***Actinoalloteichus cyanogriseus* gen. nov., sp. nov.**

Tomohiko Tamura,¹ Liu Zhiheng,² Zhang Yamei² and Kazunori Hatano¹

Author for correspondence: Tomohiko Tamura. Tel: +81 6 6300 6555. Fax: +81 6 6300 6814.
e-mail: k90089@simail.ne.jp

¹ Institute for Fermentation,
Osaka, 2-17-85 Jusohonmachi Yodogawa-ku,
Osaka 532-8686, Japan

² Institute for Microbiology,
Chinese Academy of
Sciences, Beijing, 100080,
PR China

Phylogenetic analysis of ‘*Actinoalloteichus cyanogriseus*’ based on its 16S rDNA sequence confirmed that the organism belongs to the family *Pseudonocardiaceae*. It contains glutamic acid, alanine and meso-diaminopimelic acid as cell wall amino acids, and menaquinone 9 (H₄). Its taxonomic characteristics differ from those of the previously described organisms of the family *Pseudonocardiaceae*. On the basis of these morphological, physiological, chemotaxonomic and phylogenetic analyses, a new genus, *Actinoalloteichus*, is proposed, along with a new species, namely *Actinoalloteichus cyanogriseus* sp. nov. The type strain is *A. cyanogriseus* IFO 14455^T (= AS 4.1159^T = JCM 6095^T).

Keywords: *Actinoalloteichus* gen. nov., family *Pseudonocardiaceae*, 16S rRNA

INTRODUCTION

Liu *et al.* (1984) described ‘*Actinoalloteichus cyanogriseus*’ as a new taxa having the following characteristics: blue–grey aerial mycelium with straight spore chains is present; the aerial and substrate mycelia form septa and fragment after 2 weeks incubation; bluish-violet soluble pigment is produced; the cell wall contains meso-diaminopimelic acid, glycine and lysine and whole-cell hydrolysate contains galactose without other characteristic sugars; the G + C content of DNA is 69.54 mol %.

Itoh *et al.* (1987) reported the morphological and chemotaxonomic characteristics of this organism and indicated that it contains glutamic acid, alanine and meso-diaminopimelic acid as cell wall amino acids, iso- and anteiso-branched fatty acids as the predominant cellular fatty acids, and menaquinone 9 (H₄).

Recently, we determined the phylogenetic position of ‘*Actinoalloteichus cyanogriseus*’ on the basis of its 16S rDNA sequence (Tamura & Hatano, 1998), showing that it belongs to the family *Pseudonocardiaceae* (Stackebrandt *et al.*, 1997). We also showed that it represents a line of descent distinct from those of previously described organisms.

In this paper, we describe the characterization and classification of ‘*A. cyanogriseus*’ and propose its designation as *A. cyanogriseus* gen. nov., sp. nov.

The DDBJ accession number for the 16S rRNA gene sequence of *Actinoalloteichus cyanogriseus* IFO 14455^T is AB006178.

METHODS

Micro-organisms and culture conditions. ‘*A. cyanogriseus*’ IFO 14455^T, isolated from a soil sample collected from the Yunnan province of China (Liu *et al.*, 1984), was used. To obtain cells, the organism was grown at 28 °C for 5 d in yeast extract/glucose broth (containing 10 g yeast extract and 10 g D-glucose in 1000 ml distilled water, pH 7.0) on a rotary shaker.

The culture grown on HV agar (Hayakawa & Nonomura, 1987) for 2–3 weeks at 28 °C was observed with a light microscope and a scanning electron microscope as described previously (Tamura *et al.*, 1998). Cultural and physiological characteristics were determined as described previously (Yokota *et al.*, 1993; Gordon *et al.*, 1974).

Phylogenetic analysis. The 16S rDNA sequences of ‘*A. cyanogriseus*’ and reference organisms were aligned against that of *Streptomyces ambifaciens* (Pernodet *et al.*, 1989). The CLUSTAL W version 1.7 software (Thompson *et al.*, 1994) package was used to generate the evolutionary distances (the K_{muc} value of Kimura, 1980) and the similarity values and to reconstruct the phylogenetic tree by the neighbour-joining method (Saitou & Nei, 1987) from K_{muc} values. The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. The NJplot method (Perrière & Gouy, 1996) was used for plotting the phylogenetic tree.

RESULTS AND DISCUSSION

Growth characteristics

As shown in Table 1, strain IFO 14455^T showed good growth on yeast extract/malt extract agar (ISP medium 2) and oatmeal agar (ISP medium 3) and

Table 1. Cultural characteristics of *Actinoalloteichus cyanogriseus* IFO 14455^T

Medium	Characteristics*
Yeast extract/malt extract agar (ISP-2)	
Growth	Good, brownish-grey (118) to light brownish-grey (117)
Aerial mycelium	Good, light purplish-grey (127)
Reverse colour	Black
Soluble pigment	Black
Oatmeal agar (ISP-3)	
Growth	Good, yellowish-grey (105)
Aerial mycelium	Good, pale blue (96)
Reverse colour	Yellowish-grey (105) to olive grey (122)
Soluble pigment	Light greyish-red (115)
Inorganic salts/starch agar (ISP-4)	
Growth	Poor, colourless
Aerial mycelium	Poor, colourless
Reverse colour	Colourless
Glycerol/asparagine agar (ISP-5)	
Growth	Poor, colourless to light greenish-grey (123)
Aerial mycelium	Moderate, light greenish-grey (123) to greyish-white
Reverse colour	Light greenish-grey (123)
Peptone/yeast extract/iron agar (ISP-6)	
Growth	Moderate, yellowish-grey (105) to dark reddish-brown (104)
Aerial mycelium	Poor, white
Reverse colour	Moderate yellow (64) to dark reddish-brown (104)
Soluble pigment	Black
Tyrosine agar (ISP-7)	
Growth	Poor, yellowish-grey (105)
Aerial mycelium	Moderate, white
Reverse colour	Yellowish-grey (105)
Calcium malate agar	
Growth	Poor, colourless
Aerial mycelium	Poor, white
Reverse colour	Colourless

* The colour codes correspond to those in *A Mycological Colour Chart* (Rayner, 1970).

produced a black soluble pigment on yeast extract/malt extract agar and peptone/yeast extract/iron agar (ISP medium 6), a light greyish-red pigment on oatmeal agar and a yellowish-grey pigment on tyrosine agar (ISP medium 7). It developed purplish-grey or blue aerial mycelium on yeast extract/malt extract agar, oatmeal agar and glycerol/asparagine agar (ISP medium 5).

Physiological characteristics

The biochemical properties of strain IFO 14455^T are shown in Table 2. The strain utilized D-glucose, maltose, xylose, mannitol, rhamnose, mannose and sorbitol. Decomposition of casein and urea, growth in Sabouraud dextrose broth and MacConkey agar, hydrolysis of aesculin and hippurate, utilization of sodium citrate, sodium succinate and calcium malate, gelatin liquefaction and reduction of nitrate were all negative. It grew at 20 and 37 °C, but could not grow at 15 or 45 °C.

Chemotaxonomic characteristics

Chemotaxonomic characteristics reported by Itoh *et al.* (1987) are indicated in Table 3. This organism contains MK-9(H₄) as the major menaquinone; glucose, mannose, galactose and rhamnose as the whole-cell sugars; and *meso*-diaminopimelic acid, glutamic acid and alanine as the amino acids in the cell wall. Mycolic acids are absent. The acyl type of the cell wall polysaccharides is acetyl. Phosphatidylethanolamine, phosphatidylinositol and phosphatidylglycerol were detected. The G + C content of the DNA is 72.5 mol %. The major cellular fatty acids are anteiso-C_{17:0}, iso-C_{15:0}, iso-C_{16:0} and anteiso-C_{15:0}. The cellular fatty acid pattern corresponds to type 2d (Kroppenstedt, 1985).

Analysis of 16S rDNA sequences

The phylogenetic tree obtained by applying the neighbour-joining method (Saitou & Nei, 1987) to *K_{nuc}* values of Kimura (1980) is depicted in Fig. 1.

Table 2. Phenotypic characteristics of *A. cyanogriseus* IFO 14455[†]

Characteristic	Result
Utilization of:*	
Glucose	++
Maltose	++
Inositol	—
Fructose	—
Arabinose	+ / —
Xylose	+
Mannitol	+
Rhamnose	++
Raffinose	—
Mannose	+
Saccharose	+ / —
Sorbitol	+
Decomposition of casein [†]	—
Decomposition of urea [†]	—
Growth in Sabouraud dextrose broth [†]	—
Growth on MacConkey agar [†]	—
Hydrolysis of aesculin [†]	—
Hydrolysis of hippurate [†]	—
Hydrolysis of starch [†]	w
Resistance to:†	
Methyl violet	—
Pyronin B	—
Survival at 50 °C [†]	—
Utilization of:†	
Sodium citrate	—
Sodium oxalate	w
Sodium succinate	—
Calcium malate	—
Resistance to lysozyme	+
Nitrite from nitrate [†]	—
Gelatin liquefaction [†]	—
Pigmentation in:†	
ISP-1	—
ISP-6	+
ISP-7	—
Growth temperature (°C) [†]	
15	—
20	+
25	+
30	+
37	+
45	—

* ++, Good; +, moderate; + / —, poor; —, none.

† +, Positive; —, negative; w, weakly positive.

Table 3. Chemotaxonomic characteristics of *A. cyanogriseus* IFO 14455[†]

Characteristic	
Cell wall composition	
Glutamic acid	1.0
Glycine	<0.1
Alanine	1.7
A ₂ pm	0.7
Isomer of A ₂ pm	meso-A ₂ pm
Acyl type	Acetyl
Sugar composition	Ribose, mannose, glucose, galactose
Diagnostic phospholipids	
Diphosphatidylglycerol	+
Phosphatidylmonomethyl-ethanolamine	+
Phosphatidylglycerol	+
Phosphatidylinositol	+
Phosphatidylinositol mannoside	+
Phosphatidylcholine	—
Menaquinone composition (%)	
MK-8(H ₄)	10
MK-9(H ₂)	9
MK-9(H ₁)	75
MK-9(H ₆)	2
MK-10(H ₄)	3
Mycolic acid	Absent
G + C content of DNA (mol %)	72.5
Cellular fatty acid composition (%) of:	
Anteiso-branched fatty acids	
C _{15:0}	10
C _{17:0}	20
Iso-branched fatty acids	
C _{14:0}	5
C _{15:0}	15
C _{16:0}	19
C _{16:1}	8
C _{17:0}	3
Saturated fatty acids	
C _{16:0}	2
C _{18:0}	Trace
Unsaturated fatty acid	
C _{16:1}	2
Other components	C _{15:0} , C _{17:0}

Data are from Itoh *et al.* (1987).

Analysis of the 16S rDNA confirmed that '*A. cyanogriseus*' falls into the family *Pseudonocardiaceae*. However, '*A. cyanogriseus*' represents a line of descent distinct from those of previously described organisms. '*A. cyanogriseus*' has as its closest neighbours members of the genera *Kutzneria* (Stackebrandt *et al.*, 1994) and *Streptoalloteichus* (Tomita *et al.*, 1987). The binary

similarity value for 16S rDNA of '*A. cyanogriseus*' and *Kutzneria viridogrisea* is 94.5 %, while that for '*A. cyanogriseus*' and *Streptoalloteichus hindus* is 95.1 %.

'*A. cyanogriseus*' develops aerial mycelium with straight spore chains (Fig. 2) and contains glucose, mannose, galactose and rhamnose as the whole-cell sugars and meso-diaminopimelic acid, glutamic acid and alanine as the cell wall amino acids. Itoh *et al.* (1987) reported that these morphological and cell wall components correspond to those of the genus

Fig. 1. Phylogenetic tree derived from 16S rRNA sequences for the members of the Pseudonocardiaceae. *Streptomyces ambofaciens* was used as the root organism. Bar, 0.01 K_{nuc} in nucleotide sequences. The numbers on the branches are confidence limits (expressed as percentages) estimated from a bootstrap analysis with 1000 replicates (only percentages above 50% are indicated).

Fig. 2. Scanning electron micrograph of *A. cyanogriseus* IFO 14455^T grown on HV agar for 14 d at 28 °C. Bar, 5 μm.

Nocardiopsis. However, phylogenetic analysis indicated that '*A. cyanogriseus*' is remote from the genus *Nocardiopsis* and belongs to the family Pseudonocardiaceae, its closest relation being the genus *Streptoalloteichus* (Tomita *et al.*, 1987). The taxonomic

characteristics of the members of the family Pseudonocardiaceae are shown in Table 4. The genus *Streptoalloteichus* has fatty acid type 3e and develops sporangia, while *Kutzneria* has fatty acid type 3c and develops sporangia. On the other hand, '*A. cyanogriseus*' has no 10-methylated fatty acid among the cellular fatty acids (fatty acid type 2d) and develops no sporangia or motile cells. The genera *Saccharothrix* (Labeda *et al.*, 1984), *Actinosynnema* (Hasegawa *et al.*, 1978) and *Thermocrisum* (Korn-Wendisch *et al.*, 1995) have meso-diaminopimelic acid in the cell wall (cell wall type III) and long spore chains. However, the present organism is distinguished from these genera by its morphological characteristics, fatty acid components and non-motility. Thus, in terms of its morphological and chemotaxonomic characteristics, the strain is distinct from known actinomycetes belonging to the family Pseudonocardiaceae (Table 4).

On the basis of morphological, physiological, chemotaxonomic and phylogenetic criteria, '*A. cyanogriseus*' is readily distinguishable from the actinomycetes mentioned above and warrants a new taxon. Therefore, we propose that '*A. cyanogriseus*' be classified in a new genus, *Actinoalloteichus* gen. nov., with the type species *A. cyanogriseus* sp. nov. This strain has been deposited in the Institute for Fermentation (Osaka) as strain IFO 14455^T, in the Institute of Microbiology

Table 4. Morphological and chemotaxonomic characteristics of *Actinoalloteichus* and related actinomycete genera

NT, Not tested; ND, not detected.

Genus	Morphological characteristics	Cell wall type*	Glycolyl test	Major menaquinone(s)	Fatty acid type†	Whole-cell sugar pattern‡
<i>Actinoalloteichus</i>	Long spore chain	III	Acetyl	MK-9(H ₄), 8(H ₄), 9(H ₂)	2d	C
<i>Lentzea</i>	Long hyphae, fragmentation of aerial mycelium	III	NT	MK-9	3d	C
<i>Streptoalloteichus</i>	Long hyphae and sporangia, motile spore	III	Acetyl	MK-10(H ₄ , H ₆)	3e	E
<i>Saccharothrix</i>	Spore chain, fragmentation, sporulation ('zig-zag' morphology)	III	ND	MK-10(H ₄), 9(H ₄)	3f	C (E)
<i>Amycolatopsis</i>	Long spore chain	IV	Acetyl	MK-9(H ₂), 8(H ₄)	3f	A
<i>Pseudonocardia</i>	Long spore chain	IV	Acetyl	MK-8(H ₂ , H ₄)	2	A
<i>Actinokineospora</i>	Long spore chain, motile spore	IV	Acetyl	MK-9(H ₄)	2d	A
<i>Kutzneria</i>	Sporangium	III	Acetyl	MK-9(H ₄ , H ₂ , H ₆), 8(H ₄), 10(H ₄)	3c	E
<i>Actinosynnema</i>	Spore chain, synnemata, motile spore	III	Acetyl	MK-9(H ₄), 10(H ₄)	3f	C
<i>Kibdelosporangium</i>	Sporangium-like structure	IV	Acetyl	MK-9(H ₂ , H ₄ , H ₆), 8(H ₄ , H ₆)	3c	A (B)
<i>Saccharopolyspora</i>	Beadlike spore chain	IV	Acetyl	MK-9(H ₄)	2c	A
<i>Saccharomonospora</i>	Single spore on aerial mycelium	IV	ND	MK-9(H ₄)	2a	A
<i>Actinopolyspora</i>	Long spore chain	IV	ND	MK-9(H ₄), 10(H ₄)	2c	A
<i>Thermocrisum</i>	Pseudosporangium, long chain, fragmentation	III	ND	MK-9(H ₄)	3f	C (A)
<i>Actinobispora</i>	Long hyphae, single spore or pair of spores on aerial mycelium	IV	ND	MK-7(H ₂), 9(H ₂)	ND	A

* According to the classification of Lechevalier & Lechevalier (1970). All organisms have the *meso*-diaminopimelic acid isomer.

† According to the classification of Kroppenstedt (1985).

‡ According to the classification of Lechevalier & Lechevalier (1970).

Academia Sinica as strain AS 4.1159^T and in the Japan Collection of Microorganisms as strain JCM 6095^T.

Description of *Actinoalloteichus* gen. nov.

Actinoalloteichus (Ac.ti.no.al.lo.tei'chus. Gr. n. *actis*, *actinos* a ray, beam; Gr. adj. *allos* different; Gr. n. *teichos* wall; M.L. masc. n. *Actinoalloteichus* actinomycete with a different wall).

Gram-positive, non-acid-fast, aerobic organisms with branching hyphae. Non-fragmentary substrate mycelia are present. Aerial mycelia with straight spore chains aggregate. The aerial and substrate mycelia tend to fragment. Strictly aerobic. Good growth occurs at 20–37 °C. The organism shows good growth on yeast extract/malt extract agar and oatmeal agar. Cell walls contain glutamic acid, glucosamine, alanine and *meso*-diaminopimelic acid. The wall chemotype is III, according to Lechevalier & Lechevalier (1970), and the peptidoglycan type is suggested to be A1γ according to

Schleifer & Kandler (1972). Ribose, mannose, galactose and glucose are detected in whole-cell sugars. Anteiso-C_{17:0}, iso-C_{15:0}, iso-C_{16:0} and anteiso-C_{15:0} are present as major cellular fatty acids. The G+C content of the DNA is 73 mol %. The major menaquinone is MK-9(H₄) and small amounts of MK-8(H₄) and 9(H₂) are also present. Phosphatidylethanolamine and phosphatidylmonomethylethanolamine are present as diagnostic phospholipids. The acyl type of the cell wall polysaccharides is acetyl. The type species is *Actinoalloteichus cyanogriseus*.

Description of *Actinoalloteichus cyanogriseus* sp. nov.

Actinoalloteichus cyanogriseus (cya.no.gri'se.us. L. adj. *cyaneus* deep blue; M.L. adj. *griseus* grey; M.L. adj. *cyanogriseus* blue-grey).

Morphological, chemotaxonomic and general characteristics are as given above for the genus. The veg-

etative mycelia are brown to grey in colour and the aerial mycelia are blue to grey. A black soluble pigment is produced on yeast extract/malt extract agar. Decomposition of casein and urea, growth in Sabouraud dextrose broth and MacConkey agar, hydrolysis of aesculin and hippurate, utilization of sodium citrate, sodium succinate and calcium malate, gelatin liquefaction and reduction of nitrate are all negative. Glucose, maltose, xylose, mannitol, rhamnose, mannose and sorbitol are utilized, but inositol, fructose and raffinose are not. No growth at 15 or 45 °C. The G + C content of the DNA is 73 mol %. The type strain is IFO 14455^T (= AS 4.1159^T = JCM 6095^T). The habitat is cultivated soil.

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