

## Extraction of DNA from Acidic, Hydrothermally Modified Volcanic Soils

Ruth M. Henneberger,<sup>A,B</sup> Malcolm R. Walter,<sup>A,B,C</sup> and Roberto P. Anitori<sup>A,B,D,E</sup>

<sup>A</sup> Australian Centre for Astrobiology, Macquarie University Biotechnology Research Institute, Macquarie University, North Ryde, NSW 2109, Australia.

<sup>B</sup> Macquarie University Biotechnology Research Institute, Macquarie University, North Ryde, NSW 2109, Australia.

<sup>C</sup> Department of Earth and Planetary Sciences, Macquarie University, North Ryde, NSW 2109, Australia.

<sup>D</sup> Department of Chemistry and Biomolecular Sciences, Macquarie University, North Ryde, NSW 2109, Australia.

<sup>E</sup> Corresponding author. Email: ranitori@rna.bio.mq.edu.au

**Environmental Context.** *Microorganisms are intimately involved in geochemical processes. For example, they are major players in the environmental cycling of important elements (e.g. carbon, sulfur, nitrogen, iron), rock weathering, and the formation of ores and petroleum. Identification of the environmental microbiota, commonly achieved via DNA techniques, is essential for an understanding of these processes. The main focus of this Rapid Communication is to demonstrate that endogenous DNA can be extracted from acidic, volcanic soil samples.*

**Abstract.** Acidic soils for microbial diversity studies were collected from Devil's Kitchen, a fumarolic field on Mt Hood, USA. The very dense soils, which contain clay and other minerals, are derived from andesitic and dacitic rocks altered by volcanic heat and acidic, sulfur-rich hydrothermal steam. An initial attempt to extract biomass DNA using a mechanical-based cell lysis protocol was ineffective. However, by using various other protocols, DNA was successfully extracted, leading to the identification of several acidophilic Mt Hood extremophiles. The results emphasise the importance of testing different extraction procedures when dealing with apparently intractable samples.

**Keywords.** DNA extraction — microbes — Mt Hood — soils

Manuscript received: 15 February 2006.

Final version: 24 March 2006.

### Introduction

Our research focuses on biogeochemical cycling by microbial extremophiles in hydrothermal environments, particularly sulfur cycling in acidic, sulfur-rich sites. Such locations are considered analogous to some of the earliest environments on Earth.<sup>[1]</sup> Their study can therefore provide astrobiologists with insights into the origin and evolution of terrestrial life.

A critical first step in the analysis of biogeochemical cycles is the identification of the environmental microbiota. Either culture- or molecular-based approaches are generally utilised to achieve this aim. Detailed biochemical characterisation of cultured microbes provides valuable clues to their possible biogeochemical roles. However, over 95% of environmental microbes are recalcitrant to laboratory culture.<sup>[2]</sup> Molecular techniques that target the 16S ribosomal RNA (rRNA) gene are able to access a far greater percentage of microbial diversity.<sup>[2,3]</sup> The first step in most 16S rRNA gene-based

molecular strategies is extraction of biomass DNA from the environmental sample. This procedure can be relatively simple. However, this is not always the case, as the existence in the literature of numerous DNA procedures for a large variety of different environmental sample types demonstrates. For example, many soil extraction protocols have been described.

Two factors that can complicate DNA extraction from soils are acidity, and soil–DNA interactions. DNA is unstable under acidic conditions, owing to depurination-induced degradation of DNA.<sup>[4]</sup> Several soil components can bind DNA (and cells), thereby making it difficult to extract.<sup>[5]</sup> For example, DNA binds to clay minerals such as montmorillonite and kaolinite,<sup>[6]</sup> and other soil constituents, including quartz.<sup>[7]</sup> Furthermore, DNA–clay binding increases at acidic pH.<sup>[8]</sup> We have routinely had success in extracting DNA from different environmental sample types with a FastPrep bead-beating protocol, which mechanically lyses endogenous

Table 1. Sample descriptions

Sample	Temp.	pH	Description
D6	46°C	1.6	Surface sample; grey, yellowish dense, sticky soil with very small grain size, and visible yellow and pink crystalline inclusions.
D7	86°C	1.8	Directly below sample D6; 1–10 cm depth; light to dark grey, dense but very wet soil with very small grain size, and visible black and white inclusions.
E8	39°C	1.4	Less than 10 m from F9/F10 sampling site; surface scraping from porous, grainy, sandy, soft rock; green-grey-yellow with white, black and bright yellow inclusions.
F9	39.5°C	3.3	Surface to 1–2 cm depth; homogeneous, dense, sticky, light grey soil with very small grain size and visible black and white inclusions, including thin, whitish surface crust.
F10	86.4°C	3.2	Directly below F9; 4–5 cm depth; homogeneous, dense, sticky, light grey soil with very small grain size, and visible black and white inclusions.

cells.<sup>[9]</sup> Samples have included microbial mat and biofilm from a pH-neutral hot spring<sup>[10]</sup> and from a highly acidic volcanic stream,<sup>[11]</sup> water samples (pH 7.6) from an underground aquifer,<sup>[12]</sup> microphytic tufas in slightly alkaline river water,<sup>[13]</sup> and run-off stream sediments. Bead beating is well suited for DNA extraction from soils because of its ability to disrupt spores, which are often present in soil.<sup>[14]</sup> Here we report our experiences with DNA extraction from highly acidic, hydrothermally altered Devil's Kitchen soils that did not contain visible microbial communities. We intend to demonstrate that even with apparently intractable samples with respect to DNA extraction, it is still possible to extract useable DNA.

## Experimental

Soils were collected from Devil's Kitchen (August 2004; Table 1), a hydrothermal area located at an elevation of ~3100 m on the Mt Hood volcano (Cascade Range, USA). Devil's Kitchen has been formed by a glacial moraine covering a fumarolic field, and is composed of undifferentiated andesitic volcanic flows and dacitic pyroclastic rocks.<sup>[15]</sup> Volcanic heat and acidic, sulfur-rich hydrothermal steam have altered these rocks to very dense, mineral-rich soil with visible sulfur inclusions. Samples were collected aseptically and stored on ice for 12–16 h, then frozen at –20°C until processing. DNA extractions, culturing and microscopy were conducted in Australia (1–5 months post-collection). The following DNA extraction methods were used: mechanical cell lysis—FastPrep (QBiogene Corp., Irvine, CA, USA),<sup>[9]</sup> FastPrep PEG/lysozyme modification,<sup>[16]</sup> PowerSoil™ DNA isolation kit (Mo Bio laboratories, Carlsbad, CA, USA), UltraClean™ DNA isolation kit (Mo Bio laboratories); chemical cell lysis—xanthogenate-SDS (XS) buffer, modified;<sup>[17]</sup> chemical/enzymatic cell lysis—phenol;<sup>[18]</sup> mechanical/chemical/enzymatic cell lysis—phosphate, SDS, chloroform, Bead Beater (PSC-B), modified (S. Turner, personal communication).<sup>[19]</sup> Agarose gel electrophoresis was used to assess the outcome of DNA extractions.<sup>[20]</sup> Cultured *Escherichia coli* cells for spiking of samples were enumerated using a haemocytometer. Biomass 16S rRNA genes were polymerase chain reaction (PCR)-amplified from extracted DNA samples with bacterial (PB36 and PB38)<sup>[21]</sup> or archaeal (ASF and ASR)<sup>[22]</sup> domain-specific primers (Sigma Genosys Australia Pty Ltd, Sydney, Australia), which resulted in, respectively, 1.5 kb and 1.0 kb PCR products. PCR was conducted using AmpliTaq Gold reagents (Roche Diagnostics Australia Pty Ltd, Sydney, Australia) and the following cycling conditions: 95°C for 15 min (1 cycle); 95°C for 30 s, 50°C (Bacteria and Archaea (and occasionally 55°C for the latter)) for 30 s, 72°C for 120 s (35 cycles); 72°C for 10 min (1 cycle). Template dilutions were routinely conducted to test for PCR inhibitors. Recombinant 16S rRNA gene libraries were constructed using a TA cloning kit (Invitrogen Australia Pty Ltd, Melbourne, Australia) and *E. coli* DH5 $\alpha$  cells. Individual

recombinants were screened by colony PCR and digestion with *Bsu*RI and *Hin*FI. Sequencing of selected recombinant plasmids with M13/F and M13/R primers (Sigma Genosys), and sequence database identity searches were performed as previously described.<sup>[10]</sup> Sequences have been deposited in GenBank (accession numbers DQ455568–DQ455581; www.ncbi.nlm.nih.gov/Genbank/, verified April 2006). For enrichment culturing, 5 mL of a modified mineral salt media (pH 2) was inoculated with sample material (500 mg) and incubated at 45°C, 60°C or 75°C for up to 4 weeks.<sup>[23,24]</sup> Chemical analyses (Table 2) were conducted by Beaverton Analytical, Portland, Oregon, USA. X-Ray diffraction (XRD; Table 2) was performed at the Centre of Geochemical Evolution and Metallogeny of Continents (GEMOC), Macquarie University, Sydney, Australia.

## Results and Discussion

Initial DNA extractions from samples E8, D6, D7 and F10 (Tables 1 and 2) with the FastPrep bead-beating method yielded negative results, i.e. biomass DNA was not visualised by agarose gel electrophoresis, and no PCR products were obtained using bacterial- and archaeal-specific 16S rRNA gene primers. The likelihood of PCR inhibitors affecting the result was discounted, as various template dilutions were tested. The inability to extract DNA could be explained by the fact that: (i) endogenous cells may have been absent; (ii) the number of endogenous cells was so low that any DNA extracted was below the detection limit of the techniques used; (iii) cell numbers were sufficient, but the extracted DNA was either degraded or made inaccessible (e.g. binding to clay and other minerals); or (iv) the FastPrep DNA extraction technique was unsuitable. For sample E8, reason (i) was very unlikely, as endogenous coccoid cells were present, as determined by confocal laser scanning microscopy and fluorescence *in situ* hybridisation of the fixed sample.

To ascertain whether it was actually feasible to extract DNA at all (i.e. evaluating possibility (iii) and (iv) above), samples E8 and F10 were spiked with *E. coli* cells (equivalent to 10<sup>8</sup> cells g<sup>-1</sup> sample). FastPrep-extracted genomic DNA was visible by agarose gel electrophoresis (sample E8 only), and a bacterial 16S rRNA gene PCR product was obtained for both E8 and F10 (Table 3). As PCR-amplifiable *E. coli* DNA could be extracted from spiked samples, we persisted with our extraction attempts using selected Devil's Kitchen samples.

Sample E8 was tested with several different DNA extraction methods, followed by PCR with bacterial- and archaeal-specific 16S rRNA gene primers. Although none of the six

**Table 2. Results of chemical<sup>A</sup> and X-ray diffraction (XRD) analyses**

	E8	F9	F11 <sup>B</sup>
Iron	6710	21900	25400
Nitrate-N	ND <sup>C</sup> (<10)	ND (<10)	ND (<10)
Ammonia-N	0.504	0.952	ND (<0.5)
Sulfide	118	62.9	209
Sulfate	19000	13800	1060
Chloride	ND (<50)	ND (<50)	ND (<50)
Orthophosphate-P	1.53	ND (<0.2)	ND (<0.2)
Specific conductivity	50000	7310	1150
pH	1.4	3.3	3.0
Total organic carbon	ND (<500)	718	ND (<500)
Temperature	39.0	39.5	89.1
XRD <sup>D</sup>	Sulfur, S	Tridymite, SiO <sub>2</sub>	Tridymite, SiO <sub>2</sub>
	Cristobalite, SiO <sub>2</sub>	Cristobalite, SiO <sub>2</sub>	Cristobalite, SiO <sub>2</sub>
	Mawsonite, Cu <sub>6</sub> Fe <sub>2</sub> SnS <sub>8</sub>	Quartz, SiO <sub>2</sub>	Quartz, SiO <sub>2</sub>
		Kaolinite, Al <sub>2</sub> Si <sub>2</sub> O <sub>5</sub> (OH) <sub>4</sub>	Dickite, Al <sub>2</sub> Si <sub>2</sub> O <sub>5</sub> (OH) <sub>4</sub>
		Magnetite, Fe <sub>3</sub> O <sub>4</sub>	Alunite, KAl <sub>3</sub> (SO <sub>4</sub> ) <sub>2</sub> (OH) <sub>6</sub>
			Pyrite, FeS <sub>2</sub>

<sup>A</sup> All values are given in mg kg<sup>-1</sup> wet weight, except for pH, temperature (°C) and specific conductivity (μS cm<sup>-1</sup>).

<sup>B</sup> Analyses were conducted on F11, which was from the same sampling site as F10. Both F10 and F11 were from below the surface (F10, 4–5 cm depth; F11, 8–9 cm), and had the same appearance and texture.

<sup>C</sup> ND, below limit of detection (limit given in parentheses).

<sup>D</sup> XRD was carried out at 25°C using a Siemens model D5005 diffractometer with Cu Kα radiation, set at 40 kW and 30 mA. Each run covered the range from 1.3° to 70° 2θ with a step size of 0.2° and a count time per step of 2 s.

**Table 3. DNA extraction and 16S rRNA gene polymerase chain reaction (PCR) results**

Extraction procedure	Unspiked samples									Spiked samples <sup>A</sup>								
	Sample amount (mg) used for DNA extraction			Extracted DNA visible on agarose gel			Bacterial PCR product			Archaeal PCR product			Extracted DNA visible on agarose gel			Bacterial PCR product		
Sample	E8	F9	F10	E8	F9	F10	E8	F9	F10	E8	F9	F10	E8	F9	F10	E8	F9	F10
FastPrep	490	/	520	–	/	–	–	/	–	–	/	–	+	/	–	+	/	+
FastPrep PEG/lysozyme	225	250	/	–	–	/	+	–	/	–	–	/	+	–	/	+	–	/
PSC-B	490	/	520	–	/	–	–	/	–	–	/	–	–	/	–	+	/	+
UltraClean™	210	/	210	–	/	–	–	/	–	–	/	–	+	/	–	+	/	–
PowerSoil™	250	/	240	–	/	–	–	/	–	–	/	–	+	/	–	+	/	+
XS Buffer	200	350	/	–	–	/	–	–	/	–	+	/	+	–	/	+	+	/
Phenol	520	/	550	–	/	–	–	/	–	–	/	–	–	/	–	+/-	/	–

<sup>A</sup> 10<sup>8</sup> *E. coli* cells g<sup>-1</sup> sample.

/ Not done.

extraction methods used resulted in visible biomass DNA, a bacterial 16S rRNA gene PCR product was obtained with the FastPrep PEG/lysozyme method (Table 3), suggesting successful extraction of endogenous DNA. Samples F9 and F10, collected from different horizons of the same location (Table 1), were also tested with, respectively, two and five different extraction methods. Once again, none of the methods used resulted in visible extracted biomass DNA. However, an archaeal 16S rRNA gene PCR product was obtained for F9 with the XS buffer method (Table 3). As extraction controls, duplicate samples of E8, F9 and F10 were spiked with *E. coli* cells (10<sup>8</sup> cells g<sup>-1</sup> sample). Following extraction, a bacterial 16S rRNA gene PCR product was produced in 11 of 14 spiked control reactions (Table 3).

Cloning and sequencing of the bacterial PCR product (1472–1512 base pairs) obtained with unspiked sample E8

indicated that the 16S rRNA gene sequences were predominantly (71% of 103 cloned 16S rRNA genes analysed) from *Sulfobacillus thermosulfidooxidans*,<sup>[25]</sup> or *Sulfobacillus thermotolerans*.<sup>[26]</sup> Of the remaining 29% of E8 sequences, 26% matched with *Pseudomonas fulva* and *Pseudomonas parafulva*,<sup>[27,28]</sup> and 3% to *Sporosarcina* sp. and an uncultured bacterium from acidic grassland soil.<sup>[29,30]</sup> Similar analysis of the archaeal PCR product (1019–1024 base pairs) from unspiked sample F9 indicated that virtually all recombinants (97% of 109 cloned 16S rRNA genes analysed) matched with a *Ferroplasma* sp. JTC3 and *Ferroplasma cypreacervatum* database sequences.<sup>[31,32]</sup> The remaining 3% of sequences matched with *Sulfolobus metallicus* and *Acidianus* sp.<sup>[33,34]</sup>

Enrichment cultures were obtained for several samples at different incubation temperatures (data not shown). Cloning

and sequencing of the 16S rRNA gene of microorganisms growing in an E8 enrichment culture, incubated at 45°C for 4 days, indicated the presence of sequences either virtually identical to (*Ferroplasma* sp. JTC3/F. *cypraxacervatum*, 99.9% sequence identity), or related to (*S. thermosulfidooxidans*, 91.2–95.8%) the predominant sequences identified by direct molecular-based methods. This identity supports the conclusion that our results stem not from exogenous laboratory-sourced contaminants, but from endogenous Devil's Kitchen microbes. Potential laboratory contamination of experiments with exogenous DNA or microbial cells can be an issue with 16S rRNA gene sequence studies,<sup>[35,36]</sup> especially when only small amounts of biomass DNA are extracted. Therefore, we instituted stringent precautions in the form of numerous controls: a negative control tube (i.e. water only) in all DNA extractions and subsequent PCR experiments; PCR negative controls (water); a media-only control tube during culturing; and DNA extractions and ensuing 16S rRNA gene cloning were not conducted at the same time as culturing experiments. We used standard laboratory procedures to avoid contamination, such as the use of filter tips and clean reagents. Finally, neither *Sulfobacillus* sp. nor *Ferroplasma* sp. cultures have been previously studied in our laboratory, considerably reducing this possibility as a contamination source.

Despite these strict controls and precautions, the possibility remains that the *P. fulva*/*P. parafulva* sequences identified in sample E8 represent exogenous laboratory contamination, as they are not necessarily the type of microbe expected at Devil's Kitchen. Furthermore, *Pseudomonas* sp. have been confirmed as contaminants in 16S rRNA gene experiments.<sup>[35,36]</sup> However, the following facts could support the argument that the *P. fulva*/*P. parafulva* sequences were not laboratory contaminants: (i) a *P. fulva* 16S rRNA gene sequence has been identified in a supraglacial sulfur spring,<sup>[37]</sup> (ii) the best *P. fulva*/*P. parafulva* database matches were to sequences identified on strawberry leaves and rice seeds, suggesting a link with soil; and (iii) both thermophilic and acidophilic *Pseudomonads* have been identified.

Our results with highly acidic, mineral-containing volcanic soils highlight the importance of assessing different DNA extraction methods for obtaining biomass DNA. It was also apparent that any one extraction method did not 'suit all'. For example, the FastPrep PEG/lysozyme modification was successful with sample E8, but not F9, whereas the reverse situation applied with the XS buffer method. The complexity of the soil samples with respect to DNA extraction (acidity, possible DNA–component interactions) was potentially compounded by the presence of few cells, and sample freezing and thawing before extraction. Despite these factors, it was still possible to identify the resident microbes on the basis of 16S rRNA gene sequences. Bacterial *Sulfobacillus* sp. and archaeal *Ferroplasma* sp. sequences dominated samples E8 and F9 respectively. Furthermore, similar or identical sequences were detected in an acidic (pH 2) enrichment culture of sample E8. Both *Ferroplasma* sp. and *Sulfobacillus* sp. are facultatively anaerobic, Fe<sup>2+</sup>-oxidizing acidophiles (pH optima of 1.0–1.7 and 1.9–2.4 respectively; temperature

optima of 35–42°C and 50°C) involved in sulfur cycling and the bioleaching of sulfur-containing minerals, such as pyrite, leading to acid mine drainage.<sup>[23,24,38–41]</sup> *Ferroplasma* sp. and *Sulfobacillus* sp. are microbes one would expect to find at Devil's Kitchen, a sulfur- and iron-rich acidic environment.

## Acknowledgments

RMH was funded by Macquarie University (Postgraduate Research Fund and an International Travel Scholarship). We are grateful to Prof. Peter Bergquist for advice and manuscript editing, and Dana Rogoff, Tom Bennett, Dr Steve Boyer, Bob McGown for assistance during sample collection. Tin Tin Win for XRD analyses. Paul Norman and the USDA Forest Service for the Mt Hood sampling permit. Debra Birch for microscopy. Florian Selch for assistance in the identification of cultured microbes.

## References

- [1] M. J. Van Kranendonk, F. Pirajno, *Geochem. Explor. Environ. Anal.* **2004**, *4*, 253. doi:10.1144/1467-7873/04-205
- [2] R. I. Amann, W. Ludwig, K. H. Schleifer, *Microbiol. Rev.* **1995**, *59*, 143.
- [3] P. Hugenholtz, B. Goebel, N. Pace, *J. Bacteriol.* **1998**, *180*, 4765.
- [4] R. H. Garrett, C. N. Grisham, in *Biochemistry* **1995**, p. 213 (Saunders College Publishing: USA).
- [5] A. V. Ogram, M. L. Mathot, J. B. Harsh, J. Boyle, C. A. Pettigrew, *Appl. Environ. Microbiol.* **1994**, *60*, 393.
- [6] G. Pietramellara, L. Dal Canto, C. Vettori, E. Gallori, P. Nannipieri, *Soil Biol. Biochem.* **1997**, *29*, 55. doi:10.1016/S0038-0717(96)00262-3
- [7] B. W. Aardema, M. G. Lorenz, W. E. Krumbein, *Appl. Environ. Microbiol.* **1983**, *46*, 417.
- [8] M. Khanna, G. Stotzky, *Appl. Environ. Microbiol.* **1992**, *58*, 1930.
- [9] C. Yeates, M. R. Gillings, *Lett. Appl. Microbiol.* **1998**, *27*, 49. doi:10.1046/J.1472-765X.1998.00383.X
- [10] R. P. Anitori, C. Trott, D. J. Saul, P. L. Bergquist, M. R. Walter, *Astrobiology* **2002**, *2*, 255. doi:10.1089/153110702762027844
- [11] P. Butterworth, M. R. Walter, P. L. Bergquist, H. Morgan, R. P. Anitori, *Australian Society for Microbiology Annual Scientific Meeting 2005*, Sydney, Australia.
- [12] R. Henneberger, M. R. Walter, P. L. Bergquist, R. P. Anitori, *8th International Conference of Bioastronomy 2004*, Reykjavik, Iceland.
- [13] K. D. Carthew, M. P. Taylor, R. N. Drysdale, *Geomorphology* **2006**, *73*, 78. doi:10.1016/J.GEOMORPH.2005.07.007
- [14] W. L. Nicholson, *Cell. Mol. Life Sci.* **2002**, *59*, 410. doi:10.1007/S00018-002-8433-7
- [15] T. E. C. Keith, K. E. Bargar, M. H. Beeson, *Miscellaneous Field Studies Map MF-1379-C 1982*, Department of the Interior, US Geological Survey.
- [16] M. J. Bell, N. Seymour, G. R. Stirling, A. M. Stirling, L. van Zwieten, T. Vancov, G. Sutton, P. Moody, *Aust. J. Soil Res.* **2006**, in press.
- [17] D. Tillett, B. A. Neilan, *J. Phycol.* **2000**, *36*, 251. doi:10.1046/J.1529-8817.2000.99079.X
- [18] D. Morris, PhD thesis **1998**, University of Auckland, Auckland, New Zealand.
- [19] D. N. Miller, J. E. Bryant, E. L. Madsen, W. C. Ghiorse, *Appl. Environ. Microbiol.* **1999**, *65*, 4715.
- [20] J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual 1989* (Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY).



- [21] D. J. Saul, A. G. Rodrigo, R. A. Reeves, L. C. Williams, K. M. Borges, H. W. Morgan, P. L. Bergquist, *Int. J. Syst. Bacteriol.* **1993**, *43*, 754.
- [22] R. P. Anitori, C. Trott, D. J. Saul, P. L. Bergquist, M. R. Walter, in *Bioastronomy 2002: Life Among the Stars, IAU Symposium 213* (Eds R. P. Norris, F. H. Stootman) **2004**, pp. 374–380 (Astronomical Society of the Pacific: San Francisco, CA).
- [23] M. Dopson, E. B. Lindstrom, *Appl. Environ. Microbiol.* **1999**, *65*, 36.
- [24] M. Dopson, C. Baker-Austin, A. Hind, J. P. Bowman, P. L. Bond, *Appl. Environ. Microbiol.* **2004**, *70*, 2079. doi:10.1128/AEM.70.4.2079-2088.2004
- [25] K. Goto, K. Mochida, M. Asahara, M. Suzuki, H. Kasai, A. Yokota, *Int. J. Syst. Evol. Microbiol.* **2003**, *53*, 1537. doi:10.1099/IJS.0.02546-0
- [26] T. I. Bogdanova, I. A. Tsaplina, T. F. Kondrateva, V. I. Duda, N. E. Suzina, V. S. Melamud, T. P. Tourova, G. I. Karavaiko, *Int. J. Syst. Evol. Microbiol.* **2006**, *56*, 1039.
- [27] U. Krimm, D. Abanda-Nkpwatt, W. Schwab, L. Schreiber, *FEMS Microbiol. Ecol.* **2005**, *53*, 483. doi:10.1016/J.FEMSEC.2005.02.004
- [28] M. Uchino, O. Shida, T. Uchimura, K. Komagata, *J. Gen. Appl. Microbiol.* **2001**, *47*, 247. doi:10.2323/JGAM.47.247
- [29] A. Felske, A. Wolterink, R. Van Lis, A. D. Akkermans, *Appl. Environ. Microbiol.* **1998**, *64*, 871.
- [30] B. Y. Kim, S. W. Kwon, *National Center for Biotechnology Information* **2005**, GenBank sequence accession DQ073394.
- [31] G. F. Rautenbach, C. Bowker, C. A. du Plessis, *National Center for Biotechnology Information* **2004**, GenBank sequence accession AY830840.
- [32] R. B. Hawkes, P. D. Franzmann, J. J. Plumb, in *Proceedings from the Bac-Min 2004 Conference* **2004**, pp. 11–17 (Australian Institute of Mining and Metallurgy: Carlton, Australia).
- [33] T. Fuchs, H. Huber, S. Burggraf, K. O. Stetter, *Syst. Appl. Microbiol.* **1996**, *19*, 56.
- [34] Z. He, Y. Li, P. Zhou, S. Liu, *FEMS Microbiol. Lett.* **2000**, *193*, 217.
- [35] N. Grahm, M. Olofsson, K. Ellnebo-Svedlund, H. J. Monstein, J. Jonasson, *FEMS Microbiol. Lett.* **2003**, *219*, 87. doi:10.1016/S0378-1097(02)01190-4
- [36] M. A. Tanner, B. M. Goebel, M. A. Dojka, N. R. Pace, *Appl. Environ. Microbiol.* **1998**, *64*, 3110.
- [37] S. E. Grasby, C. C. Allen, T. G. Lonzo, J. T. Lisle, D. W. Griffin, B. Beauchamp, *Astrobiology* **2003**, *3*, 583. doi:10.1089/153110703322610672
- [38] M. Dopson, E. B. Lindstrom, *Microb. Ecol.* **2004**, *48*, 19. doi:10.1007/S00248-003-2028-1
- [39] K. J. Edwards, P. L. Bond, T. M. Gihring, J. F. Banfield, *Science* **2000**, *287*, 1796. doi:10.1126/SCIENCE.287.5459.1796
- [40] P. R. Norris, D. A. Clark, J. P. Owen, S. Waterhouse, *Microbiology* **1996**, *142*, 775.
- [41] O. V. Golyshina, T. A. Pivovarova, G. I. Karavaiko, T. F. Kondrat'eva, E. R. B. Moore, W. R. Abraham, H. Lunsdorf, K. N. Timmis, *Int. J. Syst. Evol. Microbiol.* **2000**, *50*, 997.