Descriptions of *Actinoplanes ianthinogenes* nom. rev. and *Actinoplanes octamycinicus* corrig. comb. nov., nom. rev.

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Phylogenetic analysis of ‘*Actinoplanes ianthinogenes*’ Coronelli et al. 1974 and ‘*Actinoplanes ianthinogenes* subsp. *octamycinicus*’ Gauze et al. 1979 based on 16S rRNA gene sequencing data revealed that these organisms form a clade in the family *Micromonomosporaceae*. Morphological and chemotaxonomic characteristics of strains of these species were consistent with those of members of the genus *Actinoplanes*. Morphological, DNA–DNA hybridization, physiological, biochemical and chemotaxonomic data showed that ‘*A. ianthinogenes*’ and ‘*A. ianthinogenes* subsp. *octamycinicus*’ can be easily differentiated from each other and that they merit separate species status. On the basis of morphological, physiological, biochemical, chemotaxonomic and DNA–DNA hybridization data, it is concluded that ‘*A. ianthinogenes*’ and ‘*A. ianthinogenes* subsp. *octamycinicus*’ should be assigned the status of two novel species: *Actinoplanes ianthinogenes* nom. rev. (type strain NBRC 13996 T) and *Actinoplanes octamycinicus* corrig. comb. nov., nom. rev. (type strain NBRC 14524 T).

The genus *Actinoplanes* (Couch, 1950; Stackebrandt & Kroppenstedt, 1987) belongs to the family *Micromonomosporaceae* Krasil’nikov (1938) emend. Zhi et al. (2009) based on 16S rRNA gene sequencing data of strains NBRC 13996 T (=A/1668 T) and NBRC 14524 T (=INA 4041 T), respectively.

The 16S rRNA genes of these two organisms were amplified by PCR and sequenced as described previously (Tamura & Hatano, 2001). Phylogenetic neighbours were identified and pairwise 16S rRNA gene sequence similarities were calculated using the EzTaxon server (http://www.eztaxon.org/; Chun et al., 2007). 16S rRNA gene sequences were aligned with published sequences retrieved from GenBank/EMBL/DDBJ using CLUSTAL_X (Thompson et al., 1997) and were then manually edited using BioEdit version 7.0.9 (Hall, 1999). Phylogenetic trees were constructed with the neighbour-joining (NJ) (Saitou & Nei, 1987) and maximum-parsimony (MP) (Fitch, 1971) algorithms using the program MEGA 4.1 (Tamura et al., 2007). The resultant tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 replications. 16S rRNA gene sequence analysis revealed that ‘*Actinoplanes ianthinogenes*’ NBRC 13996 T and ‘*Actinoplanes ianthinogenes* subsp. *octamycinicus*’ NBRC 14524 T formed a clade in the family *Micromonomosporaceae* (Fig. 1). This clade was supported by bootstrap values of 99 and 97 % in the NJ (Fig. 1) and MP trees (Supplementary Fig. S1, available in IJSEM Online).
respectively. Strains NBRC 13996<sup>T</sup> and NBRC 14524<sup>T</sup> did not form a coherent clade with other Actinoplanes species; this clade was not supported by a high bootstrap value in the NJ tree or the branching in the MP tree. 16S rRNA gene sequence similarity between the two strains was 98.6%. The two strains showed highest sequence similarities to Actinoplanes cyanescens NBRC 14990<sup>T</sup> (AB036997; 96.4–96.5%), Actinoplanes liguriensis DSM 43865<sup>T</sup> (AJ865471; 96.3–96.4%), Actinoplanes rectilineatus NBRC 13941<sup>T</sup> (AB037010; 96.3–96.4%), Actinoplanes palleronii NBRC 14916<sup>T</sup> (AB037009; 96.2–96.4%) and Actinoplanes regularis NBRC 12514<sup>T</sup> (AB037011; 96.1–96.2%).

For chemotaxonomic analyses, cells of these two strains were grown in yeast extract-glucose broth (1% yeast extract, 1% glucose; pH 7.0) (Hatano et al., 2003) on a rotary shaker at 28 °C for 5 days. Cell wall amino acids, menaquinones, polar lipids, acyl type of peptidoglycan, mycolic acids and DNA G+C contents were analysed as described previously (Tamura et al., 1994). The isoprenoid quinone content was determined using LC-MS according to the protocol in the Shimadzu Application Data Sheet No. 010 (http://www.spectrace.com.br/analitica/aplicacoes/cromatografos/lc_ms/lcms010.pdf). Cellular fatty acid methyl esters were prepared and analysed according to the protocol of the MIDI Sherlock Microbial Identification System v. 4.0 (Sasser, 1990; MIDI, 2002). Whole-cell sugars were analysed as 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatives of the hydrolysates, which were prepared according to the method described by Honda et al. (1989) and Yang et al. (2005). The hydrolysate (10 μl) was dissolved in 50 μl 0.3 M aqueous sodium hydroxide followed by addition of 50 μl 0.3 M PMP in methanol. This mixture was allowed to react for 30 min at 70 °C, cooled to room temperature and then neutralized with 50 μl 0.3 M HCl. The resulting solution was dissolved in 1 ml chloroform. After vigorous shaking and centrifugation, the chloroform phase, which formed below the aqueous layer, was carefully discarded to remove excess reagents. After repeating the extraction process three times, the aqueous layer was filtered through a 0.20 μm membrane and the filtrate was diluted with 150 μl water before use. The PMP-labelled hydrolysates prepared in this way were analysed using a Shimadzu Prominance HPLC system equipped with LCMS-2020 (Shimadzu). The analytical column used was Shim-Pack FC-ODS (150 x 2 mm internal diameter, 3 μm particle size; Shimadzu). UV detection was performed at 250 nm. Elution was carried out at a flow rate of 0.2 ml min<sup>-1</sup> at 40 °C for 30 min. The mobile phase consisted of 5 mM formic acid (A) and acetoni trile (B); gradient elution of 15 to 30% solution B was used with a linear increase from 0 to 30 min.

The predominant menaquinone in strains NBRC 13996<sup>T</sup> and NBRC 14524<sup>T</sup> was MK-9(H<sub>4</sub>) (77 and 87%, respectively), with MK-9(H<sub>4</sub>) (18 and 11%) and MK-9(H<sub>6</sub>) (5 and 2%) as minor components. The cell-wall amino acids in these strains were meso-diaminopimelic acid, alanine and glutamic acid and the whole-cell sugars were ribose, mannose, galactose, glucose, arabinose, xylose and one unidentified O-methyl-hexose. The major fatty acids in NBRC 13996<sup>T</sup> were iso-C<sub>16:0</sub> (29.7%), anteiso-C<sub>17:0</sub> (16.1%), iso-C<sub>15:0</sub> (10.7%), C<sub>18:1ω9c</sub> (10.7%) and anteiso-C<sub>15:0</sub> (10.8%); those in NBRC 14524<sup>T</sup> were iso-C<sub>16:0</sub> (23.7%), iso-C<sub>15:0</sub> (16.1%), C<sub>18:1ω9c</sub> (11.3%) and anteiso-C<sub>17:0</sub> (10.0%) (Supplementary Table S1, available in IJSEM Online). The diagnostic phospholipid detected was phosphatidylethanolamine, a marker of phospholipid type II (Lechevalier et al., 1977) (Supplementary Fig. S2, available in IJSEM Online). Mycolic acids were not detected. The acyl type of the peptidoglycan was glycolyl. The DNA G+C contents were 71.5 and 72.1 mol% for strains NBRC 13996<sup>T</sup> and NBRC 14524<sup>T</sup>, respectively.

Morphological characteristics were observed by scanning electron microscopy as described previously (Tamura et al., 1994). ‘A. ianthinogenes’ NBRC 13996<sup>T</sup> and ‘A. ianthinogenes’ subsp. octamycini’ NBRC 14524<sup>T</sup> formed globose sporangia on short sporangiophores as reported by Coronelli et al. (1974) and Gauze et al. (1979) (Supplementary Fig. S3, available in IJSEM Online). Spores were oval with a smooth surface and 0.5–1.0 x 0.7–1.5 μm. Motile spores were also observed. Chemotaxonomic and morphological characteristics indicated that these strains belong to the genus Actinoplanes.

Cultural and physiological characteristics were determined as described previously (Gordon et al., 1974; Seino et al., 1985) by using API ZYM, API 50CH and API Coryne test strips (bioMérieux). Detailed results of these analyses are provided in Table 1 and in the species descriptions. Colonies of strain NBRC 13996<sup>T</sup> were purplish red on malt extract-yeast extract medium (ISP 2) and oatmeal medium (ISP 3), whereas colonies of strain NBRC 14524<sup>T</sup> were reddish orange on ISP 2 and yellow on ISP 3.

The microplate-hybridization method developed by Ezaki et al. (1988, 1989) was used to determine DNA–DNA relatedness. DNA–DNA relatedness values between ‘A. ianthinogenes’ NBRC 13996<sup>T</sup> and ‘A. ianthinogenes’ subsp. octamycini’ NBRC 14524<sup>T</sup> were 23–44%. These values are well below the recommended cut-off point of 70% for the delineation of bacterial species (Stackebrandt & Goebel, 1994; Wayne et al., 1987). The low levels of DNA–DNA relatedness clearly confirmed that strains NBRC 13996<sup>T</sup> and NBRC 14524<sup>T</sup> represent two different species. 16S rRNA gene sequence similarities of strains NBRC 13996<sup>T</sup> and NBRC 14524<sup>T</sup> with members of species with validly published names were lower than 96.5%, showing that NBRC 13996<sup>T</sup> and NBRC 14524<sup>T</sup> belong to a unique genospecies (Stackebrandt & Goebel, 1994; Stackebrandt & Ebers, 2006).

The phylogenetic positions of strains NBRC 13996<sup>T</sup> and NBRC 14524<sup>T</sup> in the family Micromonosporaceae may be vague. However, members of the genus Actinoplanes do not essentially form a coherent clade supported by high bootstrap values and algorithm-based analyses in the family Micromonosporaceae at this time. Therefore, on the basis of their morphological and chemotaxonomic characteristics,
Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequences of Actinoplanes ianthinogenes NBRC 13996T, Actinoplanes octamycinicus NBRC 14524T and members of the family Micromonosporaceae. The tree was constructed by using the NJ method (Saitou & Nei, 1987). The 16S rRNA gene sequence of Streptomyces ambiguofaciens ATCC 23877T was used as the outgroup. Numbers on the branches indicate the confidence limits (expressed as percentages) that were estimated by bootstrap analysis with 1000 replicates; only values >50% are provided. Bar, 0.01 K_{sub} in the nucleotide sequences.

‘A. ianthinogenes’ NBRC 13886T and ‘A. ianthinogenes subsp. octamycinicus’ NBRC 14524T should be included in the genus Actinoplanes and, on the basis of physiological, DNA–DNA relatedness and phylogenetic data, they can be classified as members of the genus Actinoplanes but can be readily distinguished from previously known members of the genus. Thus, two novel species are proposed, namely, Actinoplanes ianthinogenes nom. rev. (type strain, NBRC 13996T = A/1 16688T = ATCC 21884T = BRC 13611T = DSM 43864T = IMSNU 20032T = JCM 3249T = KCTC 9347T = KCTC 9599T = NCIMB 12639T = NRRL B-16720T; previously ‘Actinoplanes ianthinogenes’) and Actinoplanes octamycinicus corrig. comb. nov., nom. rev. (type strain, NBRC 14524T = INA 4041T = ATCC 43632T = JCM 9649T = KCTC 9593T; previously ‘Actinoplanes ianthinogenes subsp. octamycinicus’).

Description of Actinoplanes ianthinogenes nom. rev.

Actinoplanes ianthinogenes [i.an.thi.no.ge’nes. Gr. adj. ianthinos violet-blue; N.L. suff. -genes (from Gr. v. gennao) to produce) producing; N.L. masc. adj. ianthinogenes producing violet (amethyst) colour].

The description of this species is based on data reported by Coronelli et al. (1974) and data obtained in this study.

The colony surface is powdery. The substrate mycelium is purplish red on ISP 2 and ISP 3. Aerial mycelium is absent and the vegetative mycelium has branched hyphae. Sporangia on short sporangiophores arising from the vegetative mycelium, which is formed abundantly on oatmeal agar and humic acid-vitamin agar, are globose with an irregular surface and 3.0–10.0 μm in diameter. Sporangial release is observed after rupture of the sporangial wall. Subspherical spores are motile. Grows at 15–37 °C, at pH 4–8 and in a maximum NaCl concentration of 2%. N-Acetylglucosamine, aesculin ferric citrate, L-arabinose, cellobiose, dulcitol, D-fructose, D-galactose, gluconate, D-glucose, glycerol, glycogen, D-lactose, maltose, D-mannitol, D-mannose, melibiose, methyl α-D-mannopyranoside, L-rhamnose, D-sorbitol, starch, sucrose, trehalose, turanose and D-xylene can be utilized as sole carbon sources. Positive for N-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, catalase, chymotrypsin, cystine aminopeptidase, aesculin hydrolysis, esterase (C4), esterase lipase (C8), gelatin hydrolysis, β-galactosidase, α-glucosidase, β-glucosidase,

Table 1. Differential characteristics of Actinoplanes ianthinogenes and Actinoplanes octamycinicus

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<tr>
<td>pH range for growth (optimum)</td>
<td>4–8 (5–6)</td>
<td>6–9 (7–8)</td>
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<tr>
<td>Range of growth temperature (optimum) (°C)</td>
<td>15–37 (28–37)</td>
<td>15–37 (25–37)</td>
</tr>
<tr>
<td>Tolerance to NaCl (w/v, %)</td>
<td>≤2</td>
<td>≤1</td>
</tr>
<tr>
<td>Cultural characteristics (growth/colony colour*):</td>
<td>Good/purplish red (82, 83)</td>
<td>Good/reddish orange (39)</td>
</tr>
<tr>
<td>Yeast extract-malt extract agar (ISP 2)</td>
<td>Moderate/purplish red (82), orange yellow (44)</td>
<td>Moderate/yellow (47)</td>
</tr>
<tr>
<td>Oatmeal agar (ISP 3)</td>
<td>Moderate/orange (7, 8)</td>
<td>Good/yellow (12)</td>
</tr>
<tr>
<td>Inorganic salts-starch agar (ISP 4)</td>
<td>Moderate/greenish yellow (86, 88)</td>
<td>Good/brown (9, 87)</td>
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<tr>
<td>Glycerol-asparagine agar (ISP 5)</td>
<td>Moderate/yellow (12)</td>
<td>Moderate/yellow (64)</td>
</tr>
<tr>
<td>Peptone-yeast extract-iron agar (ISP 6)</td>
<td>Moderate/reddish brown (6)</td>
<td>Good/greyish brown (106)</td>
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<tr>
<td>Tyrosine agar (ISP 7)</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Esterase (C4)</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Cystine aminopeptidase</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Chymotrypsin</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Phosphohydrolase</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Urea hydrolysis</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Ribose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Methyl β-D-xylopyranoside</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Methyl α-D-mannopyranoside</td>
<td>+</td>
<td>–</td>
</tr>
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*Numerals given in parentheses correspond to the colour codes of Rayner (1970).
leucine aminopeptidase, nitrate reduction, pyrazinamidase, trypsin, urea hydrolysis and valine aminopeptidase. The major fatty acid is iso-C₁₆:0 and the major menaquinone is MK-9(H₄). The polar lipid profile contains diphosphatidylglycerol and phosphatidylethanolamine as major components but lacks phosphatidycholine and aminoglycolipids (phospholipid type PII). Additionally, moderate amounts of phosphatidylglycerol and an unknown phospholipid are detected.

The type strain is NBRC 13996T (=A/1668T=ATCC 21884T=BCRC 13611T=DSM 43864T=IMSNU 20032T=JCM 3249T=KCTC 9347T=KCTC 9592T=NCIMB 12639T=NRLB B-16720T), isolated from a soil sample collected in Blumenau, Brazil. The DNA G+C content of the type strain is 71.5 mol% (determined by HPLC).

Description of *Actinoplanes octamycinicus* corr. comb. nov., nom. rev.

*Actinoplanes octamycinicus* [oc.ta.mi.cy’ni.cus. N.L. n. *octamycinum* octamycin (name of an antibiotic); L. masc. suff. -icus suffix used with the sense of pertaining to; N.L. masc. adj. *octamycinicus* pertaining to octamycin, in reference to octamycin production).

The description of this species is based on data reported by Gauze et al. (1979) and data obtained in this study.

The colony surface is powdery. The substrate mycelium is orange, yellow or brown. Aerial mycelium is absent and vegetative mycelium has branched hyphae. Sporangia on short sporangiophores arising from the vegetative mycelium, which is formed abundantly on oatmeal agar and hemic acid-vitamin agar, are globose with an irregular surface and 3.0–10.0 μm in diameter. Sporangial release is observed after rupture of the sporangial wall. Subspherical spores are motile. Grows at 15–37°C, at pH 6–9 and in a maximum NaCl concentration of 1%. N-Acetylglucomamine, aesculin ferric citrate, L-arabinose, cellobiose, D-fructose, D-galactose, genitolios, glucosone, D-glucose, glycerol, glycogen, D-lactose, maltose, D-mannitol, D-mannose, melilobiose, methyl β-D-xylopolysanoside, L-rhamnose, D-ribose, D-sorbitol, starch, sucrose, trehalose, turanose and D-xylose can be used as sole carbon sources. Positive for N-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, catalase, aesculin hydrolysis, esterase lipase (C8), gelatin hydrolysis, β-galactosidase, α-glucosidase, β-glucosidase, leucine aminopeptidase, nitrate reduction, phosphohydrolase, pyrazinamidase, tryptophanase and valine aminopeptidase. The major fatty acid is iso-C₁₆:0 and the major menaquinone is MK-9(H₄).

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The type strain is NBRC 14524T (formerly *Actinoplanes octamycinus*), isolated from a soil sample collected in Blumenau, Brazil. The DNA G+C content of the type strain is 72.1 mol% (determined by HPLC).

References


