NOTE

***Sphingopyxis witflariensis* sp. nov., isolated from activated sludge**

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Classification of strain W-50^T, which was isolated from a wastewater treatment plant, was investigated by a polyphasic approach. Cells of strain W-50^T were Gram-negative, strictly aerobic, oxidase-positive and yellow-pigmented. Ubiquinone Q-10 was the main respiratory lipoquinone system and polar lipid fingerprints were characterized by the presence of a sphingoglycolipid, suggesting that strain W-50^T belongs to the α -4 subclass of the *Proteobacteria*. Sequencing and comparative analyses of the 16S rRNA gene of strain W-50^T supported its chemotaxonomic allocation as an α -4 proteobacterium. The most closely related established taxa were species of the genus *Sphingopyxis*, including *Sphingopyxis macrogoltabida* (97.3% similarity) and *Sphingopyxis terrae* (96.4% similarity), and *Sphingomonas taejonensis* (97.3%). These findings were supported by both the polyamine content, which consisted mainly of spermidine [12.9 μ mol (g dry wt)⁻¹], and the presence of 2-OH 14:0, 2-OH 15:0 and 2-OH 16:0 in the cellular fatty acid profile. DNA–DNA hybridization experiments resulted in similarity values of 31.9% between strain W-50^T and *Sphingopyxis macrogoltabida* IFO 15033^T, 44.9% between strain W-50^T and *Sphingopyxis terrae* IFO 15098^T and 31.0% between strain W-50^T and *Sphingomonas taejonensis* KCTC 2884^T. Based upon results obtained by detailed physiological/biochemical testing and previously published molecular evidence, strain W-50^T was clearly distinguishable from all other *Sphingopyxis* species. For these reasons, the creation of a novel species, *Sphingopyxis witflariensis* sp. nov., is proposed; strain W-50^T (= DSM 14551^T = CIP 107174^T) is the type strain.

Keywords: activated sludge, α -*Proteobacteria*, *Sphingopyxis witflariensis*, polyphasic taxonomy

In recent years, studies on bacterial isolates from various environments have resulted in a significant increase in our knowledge of organisms grouped in the genus *Sphingomonas sensu lato* (Yabuuchi *et al.*, 1990). Sphingomonads are strictly aerobic, chemoheterotrophic, Gram-negative bacteria that are of biotechnological interest not only because of their degradation capabilities for various xenobiotic substances, but also for their potential to produce exopolysaccharides (for reviews see White *et al.*, 1996; Pollock, 1993). In aquatic habitats, sphingomonads have been

found in significant numbers in drinking water (Koskinen *et al.*, 2000), but they are also rather abundant in activated sludges, accounting for 5–10% of the total cells (Neef *et al.*, 1999). The interest in these organisms has led to an increase in the number of species (> 20) in the last few years. It has also recently become obvious from 16S rDNA data that the established species of the genus *Sphingomonas* can be grouped into at least four clusters. As a consequence, a subdivision of the genus *Sphingomonas sensu lato* has been proposed very recently: the genus name *Sphingomonas* was retained for the cluster containing the type species of the genus *Sphingomonas*, *Sphingomonas paucimobilis* (Takeuchi *et al.*, 2001). For the other three clusters of *Sphingomonas*, the new genera *Sphingobium*, *Novosphingobium* and *Sphingopyxis*

Abbreviations: pNA, p-nitroanilide; pNP, p-nitrophenyl.

The EMBL accession number for the 16S rRNA gene sequence of strain W-50^T is AJ416410.

have been proposed. In addition to these phylogenetic findings, some chemotaxonomic and further phenotypic features have provided evidence that the four *Sphingomonas* clusters represent separate genera (Busse *et al.*, 1999; Takeuchi *et al.*, 2001).

To clarify the actual taxonomic position of a yellow-pigmented bacterial strain (designated W-50^T), which was previously isolated from a wastewater treatment plant and showed a positive signal with a *Sphingomonas sensu lato* group-specific oligonucleotide probe, SPH120 (Neef *et al.*, 1999), a detailed polyphasic approach was applied.

Strain W-50^T was isolated from activated sludge collected from a wastewater treatment plant in Wetzlar (Germany) by standard plating on 10-fold-diluted PTYG agar (Balkwill & Ghiorse, 1985) containing 16 mg aztreonam l⁻¹ (ICN Biomedicals). The agar plate was incubated at 20 °C for 4 days. Further cultivation was done on R2A agar (Oxoid) at 25 °C. Cell morphology of strain W-50^T was examined by light microscopy using a Zeiss Axioplan II microscope with cultures grown in liquid 10-fold-diluted PTYG medium for 24 h at 28 °C. The Gram reaction was tested according to standard procedures (Gerhardt *et al.*, 1994). Oxidase activity was tested with Bactident-Oxidase test strips (Merck). Initial phenotypic characterization was performed using the API 20NE gallery (bioMérieux), as well as by conventional testing as described previously (Denner *et al.*, 2001), including tests for: catalase activity, oxidative/fermentative glucose metabolism, nitrate reduction, H₂S production, citrate utilization, indole production, urease activity and the methyl red/Voges-Proskauer reaction. In addition, a detailed substrate utilization profile has been obtained as described previously by Kämpfer *et al.* (1991). The sensitivity of strain W-50^T to antimicrobial substances was tested in the manner described by Denner *et al.* (2001) on Iso-Sensitest agar (Oxoid) using antibiotic-impregnated disks (Oxoid). Antibiotics tested were as follows [µg (unless stated otherwise) antibiotic per disk in parentheses]: ampicillin (10), amoxycillin/clavulanic acid (30), chloramphenicol (30), colistin sulfate (10), gentamicin (10), furazolidone (10), fusidic acid (10), nitrofurantoin (100), kanamycin (30), streptomycin (10), penicillin G (10 IU), bacitracin (10), polymyxin B (300 IU) and tetracycline (30).

Standard bacteriological characterization revealed that strain W-50^T is a Gram-negative, oxidase- and catalase-positive, aerobic, non-spore-forming, motile rod (0.5–0.8 × 1.0–2.0 µm) with a respiratory metabolism. The acetone-extracted yellow pigment of strain W-50^T had the same spectral characteristics (λ_{max} at 452 and 478 nm) as those described for the carotenoid nostoxanthin (Jenkins *et al.*, 1979). After 48 h incubation at 25 °C, strain W-50^T formed circular, entire, convex, smooth and bright-yellow-pigmented colonies (diameter approx. 1 mm). No growth was observed within 14 days at 42 °C, but growth was good at 15, 20, 25, 30 and 37 °C. Strain W-50^T did not grow on

Table 1. Morphological and physiological characteristics of *Sphingopyxis* species and related organisms

Strains: 1, strain W-50^T; 2, *Sphingopyxis macrogoltabida* IFO 15033^T (data from Kämpfer *et al.*, 1997); 3, *Sphingopyxis terrae* IFO 15098^T (Kämpfer *et al.*, 1997); 4, *Sphingomonas taejonensis* JSS54^T (Lee *et al.*, 2001). All strains are positive for assimilation of D-glucose. All strains are negative for: nitrate reduction; gelatin hydrolysis; assimilation of N-acetyl D-glucosamine, adipate, citrate, gluconate, D-mannose, D-melibiose, rhamnose and salicin; and oxidative acid production from glycerol and rhamnose.

Characteristic	1	2	3	4
Colony colour*	Y	Y	Y	PY
Aesculin hydrolysis	–	+	+	+
Assimilation of:				
Caproate	–	–	–	+
L-Arabinose	–	+	–	–
Malate	–	–	+	+
Maltose	+	+	–	–

* Y, Yellow; PY, pale yellow.

MacConkey agar (Merck) or Czapek–Dox agar (Oxoid). Nitrate was not reduced to nitrite or N₂, either in the conventional test or in the API 20NE gallery. This result was essentially in accordance with the results of Takeuchi *et al.* (2001), who reported nitrate reduction by members of the genera *Sphingomonas sensu stricto* and *Novosphingobium*, but no nitrate reduction by members of the genera *Sphingobium* and *Sphingopyxis*. Furthermore, H₂S production, growth on Simmons citrate agar, indole production, urease, arginine dihydrolase and hydrolysis of gelatin, starch and DNA were all negative. Strain W-50^T did not produce acid from various sugars and related compounds, but D-glucose, D-maltose, azelate, DL-3-hydroxybutyrate, suberate, L-alanine, L-histidine, L-leucine and L-proline were utilized as sole sources of carbon. Also, some of the tested *p*-nitrophenyl (*p*NP) and *p*-nitroaniline (*p*NA) derivatives were positive, e.g. *p*NP α -D-glucopyranoside, bis-*p*NP phosphate, *p*NP phosphorylcholine, 2-deoxythymidine-2'-*p*NP phosphate, L-alanine *p*NA, L-glutamate- γ -3-carboxy *p*NA and L-proline *p*NA. Hydrolysis of ONPG was negative. The organism was susceptible to amoxycillin/clavulanic acid, ampicillin, colistin sulfate, gentamicin, kanamycin and polymyxin B. It exhibited resistance to bacitracin, chloramphenicol, furazolidone, fusidic acid, nitrofurantoin, penicillin G, streptomycin and tetracycline. Additional phenotypic features are shown in Table 1 and are included in the formal species description below.

Analysis of the cellular fatty acids of strain W-50^T by GLC according to the procedures of Kämpfer & Kroppenstedt (1996) showed that the profile consisted predominantly of 17:1 ω 6c (43.0%), 18:1 ω 7c (9.0%),

Table 2. Major fatty acid content of *Sphingopyxis* reference strains and related organisms

Values are percentages of total fatty acids. Strains: 1, strain W-50^T; 2, *Sphingopyxis macrogoltabida* IFO 15033^T (data from Kämpfer *et al.*, 1997); 3, *Sphingopyxis terrae* IFO 15098^T (Kämpfer *et al.*, 1997); 4, *Sphingomonas taejonensis* JSS54^T (Lee *et al.*, 2001). For unsaturated fatty acids, the position of the double bond can be located by counting from the methyl (ω) end of the carbon chain; *cis* is indicated by the suffix *c*; *trans* by the suffix *t*. Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains one or more of the following fatty acids: 16:1 ω 7*c* and iso 2-OH 15:0. Summed feature 7 contains one or more of the following isomers: 19:1 ω 6*c*, 19:0 cyclo ω 10*c* and/or an unknown compound with an ECL of 18.846. The unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified. ND, Not determined.

Fatty acid	1	2	3	4
Saturated fatty acids				
14:0	ND	ND	ND	ND
15:0	7.0	ND	5.9	2.3
16:0	5.8	12.8	4.5	23.0
17:0	3.8	ND	3.8	1.6
Unsaturated fatty acids				
15:1 ω 6 <i>c</i>	0.4	ND	ND	ND
16:1 ω 5 <i>c</i>	1.2	2.9	2.1	1.6
17:1 ω 8 <i>c</i>	6.2	ND	7.9	1.8
17:1 ω 6 <i>c</i>	43.0	4.5	48.0	11.8
18:1 ω 7 <i>c</i>	9.0	ND	ND	ND
18:1 ω 5 <i>c</i>	0.4	0.9	0.6	ND
11-methyl 18:1 ω 7 <i>c</i>	2.8	ND	ND	ND
Hydroxy fatty acids				
2-OH 14:0	1.0	3.0	0.8	4.3
2-OH 15:0	7.0	ND	4.9	2.3
2-OH 16:0	0.8	2.9	ND	1.5
Summed features				
Summed feature 3	11.4	31.8	8.0	21.5
Summed feature 7	ND	41.2	13.0	28.3

15:0 (7.0%), 16:0 (5.9%) and summed feature 3 (16:1 ω 7*c* and iso 2-OH 15:0, 11.4%); hydroxylated fatty acids (2-OH 14:0, 1.0%; 2-OH 15:0, 7.0%; and 2-OH 16:0, 0.8%) were also present. Takeuchi *et al.* (2001) reported that the hydroxy fatty acid profiles provide valuable information for differentiation of the genera *Sphingomonas*, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*. Species of the genus *Sphingomonas sensu stricto* contained 2-OH 14:0 and/or 2-OH 15:0 as the major components, whereas those of the genus *Sphingopyxis* were characterized by the presence of 2-OH 15:0 and/or 2-OH 16:0, in addition to 2-OH 14:0. Members of the genera *Sphingobium* and *Novosphingobium* contained 2-OH 14:0 only as the major component. The detailed fatty acid profile of strain W-50^T is shown in Table 2 and clearly indicates allocation of this strain to the genus *Sphingopyxis*. A similar fatty

acid profile has been described for other species of the genus *Sphingopyxis*, for which the major fatty acids were reported to be 18:1, saturated 16:0 and 16:1, and major hydroxylated fatty acids were 2-OH 14:0 and 2-OH 15:0 and/or 2-OH 16:0 (Busse *et al.*, 1999; Takeuchi *et al.*, 2001).

Analysis of the respiratory lipoquinones of strain W-50^T by HPLC (Tindall, 1990) gave one characteristic major peak (92%), which corresponded to ubiquinone Q-10, and a smaller peak (8%) that corresponded to ubiquinone Q-9. HPLC polyamine analysis (Busse & Auling, 1988; Busse *et al.*, 1997) with cells grown on R2A agar revealed a pattern characteristic for the genera *Sphingobium*, *Novosphingobium* and *Sphingopyxis* of the α -Proteobacteria (Busse & Auling, 1988; Busse *et al.*, 1999). Strain W-50^T contained spermidine as major component [12.9 μ mol (g dry wt)⁻¹], whereas cadaverine, putrescine and spermine were detected in only small amounts [respectively 0.1, 0.2 and 0.3 μ mol (g dry wt)⁻¹]. 1,3-Diaminopropane was detected in trace amounts.

Polar lipid fingerprinting by two-dimensional TLC according to Denner *et al.* (2001) showed that strain W-50^T possesses a sphingoglycolipid, clearly suggesting again that the bacterium belongs to the α -4 subclass of the Proteobacteria. The overall polar lipid pattern of strain W-50^T corresponded to those reported for sphingomonads (Busse *et al.*, 1999), since it was composed of phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and sphingoglycolipid. Small amounts of phosphatidylmonomethylethanolamine, which has been detected in the majority of sphingomonads, were also present in strain W-50^T. However, a detailed comparison of the polar lipid composition of strain W-50^T indicated several significant differences from those of its close relatives, *Sphingopyxis macrogoltabida* and *Sphingopyxis terrae*, including the presence of phosphatidylglycerol in small amounts and the absence of the unidentified unique polar lipid PL3 (phospholipid) and GL1 (glycolipid). The absence of unidentified glycolipids GL2 and GL4 and the occurrence of phosphatidylcholine in large amounts in strain W-50^T further distinguish it from *Sphingopyxis macrogoltabida*, whereas strain W-50^T can be distinguished from *Sphingopyxis terrae* by the lack of phosphatidylidimethylethanolamine.

The 16S rRNA gene was amplified by PCR using universal primers (5'-AGAGTTTGATYMTGGCTC-AG, positions 7–26; 5'-CAKAAAGGAGGTGAT-CC, positions 1528–1544; both from Gibco BRL) and sequenced. Phylogenetic analysis was performed using the ARB software package (Ludwig & Strunk, 1997). Distance matrix, maximum-parsimony and maximum-likelihood methods were applied for tree construction as implemented in the ARB software package (results not shown).

The 16S rRNA sequence of strain W-50^T, with a continuous stretch of 1482 bp, was used to search

tidylmonomethylethanolamine are also present. The fatty acids 17:1 ω 6c, 18:1 ω 7c, 15:0, 16:0 and summed feature 3 (16:1 ω 7c and iso 2-OH 15:0, 11.4%) are produced, in addition to the three hydroxylated fatty acids 2-OH 14:0, 2-OH 15:0 and 2-OH 16:0. Colonies are circular, opaque, slightly raised and bright yellow with entire margins on standard bacteriological media. The visible absorption spectra of the yellow pigment (acetone extract) shows two peaks, at $\lambda_{\max}$ 452 and 478 nm, and a slight inflexion at 425 nm. Growth occurs at 15–37 °C on standard media such as Tryptone soy agar, nutrient agar or Luria–Bertani medium, as well as on the nutrient-reduced media R2A agar and PTYG agar. No growth occurs on MacConkey agar and Czapek–Dox agar. Does not grow at 42 °C. Nitrate is not reduced to nitrite or N₂. Tests for indole production, arginine dihydrolase, urease and H₂S are negative. Gelatin, starch and DNA are not hydrolysed. Acid is not produced from glucose, lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, L-arabinose, raffinose, L-rhamnose, maltose, D-xylose, trehalose, cellobiose, methyl D-glucoside, erythritol, melibiose, D-arabitol or D-mannose. The following compounds are utilized as sole sources of carbon: D-glucose, D-maltose, azelate, DL-3-hydroxybutyrate, suberate, L-alanine, L-histidine, L-leucine and L-proline. The following compounds are not utilized as sole sources of carbon: N-acetylgalactosamine, N-acetylglucosamine, L-arabinose, L-arbutin, D-cellobiose, D-galactose, gluconate, D-mannose, α -D-melibiose, L-rhamnose, D-ribose, sucrose, salicin, D-trehalose, D-xylose, adonitol, *i*-inositol, D-sorbitol, glycerol, D-fructose, D-mannitol, maltitol, putrescine, *cis*-aconitate, *trans*-aconitate, 4-aminobutyrate, citrate, mesaconate, acetate, propionate, adipate, fumarate, glutarate, itaconate, DL-lactate, 2-oxoglutarate, pyruvate, L-aspartate, β -alanine, L-ornithine, L-phenylalanine, L-serine, L-histidine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate. L-Alanine pNA, γ -L-glutamate pNA, L-proline pNA, pNP α -D-glucopyranoside, bis-pNP phosphate, bis-pNP phosphorylcholine and 2-deoxythymidine-2'-pNP phosphate are hydrolysed. The following compounds are not hydrolysed: pNP β -D-galactopyranoside, ONPG, pNP β -D-glucuronide, pNP β -D-glucopyranoside and pNP β -D-xylopyranoside. Susceptible to amoxycillin/clavulanic acid, ampicillin, colistin sulfate, gentamicin, kanamycin, neomycin and polymyxin B. Resistant to bacitracin, chloramphenicol, furazolidone, fusidic acid, nitrofurantoin, penicillin G, streptomycin and tetracycline. The type strain is strain W-50^T (= DSM 14551^T = CIP 107174^T). Isolated from activated sludge of a wastewater plant in Wetzlar, Germany.

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References

- Balkwill, D. L. & Ghiorse, W. C. (1985). Characterization of subsurface bacteria associated with two shallow aquifers in Oklahoma. *Appl Environ Microbiol* **50**, 5680–5688.
- Busse, J. & Auling, G. (1988). Polyamine pattern as a chemotaxonomic marker within the *Proteobacteria*. *Syst Appl Microbiol* **11**, 1–8.
- Busse, H.-J., Bunka, S., Hensel, A. & Lubitz, W. (1997). Discrimination of members of the family *Pasteurellaceae* based on polyamine patterns. *Int J Syst Bacteriol* **47**, 698–708.
- Busse, H.-J., Kämpfer, P. & Denner, E. B. M. (1999). Chemotaxonomic characterisation of *Sphingomonas*. *J Ind Microbiol Biotechnol* **23**, 242–251.
- Denner, E. B. M., Paukner, S., Kämpfer, P., Moore, E. R. B., Abraham, W.-R., Busse, H.-J., Wanner, G. & Lubitz, W. (2001). *Sphingomonas pituitosa* sp. nov., an exopolysaccharide-producing bacterium that secretes an unusual type of sphingan. *Int J Syst Evol Microbiol* **51**, 827–841.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Jenkins, C. L., Andrews, A. G., McQuade, T. J. & Starr, M. P. (1979). The pigment of *Pseudomonas paucimobilis* is carotenoid (nostoxanthin), rather than a brominated aryl-polyene (xanthomonadin). *Curr Microbiol* **3**, 1–4.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kämpfer, P., Steiof, M. & Dott, W. (1991). Microbiological characterization of a fuel-oil contaminated site including numerical identification of heterotrophic water and soil bacteria. *Microb Ecol* **21**, 227–251.
- Kämpfer, P., Denner, E. B. M., Meyer, S., Moore, E. R. B. & Busse, H.-J. (1997). Classification of '*Pseudomonas azotocolligans*' Anderson 1955, 132, in the genus *Sphingomonas* as *Sphingomonas trueperi* sp. nov. *Int J Syst Bacteriol* **47**, 577–583.
- Koskinen, R., Ali-Vehmas, T., Kämpfer, P., Laurikkala, M., Tsitko, I., Kostyal, E., Atroshi, F. & Salkinoja-Salonen, M. (2000). Characterization of *Sphingomonas* isolates from Finnish and Swedish drinking water distribution systems. *J Appl Microbiol* **89**, 687–696.
- Lee, J.-S., Shin, Y. K., Yoon, J.-H., Takeuchi, M., Pyun, Y.-R. & Park, Y.-H. (2001). *Sphingomonas aquatilis* sp. nov., *Sphingomonas koreensis* sp. nov., and *Sphingomonas taejonensis* sp. nov., yellow-pigmented bacteria isolated from natural mineral water. *Int J Syst Evol Microbiol* **51**, 1491–1498.
- Ludwig, W. & Strunk, O. (1997). ARB – a software environment for sequence data. <http://www.mikrobiologie.tu-muenchen.de/pub/ARB/documentation/arb.ps>
- Neef, A., Witzemberger, R. & Kämpfer, P. (1999). Detection of sphingomonads and in situ identification in activated sludge using 16S rRNA-targeted oligonucleotide probes. *J Ind Microbiol Biotechnol* **23**, 261–267.
- Pollock, T. J. (1993). Gellan-related polysaccharides and the genus *Sphingomonas*. *J Gen Microbiol* **139**, 1939–1945.
- Takeuchi, M., Kawai, F., Shimada, Y. & Yokota, A. (1993). Taxonomic study of polyethylene glycol-utilizing bacteria: amended description of the genus *Sphingomonas* and new description of *Sphingomonas macroglutabidus* sp. nov., *Sphingomonas sanguis* sp. nov., and *Sphingomonas terrae* sp. nov. *Syst Appl Microbiol* **16**, 227–238.
- Takeuchi, M., Hamana, K. & Hiraishi, A. (2001). Proposal of the genus *Sphingomonas sensu stricto* and three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, on the basis of phylogenetic and chemotaxonomic analyses. *Int J Syst Evol Microbiol* **51**, 1405–1417.
- Tindall, B. J. (1990). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- White, D. C., Sutton, S. D. & Ringelberg, D. B. (1996). The genus *Sphingomonas*: physiology and ecology. *Curr Opin Biotechnol* **7**, 301–306.

Wisotzkey, J. D., Jurtshuk, P., Jr & Fox, G. E. (1990). PCR amplification of 16S rDNA from lyophilized cell cultures facilitates studies in molecular systematics. *Curr Microbiol* **21**, 325–327.

Yabuuchi, E., Yano, I., Oyaizu, H., Hashimoto, Y., Ezaki, T. & Yamamoto, H. (1990). Proposals of *Sphingomonas paucimobilis*, gen. nov. and comb. nov., *Sphingomonas parapaucimobilis* sp. nov.,

Sphingomonas yanoikuyae sp. nov., *Sphingomonas adhaesiva* sp. nov., *Sphingomonas capsulata* comb. nov., and two genospecies of the genus *Sphingomonas*. *Microbiol Immunol* **34**, 99–119.

Ziemke, F., Höfle, M. G., Lalucat, J. & Roselló-Mora, R. (1998). Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. nov. *Int J Syst Bacteriol* **48**, 179–186.