Actinoplanes liguriensis sp. nov. and *Actinoplanes teichomyceticus* sp. nov.

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The taxonomic status of '*Actinoplanes liguriae*' A/6353 and '*Actinoplanes teichomyceticus*' AB8327 was established by using a polyphasic approach. Strains A/6353 and AB8327 form distinct phylogenetic lineages in the 16S rRNA gene tree of members of the genus *Actinoplanes* and are related moderately and closely to *Actinoplanes rectilineatus* and *Actinoplanes cyaneus*, respectively. Morphological, cultural and physiological properties indicated that strains A/6353 and AB8327 represent separate, novel species of the genus *Actinoplanes*, *Actinoplanes liguriensis* sp. nov. (type strain A/6353^T = FH 2244^T = DSM 43865^T = ATCC 31048^T = BCRC 12121^T = CBS 355.75^T = IMSNU 22127^T = JCM 3250^T = KCTC 9536^T = KCC A-0250^T = NBRC 13997^T = NCIMB 12636^T = NRRL B-16723^T = SANK 62178^T) and *Actinoplanes teichomyceticus* sp. nov. (type strain AB8327^T = FH 2149^T = DSM 43866^T = ATCC 31121^T = BCRC 12106^T = FERM P-3462^T = IMSNU 20043^T = IMET 9254^T = JCM 3252^T = KCC A-0252^T = KCTC 9543^T = NBRC 13999^T = NCIMB 12640^T = NRRL B-16726^T = SANK 60479^T).

In the course of a screening programme for inhibitors of peptidoglycan biosynthesis, a new antibiotic, Ala(O)-actagardine, was isolated from strain A/6353, which was named '*Actinoplanes liguriae*' in regard of the area in Italy, Liguria, where it had been isolated (Parenti & Coronelli, 1979; Vertesy *et al.*, 1999). *Actinoplanes* strain AB8327, the producer of the antibiotic gardimycin, was described and named by Parenti and coworkers as '*Actinoplanes teichomyceticus*', referring to inhibition of cell-wall synthesis (Parenti *et al.*, 1976). Neither name was validly published. In a study on the phylogenetic relationship of *Actinoplanes* species by comparative 16S rRNA gene sequencing by Tamura & Hatano (2001), the two strains were included. In the resulting tree, derived by the neighbour-joining method, strains A/6353 and AB8327 were found to cluster within the radiation of *Actinoplanes* species, related sufficiently remotely from validly named species to warrant their description as novel species. This step is presented in this communication by providing sufficient taxonomic evidence to describe *Actinoplanes liguriensis* sp. nov. with the type strain A/6353^T and *Actinoplanes teichomyceticus* sp. nov. with the type strain AB8327^T.

The strains under study were A/6353^T (=DSM 43865^T), AB8327^T (=DSM 43866^T), *Actinoplanes palleronii* (DSM

43940^T), *Actinoplanes regularis* (DSM 43151^T) and *Actinoplanes rectilineatus* (DSM 43808^T).

The morphological and physiological characteristics of the strains were studied after incubation for 10 days at 28 °C on various media described by Shirling & Gottlieb (1966): yeast extract/malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salt/starch agar (ISP 4), glycerol/asparagine agar (ISP 5), peptone/yeast extract/iron agar (ISP 6) and tyrosine agar (ISP 7). For scanning electron microscopy, the strains were grown on different ISP media and prepared according to Grabley *et al.* (1992). Colours were determined according to the RAL colour code (RAL is the Deutsches Institut für Gütesicherung und Kennzeichnung e.V.-Reichsausschuß für Lieferbedingungen).

Utilization of carbohydrates was investigated on ISP medium 9 (Shirling & Gottlieb, 1966) by using a microtitre-plate technique with 12-well plates. A fingerprint of enzymic activities was obtained by API 20E and API ZYM test strips (bioMérieux).

Analysis of the antimicrobial spectrum was performed as described by Williams *et al.* (1989). Bacterial strains were grown on Mueller–Hinton agar and fungal strains on Czapek Dox. For metabolite production, the *Actinoplanes* strains were incubated in four different media: a soybean meal medium, a starch medium and ISP media 2 and 3 for 7 days in shaking-flask cultures at 28 °C and 180 r.p.m.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains DSM 43865^T and DSM 43866^T are AJ865471 and AJ865472, respectively.

After cultivation, the whole culture broth was extracted in methanol, evaporated to dryness and the residue was dissolved in water for antimicrobial testing.

Analysis of the whole-cell diaminopimelic acid isomers and the sugars was done by the method of Hasegawa *et al.* (1983). The phospholipids and menaquinones were analysed by the method of Minnikin *et al.* (1984). Analyses of whole-cell fatty acid pattern followed described methods using the MIDI system (Microbial ID, Inc.) (Kroppenstedt, 1985; Sasser, 1990).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of PCR products were carried out as described previously (Rainey *et al.*, 1996). The ae2 editor (Maidak *et al.*, 2001) was used to align the almost-complete 16S rRNA gene sequences of strains A/6353^T and AB8327^T against the 16S rRNA gene sequences of representatives of the main actinobacterial lineages and subsequently against members of the genus *Actinoplanes*. Pairwise evolutionary distances were computed by using the correction of Jukes & Cantor (1969). A phylogenetic dendrogram was reconstructed from a distance matrix by using the treeing algorithm of DeSoete (1983). Automated ribotyping of the isolates was accomplished by using the RiboPrinter (Qualicon) system (Bruce, 1996) and *PvuII* as restriction enzyme for cutting the genomic DNA. The patterns were compared with those included in the DSMZ RiboPrint database.

Formation of characteristic sporangia was observed in both strains (Fig. 1). Fragmentation of substrate mycelium was not found. Strain AB8327^T formed a white aerial mycelium on ISP medium 2. The surface of the spores was smooth. Only rudimentary aerial mycelium was observed for strain A/6353^T. The substrate mycelium of strain A/6353^T was yellow–orange (RAL 2000) on all tested ISP media. Strain AB8327^T formed pastel-yellow (RAL 1034) mycelium on ISP media 2, 3, 4 and 7 and beige (RAL 1001) mycelium on ISP media 5 and 6. A yellow, soluble pigment was formed by strain A/6353^T on ISP media 4 and 5 and a red one on ISP 7. Strain AB8327^T produced a red, soluble pigment on ISP 5 and a brown one on ISP 6 and Suter medium with tyrosine (Table 1).

Glucose was the only carbohydrate that was used by both strains. Xylose, inositol and raffinose could not be assimilated by either of the strains, whereas A/6353^T could grow on rhamnose and AB8327^T on arabinose, sucrose, mannose and fructose (Table 2).

Enzymic activities were analysed by using the API test systems API 20E and API ZYM (Table 3). Each strain showed a characteristic pattern with API 20E as well as with API ZYM. In the API 20E system, strains A/6353^T and AB8327^T showed urease activity, but lacked citrate utilization and indole production. In API ZYM tests, both strains were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8) and phosphohydrolase, but negative

Fig. 1. Scanning electron micrograph displaying the morphology of strains A/6353^T (a, b) and AB8327^T (c). Cultivation was carried out on ISP medium 3 at 28 °C for 14 days. (a) Sporophore and sporangia (magnification $\times 2000$). (b) Detail of spore chains by lack of sporangia envelope (magnification $\times 10\,000$). (c) Aerial mycelium and sporangia (magnification $\times 2000$).

for lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase and α -fucosidase.

Whole-cell hydrolysates contained meso-diaminopimelic acid and the sugars xylose and arabinose. Mycolic acids were missing. The characteristic menaquinones were MK-9(H₄) and MK-10(H₄). In the polar lipid extract, the diagnostic phospholipid phosphatidylethanolamine could be detected,

Table 1. Colonial characteristics of *A. palleronii* DSM 43940^T, *A. rectilineatus* DSM 43808^T, *A. regularis* DSM 43151^T, A/6353^T and AB8327^T

Formation and colour of: SM, substrate mycelium; AM, aerial mycelium; SP, soluble exopigment.

Culture medium	DSM 43940 ^T	DSM 43808 ^T	DSM 43151 ^T	A/6353 ^T	AB8327 ^T
ISP 2	SM orange–yellow	SM yellow–orange	SM brown–red	SM yellow–orange	SM pastel yellow
	AM none	AM none	AM none	AM none	AM none
	SP none	SP none	SP red	SP none	SP none
ISP 3	SM beige	SM brown–orange	SM light orange	SM yellow–orange	SM pastel yellow
	AM none	AM none	AM none	AM white	AM white
	SP none	SP none	SP red	SP none	SP none
ISP 4	SM pastel yellow	SM brown–orange	SM pure orange	SM yellow–orange	SM pastel yellow
	AM none	AM none	AM none	AM none	AM none
	SP none	SP none	SP red	SP yellow	SP none
ISP 5	SM beige	SM brown–orange	SM pure orange	SM yellow–orange	SM beige
	AM none	AM none	AM none	AM none	AM none
	SP none	SP none	SP red	SP yellow	SP red
ISP 6	SM beige	SM brown–orange	SM light orange	SM yellow–orange	SM beige
	AM none	AM none	AM none	AM none	AM none
	SP brown	SP none	SP brown	SP none	SP brown
ISP 7	SM beige	SM brown–orange	SM light orange	SM yellow–orange	SM light orange
	AM none	AM none	AM none	AM none	AM none
	SP none	SP none	SP brown–red	SP red	SP none

but phosphatidylcholine and *N*-acetylglucosamine were missing, i.e. phospholipid pattern type 2 according to Lechevalier *et al.* (1977). The fatty acid patterns were characteristic for *Actinoplanes* species, with major amounts of iso-C16:0, iso-C17:0, anteiso-C17:0, iso-C18:0 and C18:0. A detailed listing of compounds with their quantities is indicated in the species descriptions.

Strains A/6353^T and AB8327^T share 97.1 % 16S rRNA gene sequence similarity. Whilst *A. teichomyceticus* AB8327^T is related moderately to the type strain of *A. rectilineatus*, *A. liguriensis* A/6353^T shares 99.3 % similarity with the type strain of *A. cyaneus*. The binary DNA–DNA similarity value for the latter strain pair was 41.5 % [mean of two values determined in 2 × SSC, 12 % formamide at 70 °C, according

to Wink *et al.* (2003)]. 16S rRNA gene sequence similarity values with other type strains of the genus were <98.4 %. Fig. 2 displays the dendrogram of 16S rRNA gene sequence similarities. Except for the branching of strains DSM 43865^T (A/6353^T) and *A. cyaneus* DSM 46137^T, bootstrap values were <80 %, hence they are not shown. Comparison with sequences deposited in public databases showed the similarity between strain AB8327^T and '*A. teichomyceticus*' IFO 1999 (GenBank accession no. AB047513) to be 100 %, whilst the value between the two strains labelled '*A. liguriae*' (A/6353^T and IFO 13977, GenBank accession no. AB047497) was only 97.3 %. In order to check the authenticity of strain A/6353^T, originally obtained as ATCC 31048, we sequenced another culture of ATCC 31048 and confirmed its identity. Results of the sequencing

Table 2. Utilization of carbohydrates of *A. palleronii* DSM 43940^T, *A. rectilineatus* DSM 43808^T, *A. regularis* DSM 43151^T, A/6353^T and AB8327^T

All strains utilize glucose.

Carbohydrate	DSM 43940 ^T	DSM 43808 ^T	DSM 43151 ^T	A/6353 ^T	AB8327 ^T
Arabinose	–	+	+	–	+
Sucrose	+	+	+	–	+
Xylose	–	+	–	–	–
Inositol	–	+	–	–	–
Manitol	+	+	–	–	+
Fructose	+	+	+	–	+
Rhamnose	+	+	+	+	–
Raffinose	–	+	–	–	–

Table 3. Enzymic activities of *A. palleronii* DSM 43940^T, *A. rectilineatus* DSM 43808^T, *A. regularis* DSM 43151^T, A/6353^T and AB8327^T

All strains are positive for gelatinase and acid phosphatase; no strains produce H₂S or indole or show tryptophan deaminase, lipase (C14), valine arylamidase or α-fucosidase activities.

Physiological parameter	DSM 43940 ^T	DSM 43808 ^T	DSM 43151 ^T	A/6353 ^T	AB8327 ^T
API 20E					
β-Galactosidase	—	+	+	+	—
Arginine dihydrolase	—	+	+	—	+
Lysine decarboxylase	—	+	+	—	+
Ornithine decarboxylase	—	—	+	—	+
Citrate utilization	—	+	+	—	—
Urease	—	+	+	+	+
API ZYM					
Alkaline phosphatase	+	+	—	+	+
Esterase (C4)	+	+	—	+	+
Esterase lipase (C8)	+	+	—	+	+
Leucine arylamidase	+	+	—	—	—
Cystine arylamidase	—	+	—	—	—
Trypsin	—	+	—	+	—
Chymotrypsin	—	+	+	+	—
Naphthol-AS-BI-phosphohydrolase	—	+	—	+	+
α-Galactosidase	+	+	—	+	—
β-Galactosidase	+	+	—	+	—
β-Glucuronidase	—	—	—	+	—
α-Glucosidase	+	+	+	+	—
β-Glucosidase	+	+	+	+	—
N-Acetyl-β-glucoseaminidase	+	+	—	+	—
α-Mannosidase	—	+	—	+	—

studies were confirmed by RiboPrint analysis of strains A/6353^T, AB8327^T and the closest phylogenetic relatives. Strains A/6353^T and AB8327^T showed unique RiboPrint

Fig. 2. 16S rRNA gene dendrogram (DeSoete, 1983) displaying the phylogenetic position of novel members of the genus *Actinoplanes* and phylogenetically related members of the genus. Numbers indicate the percentage of bootstrap samplings, derived from 500 resamplings. More distantly related members of the genus served as a root. Numbers in parentheses are GenBank accession numbers for 16S rRNA gene sequences. Bar, 1 % sequence divergence.

patterns that were clearly separated from the patterns of the other type strains (Fig. 3) and from those of all other *Actinoplanes* type strains (not shown). Based on the phylogenetic, chemotaxonomic and phenotypic results, it can be concluded that A/6353^T and AB8327^T represent the type strains of two novel species of the genus *Actinoplanes* and we therefore propose the names *Actinoplanes liguriensis* sp. nov. (type strain A/6353^T) and *Actinoplanes teichomyceticus* sp. nov. (type strain AB8327^T).

Description of *Actinoplanes liguriensis* sp. nov.

Actinoplanes liguriensis (li.gu.ri.en'sis. N.L. masc. adj. *liguriensis* pertaining to the Italian region of Liguria).

Cells are Gram-positive. Globose to oval sporangia are formed (see Fig. 1). Rudimentary sterile mycelium is formed only on ISP medium 6. Colour of substrate mycelium is chrome yellow–orange (RAL 2000) on ISP media 2, 3, 4, 5, 6 and 7. A red, soluble pigment is formed on ISP medium 7. Colonial characteristics, carbohydrate-utilization pattern, enzyme activities and antimicrobial activities are indicated in Tables 1, 2, 3 and 4, respectively. Peptidoglycan contains meso-diaminopimelic acid; diagnostic sugars are xylose and arabinose; mycolic acids are absent; MK-9(H₄) and MK-10(H₄) are the principal menaquinones; the major phospholipid is phosphatidylethanolamine. Major fatty acid methyl

Fig. 3. Diversity of normalized *PvuII* ribotype patterns found within novel members of the genus *Actinoplanes* and their phylogenetic neighbours.

esters are (>10 %): iso-C15:0 (26.1 %), anteiso-C15:0 (13.1 %), iso-C16:0 (12.1 %), anteiso-C17:0 (11.0 %); others occur in smaller amounts (≥ 1.0 %): iso-C14:0 (1.7 %), C14:0 (1.8 %), C15:0 (1.5 %), C16:1 (5.80 %), C16:0 (6.5 %), iso-C17:1 (1.9 %), anteiso-C17:0 (1.0 %), iso-C17:0 (3.0 %), *cis*-9-C17:1 (2.2 %), *cis*-10-C17:1 (3.3 %), C17:0 (1.2 %), *cis*-9-C18:1 (2.9 %) and C18:0 (1.5 %). The antibiotics gardimycin and Ala(O)-actagardine are produced.

The type strain is A/6353^T (=FH 2244^T=DSM 43865^T=ATCC 31048^T=BCRC 12121^T=CBS 355.75^T=IMSNU 22127^T=JCM 3250^T=KCTC 9536^T=KCC A-0250^T=NBRC 13997^T=NCIMB 12636^T=NRRL B-16723^T=SANK 62178^T). Isolated from Italian soil.

Description of *Actinoplanes teichomyceticus* sp. nov.

Actinoplanes teichomyceticus [tei.cho.my.ce'ti.cus. Gr. n. *teichos* wall; Gr. n. *myces* fungus; L. adj. suff. *-icus* belonging to; *teichomyceticus* belonging to a fungus cell wall (referring to inhibition of cell-wall synthesis by teichomycin, produced by the type strain)].

Cells are Gram-positive. Spherical to oval sporangia are formed (see Fig. 1). Long, sterile hyphae are formed on ISP

media 2, 3, 4 and 5. Colour of substrate mycelium is pastel yellow (RAL 1034) on ISP media 2, 3, 4 and 7 and beige (RAL 1001) on ISP media 5 and 6. A red, soluble pigment is formed on ISP medium 5 and a brown one on ISP media 3 and 6. Colonial characteristics, carbohydrate-utilization pattern, enzyme activities and antimicrobial activities are indicated in Tables 1, 2, 3 and 4, respectively. Peptidoglycan contains meso-diaminopimelic acid; diagnostic sugars are xylose and arabinose; mycolic acids are absent; MK-9(H₄) and MK-10(H₄) are the principal menaquinones; the major phospholipid is phosphatidylethanolamine. Major fatty acid methyl esters are (>10 %): iso-C16:0 (20.7 %), iso-C15:0 (15.1 %), anteiso-C15:0 (8.8 %), anteiso-C17:0 (19.8 %); others occur in smaller amounts (≥ 1.0 %): iso-C14:0 (1.0 %), C15:0 (1.1 %), iso-C16:1 (1.6 %), *cis*-9-C16:1 (3.4 %), C16:0 (2.5 %), iso-C17:1 (2.6 %), anteiso-C17:1 (2.0 %), iso-C17:0 (3.8 %), *cis*-9-C17:1 (6.7 %), C17:0 (3.5 %), *cis*-9-C18:1 (4.3 %) and C18:0 (1.8 %). The antibiotics teichomycin A1 and A2 (renamed teicoplanin) are produced.

The type strain is AB8327^T (=FH 2149^T=DSM 43866^T=ATCC 31121^T=BCRC 12106^T=FERM P-3462^T IMSNU 20043^T=IMET 9254^T=JCM 3252^T=KCC A-0252^T=KCTC 9543^T=NBRC 13999^T=NCIMB 12640^T=NRRL B-16726^T=SANK 60479^T). Isolated from Indian soil.

Table 4. Antimicrobial activities of *A. palleronii* DSM 43940^T, *A. rectilineatus* DSM 43808^T, *A. regularis* DSM 43151^T, A/6353^T and AB8327^T

All strains showed no activity against the fungi *Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger* or the bacteria *Escherichia coli* and *Pseudomonas aeruginosa*.

Test strain	DSM 43940 ^T	DSM 43808 ^T	DSM 43151 ^T	A/6353 ^T	AB8327 ^T
<i>Staphylococcus aureus</i>	—	—	+	+	+
<i>Micrococcus luteus</i>	+	—	+	—	—
<i>Streptomyces murinus</i>	—	—	+	—	+
<i>Bacillus subtilis</i>	+	—	+	+	+

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