Dermacoccus abyssi sp. nov., a piezotolerant actinomycete isolated from the Mariana Trench

Wasu Pathom-aree,† Yuichi Nogi, Iain C. Sutcliffe, Alan C. Ward, Koki Horikoshi, Alan T. Bull and Michael Goodfellow

The monospecific genus Dermacoccus was proposed by Stackebrandt et al. (1995) to accommodate actinomycetes that had been isolated from human skin and water and previously classified as Micrococcus nishinomiyaensis Oda 1935 emend. Kocur et al. 1975. The genus belongs to the family Dermacoccaceae Stackebrandt and Schumann 2000, as do the genera Demetria Groth et al. 1997 and Kytococcus Stackebrandt et al. 1995; members of this family are usually associated with terrestrial habitats, notably cured meat products, skin and soil (De la Rosa et al., 1990; Cordero & Zumalacáregui, 2000; Papamanoli et al., 2002).

The study was designed to establish the taxonomic status of an actinobacterial strain, isolate MT1.1T, recovered from sediment collected from the Challenger Deep in the Mariana Trench and considered to be closely related to the genus Dermacoccus using 16S rRNA gene sequence data (Pathom-aree et al., 2006). The strain was the subject of a polyphasic taxonomic study, which showed that it merited recognition within a novel species of Dermacoccus.

Sediment was taken 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. Strain MT1.1T was 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. It was maintained on glucose-yeast extract agar plates (Gordon & Mihm, 1962) at room temperature and as glycerol suspensions (20%, v/v) at −20 °C.

Isolation of chromosomal DNA, PCR amplification and direct sequencing of the purified products of strain MT1.1T were carried out as described previously (Kim et al., 2000). The amplified 16S rRNA gene sequence (1443 nt) was aligned manually with corresponding sequences of representatives of genera classified in the suborder Micrococccineae that had been retrieved from DDBJ/EMBL/GenBank using the program PHYLIP (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). Stability of the resultant...
tree topologies was evaluated by bootstrap analysis (Felsenstein, 1985), based on the neighbour-joining dataset, of 1000 resamplings using the SEQBOOT and CONSENSE options from the PHYLIP package. The G + C content of the DNA of isolate MT1.1T was determined by reversed-phase HPLC (Tamaoka & Komagata, 1984).

It is evident from Fig. 1 that isolate MT1.1T formed a distinct clade in the Micrococccinae 16S rRNA gene tree together with Dermacoccus nishinomiyaiensis DSM 20448T; this association was supported by all of the tree-making algorithms and by a 100 % bootstrap value in the neighbour-joining analysis. The two organisms shared 98.5 % 16S rRNA gene sequence similarity, a value that corresponds to 21 nt differences at the 1443 locations available for alignment. The DNA G + C content of isolate MT1.1T was 65.2 mol %.

Isolate MT1.1T was examined for key chemical markers to determine whether it had a chemotaxonomic profile consistent with its classification in the genus Dermacoccus. The required biomass, derived from a 7-day-old glucose-yeast extract broth (Gordon & Mihm, 1962) shake culture grown at 28 °C, was harvested by centrifugation, washed twice with sterile distilled water and freeze-dried. 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). The peptidoglycan structure of the isolate was determined by the DSMZ identification service using established procedures (Schleifer & Kandler, 1972; Schleifer, 1985; MacKenzie, 1987).

Strain MT1.1T had chemical properties consistent with its assignment to the genus Dermacoccus Stackebrandt et al. 1995. It contains N-acetylated muramic acid, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol as major polar lipids (phospholipid type I sensu Lechevalier et al., 1977) and dihydrogenated menaquinones with eight isoprene units as the predominant isoprenologue, but lacks mycolic acids. One- and two-dimensional TLC of the total hydrolysate of the peptidoglycan (4 M HCl, 16 h at 100 °C) revealed the presence of the amino acids alanine, glutamic acid, lysine and serine. Following derivatization, the molar amino acid ratios as determined by GC were 2-0 Ala, 0-8 Ser, 2-3 Glu and 1-0 Lys; traces of hydrolytically stable peptide were also found (Lys–Ser). Partial hydrolysis (4 M HCl, 45 min at 100 °C) and two-dimensional TLC showed the presence of several peptides: L-Ala–D-Glu, D-Ala–D-Glu, L-Lys–L-Ser and D-Ala–L-Lys–L-Ser. Denitrophenylation indicated that the glutamic acid was derived from the N terminus of the interpeptide bridge. It is clear from these data that the strain has an A4α peptidoglycan type sensu Schleifer & Kandler (1972).

The fatty acids of the organism were rich in the branched-chain components 13-methyltetradecanoic (iso-C15 : 0; 7 % of total), 14-methylpentadecanoic (iso-C16 : 0; 35 % of total), 15-methylhexadecanoic (iso-C17 : 0; 10 % of total) and 14-methylhexadecanoic acids (anteiso-C17 : 0; 18 % of total); the unsaturated components heptadecanoic acid (C17 : 1; 7 % of total) and octadecenoic acid (C18 : 1; 7 % of total) were also present. Minor fatty acids detected (cumulatively 13 % of total) included iso-C14 : 0, C16 : 0, C17 : 0 and C18 : 0 (each < 4 % of total). A very similar fatty acid profile was obtained for Dermacoccus nishinomiyaiensis DSM 20448T (data not shown). Dermacoccus nishinomiyaiensis and members of the genus Kyococcus have been reported to contain significant quantities of unsaturated branched-chain fatty acids, notably 15-methylhexadecenoic acid (iso-C17 : 1, 11–33 % of total; Stackebrandt et al., 1995; Becker et al., 2002). Strain MT1.1T contained small quantities (< 5 % of total) of unidentified fatty acids that may be unsaturated branched-chain fatty acids, but a significant peak attributable to iso-C17 : 1 was
not identified in either strain MT1.1T or Dermacoccus nishinomiyaensis DSM 20448T. Production of significant quantities of unsaturated branched-chain fatty acids may thus be variable with respect to growth conditions. It is also interesting that Demetria terrae nova DSM 11295T, a representative of the third genus currently classified in the family Dermacoaceae (Stackebrandt & Schumann, 2000), was reported to contain branched-chain, though not unsaturated, fatty acids (Groth et al., 1997). Moreover, it is clearly of note that representatives of all of the genera presently grouped in the family Dermacoaceae synthesize significant quantities of branched-chain fatty acids, whereas these components are absent from members of the closely related family Dermatophilaceae (McNabb et al., 1997; Liu et al., 2002; Stackebrandt, 2003), although there have been reports of branched-chain fatty acids in some strains of Dermatophilus congolensis (Hasegawa et al., 1979; Dusch et al., 1994).

DNA–DNA hybridization experiments were carried out between isolate MT1.1T and Dermacoccus nishinomiyaensis DSM 20448T using the microplate method, as described by Ezaki et al. (1989); the mean percentage DNA–DNA relatedness value was calculated from three hybridization experiments. The mean DNA–DNA relatedness found between the two organisms was 15 ± 0·6 %, 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).

Biochemical and physiological characteristics of strain MT1.1T were examined using methods described by Kloos et al. (1974). It is clear from Table 1 that strain MT1.1T can be separated from the type strain of Dermacoccus nishinomiyaensis using a combination of phenotypic properties.

Strain MT1.1T and Dermacoccus nishinomiyaensis DSM 20448T were examined for growth at 40 MPa, according to Heald et al. (2001), in hydraulically pressurized vessels (bomb no. 4740; Parr Instruments) constructed from an Inconel 625 high-strength nickel–chromium alloy. Growth was measured as the change in total viable counts on glucose-yeast extract agar following incubation at 30 °C for 4 days; control cultures of the organisms were incubated at atmospheric pressure. The isolate grew well at 40 MPa, giving a viable count of 3·6 ± 0·4 × 10⁶ c.f.u. ml⁻¹, an increase of 60 % over that of the control culture (1·46 ± 0·7 × 10⁶ c.f.u. ml⁻¹) and can thereby be considered to be a piezotolerant actinomycete. In contrast, Dermacoccus nishinomiyaensis DSM 20448T showed a decrease in viable counts from 1·27 ± 0·4 × 10⁶ c.f.u. ml⁻¹ at atmospheric pressure to 8·1 ± 1·3 × 10⁵ c.f.u. ml⁻¹ at 40 MPa.

It can be concluded from the genotypic and phenotypic data that isolate MT1.1T represents a novel species within the genus Dermacoccus. The name proposed for this taxon is Dermacoccus abyssi sp. nov.

**Table 1. Phenotypic properties that differentiate isolate MT1.1T from Dermacoccus nishinomiyaensis DSM 20448T**

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<thead>
<tr>
<th>Character</th>
<th>MT1.1T</th>
<th>DSM 20448T</th>
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<td>Degradation of:</td>
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<tr>
<td>Aesculin</td>
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<td>Arbutin</td>
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<td>Gelatin</td>
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<td>Guanine</td>
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<td>Tween 80</td>
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<td>Growth on 10% NaCl</td>
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<td>Growth at 10°C</td>
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<td>Growth at 40 MPa</td>
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**Description of Dermacoccus abyssi sp. nov.**

Dermacoccus abyssi (a.bys’si. N.L. gen. n. abyssi of an abyss).

Non-acid–alcohol-fast, non-motile actinomycete. Forms coccoid cells (diameter 0.8–1·5 µm) that occur in irregular clusters. Cream to pale-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 optimum growth around 28 °C. Casein, cellulose, hypoxanthine, starch and uric acid are degraded. Growth occurs in the presence of 7·5 % NaCl. Additional phenotypic properties are shown in Table 1. Grows well in glucose-yeast extract broth at 40 MPa.

The type and only strain is isolate MT1.1T (= DSM 17573T = NCIMB 14084T), recovered from sediment collected from the Challenger Deep of the Mariana Trench at a depth of 10 898 m. The DNA G+C content of the type strain is 65·2 mol%.

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References


