Actinomadura namibiensis sp. nov.

Joachim Wink,¹ Reiner M. Kroppenstedt,² Gerhard Seibert¹ and Erko Stackebrandt²

Correspondence

Joachim Wink

Joachim.Wink@aventis.com

¹Aventis Pharma Deutschland, Drug Innovation and Approval, Natural Products, 65926 Frankfurt, Germany

²DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1b, 38124 Braunschweig, Germany

Strain HAG 010767^T was isolated from desert soil from Namibia during a screening programme. On the basis of analysis of 16S rDNA, the principal amino acid of the peptidoglycan, cell-wall sugars, fatty acids and polar lipids, it was possible to identify this strain as a member of the genus *Actinomadura*. Although DNA–DNA reassociation experiments revealed 72 % DNA similarity between strain HAG 010767^T and *Actinomadura kijaniata* DSM 43764^T, significant differences in the colour of the mycelium and physiological properties indicate that strain HAG 010767^T represents a novel species of this genus, for which the name *Actinomadura namibiensis* sp. nov. is proposed. The type strain is strain HAG 010767^T (=DSM 44197^T=NRRL B-24153^T).

Strain HAG 010767^T was isolated from the Namib Desert, Namibia, in 1988, using Actinomycete isolation agar. This salmon-pink strain produced aerial mycelium with spiral spore chains. The strain was compared with the type strains of all validly named *Actinomadura* species. Morphological and physiological characteristics were observed by using agar cultures of the various media described by Shirling & Gottlieb (1966), e.g. yeast extract/malt extract agar (ISP medium 2), oatmeal agar (ISP 3), inorganic salts/starch agar (ISP 4), glycerol/asparagine agar (ISP 5), peptone/yeast extract/iron agar (ISP 6) and tyrosine agar (ISP 7), incubated for 10 days at 28 °C. For scanning electron microscopy, the strain was grown on ISP 4 agar. Microscopy was done as described by Grabley *et al.* (1992).

Vegetative mycelium developed well on all ISP media tested. Aerial mycelium was formed only on media ISP 4, ISP 5 and ISP 7. No soluble pigment was produced on any of the ISP media tested. After 7–10 days on ISP 4, ISP 5 and ISP 7, the formation of spore chains could be observed. These spores exhibited a smooth surface, and the chains formed spirals that could be observed in both light and scanning electron micrographs (Fig. 1).

The vegetative mycelium was salmon pink [according to the RAL colour code (Deutsches Institut für Gütesicherung und

Kennzeichnung e.V. – Reichsausschuß für Lieferbedingungen)] on all the ISP media used except medium ISP 6. The smooth spores in the aerial mycelium are in characteristic long chains that are arranged in spirals (Fig. 1), in contrast with other *Actinomadura* species such as *Actinomadura kijaniata*, which have straight spore chains (Fig. 2).

Carbohydrate utilization was investigated on ISP 9 medium (Shirling & Gottlieb, 1966) by using a microtitre plate technique with 12-well plates. Sodium chloride tolerance was tested on microtitre plates (6-well) using a technique based on the method of Kutzner (1986). Enzymic activities were determined by using API 20E and API ZYM test strips (bioMérieux) (Humble *et al.*, 1977; Kilian, 1978; Smith *et al.*, 1972). Tests were done with type strains of all validly described *Actinomadura* species. The carbohydrate-utilization pattern and the enzymic activity pattern of strain HAG 010767^T were unique among the test strains. Table 1 displays these results in comparison with the phylogenetically neighbouring type strains and the type strain of the type species of *Actinomadura*, *Actinomadura madurae* DSM 43067^T.

Antimicrobial testing (Williams *et al.*, 1983) indicated that none of the test strains, grown either on Mueller–Hinton agar or on Czapek–Dox agar, was affected by isolate HAG 010767^T.

Analyses of whole-cell diaminopimelic acid isomers and sugars were done using the method of Hasegawa *et al.* (1983). Phospholipids and menaquinones were analysed using the method of Kutzner *et al.* (1986). Fatty acid patterns were determined as described previously (Aretz *et al.*, 2000). DL-Diaminopimelic acid is the diagnostic amino acid of the peptidoglycan; the cell wall sugars are

Published online ahead of print on 9 September 2002 as DOI 10.1099/ijs.0.02286-0.

The EMBL accession numbers for the 16S rDNA sequences of *A. namibiensis* sp. nov. DSM 44197^T, *A. echinospora* DSM 43163^T, *A. hibisca* DSM 44148^T, *A. fulvescens* DSM 43923^T, *A. atramentaria* DSM 43919^T, *A. citrea* DSM 43461^T, *A. formosensis* DSM 43997^T and *A. viridis* DSM 20745^T are respectively AJ420134–AJ420141.

Fig. 1. Spore-chain formation in *Actinomadura namibiensis* sp. nov. HAG 010767^T grown on ISP 4 for 10 days at 28 °C, viewed using light microscopy (a) and scanning electron microscopy (b, c). Original magnification: $\times 620$ (a) and $\times 10\,000$ (b, c).

galactose, glucose, ribose and madurose (chemotype III according to Lechevalier & Lechevalier, 1970). Polar lipids are of type IV, according to the phospholipid classification

Fig. 2. Spore-chain formation in *Actinomadura kijaniata* FH 2339 grown on ISP 4 for 10 days at 28 °C, viewed using scanning electron microscopy. Original magnification: $\times 5000$ (a) and $\times 7500$ (b).

of Lechevalier *et al.* (1977), and include diphosphatidylglycerol, phosphatidylinositol, phosphatidylglycerol and phosphatidylinositol mannoside. The principal menaquinones are MK-9(H₄), MK-9(H₆) and MK-9(H₈) (quinone type 4a according to Kroppenstedt & Goodfellow, 1991). The fatty acid methyl esters mainly comprised branched saturated and unsaturated fatty acids and 10-methyl C_{18:0} (tuberculostearic acid) (type 3a of Kroppenstedt & Goodfellow, 1991). Mycolic acids could not be detected.

Analysis of the almost complete 16S rDNA sequence (Rainey *et al.*, 1996) of strain HAG 010767^T in comparison with the sequences of type strains of all 27 validly described *Actinomadura* species revealed a high degree of relatedness (96–99.6% similarity). The highest value (99.6%) was found to *A. kijaniata* DSM 43764^T. A relationship dendrogram, based on neighbour-joining analysis (Felsenstein, 1993), is depicted in Fig. 3. Discrimination at a higher taxonomic level by DNA–DNA hybridization (Escara & Hutton, 1980; Huß *et al.*, 1983; Jahnke, 1992) revealed that

Table 1. Utilization of carbohydrates and enzymic reactions

Strains are listed as: 1, HAG 010767^T; 2, *Actinomadura kijaniata* ATCC 31588^T; 3, *Actinomadura atramentaria* DSM 43919^T; 4, *Actinomadura fibrosa* ATCC 49459^T; 5, *Actinomadura fulvescens* DSM 43923^T; 6, *Actinomadura madurae* CBS 3315^T; 7, *Actinomadura hibisca* IFO 15585^T; 8, *Actinomadura nitritigenes* DSM 44137^T. For utilization tests, growth is scored as: +, like positive control (basal medium with glucose); (+), better than negative control but not like positive control; –, not better than negative control (basal medium with water). All strains are positive for acid phosphatase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and β -glucosidase and negative for β -glucuronidase.

Characteristic	1	2	3	4	5	6	7	8
Utilization of:								
Glucose	+	–	+	+	+	+	+	–
Arabinose	+	–	–	+	–	+	+	–
Sucrose	+	–	–	+	–	+	+	+
Xylose	(+)	–	–	–	–	–	+	–
Inositol	(+)	–	–	+	–	+	+	+
Mannitol	(+)	–	–	–	–	–	+	+
Fructose	–	–	–	+	–	+	+	+
Rhamnose	+	–	+	+	–	(+)	+	–
Raffinose	–	–	–	+	–	–	+	+
API ZYM tests:								
Alkaline phosphatase	+	+	–	+	+	+	+	+
Esterase (C ₄)	–	+	+	+	+	–	+	–
Esterase (C ₈)	–	+	+	+	+	+	+	+
Lipase (C ₁₄)	–	+	–	–	–	–	–	–
Valine arylamidase	+	+	–	+	+	–	+	+
Cystine arylamidase	+	+	–	–	+	–	+	+
Trypsin	+	–	–	+	+	–	+	+
Chymotrypsin	+	+	–	+	+	+	+	–
α -Galactosidase	+	–	–	–	–	–	–	–
β -Galactosidase	+	+	–	–	+	–	(+)	–
α -Glucosidase	+	+	–	+	+	–	+	+
N-Acetyl- β -glucosamidase	+	+	–	+	+	–	+	+
α -Mannosidase	+	–	–	–	–	–	–	+
α -Fucosidase	–	–	–	–	–	–	(+)	–
API20E tests:								
Arginine dihydrolase	–	+	–	–	+	–	+	+
Lysine decarboxylase	–	+	–	–	+	–	+	–
Ornithine decarboxylase	–	+	–	–	+	–	+	–
Citrate utilization	–	+	–	–	+	–	+	+
Urease	–	+	+	+	+	–	–	–
Acetoin production	–	–	+	+	+	–	–	–
Gelatinase	–	–	+	+	+	+	+	+

these two strains constitute two closely related genomic taxa (72 % similarity, mean of duplicates).

On the basis of the presence of genus-specific chemotaxonomic properties, and supported by its phylogenetic position, strain HAG 010767^T is a member of the genus *Actinomadura*. Though phylogenetically highly related to *A. kijaniata*, the presence of diagnostic carbon-utilization and enzyme patterns (using APY ZYM) and the colour of the

Fig. 3. 16S rDNA dendrogram showing the positions of strain HAG 010767^T and type strains of phylogenetically closely related *Actinomadura* species. Scale bar, 2 inferred nucleotide substitutions per 100 nucleotides.

substrate mycelium (HAG 010767^T, salmon; *A. kijaniata*, green) indicate that strain HAG 010767^T represents a taxon worthy of species status. We therefore propose the name *Actinomadura namibiensis* sp. nov., with strain HAG 010767^T as the type strain.

Description of *Actinomadura namibiensis* sp. nov.

Actinomadura namibiensis (na.mi.bi.en'sis. N.L. gen. n. *namibiensis* of Namibia, where the type strain was isolated).

Gram-positive. White aerial mycelium develops only on ISP 4, ISP 5 and ISP 7 media. The substrate mycelium is salmon pink on ISP 2, ISP 3, ISP 4, ISP 5 and ISP 7 media and is colourless on ISP 6. After about 10 days, spiral spore chains, each composed of about 20 spores, are observed in the aerial mycelium. DL-Diaminopimelic acid is the diagnostic amino acid of the peptidoglycan. Cell hydrolysates contain galactose, glucose, ribose and madurose, whereas arabinose and xylose are absent. Phospholipids include diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol mannoside and phosphatidylinositol. The principal menaquinones are MK-9(H₄), MK-9(H₆) and MK-9(H₈). The fatty acid methyl esters mainly comprise iso C_{14:0} and 10-methyl C_{18:0} (tuberculostearic acid); iso C_{17:0}, 10-methyl C_{17:0} and 2-OH C_{15:0} occur in smaller amounts. Physiological reactions are indicated in Table 1. Has no antibacterial effect on *Micrococcus luteus*, *Streptomyces murinus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia*

coli, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Aspergillus niger* or *Candida albicans*. Phylogenetically related to *Actinomadura kijaniata*.

The type strain, strain HAG 010767^T (=DSM 44197^T =NRRL B-24153^T), was isolated from soil from the Namib Desert, Namibia.

References

- Aretz, W., Meiwes, J., Seibert, G., Vobis, G. & Wink, J. (2000). Friulimicins: novel lipopeptide antibiotics with peptidoglycan synthesis inhibiting activity from *Actinoplanes friuliensis* sp. nov. I. Taxonomic studies of the producing microorganism and fermentation. *J Antibiot* **53**, 807–815.
- Escara, J. F. & Hutton, J. R. (1980). Thermal stability and renaturation of DNA in dimethyl sulfoxide solutions: acceleration of the renaturation rate. *Biopolymers* **19**, 1315–1327.
- Felsenstein, J. (1993). PHYLIP – Phylogeny inference package, version 3.5.1. Distributed by the author. Department of Genetics, University of Washington, Seattle, USA.
- Grabley, S., Granzer, E., Hütter, K. & 7 other authors (1992). Secondary metabolites by chemical screening. 8. Decarestrictines, a new family of inhibitors of cholesterol biosynthesis from *Penicillium*. I. Strain description, fermentation, isolation and properties. *J Antibiot* **45**, 56–65.
- Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic *Actinomycetes*. *J Gen Appl Microbiol* **29**, 319–322.
- Humble, M. W., King, A. & Phillips, I. (1977). API ZYM: a simple rapid system for the detection of bacterial enzymes. *J Clin Pathol* **30**, 275–277.
- Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Jahnke, K.-D. (1992). BASIC computer program for evaluation of spectroscopic DNA renaturation data from Gilford System 2600 spectrophotometer on a PC/XT/AT type personal computer. *J Microbiol Methods* **15**, 61–73.
- Kilian, M. (1978). Rapid identification of *Actinomycetaceae* and related bacteria. *J Clin Microbiol* **8**, 127–133.
- Kroppenstedt, R. M. & Goodfellow, G. (1991). The family Thermomonosporaceae. In *The Prokaryotes*, 2nd edn, pp. 1085–1114. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Kutzner, H. J., Kroppenstedt, R. M. & Korn-Wendisch, F. (1986). *Methoden zur Untersuchung von Streptomyceten und einigen anderen Actinomyceten*, 4th edn. Report of the Institute for Microbiology, Technical University, Darmstadt and German Culture Collection (DSMZ), Braunschweig. Braunschweig: DSMZ.
- Lechevalier, M. P. & Lechevalier, H. A. (1970). Composition of whole cell hydrolysates as a criterion in the classification of aerobic actinomycetes. In *The Actinomycetales*, pp. 311–316. Edited by H. Prauser. Jena: Gustav Fischer.
- Lechevalier, M. P., de Bièvre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Ecol Syst* **5**, 249–260.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Smith, P. B., Thomföhrde, K. M., Rhoden, D. L. & Balows, A. (1972). API system: a multitube micromethod for identification of *Enterobacteriaceae*. *Appl Microbiol* **24**, 449–452.
- Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983). Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* **129**, 1743–1813.