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 MT1.1^T, formed a distinct clade in the *Micrococcineae* 16S rRNA gene tree together with *Dermacoccus nishinomiyaensis* DSM 20448^T. The organism had chemical and phenotypic properties consistent with its classification in the genus *Dermacoccus* and could be distinguished from *D. nishinomiyaensis* DSM 20448^T using DNA–DNA relatedness and phenotypic data. The G + C content of the DNA of isolate MT1.1^T 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 MT1.1^T (= DSM 17573^T = NCIMB 14084^T). 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 & Zumalacárregui, 2000; Papamanoli *et al.*, 2002).

The present study was designed to establish the taxonomic status of an actinobacterial strain, isolate MT1.1^T, 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 MT1.1^T 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 MT1.1^T 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 (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

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Dermacoccus abyssi* MT1.1^T is AY894323.

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 MT1.1^T was determined by reversed-phase HPLC (Tamaoka & Komagata, 1984).

It is evident from Fig. 1 that isolate MT1.1^T formed a distinct clade in the *Micrococcineae* 16S rRNA gene tree together with *Dermacoccus nishinomiyaensis* DSM 20448^T; 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 MT1.1^T was 65.2 mol%.

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 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 MT1.1^T 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 A4x 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 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).

Dermacoccus nishinomiyaensis and members of the genus *Kytococcus* 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 MT1.1^T 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

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 *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; 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 MT1.1^T or *Dermacoccus nishinomiyaensis* DSM 20448^T. 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 terrigena* DSM 11295^T, 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 MT1.1^T and *Dermacoccus nishinomiyaensis* DSM 20448^T 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 MT1.1^T were examined using methods described by Kloos *et al.* (1974). It is clear from Table 1 that strain MT1.1^T can be separated from the type strain of *Dermacoccus nishinomiyaensis* using a combination of phenotypic properties.

Strain MT1.1^T and *Dermacoccus nishinomiyaensis* DSM 20448^T 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 20448^T 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 MT1.1^T represents a novel species within the

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

+, 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(+)-mannitol, D(+)-mannose, D(+)-melezitose, D(+)-melibiose, D(+)-raffinose, D(+)-rhamnose, D(+)-salicin, sucrose, D(+)-trehalose, D(+)-sorbitol, L(+)-sorbose, xylitol or D(+)-xylose.

Character	MT1.1 ^T	DSM 20448 ^T
Degradation of:		
Aesculin	+	–
Arbutin	+	–
Gelatin	–	+
Guanine	+	–
Tween 80	+	–
Growth on 10 % NaCl	+	–
Growth at 10 °C	+	–
Growth at 40 MPa	+	–

genus *Dermacoccus*. The name proposed for this taxon is *Dermacoccus abyssi* sp. nov.

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 MT1.1^T (=DSM 17573^T=NCIMB 14084^T), 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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