Dermacoccus barathri sp. nov. and *Dermacoccus profundus* sp. nov., novel actinomycetes isolated from deep-sea mud of the Mariana Trench

Wasu Pathom-aree,^{1†} Yuichi Nogi,² Alan C. Ward,¹ Koki Horikoshi,² Alan T. Bull³ and Michael Goodfellow¹

Correspondence

Michael Goodfellow
m.goodfellow@ncl.ac.uk

¹Division of Biology, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK

²Extremobiosphere Research Center, Japan Agency for Marine–Earth Science and Technology (JAMSTEC), 2-15 Natsushima-cho, Yokosuka 237-0061, Japan

³Department of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ, UK

The taxonomic positions of two actinobacterial strains isolated from Mariana Trench sediment were established using a combination of genotypic and phenotypic data. The strains, isolates MT2.1^T and MT2.2^T, formed a distinct phyletic line in the *Micrococcineae* 16S rRNA gene tree together with *Dermacoccus abyssi* NCIMB 14084^T. The isolates had chemical and phenotypic properties typical of members of the genus *Dermacoccus* and could be distinguished sharply from one another and from the type strains of *Dermacoccus abyssi* and *Dermacoccus nishinomiyaensis* using DNA–DNA relatedness data. A range of phenotypic properties served to distinguish the two novel strains from one another and from the type strains of established *Dermacoccus* species. The G + C contents of the DNAs of strains MT2.1^T and MT2.2^T were 66.8 and 69.1 mol%, respectively. It is evident that the two isolates merit recognition as novel species within the genus *Dermacoccus*. The names proposed for these taxa are *Dermacoccus barathri* sp. nov. (type strain MT2.1^T = DSM 17574^T = NCIMB 14081^T) and *Dermacoccus profundus* sp. nov. (type strain MT2.2^T = DSM 17575^T = NCIMB 14084^T).

The genus *Dermacoccus* Stackebrandt *et al.* 1995 currently contains two species, *Dermacoccus nishinomiyaensis* (Oda 1935) Stackebrandt *et al.* 1995, a taxon that comprises strains initially classified as *Micrococcus nishinomiyaensis* Oda 1935 emend. Kocur *et al.* 1975, and *Dermacoccus abyssi* Pathom-aree *et al.* 2006a that accommodates a piezotolerant strain isolated from sediment collected from the Challenger Deep in the Mariana Trench. The genus *Dermacoccus* is classified in the family *Dermacoccaceae* Stackebrandt and Schumann 2000, together with the genera *Demetria* Groth *et al.* 1997 and *Kytococcus* Stackebrandt *et al.* 1995; isolates assigned to this family are typically associated with terrestrial habitats, notably cured meat products, skin and soil (Cordero & Zumalacárregui, 2000; De la Rosa *et al.*, 1990; Papamanoli *et al.*, 2002).

The present investigation was designed to determine the taxonomic status of two additional actinobacterial strains that were isolated from the Challenger Deep and were found

to be closely related to *Dermacoccus abyssi* (Pathom-aree *et al.*, 2006a). The isolates were the subjects of a polyphasic study, which showed that they merited classification as representing novel *Dermacoccus* species, *Dermacoccus barathri* sp. nov. and *Dermacoccus profundus* sp. nov.

Strains MT2.1^T and MT2.2^T were isolated from a sediment sample collected from the Mariana Trench (Challenger Deep; 11° 19' 911" N 142° 12' 372" E) at a depth of 10 898 m using sterilized mud samplers and the remotely operated submersible *Kaiko* (Kato *et al.*, 1997). The sample (2 ml), which was collected on 21 May 1998 during dive number 74, was transported to the UK in an insulated container at 4 °C and then stored at –20 °C. The strains were isolated from a suspension of the sediment sample used to inoculate a raffinose-histidine agar plate (Vickers *et al.*, 1984) supplemented with cycloheximide and nystatin. The isolates were maintained on glucose-yeast extract agar plates (Gordon & Mihm, 1962) at room temperature and as glycerol suspensions (20%, v/v) at –20 °C. Biomass for the chemotaxonomic and molecular systematic studies was prepared from 7-day-old glucose-yeast extract broth (Gordon & Mihm, 1962) shake cultures grown at 28 °C, washed twice with distilled water and harvested by centrifugation.

[†]Present address: Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains MT2.1^T and MT2.2^T are AY894328 and AY894329, respectively.

Isolation of chromosomal DNA, PCR amplification and direct sequencing of the purified products of strains MT2.1^T and MT2.2^T were carried out as described previously (Pathom-aree *et al.*, 2006b). Almost-complete 16S rRNA gene sequences of isolates MT2.1^T (1468 nt) and MT2.2^T (1466 nt) were aligned manually with corresponding sequences of representatives of genera classified in the suborder *Micrococcineae* that had been retrieved from the DDBJ/EMBL/GenBank databases using the program PHYDIT (available at <http://plaza.snu.ac.kr/~jchun/phydit/>). Phylogenetic trees were inferred using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms from the PHYLIP suite of programs (Felsenstein, 1993). Evolutionary distance matrices for the least-squares and neighbour-joining methods were generated after Jukes & Cantor (1969). The stability of the resultant tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings of the neighbour-joining dataset using the SEQBOOT and CONSENSE options from the PHYLIP package.

It is evident from Fig. 1 that the isolates fall within the evolutionary radiation occupied by the genus *Dermacoccus*. The two strains shared a 16S rRNA gene sequence similarity of 99.9%, a value that corresponds to a single nucleotide difference at the 1470 locations available for comparison. It is evident from the phylogenetic tree that the isolates are most closely associated with the type strain of *Dermacoccus abyssii*, a relationship that was supported by all of the tree-making algorithms and by a 100% bootstrap value.

Isolates MT2.1^T and MT2.2^T were examined for key chemical markers to determine whether they had a chemotaxonomic profile consistent with their classification within the genus *Dermacoccus*. Standard methods were used

for the extraction and analysis of fatty acids (Sutcliffe, 2000), mycolic acids (Hamid *et al.*, 1993), isoprenoid quinones (Collins, 1994), muramic acid type (Uchida *et al.*, 1999) and polar lipids (Minnikin *et al.*, 1984) from freeze-dried biomass of the isolates. The peptidoglycan structures of the cell walls of the strains were determined by the DSMZ identification service using established procedures (Schleifer & Kandler, 1972; Schleifer, 1985; MacKenzie, 1987).

The two isolates were found to have a chemotaxonomic profile consistent with their classification in the genus *Dermacoccus* (Stackebrandt *et al.*, 1995; Pathom-aree *et al.*, 2006a). They were also characterized by the presence of *N*-acetylated muramic acid, L-lysine as the diamino acid, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol as major polar lipids (phospholipid type I *sensu* Lechevalier *et al.*, 1977), dihydrogenated menaquinones with eight isoprene units as the predominant isoprenologue, fatty acids rich in branched-chain components and by the absence of mycolic acids.

One- and two-dimensional TLC of the total hydrolysate of the peptidoglycan of each strain (4 M HCl, 16 h at 100 °C) showed the presence of the amino acids alanine, glutamic acid, lysine and serine. After derivatization, the molar amino acid ratios as determined by gas chromatography were 3.0 Ala, 0.9 Ser, 2.3 Glu, 1.0 Lys and a trace of Thr for strain MT2.1^T and 3.2 Ala, 0.1 Thr, 0.9 Ser, 2.4 Glu and 1.0 Lys for strain MT2.2^T. Traces of hydrolytically stable peptide were also found for each strain (Lys-Ser). Partial hydrolysis (4 M HCl, 45 min at 100 °C) and two-dimensional TLC showed the presence of the peptides L-Ala-D-Glu, D-Ala-D-Glu, L-Lys-L-Ser, D-Ala-L-Lys-L-Ser; the patterns were identical for each strain. Denitrophenylation showed that the glutamic acid was from the N terminus of the interpeptide bridge. It is evident from these data that the strains have an A4 α peptidoglycan type *sensu* Schleifer & Kandler (1972).

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost-complete 16S rRNA gene sequences showing the relationships between isolates MT2.1^T and MT2.2^T and representatives of the suborder *Micrococcineae*. Asterisks indicate phyletic lines that were recovered using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms. Numbers at nodes indicate levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50% are given. Bar, 0.1 substitutions per nucleotide position.

The fatty acids of the two strains were very similar and notably rich in branched-chain components (>40 % total in each; 13-methyltetradecanoic, 14-methylpentadecanoic, 15-methylhexadecanoic and 14-methylhexadecanoic acids). Saturated straight-chain components (20–24 % total; hexadecanoic, heptadecanoic and octadecanoic acids) and unsaturated straight-chain components (29–36 % total; hexadecenoic, heptadecenoic and octadecenoic acids) were also present; each strain also contained a trace of 12-methyltetradecanoic acid (anteiso-15), probably as a by-product of anteiso- $C_{17:0}$ fatty acid synthesis. The fatty acid profiles were very similar to those reported previously for *Dermacoccus abyssi* NCIMB 14084^T (Pathom-aree *et al.*, 2006a). As discussed previously with respect to our analyses of fatty acid composition in *Dermacoccus abyssi* NCIMB 14084^T and *Dermacoccus nishinomiyaensis* DSM 20448^T (Pathom-aree *et al.*, 2006a), we did not detect significant quantities of branched-chain unsaturated fatty acids in isolates MT2.1^T and MT2.2^T, although these have previously been reported in *Dermacoccus nishinomiyaensis* and *Kytococcus* spp. (Stackebrandt *et al.*, 1995; Becker *et al.*, 2002). Aside from our findings, anteiso- $C_{15:0}$ was not found to be a major fatty acid for *Dermacoccus nishinomiyaensis* (Stackebrandt *et al.*, 1995) or for close relatives such as *Demetria terrigena* (Groth *et al.*, 1997), *Dermatophilus congolensis* (McNabb *et al.*, 1997) or *Kineospira limosa* (Liu *et al.*, 2002). In contrast, *Kytococcus schroeteri* and *Kytococcus sedentarius* contain minor proportions of this component (3.5 and 1.2 %, respectively; Stackebrandt *et al.*, 1995; Becker *et al.*, 2002) and *Kocuria* species significant proportions (approximately 50–70 %; Stackebrandt *et al.*, 1995; Kovács *et al.*, 1999; Schumann *et al.*, 1999).

The G+C contents of the DNAs of isolates MT2.1^T and MT2.2^T were determined by reversed-phase HPLC (Tamaoka & Komagata, 1984); an equimolar mixture of four deoxyribonucleotides in a Yamasa GC kit (Yamasa Shoyu) was used as the quantitative standard. DNA–DNA hybridization experiments were carried out between the two isolates and between them and *Dermacoccus abyssi* DSM 17573^T and *Dermacoccus nishinomiyaensis* DSM 20448^T using the microplate method, as described by Ezaki *et al.* (1989). Mean percentage DNA–DNA relatedness values were calculated from three hybridization experiments. The DNA G+C contents of isolates MT2.1^T and MT2.2^T were 66.8 and 69.1 mol%, respectively. The mean DNA–DNA relatedness found between the two isolates was 17.6 ± 6.5 %, a value well below the 70 % cut-off point recommended for the assignment of bacterial strains to the same genomic species (Wayne *et al.*, 1987). Similarly, isolates MT2.1^T and MT2.2^T had DNA–DNA relatedness values with the type strains of *Dermacoccus abyssi* and *Dermacoccus nishinomiyaensis* of 14.3 ± 4.7 and 24.6 ± 8.9 %, and 4.3 ± 2.5 and 14.6 ± 3.5 %, respectively.

The two organisms were examined for a range of biochemical and physiological characteristics using methods described by Kloos *et al.* (1974); *Williamsia marianensis* DSM 44944^T was used as a positive control for the acid from carbohydrate tests. The two isolates and the type strains of *Dermacoccus abyssi* and *Dermacoccus nishinomiyaensis* were screened for enzyme activity using API ZYM kits (bioMérieux); the results were read after 4 h at 30 °C. It is apparent from Table 1 that the two isolates can be distinguished from one another and from the type

Table 1. Phenotypic properties that differentiate isolates MT2.1^T and MT2.2^T from one another and from the type strains of *Dermacoccus abyssi* and *Dermacoccus nishinomiyaensis*

+, Positive, –, negative.

Characteristic	MT2.1 ^T	MT2.2 ^T	<i>D. abyssi</i> DSM 17573 ^T	<i>D. nishinomiyaensis</i> DSM 20448 ^T
H ₂ S production	–	+	–	–
Urea hydrolysis	+	+	–	–
Degradation of:				
Arbutin	–	–	+	–
DNA	+	–	+	+
Gelatin	–	–	–	+
Starch	–	–	+	+
Tween 80	–	–	+	–
API ZYM:				
α -Fucosidase	+	–	+	+
β -Glucosidase	+	–	+	+
Lipase (C14)	+	–	+	+
Trypsin	+	+	–	+
Growth at/on:				
10 °C	+	+	+	–
10 % NaCl	+	+	+	–
12.5 % NaCl	–	+	–	–

strains of *Dermacoccus abyssi* and *Dermacoccus nishino-miyaensis* by a range of phenotypic properties. It is particularly interesting that only the strains isolated from the Challenger Deep sediment grew at 10 °C and in the presence of high salt concentrations. The close taxonomic relationship found between the dermacocci isolated from the Mariana Trench sediment suggest that isolates MT2.1^T and MT2.2^T will prove to be piezotolerant, as *Dermacoccus abyssi* DSM 17573^T grows well at 40 MPa (Pathom-aree *et al.*, 2006a).

The genotypic and phenotypic data show that isolates MT2.1^T and MT2.2^T represent novel species within the genus *Dermacoccus*. The names proposed for these taxa are *Dermacoccus barathri* sp. nov. and *Dermacoccus profundus* sp. nov.

Description of *Dermacoccus barathri* sp. nov.

Dermacoccus barathri (ba.ra'thri. L. neut. n. *barathrum* a deep pit, an abyss; L. gen. n. *barathri* of an abyss).

Aerobic, Gram-positive, non-acid-alcohol-fast, non-motile actinomycete that forms coccoid cells (diameter 0.8–1.5 µm) that occur in irregular clusters. Light-yellow, circular, entire, convex, smooth, glistening colonies are formed on glucose-yeast extract agar after 5 days at 28 °C. Grows well on tryptic soy agar, but poorly on inorganic nitrogen agar. Growth occurs between 10 and 37 °C, with an optimum around 28 °C. Hypoxanthine, Tween 20, Tween 40 and uric acid are degraded. Acid is not formed from (+)-D-arabinose, (+)-D-arabitol, (–)-L-arabitol, (+)-D-cellobiose, dextran, *meso*-erythritol, (+)-D-fructose, (+)-D-galactose, (+)-D-glucose, glycerol, glycogen, *myo*-inositol, inulin, (+)-D-maltose, (+)-D-mannitol, (+)-D-mannose, (+)-D-melezitose, (+)-D-melibiose, (+)-D-raffinose, (–)-L-rhamnose, (+)-D-salicin, (+)-D-sucrose, (+)-D-trehalose, (+)-D-sorbitol, (+)-L-sorbose, xylitol or (+)-D-xylose. Additional phenotypic properties are shown in Table 1. Has chemical markers characteristic of the genus *Dermacoccus*.

The type strain is MT2.1^T (=DSM 17574^T=NCIMB 14081^T), which was isolated from sediment collected from the Challenger Deep of the Mariana Trench at a depth of 10 898 m. The G + C content of the DNA of the type strain is 66.8 mol%.

Description of *Dermacoccus profundus* sp. nov.

Dermacoccus profundus (pro.fun'di. L. neut. n. *profundum* depth, abyss; L. gen. n. *profundus* of an abyss).

Aerobic, Gram-positive, non-acid-alcohol-fast, non-motile actinomycete that forms coccoid cells (diameter 0.8–1.5 µm) that occur in irregular clusters. Brilliant-yellow, irregular, entire, pulvinate, rough colonies with matted surfaces are formed on glucose-yeast extract agar after 5 days at 28 °C. Grows well on tryptic soy agar, but poorly on inorganic nitrogen agar. Growth occurs between

10 and 37 °C, with an optimum around 28 °C. Hypoxanthine, Tween 20, Tween 40 and uric acid are degraded. Acid is not formed from (+)-D-arabinose, (+)-D-arabitol, (–)-L-arabitol, (+)-D-cellobiose, dextran, *meso*-erythritol, (+)-D-fructose, (+)-D-galactose, (+)-D-glucose, glycerol, glycogen, *myo*-inositol, inulin, (+)-D-maltose, (+)-D-mannitol, (+)-D-mannose, (+)-D-melezitose, (+)-D-melibiose, (+)-D-raffinose, (–)-L-rhamnose, (+)-D-salicin, (+)-D-sucrose, (+)-D-trehalose, (+)-D-sorbitol, (+)-L-sorbose, xylitol or (+)-D-xylose. Additional phenotypic properties are shown in Table 1. Has chemical markers characteristic of the genus *Dermacoccus*.

The type strain is MT2.2^T (=DSM 17575^T=NCIMB 14084^T), which was isolated from sediment collected from the Challenger Deep of the Mariana Trench at a depth of 10 898 m. The G + C content of the DNA of the type strain is 69.1 mol%.

Acknowledgements

Wasu Pathom-aree is grateful to the DPST program and the Royal Thai Government for financial support. We thank the *Kaiko* operation team and the crew of MS *Yokosuka* for collecting the sediment samples, Dr Iain Sutcliffe (University of Northumbria) for help with the fatty acid analyses, Amanda Jones (University of Newcastle) for carrying out the API tests and Dr Jean Euzéby for expert advice in naming the organisms. This work was supported by the UK Natural Environmental Research Council (grants NER/T/S/2000/00614 and NER/T/S/2000/00616). Alan Bull thanks The Leverhulme Trust for the award of an Emeritus Fellowship.

References

- Becker, K., Schumann, P., Wüllenweber, J., Schulte, M., Weil, H.-P., Stackebrandt, E., Peters, G. & von Eiff, C. (2002). *Kytococcus schroeteri* sp. nov., a novel Gram-positive actinobacterium isolated from a human clinical source. *Int J Syst Evol Microbiol* 52, 1609–1614.
- Collins, M. D. (1994). Isoprenoid quinones. In *Chemical Methods in Prokaryotic Systematics*, pp. 265–309. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.
- Cordero, M. R. & Zumalacárregui, J. M. (2000). Characterization of *Micrococcaceae* isolated from salt used for Spanish dry-cured ham. *Lett Appl Microbiol* 31, 303–306.
- De la Rosa, M. C., Mohino, M. R., Mohino, M. & Mosso, M. A. (1990). Characteristics of micrococci and staphylococci isolated from semi-preserved meat products. *Food Microbiol* 7, 207–215.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* 39, 224–229.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 17, 368–376.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Department of Genome Sciences, University of Washington, Seattle, USA.

- Fitch, W. M. & Margoliash, E. (1967). Construction of phylogenetic trees. *Science* **155**, 279–284.
- Gordon, R. E. & Mihm, J. M. (1962). Identification of *Nocardia caviae* (Erikson) nov. comb. *Ann N Y Acad Sci* **98**, 628–636.
- Groth, I., Schumann, P., Rainey, F. A., Martin, K., Schuetze, B. & Augsten, K. (1997). *Demetria terrigena* gen. nov., sp. nov., a new genus of actinomycetes isolated from compost soil. *Int J Syst Bacteriol* **47**, 1129–1133.
- Hamid, M. E., Minnikin, D. E., Goodfellow, M. & Ridell, M. (1993). Thin-layer chromatographic analysis of glycolipids and mycolic acids from *Mycobacterium farcinogenes*, *Mycobacterium senegalense* and related taxa. *Zentralbl Bakteriol* **279**, 354–367.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kato, C., Li, L., Tamaoka, J. & Horikoshi, K. (1997). Molecular analyses of the sediment of the 11,000-m deep Mariana Trench. *Extremophiles* **1**, 117–123.
- Kloos, W. E., Tornabene, T. G. & Schleifer, K. H. (1974). Isolation and characterization of micrococci from human skin, including two new species: *Micrococcus lylae* and *Micrococcus kristinae*. *Int J Syst Bacteriol* **24**, 79–101.
- Kluge, A. G. & Farris, F. G. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.
- Kocur, M., Schleifer, K. H. & Kloos, W. E. (1975). Taxonomic status of *Micrococcus nishinomiyaensis* Oda 1935. *Int J Syst Bacteriol* **25**, 290–293.
- Kovács, G., Burghardt, J., Pradella, S., Schumann, P., Stackebrandt, E. & Máriaiget, K. (1999). *Kocuria palustris* sp. nov. and *Kocuria rhizophila* sp. nov., isolated from the rhizosphere of the narrow-leaved cattail (*Typha angustifolia*). *Int J Syst Bacteriol* **49**, 167–173.
- Lechevalier, H. A., De Bièvre, C. & Lechevalier, M. P. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.
- Liu, W.-T., Hanada, S., Marsh, T. L., Kamagata, Y. & Nakamura, K. (2002). *Kineospaera limosa* gen. nov., sp. nov., a novel Gram-positive polyhydroxyalkanoate-accumulating coccus isolated from activated sludge. *Int J Syst Evol Microbiol* **52**, 1845–1849.
- MacKenzie, S. L. (1987). Gas chromatographic analysis of amino acids as the N-heptafluorobutyl isobutyl esters. *J Assoc Off Anal Chem* **70**, 151–160.
- McNabb, A., Shuttleworth, R., Behme, R. & Colby, W. D. (1997). Fatty acid characterization of rapidly growing pathogenic aerobic actinomycetes as a means of identification. *J Clin Microbiol* **35**, 1361–1368.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Oda, M. (1935). Bacteriological studies on waters used for brewing saké (part 6). I. Bacteriological studies on “miyamizu” (8) and (9). *Micrococcus* and *Actinomyces* isolated from “miyamizu”. *Jozogaku Zasshi* **13**, 1202–1228 (in Japanese).
- Papamanoli, E., Kotzekidou, P., Tzanetakis, N. & Litopoulou-Tzanetaki, E. (2002). Characterization of *Micrococcaceae* isolated from dry fermented sausage. *Food Microbiol* **19**, 441–449.
- Pathom-aree, W., Nogi, Y., Sutcliffe, I. C., Ward, A. C., Horikoshi, K., Bull, A. T. & Goodfellow, M. (2006a). *Dermacoccus abyssi* sp. nov., a novel piezotolerant actinomycete isolated from the Mariana Trench. *Int J Syst Evol Microbiol* **56**, 1233–1237.
- Pathom-aree, W., Stach, J. E. M., Ward, A. C., Horikoshi, K., Bull, A. T. & Goodfellow, M. (2006b). Diversity of actinomycetes isolated from Challenger Deep sediment (10,898 m) from the Mariana Trench. *Extremophiles* **10**, 181–189.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Schumann, P., Spröer, C., Burghardt, J., Kovacs, G. & Stackebrandt, E. (1999). Reclassification of the species *Kocuria erythromyxa* (Brooks and Murray 1981) as *Kocuria rosea* (Flügge 1886). *Int J Syst Bacteriol* **49**, 393–396.
- Stackebrandt, E. & Schumann, P. (2000). Description of *Bogoriellaceae* fam. nov., *Dermacoccaceae* fam. nov., *Rarobacteraceae* fam. nov. and *Sanguibacteraceae* fam. nov. and emendation of some families of the suborder *Micrococcineae*. *Int J Syst Evol Microbiol* **50**, 1279–1285.
- Stackebrandt, E., Koch, C., Gvozdiak, O. & Schumann, P. (1995). Taxonomic dissection of the genus *Micrococcus*: *Kocuria* gen. nov., *Nesterenkonia* gen. nov., *Kytococcus* gen. nov., *Dermacoccus* gen. nov., and *Micrococcus* Cohn 1872 gen. emend. *Int J Syst Bacteriol* **45**, 682–692.
- Sutcliffe, I. C. (2000). Characterisation of a lipomannan lipoglycan from the mycolic acid containing actinomycete *Dietzia maris*. *Antonie van Leeuwenhoek* **78**, 195–201.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Uchida, K., Kudo, T., Suzuki, K. I. & Nakase, T. (1999). A new rapid method of glycolate test by diethyl ether extraction, which is applicable to a small amount of bacterial cells of less than one milligram. *J Gen Appl Microbiol* **45**, 49–56.
- Vickers, J. C., Williams, S. T. & Ross, G. W. (1984). A taxonomic approach to selective isolation of streptomycetes from soil. In *Biological, Biochemical and Biomedical Aspects of Actinomycetes*, pp. 553–561. Edited by L. Ortiz-Ortiz, L. F. Bojalil & V. Yakoleff. Orlando: Academic Press.
- 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.