Dermacoccus barathri sp. nov. and Dermacoccus profundi 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

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 MT2.1T and MT2.2T, formed a distinct phyletic line in the Micrococccineae 16S rRNA gene tree together with Dermacoccus abyssi NCIMB 14084T. 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 MT2.1T and MT2.2T 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 MT2.1T = DSM 17574T = NCIMB 14081T) and Dermacoccus profundi sp. nov. (type strain MT2.2T = DSM 17575T = NCIMB 14084T).

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 Kyttococcus 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 profundi sp. nov.

Strains MT2.1T and MT2.2T 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.

1Present 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 MT2.1T and MT2.2T are AY894328 and AY894329, respectively.
Isolation of chromosomal DNA, PCR amplification and direct sequencing of the purified products of strains MT2.1T and MT2.2T were carried out as described previously (Pathom-aree et al., 2006b). Almost-complete 16S rRNA gene sequences of isolates MT2.1T (1468 nt) and MT2.2T (1466 nt) were aligned manually with corresponding sequences of genera classified in the suborder Micrococccinae 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 (Pathom-aree et al., 2006b) 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 abyssi*, a relationship that was supported by all of the tree-making algorithms and by a 100% bootstrap value.

Isolates MT2.1T and MT2.2T 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 MT2.1T and 3:2 Ala, 0:1 Thr, 0:9 Ser, 2:4 Glu and 1:0 Lys for strain MT2.2T. Traces of hydrostablycceptable 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-Glu, D-Ala–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 A42 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 MT2.1T and MT2.2T and representatives of the suborder Micrococccinae. 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.](image-url)
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, 13-methyltetradecanoic, 14-methylpentadecanoic, and 14-methylhexadecanoic acids). Notably, branched-chain components (anteiso-C15:0 and anteiso-C17:0) were also present; each strain also contained a trace of 12-methyltetradecanoic, 13-methyltetradecanoic, 14-methylpentadecanoic, and 14-methylhexadecanoic acids). The fatty acid methyltetradecanoic acid (anteiso-15), probably as a by-product of anteiso-C15:0 fatty acid synthesis. The fatty acid profiles were very similar to those reported previously for *Dermacoccus abyssi* NCIMB 14084\textsuperscript{T} (Pathom-aree et al., 2006a). As discussed previously with respect to our analyses of fatty acid composition in *Dermacoccus abyssi* NCIMB 14084\textsuperscript{T} and *Dermacoccus nishinomiyaensis* DSM 20448\textsuperscript{T} (Pathom-aree et al., 2006a), we did not detect significant quantities of branched-chain unsaturated fatty acids in isolates MT2.1\textsuperscript{T} and MT2.2\textsuperscript{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-C15:0 was not found to be a major fatty acid for *Dermacoccus nishinomiyaensis* (Stackebrandt et al., 1995) or for close relatives such as *Demetria terragenae* (Groth et al., 1997), *Dermatophilus congoensis* (McNabb et al., 1997) or *Kineosphaera 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\textsuperscript{T} and MT2.2\textsuperscript{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\textsuperscript{T} and *Dermacoccus nishinomiyaensis* DSM 20448\textsuperscript{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\textsuperscript{T} and MT2.2\textsuperscript{T} were 66±8 and 69±1 mol%, respectively. The mean DNA–DNA relatedness found between the two isolates was 17±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\textsuperscript{T} and MT2.2\textsuperscript{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\textsuperscript{T} was used as a positive control for the acid from salmon. 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 strains of *Dermacoccus abyssi* and *Dermacoccus nishinomiyaensis*.

### Table 1. Phenotypic properties that differentiate isolates MT2.1\textsuperscript{T} and MT2.2\textsuperscript{T} from one another and from the type strains of *Dermacoccus abyssi* and *Dermacoccus nishinomiyaensis*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MT2.1\textsuperscript{T}</th>
<th>MT2.2\textsuperscript{T}</th>
<th><em>D. abyssi</em> DSM 17573\textsuperscript{T}</th>
<th><em>D. nishinomiyaensis</em> DSM 20448\textsuperscript{T}</th>
</tr>
</thead>
<tbody>
<tr>
<td>H\textsubscript{2}S production</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Urea hydrolysis</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<td>Degradation of:</td>
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<tr>
<td>Arbutin</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td>DNA</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Gelatin</td>
<td>−</td>
<td>−</td>
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<td>Starch</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<td>Tween 80</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<td>API ZYM:</td>
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<tr>
<td>α-Fucosidase</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>β-Glucosidase</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Lipase (C14)</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Trypsin</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<td>Growth at/on:</td>
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<tr>
<td>10 °C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>10% NaCl</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>12.5% NaCl</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
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</table>
strains of *Dermacoccus abyssi* and *Dermacoccus nishinomiyensis* 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 *Dermacoccus* 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 profundi* 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 coccolid cells (diameter 0.5–1 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-xylene. 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%.

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**References**


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