Winogradskyella rapida sp. nov., isolated from protein-enriched seawater

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Flavobacteria are emerging as an important group of organisms associated with the degradation of complex organic matter in aquatic environments. A novel Gram-reaction-negative, heterotrophic, rod-shaped, aerobic, yellow-pigmented and gliding bacterium, strain SCB36^T, was isolated from a protein-enriched seawater sample, collected at Scripps Pier, Southern California Bight (Eastern Pacific). Analysis of the 16S rRNA gene sequence showed that the bacterium was related to members of the genus *Winogradskyella* within the family *Flavobacteriaceae*, phylum *Bacteroidetes*. 16S rRNA gene sequence similarity to the other *Winogradskyella* species was 94.5–97.1 %. DNA–DNA relatedness between strain SCB36^T and *Winogradskyella thalassocola* KMM 3907^T, its closest relative in terms of 16S rRNA gene sequence similarity, was 20 %. On the basis of the phylogenetic and phenotypic data, strain SCB36^T represents a novel species of the genus *Winogradskyella*, for which the name *Winogradskyella rapida* sp. nov. is proposed. The type strain is SCB36^T (=CECT 7392^T =CCUG 56098^T).

Members of the phylum *Bacteroidetes* make up a significant portion (up to 30 %) of marine bacterioplankton communities (Glöckner *et al.*, 1999). Notably, members of the family *Flavobacteriaceae* account for a majority of the phylogenetic diversity found among marine *Bacteroidetes* (Alonso *et al.*, 2007). Members of the phylum *Bacteroidetes* in general, and members of the family *Flavobacteriaceae* in particular, have been implicated in the degradation of complex organic matter in the sea (Abell & Bowman, 2005; Kirchman, 2002; Pinhassi *et al.*, 2004). Cottrell & Kirchman (2000) highlighted that marine members of the *Bacteroidetes* have a preference for the utilization of protein over that of simple monomers. Understanding the taxonomy and the phenotypic characteristics of bacteria that potentially participate in the degradation of organic matter in the sea is an important step in understanding how biogeochemical processes are regulated.

Strain SCB36^T was isolated from a seawater mesocosm experiment designed to investigate how a natural bacterio-

plankton community would respond to protein enrichment (Pinhassi *et al.*, 1999). Enrichment with protein (BSA) was chosen in an attempt to simulate the release of organic matter during the decay phase of a phytoplankton bloom. Surface seawater was collected on 7 December 1995 from Scripps Pier, Southern California Bight (La Jolla, CA, USA; 32° 53' N 117° 15' W). The sample was incubated for 3 days at 15 °C with a 12 h dark/12 h light cycle, after which agar plates were prepared for strain isolation as described previously by Pinhassi *et al.* (1999). Strain SCB36^T was one of the bacteria that exhibited the most pronounced positive growth response to enrichment with protein and reached net growth rates of nearly 1.5 day⁻¹ (see Supplementary Fig. S1, available in IJSEM Online), compared to net growth rates below 0.4 day⁻¹ for the total bacterial community (Pinhassi *et al.*, 1999). Further, its growth rate closely followed the increase in hydrolytic ectoenzyme activity measured in the mesocosms, particularly that of protease activity (Supplementary Fig. S1). Together with some other flavobacteria species, strain SCB36^T increased in abundance even though it was in competition with other members of the bacterial community and exposed to active protozoan grazing, indicating its high growth potential when supplied with protein.

For strain isolation, 0.1 ml of 1/100-diluted sample water from the protein-enriched seawater mesocosm was spread

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SCB36^T is U64013.

Rates of growth and protease activity for strain SCB36^T and a scanning electron micrograph of bacterial cells are available as supplementary material with the online version of this paper.

onto ZoBell agar plates prepared from seawater from the Southern California Bight. Yellow colonies grew on these plates with an abundance of approximately 1×10^3 c.f.u. ml^{-1} . After primary isolation and purification, strain SCB36^T was cultivated at 23 °C on the same medium and stored at –80 °C in ZoBell medium with 25 % (v/v) glycerol. For subsequent culturing of strain SCB36^T, marine broth/agar 2216 (MB/MA; Difco) was used unless otherwise stated.

Determination of growth at different temperatures, nitrate reduction, acid production from carbohydrates, production of hydrogen sulfide, indole and acetoin (Voges–Proskauer reaction), hydrolysis of casein, gelatin, starch, Tweens 20, 40 and 80, agar (1.5 %, w/v), DNA, urea and cellulose (CM-cellulose and filter paper) and oxidase, catalase, β -galactosidase and alkaline phosphatase activities were carried out according to standard procedures (Gerhardt *et al.*, 1994). Requirement of NaCl for growth was assessed on a solid medium, consisting of (1^{-1} distilled water): 5 g Bacto peptone (Difco), 2 g Bacto yeast extract (Difco), 1 g glucose, 0.02 g KH_2PO_4 , 0.05 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 20 g Bacto agar (Difco), supplemented with 0, 1, 2, 3, 4, 5, 6, 8, 10 or 12 % (w/v) NaCl; growth was determined after 7 days of incubation at 28 °C. Utilization of carbon sources was examined as described previously (Nedashkovskaya *et al.*, 2003). Hydrolysis of chitin (1 %, w/v) was determined by the appearance of clear zones around colonies on chitin agar. The presence of flexirubin pigments in strain SCB36^T was determined by the method of Fautz & Reichenbach (1980). Gliding motility was determined as described previously (Bowman, 2000). For studies of cell morphology, cells were grown at 21 °C in MB until early exponential growth phase (35 h of incubation), when cells were fixed with glutaraldehyde and filtered onto 0.2 μm -pore-size polycarbonate filters (Nuclepore). Samples were treated by sequential ethanol-dehydration steps, critical-point drying with CO_2 and silver coating and viewed with a Hitachi S-3500N scanning electron microscope. Susceptibility to antibiotics was examined by the routine disc-diffusion plate method after 7 days of incubation on MA at 28 °C. Discs were impregnated with the following antibiotics (μg unless otherwise stated): ampicillin (10), benzylpenicillin (10 U), carbenicillin (100), chloramphenicol (30), doxycycline (10), erythromycin (15), gentamicin (10), kanamycin (30), lincomycin (15), neomycin (30), oleandomycin (15), polymyxin B (300 U), streptomycin (30) and tetracycline (30). Phenotypic characteristics of strain SCB36^T were determined in the same laboratory under the same conditions and using the same methodology as described previously for the type strains of three other *Winogradskyella* species (Nedashkovskaya *et al.*, 2005). Cells of strain SCB36^T were Gram-reaction-negative single rods, approximately 0.3–0.4 μm in diameter and 0.8–1.8 μm in length (Supplementary Fig. S2), motile by gliding, with a chemo-organotrophic respiratory type of metabolism. The abilities of strain SCB36^T to produce

hydrogen sulfide and to form acid from glycerol distinguished it from other members of the genus *Winogradskyella*. Also, unlike most other *Winogradskyella* species, it was unable to degrade agar. Other physiological characteristics are given in Table 1 and in the species description.

Table 1. Differentiating phenotypic features of type strains of some *Winogradskyella* species

Strains: 1, *Winogradskyella rapida* sp. nov. SCB36^T; 2, *W. thalassocola* KMM 3907^T; 3, *W. epiphytica* KMM 3906^T; 4, *W. eximia* KMM 3944^T; 5, *W. poriferorum* UST030701-295^T. Data for reference strains were taken from Lau *et al.* (2005) (*W. poriferorum* UST030701-295^T) or Nedashkovskaya *et al.* (2005) (remaining strains). All strains were positive for the following tests: chemo-organotrophic respiratory type of metabolism; gliding motility; oxidase, catalase and alkaline phosphatase activities; requirement for Na^+ ions for growth; hydrolysis of gelatin and Tween 40. All strains were negative for the following tests: nitrate reductase and β -galactosidase activities; flexirubin-type pigment production; indole and acetoin production; hydrolysis of urea and chitin; acid production from L-arabinose, D-galactose, lactose, melibiose, L-rhamnose, DL-xylose, adonitol, dulcitol, inositol, sorbitol and citrate; and utilization of L-arabinose, lactose, inositol, sorbitol, malonate and citrate. All strains were susceptible to carbenicillin and lincomycin and resistant to benzylpenicillin, gentamicin, kanamycin, neomycin, polymyxin B and streptomycin. +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5
Growth at/with:					
37 °C	–	–	+	–	+
44 °C	–	–	–	–	+
8 % NaCl	+	+	+	–	–
Hydrogen sulfide production	+	–	–	–	–
Degradation of:					
Agar	–	+	+	+	–
Casein, starch	+	–	–	+	–
Tween 20	+	–	+	+	+
Tween 80	+	–	+	–	+
DNA	–	–	+	–	+
Acid formation from:					
D-Glucose, maltose	+	+	–	+	–
Cellobiose	–	+	–	–	–
Sucrose	–	–	–	+	–
Glycerol	+	–	–	–	–
Mannitol	+	–	–	+	–
Utilization of:					
D-Glucose, D-mannose	+	+	–	+	–
Sucrose	–	–	–	+	–
Mannitol	+	–	–	+	–
Susceptibility to:					
Ampicillin	+	–	+	–	+
Oleandomycin	+	+	+	–	ND
Tetracycline	+	–	+	+	+
DNA G + C content (mol%)	36.3	34.6	35.2	36.1	32.8

The API ZYM (bioMérieux) gallery was used to test for enzyme activities of the strain according to the manufacturer's instructions except that cells were suspended in a 3.5 % sea-salt solution before inoculation of the API ZYM strips and the strips were incubated for 20 h at 20 °C. Strain SCB36^T was positive for 10 of the 19 enzyme activities tested. The highest activities (value of 5 on a scale from 1 to 5) were recorded for leucine and valine arylamidases as well as alkaline phosphatase. The high values for arylamidases, particularly valine arylamidase, are notable considering that several gammaproteobacteria show lower activities, if any, for these enzymes (Arahal *et al.*, 2007). We note that recent genome analyses of marine flavobacteria reveal comparatively large numbers of genes encoding peptidases (Bauer *et al.*, 2006; González *et al.*, 2008). These results suggest that the potential to hydrolyse polypeptides could help to explain the success of strain SCB36^T following the enrichment of seawater with dissolved protein.

For analysis of the fatty acid composition, strain SCB36^T was grown on MA at 28 °C. When dense growth had developed (24 h), cells were harvested and the fatty acid composition was determined according to the standard protocol of the Microbial Identification System. Fatty acid analysis of strain SCB36^T was carried out in the same laboratory and under the same conditions and methodology as described previously for three other *Winogradskyella* species (Nedashkovskaya *et al.*, 2005). The cellular fatty acid profile of strain SCB36^T differed markedly from those of the other *Winogradskyella* species in that the four dominant fatty acids, anteiso-C_{15:0}, iso-C_{15:0}, iso-C_{15:0} 3-OH and iso-C_{15:1}, together accounted for 62 % of the total fatty acids (each accounting for 14–18 % of total), while iso-C_{16:0} 3-OH was only a minor component (Table 2).

For phylogenetic analysis, the genomic DNA was isolated following a standard phenol extraction protocol (Wilson, 1994). The 16S rRNA gene sequence was amplified by means of PCR using the primers 27F and 1492R (Giovannoni, 1991). Sequencing of the 16S rRNA gene was carried out by Macrogen (Korea), using primers 27F and 1492R and the internal primer 907RM (Muyzer *et al.*, 1998). The nearly complete 16S rRNA gene sequence of strain SCB36^T was 1478 nucleotides in length. CLUSTAL W was used to align the sequence with those from members of the family *Flavobacteriaceae* and a neighbour-joining phylogenetic tree was constructed with the MEGALIGN software in the DNASTAR package (version 7.0). As seen in Fig. 1, strain SCB36^T clustered with members of the genus *Winogradskyella*. The topology of the maximum-likelihood tree was essentially the same (data not shown). The 16S rRNA gene sequence similarities of strain SCB36^T to *Winogradskyella thalassocola* KMM 3907^T and to the other *Winogradskyella* type strains were 97.1 and 94.5–96.5 %, respectively. Sequence similarities to members of other genera of the *Flavobacteriaceae* ranged from 88.0 to 93.5 %. Thus, 16S rRNA gene sequence analysis suggested that

Table 2. Whole-cell fatty acid profiles of strain SCB36^T and the type strains of some other *Winogradskyella* species

Strains: 1, *W. rapida* sp. nov. SCB36^T; 2, *W. thalassocola* KMM 3907^T; 3, *W. epiphytica* KMM 3906^T; 4, *W. eximia* KMM 3944^T; 5, *W. poriferorum* UST030701-295^T. Data for reference strains were taken from Lau *et al.* (2005) (*W. poriferorum* UST030701-295^T) or Nedashkovskaya *et al.* (2005) (remaining strains). Fatty acids that comprise <1 % of the total for all strains are not given. tr, Traces (<1 % of total fatty acids).

Fatty acid	1	2	3	4	5
iso-C _{13:0}	tr	—	—	—	2.6
iso-C _{14:0}	—	2.6	4.5	1.4	1.2
iso-C _{14:0} 3-OH	—	tr	1.6	—	tr
iso-C _{14:1}	—	—	1.4	—	2.0
C _{15:0}	—	7.9	1.2	6.7	—
anteiso-C _{15:0}	14.1	4.9	15.9	7.0	—
iso-C _{15:0}	18.5	8.7	6.7	25.6	12.6
iso-C _{15:0} 3-OH	14.8	11.9	2.9	2.6	9.8
C _{15:0} 2-OH	2.2	1.8	3.3	1.0	3.1
C _{15:0} 3-OH	2.1	2.5	—	—	2.4
C _{15:1} ω6c	3.8	6.5	—	—	—
anteiso-C _{15:1}	2.9	1.6	6.3	1.4	1.5
iso-C _{15:1}	14.6	11.4	8.1	10.4	20.9
10-Methyl C _{16:0}	—	—	—	6.3	—
C _{16:0} 3-OH	tr	1.0	—	—	1.3
iso-C _{16:0}	—	tr	3.7	5.7	tr
iso-C _{16:0} 3-OH	3.3	18.1	17.1	3.2	11.4
iso-C _{16:1}	tr	2.7	3.5	4.7	tr
C _{17:0} 2-OH	2.7	tr	5.2	1.0	tr
C _{17:0} cyclo	—	—	—	2.3	—
iso-C _{17:0} 3-OH	8.2	5.4	7.3	6.7	10.2
anteiso-C _{17:1}	—	—	—	2.3	—
iso-C _{17:1} ω9c	1.5	tr	1.1	—	—
C _{17:1} ω6c	tr	tr	1.9	—	tr
Summed feature 3*	—	4.2	5.1	6.1	9.8

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained iso-C_{15:0} 2-OH and/or C_{16:1} ω7c.

strain SCB36^T represents a novel species in the genus *Winogradskyella*.

For G + C content determination and DNA–DNA hybridization, genomic DNA was extracted using the technique of Marmur (1961). DNA G + C content was determined by the thermal denaturation method (Marmur & Doty, 1962). The DNA G + C content of strain SCB36^T was 36.3 mol%, which is similar to or slightly higher than the values reported for the other *Winogradskyella* species (32.8–36.1 mol%; Nedashkovskaya *et al.*, 2005; Lau *et al.*, 2005). DNA–DNA hybridization experiments were performed spectrophotometrically using the initial renaturation rate method (De Ley *et al.*, 1970). The level of DNA–DNA relatedness between strain SCB36^T and its closest relative in terms of 16S rRNA gene sequence

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequence analysis of representative members of the family *Flavobacteriaceae*, showing the position of strain SCB36^T. The sequence of *Flexibacter flexilis* ATCC 23079^T (GenBank accession no. M62794) was used as the outgroup (not shown). Bootstrap values (>50%) based on 1000 resamplings are shown at branch nodes. Bar, 0.03 substitutions per nucleotide position.

similarity, *W. thalassocola* KMM 3907^T, was 20%. This substantiated the phylogenetic analysis and further supported our proposal of strain SCB36^T as belonging to a novel *Winogradskyella* species.

A polyphasic approach showed that strain SCB36^T could be distinguished from recognized species of the genus *Winogradskyella* by a combination of genetic and phenotypic data and phylogenetic inference. We therefore conclude that this bacterium represents a novel species, for which the name *Winogradskyella rapida* sp. nov. is proposed.

Description of *Winogradskyella rapida* sp. nov.

Winogradskyella rapida (ra.pi'da. L. fem. adj. *rapida* fast, referring to the capacity for rapid growth in protein-enriched seawater).

Cells are 0.3–0.4 µm in diameter and 0.8–1.8 µm long. Colonies on MA are round, 2–3 mm in diameter and light-yellow-pigmented with a shiny surface. Na⁺ ions are required for growth. Grows with 1–8% NaCl (optimum 2–3%) and at 4–36 °C (optimum 23–25 °C). β-Galactosidase-negative. Nitrate is not reduced. Hydrogen sulfide is produced but indole and acetoin (Voges-Proskauer reaction) are not produced. Flexirubin-type pigments are not detected. Casein, gelatin, starch and Tweens 20, 40 and 80 are hydrolysed but agar, DNA, urea, cellulose (CM-cellulose and filter paper) and chitin are not hydrolysed. Acid is produced from D-glucose, maltose, D-fructose, glycerol and mannitol, but not from L-arabinose,

cellobiose, D-galactose, lactose, melibiose, raffinose, L-rhamnose, sucrose, L-sorbose, trehalose, DL-xylose, N-acetylglucosamine, adonitol, dulcitol, inositol, sorbitol or citrate. D-Mannose is utilized, but L-arabinose, lactose, inositol and sorbitol are not. According to the API ZYM gallery (bioMérieux), acid and alkaline phosphatases, esterase (C4), esterase lipase (C8), lipase (C14), leucine, valine and cystine arylamidases, trypsin, naphthol-AS-BI-phosphohydrolase and α-glucosidase activities are present, but α-chymotrypsin, α- and β-galactosidases, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. Susceptible to ampicillin, carbenicillin, chloramphenicol, doxycycline, erythromycin, lincomycin, oleandomycin and tetracycline. Resistant to benzylpenicillin, gentamicin, kanamycin, neomycin, polymyxin B and streptomycin. The main cellular fatty acids are anteiso-C_{15:0}, iso-C_{15:0}, iso-C_{15:0} 3-OH, iso-C_{15:1} and iso-C_{17:0} 3-OH. The DNA G+C content of the type strain is 36.3 mol%.

The type strain, SCB36^T (=CECT 7392^T =CCUG 56098^T), was isolated from a protein-enriched surface seawater sample from Scripps Pier (La Jolla, CA, USA), in the Eastern Pacific Ocean.

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References

- Abell, G. C. J. & Bowman, J. P. (2005). Colonization and community dynamics of class *Flavobacteria* on diatom detritus in experimental mesocosms based on Southern Ocean seawater. *FEMS Microbiol Ecol* **53**, 379–391.
- Alonso, C., Warnecke, F., Amann, R. & Pernthaler, J. (2007). High local and global diversity of *Flavobacteria* in marine plankton. *Environ Microbiol* **9**, 1253–1266.
- Arahal, D. R., Lekunberri, I., Gonzalez, J. M., Pascual, J., Pujalte, M. J., Pedros-Alio, C. & Pinhassi, J. (2007). *Neptuniibacter caesariensis* gen. nov., sp. nov., a novel marine genome-sequenced gammaproteobacterium. *Int J Syst Evol Microbiol* **57**, 1000–1006.
- Bauer, M., Kube, M., Teeling, H., Richter, M., Lombardot, T., Allers, E., Würdemann, C. A., Quast, C., Kuhl, H. & other authors (2006). Whole genome analysis of the marine *Bacteroidetes* '*Gramella forsetii*' reveals adaptations to degradation of polymeric organic matter. *Environ Microbiol* **8**, 2201–2213.
- Bowman, J. P. (2000). Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. *Int J Syst Evol Microbiol* **50**, 1861–1868.

- Cottrell, M. T. & Kirchman, D. L. (2000).** Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. *Appl Environ Microbiol* **66**, 1692–1697.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970).** The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Fautz, E. & Reichenbach, H. (1980).** A simple test for flexirubin-type pigments. *FEMS Microbiol Lett* **8**, 87–91.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994).** *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Giovannoni, S. J. (1991).** The polymerase chain reaction. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 177–201. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Glöckner, F. O., Fuchs, B. M. & Amann, R. (1999).** Bacterioplankton composition of lakes and oceans: a first comparison based on fluorescence in situ hybridization. *Appl Environ Microbiol* **65**, 3721–3726.
- González, J. M., Fernández-Gómez, B., Fernández-Guerra, A., Gómez-Consarnau, L., Sánchez, O., Coll-Lladó, M., Del Campo, J., Escudero, L., Rodríguez-Martínez, R. & other authors (2008).** Genome analysis of the proteorhodopsin-containing marine bacterium *Polaribacter* sp. MED152 (*Flavobacteria*). *Proc Natl Acad Sci U S A* **105**, 8724–8729.
- Kirchman, D. L. (2002).** The ecology of *Cytophaga-Flavobacteria* in aquatic environments. *FEMS Microbiol Ecol* **39**, 91–100.
- Lau, S. C. K., Tsoi, M. M. Y., Li, X. C., Plakhotnikova, I., Dobretsov, S., Lau, K. W., Wu, M., Wong, P. K., Pawlik, J. R. & other authors (2005).** *Winogradskyella poriferorum* sp. nov., a novel member of the family *Flavobacteriaceae* isolated from a sponge in the Bahamas. *Int J Syst Evol Microbiol* **55**, 1589–1592.
- Marmur, J. (1961).** A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Marmur, J. & Doty, P. (1962).** Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Muyzer, G., Brinkhoff, T., Nübel, U., Santegoeds, C., Schäfer, H. & Wawer, C. (1998).** Denaturing gradient gel electrophoresis (DGGE) in microbial ecology. In *Molecular Microbial Ecology Manual*, vol. 3.4.4, pp. 1–27. Edited by A. D. L. Akkermans, J. D. van Elsas & F. J. de Bruijn. Dordrecht: Kluwer.
- Nedashkovskaya, O. I., Suzuki, M., Vysotski, M. V. & Mikhailov, V. V. (2003).** *Reichenbachia agariperforans* gen. nov., sp. nov., a novel marine bacterium in the phylum *Cytophaga-Flavobacterium-Bacteroides*. *Int J Syst Evol Microbiol* **53**, 81–85.
- Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Snauwaert, C., Vancanneyt, M., Swings, J., Kim, K. O., Lysenko, A. M., Rohde, M. & other authors (2005).** *Winogradskyella thalassocola* gen. nov., sp. nov., *Winogradskyella epiphytica* sp. nov. and *Winogradskyella eximia* sp. nov., marine bacteria of the family *Flavobacteriaceae*. *Int J Syst Evol Microbiol* **55**, 49–55.
- Pinhassi, J., Azam, F., Hemphälä, J., Long, R. A., Martinez, J., Zweifel, U. L. & Hagström, Å. (1999).** Coupling between bacterioplankton species composition, population dynamics, and organic matter degradation. *Aquat Microb Ecol* **17**, 13–26.
- Pinhassi, J., Sala, M. M., Havskum, H., Peters, F., Guadayol, Ò., Malits, A. & Marrasé, C. (2004).** Changes in bacterioplankton composition under different phytoplankton regimens. *Appl Environ Microbiol* **70**, 6753–6766.
- Wilson, K. (1994).** Preparation of genomic DNA from bacteria. In *Current Protocols in Molecular Biology*, pp. 2.4.1–2.4.2. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Green Publishing & Wiley-Interscience.