Delftia lacustris sp. nov., a peptidoglycan-degrading bacterium from fresh water, and emended description of *Delftia tsuruhatensis* as a peptidoglycan-degrading bacterium

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Extracellular peptidoglycan is commonly found in natural environments, yet little is known about its biodegradation in nature. We here describe a novel peptidoglycan-degrading bacterium, designated strain 332^T, isolated from mesotrophic lake water in Denmark. The strain was a Gram-negative-staining, motile rod. It had chitinase and lysozyme activities, which are relevant to peptidoglycan degradation, and was capable of utilizing several mono- and disaccharides, amino acids and organic acids. Phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain 332^T belonged to the genus *Delftia*. Fatty acids of the strain included C_{8:0} and C_{10:0}, which are characteristic of the genus *Delftia*. The DNA G+C content of the strain was 65.3 mol%. A DNA–DNA hybridization value of 66.2% was found between strain 332^T and *Delftia tsuruhatensis* DSM 17581^T. Based on differences in physiological and biochemical characteristics, the strain is considered to represent a novel species, for which the name *Delftia lacustris* sp. nov. is proposed. The type strain is 332^T (=DSM 21246^T =LMG 24775^T). An emended description of *Delftia tsuruhatensis* is also presented.

Extracellular peptidoglycan (PG) components commonly occur in nature (Benner & Kaiser, 2003). These cell-wall compounds have been found to be degradable by indigenous bacteria, but at a lower rate than other polymers of bacterial origin, such as proteins (Nagata *et al.*, 2003). During growth, large portions of PG in Gram-negative bacteria are recycled by being degraded and reassimilated (Park & Uehara, 2008), but it is not known whether the enzymes involved in this recycling are also engaged in utilization of extracellular PG. Degradation of PG and chitin may involve related enzymic processes, because the glycan backbones of PG and chitin possess similar $\beta(1,4)$ bonds, and some bacterial chitinases also have lysozyme activity (Wang & Chang, 1997). To our knowledge, bacterial species that are capable of degrading extracellular PG have not previously been described. We report here the characterization of a novel strain that degrades extracellular PG. The strain, designated strain

332^T, was isolated from mesotrophic lake water and cultivated on PG-enriched media [tryptic soy agar (TSA) or dilute tryptic soy broth (TSB) (0370-17-1; Difco) amended with 2 mg PG l⁻¹]. Analysis of its 16S rRNA gene sequence revealed that strain 332^T clustered within the genus *Delftia*. The genus *Delftia* includes two recognized species, *Delftia acidovorans* (isolated from soil) and *Delftia tsuruhatensis* (isolated from sludge) (Wen *et al.*, 1999; Shigematsu *et al.*, 2003). On the basis of phenotypic and molecular analysis, we propose that strain 332^T represents a novel species within the genus *Delftia*.

Lake-water microcosms were established with filtered water (0.8 μ m pore size) collected from the mesotrophic Lake Sjølsø, northern Zealand, Denmark. The microcosms were supplemented with PG from *Bacillus* sp. strain A6 (Frette *et al.*, 2004). PG was purified according to the method of Pelz *et al.* (1998) (see details below) and added to a final concentration of 2 mg l⁻¹. After incubation for 7 days at 20 °C, bacteria from the microcosms were cultivated on 1/10 LB agar (Eisenstadt *et al.*, 1994). Subsequently, isolates were transferred to 1/10 LB agar supplemented with PG (or with heat-denatured cell-wall material) from *Bacillus* sp. strain A6 and isolates that formed clearing zones were

Abbreviations: MUF, methyl umbelliferyl; PG, peptidoglycan; SEM, scanning electron microscopy.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 332^T is EU888308.

selected for further analysis. Strain 332^T was selected because of its capacity to form distinct but opaque clearing zones. The strain was routinely cultured in LB broth or TSB in shake flasks incubated overnight at 22 °C.

Standard physiological analyses were performed on cells grown at 22 °C in TSB or on 1/10 TSA at pH 7.2, using individual tests and multi-substrate kits. Tests for strain 332^T, *D. tsuruhatensis* DSM 17581^T and *D. acidovorans* DSM 39^T were performed under identical conditions with parallel cultures. The individual tests included the Gram designation (KOH method), oxidase activity (BBL Dryslide Oxidase kit; BD), catalase activity (H₂O₂ addition), oxidation or fermentation of glucose (test according to Hugh & Leifson, 1953), casein hydrolysis (detection of clearing zones on LB agar enriched with 3 % skimmed milk), lysozyme activity (EnzChek lysozyme assay kit; Invitrogen), DNase activity [detection of clearing zones in TSA (20 g agar l⁻¹) enriched with 2 g DNA l⁻¹, made visible after addition of 1 M HCl (Jeffries *et al.*, 1957)] and chitinase, cellulose and starch degradation using the methyl umbelliferyl (MUF) substrate analogues MUF *N*-acetyl- β -glucosaminide, MUF β -glucosidase and MUF α -glucosidase (Sigma-Aldrich). The MUF substrates were applied as described elsewhere (O'Brien & Colwell, 1987; Cody, 1989). Briefly, 30 μ l MUF substrate was added to 4 cm filter-paper discs, after which material from several colonies was rubbed onto the surface of the discs. The discs were incubated at 28 °C for 30 min and then 50 μ l saturated sodium bicarbonate solution was added to each disc. The discs were exposed to UV light and appearance of blue fluorescence indicated a positive reaction.

Because of the close phylogenetic relationship between strain 332^T and *D. tsuruhatensis* relative to *D. acidovorans*, most tests were only performed on strain 332^T and *D. tsuruhatensis* DSM 17581^T. The multi-substrate kits that were used were API 20NE, API 50 CH (carbohydrate utilization) and API ZYM (various enzymes) (bioMérieux) and GN2 MicroPlate and EcoPlate (Biolog), according to the manufacturers' instructions. For analysis of whole-cell fatty acids and DNA–DNA hybridization, cells of *D. tsuruhatensis* DSM 17581^T, *D. acidovorans* DSM 39^T (DNA–DNA hybridization only) and strain 332^T were grown in TSB under identical growth conditions. Genomic G+C content and whole-cell fatty acids were analysed by HPLC analysis and GC-MS, respectively, at the DSMZ. DNA–DNA hybridization was performed in duplicate according to De Ley *et al.* (1970) at the DSMZ.

Degradation of extracellular PG was tested with liquid and solid media, each with a different method of quantification. PG from *Erythrobacter* sp. (class *Alphaproteobacteria*) was purified according to Pelz *et al.* (1998). Briefly, the extraction included SDS treatment at 100 °C for 1 h to obtain cell lysis, sonication for 1 min for further cell destruction, digestion with trypsin to remove proteins and extraction of lipids with organic solvents of decreasing polarity. The purified PG was added to 1/25 TSB and 1/25

TSA that was poured on top of 1/25 TSA without PG. The final PG concentration was 2 mg l⁻¹ in both the liquid medium and the top layer. In the 1/25 TSB, growth of inoculated cells was measured as the increase in cell density relative to controls (1/25 TSB without PG). At daily intervals, 50 μ l of a 1:10 000-diluted stock solution of SYBR Green I stain (Invitrogen) was added to triplicate subsamples of 2 ml, after which the fluorescence intensity (excitation 490 nm; emission 520 nm) was determined. For tests on solid medium, cells were spread on 1/25 TSA plates with and without a PG-containing top layer. Utilization (hydrolysis) of PG was determined from clearing (opaque) zones around the colonies formed on the almost-transparent PG-containing agar.

Capacity for growth was tested at different temperatures (3, 5, 10, 20, 25, 30 and 37 °C), salinities (0, 1, 3, 6 and 10 %, w/v, NaCl) and pH (pH 5, 6, 7, 8, 9 and 10) in 10 ml of 1/10 or full-strength TSB incubated on a shaking table at 20 °C (3–37 °C for analysis of temperature preference). Similarly, the growth rate was determined at 20 °C in both 1/10 and full-strength TSB.

Cellular morphology and size of strain 332^T were determined by scanning electron microscopy (SEM) of an overnight culture grown in 1/10 TSB and filtered onto 0.2 μ m pore-size polycarbonate filters. The filters were fixed and dehydrated according to Watson *et al.* (1980), mounted on aluminium stubs and sputter-coated with gold/palladium. The cells were observed with a Quanta 200 SEM microscope (FEI Company). Sizes of the cells were measured using the program LCS LITE (Leica Microsystems).

Genomic DNA was isolated from strain 332^T after cultivation of cells in TSB overnight as described previously (Brandt *et al.*, 2006). The 16S rRNA gene sequence was amplified by PCR using primers Fd1 and Rd1 (Brandt *et al.*, 1999) and purified by a QIAquick PCR purification kit (Qiagen). The nearly complete 16S rRNA gene sequence (1543 bp) was obtained by sequencing the gene in both directions (GATC Biotech).

Strain 332^T was a Gram-negative-staining, non-spore-forming, motile (although no flagella were observed by SEM) rod-shaped bacterium. Cells were 2.3 ± 0.7 μ m long and 0.7 ± 0.1 μ m wide. Cells grew between 3 and 37 °C, with optimum growth at 25 °C. Growth was observed at pH 5–10, with optimum growth at pH 6–7. Growth was observed at salinities from 0 to 6 g NaCl l⁻¹, with optimum growth at 1 g l⁻¹. The cells were oxidase- and catalase-positive. Growth occurred on the following compounds with oxygen as the electron acceptor: phenylacetic acid, L-asparagine, adipic acid, 4-hydroxybenzoic acid, α -ketobutyric acid, caproic acid, citrate, gluconic acid, 2- and 5-ketogluconate, itaconic acid, D-malic acid, malonic acid, L-phenylalanine, L-threonine, L-arabinose, *N*-acetyl-D-glucosamine (weak reaction), D-mannose, glycogen, D-mannitol, gelatin, pyruvic acid methyl ester and Tweens 40 and 80. Nitrate reduction to nitrite occurred, but no anaerobic growth or fermentation was observed, as

determined from the O-F reaction (Hugh & Leifson, 1953). Doubling times at 20 °C were 6.0 and 1.7 h in 1/10 and full-strength TSB, respectively. The following compounds did not support growth: D-glucose, cellobiose, aesculin, galactopyranoside, glucose 1-phosphate, glycogen, maltose, α -cyclodextrin, methyl β -D-glucoside, D-xylose, i-erythritol, D-galactonic acid γ -lactone, L-arginine, D-galacturonic acid, 2-hydroxybenzoic acid, L-serine, D-glucosaminic acid, glycyl L-glutamic acid, phenylethylamine, α -lactose, DL- α -glycerol phosphate, putrescine and tryptophan. Enzyme activities of strain 332^T included acid and alkaline phosphatases, chitinase, esterase (C4), esterase lipase (C8), α - and β -glucosidases, leucine and valine arylamidases, lysozyme and naphthol-AS-BI-phosphohydrolase. Strain 332^T exhibited weak proteolytic activity (hydrolysis of casein) but did not degrade starch.

PG in liquid medium supported growth of both strain 332^T and *D. tsuruhatensis* DSM 17581^T. After 3 days, cell densities of strain 332^T and *D. tsuruhatensis* DSM 17581^T were 1.9- to 3.2-fold higher in the PG-enriched cultures than in cultures without PG. At day 5, the cell density had doubled again in the cultures of strain 332^T enriched with PG, while no additional growth was found for *D. tsuruhatensis* DSM 17581^T in the PG-enriched medium after day 3. On solid medium enriched with PG, 3–4 mm opaque zones were formed around the colonies, indicating some reaction with PG; no clear zones were observed. No DNase activity was observed for strain 332^T, *D. tsuruhatensis* DSM 17581^T or *D. acidovorans* DSM 39^T. Differences between strain 332^T, *D. tsuruhatensis* DSM 17581^T and *D. acidovorans* DSM 39^T with respect to utilization of organic compounds and enzyme activities are given in Table 1.

The chitinase and lysozyme activities indicate that strain 332^T has the capacity to break β (1,4) glycosidic bonds in chitin and PG, and this may explain its success in utilizing bacterial cell-wall material from Gram-positive as well as Gram-negative bacteria, as observed during the initial screening on PG-enriched agar. The capacity to degrade chitin and PG has not previously been reported for the genus *Delftia*. The observed opaque, rather than clear, zones around the colonies on PG-enriched agar may indicate that PG was not completely degraded and assimilated by strain 332^T. The weak reaction on N-acetyl-D-glucosamine (Biolog and API kits) further suggests slow utilization of some PG components.

The dominant fatty acids in strain 332^T were C_{16:1} ω 7*c*/iso-C_{15:0} 2-OH (36.9 % of total fatty acid esters), C_{16:0} (34.3 %), C_{18:1} ω 7*c* (15.4 %) and C_{17:0} cyclo (6.1 %) (data not shown). Two fatty acids, C_{15:1} ω 6*c* and C_{17:1} ω 8*c*, were not found in strain 332^T, but only in *D. tsuruhatensis* DSM 17581^T. The sum of the C_{17:0} and C_{19:0} cyclopropane fatty acids, which are produced mainly during the late growth phase, and their precursors, C_{17:1} ω 8*c* + C_{16:1} ω 5*c* and C_{19:0} cyclo ω 8*c* + C_{18:1} ω 7*c*, was higher in strain 332^T (42.9 and 16.1 %, respectively) than in *D. tsuruhatensis* DSM 17581^T (41.0 and 14.8 %, respectively) (Table 1). C_{8:0} 3-OH and

C_{10:0} 3-OH, which are characteristic fatty acids of the genus *Delftia* (Wen *et al.*, 1999), were also found in strain 332^T, although C_{8:0} 3-OH occurred in only trace amounts (0.2 % of the total fatty acid esters). The DNA G + C content of strain 332^T was 65.3 mol%.

BLAST (Johnson *et al.*, 2008) and SEQMATCH (RDP 9 website; Cole *et al.*, 2007) comparisons of 16S rRNA gene sequences both showed that strain 332^T grouped within the family *Comamonadaceae* within the order *Burkholderiales* of the class *Betaproteobacteria*. Hence, all 55 published type-strain sequences within this family were retrieved from the RDP 9 database and used for initial phylogenetic analysis (Treebuilder; RDP 9) with *Escherichia coli* as the outgroup (data not shown). Based on taxonomic and sequence-quality criteria, a subset of the 55 sequences was selected. These sequences were aligned (RDP 9) with subsequent manual inspection and minor corrections being performed in BioEdit (Hall, 1999) version 7.0.9.0, and CLUSTAL X 2.0 software (Larkin *et al.*, 2007) was finally used for the calculation of pairwise nucleotide similarity values based on unambiguous nucleotides (Jukes–Cantor algorithm), for construction of phylogenetic trees by the neighbour-joining method and for validation of the tree topology by bootstrapping (1000 resamplings).

Phylogenetic analysis of the 16S rRNA gene sequences (Fig. 1) clearly showed that strain 332^T belonged to the genus *Delftia* and further indicated that *D. tsuruhatensis* T7^T was the closest relative, with a nucleotide similarity of 99.9 %. This finding was substantiated in a subsequent phylogenetic analysis including 32 *Delftia* 16S rRNA gene sequences present within the RDP 9 database (not shown). Hence, all *Delftia* sequences could be divided into two phylogenetic clusters, supported by a bootstrap value of 57 %, with *D. acidovorans* sequences in one cluster and *D. tsuruhatensis* sequences and strain 332^T in the other cluster.

DNA–DNA hybridization experiments performed at the DSMZ using standard protocols confirmed the close taxonomic relationship between strain 332^T and *D. tsuruhatensis* DSM 17581^T and, to a lesser extent, also with *D. acidovorans* DSM 39^T (Table 1). However, the DNA–DNA relatedness values fell below the 70 % threshold value for the delineation of novel species (Wayne *et al.*, 1987). Distinct phenotypic characteristics were observed for strain 332^T (capacity to utilize D-mannitol, D-malic acid, D-galactonic acid lactone and D-gluconic acid, as well as chitinase activity and a significantly higher growth with PG in liquid medium; Table 1), and therefore we propose that the PG-degrading strain that has been isolated from lake water represents a novel species within the genus *Delftia*, for which the name *Delftia lacustris* sp. nov. is proposed.

Emended description of *Delftia tsuruhatensis* Shigematsu *et al.* 2003

In the original description by Shigematsu *et al.* (2003), information on degradation of PG was not included. Tests

Table 1. Differential characteristics of strain 332^T and the type strains of *Delftia* species

Strains: 1, *Delftia lacustris* sp. nov. 332^T; 2, *D. tsuruhatensis* DSM 17581^T; 3, *D. acidovorans* DSM 39^T. +, Positive; w, weak; –, negative; ND, not reported/not determined. Data were obtained in this study unless indicated otherwise.

Characteristic	1	2	3
Use of carbon sources for growth			
D-Mannitol	+	–	+*
D-Malic acid	+	–	+*
D-Galactonic acid lactone	w	–	ND
D-Gluconic acid	w	–	+*
γ-Hydroxybutyric acid	+	+	–
L-Serine	–	–	w†
Enzyme activities			
Chitinase	+	–	ND
Lysozyme	+	+‡	ND
Proteolytic (casein)	w	w	–*§
Degradation of PG in liquid culture and agar	+	+	ND
Major 3-OH fatty acids	10:0, 8:0	10:0	10:0, 8:0*
Cellular lipids (%)			
C _{15:0}	0.69	4.22	ND
C _{17:0}	0.31	1.79	ND
C _{17:1ω8c} +C _{16:1ω5c}	42.9	41.0	ND
C _{19:0 cyclo ω8c} +C _{18:1ω7c}	16.1	14.8	ND
DNA G+C content (mol%)	65.3	66.2	67*
DNA–DNA relatedness to strain 332 ^T	ND	66.2±0.6	36.0±4.2

*Data from Wen *et al.* (1999).

†Wen *et al.* (1999) did not observe growth on L-serine.

‡Lysozyme activity was about 25 % higher for *D. tsuruhatensis* DSM 17581^T than for strain 332^T.

§Liquefaction of gelatin was negative.

||Data from Shigematsu *et al.* (2003).

demonstrate that *D. tsuruhatensis* DSM 17581^T is capable of degrading PG in both liquid and solid media, and that it possesses extracellular lysozyme activity (Table 1). In

contrast to strain 332^T, no chitinase activity is found in *D. tsuruhatensis* DSM 17581^T.

Description of *Delftia lacustris* sp. nov.

Delftia lacustris [la.cus'tris. N.L. fem. adj. *lacustris* (L. n. *lacus* a lake) growing by lakes or ponds].

Gram-negative-staining, motile rods ($2.3 \pm 0.7 \times 0.7 \pm 0.1 \mu\text{m}$). Growth occurs at 3–37 °C; optimum growth at 25 °C. Growth occurs at pH 5–10; optimum growth at pH 6–7. Growth occurs at salinities of 0–6 g NaCl l^{–1}; optimum growth at 1 g l^{–1}. Doubling time is 1.7 h at 20 °C with full-strength TSB. Non-fermentative. Positive for acid and alkaline phosphatases, chitinase, esterase (C4), esterase lipase (C8), α- and β-glucosidases, leucine and valine arylamidases, lysozyme and naphthol-AS-BI-phosphohydrolase. Denitrification ability is not observed, but nitrate reduction is observed. The following compounds can be utilized as energy and carbon sources: phenylacetic acid, L-asparagine, adipic acid, 4-hydroxybenzoic acid, α-ketobutyric acid, caproic acid, citrate, gluconic acid, 2- and 5-ketogluconate, itaconic acid, D-malic acid, malonic acid, L-phenylalanine, L-threonine, L-arabinose, N-acetyl-D-glu-

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences from strain 332^T and related type strains. *Neisseria gonorrhoeae* NCTC 8375^T was used as the outgroup. Bootstrap values (≥50 %) based on 1000 resamplings are shown at branch nodes. Bar, 0.01 substitutions per site.

cosamine, D-glucose, D-mannose, glycogen, D-mannitol, gelatin, peptidoglycan, pyruvic acid methyl ester and Tweens 40 and 80. The following compounds cannot be utilized: D-glucose, cellobiose, aesculin, galactopyranoside, glucose 1-phosphate, glycogen, maltose, α -cyclodextrin, methyl β -D-glucoside, D-xylose, D-erythritol, D-galactonic acid γ -lactone, L-arginine, D-galacturonic acid, 2-hydroxybenzoic acid, L-serine, D-glucosaminic acid, glycyl L-glutamic acid, phenylethylamine, α -lactose, DL- α -glycerol phosphate, putrescine and tryptophan. The dominant cellular fatty acids are C_{16:1} ω 7*c*/iso-C_{15:0} 2-OH, C_{16:0}, C_{18:1} ω 7*c* and C_{17:0} cyclo; C_{10:0} 3-OH, C_{12:0}, C_{14:0}, C_{15:0} and C_{8:0} 3-OH are found in smaller amounts. The DNA G+C content of the type strain is 65.3 mol%.

The type strain is 332^T (=DSM 21246^T =LMG 24775^T), isolated from mesotrophic lake water.

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References

- Benner, R. & Kaiser, K. (2003). Abundance of amino sugars and peptidoglycan in marine particulate and dissolved organic matter. *Limnol Oceanogr* **48**, 118–128.
- Brandt, K. K., Patel, B. K. C. & Ingvorsen, K. (1999). *Desulfocella halophila* gen. nov., sp. nov., a halophilic fatty acid-oxidizing sulfate-reducing bacterium isolated from sediments of the Great Salt Lake. *Int J Syst Bacteriol* **49**, 193–200.
- Brandt, K. K., Petersen, A., Holm, P. E. & Nybroe, O. (2006). Decreased abundance and diversity of culturable *Pseudomonas* spp. populations with increasing copper exposure in the sugar beet rhizosphere. *FEMS Microbiol Ecol* **56**, 281–291.
- Cody, R. M. (1989). Distribution of chitinase and chitobiase in *Bacillus*. *Curr Microbiol* **19**, 201–205.
- Cole, J. R., Chai, B., Farris, R. J., Wang, Q., Kulam-Syed-Mohideen, A. S., McGarrell, D. M., Bandela, A. M., Cardenas, E., Garrity, G. M. & Tiedje, J. M. (2007). The Ribosomal Database Project (RDP-II): introducing myRDP space and quality controlled public data. *Nucleic Acids Res* **35**, D169–D172.
- De Ley, J., Cattoir, H. & Reynaert, A. (1970). Quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Eisenstadt, E., Carlton, B. C. & Brown, B. J. (1994). Gene mutation. In *Methods for General and Molecular Bacteriology*, pp. 297–316. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Frette, L., Johnsen, K., Jørgensen, N. O. G., Nybroe, O. & Kroer, N. (2004). Functional characteristics of culturable bacterioplankton from marine and estuarine environments. *Int Microbiol* **7**, 219–227.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Hugh, R. & Leifson, E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. *J Bacteriol* **66**, 24–26.
- Jeffries, C. D., Holtman, D. F. & Guse, D. G. (1957). Rapid method for determining the activity of microorganisms on nucleic acids. *J Bacteriol* **73**, 590–591.
- Johnson, M., Zaretskaya, I., Raytselis, Y., Merezuk, Y., McGinnis, S. & Madden, T. L. (2008). NCBI BLAST: a better web interface. *Nucleic Acids Res* **36**, W5–W9.
- Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A. & other authors (2007). CLUSTAL W and CLUSTAL_X version 2.0. *Bioinformatics* **23**, 2947–2948.
- Nagata, T., Meon, B. & Kirchman, D. L. (2003). Microbial degradation of peptidoglycan in seawater. *Limnol Oceanogr* **48**, 745–754.
- O'Brien, M. & Colwell, R. R. (1987). A rapid test for chitinase activity that uses 4-methylumbelliferyl-N-acetyl-beta-D-glucosaminide. *Appl Environ Microbiol* **53**, 1718–1720.
- Park, J. T. & Uehara, T. (2008). How bacteria consume their own exoskeletons (turnover and recycling of cell wall peptidoglycan). *Microbiol Mol Biol Rev* **72**, 211–227.
- Pelz, O., Cifuentes, L. A., Hammer, B. T., Kelley, C. A. & Coffin, R. B. (1998). Tracing the assimilation of organic compounds using $\delta^{13}\text{C}$ analysis of unique amino acids in the bacterial peptidoglycan cell wall. *FEMS Microbiol Ecol* **25**, 229–240.
- Shigematsu, T., Yumihara, K., Ueda, Y., Numaguchi, M., Morimura, S. & Kida, K. (2003). *Delftia tsuruhatensis* sp. nov., a terephthalate-assimilating bacterium isolated from activated sludge. *Int J Syst Evol Microbiol* **53**, 1479–1483.
- Wang, S. L. & Chang, W. T. (1997). Purification and characterization of two bifunctional chitinases/lysozymes extracellularly produced by *Pseudomonas aeruginosa* K-187 in a shrimp and crab shell powder medium. *Appl Environ Microbiol* **63**, 380–386.
- Watson, L. P., McKee, A. E. & Merrell, B. R. (1980). Preparation of microbiological specimens for scanning electron microscopy. *Scan Electron Microsc* **2**, 45–56.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Wen, A., Fegan, M., Hayward, C., Chakraborty, S. & Sly, L. I. (1999). Phylogenetic relationships among members of the *Comamonadaceae*, and description of *Delftia acidovorans* (den Dooren de Jong 1926 and Tamaoka *et al.* 1987) gen. nov., comb. nov. *Int J Syst Bacteriol* **49**, 567–576.