Proposal to transfer *Actinomadura carminata* to a new subspecies of the genus *Nonomuraea* as *Nonomuraea roseoviolacea* subsp. *carminata* comb. nov.

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An anthracycline-producing actinomycete (strain SF2303) was compared with two other anthracycline producers, *Actinomadura carminata* IFO 15903^T and *Nonomuraea roseoviolacea* IFO 14098^T, using morphological, physiological, chemotaxonomic and molecular-genetic criteria. The morphological and cultural characteristics of these three strains are similar. The physiological properties of strain SF2303 and *N. roseoviolacea* IFO 14098^T are very similar, but are different from those of *A. carminata* IFO 15903^T in the utilization of some sugars and the reduction of nitrate. Their chemotaxonomic properties [cell wall chemotype, IIIB; major menaquinone, MK-9 (III, VIII-H₄); phospholipid type, PIV; cellular fatty acids 10M-17:0/17:1 and iso-16:0 as major components and 2-hydroxy fatty acids as minor components; mycolic acid, absent] were identical and indicated that these three strains belong to the family *Streptosporangiaceae*. On the basis of 16S rDNA sequences and phylogenetic analysis, they were all included in the cluster formed by species of *Nonomuraea*. The levels of DNA relatedness between strain SF2303 and *N. roseoviolacea* IFO 14098^T ranged from 71 to 78%; however, the levels of relatedness between the two strains and *A. carminata* IFO 15903^T were lower (49–60%). Therefore, strain SF2303 was identified as a member of the species *N. roseoviolacea* and it is proposed that *Actinomadura carminata* Gauze *et al.* 1973 should be transferred to a new subspecies of the genus *Nonomuraea* Zhang *et al.* 1998 as *N. roseoviolacea* subsp. *carminata* comb. nov.

Keywords: *Actinomadura carminata*, family *Streptosporangiaceae*, anthracycline-producing actinomycetes

INTRODUCTION

In our screening programme for new anti-tumour antibiotics, we isolated an anthracycline-producing actinomycete, strain SF2303. For identification, it was compared with two anthracycline-producing organisms, *Actinomadura carminata* IFO 15903^T and *Nonomuraea roseoviolacea* IFO 14098^T. Morphological, physiological, chemotaxonomic (including the HPLC patterns of secondary metabolites) and

molecular-genetic (16S rDNA sequence analysis and DNA–DNA hybridization) criteria were used.

Taxonomic reviews of the genera of wall-chemotype-III sporoactinomycetes such as *Actinomadura*, *Microbispora*, *Microtetraspora*, *Nocardiopsis* and *Nonomuraea* have been carried out since the 1980s (Fischer *et al.*, 1983; Athalye *et al.*, 1984; Poschner *et al.*, 1985; Miyadoh *et al.*, 1989, 1990; Grund & Kroppenstedt, 1989). Kroppenstedt *et al.* (1990) revised the genera *Actinomadura* and *Microtetraspora* by using chemotaxonomic and molecular-genetic techniques and proposed to transfer the *Actinomadura pusilla* group to the emended genus *Microtetraspora*. However, they did not include the type strain of *A. carminata* in their study. Kroppenstedt & Goodfellow (1992) and

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences described in this work are AB039960 (strain SF2303), AB039961 (*Actinomadura carminata* IFO 15903^T) and AB039959 (*Nonomuraea roseoviolacea* IFO 14098^T).

Goodfellow (1992) comprehensively reviewed the taxonomy of the families *Thermomonosporaceae* and *Streptosporangiaceae*. Miyadoh *et al.* (1997) showed the morphological diversity in these families in the *Atlas of Actinomycetes*. Zhang *et al.* (1998) established the genus *Nonomuria*, on the basis of 16S rDNA sequence analysis, and transferred the *Microtetraspora pusilla* group to the new taxon. Chiba *et al.* (1999) later corrected the genus name from *Nonomuria* to *Nonomuraea* to conform with the rules of the International Code of Nomenclature of Bacteria.

In the present paper, the taxonomic position of strain SF2303 is evaluated by comparing it with two anthracycline-producing type strains; we propose the transfer of *A. carminata* to a new subspecies of the genus *Nonomuraea* as *N. roseoviolacea* subsp. *carminata* comb. nov.

METHODS

Bacterial strains. Strain SF2303 (= IFO 16417 = NRRL B-24114) was isolated by the dry heating method developed by Nonomura & Ohara (1969) from a soil sample collected at Yokohama, Japan, in 1980. The type strains used in this study were obtained from the Japan Collection of Micro-organisms (Saitama, Japan) and the Institute for Fermentation (Osaka, Japan).

Morphological, cultural and physiological characteristics. The methods adopted by the International *Streptomyces* Project (Shirling & Gottlieb, 1966) were used for morphological, cultural and physiological characterization. Observations were made after incubation at 28 °C for 2–4 weeks. Colour determinations were made by comparing the cultures with colour chips from the *Color Harmony Manual*, 4th edn (1958). For scanning electron microscopy, a spore-bearing agar block was fixed overnight in osmium tetroxide fumes, frozen in liquid nitrogen and then freeze-dried. Each specimen was coated with gold–palladium by using an ion-sputter instrument and was then observed with a scanning electron microscope (model JSM6300F; Japan Electron Optics Laboratory).

Chemical analysis of cells. Cell walls were prepared by disrupting about 1–2 g freeze-dried cells with glass beads (0.1 mm in diameter) by using a sonic oscillator (model Insonator 201M; Kubota) at 180 W for 30 min. Non-disrupted cells were removed by centrifugation at 3000 *g* for 30 min and then a crude wall fraction was recovered from the supernatants at 18000 r.p.m. (26000 *g*) for 30 min. The pellets were suspended in 4% SDS solution and boiled for 40 min. Cell walls were collected by spinning at 18000 r.p.m. for 30 min at 25 °C, washed twice with purified water at 25 °C and then freeze-dried. The presence of amino acids and sugars in cell wall and whole-cell hydrolysates was determined by the methods of Becker *et al.* (1965) and Lechevalier (1968). The ratio of diaminopimelic acid stereoisomers was determined by using 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl isothiocyanate as a chiral reagent (Takahashi *et al.*, 1989). Phospholipids were analysed by the procedure of Lechevalier & Lechevalier (1970). Menaquinone samples were prepared by extraction with chloroform/methanol (2:1) from freeze-dried cells and subsequent purification by TLC. They were analysed by using a reverse-phase HPLC–mass spectrometer (models HP1090 and HP5989A with an interface of particle beam-

EI; Hewlett Packard). Cellular fatty acids extracted with *n*-hexane were transformed to methyl ester derivatives with anhydrous methanolic HCl and were analysed by using a GLC–mass spectrometer (models HP5890 and HP5989A; Hewlett Packard).

Fermentation, extraction and analysis of secondary metabolites. The method for fermentation, extraction and analysis of secondary metabolites, including anthracycline antibiotics, was as follows. Well-grown slant cultures of strain SF2303, *A. carminata* IFO 15903^T and *N. roseoviolacea* IFO 14098^T were used to inoculate 20 ml of a seed medium consisting of soluble starch (2.0%), glucose (1.0%), wheat germ (0.6%), peptone (0.5%), yeast extract (0.3%), soybean meal (0.2%) and CaCO₃ (0.1%) (pH 7.0) in 100 ml Erlenmeyer flasks. The flasks were shaken at 28 °C for 3 d on a rotary shaker (220 r.p.m.) and then transferred into 80 ml production medium consisting of maltose syrup (3.0%), soybean meal (1.0%), soluble vegetable protein (0.5%), wheat germ (0.5%) and CaCO₃ (0.1%) (pH 7.0) in 500 ml Erlenmeyer flasks. The flasks were shaken at 28 °C for 5 d. After fermentation, 80 ml acetone was added to each flask; the flasks were shaken violently before removal of the mycelial mass by centrifugation. These acetone extracts were analysed by using an HPLC–mass spectrometer (models HP1090L and HP5989A; Hewlett Packard).

Preparation of DNA. Total DNA was isolated from cells grown in yeast extract/glucose broth according to the method of Saito & Miura (1963), with modifications. Wet biomass (approx. 3 g) was suspended in 10 ml TE buffer (10 mM Tris/HCl, 1 mM EDTA; pH 8.0) supplemented with lysozyme (10 mg ml⁻¹) and incubated for 15 min at 37 °C before the addition of 1 ml 20% (w/v) SDS, 10 ml Tris/HCl (pH 8.0)-saturated phenol and 1.5 ml 5 M NaCl. The resulting preparation was centrifuged at 5000 *g* for 10 min, the supernatant was extracted twice with equal volumes of chloroform and the crude DNA precipitate was recovered with an equal volume of 2-propanol. The DNA was resuspended in 10 ml TE buffer and incubated first with RNase A (20 µg ml⁻¹; Sigma) for 60 min at 50 °C and then with proteinase K (100 µg ml⁻¹; Sigma) for 60 min at 37 °C. Thereafter, the DNA was extracted once with phenol and twice with chloroform. It was then precipitated with 2-propanol. The precipitate was rinsed with 70% ethanol, dried and then dissolved in TE buffer. The concentration and quality of each DNA preparation were determined by using a spectrophotometer (model DU 68; Beckman).

16S rDNA sequence analysis. The 16S rDNA was amplified by using the PCR method with *Taq* DNA polymerase (Takara) and a pair of primers (5'-GAGTTTGATCCTGGCTCAG-3' and 5'-AAGGAGGTGATCCAGCC-3', positions 9–27 and 1541–1525 according to *Escherichia coli* numbering). The conditions for thermal cycling were as follows: denaturation of the target DNA at 96 °C for 2 min followed by 30 cycles of denaturation at 95 °C for 40 s, primer annealing at 53 °C for 1 min and primer extension at 72 °C for 2 min. At the end of the cycling, the reaction mixture was held at 72 °C for 5 min and then cooled to 4 °C.

The 1.5 kb amplified 16S rDNA was subjected to cycle sequencing with a dRhodamine terminator cycle sequencing kit (PE Applied Biosystems), according to the manufacturer's protocol, with the following primers: 5'-GAGTTTGATCCTGGCTCAG-3' (9–17), 5'-CTCCTACGGGAG-GCAGCAG-3' (339–357), 5'-GTATTACCGCGGCTGCTG-3' (536–519), 5'-GCAACGAGCGCAACCC-3' (1099–1114), 5'-GCGGTGTGTACAAGGCC-3' (1382–1365)

Fig. 1. Scanning electron micrographs of *Actinomadura carminata* IFO 15903^T on humic acid–vitamin agar (Hayakawa & Nonomura, 1987) after incubation at 28 °C for 5 d (a) and for 2 weeks (b). Bar, 1.0 µm.

and 5'-GGCTACCTTGTTACGACTT-3' (1492–1474). Thermal cycling was performed as follows: denaturation of the target DNA at 96 °C for 30 s followed by 25 cycles of denaturation at 96 °C for 30 s, primer annealing at 50 °C for 15 s and primer extension at 60 °C for 4 min. The products were analysed with a DNA sequencer (ABI PRISM model 377) according to the manufacturer's protocol.

Phylogenetic analysis. The 16S rDNA sequences of the strains and reference organisms were aligned against that of

Streptomyces ambofaciens (Pernodet *et al.*, 1989). The CLUSTAL W software package version 1.6 (Thompson *et al.*, 1994) was used to generate evolutionary distances (the K_{nuc} value of Kimura, 1980) and similarity values; the phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) from K_{nuc} values. The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

DNA–DNA hybridization. DNA probes (10–20 ng purified DNA per labelling reaction) were radiolabelled with [α -³²P]dCTP (Redivue; Amersham Pharmacia Biotech) using a random prime DNA-labelling system (Rediprime II; Amersham Pharmacia Biotech). Hybridizations were done on nylon membranes (Hybond-N+; Amersham Pharmacia Biotech) according to the dot-blotting protocol of the supplier. Unlabelled target DNAs (1.0 µg) were spotted onto a membrane, denatured with 0.4 M NaOH for 15 min and then neutralized and fixed to the membrane with 1.5 M NaCl/0.5 M Tris (pH 7.5) for 2 min. The blotted membranes were prehybridized with 10 ml hybridization solution (3 × SSC/0.1% SDS containing denatured non-homologous DNA from calf thymus at a final concentration of 100 µg ml⁻¹ and 4 × Denhardt's solution) in a plastic bag at 70 °C for 1 h as a prehybridization. Heat-denatured probe was then added to the bag. The reassociation reaction between the membrane-fixed DNA and ³²P-labelled DNA was performed at 70 °C for 16–20 h. After hybridization, the membranes were washed twice with 2 × SSC/0.1% SDS for 10 min at room temperature and once each with 1 × SSC/0.1% SDS for 10 min at 70 °C and 0.1 × SSC/0.1% SDS for 10 min at 70 °C. The membranes were then air-dried. The radioactivity of each individual dot was measured using a Fujix bio-imaging analyser (model BAS3000 II; Fuji Photo Film). Hybridization experiments were performed in triplicate.

RESULTS AND DISCUSSION

Morphological characteristics

The morphological characteristics of strain SF2303, *A. carminata* IFO 15903^T and *N. roseoviolacea* IFO 14098^T were almost the same. Their substrate hyphae were long, irregularly branched and did not fragment into short elements. The aerial hyphae branched monopodially and were 0.3–0.4 µm in diameter. The spore chains were formed on the aerial hyphae in tightly closed spirals. As shown in Fig. 1(b), well-

Table 1. Cultural characteristics of *A. carminata* IFO 15903^T and related strains

Colour designations taken from the *Color Harmony Manual*, 4th edn (Chicago: Container Corporation of America, 1958).

Medium	Strain SF2303		<i>N. roseoviolacea</i> IFO 14098 ^T		<i>A. carminata</i> IFO 15903 ^T	
	Reverse colour	Aerial mycelium	Reverse colour	Aerial mycelium	Reverse colour	Aerial mycelium
Yeast extract/malt extract agar (ISP no. 2)	Old wine	Abundant alabaster tint	Old wine	Abundant alabaster tint	Old wine	Abundant alabaster tint
Oatmeal agar (ISP no. 3)	Rose grey	Moderate shell pink	Rose grey	Moderate shell pink	Apricot	Moderate shell pink
Inorganic salts/starch agar (ISP no. 4)	Apricot	Abundant shell pink	Apricot	Moderate shell pink	Apricot	Poor shell pink
Glycerol/asparagine agar (ISP no. 5)	Melon yellow	None	Melon yellow	None	Melon yellow	None
Peptone/yeast extract agar (ISP no. 6)	Light amber	None	Beaver	None	Beaver	None
Tyrosine agar (ISP no. 7)	Light tan	None	Light tan	None	Light tan	None
Yeast extract/starch agar	Rose grey	Poor cedar rose	Rose grey	Poor cedar rose	Light fawn	Poor alabaster tint
Maltose Bennett agar	Cedar	Abundant flesh pink	Old wine	Abundant flesh pink	Old wine	Abundant flesh pink

Table 2. Physiological characteristics of *A. carminata* IFO 15903^T and related strains

All strains listed were positive for hydrolysis of starch, utilization of D-glucose, L-arabinose, D-fructose, L-rhamnose and D-galactose, growth at 15 and 42 °C and tolerance of 3% NaCl in yeast extract/starch agar. All strains were negative for coagulation and peptonization of milk, utilization of D-mannitol and raffinose and production of melanoid pigment on tyrosine agar (ISP no. 7).

Physiological property	SF2303	<i>N. roseoviolacea</i> IFO 14098 ^T	<i>A. carminata</i> IFO 15903 ^T
Liquefaction of gelatin	—	+	—
Reduction of nitrate	+	+	—
Utilization of:			
D-Xylose	+ / —	+ / —	+
myo-Inositol	—	+ / —	—
Sucrose	—	—	+ / —

developed spore chains were enveloped in a slimy mass, giving the appearance of sporangia (pseudo-sporangia). The spores were oval to cylindrical in

shape and 0.6–1.2 µm in size, with smooth surfaces (Fig. 1).

Cultural and physiological characteristics

As summarized in Table 1, the cultural characteristics of the three strains are very similar. On ISP medium 2, these strains formed wine-red-coloured pigments (pH indicator) due to the production of anthracycline antibiotics. As shown in Table 2, the physiological characteristics of strain SF2303 and *N. roseoviolacea* strain IFO 14098^T were very similar but were different from those of *A. carminata* IFO 15903^T in terms of the utilization of sucrose and D-xylose and the reduction of nitrate.

Chemotaxonomic characteristics

The chemotaxonomic characteristics of strain SF2303, *A. carminata* IFO 15903^T and *N. roseoviolacea* IFO 14098^T were basically identical. The cell wall of the strains contained *meso*-diaminopimelic acid, glutamic acid and alanine, with small amounts of LL-diaminopimelic acid and glycine. As shown in Table 3, the proportion of *meso*-diaminopimelic acid in the three strains was 71–73%. The proportions in other species of the family *Streptosporangiaceae* (*Herbido-*

Table 3. Proportions of diaminopimelic acid stereoisomers in the cell walls of *A. carminata* IFO 15903^T and related strains

Proportions were determined as follows: 20 µl 5% (v/v) triethylamine solution and 10 µl 2% (v/v) 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate solution in acetonitrile were added to 10 µl cell wall hydrolysate in 0.1 M HCl. The resulting mixture was allowed to stand at room temperature for 25 min. The derivatization reaction was stopped by the addition of 20 µl 50 mM aqueous taurine solution. Amino acid derivatives were analysed by using an HPLC–mass spectrometer. A₂pm, Diaminopimelic acid.

Strain	<i>meso</i> -A ₂ pm (%)	LL-A ₂ pm (%)
Family <i>Streptosporangiaceae</i>		
Strain SF2303	71	29
<i>A. carminata</i> IFO 15903 ^T	71	29
<i>N. roseoviolacea</i> IFO 15238 ^T	73	27
<i>Herbidospira cretacea</i> JCM 8554	75	25
<i>Microbispora rosea</i> IFO 14044 ^T	77	23
<i>Microtetraspora glauca</i> IFO 14761 ^T	75	25
<i>Nonomuraea helvata</i> IFO 14681 ^T	73	27
<i>Nonomuraea pusilla</i> IFO 14684 ^T	76	24
<i>Nonomuraea spiralis</i> JCM 3286 ^T	74	26
<i>Streptosporangium roseum</i> IFO 3776 ^T	71	29
Family <i>Thermomonosporaceae</i>		
<i>Actinomadura citrea</i> IFO 14678 ^T	83	17
<i>Actinomadura kijaniata</i> IFO 14229 ^T	87	13
<i>Actinomadura madurae</i> IFO 14623 ^T	80	20
<i>Actinomadura viridis</i> IFO 15238 ^T	88	12
Others		
<i>Streptomyces griseus</i> IFO 12875 ^T	0	100
<i>Rhodococcus erythropolis</i> JCM 3011	100	0

Table 4. Cellular fatty acid composition of *A. carminata* IFO 15903^T and related strains

Strains are indicated as: 1, strain SF2303; 2, *A. carminata* IFO 15903^T; 3, *N. roseoviolacea* IFO 15238^T; 4, *H. cretacea* JCM 8554; 5, *Microbispora rosea* IFO 14044^T; 6, *Microtetraspora glauca* IFO 14761^T; 7, *Nonomuraea ferruginea* IFO 14094^T; 8, *N. helvata* IFO 14681^T; 9, *N. pusilla* IFO 14684^T; 10, *N. spiralis* JCM 3286^T; 11, *Streptosporangium roseum* IFO 3776^T; 12, *A. citrea* IFO 14678^T; 13, *Actinomadura coerulea* IFO 14679^T; 14, *A. kijaniata* IFO 14229^T; 15, *Actinomadura luteofluorescens* JCM 4203^T; 16, *A. madurae* IFO 14623^T; 17, *Actinomadura vinacea* JCM 3325^T; and 18, *A. viridis* IFO 15238^T. Fatty acid abbreviations are shown according to the following example: iso-16:0, 14-methylpentadecanoic acid; 10M-18:0, 10-methyloctadecanoic acid (tuberculoheptanoic acid); 18:1, oleic acid.

Fatty acid	Family Streptosporangiaceae											Family Thermomonosporaceae						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
14:0	—	—	—	4	2	2	—	5	2	—	—	—	—	—	—	2	1	—
15:0	4	7	10	13	3	6	3	3	5	7	9	—	1	1	1	2	4	—
16:0	7	5	6	8	15	6	3	17	5	2	5	39	44	36	38	33	37	28
17:0	11	15	12	8	2	2	6	2	3	5	8	—	1	2	1	3	8	—
18:0	—	—	—	—	—	—	—	—	—	—	—	5	5	19	4	8	10	11
iso-14:0	—	—	—	4	2	2	2	—	1	4	7	—	—	—	—	—	1	—
iso-15:0	5	3	3	2	3	3	—	4	3	2	1	—	—	—	—	—	—	—
iso-16:0	19	25	18	8	23	21	18	7	20	40	8	1	9	2	2	8	4	11
16:1	1	3	1	3	15	5	3	22	3	—	2	11	4	2	21	2	8	6
17:1	5	4	5	18	5	12	26	10	10	3	19	—	—	—	—	—	2	—
18:1	—	1	—	2	3	3	5	6	2	—	3	27	11	22	18	23	14	18
iso-16:1	—	—	—	—	—	3	1	—	2	2	—	—	—	—	—	—	—	—
iso-18:1	—	—	—	—	—	2	4	—	4	1	—	—	—	—	—	1	—	1
10M-16:0	6	4	5	—	9	4	—	6	4	1	2	—	2	—	2	—	2	—
10M-17:0	24	20	27	6	3	9	6	2	11	14	15	—	2	—	—	—	—	—
10M-18:0	4	5	5	—	1	1	—	1	2	—	2	16	14	14	10	13	7	18
2OH-iso-16:0	7	3	3	5	8	11	15	3	13	14	5	—	—	—	—	—	—	—
2OH-15:0	—	—	—	4	—	2	—	1	2	3	3	—	—	—	—	—	—	—
2OH-16:0	3	2	2	6	3	4	3	8	3	—	5	—	—	—	—	—	—	—

Fig. 2. HPLC patterns of secondary metabolites, including anthracyclines. Arrows indicate anthracycline peaks. (a) *A. carminata* IFO 15903^T; (b) *N. roseoviolacea* IFO 14098^T.

Fig. 3. Phylogenetic tree of the family Streptosporangiaceae, including *A. carminata* IFO 15903^T and related strains, based on 16S rDNA sequence analysis by the neighbour-joining method. The accession numbers for the sequences used for reference are given after the strain numbers. *Actinomadura madurae* (D85468) and *Streptomyces ambofaciens* (M27245) were used as the root organisms. Bar, 0.01 K_{nuc} .

Table 5. DNA relatedness of *A. carminata* IFO 15903^T and related strains

Source of unlabelled DNA	Hybridization (%) with ³² P-labelled DNA from:					
	1	2	3	4	5	6
1. Strain SF2303	100	60	71	11	19	6
2. <i>A. carminata</i> IFO 15903 ^T	54	100	49	9	12	7
3. <i>N. roseoviolacea</i> IFO 14098 ^T	78	52	100	11	12	7
4. <i>N. helvata</i> IFO 14681 ^T	24	18	10	100	21	7
5. <i>Nonomuraea roseola</i> IFO 14685 ^T	20	15	13	9	100	6
6. <i>Nonomuraea fastidiosa</i> IFO 14680 ^T	9	16	6	5	14	2
7. <i>Streptomyces griseus</i> IFO 12875 ^T	20	8	10	3	5	100

spora cretacea, *Microbispora rosea*, *Microtetraspora glauca*, three *Nonomuraea* species and *Streptosporangium roseum*) and in the four *Actinomadura* species were 71–77% and 80–88%, respectively. Whole-cell hydrolysates of the three strains contained glucose, mannose, galactose, ribose and madurose. These data indicate that they have a type III cell wall and a type B whole-cell sugar pattern. In the process of these analyses, we reconfirmed that the cell wall of *A. carminata* IFO 15903^T contained non-peptidoglycan amino acids, as reported by Naumova *et al.* (1986). These authors reported that these amino acids were a specific cell wall component of this strain only and were removed completely from the wall by trypsin digestion. However, we found that this phenomenon was common in all of the genera belonging to the family *Streptosporangiaceae*. This characteristic might be a new chemotaxonomic marker for actinomycetes, and we are planning to report the data elsewhere. The major menaquinone was MK-9 (H₄), with saturated isoprene units at sites III and VIII. This saturation pattern of isoprenoids is characteristic of the family *Streptosporangiaceae*, as reported by Kroppenstedt *et al.* (1990). Strain SF2303, *A. carminata* IFO 15903^T and *N. roseoviolacea* IFO 14098^T contained both glucosamine-containing phospholipid and phosphatidylethanolamine as cellular phospholipids (a type PIV phospholipid pattern; Lechevalier *et al.*, 1981). As shown in Table 4, the cellular fatty acids of members of the family *Streptosporangiaceae*, represented by three strains, were very complex and were characterized by the presence of 17:1/10M-17:0 (10-methylheptadecanoic acid) and 2-hydroxy fatty acids. In contrast, the cellular fatty acid profiles of strains of the *Thermomonosporaceae* were relatively simple, containing 16:0 (palmitic acid) and 18:1 (oleic acid)/10M-18:0 (tuberculosic acid) as major fatty acids. Lennarz *et al.* (1962) demonstrated that 18:1 fatty acid was the precursor of 10M-18:0 fatty acid in *Mycobacterium phlei*. Suzuki & Komagata (1983) noted an increase in methylation as cultures aged. Mycolic acids were not detected. The acyl type of the cell wall peptidoglycan was the acetyl type. These chemotaxonomic characteristics of the three strains were identical to those of the family *Streptosporangiaceae*. Therefore, *A.*

carminata IFO 15903^T should be transferred from the genus *Actinomadura*, belonging to the family *Thermomonosporaceae*, to the family *Streptosporangiaceae*. As shown in Fig. 2, HPLC analysis revealed that the secondary metabolites, including anthracyclines, of *A. carminata* IFO 15903^T were essentially identical to those of *N. roseoviolacea* IFO 14098^T. This result strongly supported the above conclusion.

16S rDNA sequence analysis

Fig. 3 shows a neighbour-joining phylogenetic tree of type strains of the family *Streptosporangiaceae*, including strain SF2303 and strains that are related on the basis of 16S rDNA sequences. The three strains fell into the genus *Nonomuraea* cluster. Thus, in phylogenetic terms, *A. carminata* IFO 15903^T does not belong to the genus *Actinomadura*.

DNA–DNA hybridization

The results of DNA–DNA hybridization among these three strains, the other three type strains of the genus *Nonomuraea* and *Streptomyces griseus* IFO 12875^T are shown in Table 5. The levels of DNA relatedness between strain SF2303 and *N. roseoviolacea* IFO 14098^T ranged from 71 to 78%; however, the levels between these two strains and *A. carminata* IFO 15903^T were lower (49–60%). In contrast, low levels (2–24%) of relatedness were found between these three strains and the other four strains. Therefore, we identified strain SF2303 (IFO 16417) as a member of the species *Nonomuraea roseoviolacea*, and we propose the transfer of *A. carminata* Gauze *et al.* 1973 to a new subspecies of genus *Nonomuraea* Zhang *et al.* 1998 as *N. roseoviolacea* subsp. *carminata* comb. nov.

Description of *Nonomuraea roseoviolacea* subsp. *carminata* (Gauze *et al.* 1973) comb. nov.

Basonym *Actinomadura carminata* Gauze *et al.* 1973. Substrate hyphae are long, irregularly branched and do not fragment into short elements. Aerial hyphae branch monopodially and are 0.3–0.4 µm in diameter. Spore chains are formed on the aerial hyphae in tightly

closed spirals. Well-developed spore chains are enveloped in a slimy mass, giving the appearance of sporangia (pseudosporangia). Spores are oval to cylindrical in shape and 0.6–1.2 µm in size, with smooth surfaces. The colour of the aerial hyphal mass is pink, and a wine-red soluble colour is formed in ISP medium 2. Zoospores are not formed. Hydrolysis of starch and growth at 42 °C are positive. Reduction of nitrate, liquefaction of gelatin, peptonization of milk and production of melanoid pigment are negative. Grows in the presence of 3 % NaCl. Utilizes D-glucose, L-arabinose, D-xylose, D-fructose, L-rhamnose, sucrose and D-galactose, but not *myo*-inositol, D-mannitol or raffinose. Cell wall chemotype IIIB. Phospholipid type PIV. MK-9 (III, VIII-H₄) is the major menaquinone. Cellular fatty acids are characterized by the presence of 10M-17:0/17:1 and iso-16:0 as major components and 2-hydroxy fatty acids as minor components. Mycolic acids are not produced. The cell wall peptidoglycan is of the acetyl type. Aerobic. Mesophilic. Antagonistic properties: produces anthracyclines. The habitat is soil. The type strain is IFO 15903^T (= INA 4281^T).

Description of *Nonomuraea roseoviolacea* subsp. *roseoviolacea* (Nonomura and Ohara 1971) Zhang et al. 1998 comb. nov.

According to Rule 40d (formerly Rule 46) of the International Code of Nomenclature of Bacteria, the valid publication of *Nonomuraea roseoviolacea* subsp. *carminata* automatically creates another subspecies, *Nonomuraea roseoviolacea* subsp. *roseoviolacea* (Nonomura and Ohara 1971) Zhang et al. 1998.

The original description of *Nonomuraea roseoviolacea* subsp. *roseoviolacea* given by Nonomura & Ohara (1971) is unchanged. In addition, this subspecies is characterized by the following chemotaxonomic properties. Cell wall chemotype IIIB. Phospholipid type PIV. MK-9 (III, VIII-H₄) is the major menaquinone. Cellular fatty acids are characterized by the presence of 10M-17:0/17:1 and iso-16:0 as major components and 2-hydroxy fatty acids as minor components. Mycolic acids are not produced. The cell wall peptidoglycan is of the acetyl type. The type strain is IFO 14098^T.

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