Amycolatopsis palatopharyngis sp. nov., a potentially pathogenic actinomycete isolated from a human clinical source

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The taxonomic position of an actinomycete, strain 1BDZ^T, isolated from a clinical human source was determined using a polyphasic approach. Phylogenetic analysis based on almost complete 16S rDNA sequences showed that this organism consistently formed a distinct line with the *Amycolatopsis methanolica* subclade within the genus *Amycolatopsis*, and shared moderately low 16S rDNA similarity (<96.5%) with other species. The organism was also found to have chemical and morphological properties typical of members of the genus *Amycolatopsis*.

A range of phenotypic characteristics readily distinguished this strain from representatives of all species of *Amycolatopsis* with validly published names. On the basis of these data, a novel species, *Amycolatopsis palatopharyngis* sp. nov., is proposed to accommodate strain 1BDZ^T (=AS 4.1729^T=PCM 2600^T).

The genus *Amycolatopsis* was established by Lechevalier *et al.* (1986) and was assigned to the family *Pseudonocardiaceae* (Embley *et al.*, 1988; Warwick *et al.*, 1994), which also contains the genera *Actinopolyspora*, *Kibdelosporangium*, *Prauserella*, *Pseudonocardia*, *Saccharomonospora*, *Saccharopolyspora* and *Thermocristum* (Kim & Goodfellow, 1999; Labeda & Kroppenstedt, 2000; Huang *et al.*, 2002). Recently, increasing interest has been shown in *Amycolatopsis*, as it contains strains that produce commercially significant bioactive compounds. Eight novel species with validly published names have been described during the last 3 years (Goodfellow *et al.*, 2001; Huang *et al.*, 2001; Lee & Hah, 2001; Kim *et al.*, 2002; Al-Musallam *et al.*, 2003; Labeda *et al.*, 2003). The 18 species of this genus phylogenetically form two groups, the *Amycolatopsis methanolica* and *Amycolatopsis orientalis* subclades (Kim *et al.*, 2002). The *A. orientalis* subclade comprises 11 mesophilic species, *Amycolatopsis alba*, *Amycolatopsis albidoflavus*, *Amycolatopsis azurea*, *Amycolatopsis coloradensis*, *Amycolatopsis japonica*, *Amycolatopsis keratiniphila*, *Amycolatopsis mediterranei*, *A. orientalis*, *Amycolatopsis pretoriensis*, *Amycolatopsis rubida* and *Amycolatopsis sulphurea* (Huang *et al.*,

2001; Lee & Hah, 2001; Kim *et al.*, 2002; Al-Musallam *et al.*, 2003; Labeda *et al.*, 2003), and three moderately thermophilic species, *Amycolatopsis kentuckyensis*, *Amycolatopsis lexingtonensis* and *Amycolatopsis sacchari*, which grow at 45 °C (Goodfellow *et al.*, 2001; Labeda *et al.*, 2003). The *A. methanolica* subclade comprises three thermophilic species, *Amycolatopsis eurytherma*, *A. methanolica* and *Amycolatopsis thermoflava*, which grow at temperatures above 50 °C (de Boer *et al.*, 1990; Chun *et al.*, 1999; Kim *et al.*, 2002). The remaining thermophilic species, *Amycolatopsis fastidiosa*, which grows over a wide temperature range (10–60 °C) (Henssen *et al.*, 1987), is loosely associated with the others and forms a distinct phyletic line at the foot of the *Amycolatopsis* phylogenetic tree (Goodfellow *et al.*, 2001; Huang *et al.*, 2001; Lee & Hah, 2001; Kim *et al.*, 2002). In addition, Barreiro *et al.* (2000) also proposed that the cephamycin producer '*Nocardia lactamdurans*' should be incorporated into the genus *Amycolatopsis* as '*Amycolatopsis lactamdurans*', which falls into the *A. orientalis* subclade (Kim *et al.*, 2002). Members of the genus *Amycolatopsis* can be distinguished from all other genera belonging to the family *Pseudonocardiaceae* by a combination of chemical and morphological properties (Kim & Goodfellow, 1999).

In the present investigation, a polyphasic study was undertaken to determine the taxonomic position of a Gram-positive, facultatively anaerobic strain, 1BDZ^T, which was isolated from a clinical sample of the palatopharyngeal

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mucosa of an elderly human patient. Genotypic and phenotypic data indicated that this strain should be classified as a novel species of the genus *Amycolatopsis*, for which we propose the name *Amycolatopsis palatopharyngis* sp. nov.

Strain 1BDZ^T was isolated on a brain heart infusion agar plate that had been seeded using swabs and incubated at 37 °C for 5 days under microaerophilic conditions. The clinical sample was collected from the infected palatopharyngeal mucosa of a 70-year-old male patient. The isolate was maintained on modified Bennett's agar (Jones, 1949) slants at 4 °C and as suspensions of mycelial fragments in glycerol (20 %, v/v) at -20 °C. Biomass for chemical and molecular systematic analyses was prepared as described previously (Huang *et al.*, 2002) with the modification that the strain was grown at 37 °C for 3–5 days and cells were then killed by shaking with formalin (1 %, v/v).

Cultural and morphological properties were observed using a Zeiss Axioskop 20 light microscope and a Hitachi S-570 scanning electron microscope after growth on modified Bennett's, glucose/yeast extract/malt extract (International *Streptomyces* Project medium 2; Shirling & Gottlieb, 1966) and inorganic salt/starch agars (International *Streptomyces* Project medium 4; Shirling & Gottlieb, 1966) for 7–14 days at 28 °C.

Phenotypic tests were carried out by following the procedures of Goodfellow *et al.* (1997) and Gordon *et al.* (1974). Antibiotic-resistance tests were performed using Bennett's medium and the method described previously (Huang *et al.*, 2001).

Isomers of diaminopimelic acid and whole-cell sugars were analysed according to the procedures developed by Hasegawa *et al.* (1983) and Lechevalier & Lechevalier (1980). Mycolic acids were checked by the acid methanolysis method of Minnikin *et al.* (1980). Polar lipids were examined by two-dimensional TLC and identified using the method of Minnikin *et al.* (1984). Menaquinones were extracted and purified as described by Collins (1985) and were analysed by HPLC (Wu *et al.*, 1989). Cellular fatty acids were determined by treating dry cell mass (2 mg) with 1 M methanolic HCl (0.5 ml) at 80 °C for 1 h and then extracting the fatty acid methyl esters liberated and analysing them directly using GLC-MS (Paściak *et al.*, 2002). The G+C content of the DNA was determined by using the thermal denaturation (*T_m*) method (Marmur & Doty, 1962) with *Escherichia coli* AS 1.365 as the standard.

Genomic DNA preparation and PCR amplification of 16S rDNA were performed by employing the method of Chun & Goodfellow (1995). The amplified product was sequenced as described previously (Huang *et al.*, 2001). The nucleotide sequence was obtained automatically by using an Applied Biosystems DNA sequencer (model 377) and software provided by the manufacturer. The 16S rDNA sequence of strain 1BDZ^T was aligned manually against

corresponding sequences retrieved from the GenBank database using the CLUSTAL X 1.8 program (Thompson *et al.*, 1997). Phylogenetic trees were inferred by using the neighbour-joining (Saitou & Nei, 1987), least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981) treeing algorithms. Evolutionary distance matrices were generated as described by Kimura (1980). The resultant unrooted tree topologies were evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining method, based on 1000 resamplings. The PHYLIP package (Felsenstein, 1993) was used for all phylogenetic analyses.

An almost complete 16S rDNA sequence was obtained for strain 1BDZ^T (1427 nt). Phylogenetic analysis in which this sequence was compared with corresponding sequences of representatives of the suborder *Pseudonocardineae* indicated that this strain belongs to the family *Pseudonocardiaceae* (data not shown). Further analysis, supported by a high bootstrap value of 100 %, showed that strain 1BDZ^T is a member of the genus *Amycolatopsis* (Fig. 1); a bootstrap value of 89 % supports the assessment that it forms a distinct line with the *A. methanolica* subclade. The 16S rDNA sequence similarities between strain 1BDZ^T and

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost complete 16S rDNA sequences showing the phylogenetic position of strain 1BDZ^T within the radiation of *Amycolatopsis* species. Branches that were also recovered using the least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981) methods are respectively indicated by the letters f and m. Numbers at nodes indicate percentage levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values over 50 % are given. Bar, 0.01 substitutions per nucleotide position.

its nearest neighbours, the three thermophilic species *A. methanolica*, *A. thermoflava* and *A. eurytherma*, were respectively 96.40, 96.32 and 96.16 %. Similarity values with other members of the genus *Amycolatopsis* were in the range 93.86–95.99 %. Thus, the unique phylogenetic position and moderately low 16S rDNA sequence similarities indicate that strain 1BDZ^T represents a novel taxon within the genus *Amycolatopsis*.

The chemotaxonomic data for strain 1BDZ^T are consistent with its assignment to the genus *Amycolatopsis*. It contained *meso*-diaminopimelic acid as the wall diamino acid and arabinose and galactose as major wall sugars (type IV cell wall, according to Lechevalier & Lechevalier, 1980); tetrahydrogenated menaquinones with nine isoprene units were the predominant isoprenoid quinones. The phospholipid pattern consisted of major amounts of phosphatidylethanolamine (a taxonomically significant phospholipid) and minor amounts of phosphatidylglycerol and phosphatidylinositol. The cellular fatty acid profiles included a large amount of 14-methylpentadecanoic acid (i-C16:0; 50.3 % of total). Considerable amounts of 13-methylpentadecanoic acid (ai-C16:0; 3.6 %), hexadecanoic acid (C16:0; 21.7 %), 14-methylhexadecanoic acid (ai-C17:0; 12.4 %), heptadecanoic acid (C17:0; 8.5 %) and octadecanoic acid (C18:0; 3.7 %) were also present. The organism did not contain mycolic acids.

The classification of strain 1BDZ^T in the genus *Amycolatopsis* is also supported by phenotypic and morphological characteristics. The organism is a non-motile, Gram-positive, not acid–alcohol-fast, catalase-positive actinomycete; it forms branched substrate mycelium that fragments into rod-like elements and carries sparse to moderate, white aerial hyphae that differentiate into long chains of spore-like structures. A combination of phenotypic properties easily distinguishes this strain from representatives of its nearest neighbours, the *A. methanolica* subclade (Table 1), and also from those of all *Amycolatopsis* species with validly published names. It is mesophilic, growing well in both aerobic and anaerobic conditions over a wide pH range (pH 6.0–10.0) and over a wide range of NaCl concentrations (0.5–7 %, w/v). Weak growth also occurs on 10 % NaCl. Additional physiological characteristics are shown in Table 1.

On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain 1BDZ^T merits recognition as a novel species of *Amycolatopsis*. We propose the name *Amycolatopsis palatopharyngis* sp. nov. for this taxon.

Description of *Amycolatopsis palatopharyngis* sp. nov.

Amycolatopsis palatopharyngis (pa.la.to.pha.ryn'gis. N.L. gen. n. *palatopharyngis* of the palatopharynx).

The organism forms branched yellow to yellow-brown substrate mycelium that fragments into rod-like elements.

Table 1. Differential phenotypic characteristics of *A. palatopharyngis* sp. nov. 1BDZ^T and its nearest phylogenetic neighbours

Strains: 1, strain 1BDZ^T; 2, *A. eurytherma* DSM 44348^T; 3, *A. methanolica* IMSNU 20055^T; 4, *A. thermoflava* IFO 14333^T. Data for reference type strains were taken from Kim *et al.* (2002). +, Positive or present; w, weakly positive; –, negative or absent. All strains were positive for acid production from adonitol, *meso*-erythritol, (+)-D-fructose, (+)-D-galactose, (+)-D-trehalose and (+)-D-xylose and growth on 5 % NaCl (w/v). All of the strains were negative for acid production from (+)-D-maltose and production of amylase.

Characteristic	1	2	3	4
Anaerobic growth	+	–	–	–
Production of soluble pigment	–	–	–	+
Acid production from:				
(+)-L-Arabinose	+	+	–	+
(+)-D-Cellobiose	–	+	+	+
Dextrin	–	w	–	–
<i>meso</i> -Inositol	+	+	–	–
(+)-D-Lactose	–	w	–	+
(–)-D-Mannitol	–	+	+	+
(+)-D-Melibiose	–	–	–	+
Methyl α -D-glucoside	–	–	–	+
(+)-D-Raffinose	+	–	–	+
(+)-L-Rhamnose	–	+	+	–
Salicin	–	–	w	+
(–)-D-Sorbitol	–	+	+	+
(–)-D-Sucrose	–	–	+	–
Decomposition of:				
Aesculin	–	–	w	+
Allantoin	–	+	+	+
Casein	+	+	–	+
Gelatin	+	+	+	–
Hypoxanthine	+	w	+	+
Xanthine	+	–	–	+
Growth at:				
10 °C	+	–	–	–
45 °C	–	+	+	+
Production of:				
Nitrate reductase	+	+	+	–
Urease	w	+	–	+

White aerial mycelium is produced, sparsely on Bennett's, glucose/yeast extract/malt extract and brain heart infusion agars and moderately on inorganic salts/starch agar. The aerial mycelium differentiates into long chains of spore-like structures. Diffusible pigments are not produced. Facultatively anaerobic and catalase-positive. Growth occurs between pH 6 and 10, between 0.5 and 10 % NaCl and between 10 and 40 °C, but not at 45 °C. Acid is produced from adonitol, (+)-L-arabinose, *meso*-erythritol, (+)-D-fructose, (+)-D-galactose, *meso*-inositol, (+)-D-raffinose, (+)-D-trehalose and (+)-D-xylose, but not from (+)-D-cellobiose, dextrin, (+)-D-lactose, (+)-D-maltose,

(-)-D-mannitol, (+)-D-melezitose, (+)-D-melibiose, methyl α -D-glucoside, (+)-L-rhamnose, salicin, (-)-D-sorbitol or (-)-D-sucrose. Casein, gelatin, hypoxanthine, L-tyrosine and xanthine are degraded, but aesculin, allantoin and starch are not. Exhibits nitrate reductase and weak urease activity. Resistant to ampicillin (10 μ g), carbenicillin (100 μ g) and cephalothin (30 μ g), but not to acetylspiramycin (15 μ g), chloramphenicol (30 μ g), clarithromycin (15 μ g), doxycycline (30 μ g), gentamicin (10 μ g), kanamycin (30 μ g), midecamycin (15 μ g), minocycline (30 μ g), novobiocin (5 μ g), penicillin (10 IU), rifampicin (5 μ g), streptomycin (10 μ g) or tobramycin (10 μ g). Chemotaxonomic characteristics are typical of those for the genus *Amycolatopsis*. The G + C content of the DNA is 65.8 mol% (T_m method).

The type strain, 1BDZ^T [=AS 4.1729^T (Chinese General Microbiological Collection Centre)=PCM 2600^T (Polish Collection of Microorganisms)], was isolated from the infected palatopharyngeal mucosa of an elderly human patient.

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