Methanococcus aeolicus sp. nov., a mesophilic, methanogenic archaeon from shallow and deep marine sediments

Melissa M. Kendall,¹† Yitai Liu,¹ Magdalena Sieprawska-Lupa,³
Karl O. Stetter,² William B. Whitman³ and David R. Boone¹†

Correspondence

Melissa M. Kendall
melissa.kendall@utsouthwestern.
edu

¹Biology Department, Portland State University, PO Box 751, Portland, OR 97207-0751, USA

²Universität Regensburg, Lehrstuhl für Mikrobiologie, Universitätstraße 31, 93343 Regensburg, Germany

³Department of Microbiology, University of Georgia, 541 Biological Sciences Building, Athens, GA 30602-2605, USA

Three strains of CO₂-reducing methanogens were isolated from marine sediments. Strain PL-15/H^P was isolated from marine sediments of the Lipari Islands, near Sicily and the other two strains, Nankai-2 and Nankai-3^T, were isolated from deep marine sediments of the Nankai Trough, about 50 km from the coast of Japan. Analysis of the cellular proteins and 16S rRNA gene sequences indicated that these three strains represented a single novel species that formed a deep branch of the mesophilic methanococci. Phylogenetic analysis indicated that the three strains were most closely related to *Methanothermococcus okinawensis* (95 % 16S rRNA gene sequence similarity). However, strains PL-15/H^P, Nankai-2 and Nankai-3^T grew at temperatures that were more similar to those of recognized species within the genus *Methanococcus*. Strain Nankai-3^T grew fastest at 46 °C. Results of physiological and biochemical tests allowed the genotypic and phenotypic differentiation of strains PL-15/H^P, Nankai-2 and Nankai-3^T from closely related species. The name *Methanococcus aeolicus* sp. nov. is proposed, with strain Nankai-3^T (= OCM 812^T = DSM 17508^T) as the type strain.

In 1984, novel restriction enzymes were found in a methanogenic archaeon, strain PL-15/H^P, which had been isolated from the Mediterranean Sea near Sicily (Schmid *et al.*, 1984). At that time, it was suggested that this strain might represent a novel species, '*Methanococcus aeolicus*'. Because the strain was not freely available due to patent restrictions, this species was not formally described. Nevertheless, the genetic and phylogenetic distinctiveness of strain PL-15/H^P from other *Methanococcus* species was confirmed by electrophoretic analysis of cellular proteins, DNA–DNA hybridization and 16S rRNA gene sequence analysis (Keswani *et al.*, 1996). However, because of the

absence of a type strain deposited in culture collections, the species '*Methanococcus aeolicus*' has lacked standing in nomenclature (Whitman *et al.*, 2001). Subsequently, sequences of 16S rRNA genes very similar to that of strain PL-15/H^P have been found in marine hydrothermal vent fluids (Huber *et al.*, 2002), supporting the importance of this phylogenetic group in marine sediments.

We report here the isolation and description of two novel methanogenic strains (Nankai-2 and Nankai-3^T) from deep marine sediments of the Nankai Trough off the coast of Japan, which are phylogenetically and phenotypically similar to strain PL-15/H^P. We also report the description and isolation of strain PL-15/H^P, accomplished in the early 1980s. We propose that the three strains represent a novel species, *Methanococcus aeolicus* sp. nov., with strain Nankai-3^T as the type strain and strains PL-15/H^P and Nankai-2 as reference strains.

Strain PL-15/H^P was isolated from shallow marine sediment collected from the Lipari Islands near Sicily. Strains Nankai-2 and Nankai-3^T were enriched and isolated from sediment taken from the Nankai Trough near the coast of Japan in November 1997. *Methanoculleus submarinus* Nankai-1^T had

†Deceased 27 May 2005.

‡Present address: Department of Microbiology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390-9048, USA.

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of strains PL-15/H^P, Nankai-2 and Nankai-3^T are DQ136171, DQ195165 and DQ195164, respectively.

A figure showing the effects of temperature, pH and salinity on the growth of strain Nankai-3^T is available as supplementary material in IJSEM Online.

been isolated previously from the same sample (Mikucki *et al.*, 2003). At the sampling site, the water was 950 m deep, and the sample was obtained from a core at a depth of 247 m below the sediment surface, near the zone where methane hydrates occurred (Mikucki *et al.*, 2003). The sample was placed in a canning jar, flushed with argon, sealed and maintained at 2–4 °C for 5 days until it was processed at the Idaho Environmental and Engineering Laboratory by Mark Delwiche (Mikucki *et al.*, 2003). The core was then placed in an anaerobic chamber and pared, and 10 g from the interior of the core was added to 90 ml MSH medium in a serum bottle. The bottle was pressurized with H₂ and incubated at room temperature. After 2 months, increases in CH₄ in the headspace of the bottle ceased (Mikucki *et al.*, 2003). This enrichment culture was then transported to Portland State University and inoculated into roll tubes in MSH enrichment medium under H₂/CO₂ (Mikucki *et al.*, 2003). To distinguish colonies of methanogens from those of non-methanogens, unopened roll tubes were observed by epifluorescence microscopy (Zeiss O2 filter set). Two epifluorescent colonies were picked and named Nankai-2 and Nankai-3^T. Each strain grew at 37 °C and was further purified by colony picks from roll-tube cultures incubated at 37 °C.

Unless indicated otherwise, the culture medium used in this study was MSH medium (Boone *et al.*, 1989; Ni & Boone, 1991) at pH 7.2, with an overpressure of 1 atm H₂. Incubations were at 37 °C with shaking. MSH enrichment medium was the same as MSH medium except that the amounts of Trypticase peptones and yeast extract were reduced to 0.25 g l⁻¹, mercaptoethane sulfonic acid was omitted and the sodium sulfide concentration was increased to 0.5 g l⁻¹. For experiments on the effect of salinity, NaCl was added to MSH medium to give various Na⁺ concentrations. For preparation of media at different pH values, lower pH values were obtained by adjustment with HCl. Higher pH values were obtained by using a mixture of N₂ and CO₂ for the gas phase (Chong *et al.*, 2002). pH values above 7.8 were obtained by using a 100 % CO₂ gas phase and adjusting the pH with NaOH. The anaerobic Hungate technique with serum-tube modifications was used throughout (Hungate, 1969). Substrate tests were conducted by adding acetate (40 mM), trimethylamine (20 mM), methanol (20 mM), ethanol (1 %, v/v), 1-propanol (1 %), 2-propanol (1 %), 1-butanol (1 %) or 2-butanol (1 %) to the medium. Growth was estimated from the accumulation of methane in the headspace gas, as measured by gas chromatography (Maestrojuán & Boone, 1991), taking into account the methane produced during the growth of the inoculum (Powell, 1983). The specific growth rate was calculated by fitting the Gompertz equation (Zwietering *et al.*, 1990) to these data. All growth experiments were performed in triplicate.

The 16S rRNA gene sequences were determined after extraction of DNA from cell pellets of pure cultures of strains PL-15/H^P, Nankai-2 and Nankai-3^T by using QIAamp DNA

Mini kit procedures (Qiagen). The 16S rRNA genes were amplified by PCR, purified and sequenced as described previously (Singh *et al.*, 2005). The sequences were aligned manually with related sequences obtained from the Ribosomal Database Project (Maidak *et al.*, 2001). Regions where alignment was ambiguous were not used in the phylogenetic analysis. Phylogenetic relationships were determined with maximum-likelihood analysis using the HKY-85 model of evolution in PAUP* 4.0 (Swofford, 2002). A bootstrap analysis was performed using 1000 trial replications to provide confidence estimates for branch support. The sequence we obtained for strain PL-15/H^P was the same as the sequence already in GenBank (accession no. U39016), except that many of the unknown bases in the earlier sequence were successfully resolved. Whole proteins of strains PL-15/H^P, Nankai-2 and Nankai-3^T were compared using SDS-PAGE of cellular proteins, as described previously (Franklin *et al.*, 1988).

Colonies of strains PL-15/H^P, Nankai-2 and Nankai-3^T were almost transparent and whitish, with smooth edges when observed by the naked eye or hand lens and 1 mm in diameter after 40 days of incubation at 37 °C. Cells of all three strains were motile irregular coccoids, 1.5–2 µm in diameter, and occurred singly. When viewed by epifluorescence microscopy with a Zeiss O2 filter set, cells were autofluorescent, as is typical of methanogens. Each of the three strains was susceptible to lysis with 0.01 % SDS, suggesting the presence of a protein cell wall (Boone & Whitman, 1988).

Each of the three strains grew with H₂ (1 atm) or formate (80 mM) as electron donor for reduction of CO₂. Cells did not form methane from acetate, trimethylamine or methanol, either alone or in combination with formate. In addition, in the mineral medium McN (Whitman *et al.*, 1986), strain Nankai-3^T failed to form methane or grow in the presence of ethanol, 1-propanol or 2-propanol after 5 days of incubation. The concentrations of these potential catabolic substrates did not appear to be inhibitory as, when cultures were incubated with these concentrations plus 5 or 20 mM formate, methane was formed in the amount expected from formate alone. In contrast, 1 % 1-butanol and 2-butanol completely inhibited methanogenesis from formate.

Each strain grew autotrophically in MSH medium plus H₂ when all organic compounds were omitted. Growth occurred even after four successive transfers with 2.5 % (v/v) inoculum in mineral medium. In the McN mineral medium, growth of strain PL-15/H^P was stimulated by the addition of 11 µM sodium selenite. Strain PL-15/H^P could also use either S⁰ or H₂S as the sole sulfur source and either NH₃ or N₂ as the sole nitrogen source. Amino acids were not used as nitrogen sources (Whitman *et al.*, 1987).

Strain PL-15/H^P grew at 20–45 °C, but did not grow at 50 °C. Temperatures below 20 °C were not tested. Strain Nankai-2 grew at 10–50 °C, with no growth at 55 °C (temperatures

Table 1. Comparison of physiological characteristics of isolates PL-15/H^P, Nankai-2, Nankai-3^T and other recognized species of *Methanococcus* and *Methanothermococcus*

Species: 1, *Methanococcus aeolicus* PL-15/H^P; 2, *Methanococcus aeolicus* Nankai-2; 3, *Methanococcus aeolicus* Nankai-3^T; 4, *Methanococcus vannielii* SB^T (data from Stadtman & Barker, 1951); 5, *Methanococcus voltae* PS^T (Balch *et al.*, 1979; Whitman *et al.*, 1982); 6, *Methanococcus maripaludis* JJ^T (Jones *et al.*, 1983); 7, *Methanothermococcus okinawensis* IH1^T (Takai *et al.*, 2002); 8, *Methanothermococcus thermolithotrophicus* SN-1^T (Huber *et al.*, 1982). ND, Not determined.

Characteristic	<i>Methanococcus aeolicus</i>			4	5	6	7	8
	1	2	3					
DNA G+C content (mol%)	32*	ND	ND	31–33	30.7	33	33.5	32.5
Cell diameter (µm)	1.5–2.0	1.5–2.0	1.5–2.0	1.3	1.3–1.7	0.9–1.3	1.0–1.5	1.5
Autotrophic growth	+	+	+	+	–	+	+	
Nitrogen source(s)	NH ₃ , N ₂	ND	ND	NH ₃	NH ₃	NH ₃ , N ₂ , alanine	NH ₃	N ₂ , NO ₃ [–] , NH ₃
Temperature range (°C)	≤20–45	≤10–50	≤20–55	≤20–45	≤20–45	18–47	40–75	17–70
pH range	6.5–8.0	4.3–7.5	5.5–7.5	6.5–8.0	6.5–8.0	6.4–8.2	4.5–8.5	4.9–9.8
Na ⁺ range (M)	0.05–0.8	0.05–1.0	0.05–1.0	0.05–0.8	0.08–0.8	0.05–0.8	0.08–1.0	0.1–1.6

*Data from Whitman (2001).

below 10 °C were not tested). Strain Nankai-3^T grew at 20–55 °C, but not at 10 or 60 °C. The growth response of strain Nankai-3^T to temperature was fitted to the square-root equation of Ratkowsky *et al.* (1982, 1983). Using this method, the theoretical optimum temperature for growth was determined to be 46.0 °C, and the minimum and maximum temperatures were 7.1 and 54.7 °C, respectively (Supplementary Fig. S1a in IJSEM Online). This temperature response was somewhat more thermophilic than that found in other methanococci. For instance, in McN medium, the temperature optima of *Methanococcus maripaludis* strains JJ^T and S2 were 37 °C and the maximum growth temperatures were 43 °C (no growth at 45 °C) and 47 °C, respectively (W. B. Whitman, unpublished data; Jones *et al.*, 1983). In contrast, the temperature optima and maxima for *Methanothermococcus* species are much more extreme, being greater than 60 and 70 °C, respectively (Table 1). Salinity and pH ranges for strains Nankai-3^T, Nankai-2 and PL-15/H^P are given in Table 1 and are

shown for strain Nankai-3^T (see Supplementary Fig. S1b, c in IJSEM Online).

The 16S rRNA gene sequences of each of the three strains were more similar to each other (>99 %) than to any other sequence in GenBank. Fig. 1 shows an evolutionary tree based on a maximum-likelihood-generated comparison of the 16S rRNA gene sequences. As noted previously by Takai *et al.* (2002), *Methanococcus aeolicus* is more closely related using this method to the thermophile *Methanothermococcus okinawensis* (95 % sequence similarity) than to the other mesophilic methanococci (91–93 % sequence similarity). In 2001, the genus *Methanococcus* was reorganized to include all mesophilic species, which at that time formed a monophyletic group of the *Methanococcales* (Whitman *et al.*, 2001). The thermophile *Methanococcus thermolithotrophicus* was also transferred to a new genus, *Methanothermococcus*. The discovery of *Methanothermococcus okinawensis* suggested that this classification scheme might be too simplistic.

Fig. 1. Maximum-likelihood phylogenetic tree of *Methanococcus* species and strains PL-15/H^P, Nankai-2 and Nankai-3^T, based on 16S rRNA gene sequences. Numbers at nodes are percentage bootstrap values based on 1000 iterations; only values >50 % are shown. Sequences of *Thermococcus kodakarensis* strain IC-125^T (GenBank accession no. AB005296) and *Acidianus infernus* So4a^T (accession no. D85505) were used as outgroups (not shown). Bar, 0.05 substitutions per site.

Fig. 2. SDS-PAGE of cellular proteins of strains PL-15/H^P, Nankai-2 and Nankai-3^T. Lanes: 1, molecular mass markers (in kDa); 2, *Methanococcus maripaludis* strain S2; 3, strain PL-15/H^P; 4, strain Nankai-2; 5, strain Nankai-3^T.

Because this thermophile forms a new clade with the mesophile *Methanococcus aeolicus* in rRNA gene trees, growth temperature may not prove to be a reliable discriminatory factor within this group. Since *Methanothermococcus okinawensis* and *Methanococcus aeolicus* both possess low sequence similarity to other *Methanothermococcus* species as well as to *Methanococcus* species, a new genus for these species may be warranted. This conclusion is supported by the elevated temperature optimum of *Methanococcus aeolicus* and the fact that the two species are present in submarine sediments. Nevertheless, in the absence of additional unifying characteristics, these criteria are judged insufficient in themselves to justify formation of a novel genus.

The overall similarity of the protein profiles of strains PL-15/H^P, Nankai-2 and Nankai-3^T was within the range observed for members of the same species of other methanococci and was quite different from the protein profile of *Methanococcus maripaludis* (Fig. 2; Keswani *et al.*, 1996). The protein profiles of strains Nankai-2 and Nankai-3^T were very similar; however, several physiological differences existed between these strains (Table 1).

Based on their cellular protein profiles, 16S rRNA gene sequences and phenotypic properties, strains PL-15/H^P, Nankai-2 and Nankai-3^T are all closely related and belong in the same species. Nevertheless, these strains are physiologically distinct, as shown in Table 1, and small differences are observed in their cellular protein profiles. DNA–DNA hybridization and cellular protein profiles also confirm that strain PL-15/H^P is distinct from other described species of *Methanococcus* (Keswani *et al.*, 1996). Thus, we propose that strains PL-15/H^P, Nankai-2 and Nankai-3^T represent a

novel species, with the name *Methanococcus aeolicus* sp. nov.

Description of *Methanococcus aeolicus* sp. nov.

Methanococcus aeolicus (ae.o'li.cus. L. masc. adj. *aeolicus* Æolian, referring to the Lipari Islands near Sicily, the source of the first isolated strain).

Coccoid cells, 1.5–2 µm in diameter and occurring singly, motile and sensitive to lysis by detergent. Strictly anaerobic, growing autotrophically by reduction of CO₂ to CH₄, with H₂ (or formate) as electron donor. Acetate, methanol, methylamines, ethanol, 1-propanol and 2-propanol are not catabolized. 1-Butanol and 2-butanol inhibit methane production. N₂ or NH₃ may serve as nitrogen source; H₂S or S⁰ may serve as sulfur source. Selenium stimulates growth. Grows fastest at mesophilic temperatures or slightly above (46 °C), near neutral pH and at salinities of 0.2–0.4 M Na⁺. Maximum specific growth rate is approximately 0.5 h⁻¹. The G+C content of the DNA of strain PL-15/H^P is 32 mol% (by chromatographic analysis of bases). Habitat is marine sediments.

The type strain is strain Nankai-3^T (= OCM 812^T = DSM 17508^T); strains PL-15/H^P (= OCM 836^P = DSM 17251^P) and Nankai-2 (= OCM 811) are reference strains.

Acknowledgements

We thank T. Uchida (Japanese Petroleum Exploration Company), Mark Delwiche and Fredrick S. Colwell (both Idaho National Environmental and Engineering Laboratory) for providing the core samples from the Nankai Trough. We also acknowledge J. P. Euzéby for help with the etymology of the species name. This work was supported by grants from the US Department of Energy's Subsurface Science program to the Subsurface Microbial Culture Collection (DOE prime contract DE-FG02-96ER62210 through purchase order G09965 from Florida State University), the National Science Foundation's LExEn program (prime contract OCE-0085607 via subcontract from University of California at Irvine, L00OCE0085607) and an IGERT Environmental Subsurface Biosphere fellowship to M. M. K.

References

- Balch, W. E., Fox, G. E., Magrum, L. J., Woese, C. R. & Wolfe, R. S. (1979). Methanogens: reevaluation of a unique biological group. *Microbiol Rev* **43**, 260–296.
- Boone, D. R. & Whitman, W. B. (1988). Proposal of minimal standards for describing new taxa of methanogenic bacteria. *Int J Syst Bacteriol* **38**, 212–219.
- Boone, D. R., Johnson, R. L. & Liu, Y. (1989). Diffusion of the inter-species electron carriers H₂ and formate in methanogenic ecosystems and its implications in the measurement of K_m for H₂ or formate uptake. *Appl Environ Microbiol* **55**, 1735–1741.
- Chong, S. C., Liu, Y., Cummins, M., Valentine, D. L. & Boone, D. R. (2002). *Methanogenium marinum* sp. nov., a H₂-using methanogen from Skan Bay, Alaska, and kinetics of H₂ utilization. *Antonie van Leeuwenhoek* **81**, 263–270.

- Franklin, M. J., Wiebe, W. J. & Whitman, W. B. (1988). Populations of methanogenic bacteria in a Georgia salt marsh. *Appl Environ Microbiol* **54**, 1151–1157.
- Huber, H., Thomm, M., König, H., Thies, G. & Stetter, K. O. (1982). *Methanococcus thermolithotrophicus*, a novel thermophilic lithotrophic methanogen. *Arch Microbiol* **132**, 47–50.
- Huber, J. A., Butterfield, D. A. & Baross, J. A. (2002). Temporal changes in archaeal diversity and chemistry in a mid-ocean ridge subseafloor habitat. *Appl Environ Microbiol* **68**, 1585–1594.
- Hungate, R. E. (1969). A roll-tube method for the cultivation of strict anaerobes. *Methods Microbiol* **3B**, 117–132.
- Jones, W. J., Paynter, M. J. B. & Gupta, R. (1983). Characterization of *Methanococcus maripaludis* sp. nov., a new methanogen isolated from salt marsh sediment. *Arch Microbiol* **135**, 91–97.
- Keswani, J., Orkand, S., Premachandran, U., Mandelco, L., Franklin, M. J. & Whitman, W. B. (1996). Phylogeny and taxonomy of mesophilic *Methanococcus* spp. and comparison of rRNA, DNA hybridization, and phenotypic methods. *Int J Syst Bacteriol* **46**, 727–735.
- Maestrojuán, G. M. & Boone, D. R. (1991). Characterization of *Methanosarcina barkeri* MS^T and 227, *Methanosarcina mazei* S-6^T, and *Methanosarcina vacuolata* Z-761^T. *Int J Syst Bacteriol* **41**, 267–274.
- Maidak, B. L., Cole, J. R., Lilburn, T. G. & 7 other authors (2001). The RDP-II (Ribosomal Database Project). *Nucleic Acids Res* **29**, 173–174.
- Mikucki, J. A., Liu, Y., Delwiche, M., Colwell, F. S. & Boone, D. R. (2003). Isolation of a methanogen from deep marine sediments that contain methane hydrates, and description of *Methanoculleus submarinus* sp. nov. *Appl Environ Microbiol* **69**, 3311–3316.
- Ni, S. & Boone, D. R. (1991). Isolation and characterization of a dimethyl sulfide-degrading methanogen, *Methanobolus siciliae* HI350, from an oil well, characterization of *M. siciliae* T4/M^T, and emendation of *M. siciliae*. *Int J Syst Bacteriol* **41**, 410–416.
- Powell, G. E. (1983). Interpreting gas kinetics of batch culture. *Biotechnol Lett* **5**, 437–440.
- Ratkowsky, D. A., Olley, J., McMeekin, T. A. & Ball, A. (1982). Relationship between temperature and growth rate of bacterial cultures. *J Bacteriol* **149**, 1–5.
- Ratkowsky, D. A., Lowry, R. K., McMeekin, T. A., Stokes, A. N. & Chandler, R. E. (1983). Model for bacterial culture growth rate throughout the entire biokinetic temperature range. *J Bacteriol* **154**, 1222–1226.
- Schmid, K., Thomm, M., Laminet, A., Laue, F. G., Kessler, C., Stetter, K. O. & Schmitt, R. (1984). Three new restriction endonucleases *MaeI*, *MaeII* and *MaeIII* from *Methanococcus aeolicus*. *Nucleic Acids Res* **12**, 2619–2628.
- Singh, N., Kendall, M. M., Liu, Y. & Boone, D. R. (2005). Isolation and characterization of methylophilic methanogens from anoxic marine sediments in Skan Bay, Alaska: description of *Methanococcoides alaskense* sp. nov., and emended description of *Methanosarcina baltica*. *Int J Syst Evol Microbiol* **55**, 2531–2538.
- Stadtman, T. C. & Barker, H. A. (1951). Studies on the methane fermentation. X. A new formate-decomposing bacterium, *Methanococcus vannielii*. *J Bacteriol* **62**, 269–280.
- Swofford, D. L. (2002). PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods). Sunderland, MA: Sinauer Associates.
- Takai, K., Inoue, A. & Horikoshi, K. (2002). *Methanothermococcus okinawensis* sp. nov., a thermophilic, methane-producing archaeon isolated from a Western Pacific deep-sea hydrothermal vent system. *Int J Syst Evol Microbiol* **52**, 1089–1095.
- Whitman, W. B. (2001). Genus I *Methanococcus* Kluyver and van Niel 1936, 400, emend. Barker 1936, 430,^{AL} Mah and Kuhn 1984b, 264 (Nom. Cons., Opinion 62 of the Jud. Comm. 1986a, 491) emend. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 236–242. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.
- Whitman, W. B., Ankwarda, E. & Wolfe, R. S. (1982). Nutrition and carbon metabolism of *Methanococcus voltae*. *J Bacteriol* **149**, 852–863.
- Whitman, W. B., Shieh, J., Sohn, S., Caras, D. S. & Premachandran, U. (1986). Isolation and characterization of 22 mesophilic methanococci. *Syst Appl Microbiol* **7**, 235–240.
- Whitman, W. B., Sohn, S., Kuk, S. & Xing, R. (1987). Role of amino acids and vitamins in nutrition of mesophilic *Methanococcus* spp. *Appl Environ Microbiol* **53**, 2373–2378.
- Whitman, W. B., Boone, D. R., Koga, Y. & Keswani, J. (2001). Taxonomy of methanogenic *Archaea*. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 211–213. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.
- Zwietering, M. H., Jongenburger, I., Rombouts, F. M. & van't Riet, K. (1990). Modeling of the bacterial growth curve. *Appl Environ Microbiol* **56**, 1875–1881.