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

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The taxonomic status of an actinobacterial strain isolated from Mariana Trench sediment was determined using a polyphasic taxonomic approach. The strain, isolate MT1.1T, formed a distinct clade in the Micrococcineae 16S rRNA gene tree together with Dermacoccus nishinomiyaensis DSM 20448T. The organism had chemical and phenotypic properties consistent with its classification in the genus Dermacoccus and could be distinguished from D. nishinomiyaensis DSM 20448T using DNA–DNA relatedness and phenotypic data. The G+C content of the DNA of isolate MT1.1T was 65.2 mol%. It is evident that the organism merits recognition as a novel species in the genus Dermacoccus. The name proposed for this taxon is Dermacoccus abyssi sp. nov.; the type strain is MT1.1T (=DSM 17573T = NCIMB 14084T). The organism grows well at 40 MPa and hence is piezotolerant.

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 present 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.

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 Micrococcineae that had been retrieved from DDBJ/EMBL/GenBank 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 (Klug & 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
Strain MT1.1^T had chemical properties 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).

Isolate MT1.1^T was examined for key chemical markers to determine whether it had a chemotaxonomic profile consistent with its classification in the genus *Dermacoccus*. 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-methylhexadecenoic acids (anteiso-C17 : 0; 18 % of total); the unsaturated components heptadecenoic 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 nishinomiyaensis* DSM 20448^T^ (data not shown).

*Fig. 1.* Neighbour-joining tree (Saitou & Nei, 1987) based on almost complete 16S rRNA gene sequences showing relationships between isolate MT1.1^T^ and representatives of the suborder *Micrococccineae*. 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; f and p indicate branches formed using the least-squares and maximum-parsimony methods, respectively. Numbers at nodes show percentages of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50 % are given. Bar, 0-02 substitutions per nucleotide position.
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 terraeagenta* DSM 11295T, a representative of the third genus currently classified in the family *Dermacoccaceae* (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 *Dermacoccaceae* 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). The families *Dermacoccaceae* and *Dermatophilaceae* were defined on the basis of signature nucleotides in their 16S rRNA gene sequences (Stackebrandt & Schumann, 2000); hence, the fatty acid data provide additional evidence for the distinction of these taxa.

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 ± 0.4 × 10^8 c.f.u. ml^-1, an increase of 60 % over that of the control culture (1 ± 0.7 × 10^6 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 ± 0.4 × 10^6 c.f.u. ml^-1 at atmospheric pressure to 8 ± 1.3 × 10^5 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.

**Description of *Dermacoccus abyssi* sp. nov.**

*Dermacoccus abyssi* (a.bys’i. 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 7–9 % 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^T), recovered from sediment collected from the Challenger Deep of the Mariana Trench at a depth of 10098 m. The DNA G+C content of the type strain is 65 ± 2 mol%.

**Acknowledgements**

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<th>Character</th>
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<th>DSM 20448T</th>
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<tr>
<td>Degradation of:</td>
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<tr>
<td>Aesculin</td>
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<td>Growth at 40 MPa</td>
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**Table 1. Phenotypic properties that differentiate isolate MT1.1T from *Dermacoccus nishinomiyaensis* DSM 20448T**

+, Positive, −, negative. Neither strain formed acid from adonitol, D(+)←arabinose, D(+)←arabitol, L(−)←arabitol, D(+)←cellobiose, dextran, dextrin, meso-erythritol, D(+)-fructose, D(+)-galactose, D(+)←glucose, D(+)←glycerol, glycogen, myo-inositol, inulin, D(+)←maltose, D(+)←mannose, D(+)←melezitose, D(+)←melibiose, D(+)←raffinose, D(+)←rhamnose, D(+)←salicin, sucrose, D(+)←trehalose, D(+)←sorbitol, L(+)←sorbose, xylitol or D(+)←xylose.
