Idiomarina loihiensis sp. nov., a halophilic *γ-Proteobacterium* from the Lō'ihi submarine volcano, Hawai'i

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During an investigation of bacterial diversity at hydrothermal vents on the Lō'ihi Seamount, Hawai'i, a novel bacterium (designated L2-TR^T) was cultivated, which shares 99·9 % 16S rRNA gene sequence similarity over 1415 nt with an uncultured eubacterium from sediment at a depth of 11 000 m in the Mariana Trench. The nearest cultivated neighbour of L2-TR^T, however, is *Idiomarina abyssalis* KMM 227^T, with which it shares 98·9 % 16S rRNA sequence similarity. L2-TR^T differed from *I. abyssalis* KMM 227^T in several phenotypic respects, including growth at 46 °C and in medium that contained 20 % (w/v) NaCl. DNA–DNA hybridization data showed that L2-TR^T did not belong to the species *I. abyssalis* (43·4 % DNA–DNA reassociation). Cells of L2-TR^T were Gram-negative rods, 0·35 μm wide and 0·7–1·0 μm long, which were occasionally up to 1·8 μm in length. Cells were motile by a single polar or subpolar flagellum. The major fatty acid in L2-TR^T was iso-$C_{15:0}$ (32·6 %). The DNA G+C content was 47·4 mol%. Phenotypic and genotypic analyses indicated that L2-TR^T could be assigned to the genus *Idiomarina* but, based on significant phenotypic and genotypic differences, constituted a novel species within this genus, *Idiomarina loihiensis* sp. nov., of which L2-TR^T (=ATCC BAA-735^T=DSM 15497^T) is the type strain.

Steep thermal and chemical gradients at active, submarine hydrothermal vents affect the composition and metabolic activities of bacterial communities at these sites (Karl *et al.*, 1989). Early perceptions of microbial community structure at such vents were that *Archaea* would dominate under *in situ* conditions of high pressure and temperature. Widespread occurrence of *Bacteria* at hydrothermal vents has since been demonstrated (Ruby *et al.*, 1981; Harwood *et al.*, 1982; Moyer *et al.*, 1994, 1995; Miroschnichenko *et al.*, 1999) and there is compelling evidence that they, rather than *Archaea*, dominate vent microbial communities (Guezennec *et al.*, 1996; Sievert *et al.*, 2000).

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of L2-TR^T is AF288370.

Details of the growth of *Idiomarina loihiensis* sp. nov. in different NaCl concentrations and its full fatty acid composition are available as supplementary material in IJSEM Online.

The Lō'ihi Seamount, located 35 km off the south-east coast of the island of Hawai'i, covers approximately 40 km^2 and rises 3500 m from the sea floor to within 1300 m of the surface of the Pacific Ocean. The area is volcanically active, with localized venting of hydrothermal fluids, lava ejections, earthquakes and landslides (Klein, 1982; Malahoff, 1987; Karl *et al.*, 1988). Karl *et al.* (1989) detected metabolically active bacteria in vent fluids at Lō'ihi. Moyer *et al.* (1994, 1995) subsequently described microbial community structure in microbial mats at Lō'ihi by using amplified partial 16S rRNA gene sequences, but only recently has a strain cultivated from Lō'ihi been described (Emerson & Moyer, 2002). As molecular methods such as 16S rDNA clone libraries do not fully describe microbial diversity (Palleroni, 1997; Suzuki *et al.*, 1997; Donachie *et al.*, 2002), nor do they elucidate physiological features that might reveal biogeochemical potential, we cultivated aerobic heterotrophic bacteria from Lō'ihi in order to provide the first insight into these bacteria at the site. Here, we describe the first novel micro-organism isolated from the Lō'ihi Seamount.

During dive no. PV421 of DSRV *Pisces V* (10 September

1999) into Pele's Pit, Lō'ihi Seamount, we collected hydrothermal fluids (163 °C) venting into sea water (4 °C) at a depth of 1296 m by using a 'suction bucket'. Combined fluids were returned to the RV *Ka'imikai-o-Kanaloa* within 2 h, during which time the container passed through sea water that ranged from 4 to 26 °C. A subsample (1 l) was transferred aboard ship into a sterile 1 l Nalgene bottle and stored at 4 °C until delivery to a shore laboratory (72 h).

In the laboratory, water was centrifuged (30 min, 4500 ***g***) in an ethanol-rinsed, autoclaved and UV-irradiated 1 l bottle in a KA-9 high-speed composite rotor assembly (Composite Rotor). Spread plates were prepared with 200 µl of the pellet on marine agar (MA; Difco). A translucent beige colony, designated L2-TR^T, which arose after 24 h at 30 °C was transferred to MA for isolation and incubated at 30 °C. We selected this incubation temperature because many bacteria cultivated from permanently cold marine waters are in fact mesophilic (Donachie, 1996). Strain purity was checked after 24 h by Gram-stain and further transfers to ensure colony uniformity. L2-TR^T was thereafter maintained on MA or in marine broth (MB; Difco). Stock cultures were stored in MB with 30 % glycerol (final concentration) at −80 °C.

Tolerance or requirement of NaCl by L2-TR^T was tested on tryptic soy agar (TSA; BBL) with 0·5–20·0 % (w/v) NaCl at 30 °C for 10 days. Optimum salinity for growth was determined by changes in turbidity with time in 50 % strength MB with a range of NaCl concentrations from 1 to 20 % (w/v). Temperature range for growth was determined on MA plates that were incubated at 4–50 °C. Anaerobic growth was checked on MA in the BBL GasPak Pouch system, with oxygen and carbon dioxide concentrations of <2 and >4 %, respectively.

Motility was observed in a hanging-drop preparation under a 1000× objective lens with oil immersion after 24 h incubation in MB. Single colonies removed from MA were tested for catalase and cytochrome *c* oxidase activities with 3 % hydrogen peroxide (Sigma) and tetramethyl *p*-phenylenediamine (BBL), respectively. The presence of nitrate reductase was tested in nitrate broth that contained 0, 3·2 and 7·5 % (w/v) NaCl. Amylase activity was tested on starch medium (Difco) with a range of NaCl concentrations from 0 to 7·5 % (w/v) by flooding plates with iodine after 7 days growth at 30 °C. DNA hydrolysis was determined on DNase test agar with methyl green (Difco) and gelatinase activity was checked in gelatinase nutrient medium (Difco), both with 2 and 7 % (w/v) NaCl.

Growth and acidification of carbohydrates were determined in API 50CH (bioMérieux Vitek) after 5 days incubation in CHB/E medium prepared according to the manufacturer's instructions, except that SL-8 trace elements solution (Atlas, 1997) was used instead of Cohen-Bazire mineral base and salinity was adjusted to 2·5 % (w/v) NaCl. Constitutive enzyme activities were assayed by using the API ZYM system (bioMérieux Vitek). The Biolog GN system was used to determine oxidation by L2-TR^T of carbohydrates, alcohols, organic acids, amino acids and nucleosides presented as single carbon sources. Assimilation and enzyme activity tests were each conducted at least three times. Fatty acids in whole cells grown on MA (48 h, 30 °C) were determined by using the MIDI system (Sasser, 1997). Cells of L2-TR^T in MB (48 h, 30 °C) were prepared for scanning electron microscopy as described previously (Donachie *et al.*, 2002).

Genomic DNA was extracted from MB cultures (after 48 h) by using the GNOME DNA Isolation kit (Qbiogene). A ~1·4 kbp fragment of the 16S rRNA gene was amplified by PCR with *Pfu* DNA polymerase and primers 27F and 1492R (Mullis & Faloona, 1987; Lane, 1991). The PCR product was purified by using a QIAquick PCR Purification kit (Qiagen) and used as the template in dye terminator sequencing PCRs (Beckman Instruments). PCR products were sequenced by using a Beckman CEQ 2000 DNA analyser. rDNA sequences were assembled and edited in Seqman (DNASTAR). DNA–DNA hybridization was carried out by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ); genomic DNA was isolated in a French pressure cell (Thermo Spectronic) from ~3 g (wet wt) of cells grown in MB at 30 °C. DNA was then purified by chromatography on hydroxyapatite (Cashion *et al.*, 1977) and hybridized with DNA extracted in the same manner from *I. abyssalis* ATCC BAA-312^T (Ivanova *et al.*, 2000), the closest cultivated neighbour of L2-TR^T on the basis of 16S rRNA gene sequence similarity. Hybridization procedures followed De Ley *et al.* (1970), with modifications after Huß *et al.* (1983) and Escara & Hutton (1980). A model 2600 spectrophotometer equipped with a model 2527-R thermoprogrammer and plotter (Gilford Instrument Laboratories) was used in the analysis. Renaturation rates were computed with the TRANSFER.BAS program (Jahnke, 1992). DNA G+C content was calculated directly from the ~2·8 Mbp we have sequenced of the estimated 3 Mbp genome (data not shown). The relationship of L2-TR^T with its nearest cultivated and uncultured neighbours and representative members of the family *Alteromonadaceae* was visualized in a phylogenetic tree based on a CLUSTALX alignment (Thompson *et al.*, 1997) of the respective 16S rRNA gene nucleotide sequences downloaded from GenBank (Altschul *et al.*, 1997).

Colonies of L2-TR^T on MA were translucent beige to yellow, 2 mm in diameter, circular, low convex to raised, smooth, shiny and entire. Older colonies (>72 h) were sticky to butyrous. Cells stained Gram-negative and presented as straight to slightly curved rods of 0·35 µm wide and 0·7–1·8 µm in length, which were exceptionally up to several tens of micrometres long, after 2 days in MB at 30 °C (Fig. 1). L2-TR^T grew on TSA that contained 0·5 and 20 % (w/v) NaCl. Optimum salinity for growth in 50 % MB was 10 % (w/v) (see supplementary material in IJSEM Online). Colonies appeared on MA after 2–3 days at 4 °C

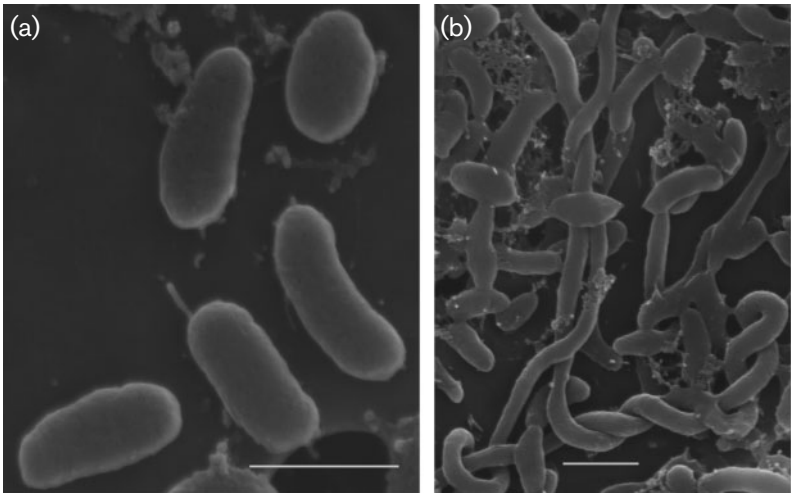

Fig. 1. Scanning electron micrographs of *Idiomarina loihiensis* L2-TR^T. (a) Cells of 0·35 × ~1·0 μm, one with a single polar flagellum; (b) an exceptionally long cell of L2-TR^T amid others displaying a regular, slightly ovoid rod morphotype. Bars, 1 μm.

and overnight at 43 °C. Growth at 46 °C was very weak; there was no growth at 50 °C. The strain did not grow in a CO_2-enriched, anoxic atmosphere. L2-TR^T was motile, catalase-positive and cytochrome *c* oxidase-positive. Nitrate was not reduced either in the absence of NaCl or in the presence of 3·2 % (w/v) NaCl; however, nitrate reduction did proceed with 7·5 % (w/v) NaCl in the medium. DNA and gelatin were hydrolysed, but starch was not. Constitutive enzymes expressed by L2-TR^T were alkaline phosphatase, esterase (C_4), esterase lipase (C_8), leucine arylamidase, acid phosphatase and phosphohydrolase. Several carbon sources were oxidized in the Biolog GN system (Table 1). The major fatty acid was 13-methyl tetradecanoic acid (iso-$C_{15:0}$) (Table 2).

The nucleotide sequence of the 16S rRNA gene in L2-TR^T shared 98·9 % similarity over 1415 nt with that of *I. abyssalis* ATCC BAA-312^T, its nearest cultivated neighbour in this respect. L2-TR^T fell firmly in the genus *Idiomarina* in the family *Alteromonadaceae* in the domain *Bacteria* (Fig. 2). The 16S rRNA gene sequence of L2-TR^T did not share ⩾97 % similarity with any others reported from Lō'ihi (Moyer *et al.*, 1994, 1995), although it was related to cultured and uncultured deep-sea sediment and/or hydrothermal vent bacteria (cf. Fig. 2). As DNA–DNA hybridization showed that reassociation of genomic DNA from L2-TR^T and *I. abyssalis* ATCC BAA-312^T was only 47·3 %, L2-TR^T does not belong to the species *I. abyssalis* when the recommendation of Wayne *et al.* (1987) is considered. Placement of L2-TR^T in the genus *Idiomarina*, however, was supported by the DNA G+C content of 47·4 mol%, which is in the range reported for other members of this genus.

L2-TR^T differed phenotypically and genotypically from the two species for which the genus *Idiomarina* was first established, *I. abyssalis* and *Idiomarina zobellii*. Phenotypic differences included the maximum growth temperature, with L2-TR^T growing above 40 °C compared to a maximum of 30 °C reported for these two species (Ivanova *et al.*, 2000). Significant phenotypic differences extended to cell morphology, carbon sources utilized and NaCl tolerance (Tables 1 and 2). The response of L2-TR^T to salinity differed markedly from those of the three described *Idiomarina* species (Ivanova *et al.*, 2000; Brettar *et al.*, 2003), each of which has an optimum salinity range for growth of 3–6 % (w/v). Although *Idiomarina baltica* was tested only to 10 % (w/v) NaCl (Brettar *et al.*, 2003), strain L2-TR^T appeared to have the highest optimum salinity for growth and to be the species in this genus with the broadest salinity range for growth.

The fatty acid profile of L2-TR^T displayed the same dominance by iso-branched fatty acids that characterizes other members of the genus *Idiomarina*. Indeed, anteiso-branched fatty acids comprised <1 % of the total fatty acid pool. The taxonomic significance of the fatty acid composition of *Idiomarina* species is discussed by Brettar *et al.* (2003). L2-TR^T appears to be unique in this genus, however, with twice the percentage of saturated fatty acids that has been reported for *I. zobellii* and *I. baltica* and ~50 % more than that reported for *I. abyssalis* (Table 2).

Much discussion has centred on which parameters to consider when designating novel species (Stackebrandt & Goebel, 1994). Limited consensus exists on what level of 16S rRNA gene sequence similarity might distinguish one species from another. Stackebrandt & Pukall (1999), however, advised that even a 16S rRNA similarity level of >99·5 % is insufficient evidence to affiliate an isolate to a particular species. We have demonstrated that two cultures that share almost 99 % 16S rRNA gene sequence similarity, L2-TR^T and *I. abyssalis*, can be distinguished at the species level by phenotypic characteristics and DNA hybridization. In light of the differences described above between L2-TR^T and other members of the genus *Idiomarina*, we propose that L2-TR^T is a novel species within this genus and that it be assigned the designation *Idiomarina loihiensis* sp. nov., of which L2-TR^T (=ATCC BAA-735^T=DSM 15497^T) is the type strain.

Table 1. Selected phenotypic characteristics for the differentiation of *Idiomarina loihiensis* L2-TR^T from type strains of other species of the genus *Idiomarina*

Species: 1, *I. loihiensis*; 2, *I. abyssalis* [data from Ivanova *et al.* (2000)]; 3, *I. zobellii* [data from Ivanova *et al.* (2000)]; 4, *I. baltica* [data from Brettar *et al.* (2003)].

Characteristic	1	2	3	4
Cell length (μm)	0·7–1·8	1–1·8	1–1·8	0·7–1·6
Cell diameter (μm)	0·35–0·45	0·7–0·9	0·7–0·9	0·4–0·7
Flagella*	sp or subp	sp	sp and f	sp
Growth with NaCl (w/v, %)	0·5–20	0·6–15	1·0–10	1–10
NaCl optimum (w/v, %)	7·5–10	3–6	3–6	3–6
DNA G+C content (mol%)	47·4	50·4	48	49·9
Growth range (°C)	4–46	4–30	4–30	8–46
α-Cyclodextrin	−	+	−	−
Dextrin	+	+	−	−
Glycogen	+	+	+	−
Tween 40	+	−	−	+
Tween 80	+	−	−	+
Maltose	+	−	−	−
Methyl pyruvate	+	+	−	−
Monomethyl succinate	+	+	+	−
Acetic acid	+	+	−	+
cis-Aconitic acid	+	−	−	−
Citric acid	+	−	−	−
D-Gluconic acid	+	−	−	−
β-Hydroxybutyric acid	+	−	−	−
α-Ketobutyric acid	+	+	+	+
α-Ketoglutaric acid	+	−	−	−
α-Ketovaleric acid	−	+	+	+
DL-Lactic acid	+	−	−	−
Malonic acid	+	−	−	−
Propionic acid	+	+	−	−
Succinic acid	−	+	+	−
L-Alaninamide	−	+	+	−
D-Alanine	+	−	−	−
L-Alanine	+	+	+	−
L-Alanylglycine	−	+	+	−
L-Asparagine	+	−	−	−
L-Glutamic acid	+	−	−	−
Glycyl L-glutamic acid	−	+	+	−
L-Ornithine	−	−	+	−
L-Proline	+	+	−	−
D-Serine	+	−	−	−
Inosine	+	−	−	−
2,3-Butanediol	+	−	−	−
Glycerol	+	+	−	−
Glucose 6-phosphate	−	+	−	−

*sp, Single polar; subp, subpolar; f, fimbrae.

Description of *Idiomarina loihiensis* sp. nov.

Idiomarina loihiensis (lo.i.hi.en′sis. N.L. fem. adj. *loihiensis* originating from Lō'ihi, the site of isolation of the type strain).

Gram-negative rods, 0·35 μm wide and 0·7–1·0 μm long, which are occasionally up to 1·8 μm in length. Cells are motile by a single polar or subpolar flagellum. Growth occurs at 4–46 °C. Optimum salinity for growth is 7·5–10·0 % (w/v). Growth occurs aerobically on the following single carbon sources: methyl pyruvate, acetic acid, α-ketobutyric acid, propionic acid, L-alanine, L-alanylglycine,

Table 2. Fatty acid composition of *Idiomarina loihiensis* L2-TR^T, *I. abyssalis*, *I. zobellii* and *I. baltica*

Species: 1, *I. loihiensis*; 2, *I. abyssalis* [data from Ivanova *et al.* (2000)]; 3, *I. zobellii* [data from Ivanova *et al.* (2000)]; 4, *I. baltica* [data from Brettar *et al.* (2003)]. Only those fatty acids detected in amounts >1% of the total in each strain are shown. Fatty acids that comprised <1% each are available in a supplementary table in IJSEM Online. –, Not detected.

Fatty acid	1	2	3	4
$C_{16:0}$	7·6	6·3	4·6	4·8
$C_{18:0}$	1·6	1·8	0·8	0·9
Total saturated	9·2			5·7
$C_{11:0}$ iso	2·0	–	–	2·5
$C_{13:0}$ iso	1·8	1·0	1·1	0·8
$C_{15:0}$ iso	32·6	33·7	40·6	36·9
$C_{15:1}$ iso	1·3	2·3	1·6	1·5
$C_{15:1}\omega 8c$	–	1·3	1·1	
$C_{16:1}\omega 7c$	6·0	7·0	8·3	8·4
$C_{17:0}$ iso	11·0	11·9	12·5	11·2
$C_{17:1}\omega 6c$	–	1·5	3·4	
$C_{17:1}\omega 8c$	0·9	0·8	1·1	0·7
$C_{17:1}\omega 9c$ iso	11·9	–	–	10·0
$C_{18:1}\omega 7c$	5·5	6·7	5·9	6·0
$C_{18:1}\omega 7c$ 11-methyl	–	–	–	1·8
$C_{18:1}\omega 9c$	1·0	1·4	0·9	0·9
Total monounsaturated	74			81·1
$C_{10:0}$ 3-OH	0·8	–	–	1·2
$C_{11:0}$ iso 3-OH	4·1	–	–	3·7
$C_{13:0}$ iso 3-OH	3·3	–	–	3·2
Total hydroxy fatty acids	8·2			8·1
$C_{17:0}$ cyclo	1·7	–	–	0·6
Total named	99·6			95·5

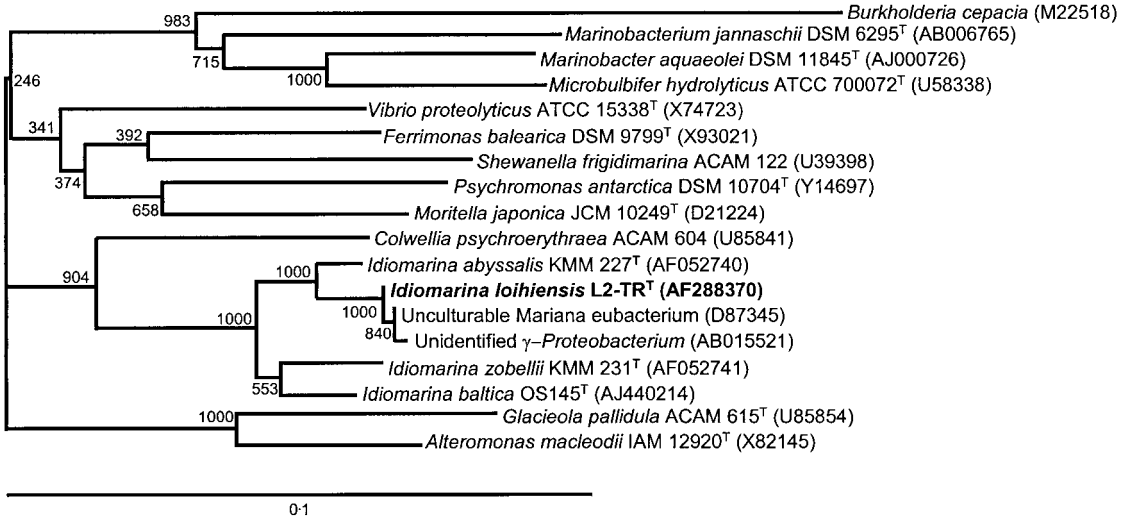

Fig. 2. Phylogenetic tree showing the relationship of *Idiomarina loihiensis* L2-TR^T with representatives of the family *Alteromonadaceae*, on the basis of 1350 nt of the 16S rRNA gene sequence aligned in CLUSTALX. Bootstrap values for 1000 replicates are shown. The tree is based on the neighbour-joining method of Saitou & Nei (1987), corrected for multiple substitutions and rendered in TREEVIEW (Page, 1996). Bar, 0·1 nucleotide substitutions per site.

glycyl L-glutamic acid, L-proline, glycerol, alaninamide, Tween 40, Tween 80 and inositol. The following constitutive enzyme activities are expressed: alkaline phosphatase, esterase (C_4), esterase lipase (C_8), leucine arylamidase, acid phosphatase and phosphohydrolase. Major fatty acid is 13-methyl tetradecanoic acid (iso-$C_{15:0}$); most fatty acids are iso-branched. DNA G+C content is 47·4 mol%. Phylogenetic placement based on 16S rRNA gene sequence affiliates the strain to the genus *Idiomarina*, but there is evidence for divergence from the three previously recognized species in this genus, *I. abyssalis* ATCC BAA-312^T, *I. zobellii* ATCC BAA-313^T and *I. baltica* DSM 15154^T.

The type strain is L2-TR^T (=ATCC BAA-735^T=DSM 15497^T).

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References

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.

Atlas, R. M. (1997). *Handbook of Microbiological Media*, 2nd edn, p. 7. Edited by L. C. Parks. Boca Raton, FL: CRC Press.

Brettar, I., Christen, R. & Höfle, M. G. (2003). *Idiomarina baltica* sp. nov., a marine bacterium with a high optimum growth temperature isolated from surface water of the central Baltic Sea. *Int J Syst Evol Microbiol* **53**, 407–413.

Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Donachie, S. P. (1996). A seasonal study of marine bacteria in Admiralty Bay (Antarctica). *Proc NIPR Symp Polar Biol* **9**, 111–124.

Donachie, S. P., Christenson, B. W., Kunkel, D. D., Malahoff, A. & Alam, M. (2002). Microbial community in acidic hydrothermal waters of volcanically active White Island, New Zealand. *Extremophiles* **6**, 419–425.

Emerson, D. & Moyer, C. L. (2002). Neutrophilic Fe-oxidizing bacteria are abundant at the Loihi Seamount hydrothermal vents and play a major role in Fe oxide deposition. *Appl Environ Microbiol* **68**, 3085–3093.

Escara, J. F. & Hutton, J. R. (1980). Thermal stability and renaturation of DNA in dimethyl sulfoxide solutions: acceleration of the renaturation rate. *Biopolymers* **19**, 1315–1327.

Guezennec, J. G., Dussauze, J., Bian, M., Rocchiccioli, F., Ringelberg, D., Hedrick, D. B. & White, D. C. (1996). Bacterial community structure in sediments from Guaymas Basin, Gulf of California, as determined by analysis of phospholipid ester-linked fatty acids. *J Mar Biotechnol* **4**, 165–175.

Harwood, C. S., Jannasch, H. W. & Canale-Parola, E. (1982). Anaerobic spirochete from a deep-sea hydrothermal vent. *Appl Environ Microbiol* **44**, 234–237.

Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

Ivanova, E. P., Romanenko, L. A., Chun, J. & 7 other authors (2000). *Idiomarina* gen. nov., comprising novel indigenous deep-sea bacteria from the Pacific Ocean, including descriptions of two species, *Idiomarina abyssalis* sp. nov. and *Idiomarina zobellii* sp. nov. *Int J Syst Evol Microbiol* **50**, 901–907.

Jahnke, K.-D. (1992). Basic computer program for evaluation of spectroscopic DNA renaturation data from GILFORD system 2600 spectrometer on a PC/XT/AT type personal computer. *J Microbiol Methods* **15**, 61–73.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Karl, D. M., McMurtry, G. M., Malahoff, A. & Garcia, M. O. (1988). Loihi Seamount, Hawaii: a mid-plate volcano with a distinctive hydrothermal system. *Nature* **335**, 532–535.

Karl, D. M., Brittain, A. M. & Tilbrook, B. D. (1989). Hydrothermal and microbial processes at Loihi Seamount, a mid-plate hot-spot volcano. *Deep-Sea Res* **36**, 1655–1673.

Klein, F. W. (1982). Earthquakes at Loihi submarine volcano and the Hawaiian hot spot. *J Geophys Res* **87**, 7719–7726.

Malahoff, A. (1987). Geology of the summit of Loihi submarine volcano. *U S Geol Surv Prof Pap* **1350**, 133–144.

Miroschnichenko, M. L., Rainey, F. A., Rhode, M. & Bonch-Osmolovskaya, E. A. (1999). *Hippea maritima* gen. nov., sp. nov., a new genus of thermophilic, sulfur-reducing bacterium from submarine hot vents. *Int J Syst Bacteriol* **49**, 1033–1038.

Moyer, C. L., Dobbs, F. C. & Karl, D. M. (1994). Estimation of diversity and community structure through restriction fragment length polymorphism distribution analysis of bacterial 16S rRNA genes from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. *Appl Environ Microbiol* **60**, 871–879.

Moyer, C. L., Dobbs, F. C. & Karl, D. M. (1995). Phylogenetic diversity of the bacterial community from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. *Appl Environ Microbiol* **61**, 1555–1562.

Mullis, K. B. & Faloona, F. A. (1987). Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. *Methods Enzymol* **155**, 335–350.

Page, R. D. M. (1996). TREEVIEW: an application to display phylogenetic trees on personal computers. *Comp Appl Biosci* **12**, 357–358.

Palleroni, N. J. (1997). Prokaryotic diversity and the importance of culturing. *Antonie van Leeuwenhoek* **72**, 3–19.

Ruby, E. G., Wirsen, C. O. & Jannasch, H. W. (1981). Chemolithotrophic sulfur-oxidizing bacteria from the Galapagos Rift hydrothermal vents. *Appl Environ Microbiol* **42**, 317–324.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sasser, M. (1997). *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids* (MIDI Technical Note 101). Newark, DE: MIDI.

Sievert, S. M., Ziebis, W., Kuever, J. & Sahm, K. (2000). Relative abundance of *Archaea* and *Bacteria* along a thermal gradient of a shallow-water hydrothermal vent quantified by rRNA slot-blot hybridization. *Microbiology* **146**, 1287–1293.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Stackebrandt, E. & Pukall, R. (1999). Response to Althoff *et al.* (*Mar Biol* 130: 529–536). Deriving taxonomic decisions from 16S rDNA: a case study. *Mar Biol* **133**, 159–161.

Suzuki, M. T., Rappé, M. S., Haimberger, Z. W., Winfield, H., Adair, N., Ströbel, J. & Giovannoni, S. J. (1997). Bacterial diversity among small-subunit rRNA gene clones and cellular isolates from the same seawater sample. *Appl Environ Microbiol* **63**, 983–989.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 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.