NOTE

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The taxon '*Sebekia benihana*', an actinomycete of industrial significance, has been subjected to a polyphasic taxonomic study. 16S rDNA sequence analyses of strain DSM 44320 (= NRRL 11111) revealed 100% similarity to the 16S rDNA sequence of *Nonomuraea roseola* DSM 43767^T and high values (>98.5%) to some other species of this genus. DNA–DNA similarity values of less than 31% observed between strain DSM 44320 and *N. roseola* DSM 43767^T, *Nonomuraea recticatena* DSM 43937^T and *Nonomuraea africana* DSM 43748^T indicate that this strain represents an individual genospecies. Differences between strain DSM 44320^T and the 16 validly described *Nonomuraea* species occur in the primary structure of the 16S rDNA, the physiological test profile, the morphology of mycelium and the spore surface and the colour of substrate mycelium. The only strain of '*S. benihana*', DSM 44320^T (= NRRL 11111^T), is proposed as the type strain of a new species of the genus *Nonomuraea*, *Nonomuraea dietzii* sp. nov.

Keywords: '*Sebekia benihana*', *Nonomuraea dietzii*, polyphasic taxonomy

In 1977, Sebek & Dolak reported on the biomodification of novobiocin by a novel actinomycete, strain NRRL 11111 (UC 5762; Sebek & Dolak, 1977), which was deposited as a patent strain (US patent 4 128 563 from 5 December, 1978). This strain was later classified informally as '*Sebekia benihana*' (Dietz & Li, 1982). The chemical background to this pharmaceutically important reaction was provided in a subsequent publication (Sebek & Dolak, 1984). The same strain was also used efficiently in the conversion, and hence detoxification, of the carboxylic polyether nigericin (Delort *et al.*, 1988).

'*S. benihana*' is a Gram-positive, aerobic, thermophilic actinomycete isolated from soil. Scanning electron microscopy revealed two types of spore-forming structures, straight chains of spores with cross-ridged surfaces and pseudosporangial structures, from which smooth- and rough-surfaced spores were ejected. The strain is melanin-negative and belongs to the red colour group (Dietz & Li, 1982). In the generic key of the actinomycetes and related organisms (Dietz & Thayer, 1980), the following phenotypic properties led

to the identification of '*S. benihana*': spores formed; meso-A₂pm (diaminopimelic acid) present; sporangia absent; spores in chains; no motile spores; madurose and mannose present.

In recent years, there has been increased interest in the development of a phylogenetic framework for actinomycetes, as the rapid affiliation of isolates to well-defined species facilitates the making of decisions as to whether or not these isolates should be included in programmes designed to screen for pharmaceutically interesting enzymes and secondary metabolites. The industrial importance of '*S. benihana*' led us to investigate its phylogenetic position, the result of which was subsequently supported by morphological and chemotaxonomic data.

'*S. benihana*' DSM 44320 (= NRRL 11111) was obtained from the DSMZ. Suspensions of mycelial fragments and spores were stored in glycerol (50%, v/v) at 25 °C until use (Zippel & Neigenfind, 1988). To obtain good sporulation and to stimulate the production of sporangia strains, the strain was cultivated on International *Streptomyces* Project (ISP) media 2 to 7 at 28 °C for 2–3 weeks (pH 7.2–7.5) (Shirling & Gottlieb, 1966).

Cell material for the chemotaxonomic analyses was obtained by growing the strains in trypticase-soy

The EMBL accession numbers for the 16S rDNA sequences determined in this study are AJ269555 (DSM 43748^T), AJ278220 (DSM 44320^T), AJ278221 (DSM 43767^T) and AJ404230 (DSM 43937^T).

(30 g l⁻¹) broth (Merck 5459) on a rotary shaker for 3–7 d at 28 °C. The cells were harvested by filtration or centrifugation, washed twice with distilled water and either freeze-dried or used immediately.

The chemotaxonomic features analysed included the cell wall amino acids and sugars (Lechevalier & Lechevalier, 1970a, c; modified by Stanek & Roberts, 1974), the acyl type of the cell wall (Uchida & Aida, 1977), the isoprenoid quinones (Minnikin *et al.*, 1984; Kroppenstedt *et al.*, 1981; Kroppenstedt, 1982, 1985), the polar lipids (Lechevalier *et al.*, 1981; Minnikin *et al.*, 1984) and the fatty acids (Miller & Berger, 1984). Analysis of the 16S rDNA sequence and phylogenetic comparisons were performed as described by Rainey *et al.* (1996). DNA was isolated as described by Cashion *et al.* (1977) and Escara & Hutton (1980). DNA–DNA hybridization, performed under optimal conditions (2 × SSC, 10% DMSO at 69 °C), was carried out according to methods described previously (Huß *et al.*, 1983; Jahnke, 1992). DNA-reassociation studies, using optimal renaturation conditions (2 × SSC, 10% DMSO at 69 °C) were performed using two different spectrophotometers (Gilford 2600 and Kontron 943).

Analysis of the 16S rDNA revealed that '*S. benihana*' DSM 44320 did not represent a novel lineage within the mycelium-forming actinomycetes, but high levels of similarity between the 16S rDNA of strain DSM 44320 (AJ278220) and those of some type strains of *Nonomuraea*, *Nonomuraea roseola* DSM 43767^T (100%, AJ278221), *Nonomuraea recticatena* DSM 43937^T (99.8%, AJ404230) and *Nonomuraea africana* DSM 43748^T (99.0%, AJ269555), indicated that this strain is a member of the genus *Nonomuraea*. Similarity values obtained for the type strains of the three species range between 98.9 and 99.8%, but no signature nucleotides could be identified that would support the specific phylogenetic clustering of these organisms. A slightly lower range of similarity values (97.5–98.0%) for these three type strains has been reported by Wang *et al.* (1996). The data support the finding from a previous 16S rDNA analysis by Tamura & Hatano (1998), who found '*S. benihana*' grouping with members of the genus *Microtetraspora* (species of which were subsequently reclassified as *Nonomuraea* species). Pairwise DNA–DNA reassociation values obtained with the Gilford spectrophotometer for the four organisms ranged between 45 and 10%, those obtained with the Kontron photometer ranged between 48 and 6% and pairwise deviation was lower than 10%. The highest value was found for the pair DSM 43937^T and DSM 43748^T (45 and 48%, respectively), whereas the values determined for '*S. benihana*' DSM 44320 and the three type strains ranged only between 31 and 11%.

'*S. benihana*' showed good growth on all ISP media used. The aerial mycelium developed different colours depending upon the ISP medium used. The colours were described by using the RAL code (Reichsausschuß für Lieferbedingungen – Deutsches Institut

für Gütesicherung und Kennzeichnung e.V.): red/brown (ISP2), beige (ISP3), pink (ISP4 and ISP7), antique rose (ISP5) and white (ISP6). After approximately 14 d, the aerial hyphae developed masses of long spore chains, each comprising up to 30 spores. The spore chains showed some similarities to those of *Herbidospora creata* (Kudo *et al.*, 1993). Sporangia could not be detected. Depending upon the ISP medium, the non-fragmenting substrate mycelium was either brown (ISP2), beige (ISP3), pink (ISP3, ISP7) or rose (ISP5, ISP6). A yellow soluble pigment was produced on ISP medium. The mycelium of *N. roseola* DSM 43767^T developed on any of the ISP media used is salmon/orange (RAL 2012) in colour, while that of *N. recticatena* DSM 43937^T is either colourless, purple/red (RAL 3004 on ISP3) or red/brown (RAL 8012 on ISP7).

Hydrolysates of whole cells contained DL-diaminopimelic acid and the sugars galactose, glucose and ribose; arabinose, xylose and rhamnose were absent and only minor amounts of madurose were detected. This combination of the cell wall diamino acid and sugars represented chemotype III *sensu* Lechevalier & Lechevalier (1970b). The absence of significant quantities of madurose is in contrast to the scenario described by Dietz & Thayer (1980), who used the presence of this sugar in the diagnostic identification of mycelium-forming actinomycetes. However, madurose (3-*O*-methyl D-galactose) can be easily hydrolysed, resulting in the formation of galactose.

Chemotaxonomic data confirm the affiliation of '*S. benihana*' to the family *Streptosporangiaceae*, to which *Nonomuraea* belongs (Collins *et al.*, 1988; Kroppenstedt, 1982; Kudo *et al.*, 1993). The polar lipids are of type PIV, according to the phospholipid classification of Lechevalier *et al.* (1977), and include diphosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, methyl-phosphatidylethanolamine and the diagnostic *N*-acetylglucosamine phospholipid. In addition, some unspecified glycolipids could be found. The principal menaquinones were MK-9(III, VIII H₄) (58%) and MK-9(H₂) (30%). Small amounts of MK-9 (3%) and MK-8(H₄) (9%) could also be found (representing quinone type 4A2, according to Kroppenstedt & Goodfellow, 1991). The qualitative and quantitative composition of the fatty acids placed '*S. benihana*' in a tight cluster with the type strains of *N. roseola*, *N. recticatena* and *N. africana*. The pattern of fatty acid methyl esters mainly comprised iso- and 10-heptadecanoic branched fatty acids. In addition, smaller amounts of unbranched saturated and unsaturated fatty acids and 2-hydroxy fatty acid methyl esters could be found (representing the 3c type of Kroppenstedt, 1985). A diagnostic feature of this fatty acid type is the occurrence of large amounts of 10-methyl 17:0 fatty acids (34%) in combination with 2-hydroxy fatty acids (9%).

'*S. benihana*' DSM 44320 differs from the 16 validly described *Nonomuraea* species not only in the primary

Table 1. Differential physiological characteristics of '*S. benihana*' and type strains of *Nonomuraea* species

Characteristics are scored as: +, positive; –, negative; w, weak; ND, not determined. All organisms utilized D-glucose and were negative in the tests for tryptophan deaminase, indole production and the formation of H₂S (no data available for *Nonomuraea longicatena*). Species: 1, *N. africana* DSM 43748^T; 2, *Nonomuraea angiospora* DSM 43173^T; 3, *Nonomuraea fastidiosa* DSM 43674^T; 4, *Nonomuraea ferruginea* DSM 43553^T; 5, *Nonomuraea flexuosa* DSM 43186^T; 6, *Nonomuraea helvata* DSM 43142^T; 7, *N. longicatena* NRRL 15532^T (data from Chiba *et al.*, 1999); 8, *Nonomuraea polychroma* DSM 43925^T; 9, *Nonomuraea pusilla* DSM 43357^T; 10, *N. recticatena* DSM 43937^T; 11, *N. roseola* DSM 43767^T; 12, *Nonomuraea roseoviolacea* DSM 43144^T; 13, *Nonomuraea rubra* DSM 43768^T; 14, *Nonomuraea salmonea* DSM 43678^T; 15, *Nonomuraea spiralis* DSM 43555^T; 16, *Nonomuraea turkmeniaca* DSM 43926^T.

Characteristic	DSM 44320 ^T	1	2	3	4	5*	6	7	8	9	10	11	12	13	14	15	16
Utilization of:																	
L-Arabinose	w	+	–	–	+	–	–	+	–	–	+	–	+	+	–	–	+
Sucrose	–	+	–	–	+	–	+	ND	–	–	–	+	+	+	+	+	+
D-Xylose	–	+	+	+	+	–	+	ND	–	–	–	–	–	+	–	+	+
m-Inositol	–	+	+	+	–	–	+	–	–	–	+	+	+	+	–	–	+
D-Mannose	–	+	–	–	+	–	–	+	–	–	–	–	+	+	–	+	+
D-Fructose	+	+	–	–	+	–	–	ND	–	–	–	–	+	+	–	–	+
L-Rhamnose	+	+	–	–	+	–	–	–	–	–	+	+	+	+	+	+	+
D-Raffinose	–	+	+	+	–	–	+	+	–	–	–	–	+	+	–	–	+
Cellobiose	–	+	–	–	–	+	–	–	–	–	–	–	–	+	–	–	+
Production of:																	
Gelatinase	+	+	+	+	+	–	+	–	+	+	–	+	+	+	+	–	+
Citrate lyase	+	+	–	+	+	+	+	ND	–	–	–	+	–	+	+	–	+
Urease	+	+	+	+	+	–	+	+	–	+	+	+	+	+	+	–	+
Arginine dihydrolase	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	–	+
β-Galactosidase	+	+	–	+	+	–	+	+	–	–	–	+	–	+	+	+	+
Lysine decarboxylase	–	+	+	+	+	–	+	+	+	–	–	+	+	+	+	–	+
Ornithine decarboxylase	+	+	+	+	+	–	+	+	+	–	–	+	+	+	+	–	–
Acetoin production	+	+	–	+	+	–	+	ND	+	+	+	+	+	+	+	+	+

* Physiological reactions of *N. flexuosa* DSM 43186^T towards the API 20E substrate panel were obtained at 45 °C.

structure of the 16S rDNA but also in the physiological test profile, as determined according to Shirling & Gottlieb (1966) and using the API 20E system (Table 1), the morphology of mycelia and spore surfaces and the colour of substrate mycelium (Chiba *et al.*, 1999). Of the three type strains that are most closely related phylogenetically, the highest similarity to strain DSM 44320 was scored by *N. roseola* DSM 4376^T, which utilized D-glucose, citrate and L-rhamnose but not sucrose, xylose, D-mannose, D-raffinose or cellobiose. Differences were determined in the utilization of sucrose and m-inositol, which gave negative results for strain DSM 44320, and D-fructose, which was utilized by strain DSM 44320. A higher degree of similarity was detected in terms of the activities of enzymes, which were positive for gelatinase, urease, arginine dihydrolase, β-galactosidase and ornithine decarboxylase, and for the production of acetoin and H₂S; the two strains were negative for tryptophan deaminase and lysine decarboxylase and for indole production.

On the basis of phylogenetic, chemotaxonomic and physiological evidence, the taxon '*S. benihana*' should be considered a new species of *Nonomuraea*. Besides

the low DNA–DNA reassociation values, the main arguments supporting this assignment are the differences in some physiological properties, the morphology of the mycelium and spore surfaces, the colour of substrate mycelium, the ability to transform novobiocin and the detoxification of nigericin; the latter two properties have not yet been detected in other strains of *N. roseola* or in other *Nonomuraea* species. The species name used originates from the Japanese word '*benihana*', relating to a three-petalled red flower, as this reflects the appearance of '*S. benihana*' colonies. As this epithet is not specific to an individual species of *Nonomuraea*, we propose the epithet *dietzii* to honour the scientific work of Alma Dietz.

Description of *Nonomuraea dietzii* sp. nov.

Nonomuraea dietzii (diet'zi.i. N.L. gen. n. *dietzii* of Dietz, named in honour of the American microbiologist Alma Dietz).

Gram-positive. The aerial mycelium develops different colours depending upon the ISP medium used: red/brown (ISP2), beige (ISP3), pink (ISP4 and ISP7), antique rose (ISP5) and white (ISP6); after approxi-

mately 14 d, the aerial hyphae develop masses of long spore chains, each containing up to 30 spores. Spore chains may be stalked or may form clusters emerging directly from the agar. Sporangia are not detected. Depending upon the ISP medium used, the non-fragmenting substrate mycelium is brown (ISP2), beige (ISP3), pink (ISP3, ISP 7) or rose (ISP5, ISP6) in colour. DL-Diaminopimelic acid is the diagnostic amino acid of the peptidoglycan. Cell hydrolysates contain galactose, glucose and ribose but not arabinose, xylose or rhamnose; madurose occurs in minor amounts. The phospholipids include diphosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, methyl-phosphatidylethanolamine and *N*-acetylglucosamine phospholipid; some unspecified glycolipids also occur. The principal menaquinones are MK-9(III, VIII H₄) and MK-9(H₂); small amounts of MK-9 and MK-8(H₄) occur. The pattern of fatty acid methyl esters is composed mainly of iso- (iso-C_{14:0} and iso-C_{16:0}) and 10-heptadecanoic (10-methyl-C_{17:0}) branched fatty acids. Smaller amounts of fatty acids are unbranched and saturated (C_{15:0}; C_{17:0}) and unsaturated fatty acids (*cis*9-C_{17:1}) and 2-hydroxy (iso-C_{16:0} 2-OH) fatty acid methyl esters can be found. L-Arabinose, D-fructose, D-glucose, citrate and L-rhamnose are utilized, but not *m*-inositol, sucrose, xylose, D-mannose, D-raffinose or cellobiose. Positive for gelatinase, urease, arginine dihydrolase, β -galactosidase and ornithine decarboxylase activities. Positive for acetoin and H₂S production; negative for tryptophan deaminase, lysine decarboxylase and indole production. Shows no antibacterial effect on *Micrococcus luteus*, *Streptococcus murinus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Aspergillus niger* or *Candida albicans*. The isolation source is not recorded. The type strain is DSM 44320^T (= NRRL 11111^T).

References

- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- Chiba, S., Suzuki, M. & Ando, K. (1999). Taxonomic re-evaluation of '*Nocardopsis*' sp. K-252^T (= NRRL 15532^T): a proposal to transfer this strain to the genus *Nonomuraea* as *Nonomuraea longicatena* sp. nov. *Int J Syst Bacteriol* **49**, 1623–1630.
- Collins, M. D., Kroppenstedt, R. M., Tamaoka, J., Komagata, K. & Kinoshita, T. (1988). Structures of the tetrahydrogenated menaquinones *Faenia rectivirgula* and *Saccharothrix australiensis*. *Curr Microbiol* **17**, 275–279.
- Delort, A. M., Jeminet, G., Sancelme, M. & Dauphin, G. (1988). Microbial conversion of nigericin in three successive steps, by *Sebekia benihana*. *J Antibiot* **41**, 916–924.
- Dietz, A. & Li, G. P. (1982). *Sebekia*, a new genus of the family *Actinoplanaceae*. In *Abstracts of the Annual Meeting of the American Society for Microbiology*, abstract I63. Washington, DC: American Society for Microbiology.
- Dietz, A. & Thayer, D. W. (1980). *Actinomycete Taxonomy*. SIM Special Publication no. 6. Los Angeles, CA: Society for Industrial Microbiology.
- 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.
- HuB, 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.
- Kroppenstedt, R. M. (1982). Separation of bacterial menaquinones by HPLC using reverse phase (RP-18) and a silver loaded ion exchanger. *J Liquid Chromatogr* **5**, 2359–2367.
- Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics*, pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Kroppenstedt, R. M. & Goodfellow, M. (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.
- Kroppenstedt, R. M., Korn-Wendisch, F., Fowler, V. J. & Stackebrandt, E. (1981). Biochemical and molecular genetic evidence for a transfer of *Actinoplanes armeniacus* into the family *Streptomycetaceae*. *Zentbl Bakteriol Hyg 1 Abt Orig C* **2**, 254–262.
- Kudo, T., Itoh, T., Miyadoh, S., Shomura, T. & Seino, A. (1993). *Herbidospira* gen. nov., a new genus of the family *Streptosporangiaceae* Goodfellow et al. 1990. *Int J Syst Bacteriol* **43**, 319–328.
- Lechevalier, M. P. & Lechevalier, H. A. (1970a). 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, H. A. & Lechevalier, M. P. (1970b). A critical evaluation of the genera of aerobic actinomycetes. In *The Actinomycetales*, pp. 393–405. Edited by H. Prauser. Jena: Gustav Fischer.
- Lechevalier, M. P. & Lechevalier, H. (1970c). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435–443.
- Lechevalier, M. P., de Bièvre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Ecol Syst* **5**, 249–260.
- Lechevalier, M. P., Stern, A. E. & Lechevalier, H. A. (1981). Phospholipids in the taxonomy of actinomycetes. In *Actinomycetes*, pp. 111–116. Edited by K. P. Schaal & G. Pulverer. Stuttgart: Gustav Fischer.
- Miller, L. & Berger, T. (1984). *Bacterial Identification by Gas Chromatography and Whole Cell Fatty Acids*. Gas Chromatography Application Note 228–41. Palo Alto, CA: Hewlett-Packard.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M. & Parlett, J. H. (1984). An integrated procedure for extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Sebek, O. K. & Dolak, L. A. (1977). Further studies on the

biomodification of novobiocin. In *Abstracts of the Annual Meeting of the American Society for Microbiology*, abstract O26. Washington, DC: American Society for Microbiology.

Sebek, O. K. & Dolak, L. A. (1984). Microbial hydroxylation of novobiocin and related compounds. *J Antibiot* **37**, 136–142.

Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.

Stanek, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin layer chromatography. *Appl Microbiol* **28**, 226–231.

Tamura, T. & Hatano, K. (1998). Phylogenetic analyses on the

strains belonging to invalidated genera of the order *Actinomycetales*. *Actinomycetologica* **12**, 15–28.

Uchida, K. & Aida, K. (1977). Acyl type of bacterial cell wall: its simple identification by colorimetric method. *J Gen Appl Microbiol* **23**, 249–260.

Wang, Y., Zhang, Z. & Ruan, J. (1996). Phylogenetic analysis reveals new relationships among members of the genera *Microtetraspora* and *Microbispora*. *Int J Syst Bacteriol* **46**, 658–663.

Zippel, M. & Neigenfind, M. (1988). Preservation of streptomycetes. *J Gen Appl Microbiol* **34**, 7–14.