Taxonomic re-evaluation of '*Nocardiosis*' sp. K-252^T (= NRRL 15532^T): a proposal to transfer this strain to the genus *Nonomuraea* as *Nonomuraea longicatena* sp. nov.

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Re-evaluation of the taxonomic position of strain K-252^T, which produces the compound K-252a, showed that the strain does not belong to the genus *Nocardiosis* suggested previously. Strain K-252^T formed aerial mycelia with long spore chains, and hyphal fragmentation was not observed. The cell wall chemotype of the strain was III/B, the major menaquinone was MK-9 (III, VIII-H₂), the phospholipid pattern was PIV and the major cellular fatty acids were 10Me-C_{17:0}, iso-C_{16:0} and C_{16:0}. Phylogenetic analysis of the 16S rRNA sequence showed that strain K-252^T was clustered in the *Nonomuraea* group. Furthermore, on the basis of DNA–DNA reassociation and phenotypic data, strain K-252^T (= NRRL 15532^T) was classified as a new species of the genus *Nonomuraea*. This strain is proposed as *Nonomuraea longicatena* sp. nov.

Keywords: *Nonomuraea longicatena* sp. nov., *Nocardiosis*, K-252a, protein kinase C inhibitor

INTRODUCTION

Until the mid-1980s, the taxonomic handling of spore chain-forming, non-motile, wall chemotype III actinomycetes was not consistent. Because of a deficiency of appropriate data sets, the taxonomic positions of many species belonging to the genera *Actinomadura*, *Microtetrastroma*, '*Micropolyspora*' or *Nocardiosis* were mixed up; as a result, they were assigned temporary status (Cross & Goodfellow, 1973; Galatenko *et al.*, 1981; Gause *et al.*, 1974, 1984; Lavrova & Preobrazhenskaya, 1975; Lechevalier & Lechevalier, 1970a; Meyer, 1979; Meyer & Sveshnikova, 1974; Nonomura & Ohara, 1971a, b; Preobrazhenskaya *et al.*, 1975, 1976, 1982; Preobrazhenskaya & Sveshnikova, 1974; Soina *et al.*, 1975; Terekhova *et al.*, 1982; Zhukova *et al.*, 1968). The current taxonomic view on these species is based on numerical taxonomy (Athalye *et al.*, 1985; Grund & Kroppenstedt, 1989, 1990), chemotaxonomic studies (especially on lipids; Athalye *et al.*, 1984; Fischer *et al.*, 1983; Grund & Kroppenstedt, 1989, 1990; Kroppenstedt *et al.*,

1990; Miyadoh *et al.*, 1989; Poschner *et al.*, 1985) and phylogenetic approaches including DNA–rRNA cistron similarity (Fischer *et al.*, 1983), the analysis of ribosomal proteins (Ochi, 1992; Ochi *et al.*, 1991), 16S rRNA cataloguing (Fowler *et al.*, 1985) and the study of 16S rRNA gene sequences (Wang *et al.*, 1996; Rainey *et al.*, 1996; Zhang *et al.*, 1998). The descriptions of the genera *Actinomadura* and *Microtetrastroma* were emended as a result of the accumulation of such taxonomic data (Kroppenstedt *et al.*, 1990; Zhang *et al.*, 1998): the new genera *Saccharothrix* (Labeda *et al.*, 1984) and *Nonomuria* (Zhang *et al.*, 1998) were proposed. [The name '*Nonomuria*', proposed as a new genus by Zhang *et al.* (1998), would be a misnomer according to the International Code of Nomenclature of Bacteria (Rule 57a, Appendix 9); the name *Nonomuraea* should be proposed as the correct spelling.]

In 1983, K-252a, an inhibitor of protein kinase C, was discovered in culture broth from an actinomycete (Matsuda *et al.*, 1985; Kase *et al.*, 1986). The producing strain, K252^T, showed fragmented substrate mycelia and had well-developed spore chains on whole aerial mycelia. It possessed a chemotype III/C cell wall (Lechevalier & Lechevalier, 1970b); *meso*-diaminopimelic acid was present but diagnostic sugars such as

The GenBank/EMBL/DBJ accession number for the almost complete 16S rDNA sequence (1499 nucleotides) of strain K-252^T is AB018787.

arabinose, xylose and madurose were not detected in whole-cell hydrolysate. This strain was considered to belong to the genus *Nocardiopsis* Meyer 1976 (Meyer, 1976). Strain K-252^T was deposited in the Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, US Department of Agriculture (as NRRL 15532^T).

In this paper, the taxonomic position of strain K-252^T is re-evaluated using the current taxonomic view and we propose a new species of the genus *Nonomuraea* for this strain.

METHODS

Organisms and culture conditions. The organisms used in this study are listed in Table 1. Strain K-252^T was isolated from a soil sample collected in Tokyo, Japan. For activation and biomass preparations, all strains except *Nonomuraea flexuosa* IFO14349 were cultured in shaking flasks of SR-3 broth [composed of (% v/v) 1% glucose, 1% soluble starch, 0.3% beef extract, 0.5% yeast extract, 0.5% Bacto tryptone (Difco), 0.2% CaCO₃ (pH 7.2 before sterilization)] at 28 °C for 3–7 d. *N. flexuosa* IFO14349 was cultured at 45 °C in the same manner for 5 d.

Cultural and morphological properties. For cultural and morphological characterization of strain K-252^T, the methods of Shirling & Gottlieb (1966) were employed. The morphological structures were observed at high magnification using a light microscope (Biophoto model; Nikon). Additional morphological data were obtained using scanning electron microscopy (model S-570; Hitachi) on dehydrated, gold/platinum-coated specimens of the strain.

Biochemical and physiological characteristics. Strain K-252^T was examined for the ability to degrade casein (1%, w/v), elastin (0.3%, w/v), aesculin (0.1%, w/v), gelatin (12%, w/v), hypoxanthine (0.5%, w/v), testosterone (1.0%, w/v), tyrosine (0.5%, w/v) and xanthine (0.4%, w/v). NaCl tolerance was assessed using nutrient agar (peptone, 5 g; beef extract, 3 g; agar, 15 g; distilled water, 1000 ml; pH 7.0) as the basal medium. DNA- and starch degradation were determined on Bacto DNase Test agar (Difco) and inorganic/starch agar by flooding with 1 M HCl and iodine solution, respectively. Utilization of carbon sources was tested by using modified Pridham & Gottlieb medium (Shirling & Gottlieb, 1966). Each carbon source was added to the basal medium to give a final concentration of 1% (w/v).

Chemotaxonomic properties. The conformation of diaminopimelic acid in the cell wall was determined according to the method of Hasegawa *et al.* (1983). For analysis of whole-cell sugar, freeze-dried biomass (100 mg) was hydrolysed with 2 ml 0.5 M H₂SO₄ at 100 °C for 4 h in a screw-capped test tube and the hydrolysate neutralized by saturated Ba(OH)₂ solution. Precipitated BaSO₄ was removed by centrifugation and the supernatant concentrated by freeze-drying. The freeze-dried sample was rehydrated in 100 µl distilled H₂O and analysed by the method of Yokota & Hasegawa (1988) using HPLC. Menaquinones were extracted from lyophilized biomass (200 mg) by shaking in 20 ml chloroform/methanol (2:1, v/v) overnight in the dark followed by purification with silica-gel TLC (silica gel 60 F254, 2020 cm; Merck) using a solvent system of hexane/diethylether (85:15, v/v). The purified menaquinone was analysed by HPLC with a Nova-Pak C-18 column (3.9150 mm; Waters), eluted with methanol-2-propanol (11:4, v/v) at 1 ml min⁻¹ at 35 °C. The components were detected with a UV spectrophotometric detector (model SPD-6A; Shimadzu) at

Table 1. Strains used in this study and DNA–DNA homologies between strain K-252^T and *Nonomuraea* spp.

Organism	Source/strain no.	Homology with DNA of strain K-252 ^T (%)
Strain K-252 ^T (= NRRL 15532 ^T)	Soil	100
<i>Nonomuraea africana</i>	JCM 6240 ^T	29
<i>Nonomuraea angiospora</i>	KCC-A-0109 ^T	24
<i>Nonomuraea fastidiosa</i>	JCM 3321 ^T	27
<i>Nonomuraea ferruginea</i>	KCC-A-0283 ^T	10
<i>Nonomuraea flexuosa</i>	IFO 14349 ^T	16
<i>Nonomuraea helvata</i>	A-105 ^T (Nonomura)	16
<i>Nonomuraea polychroma</i>	IFO 14345 ^T	24
<i>Nonomuraea pusilla</i>	A-118 ^T (Nonomura)	21
<i>Nonomuraea recticatena</i>	IFO 14525 ^T	25
<i>Nonomuraea roseola</i>	JCM 3323 ^T	30
<i>Nonomuraea roseoviolacea</i>	A-5 ^T (Nonomura)	22
<i>Nonomuraea rubra</i>	DSM 43768 ^T	14
<i>Nonomuraea salmona</i>	JCM 3324 ^T	37
<i>Nonomuraea spiralis</i>	KCC-A-0286 ^T	23
<i>Nonomuraea turkmeniaca</i>	IFO 14348 ^T	16
<i>Microtetraspora fusca</i>	DSM 43841 ^T	
<i>Microtetraspora glauca</i>	DSM 43311 ^T	
<i>Microtetraspora niveoalba</i>	KCC-A-0149 ^T	

270 nm. The identity of each component was determined by comparing retention times with those for standard menaquinones extracted from strains whose quinone profiles are well characterized (Kroppenstedt *et al.*, 1990; Kudo *et al.*, 1993). Phospholipids were extracted and purified by the method of Yassin *et al.* (1993) and analysed by the method of Lechevalier & Lechevalier (1980), with TLC (aluminium sheet silica gel 60 F254, precorted, 10 × 10 cm; Merck). Fatty acids were extracted from lyophilized biomass (100 mg) as the methyl ester form with 5% methanolic hydrochloric acid solution at 100 °C for 3 h and analysed by GC/MS (model QP-5000; Shimadzu) with a DB-1 capillary column (0.25 mm × 60 m, 0.25 µm film thickness; J&W Scientific).

Extraction of DNA. Biomass at mid-exponential phase cultivated in SR-3 broth was supplemented with glycine and mannitol to final concentrations of 1% (w/v) and 5% (w/v), respectively. After further cultivation, cells of late log to early stationary phase were harvested, suspended in 5 ml 10 mM EDTA/150 mM NaCl (pH 8.5), incubated at 80 °C for 30 min and the suspensions repeatedly frozen and melted. After treatment with lysozyme (incubated overnight at 37 °C), proteinase K (37 °C for 30 min) and achromopeptidase (55 °C for 30 min), 500 µl 20% SDS/0.25 M NaOH solution was added and the suspensions were then incubated at 70 °C for 30 min. An equal volume of phenol solution [equilibrated with 1 M Tris (pH 8) and containing 0.1% (w/v) 8-hydroxyquinoline] was added; this was followed by vigorous shaking and then centrifugation (10000 r.p.m., 10 min). The supernatant was removed carefully and the precipitated DNA was then washed with ice-cold ethanol and dried. Further purification steps, including RNase treatment, were carried out according to the method of Saito & Miura (1963).

PCR amplification and sequencing of the 16S rRNA gene. PCR amplification of the 16S rRNA gene (16S rDNA) from strain K-252^T was performed with primers Pf (5'-AGAGT-TTGATCCTGGCTCAG-3', positions 7–26) and Pr (5'-AAGGAGGTGATCCAGCCGCAC-3', positions 1525–1505). The numbers of primer regions in parentheses correspond to those for 16S rRNA in *Streptomyces ambofaciens*. The amplified 16S rDNA fragment was purified by electrophoresis and electroelution from the gel. The sequencing reaction was performed using the *Taq* DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems). The sequencing results were analysed with an ABI 377 DNA sequencer (Perkin-Elmer) on 48 cm, 5% polyacrylamide gels. The following sequencing primers were used: B1m (5'-CTCCTACGGGAGGCAGCAGT-3', positions 331–350), B3R (5'-TATTACCGCGGCTGCTGGCA-3', positions 507–488), B4m (5'-GTGTAG(AC)(AG)GTGAAAT(GT)-CGTAGA-3', positions 655–676), B5R (5'-CCCCGTCAA-TTCATTTGAGT-3', positions 902–883), B6m (5'-ACAGGTGTCATGGCTGTCG-3', positions 1016–1036) and BR2 (5'-TACGGTTACCTTGTTACGACTT-3', positions 1497–1476).

Phylogenetic analysis. The nucleotide sequences of strain K-252^T and reference strains were aligned by using the CLUSTAL W program (Higgins *et al.*, 1991). The gaps were eliminated manually. The similarity values and the evolutionary distances were calculated by using PAUP (Swofford & Begle, 1993) and the PHYLIP package (Felsenstein, 1993), respectively. A phylogenetic tree was constructed by using the neighbour-joining method of Saitou & Nei (1987).

Nucleotide sequence accession numbers. The accession

numbers of the nucleotide sequences of reference strains used in this study are as follows: U48842 (*Nonomuraea africana* = '*Microtetraspora africana*'), U48843 (*Nonomuraea angiospora* = '*Microtetraspora angiospora*'), U48844 (*Nonomuraea fastidiosa* = '*Microtetraspora fastidiosa*'), U48845 (*Nonomuraea ferruginea* = '*Microtetraspora ferruginea*'), U48975 (*Nonomuraea helvata* = '*Microtetraspora helvata*'), U48977 (*Nonomuraea polychroma* = '*Microtetraspora polychroma*'), U48978 (*Nonomuraea pusilla* = '*Microtetraspora pusilla*'), U48979 (*Nonomuraea recticatena* = '*Microtetraspora recticatena*'), U48980 (*Nonomuraea roseola* = '*Microtetraspora roseola*'), U48982 (*Nonomuraea salmonea* = '*Microtetraspora salmonea*'), U48983 (*Nonomuraea spiralis* = '*Microtetraspora spiralis*'), U48973 (*Microtetraspora fusca*), U48974 (*Microtetraspora glauca*), U48976 (*Microtetraspora niveoalba*), U48984 (*Microbispora aerata*), U48990 (*Microbispora diastatica*), U48985 (*Microbispora parva*), D86936 (*Microbispora rosea*), U48986 (*Microbispora thermodiaestatica*), U48987 (*Microbispora thermorosea*), D85495 (*Planomonospora parontospora*), U58523 (*Thermobispora bispora*), X97886 (*Nocardopsis dassonvillei*), X97887 (*Nocardopsis listeri*), M27245 (*Streptomyces ambofaciens*), X89944 (*Streptosporangium longisporum*) and X89947 (*Streptosporangium roseum*).

DNA base composition. The G + C content of the DNA was determined according to the method of Tamaoka & Komagata (1984), using HPLC.

DNA-DNA hybridization. Levels of DNA relatedness among strain K-252^T and related organisms were determined with a method modified from that of Ezaki *et al.* (1989), using peroxidase/[2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) as the enzyme/chromogen system instead of the β-galactosidase/(4-methylumbelliferyl-β-galactosidase) system. After overnight hybridization, microtitre plates (MaxiSorp; Nalge Nunc International) were washed with 0.01 × SSC (1 × SSC is 0.15 M NaCl/0.015 M sodium citrate). A 100 µl portion of a streptavidin-horseradish peroxidase conjugate solution [10 µl streptavidin-horseradish peroxidase conjugate (Gibco-BRL) plus 200 µl BSA in 10 ml PBS buffer (PBS; 8 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 137 mM NaCl, 2.7 mM KCl, pH 7.2)] was added to the wells; the microtitre plates were then incubated at 37 °C for 30 min. After incubation, the microtitre plates were washed with 0.01 × SSC. A 100 µl volume of a chromogen solution (0.03% H₂O₂ in 2 mM ABTS/2% sodium citrate, pH 4.2) was added to the wells; the microtitre plates were then incubated at 37 °C for 30–60 min. The enzyme reaction was stopped by adding 10 µl 20% SDS solution. The intensity of the green colour was measured at 415 nm with a microplate reader (Benchmark model; Bio-Rad).

RESULTS

Morphology

The vegetative hyphae of strain K-252^T were well developed and branched. The mycelia were cream to ochre in colour on various agar media. Hyphal fragmentation was not observed. Aerial mycelia were formed on agar media and spore chains developed at the greater part of the mycelia. Spore chains were straight, curved or irregularly spiral with rows of 10–30 spores. Spores were ovoid to cylindrical (0.4–0.8

Fig. 1. Light micrograph of strain K-252^T cultured on yeast extract/malt extract agar (ISP no. 2) at 28 °C for 2 weeks. Bar, 10 µm.

Fig. 2. Scanning electron micrograph of strain K-252^T cultured on yeast extract/malt extract agar (ISP no. 2) at 28 °C for 2 weeks. Bar, 2 µm.

× 0.7–1.5 µm), smooth-surfaced and non-motile (Figs 1 and 2). The formation of sporangia-like structures or synnema was not observed.

Chemotaxonomic properties

The diaminopimelic acid in whole-cell hydrolysates of strain K-252^T was in the *meso* configuration and the diagnostic sugar madurose was detected in whole-cell hydrolysates. These data suggested that strain K-252^T belongs to the wall chemotype III/B group. The main menaquinone was MK-9 (III, VIII-H₄), with MK-9(H₂) and MK-9(H₆) as minor components. The phospholipids diphosphatidylglycerol, phosphatidylethanolamine and hydroxylated phosphatidylethanolamine were present, as were uncharacterized ninhydrin-positive glycolipids. The phospholipid pattern of strain K-252^T corresponds to phospholipid pattern IV (Lechevalier *et al.*, 1981). The largest proportion of the cellular fatty acids comprised 14-methylpentadecanoic acid (iso-C_{16:0}) and 10-methylheptadecanoic acid (10Me-C_{17:0}). The G + C content of the DNA was 71.7 mol %.

Phylogenetic analysis

In phylogenetic analysis, using 16S rDNA sequences of 11 *Nonomuraea* species and related actinomycetes, strain K-252^T was placed in the cluster composed of *Nonomuraea* spp. with bootstrap values of 804/1000 (Fig. 3). Although 15 species of this genus had been validly published, a sequence identical to that of strain K-252^T had not been found. The sequences of *Nonomuraea* spp., including strain K-252^T, share a high level of similarity ranging from 96.9 to 99.4 %. Strain K-252^T appeared to be more closely related to *Nonomuraea* spp. than to other closely related actinomycetes, sharing a sequence similarity ranging from 96.6 to 97.1 % with the former and less than 95.9 % with latter, respectively (Table 2).

DNA–DNA hybridization

The DNA of *Nonomuraea* sp. K-252^T exhibited homology values of 10–37 % with DNAs from type strains of 15 *Nonomuraea* species (Table 1).

DISCUSSION

In 1983, strain K-252^T, the producer of K-252a, was discovered and identified as *Nocardiopsis* sp. on the basis of its morphological and wall chemotype properties. At that time, *Nocardiopsis* was the only long spore-chain-forming, 'fragmenting', non-motile, wall chemotype III, whole-cell sugar pattern 'C' actinomycete genus. However, because of the accumulation of chemotaxonomic and phylogenetic data on wall chemotype III actinomycete genera, their taxonomic status been changing profoundly. Techniques of chemotaxonomic analysis have also progressed. On the basis of the results in this study and the current taxonomic view, we have concluded that strain K-252^T belongs to the genus *Nonomuraea*.

Strain K-252^T is distinguishable from other validly

Fig. 3. Neighbour-joining tree based on 16S rRNA gene sequences (1348 bases) showing relationships among strain K-252^T, *Nonomuraea* spp. and representative species of related taxa. The numbers on branches that divide major clusters indicate bootstrap values of 1000 sets of resampling. The accession numbers of sequences used in this study are listed in Methods. T, Type strain. Bar, 0.01 substitutions per nucleotide position.

Table 2. Percentage similarity of the 16S rRNA sequence (1348 bases)

Strain	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1 Strain K-252 ^T	96.7	97.1	96.7	96.7	97.1	97.0	96.6	96.7	96.6	97.3	96.8	95.2	95.0	94.7	95.5	95.4	95.7	95.6	95.4	95.9	91.0	95.6	95.5	96.1	92.4	91.8	90.8
2 <i>Nonomuraea africana</i>		97.7	97.6	97.4	98.4	97.9	97.0	99.0	99.0	97.8	97.5	96.4	96.4	96.0	96.1	96.2	96.3	95.6	95.8	95.6	91.4	97.0	96.7	96.3	92.7	92.4	91.5
3 <i>Nonomuraea angiospora</i>			98.4	97.6	98.8	98.1	98.4	97.8	97.8	98.7	98.5	96.7	96.7	96.2	96.2	97.0	96.4	95.8	96.1	96.4	91.2	96.0	95.8	96.4	93.0	92.2	91.2
4 <i>Nonomuraea fastidiosa</i>				97.5	98.7	98.2	97.8	97.3	97.3	98.3	98.0	96.5	96.7	96.2	96.7	96.7	96.4	95.6	96.5	96.4	91.2	95.8	95.5	96.2	92.1	92.2	90.9
5 <i>Nonomuraea ferruginea</i>					97.9	97.9	97.6	97.3	97.3	98.0	97.8	96.8	96.7	96.5	96.3	96.2	96.1	95.8	96.1	95.4	91.2	96.4	96.5	96.3	92.4	92.1	91.1
6 <i>Nonomuraea helvata</i>						99.0	98.2	98.1	98.1	98.6	98.6	97.3	97.3	96.9	97.0	96.9	96.9	96.2	96.8	96.6	91.9	96.1	96.0	96.7	92.2	92.1	91.1
7 <i>Nonomuraea polychroma</i>							97.7	97.7	97.6	98.1	97.9	97.0	96.7	96.5	96.8	96.5	96.6	95.9	96.5	95.9	91.4	95.9	96.1	96.1	92.2	91.8	90.7
8 <i>Nonomuraea pusilla</i>								96.9	96.9	98.1	97.8	97.3	97.4	97.0	96.2	97.0	96.1	95.5	96.1	96.7	91.4	96.1	95.8	96.7	91.8	91.8	91.3
9 <i>Nonomuraea recticatena</i>									99.4	97.6	97.2	96.3	96.3	95.8	95.9	96.1	96.1	95.5	95.6	95.4	91.0	97.0	96.8	96.2	92.7	92.4	91.6
10 <i>Nonomuraea roseola</i>										97.6	97.2	96.3	96.3	95.8	95.9	96.1	96.1	95.5	95.6	95.4	91.0	97.0	96.8	96.2	92.8	92.6	91.5
11 <i>Nonomuraea salmonea</i>											98.7	96.4	96.4	96.0	96.1	96.8	96.1	95.5	95.8	96.2	91.1	96.0	95.6	96.3	91.9	92.2	91.2
12 <i>Nonomuraea spiralis</i>												96.1	96.3	95.8	95.7	96.4	95.8	95.4	95.5	95.9	90.9	95.8	95.4	96.3	91.8	92.1	91.2
13 <i>Microtetraspora fusca</i>													99.6	99.4	98.0	98.1	97.9	97.5	97.9	97.3	92.2	95.8	95.9	96.0	91.3	91.0	91.9
14 <i>Microtetraspora glauca</i>														99.3	97.8	98.1	97.6	97.3	97.6	97.4	92.0	95.8	95.7	95.8	91.0	91.0	91.8
15 <i>Microtetraspora niveoalba</i>															97.6	97.8	97.6	97.2	97.6	97.2	92.0	95.5	95.5	95.5	90.9	90.9	91.5
16 <i>Microbispora aerata</i>																98.2	99.1	98.3	99.7	98.7	92.4	95.5	95.5	95.8	91.7	91.2	90.8
17 <i>Microbispora diastatica</i>																	98.4	97.9	98.1	98.6	91.9	95.7	95.6	95.9	91.5	91.8	91.5
18 <i>Microbispora parva</i>																		98.9	99.1	98.2	92.1	95.4	95.5	95.4	91.8	91.4	91.1
19 <i>Microbispora rosea</i>																			98.3	97.6	91.6	95.8	95.8	95.6	92.0	91.5	91.3
20 <i>Microbispora thermodiastatica</i>																				98.7	92.5	95.3	95.3	95.6	91.6	91.2	90.8
21 <i>Microbispora thermorosea</i>																					92.0	95.4	94.7	96.0	91.4	91.5	91.2
22 <i>Thermobispora bispora</i>																						90.3	90.8	90.8	88.9	88.4	88.2
23 <i>Streptosporangium longisporum</i>																							98.7	97.5	92.8	92.9	92.4
24 <i>Streptosporangium roseum</i>																								97.3	93.2	92.5	92.2
25 <i>Planomonospora parontospora</i>																									92.5	92.4	92.4
26 <i>Nocardioopsis dassonvillei</i>																										98.2	89.2
27 <i>Nocardioopsis listeri</i>																											89.3
28 <i>Streptomyces ambifaciens</i>																											

Table 3. Differential characteristics of strain K-252^T and *Nonomuraea* spp.

Data for organisms other than strain K-252^T and details of spore-chain morphologies of *Nonomuraea* spp. are taken from *Bergey's Manual of Determinative Bacteriology*, 9th edn and from Tomita *et al.* (1991). Species: 1, *N. africana*; 2, *N. angiospora*; 3, *N. fastidiosa*; 4, *N. ferruginea*; 5, *N. flexosa*; 6, *N. helvata*; 7, *N. polychroma*; 8, *N. pusilla*; 9, *N. recticatena*; 10, *N. roseola*; 11, *N. roseoviolacea*; 12, *N. rubra*; 13, *N. salmonea*; 14, *N. spiralis*; 15, *N. turkmeniaca*; 16, *Microtetrastora tyrhenii*. +, Positive; –, negative; w, weak; ND, not determined; ps, pseudosporangia.

Characteristic	K-252 ^T	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
No. of spores/sporophores	10–30	4–10	4–15	4–10	4–10	4–10	4–10	4–10	>10(ps)	4–20	6–20	4–20(ps)	4–20	4–30	4–20	10–20	5–15
Growth on ISP medium 3																	
Aerial mycelium	White	Grey/blue	White	White/pink	White/pink	White/yellow	'Trace, white'	Trace	White/cream	White/cream	Pink	Pink/violet	Trace	Pink	White/yellow	Trace	White
Substrate mycelium	Ochre	Yellow	White/ochre	Colourless	Pink	Brown	Yellow/brown	'Colourless, brown'	Grey/brown	Dark yellow/brown	Brown/red	Violet	'Orange, red'	Red	Yellow/brown	Violet/red	Light yellow
Soluble pigment	–	Yellow/brown	–	–	–	–	–	–	–	–	–	Violet	Red	–	–	Pink/violet	–
Decomposition of:																	
Casein	+	ND	+	+	+	+	–	–	–	ND	–	–	–	+	–	+	+
DNA	–	ND	+	+	+	+	–	–	+	ND	–	+	–	+	+	–	ND
Elastin	+	ND	+	ND	+	ND	ND	ND	–	ND	ND	–	+	+	ND	ND	ND
Aesculin	w	ND	+	+	–	+	+	+	+	ND	+	+	–	+	+	+	+
Gelatin	–	ND	+	+	–	+	–	+	+	ND	+	+	+	+	–	ND	–
Hypoxanthine	+	ND	+	+	+	–	–	+	+	ND	+	+	+	+	–	+	+
Starch	+	ND	–	–	+	+	–	–	–	ND	–	–	+	–	–	–	+
Testosterone	w	ND	+	–	+	+	+	–	+	ND	+	–	+	+	+	–	ND
Tyrosine	–	ND	+	–	+	+	–	–	+	ND	+	–	+	+	+	–	+
Xanthine	–	ND	–	–	–	–	–	–	–	ND	–	–	–	–	–	–	–
Nitrate reduction	–	ND	–	+	+	+	+	–	+	ND	+	+	+	+	+	–	+

published *Nonomuraea* spp. not only by DNA re-association but also by phenotypic properties (Table 3). Of the type strains of the genus *Nonomuraea* and related actinomycetes, *Microtetrastora tyrhenii* was unobtainable. Although the taxonomic status of *M. tyrhenii* was not mentioned in the study by Zhang *et al.* (1998), it is likely that this species belongs to the genus *Nonomuraea* because of its morphological properties (Tomita *et al.*, 1991). Despite the lack of DNA relatedness data, strain K-252^T is also phenotypically distinguishable from *M. tyrhenii*. Therefore it is proposed that strain K-252^T is classified as a new species of the genus *Nonomuraea*, namely *Nonomuraea longicatena* sp. nov.

Description of *Nonomuraea longicatena* sp. nov. Chiba, Suzuki and Ando

Nonomuraea longicatena (lon.gi.ca.te'na. L. adj. *longi*-long; L. n. *catena* chain; *longicatena* long chain, referring to long spore chains).

Spore chains straight, curved, or irregular spiral with rows of 10–30 spores on aerial hyphae. Spores ovoid to cylindrical with a smooth surface. Oatmeal agar (ISP no. 3): poor growth, white aerial mycelium, colourless to ochre substrate mycelium, no diffusible pigment. Yeast extract/malt extract agar (ISP no. 2): abundant growth, white aerial mycelium, ochre substrate mycelium, pale yellow diffusible pigment. Inorganic salt/starch agar (ISP no. 4): poor to moderate growth, white aerial mycelium, parchment substrate mycelium, no diffusible pigment. D-Glucose, D-ribose, L-rhamnose, trehalose, adonitol, glycerol and inositol are utilized as sole carbon sources but D-arabinose, L-

arabinose, D-xylose, cellobiose, galactose, inulin, lactose, maltose, raffinose, salicin, sucrose, dulcitol and mannitol are not. Casein, elastin, hypoxanthin and tyrosine are degraded but DNA, gelatin and xanthine are not. Grows in the presence of up to 3 % NaCl. No growth at 45 °C. The G+C content of the DNA is 71.7 mol %. The type strain is NRRL 15532^T.

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