18:1}\omega7c$, with $C_{10:1}$ 3-OH, $C_{10:0}$ 3-OH, $C_{12:0}$ 3-OH, $C_{14:1}$ 3-OH, $C_{14:0}$ 3-OH and $C_{19:1}$ present in smaller amounts. The main polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylmonomethylamine. The DNA base composition of the strains is 54–55 mol% G + C. 16S rRNA gene sequence comparisons show that the isolates are related to the genera *Oceanospirillum*, *Pseudospirillum*, *Marinospirillum*, *Halomonas* and *Chromohalobacter* in the γ-*Proteobacteria*. Morphological, physiological and genotypic differences from these previously described genera support the description of a novel genus and species, *Saccharospirillum impatiens* gen. nov., sp. nov. The type strain is EL-105[T] (= DSM 12546[T] = CECT 5721[T]).

The ice-free Vestfold Hills (East Antarctica) abound with hypersaline and meromictic lakes of various sizes, which were previously open to the ocean (Labrenz *et al.*, 1998). As 'marine relicts' (Perriss & Laybourn-Parry, 1997), sea water determined their initial salt concentration and composition and most likely, also the composition of their microbial communities. One such lake is the meromictic, heliothermal and hypersaline Ekho Lake. Two hundred and fifty pure bacterial cultures were isolated from the lake's aerobic zone. Of these, 51 isolates represented the distribution of colony-forming units and cell/colony diversity, and were studied for physiological adaptations to the depth layer from which they were obtained. Several of these isolates have already been described as new genera or species: among these were the α-*Proteobacteria Antarctobacter heliothermus* (Labrenz *et al.*, 1998), *Roseovarius tolerans* (Labrenz *et al.*, 1999), *Staleya guttiformis*

and *Sulfitobacter brevis* (Labrenz *et al.*, 2000), as well as the Gram-positive bacteria *Friedmanniella lacustris*, *Nocardioides aquaticus* (Lawson *et al.*, 2000) and *Nesterenkonia lacusekhoensis* (Collins *et al.*, 2002). It was also shown that, with respect to actively heterotrophic micro-organisms, the aerobic zone of Ekho Lake (0–24 m) appeared to be divided into two different layers (Labrenz & Hirsch, 2001): (i) the 0–3 m layer, with water turnover and extreme Antarctic temperature conditions, supported halotolerant α-*Proteobacteria* as well as representatives of Gram-positive taxa, and (ii) the hypersaline and heliothermally heated layer below (4–24 m), with marine and halophilic γ-*Proteobacteria*. The presence of two *Halomonas* species in Ekho Lake and various other hypersaline lakes of the Vestfold Hills was shown by James *et al.* (1994), who employed immunofluorescence with specific antibodies. The *Halomonas* species represented up to 40 % of the total bacterial numbers in Ekho and Organic Lakes. Of our bacterial Ekho Lake isolates, 35 % were also members of the γ-*Proteobacteria*, mostly related to members of the family *Halomonadaceae* and the genus *Oceanospirillum*. In the present publication, we characterize and describe five bacterial isolates from Ekho Lake that are members of the γ-*Proteobacteria*, which are related to the *Oceanospirillum* group as well as to members of the family *Halomonadaceae*.

Table 1. Original Ekho Lake conditions

Characteristic	EL-105[T]	EL-143	EL-166	EL-176	EL-195
Depth (m)	9	4	16	14	15
Salinity (‰)	72	59	76	76	ND
Temperature (°C)	14·8	12·1	16·4	14·4	ND
pH	8·2	8·3	7·8	8·1	ND

ND, Not determined.

Enrichment and isolation of Ekho Lake strains were performed according to Labrenz et al. (1998). Enrichment conditions followed the characteristics of the original water samples (Table 1). Pure cultures were kept either as serial transfers on slants, or lyophilized, or deep-frozen at −72 °C in the growth medium. Analysis of morphological, physiological and metabolic properties were performed as described previously (Labrenz et al., 1998, 1999, 2000). The aerobic dissimilation of carbon sources was investigated with the GN system (Biolog) and the API 50CH system (bioMérieux), as well as with a minimal medium (Labrenz et al., 1998).

Analysis of fatty acid methyl esters was carried out with 20 mg freeze-dried biomass, employing methods which allowed selective hydrolysis of ester- and amide-linked fatty acids as described previously (Labrenz et al., 2000). Respiratory lipoquinones and polar lipids were extracted from 100 mg freeze-dried material using the two-stage method, and analysed according to Tindall (1990a, b). Diamino acids of cell walls were separated by one-dimensional TLC on cellulose plates, using the solvent system of Rhuland et al. (1955).

DNA G + C contents were analysed according to Mesbah et al. (1989), and dot-blot hybridization experiments were carried out with the DIG DNA Labeling and Detection kit (Boehringer Mannheim), as described previously (Labrenz et al., 1998). DNA probes were prepared from strains EL-105[T] and EL-166; hybridization occurred against chromosomal DNA from the Ekho Lake strains and negative control Roseobacter denitrificans DSM 7001[T]. The stringency values of 70 and 75 % were calculated according to Sambrook et al. (1989).

16S rRNA gene fragments were generated by PCR, using universal primers pA (positions 8–28, Escherichia coli numbering) and pH* (1542–1522). The amplified products were purified by using a QIAquick PCR Purification kit (Qiagen) and sequenced directly using primers to conserved regions of the rRNA. Sequencing was performed using an ABI PRISM Taq DyeDeoxy Terminator Cycle Sequencing kit and a model 373A automatic DNA sequencer (both from Applied Biosystems). To determine the closest relatives of the EL strains, preliminary searches in the EMBL database were performed with the program FASTA (Pearson

& Lipman, 1988). Closely related sequences were retrieved from EMBL and aligned with the newly determined sequences, using the program DNATools (Rasmussen, 1995). Approximately 100 bases at the 5′ end of the resulting multiple sequence alignment were omitted from further analysis, because of alignment uncertainties due to the highly variable region V1, using the program GeneDoc (Nicholas et al., 1997). A phylogenetic tree was reconstructed according to the neighbour-joining method (Saitou & Nei, 1987) with the programs DNATools and TreeView (Page, 1996), and the stability of the groupings was estimated by bootstrap analysis (1000 replications). 16S rRNA gene signature nucleotides, characteristic of the family Halomonadaceae and its relatives, were analysed in ARB (Strunk & Ludwig, 1995).

Five bacterial isolates were obtained from Ekho Lake samples, taken from depths of 4, 9, 14, 15 and 16 m (Table 1). These isolates are referred to as EL-105[T], EL-143, EL-166, EL-176 and EL-195, respectively. All of the isolates were Gram-negative spirilla (Fig. 1a) and were motile, with one or two monopolar flagella (Fig. 1b). Cell size was 0·48–1·0 × 2·0–12·0 μm, with a mean size of
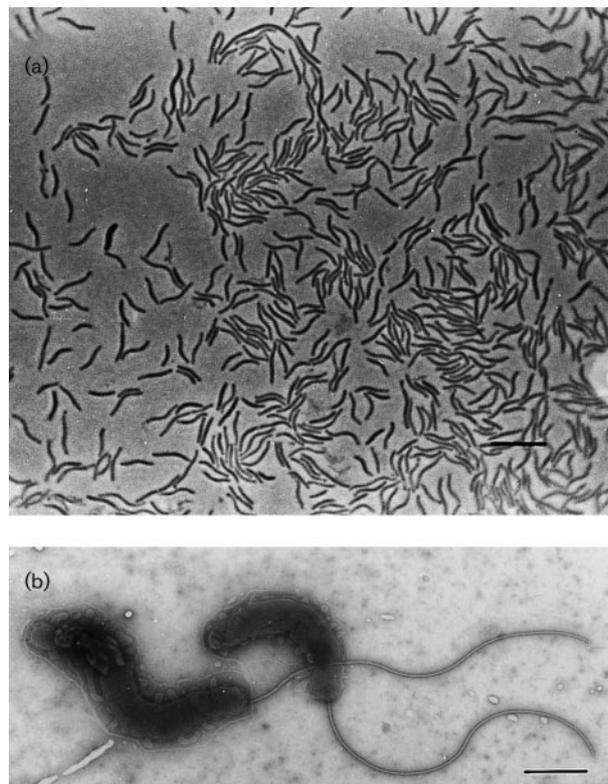
Fig. 1. (a) Phase-contrast light micrograph of strain EL-143 on an agar-coated slide (Pfennig & Wagener, 1986). Bar, 10 μm. (b) Electron micrograph of cells of strain EL-105[T], negatively stained with phosphotungstic acid, showing monopolar flagella. Bar, 1 μm.

0·53–0·87 × 3·79–6·24 μm. In older stages, coccoid bodies were observed.

Aerobic to microaerophilic growth was visible after 3–5 days at 20 °C on PYGV medium with 25‰ artificial sea water (ASW). Colonies were 3–5 mm in diameter, circular with irregular edges, smooth, convex and whitish (EL-105[T], EL-176), whitish-beige (EL-166, EL-195) or whitish-yellow (EL-143). The temperature range for growth was <2·5–43 °C. Optimal growth occurred between 16 and 30 °C at pH values of 5·5–9·5. Optimal pH was 6·5–8·6. The five isolates had an absolute requirement for Na$^+$; the ions K$^+$, Mg^{2+}, Ca^{2+}, Cl$^-$ and SO$_4^{2-}$ could all be replaced by other ions. The osmotolerance ranged from <10 to 150‰ ASW, with an optimum between 10 and 130‰. The NaCl tolerance ranged from <1 to <13 %, with an optimum between 2·0 and 6·0 % NaCl. No anaerobic growth occurred on glucose in the absence of nitrate. The cells did not grow photoautotrophically with H$_2$/CO$_2$ (80:20) or photoorganotrophically with acetate or glutamate. Differences in growth characteristics of the EL strains are available as supplementary data in IJSEM Online.

All five EL strains exhibited peroxidase, catalase and cytochrome oxidase activities. They did not produce acetoin from glucose. They were susceptible to chloramphenicol (30 μg), streptomycin (10 μg), polymyxin B (300 U), penicillin G (10 U) and tetracycline (30 μg). Biotin, nicotinic acid and pantothenate stimulated the growth of some strains. All strains were able to reduce nitrate to nitrite by assimilation, and four anaerobically. Sulfide was produced, but indole was not. All strains had DNase activity and hydrolysed gelatin. Alginate was not hydrolysed. Differences in physiological characteristics of the EL strains are available as supplementary data in IJSEM Online.

The isolates grew with 0·2 % (w/v) pyruvate, malate, succinic, citric and glutamic acids as sole carbon sources. Differences in carbon metabolism between strains are available as supplementary data in IJSEM Online. With the API 50CH system, the following carbon compounds were metabolized: glycerol, ribose, galactose, D-glucose, D-fructose, D-mannose, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, starch, glycogen and D-turanose. The isolates did not metabolize erythritol, D-arabinose, L-arabinose, L-xylose, adonitol, methyl β-D-xyloside, L-sorbose, dulcitol, mannitol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, inulin, melezitose, D-raffinose, xylitol, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconic acid or 2-ketogluconic acid. Differences in metabolism of the EL strains are available as supplementary data in IJSEM Online. In the Biolog system, the isolates metabolized D-fructose, D-galactose, D-melibiose, acetic acid and propionic acid. With the exception of EL-166, all isolates metabolized L-fucose, gentiobiose, α-D-glucose, α-lactose, α-lactose-lactulose, maltose, D-mannose, methyl β-D-glucoside, psicose, D-raffinose, L-rhamnose, sucrose, D-trehalose, xylitol, glucuronamide,

L-asparagine, uridine and thymidine. They did not metabolize α-cyclodextrin, dextrin, glycogen, Tween 40, N-acetylglucosamine, L-arabinose, i-erythritol, methyl pyruvate, monomethyl succinate, cis-aconitic acid, citric acid, D-galactonic acid lactone, D-glucuronic acid, β-hydroxybutyric acid, p-hydroxyphenylacetic acid, itaconic acid, α-oxoglutaric acid, DL-lactic acid, malonic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, L-aspartic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-histidine, L-leucine, L-phenylalanine, L-proline, γ-aminobutyric acid, urocanic acid, putrescine, 2-aminoethanol, glycerol, DL-α-glycerophosphate, glucose 1-phosphate or glucose 6-phosphate. Differences in metabolism are available as supplementary data in IJSEM Online.

The peptidoglycan of all five isolates contained m-diaminopimelic acid. Ubiquinones were the sole respiratory quinones detected, with Q-8 (94–95 %) predominating and Q-9 (4–6 %) present in minor amounts. All strains contained diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylmonomethylamine. Additionally, cells contained 1–3 unidentified amino- and phospholipids, respectively. The fatty acid composition of the strains is shown in comparison to related genera (Table 2). Approximately 25–30 % of the hydroxy fatty acids were released by methods which indicated that they were amide-linked. The ratio of non-polar fatty acids to hydroxy fatty acids was 90:5. The DNA G+C contents of the newly isolated strains were found to be 53·7–55·2 mol%. DNA–DNA hybridization values between EL-105[T] and the other EL-strains were >75 %, which exceeds the value of 70 % normally considered for strains to be members of the same species (Wayne *et al.*, 1987).

To establish the phylogenetic affinities of the isolates, the almost-complete 16S rRNA gene sequences of strains EL-105[T] and EL-166 were determined. Partial 16S rRNA gene sequences of approximately 800 bp were determined for strains EL-143, EL-176 and EL-195. Among the five strains, 100 % sequence similarity was exhibited, thereby demonstrating their genealogical homogeneity. Sequence searches of the EMBL database revealed that the novel organism was related to the γ-subclass of the *Proteobacteria* (data not shown). Pairwise analysis revealed that the novel isolates displayed the highest 16S rRNA gene sequence similarity (88–89 %) to species belonging to the genera *Chromohalobacter*, *Halomonas* and *Oceanospirillum*. However, significant differences exist between the novel organism and members of the family *Halomonadaceae* [which currently includes the genera *Halomonas*, *Chromohalobacter*, *Zymobacter*, and in the future possibly the genus *Carnimonas* (Arahal *et al.*, 2002), Tables 2 and 3]. The *Halomonadaceae* comprise bacteria that produce Q-9 as a major respiratory lipoquinone and C$_{18:1}$, C$_{19:0}$cyc and C$_{16:0}$ as major fatty acids. The major hydroxy fatty acid within the genera *Halomonas* and *Chromohalobacter* is C$_{12:0}$

Table 2. Fatty acid composition of EL-105[T] in comparison with *Marinospirillum minutulum*, *Oceanospirillum* species, *Marinobacterium jannaschii* and *Marinobacter hydrocarbonoclasticus* as well as the type species of related genera

Taxa: 1, EL-105[T]; 2, *M. minutulum* NBRC 15450[T]; 3, *Pseudospirillum japonicum* NBRC 15446[T]; 4, *O. multiglobuliferum* NBRC 13614[T]; 5, *O. maris* NBRC 15468[T]; 6, *O. beijerinckii* NBRC 15445[T]; 7, *O. linum* NBRC 15448[T]; 8, *Oceanobacter kriegii* NBRC 15467[T]; 9, *M. jannaschii* NBRC 15466[T]; 10, *Halomonas elongata* DSM 2581[T]; 11, *Chromohalobacter marismortui* DSM 6770[T]; 12, *Zymobacter palmae* IAM 14233[T]; 13, *Carnimonas nigrificans* CTCBS1[T]; 14, *M. hydrocarbonoclasticus* DSM 8798. Data from this study, Sakane & Yokota (1994), Franzmann & Tindall (1990), Okamoto *et al.* (1993) and Garriga *et al.* (1998).

Fatty acid	1*	2	3	4	5	6	7	8	9	10	11	12	13	14
Non-polar†														
$C_{12:0}$		2	3	3	4	4	3	7	2		3	5		4
$C_{12:1}$			2	4			2	4						
$C_{14:0}$			1	2	2	4	1	1	1			4		2
$C_{14:1}$		4						1						
$C_{15:0}$						1		2				<0·5		
$C_{16:0}$	20 (2·0)	35	25	28	31	32	16	16	19	26	14	53	41	26
$C_{16:1}$	23 (1·9)	26	57	44	47	50	48	36	46	4	27	<0·5	7	
$C_{17:0}$								2				<0·5		
$C_{17}cyc$										5	4			2
$C_{17:1}$								3						
$C_{18:0}$					1			1	1			5		2
$C_{18:1}$	54 (1·3)	32	14	20	11	9	30	27	31	26	29	8	21	38
$C_{19}cyc$										37	15	25	22	
$C_{19:1}$	2 (0·9)													
3-Hydroxy‡														
$C_{10:0}$	12 (1·8)		4	100	100	63	100	19	100			<0·5		
$C_{10:1}$	4 (1·0)													
$C_{12:0}$	7 (1·6)	61	96					54		100	100	>99		100
$C_{12:1}$													80	
$C_{14:0}$	31 (2·5)	3				30								
$C_{14:1}$	44 (6·5)	36												
$C_{16:0}$						6		27					20	

*Standard error in parentheses.
†Numbers refer to the percentage of an acid relative to the total non-polar fatty acids.
‡Numbers refer to the percentage of an acid relative to the total hydroxy fatty acids.

3-OH, the majority of which is ester-linked (about 75 %). It should be noted that members of the genus *Marinobacter* also produce the ubiquinone Q-9 and $C_{12:0}$ 3-OH as the major hydroxy fatty acid, but this hydroxy fatty acid is almost exclusively amide-linked (Spröer *et al.*, 1998). Members of the *Halomonadaceae* have 15 signature characteristics in their 16S rRNA gene sequence, including a very rare cytosine residue at position 486 (Dobson & Franzmann, 1996; Franzmann *et al.*, 1989). Only seven signature characteristics in the 16S rRNA gene sequence of the novel EL strains are shared with the *Halomonadaceae*, excluding the rare cytosine residue at position 486 (Table 3; detailed 16S rDNA signature nucleotide characteristics are available as supplementary data in IJSEM Online). Moreover, the major respiratory lipoquinone of the Antarctic EL strains is Q-8, while the fatty acid $C_{19:0}cyc$ is not produced. Major hydroxy fatty acids are $C_{14:0}$ 3-OH and $C_{14:1}$ 3-OH. These significant differences separate the EL strains from the family *Halomonadaceae*.

A neighbour-joining tree shows the phylogenetic position of the novel bacterium (as exemplified by strain EL-105[T]) amongst the *Proteobacteria*, defined by the family *Halomonadaceae* and the *Oceanospirillum* group (Fig. 2). All associations showing bootstrap resampling values of 90 % or more in the neighbour-joining tree were confirmed by parsimony analysis. Treeing analyses demonstrated that the Ekho Lake strain EL-105[T] formed a distinct line clustering with *Pseudospirillum japonicum*; however, EL-105[T] did not display a particularly close nor statistically significant association (as shown by bootstrap resampling) with any recognized taxon (Fig. 2). Indeed, from sequence divergence (>10 %) and tree topology considerations, the EL bacterium appears to be equivalent in rank to the genera *Halomonas*, *Carnimonas*, *Marinospirillum*, *Pseudospirillum*

Table 3. Differential characteristics of strain EL-105[T] and the genera *Oceanospirillum*, *Marinospirillum*, *Pseudospirillum*, *Carnimonas* and the genera of the family *Halomonadaceae*

Taxa: 1, EL-105[T]; 2, *Oceanospirillum*; 3, *Marinospirillum*; 4, *P. japonicum* ATCC 19191[T]; 5, *Halomonas*; 6, *Chromohalobacter*; 7, *Zymobacter*; 8, *C. nigrificans* CTCBS1[T]. Data from this study, Dobson & Franzmann (1996), Franzmann & Tindall (1990), Garriga *et al.* (1998), Hylemon *et al.* (1973), Krieg (1984), Okamoto *et al.* (1993), Sakane &Yokota (1994), Satomi *et al.* (1998, 2002) and Ventosa *et al.* (1989). +, Positive; −, negative; ND, not determined; D, different between organisms.

Characteristic	1	2	3	4	Family *Halomonadaceae*			8
					5	6	7	
Cell morphology	Spirillum	Spirillum	Spirillum	Curved, straight or S-shaped	Straight or curved rods	Rods	Rods	Straight or slightly curved rods
Motility	+	+	+	+	D	+	+	−
Flagella	Polar	Bipolar	Bipolar	Bipolar	Peritrichous/ polar	Peritrichous	Peritrichous	None
Relation to oxygen	Aerobic/ microaerophilic	Aerobic	Aerobic/ microaerophilic	Aerobic	Aerobic*	Aerobic	Facultatively anaerobic	Aerobic
Growth at 12·75 % (w/v) NaCl	−	−	−	−	+	+	ND	−
Aesculin hydrolysis	+	−	−	−	D	−	ND	+
Utilization of sugars	+	−	−	−	+	+	+	+
Nitrate to nitrite	+	−	D	−	D	D	−	−
Oxidase	+	+	+	+	D	−	−	+
DNA G+C content (mol%)	54·5–54·8	42–50	42·5–45	45	60·5–66·7	62–64·9	55·4–56·2	56±0·3
Major respiratory lipoquinone	Ubiquinone-8	Ubiquinone-8	Ubiquinone-8	Ubiquinone-8	Ubiquinone-9	ND	Ubiquinone-9	Ubiquinone-9
Number of 16S rRNA gene signature characteristics shared with *Halomonadaceae*	7	4†	9‡	3	15	15	15	13

*Some strains are capable of anaerobic growth in the presence of nitrate.

†With respect to *Oceanospirillum linum* ATCC 11336[T]. However, Garriga *et al.* (1998) reported 10 characteristics shared with *Halomonas*.

‡With respect to *Marinospirillum minutulum* NBRC 15450[T].

Saccharospirillum gen. nov.
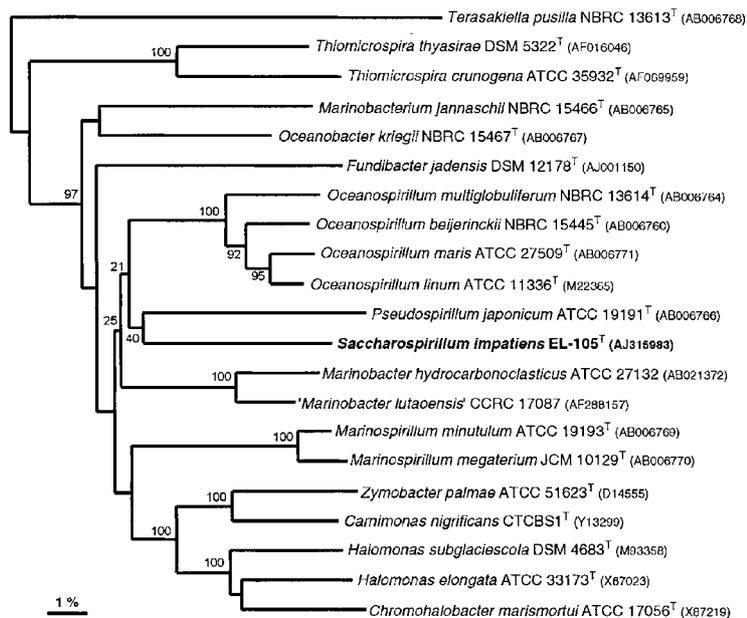
Fig. 2. Unrooted neighbour-joining tree showing phylogenetic relationships of isolate EL-105[T] and closely related *Proteobacteria*. The tree was based on a comparison of approximately 1320 nt. Bootstrap values, expressed as percentages of 1000 replications, are given at branching points. Bar, 1 % sequence divergence.

and *Oceanospirillum*. It is pertinent to note that the phylogenetic separateness of the novel bacterium is strongly supported by phenotypic considerations. According to Holt *et al.* (1994), all helical, halophilic, chemo-organotrophic and aerobic bacteria belong to the genus *Oceanospirillum*. As currently defined, *Oceanospirillum* is based on a pattern or core of phenotypic characteristics that include bipolar flagella tufts or at least flagella; helical cellular shape; a predominance of coccoid bodies in older cultures; an inability to catabolize sugars, starch or casein; formation of poly-β-hydroxybutyrate; a strictly respiratory metabolism with oxygen as the terminal electron acceptor; a negative indole test; a requirement for sea water; an optimum temperature of 30–32 °C; and simple heterotrophic nutrition (Krieg, 1984). However, considerable interspecies diversity of *Oceanospirillum* (Fig. 2) was evident from rRNA–DNA hybridization experiments (Pot *et al.*, 1989), fatty acid analysis (Table 2) and isoprenoid quinone analysis (Sakane & Yokota, 1994), as well as polyamine composition (Hamana *et al.*, 1994). Using chemotaxonomic criteria in concert with phylogenetic evidence, Satomi *et al.* (1998) proposed the transfer of *Oceanospirillum minutulum* to the genus *Marinospirillum*. Based on phylogenetic studies using the 16S rDNA and *gyrB* genes, Satomi *et al.* (2002) have recently rearranged the genus *Oceanospirillum*, with the transfer of *Oceanospirillum japonicum* to the genus *Pseudospirillum*, *Oceanospirillum kriegii* to the genus *Oceanobacter* and *Oceanospirillum pusillum* to the genus *Terasakiella* (as *Terasakiella pusilla*). *Oceanospirillum sensu stricto* was defined to consist of *Oceanospirillum linum*, *Oceanospirillum maris*, *Oceanospirillum beijerinckii* and *Oceanospirillum multiglobuliferum*. Finally, these authors

combined the subspecies of *O. maris* (*O. maris* subsp. *maris*, *O. maris* subsp. *hiroshimense* and *O. maris* subsp. *williamsae*) and *O. beijerinckii* (*O. beijerinckii* subsp. *beijerinckii* and *O. beijerinckii* subsp. *pelagicum*) as *O. maris* and *O. beijerinckii*, respectively. It is also evident from the present 16S rDNA sequence analysis that the former members of the genus *Oceanospirillum* did not constitute a monophyletic group (Fig. 2).

It is also pertinent to note that, by utilizing sugars and forming monopolar flagella, the EL bacterium does not fulfil two of Krieg's typical core characteristics (Krieg, 1984) for the genus *Oceanospirillum* (Table 3), including recently redescribed *Oceanospirillum* species. Additionally, the Ekho Lake strains differ from *Oceanospirillum* in reducing nitrate (Satomi *et al.*, 2002). Major 3-hydroxy fatty acids are $C_{14:1}$ and $C_{14:0}$, instead of $C_{10:0}$ as for *Oceanospirillum*. In fact, with $C_{12:0}$ 3-OH, $C_{14:0}$ 3-OH and $C_{14:1}$ 3-OH, the fatty acid profile of the EL strains seems to be more closely related to that of *Marinospirillum minutulum* than *Oceanospirillum* spp. (Table 2). Using differential hydrolysis of ester- and amide-linked hydroxy fatty acids, it is interesting to note that members of the family *Halomonadaceae* and the novel isolates contain predominantly ester-linked 3-OH fatty acids, whereas *Marinobacter hydrocarbonoclasticus* produces predominantly amide-linked 3-OH fatty acids. Such differences are probably due to structural differences in the lipopolysaccharides, and the application of such methods to other members of this group may prove to be fruitful in elucidating the taxonomic and evolutionary diversity in this group. These differences, together with 16S rRNA considerations, preclude the inclusion of the EL bacterium in the genus *Oceanospirillum*.

From the combination of physiological characteristics, chemotaxonomic and biochemical tests, respiratory lipoquinones, fatty acid profiles, polar lipid data and 16S rRNA gene analyses, it is evident that these strains are a hitherto unknown lineage related to, but separate from, *Oceanospirillum* and members of the *Halomonadaceae*. Therefore, based on both phenotypic and genetic evidence, we propose that the novel EL strains should be classified in a new genus, *Saccharospirillum* gen. nov., as *Saccharospirillum impatiens* sp. nov.

Description of *Saccharospirillum* gen. nov.

Saccharospirillum (Sac.cha.ro.spi.ril′lum. Gr. n. *sakkharos* sugar; Gr. n. *spira* a spiral; N.L. dim. neut. n. *spirillum* a small spiral; N.L. n. *Saccharospirillum* a small spiral that catabolizes sugars).

Gram-negative spirilla, motile by monopolar flagella. Coccoid bodies may be formed in older cultures. The cells contain poly-β-hydroxybutyrate and do not form spores. The temperature range for growth is <2.5 to $43\,^{\circ}$C. The cells have an absolute requirment for Na^+ and grow in the range of <1.0 to $15.0\,\%$ (w/v) NaCl. They grow in the presence of <10 to $150\,$‰ ASW. The pH tolerance range is >5.5 to <9.5. Aerobic to microaerophilic heterotrophs; grow on various sugars and carboxylic acids. No anaerobic growth occurs on glucose in the absence of nitrate. They do not grow photoautotrophically with H_2/CO_2 (80 : 20) or photoorganotrophically with acetate or glutamate. The cells exhibit peroxidase, catalase and cytochrome oxidase activities. The peptidoglycan contains meso-diaminopimelic acid. Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylmonomethylamine are present. Predominant cellular fatty acids are $C_{16:1}\omega7c$, $C_{16:0}$ and $C_{18:1}\omega7c$, with $C_{10:1}$ 3-OH, $C_{10:0}$ 3-OH, $C_{12:0}$ 3-OH, $C_{14:1}$ 3-OH, $C_{14:0}$ 3-OH and $C_{19:1}$ present in smaller amounts. The major respiratory quinone is Q-8. The type species of the genus, *Saccharospirillum impatiens*, was isolated from water samples from Ekho Lake, Antarctica (Vestfold Hills).

Description of *Saccharospirillum impatiens* sp. nov.

Saccharospirillum impatiens [im.pa′ti.ens. L. adj. *impatiens* unable to tolerate (antibiotics)].

Cell size is 0.48–1.0×2.0–12.0 μm, with a mean size of 0.53–0.87×3.79–6.24 μm. Colonies on PYGV agar with ASW are circular with irregular edges, smooth, convex and whitish, whitish-beige or whitish-yellow. Optimal growth occurs between 16 and $33\,^{\circ}$C with concentrations of 2.0–$6.0\,\%$ NaCl or 10–$130\,$‰ ASW. The optimum pH is 6.5–8.6. Biotin and nicotinic acid stimulate growth. The cells are susceptible to chloramphenicol, streptomycin, penicillin G, tetracycline and polymyxin B. DNA and gelatin are hydrolysed. Tween 80 and starch are variably hydrolysed. Alginate is not hydrolysed. Growth occurs on pyruvate, succinic acid, malate, citric acid, glutamic acid, D-propionic acid, glycerol, ribose, galactose, D-fructose, D-mannose, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, glycogen and D-turanose. Nitrate is reduced to nitrite. H_2S is produced, but indole is not. The DNA G + C content is 53.7–55.2 mol%. Chemotaxonomic properties and other characteristics are as given for the genus.

The type strain of *Saccharospirillum impatiens* is EL-105[T] = DSM 12546[T] = CECT 5721[T]. Reference strains are EL-166 (= DSM 12548), EL-143, EL-176 and EL-195.

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