***Catenuloplanes crispus* (Petrolini et al. 1993)
comb. nov.: incorporation of the genus
Planopolyspora Petrolini 1993 into the genus
Catenuloplanes Yokota et al. 1993 with an
amended description of the genus
*Catenuloplanes***

Takuji Kudo, Yuki Nakajima and Ken-ichiro Suzuki

Author for correspondence: Takuji Kudo. Tel: +81 48 462 1111. Fax: +81 48 462 4617.
e-mail: kudo@jcm.riken.go.jp

Japan Collection of
Microorganisms, The
Institute of Physical and
Chemical Research (RIKEN),
Wako, Saitama 351-0198,
Japan

The taxonomic position of the genus *Planopolyspora* comprising a single species, *Planopolyspora crispa*, was reviewed. This genus was originally characterized by formation of long, curly and sometimes branching sporangia containing numerous zoospores arranged in a single row and by the presence of meso-diaminopimelic acid and madurose (3-O-methyl-D-galactose) in whole-cell hydrolysates. However, our chemotaxonomic analyses of the type strain of *P. crispa* did not agree with the original description. The peptidoglycan contained L-lysine but not meso-diaminopimelic acid, and the whole-cell hydrolysate contained xylose as the characteristic sugar but not madurose. These characteristics and other chemotaxonomic profiles (e.g. menaquinone, phospholipid and cellular fatty acid compositions) of the genus *Planopolyspora* coincided with those of the genus *Catenuloplanes*. These two genera also had very similar morphological characteristics, but in the original description of the genus *Catenuloplanes* the presence of sporangia was not referred to. This difference is considered to originate from a divergence of views owing to the ambiguity of the definition of the term 'sporangium' in actinomycete morphology. Phylogenetic analysis based on 16S rDNA sequences also supported the proposal that the genera *Planopolyspora* and *Catenuloplanes* should be combined into one genus. Levels of DNA relatedness among the type strains of *P. crispa* and six *Catenuloplanes* species and their cultural, physiological and biochemical characteristics indicated that *P. crispa* should be classified as an independent species of the genus *Catenuloplanes*, which has priority over the genus *Planopolyspora*. Therefore, it is proposed that *Planopolyspora crispa* be transferred to the genus *Catenuloplanes* as *Catenuloplanes crispus* comb. nov.

Keywords: *Catenuloplanes crispus*, *Planopolyspora crispa*, Actinomycetales, Micromonosporaceae, actinomycetes

INTRODUCTION

The genus *Planopolyspora* and its type species *Planopolyspora crispa* were described by Petrolini et al.

Abbreviation: A₂pm, 2,6-diaminopimelic acid.

The DDBJ/EMBL/GenBank accession number for the 16S rDNA sequence of *Planopolyspora crispa* JCM 9312^T is AB024701.

(1993a) for a single actinomycete strain isolated from decomposed leaf litter of *Prunus persica*. This genus was characterized by production of tubular, curly, twisted and sometimes branched sporangia that developed in clusters on the colony surface. The sporangia contained numerous spores arranged in a single row and the sporangiospores became motile by subpolar flagellation after release into water. According to chemotaxonomic analyses in the original de-

Table 1. Cultural, physiological and biochemical characteristics of the type strains of *Planopolyspora* and *Catenuloplanes* species

All strains decomposed casein, aesculin and urea; none decomposed adenine, DNA, hypoxanthine, tyrosine and xanthine. All strains utilized L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, glycerol, *myo*-inositol, D-lactose, maltose, D-mannitol, D-mannose, L-rhamnose, salicin, starch, sucrose, D-trehalose and D-xylose; none utilized adonitol, dulcitol, *i*-erythritol and D-melezitose. All strains utilized fumarate, L-malate and succinate; none utilized benzoate, citrate, mucate, oxalate and L-tartrate. All strains produced acid from L-arabinose, D-fructose, D-galactose, D-glucose, *myo*-inositol, L-rhamnose, sucrose and D-xylose; none produced acid from D-melezitose, methyl α -D-glucoside and L-sorbose. All strains were susceptible to novobiocin and kanamycin at 20 μ g ml⁻¹.

Characteristic	<i>P. crispa</i> JCM 9312 ^T	<i>C. atrovinosus</i> JCM 9535 ^T	<i>C. castaneus</i> JCM 9537 ^T	<i>C. indicus</i> JCM 9534 ^T	<i>C. japonicus</i> JCM 9106 ^T	<i>C. nepalensis</i> JCM 9536 ^T	<i>C. niger</i> JCM 9533 ^T
Colour of colony on:							
Oatmeal agar (ISP-3)	3 ne (topaz)	4 lc (dusty orange)	3 nc (amber)	4 ng (light brown)	3 lc (amber)	4 nc (russet orange)	4 ng (light brown)
Inorganic salts/starch agar (ISP-4)	4 ca (pearl pink)	3 gc (light tan)	4 ca (pearl pink)	4 ic (pastel orange)	4 ca (pearl pink)	4 ic (pastel orange)	4 le (maple)
Glycerol/asparagine agar (ISP-5)	3 ga (melon yellow)	4 le (maple)	4 na (bright orange)	4 ia (light orange)	4 na (bright orange)	4 na (bright orange)	4 na (bright orange)
Yeast extract/starch agar	2 gc (bamboo)	2 ec (sand)	3 gc (light tan)	4 ne (luggage tan)	3 gc (light tan)	3 gc (light tan)	3 gc (light tan)
Bennett's agar containing maltose	4 ca (pearl pink)	2 gc (bamboo)	3 gc (light tan)	4 ec (light rose beige)	4 ca (pearl pink)	Colourless	3 gc (light tan)
Soluble pigment in:							
Glycerol/asparagine agar (ISP-5)	Yellowish	None	None	Yellowish	None	None	None
Nutrient agar	None	Brownish	None	None	None	None	Brownish
Decomposition of:							
Elastin	+	±	—	+	+	—	+
Testosterone	+	+	—	+	+	—	+
Utilization of:							
Methyl α -D-glucoside	—	—	—	—	+	—	+
D-Raffinose	—	—	—	—	+	+	+
D-Ribose	±	+	—	+	±	±	+
Acid from:							
Melibiose	—	+	+	+	—	+	+
D-Ribose	—	+	—	—	—	—	—
Tolerant to (20 μ g ml ⁻¹):							
Ampicillin	—	+	—	—	—	—	+
Benzylpenicillin	—	+	+	—	—	+	—
Cephalexin	—	+	—	+	—	—	+
Growth in 2% (w/v) NaCl	—	—	—	+	+	—	+

scription, *meso*-2,6-diaminopimelic acid (*meso*-A₂pm) and madurose (3-*O*-methyl-D-galactose) were detected in the whole-cell hydrolysate; the authors classified their new genus into the 'maduromycete' group, the tentative name for the madurose-containing taxa (Goodfellow, 1989). Other chemotaxonomic properties of this genus such as menaquinone, phospholipid and cellular fatty acid compositions have not been determined so far. The genus *Planopolyspora* and its type species *P. crispa* were cited in list no. 47 for validation of the publication of new names and new combinations previously effectively published outside the *International Journal of Systematic Bacteriology* (Petrini *et al.*, 1993b). At present, *P. crispa* is still the single member of the genus *Planopolyspora*.

Prior to the validation of the genus *Planopolyspora*, the genus *Catenuloplanes* was proposed by Yokota *et al.* (1993) as a new genus and revived name. Strains belonging to this genus bore arthrospores arranged in chains arising from the substrate mycelium or the aerial mycelium but did not produce sporangia. The spores showed motility by peritrichous flagellation after release into water. The cell wall contained L-lysine but not A₂pm as the diamino acid in the peptidoglycan. The most diagnostic cell wall sugar of the genus was xylose but madurose was not detected.

The major menaquinones were reported to be MK-9(H₈) and MK-10(H₈) and characteristic phospholipids were phosphatidylethanolamine and phosphatidylcholine [type PIII of Lechevalier *et al.* (1977)]. In addition to the type species *Catenuloplanes japonicus*, five species *Catenuloplanes niger*, *Catenuloplanes indicus*, *Catenuloplanes atrovinosus*, *Catenuloplanes castaneus* and *Catenuloplanes nepalensis* were proposed by Tamura *et al.* (1995). Subsequently, the genus *Catenuloplanes* was classified in the family *Micromonosporaceae* Krasil'nikov 1938 emend. Koch *et al.*, 1996 on the basis of 16S rDNA sequences (Koch *et al.*, 1996).

Although the genera *Planopolyspora* and *Catenuloplanes* were different in terms of formation of sporangia and compositions of cell wall amino acids and whole-cell sugars, scanning electron micrographs associated with the original descriptions of both genera indicated that these two genera had quite similar morphological properties. In this paper, we review the taxonomic positions of the type strains of *P. crispa* and *Catenuloplanes* species from morphological, chemotaxonomic and phylogenetic points of view and propose incorporation of the genus *Planopolyspora* into the genus *Catenuloplanes* as *Catenuloplanes crispus* comb. nov.

METHODS

Bacterial strains and cultivation. The following strains were used in this study: *P. crispa* JCM 9312^T (= ATCC 51431^T = DSM 44128^T = IFO 15622^T = IPV 2867^T = NCB 1173^T), *C. japonicus* JCM 9106^T (= ATCC 31637^T = DSM 44102^T = IFO 14176^T = NCIMB 13394^T = VKM Ac-875^T), *C. atrovinosus* JCM 9535^T (= ATCC 700015^T = IFO 15579^T), *C. castaneus* JCM 9537^T (= ATCC 700016^T = IFO 15584^T), *C. indicus* JCM 9534^T (= ATCC 700014^T = IFO 15575^T), *C. nepalensis* JCM 9536^T (= ATCC 700017^T = IFO 15583^T) and *C. niger* JCM 9533^T (= ATCC 31638^T = IFO 14177^T). These organisms were routinely cultivated on yeast extract/starch agar containing 2 g yeast extract (Difco), 10 g soluble starch and 15 g agar in 1000 ml distilled water (pH 7.3) at 28 °C for 14 d and maintained at 8 °C. Biomasses for chemotaxonomic analyses and DNA preparation were obtained from cultures grown in yeast extract/glucose broth containing 10 g yeast extract and 10 g D-glucose in 1000 ml distilled water (pH 7.2) on a rotary shaker at 30 °C.

Morphology. Cultures were grown on oatmeal/nitrate agar containing 3 g powdered oatmeal, 0.2 g KNO₃, 0.5 g K₂HPO₄, 0.2 g MgSO₄·7H₂O and 15 g agar in 1000 ml distilled water (pH 7.0) for 14 d at 28 °C and observed with light and scanning electron microscopes. The specimens for scanning electron microscopy were prepared as described previously (Itoh *et al.*, 1989).

Cultural, physiological and biochemical characteristics. Cultural characteristics were tested by using 21 d cultures grown at 28 °C on four selected agar media (Table 1). Colour designations and colour names of colonies were described by comparison with colour chips from the *Colour Harmony Manual* (Jacobson *et al.*, 1958). Decomposition of urea was examined on Christensen agar (Difco) supplemented with 2% (w/v) urea and decomposition of the other compounds was examined on the basal medium of Gordon *et al.* (1974). Utilization of carbohydrates as sole carbon sources was tested by using neutralized yeast nitrogen base without amino acids (Difco) as a basal medium according to the method of Stevenson (1967). Utilization of organic acids was examined on the defined medium of Gordon *et al.* (1974). Resistance to NaCl was determined on yeast extract/starch agar supplemented with various concentrations (0–10%, w/v) of NaCl at 28 °C. Susceptibility to various antibiotics was also determined on the same agar media supplemented with antibiotics (20 µg ml⁻¹).

Chemotaxonomy. Cell walls were purified and hydrolysed by the methods of Kawamoto *et al.* (1981) and their amino acid composition was analysed with an automatic amino acid analyser. To determine the configurations of the amino acids, hydrolysates of purified cell walls were reacted with a chiral reagent [(+)-1-(9-fluorenyl)ethyl chloroformate] and analysed by HPLC as described by Einarsson & Josefsson (1987). The N-acyl group of muramic acid in the peptidoglycan was determined by the method of Uchida & Aida (1984). Samples for analysis of whole-cell sugars were prepared as described by Lechevalier & Lechevalier (1970) and were analysed by the HPLC method of Mikami & Ishida (1983). Phospholipids were extracted and identified by the method of Minnikin *et al.* (1984). Methyl esters of cellular fatty acids were prepared by the direct transmethylation method with methanolic hydrochloride and were analysed by GLC as described previously (Suzuki & Komagata, 1983). This methyl ester preparation was also used for detection of mycolic acids by application to silica gel TLC developed with n-hexane/dimethylether (4:1). Isoprenoid

Fig. 1. Phylogenetic relationship of *Planopolyspora crispa* JCM 9312^T and some members of the family *Micromonosporaceae* based on 16S rDNA sequence comparison. The numbers on the tree indicate bootstrap values greater than 50%. The numbers in parentheses are accession numbers for the DDBJ/EMBL/GenBank nucleotide sequence databases.

quinones were extracted as described by Collins *et al.* (1984) and were analysed by HPLC and MS (Tamaoka *et al.*, 1983; Kudo *et al.*, 1993).

16S rDNA sequence analysis. 16S rDNA was amplified by the PCR method using a *Taq* DNA polymerase (Takara) and primers 20F [positions 10–25, *Escherichia coli* numbering (Brosius *et al.*, 1978)] and 1540R (positions 1542–1525). Amplification was carried out with a DNA thermal cycler (GeneAmp PCR system 9700; Applied Biosystems) using the following programme: 94 °C for 5 min followed by 30 cycles of denaturation (94 °C for 1 min), primer annealing (55 °C for 1.5 min) and primer extension (72 °C for 2.5 min). At the end of the cycle, the reaction mixture was kept at 72 °C for 10 min and then cooled to 4 °C. The amplified 16S rDNA fragment was purified and subjected directly to cycle sequencing by using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) according to the manufacturer's protocol with the following primers: 27F, 11–27; 350F, 343–357; 350R, 357–342; 780F, 786–803; 780R, 803–785; 1100F, 1099–1114; and 1492R, 1510–1492. The conditions for thermal cycling were 96 °C for 30 s followed by 25 cycles of denaturation (96 °C for 10 s), primer annealing (50 °C for 5 s) and primer extension (60 °C for 4 min). The products were analysed with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The sequence was multiple-aligned with the selected sequences

(Fig. 1) obtained from the DDBJ/EMBL/GenBank databases by using the CLUSTAL W program package (Thompson *et al.*, 1994); the alignment was manually verified and adjusted prior to construction of a phylogenetic tree. The phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) in the PHYLIP package (Felsenstein, 1995). The confidence values of branches of the phylogenetic tree were determined by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

Preparation of DNA, DNA base composition and DNA-DNA hybridization. DNA was isolated from biomass by the method of Saito & Miura (1963). Lysis of cells was performed by using achromopeptidase, crude (Wako Pure Chemical Industries) and *N*-acetylmuramidase SG (Seikagaku Kogyo) as well as lysozyme. The DNA G+C content was determined by the HPLC method after digestion by nuclease P1 (Yamasa Shoyu) and bacterial alkaline phosphatase (Sigma) (Tamaoka & Komagata, 1984). An equimolar mixture of nucleotides for analysis of DNA base composition (Yamasa Shoyu) was used as the quantitative standard. The DNA-DNA hybridization experiment was carried out as described by Ezaki *et al.* (1989).

RESULTS AND DISCUSSION

Morphological observation

Morphology of the genera *Planopolyspora* and *Catenuloplanes* was discussed in detail in each original description (Petroli *et al.*, 1993a; Yokota *et al.*, 1993). Our observations of these two genera with light and scanning electron microscopy were consistent with those in the original descriptions. Both of the type strains of *P. crispa* and *C. japonica* produced curly and branching sporogenous hyphae arising from the agar surface. The spores were arranged in a chain and the spore chains were aggregated into clusters. Each spore could be recognized by septation of the sporogenous

hyphae in the old cultures. In these points, the modes of sporulation of the two genera were identical. A point of difference between the original descriptions of the two genera is whether the spore chains are enveloped in sporangial walls or not. Petroli *et al.* (1993a) made an effort to elucidate the existence of the sporangial wall, but they mentioned that it was difficult to see the sporangial wall because the spores completely filled the sporangia and the wall tightly adhered to the spores. Only residues of the sporangial envelopes were observed after release of spores. On the other hand, Yokota *et al.* (1993) regarded the spores as arthrospores and did not refer to the outer sheath. As pointed out by Williams *et al.* (1973), definition of the term 'sporangium' is ambiguous in actinomycete morphology and even actinomycetes with spore chains (e.g. *Streptomyces* species) have a thin outer sheath around the spores. Also, in the case of the genera *Planopolyspora* and *Catenuloplanes*, formation of 'sporangia' remains controversial and the difference between the original descriptions of the two genera originated from a divergence of views. Therefore, we conclude that the two genera are completely identical in terms of their morphological characteristics.

Chemotaxonomic characteristics

Table 2 shows the amino acid composition in cell wall peptidoglycan, *N*-glycolyl residue contents released from muramic acid in the cell walls and whole-cell sugar patterns of the type strains of *Planopolyspora* and *Catenuloplanes* species. In the original description (Petroli *et al.*, 1993a), the genus *Planopolyspora* was characterized by the presence of *meso*-A₂pm as a cell wall component. However, in this study, *P. crispa* JCM 9312^T did not contain *meso*-A₂pm but L-lysine

Table 2. Amino acid composition of cell walls, content of glycolyl residue in whole cells and whole-cell sugar patterns of the type strains of *Planopolyspora* and *Catenuloplanes* species

Gly, Glycine; Glu, glutamate; Lys, lysine; Ala, alanine; Ser, serine; Rha, rhamnose; Rib, ribose; Xyl, xylose; Mad, madurose; Ara, arabinose; Man, mannose; Glc, glucose; Gal, galactose.

Strain	Amino acids in cell wall (molar ratios*)					Glycolyl residue (nmol per mg dried cell)	Whole-cell sugar							
	Gly	D-Glu	L-Lys	D-Ala	L-Ser + D-Ser†		Rha	Rib	Xyl	Mad	Ara	Man	Glc	Gal
<i>P. crispa</i> JCM 9312 ^T	1.04	1.00	0.99	0.80	0.95	103	—	+	++	—	±	++	++	±
<i>C. atrovinosus</i> JCM 9535 ^T	1.03	1.00	0.99	0.79	0.91	98	—	+	++	—	—	+	++	+
<i>C. castaneus</i> JCM 9537 ^T	1.07	1.00	0.99	0.79	0.99	90	±	++	++	—	±	++	++	±
<i>C. indicus</i> JCM 9534 ^T	1.02	1.00	0.91	0.78	1.03	73	—	+	++	—	±	+	+	±
<i>C. japonicus</i> JCM 9106 ^T	1.07	1.00	0.99	0.80	0.96	94	±	++	++	—	±	++	±	±
<i>C. nepalensis</i> JCM 9536 ^T	1.04	1.00	0.97	0.88	1.05	79	—	+	++	—	±	+	±	±
<i>C. niger</i> JCM 9533 ^T	1.07	1.00	0.99	0.87	1.10	69	—	+	++	—	—	+	++	±

* Molar ratios were represented with glutamic acid as a unit.

† The molar ratio of D-serine to L-serine was ca. 0.3.

Table 3. Menaquinone composition of the type strain of *Planopolyspora* and *Catenuloplanes* species

Strain	Menaquinone composition (%)							
	MK-10	MK-10(H ₂)	MK-10(H ₄)	MK-10(H ₆)	MK-10(H ₈)	MK-11(H ₂)	MK-11(H ₄)	MK-11(H ₆)
<i>P. crispa</i> JCM 9312 ^T	2	7	55	13	2	3	14	4
<i>C. atrovinosus</i> JCM 9535 ^T	2	8	51	6	1	5	23	4
<i>C. castaneus</i> JCM 9537 ^T	3	5	68	6	0	1	14	2
<i>C. indicus</i> JCM 9534 ^T	2	10	61	4	0	3	17	2
<i>C. japonicus</i> JCM 9106 ^T	6	14	55	8	1	4	11	2
<i>C. nepalensis</i> JCM 9536 ^T	4	12	22	0	0	29	29	1
<i>C. niger</i> JCM 9533 ^T	2	3	56	7	1	2	25	4

Table 4. Cellular fatty acid composition of the type strains of *Planopolyspora* and *Catenuloplanes* species

Strain	Fatty acid composition (%)*															
	Normal									Iso-branched						Anteiso-branched
	14:0	15:0	16:0	16:1	17:0	18:0	18:1	19:0	19:1	15:0	16:0	17:0	17:1	18:1	19:0	17:0
<i>P. crispa</i> JCM 9312 ^T	1	1	14	2	11	5	25	TR	1	TR	15	4	0	1	TR	19
<i>C. atrovinosus</i> JCM 9535 ^T	TR	1	9	1	8	6	17	TR	1	TR	9	9	1	1	1	34
<i>C. castaneus</i> JCM 9537 ^T	1	TR	35	10	2	7	32	TR	TR	TR	5	TR	0	0	0	8
<i>C. indicus</i> JCM 9534 ^T	1	1	13	1	14	7	27	1	2	1	6	6	TR	TR	1	18
<i>C. japonicus</i> JCM 9106 ^T	1	1	40	8	5	9	29	TR	0	TR	1	1	0	0	0	5
<i>C. nepalensis</i> JCM 9536 ^T	1	1	21	2	10	16	36	TR	1	TR	TR	3	0	TR	TR	7
<i>C. niger</i> JCM 9533 ^T	TR	TR	12	1	7	11	33	1	2	TR	3	9	TR	TR	1	19

TR, Trace amount (<0.5%).

* Fatty acid nomenclature: number of carbon atoms: number of double bonds.

and L-serine with a small amount of D-serine, which have been reported as peculiarities of the genera *Catenuloplanes* (Yokota *et al.*, 1993) and *Couchioplanes* (Tamura *et al.*, 1994). *P. crispa* JCM 9312^T had N-glycolated muramic acid in the peptidoglycan in common with members of the genus *Catenuloplanes* and other genera belonging to the family *Micromonosporaceae*. A diagnostic sugar of *P. crispa* JCM 9312^T was xylose, which is also characteristic of members of the *Micromonosporaceae*; madurose, which was reported to be present in the original description, was not detected. This fact means that the basis on which the original authors classified the genus *Planopolyspora* into the 'maduromycete' group is lost. The isoprenoid quinones of *P. crispa* JCM 9312^T were mainly composed of MK-10(H₄) and MK-11(H₄), as shown in Table 3. Although this menaquinone profile does not coincide with profiles of *Catenuloplanes* species, which were reported to contain MK-9(H₈) and MK-10(H₈) by Yokota *et al.* (1993) and Tamura *et al.* (1995), our MS analyses revealed that all *Catenuloplanes* strains also had MK-10(H₄) and MK-11(H₄) as major components (Table 3). Table 4 shows the cellular

fatty acid composition of the type strains of *P. crispa* and *Catenuloplanes* species and all of the strains tested showed the similar patterns with minor variations. *P. crispa* JCM 9312^T had phosphatidylcholine as a diagnostic phospholipid [phospholipid type PIII of Lechevalier *et al.* (1977)] and no mycolic acids.

The type strain of *P. crispa* shared cell wall amino acid and whole-cell sugar compositions with the genera *Catenuloplanes* and *Couchioplanes*, but the genus *Couchioplanes* obviously differs from the other two genera in morphological characteristics and profiles of menaquinones and phospholipids (*Couchioplanes* strains have no phosphatidylcholine).

16S rDNA sequence analysis

The 1440 nt of the 16S rDNA of *Planopolyspora crispa* JCM 9312^T were sequenced and compared with sequences of selected members of the family *Micromonosporaceae* (Fig. 1). The data set used for construction of the phylogenetic tree contained 1411 nt positions of each sequence as a result of elimination of

Table 5. DNA base composition and DNA–DNA relatedness among the type strains of *Planopolyspora crispa* and *Catenuloplanes* species

Strain	G + C content (mol %)	DNA complementarity (%) with labelled DNA from:					
		JCM 9312 ^T	JCM 9106 ^T	JCM 9533 ^T	JCM 9534 ^T	JCM 9535 ^T	JCM 9536 ^T JCM 9537 ^T
<i>P. crispa</i> JCM 9312 ^T	70.0	100	59	42	28	47	44 47
<i>C. japonicus</i> JCM 9106 ^T	70.0	42	100	34	24	37	40 42
<i>C. niger</i> JCM 9533 ^T	71.2	45	47	100	39	54	56 49
<i>C. indicus</i> JCM 9534 ^T	70.4	37	37	52	100	44	48 39
<i>C. atrovinosus</i> JCM 9535 ^T	71.5	40	44	42	31	100	50 50
<i>C. nepalensis</i> JCM 9536 ^T	70.3	37	44	45	34	46	100 50
<i>C. castaneus</i> JCM 9537 ^T	71.3	39	48	38	28	46	41 100

gaps and ambiguous nucleotides from the sequences between positions 34 and 1491 (*Escherichia coli* position number). As shown in Fig. 1, *P. crispa* JCM 9312^T was closely related to *C. japonicus* and these two organisms formed a coherent cluster supported by a high bootstrap value (98%). The level of 16S rDNA sequence similarity between *P. crispa* JCM 9312^T and *C. japonicus* was 98.0%.

Coincidence of the morphological and chemotaxonomic characteristics between the genera *Planopolyspora* and *Catenuloplanes* and the result of phylogenetic analysis indicate that these two genera should be incorporated into one genus, *Catenuloplanes*, which has priority over the genus *Planopolyspora* [*Bacteriological Code* 1990 Revision, rule 24b (2)].

DNA hybridization and physiology

Table 5 shows comparisons of DNA base composition and levels of DNA relatedness among the type strains of the genera *Planopolyspora* and *Catenuloplanes*. The levels of the DNA relatedness between *P. crispa* JCM 9312^T and *Catenuloplanes* strains were 28–59%. These values correspond to those observed among the previously described species of the genus *Catenuloplanes* (24–56%). As shown in Table 1, *P. crispa* JCM 9312^T was distinguishable from the type strains of the genus *Catenuloplanes* by physiological and biological aspects, particularly decomposition of elastin and testosterone, utilization of methyl α -D-glucoside and D-raffinose, tolerance to ampicillin, benzylpenicillin and cephalixin, and acid production from melibiose and D-ribose. Therefore, *P. crispa* should be transferred to the genus *Catenuloplanes* as an independent species for which the name *Catenuloplanes crispus* comb. nov. is proposed.

Amended description of the genus *Catenuloplanes* Yokota et al. 1993

The amended description below is based on data from Yokota et al. (1993), Petrolini et al. (1993a) and this study. Aerobic, Gram-positive and non-acid-fast.

Forms non-fragmenting and branched vegetative hyphae. Aerial mycelium is rudimentarily developed or absent. Spores, if produced, are arranged in chains, which arise from the vegetative hyphae or are formed on the rudimentary aerial hyphae. The spore chains are aggregated into clusters and may be enveloped by outer sheaths. The configuration of spore chains is curly or spiral with one or two turns and sometimes branched. The spores are rod-shaped, straight or curved with smooth surfaces, and become motile by peritrichous flagella after release into water. The cell wall peptidoglycan contains glycine, D-glutamate, L-lysine, D-alanine and L-serine in a ratio of 1:1:1:0.8:1 and lacks diaminopimelic acid. The muramic acid in the glycan moiety is N-glycolated. The whole-cell hydrolysate contains xylose, mannose, glucose and ribose, and lacks madurose. The predominant menaquinones are MK-10(H₄) and MK-11(H₄) and, in some strains, MK-10, MK-10(H₂), MK-10(H₆), MK-10(H₈), MK-11(H₂) and MK-11(H₆) are also present as minor components. The diagnostic phospholipid is phosphatidylcholine. The cellular fatty acid was composed of hexadecanoic, octadecanoic, octadecenoic and 14-methylhexadecanoic acids. Hexadecenoic, heptadecanoic and 14-methylpentadecanoic acids are also detected as major components in some strains. Mycolic acids are absent. The G+C content of the DNA is 70–72 mol%. The type species is *Catenuloplanes japonicus*.

Description of *Catenuloplanes crispus* (Petrolini et al. 1993) comb. nov. (basonym *Planopolyspora crispa*)

Catenuloplanes crispus (cri'spus. L. adj. *crispus* curly).

Vegetative mycelia are yellow to brown on most media. Yellowish diffusible pigment is produced in glycerol/asparagine agar. Decomposes casein, elastin, aesculin, testosterone and urea, but not adenine, DNA, hypoxanthine, tyrosine or xanthine. Utilizes L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, glycerol, myo-inositol, D-lactose, maltose, D-mannitol, D-mannose, L-rhamnose, salicin, starch, sucrose, D-trehalose and D-xylose as a sole carbon source, but not

adonitol, dulcitol, *i*-erythritol, D-melezitose, methyl α -D-glucoside or D-raffinose. Utilizes fumarate, L-malate and succinate, but not benzoate, citrate, mucate, oxalate or L-tartrate. Produces acids from L-arabinose, D-fructose, D-galactose, D-glucose, *myo*-inositol, L-rhamnose, sucrose and D-xylose, but not from D-melezitose, melibiose, methyl α -D-glucoside, D-raffinose, D-ribose or L-sorbose. No growth in the presence of 2% (w/v) NaCl. Susceptible to antibiotics (20 μ g ml⁻¹): ampicillin, benzylpenicillin, cephalixin, novobiocin and kanamycin. Mesophilic. Monotypic. The DNA G + C content is 70 mol%. The type strain is strain JCM 9312^T (= ATCC 51431^T = DSM 44128^T = IFO 15622^T = IPV 2867^T = NCB 1173^T).

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