Nocardiopsis metallicus sp. nov., a metal-leaching actinomycete isolated from an alkaline slag dump

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A taxonomic study was carried out on a metal-mobilizing, alkaliphilic bacterium from an alkaline slag dump, strain KBS6T. The strain produced substrate and aerial mycelia. Growth occurred in the pH range 7.0–10.5, with an optimum at pH 8.5. A salt concentration of up to 10% was tolerated, and various organic substrates were used for growth. The results of a 16S rDNA sequence comparison revealed that strain KBS6T belongs to the genus Nocardiopsis. DNA–DNA hybridization with the two closest relatives, Nocardiopsis exhalans and Nocardiopsis prasina, gave similarity values of 18.2 and 44.1%, respectively, which indicated that strain KBS6T represents a novel species of the genus Nocardiopsis. This is consistent with the morphological, physiological and chemotaxonomic data. Because of the ability of this microorganism to solubilize metals, the name Nocardiopsis metallicus sp. nov. is proposed for strain KBS6T (= DSM 44598T = NRRL B-24159T), this being the type strain.

Keywords: Nocardiopsis, Nocardiopsis metallicus sp. nov.

INTRODUCTION

Acidophilic and chemolithotrophic micro-organisms are well known for their bioleaching activities, processes in which metals are mobilized from soils, ores and solid-waste material (Bosecker, 1997; Johnson, 1998; Brandl, 2001). Recently, Willscher & Bosecker (2001) showed that several alkaliphilic, heterotrophic microorganisms show bioleaching activities. One such organism, which prefers light, alkaline conditions (pH 8.5) for growth, is a member of the genus Nocardiopsis (Kroppenstedt, 1992). Although Nocardiopsis strains have been isolated from indoor environments (Peltola et al., 2001), a composting facility (Kämpfer et al., 2002) and even from clinical materials (Yassin et al., 1997), they are frequently isolated from alkaline soils (Mikami et al., 1992; Al-Tai & Ruan, 1994; Yassin et al., 1993a; Chun et al., 2000; Evtushenko et al., 2000; Al-Zarban et al., 2002). In this study, strain KBS6T, isolated from an alkaline dump associated with metallurgical processing in Germany, was subjected to taxonomic study and was found to represent a novel species of the genus Nocardiopsis. In this paper, a description of this species is given.

METHODS

Strain and culture conditions. Strain KBS6T was isolated from an alkaline slag dump associated with metallurgical processing in Germany. Isolation was done at 30 °C by dilution plating on R2A agar for enrichment of soil bacteria (Reasner & Geldreich, 1985). The agar medium contained the following (l−1 deionized water): 0.5 g yeast extract, 0.5 g protease peptone, 0.5 g Casamino acids, 0.5 g soluble starch, 0.3 g sodium pyruvate, 0.3 g K2HPO4, 0.05 g MgSO4, 5H2O, 0.5 g glucose and 15 g agar. The pH was adjusted to 7.2 using crystalline K2HPO4 or KH2PO4.

For morphological studies, the organism was grown on R2A agar and standard nutrient agar I (Merck) amended with 10 g sucrose l−1 at 30 °C. For physiological studies and chemotaxonomic analyses, the organism was grown in R2A medium, which consists of R2A agar but without agar. For chemotaxonomic analyses, the cells were collected by filtration or centrifugation, washed twice with water and freeze-dried.

Physiology. All physiological tests were carried out in triplicate at 30 °C unless other temperatures are indicated. The growth temperatures (10, 30 and 45 °C) and salt concentrations (0–10%) were tolerated by the strain.

The EMBL accession number for the 16S rDNA sequence of Nocardiopsis metallicus sp. nov. KBS6T is AJ420769.
tolerance (at 5, 10, 15 and 20%, w/v, NaCl) were checked using R2A agar. For determination of the pH range, R2A medium was adjusted to different pH values using HCl or NaOH. After sterilization, the pH was checked again and adjusted before inoculation. For each pH value, three parallel assays were incubated on a rotary shaker. Optical density and pH were measured weekly after inoculation. For the carbon-utilization tests, a basal agar, described by Chun et al. (2000), was used and contained the following (1 l): deionized water: 1 g (NH₄)₂SO₄, 7 g KH₂PO₄, 2 g K₂HPO₄, 0.1 g MgSO₄, 10 g NaCl and 12 g agar. The pH was adjusted to 9.0 using NaOH or HCl before sterilization. Each sterile, filtered substrate was added to a final concentration of 1%. In the case of the nitrogen compounds t-alanine, gelatin, proline and serine, the (NH₄)₂SO₄ in the basal agar was omitted. The plates were checked weekly for growth.

Chemotaxonomy. Amino acid and sugar analyses of whole-cell hydrolysates were performed as described by Stanek & Roberts (1974). The occurrence of mycolic acids was checked by TLC according to the procedure of Minnikin et al. (1975). Polar lipids were extracted, examined by two-dimensional TLC and identified using published procedures (Minnikin et al., 1984). Fatty acid methyl esters were prepared from 40–80 mg wet cells (Miller, 1982; Sasser, 1990). Mixtures of fatty acid methyl esters were analysed by capillary GC, using a Hewlett Packard model 5898A gas chromatograph run using Microbial Identification software (Microbial ID).

16S rDNA sequence determination and phylogenetic analyses. Genomic DNA extraction, PCR-mediated amplification of the 16S rDNA and purification of PCR products were carried out as described previously (Rainey et al., 1996). Purified PCR products were sequenced with Taq Dye-deoxy terminator cycle sequencing kits (Applied Biosystems) according to the manufacturer’s protocol. An Applied Biosystems 373A DNA sequencer was used for electrophoresis of the sequence reaction products. The ae2 editor (Maidak et al., 1999) was used to align the 16S rDNA sequence determined in this study against 16S rDNA sequences of representatives of the main bacterial lineages available from public databases. Pairwise evolutionary distances were computed using the correction of Jukes & Cantor (1969). The least-squares distance method of De Soete (1983) was used in construction of the phylogenetic dendrogram from distance matrices. Bootstrap analysis was done as described by Felsenstein (1993).

Determination of DNA–DNA similarities and G + C content. DNA was isolated by chromatography on hydroxypatite by the procedure of Cashion et al. (1977). DNA–DNA hybridization was carried out as described by De Ley et al. (1970), with the modification described by Huß et al. (1983) and Escara & Hutton (1980). A Gilford System model 2600 spectrometer equipped with a Gilford model 2527-R thermostprogrammer and plotter was used. Renaturation rates were computed with the TRANSFER.BAS program of Jahnke (1992). The G + C content was determined by HPLC of deoxyribonucleosides by using the method of Mesbah et al. (1989).

RESULTS AND DISCUSSION

Morphological characteristics

Strain KBS6T showed weak growth on agar plates and in liquid medium. On agar plates, a yellow-brown substrate mycelium formed, and white aerial mycelium

![Fig. 1. Phylogenetic dendrogram based on 16S rDNA sequence analysis, showing the phylogenetic position of N. metallicus sp. nov. KBS6T compared with the other species of the genus Nocardiopsis. The sequences of Actinomadura madurae DSM 43067T (X97889) and Micromonospora coerulea DSM 43143T (X92598) served as outside references. Bootstrap values > 85% are indicated at the relevant branching points. Bar, 5 nucleotide substitutions per 100 nucleotides.](image-url)

with straight spore chains occurred. These morphological characteristics are consistent with those described for Nocardiopsis species (Kroppenstedt, 1992).

Phylogenetic analysis

The almost complete 16S rDNA sequence of strain KBS6T, consisting of 1488 nt, was compared with sequences of members of the order Actinomycetales. Members of the genus Nocardiopsis were the closest phylogenetic neighbours. Pairwise similarity values ranged between 95.6% (Nocardiopsis trehalosi NRRL 12026T and 99.4% (Nocardiopsis exhalans DSM 44407T). Pairwise similarity values of > 97% were also found for Nocardiopsis prasina DSM 43845T (99.3%), Nocardiopsis alba DSM 43377T (98.6%), Nocardiopsis listeri DSM 40297T (98.4%), Nocardiopsis lucentensis DSM 44048T (98.2%), Nocardiopsis umidaschalea DSM 44362T (98.1%), Nocardiopsis synemataformans DSM 44143T (97.7%), Nocardiopsis dassonvillii DSM 43111T (97.7%), Nocardiopsis tropica VKM Ac-1457T (97.6%) and Nocardiopsis halotolerans DSM 44410T (97.0%). The phylogenetic tree of Nocardiopsis species is shown in Fig. 1. The closest phylogenetic neighbours of strain KBS6T are N. exhalans DSM 44407T and N. prasina DSM 43845T. The DNA of these two strains was hybridized against DNA of strain KBS6T. The DNA–DNA relatedness of strain KBS6T to N. exhalans DSM 44407T was 18.2% and to N. prasina DSM 43845T was 44.1%. These values were far below the value of 70% recommended by Wayne et al. (1987) for strains of the same species. The G + C content of strain KBS6T was 70.8 mol%.
Table 1. Substrate utilization by N. metallicus sp. nov. and the phylogenetically most closely related species

Data for N. exhalans and N. prasina were taken from Yassin et al. (1997) and Peltola et al. (2001). +, Substrate utilized for growth; −, substrate not utilized for growth; ND, not determined. All three strains utilized acetate, gluconate, d-glucose, d-maltose, l-proline, d-trehalose and d-xylose. None of the strains utilized 3-hydroxybenzoate or inositol.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>N. metallicus KBS6&lt;sup&gt;T&lt;/sup&gt;</th>
<th>N. exhalans DSM 44407&lt;sup&gt;T&lt;/sup&gt;</th>
<th>N. prasina DSM 43845&lt;sup&gt;T&lt;/sup&gt;</th>
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<tr>
<td>l-Alanine</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Cellobiose</td>
<td>+</td>
<td>ND</td>
<td>−</td>
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<tr>
<td>d-Galactose</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>ND</td>
<td>−</td>
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<tr>
<td>Raffinose</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>l-Serine</td>
<td>−</td>
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Physiological characteristics

Strain KBS6<sup>T</sup> was able to dissolve metals from siliceous, alkaline dump material (Willscher & Bosecker, 2001). This ability has not been described previously for any other Nocardiopsis strain (Kroppenstedt & Evtushenko, 2002). Growth occurred in the pH range 7.0–10.5, with optimal growth at pH 8.5. Strain KBS6<sup>T</sup> grew on R2A agar supplemented with up to 10% (w/v) NaCl. No growth occurred at 15% (w/v) NaCl. Optimal growth was observed at 30 °C. Scant growth was obtained at 10 °C, but there was no growth at 40 °C. Further physiological characteristics of strain KBS6<sup>T</sup> and of the phylogenetically closest related species N. exhalans and N. prasina are given in Table 1. The substrate-utilization pattern of strain KBS6<sup>T</sup> is different from those of the other two Nocardiopsis species.

Chemotaxonomic characteristics

Whole-cell hydrolysates contained meso-diaminopimelic acid as the only diamino acid of the peptidoglycan and ribose as the only sugar. Diagnostic sugars such as arabinose, xylose and maltriose (Lechevalier et al., 1971), rhamnose (Labeleda et al., 1984) and mycolic acids were not detected.

The polar lipid pattern was composed of the diagnostic diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylcholine and unknown phospholipids with high R<sub>f</sub> values (above diphasatidylglycerol). The phospholipid pattern is type PII, with phosphatidylcholine as the characteristic phospholipid (Lechevalier et al., 1977). This phospholipid pattern is also found in species of the genera Actinopolyspora, Saccharopolyspora and Pseudonocardia. However, Nocardiopsis strains are easily differentiated from these taxa by the occurrence of phosphatidylethanolamine, large amounts of phosphatidylglycerol and the lack of hydroxyphosphatidylethanolamine. In addition, unknown phospholipids with high R<sub>f</sub> values (above diphosphatidylglycerol) were detected. These unknown phospholipids are of diagnostic value and, to date, have only been found in Nocardiopsis species (Kroppenstedt, 1992). The taxonomic value of other ‘non-diagnostic’ phospholipids has been mentioned by Yassin et al. (1993b).

The whole-cell fatty acid compositions of strain KBS6<sup>T</sup> and of the phylogenetically most closely related species, N. exhalans and N. prasina, are given in Table 2. In general, the fatty acid profile of strain KBS6<sup>T</sup> is characteristic of members of the genus Nocardiopsis (type 3d fatty acids according to Kroppenstedt, 1985), but the composition of the fatty acids of strain KBS6<sup>T</sup> is different from those of the other two Nocardiopsis species.

In summary, the chemotaxonomic properties of strain KBS6<sup>T</sup> are consistent with its classification in the genus Nocardiopsis (Kroppenstedt, 1992). On the basis of all the results, we propose strain KBS6<sup>T</sup> as the type strain of a novel species of the genus Nocardiopsis.
Description of *Nocardiopsis metallicus* sp. nov.

*Nocardiopsis metallicus* (me.ta'lli.cus. L. masc. n. *metallicus* the miner, referring to the ability to mobilize metals from slag).

Aerobic actinomycete producing a yellow-brown substrate mycelium and a white aerial mycelium. Optimal growth occurs at pH 8.5; the pH range is 7.0–10.5. Grows well at 30 °C, weakly at 10 °C and not at 40 °C. Growth occurs on media supplemented with up to R-phatidylcholine and unknown phospholipids with high amine, phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylethanol acid of the peptidoglycan and ribose as the only sugar. used as substrates for growth. Whole-cell hydrolysates halose, gluconate, cellobiose, maltose, mannitol, sucrose, trehalose, L-rhamnose, D-xylene, gelatin and proline are used as substrates for growth. Whole-cell hydrolysates contain meso-diaminopimelic acid as the only diamino acid of the peptidoglycan and ribose as the only sugar. The polar lipid pattern is as follows: diphostatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylglycerol and mannoside, phosphatidylcholine and unknown phospholipids with high Rf values (above diphostatidylglycerol). The fatty acids comprise iso-, anteiso- and 10-methyl-branched fatty acids. The DNA G + C content of the type strain is 70.8 mol%. The type strain is strain KBS6T (= DSM 44598T = NRRL B-24159T). It was isolated from an alkaline slag dump associated with metallurgical processing in Germany.

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**REFERENCES**


