Catenulispora subtropica sp. nov. and *Catenulispora yoronensis* sp. nov.

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Three strains of actinomycetes that formed spore chains were isolated from a paddy field and forest soil and subjected to a polyphasic taxonomic characterization study. The cell wall peptidoglycan contained LL-diaminopimelic acid and glycine. The major isoprenoid quinones were MK-9(H₈) and MK-9(H₆), and the major cellular fatty acids were iso-C_{16:0} and anteiso-C_{17:0}. Phylogenetic analysis based on 16S rRNA gene sequences showed that these organisms belonged in a monophyletic cluster with members of the genus *Catenulispora*. According to the physiological, biochemical and chemotaxonomic data, the novel organisms could be differentiated from recognized members of the genus *Catenulispora*. Based on the phenotypic and genotypic data, the three strains are considered to represent two novel species of the genus *Catenulispora*, for which the names *Catenulispora subtropica* sp. nov. for strains TT 99-48^T (type strain; =NBRC 103395^T=KCTC 19328^T) and TT 01-19 (=NBRC 103396), and *Catenulispora yoronensis* sp. nov. (type strain TT N02-20^T=NBRC 103397^T=KCTC 19327^T) are proposed. An emended description of the genus *Catenulispora* is given.

The genus *Catenulispora* (Busti *et al.*, 2006) in the suborder *Catenulisporineae* (Cavaletti *et al.*, 2006) has been well characterized based on acidophilic properties and chemotaxonomic features as well as on 16S rRNA gene-based phylogenetic analysis. The genus currently contains two species with validly published names, *Catenulispora acidiphila* (Busti *et al.*, 2006) and *Catenulispora rubra* (Tamura *et al.*, 2007).

In the course of research on the diversity of actinomycetes in Japan, three actinomycete strains were isolated from paddy-field soil samples and a forest soil sample. The strains contained LL-diaminopimelic acid (LL-A₂pm) and glycine in the peptidoglycan, and phylogenetically belonged to the genus *Catenulispora*. Based on the data from a polyphasic taxonomic study, we propose that the three strains represent two novel species of the genus *Catenulispora*.

Strains TT 99-48^T and TT 01-19 were isolated from paddy-field soil from Iriomote Island, Okinawa, in 1999, and Ishigaki Island, Okinawa, in 2001, respectively; strain TT N02-20^T was isolated from forest soil in Yoro Valley,

Chiba, in 2002. The strains were all isolated using the yeast extract-SDS method (Hayakawa & Nonomura, 1989) on HV medium (Hayakawa & Nonomura, 1987) containing 20 mg nalidixic acid l⁻¹ and 50 mg cycloheximide l⁻¹.

PCR amplification, sequencing of the 16S rRNA genes and phylogenetic analysis were performed as described previously (Tamura & Hatano, 2001). Distances (distance options according to the Kimura two-parameter model) were calculated, and clustering was performed using the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods; bootstrap values (Felsenstein, 1985) based on 1000 replications were used for this purpose. The 16S rRNA gene analysis showed that strains TT 99-48^T, TT 01-19 and TT N02-20^T formed a monophyletic cluster with recognized members of the genus *Catenulispora* (Fig. 1). The 16S rRNA gene sequence of strain TT 99-48^T was identical to that of strain TT 01-19. The binary similarity values of the 16S rRNA gene sequences among strains TT 99-48^T, TT N02-20^T, *C. acidiphila* NBRC 102108^T and *C. rubra* Aac-30^T ranged from 97.4 to 98.7%.

Cell-wall amino acids, the acyl type of peptidoglycan, whole-cell sugars, cellular fatty acids, mycolic acids, menaquinones and DNA base composition were analysed as described previously (Tamura *et al.*, 1994). Freeze-dried cells for chemotaxonomic analyses were prepared from cultures grown in yeast extract–glucose broth (1.0% yeast extract, 1.0% D-glucose, pH 7.2) on a rotary shaker at 28 °C. The predominant menaquinones were MK-9(H₈)

Abbreviation: LL-A₂pm, LL-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains TT 99-48^T and TT N02-20^T are AB327248 and AB327250, respectively.

A table showing the cellular fatty acid profiles of strains TT 99-48^T and TT N02-20^T and the type strains of recognized *Catenulispora* species is available as supplementary material with the online version of this paper.

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences of strains TT 99-48^T and TT N02-20^T and members of the suborder *Catenulisporineae*. The tree was constructed using the neighbour-joining method. The 16S rRNA gene sequences of *Actinospica acidiphila* GE134766^T (AJ865861) and *Actinospica robiniae* GE134769^T (AJ865863) were used as an outgroup. Bar, 0.01 K_{nuc} . Numbers at branch points are confidence limits (expressed as percentages) estimated from a bootstrap analysis of 1000 replicates.

and MK-9(H₆) with MK-9(H₄) and MK-9(H₁₀) as minor components. The three strains contained LL-A₂pm, glycine, alanine and glutamic acid in the cell-wall, and mannose, arabinose and galactose as whole-cell sugars. The major cellular fatty acids of strains TT 99-48^T, TT 01-19 and TT N02-20^T were iso-C_{16:0} (46–63 %) and anteiso-C_{17:0} (14–28 %) (see Supplementary Table S1, available in IJSEM online). Diphosphatidylglycerol was detected, whereas phosphatidylcholine, phosphatidylethanolamine and some other phospholipids containing glucosamine were not

detected (phospholipid type PI *sensu*; Lechevalier *et al.*, 1977). The DNA G+C contents were 69–71 mol%. Mycolic acids were not present. The acyl type of the cell-wall muramic acid was acetyl type.

Morphological characteristics were observed using scanning electron microscopy as described previously (Tamura *et al.*, 1994). Strains TT 99-48^T, TT 01-19 and TT N02-20^T formed rectiflexible or straight spore chains comprising rod-shaped spores, 0.5–0.8 µm in diameter and 0.8–1.2 µm in length. Determination of the cultural and physiological characteristics was performed as described by Seino *et al.* (1985) and Shirling & Gottlieb (1966) and by using API ZYM, API 50 CH and API Coryne (bioMérieux). The pH for growth was analysed by using filter-sterilized, yeast extract–glucose broth adjusted to various pH values with sulfuric acid. Detailed results of these analyses are provided in the species description and in Tables 1 and 2. Strains TT 99-48^T and TT 01-19 shared very similar characteristics, but strain TT N02-20^T showed obvious differences. They all grew optimally at pH 6–7; strains TT 99-48^T and TT 01-19 grew at pH 8, but strain TT N02-20^T did not. The physiological characteristics of these three strains were clearly different from those of *C. acidiphila* NBRC 102108^T and *C. rubra* Aac-30^T (Table 2).

The microplate hybridization method developed by Ezaki *et al.* (1988, 1989) was used to determine DNA–DNA relatedness. The DNA–DNA relatedness values between

Table 1. Cultural characteristics of strains TT 99-48^T (*Catenulispora subtropica* sp. nov.) and TT N02-20^T (*Catenulispora yoronensis* sp. nov.)

Colour codes in parentheses correspond to those given by Rayner (1970). The diffusible pigment was not produced in all the media tested without tyrosine agar. –, Negative for diffusible pigment.

Characteristic	Strain TT 99-48 ^T	Strain TT N02-20 ^T
Yeast extract-malt extract agar (ISP 2)		
Growth	Moderate	Good
Colour of colony	Greyish reddish-orange (59)	Light to moderate yellowish brown (88), moderate yellow (64)
Oatmeal agar (ISP 3)		
Growth	Moderate	Good
Colour of colony	Greyish white	Moderate yellow (64), light to moderate yellowish brown (88)
Inorganic salts-starch agar (ISP 4)		
Growth	Poor	Good
Colour of colony	Pale yellow (45)	Pale yellow (45)
Glycerol-asparagine agar (ISP 5)		
Growth	Poor	Poor
Colour of colony	Greyish-white	Greyish-white
Peptone-yeast-iron agar (ISP 6)		
Growth	Moderate	Poor
Colour of colony	Greyish-white	Greyish-white
Tyrosine agar (ISP 7)		
Growth	Moderate	Poor
Colour of colony	Greyish-white	Greyish-white
Diffusible pigment	Greyish reddish-orange (59)	–

Table 2. Differential characteristics of strains TT 99-48^T and TT 01-19 (*Catenulispora subtropica* sp. nov.), TT N02-20^T (*Catenulispora yoronensis* sp. nov.) and recognized species of the genus *Catenulispora*

Strains: 1, TT 99-48^T; 2, TT 01-19; 3, TT N02-20^T; 4, *C. acidiphila* NBRC 102108^T; 5, *C. rubra* Aac-30^T. All strains utilize D-xylose, D-glucose, D-mannose, arbutin, aesculin, ferric citrate, D-cellobiose, maltose, trehalose, starch, glycogen, gentiobiose and L-fucose, but not erythritol, D-arabinose, D-ribose, L-xylose, D-adonitol, L-sorbose, dulcitol, inositol, D-sorbitol, methyl α -D-glucopyranoside, inulin, D-melezitose, xylitol, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, 2-ketogluconate or 5-ketogluconate. All strains are positive for pyrazinamidase, alkaline phosphatase, α -glucosidase, N-acetyl- β -glucosaminidase, aesculin hydrolysis, leucine aminopeptidase, valine aminopeptidase, acid phosphatase, phosphohydrolase, β -glucosidase and α -mannosidase; and negative for pyrrolidonyl arylamidase, urea hydrolysis, lipase, cystine aminopeptidase and α -galactosidase. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3	4	5
Nitrate reduction	+	+	–	–	–
β -Glucuronidase	–	–	–	–	+
β -Galactosidase	+	+	–	+	w
Gelatin hydrolysis	+	+	–	+	–
Catalase	+	+	+	+	–
Trypsin	+	+	–	–	+
α -Fucosidase	+	+	+	–	–
Utilization of:					
Glycerol	+	+	–	–	+
L-Arabinose	+	+	–	–	+
Methyl β -D-xylopyranoside	w	+	–	–	–
D-Galactose	+	+	–	+	–
D-Fructose	–	–	–	+	+
D-Mannitol	–	–	–	–	+
Methyl α -D-glucopyranoside	–	–	–	–	+
N-Acetylglucosamine	+	+	–	w	+
Salicin	+	+	–	+	w
Sucrose	–	–	w	–	+
Gluconate	+	+	+	+	–
pH for growth	5–8	5–8	5–7	5–8	4–6.5

strain TT 99-48^T and strain TT N02-20^T, *C. rubra* Aac-30^T and *C. acidiphila* NBRC 102108^T were below 36%; however, the level of relatedness with strain TT 01-19 was 74%. The DNA–DNA relatedness between strain TT N02-20^T and strains TT 99-48^T and TT 01-19 and *C. rubra* Aac-30^T and *C. acidiphila* NBRC 102108^T was below 36%. Strain TT 01-19 was identified as representing the same species as TT 99-48^T. Strains TT 99-48^T and TT N02-20^T could also be distinguished from recognized *Catenulispora* species based on several phenotypic properties (Table 2). Therefore, based on the morphological, physiological and chemotaxonomic data, DNA–DNA relatedness and phylogenetic distinctiveness, the three strains should be assigned as representing two novel species for which the names *Catenulispora subtropica* sp. nov. and *Catenulispora yoronensis* sp. nov. are proposed.

The two recognized species of the genus *Catenulispora*, *C. acidiphila* and *C. rubra* are acidophilic. Consequently, *C. acidiphila* was characterized as an organism that possessed glucose, xylose, ribose, rhamnose and arabinose as whole-cell sugars in the genus description (Busti *et al.*, 2006). Conversely, the novel isolates are non-acidophilic and possess mannose, arabinose and galactose. These facts justify an emendation of the description of the genus *Catenulispora*.

Emended description of the genus *Catenulispora* Busti *et al.* 2006

Catenulispora (Ca.te.nu.li.spo'ra. L. fem. n. *catenula* small chain; Gr. fem. n. *spora* seed; N.L. fem. n. *Catenulispora* a thin chain of spores).

Gram-positive, acidophilic or non-acidophilic, non-acid-fast, aerobic organisms that form branching hyphae. Non-fragmentary vegetative mycelium and aerial hyphae starting to septate in chains of cylindrical arthrospores are produced. Motile elements are not produced. Peptidoglycan contains LL-A₂pm, glycine, glutamic acid and alanine. Mannose, arabinose and galactose are detected as whole-cell sugars; xylose, rhamnose, ribose and glucose are also detected. Iso-C_{16:0} and anteiso-C_{17:0} are present as major cellular fatty acids. Menaquinones MK-9(H₆), MK-9(H₄) and MK-9(H₈) are predominant. Diphosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides are present, whereas phosphatidylethanolamine and phosphatidylcholine are absent. The G+C content of the DNA is 69–72 mol%. The type species is *Catenulispora acidiphila*. Phylogenetically, the genus *Catenulispora* is a member of the family *Catenulisporaceae* within the suborder *Catenulisporineae* of the order *Actinomycetales*.

Description of *Catenulispora subtropica* sp. nov.

Catenulispora subtropica (sub.tro.pi'ca. N.L. fem. adj. *subtropica* pertaining to subtropical zone, the origin of the soil sample from which the type strain was isolated).

Gram-positive, aerobic actinomycete that forms extensively branched substrate hyphae. Vegetative hyphae are finely branched and do not fragment. Monopodially or dichotomously branching aerial mycelia develop sparsely. Greyish reddish-orange colonies are formed on ISP 2. Temperature range for growth is 10–37 °C, with optimum growth at 25–30 °C. pH range for growth is 5 or 6 to 8, with optimum growth at pH 6–7. Glycerol, L-arabinose, D-xylose, methyl β -D-xylopyranoside, D-galactose, D-glucose, D-mannose, N-acetylglucosamine, arbutin, aesculin, ferric citrate, salicin, D-cellobiose, maltose, starch, glycogen, gentiobiose, L-fucose and gluconate are used as sole carbon sources. Positive for catalase, alkaline phosphatase, nitrate reduction, pyrazinamidase, leucine aminopeptidase, valine aminopeptidase, trypsin, acid phosphatase, phosphohydrolase, β -galactosidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosamini-

dase, α -mannosidase and α -fucosidase; negative for pyrrolidonyl arylamidase, esterase (C4), esterase lipase (C8), lipase (C14), cystine aminopeptidase, chymotrypsin, α -galactosidase and β -glucuronidase. Aesculin and gelatin are hydrolysed, but not urea. Predominant menaquinones are MK-9(H₈) and MK-9(H₆). Predominant cellular fatty acids are iso-C_{16:0} and anteiso-C_{17:0}. Cell wall contains LL-A₂pm, glycine, glutamic acid and alanine (peptidoglycan type A3 γ sensu; Schleifer & Kandler, 1972). Contains mannose, arabinose and galactose as whole-cell sugars. The DNA G + C content is 70–71 mol%.

The type strain, TT 99-48^T (=NBRC 103395^T=KCTC 19328^T), was isolated from a paddy rice soil from Iriomote Island, Japan. Strain TT 01-19 (=NBRC 103396) is a reference strain.

Description of *Catenulispora yoronensis* sp. nov.

Catenulispora yoronensis (yo.ro.nen'sis. N.L. fem. adj. *yoronensis* pertaining to Yoro Valley, Chiba, Japan, the origin of the soil sample from which the type strain was isolated).

Gram-positive, aerobic actinomycete that forms extensively branched substrate hyphae. Vegetative hyphae are finely branched and do not fragment. Monopodially or dichotomously branching aerial mycelia develop sparsely. Light to moderate yellowish brown and/or moderate yellow colonies are formed on ISP 2. Temperature range for growth is 10–37 °C, with optimum growth at 25–30 °C. pH range for growth is 5–7, with optimum growth at pH 6–7. D-Xylose, D-glucose, D-mannose, arbutin, aesculin, ferric citrate, D-cellobiose, maltose, D-melibiose, trehalose, starch, glycogen, L-fucose and gluconate are used as sole carbon sources. Positive for catalase, pyrazinamidase, alkaline phosphatase, leucine aminopeptidase, valine aminopeptidase, acid phosphatase, phosphohydrolase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase; negative for nitrate reduction, pyrrolidonyl arylamidase, esterase (C4), esterase lipase (C8), lipase (C14), cystine aminopeptidase, α -galactosidase, trypsin, chymotrypsin, β -galactosidase and β -glucuronidase. Aesculin is hydrolysed, but gelatin and urea are not. Predominant menaquinones are MK-9(H₈) and MK-9(H₆). Predominant cellular fatty acids are iso-C_{16:0} and anteiso-C_{17:0}. Cell wall contains LL-A₂pm, glycine, glutamic acid and alanine (peptidoglycan type A3 γ sensu; Schleifer & Kandler, 1972). Contains mannose, arabinose and galactose as whole-cell sugars. The DNA G + C content of the type strain is 69 mol%.

The type strain, TT N02-20^T (=NBRC 103397^T=KCTC 19327^T), was isolated from forest soil from Yoro Valley, Japan.

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