Catenulispora rubra sp. nov., an acidophilic actinomycete isolated from forest soil

Tomohiko Tamura, Yuumi Ishida, Takeshi Sakane and Ken-ichiro Suzuki

Correspondence

Tomohiko Tamura

tamura-tomohiko@nite.go.jp

NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation,
2-5-8 Kazusakamatari, Kisarazu, Chiba 292-0818, Japan

In the course of screening novel secondary metabolites, an acidophilic actinomycete strain, designated Aac-30^T, was isolated from forest soil and subjected to a polyphasic taxonomic characterization study. It grew well on media in which the pH ranged from 4 to 6, but not on medium with pH adjusted to 7. It possessed LL-diaminopimelic acid and glycine in the cell-wall peptidoglycan, MK-9(H₆) and MK-9(H₈) as major isoprenoid quinones, iso-C_{16:0} and anteiso-C_{17:0} as major cellular fatty acids, and phosphatidylglycerol and phosphatidylinositol as polar lipids. The G+C content of the genomic DNA was 69.1 mol%. Phylogenetic analyses based on 16S rRNA gene sequences showed that the organism belonged to the family *Catenulisporaceae* and consistently formed a monophyletic cluster with members of the genus *Catenulispora*. Physiological, biochemical and chemotaxonomic data revealed that this novel organism could be readily differentiated from recognized members of the genus *Catenulispora* and that it merits separate species status. Based on the phenotypic and genetic evidence presented, strain Aac-30^T is considered to represent a novel species of the genus *Catenulispora*, for which the name *Catenulispora rubra* sp. nov. is proposed. The type strain is Aac-30^T (=NBRC 101179^T=DSM 44948^T).

The genus *Catenulispora* was proposed by Busti *et al.* (2006) to accommodate a group of actinomycetes containing LL-diaminopimelic acid (LL-A₂pm) and glycine in the cell-wall peptidoglycan. The type, and only, species of the genus, *Catenulispora acidiphila*, is reported to be acidophilic. In the course of isolating acidophilic microorganisms for screening bioactive substances, strain Aac-30^T was recovered from a forest soil sample collected at Oodate, Akita Prefecture, Japan, on an acidic medium for isolation. The isolation medium was prepared by mixing separately prepared solutions A (4 g glucose, 2 g Difco yeast extract and 18 g agar in 500 ml distilled water; pH adjusted to 5.0 with H₂SO₄) and B [2 g (NH₄)₂SO₄, 0.5 g K₂HPO₄, 0.5 g MgSO₄·7H₂O and 0.1 g KCl in 500 ml distilled water; pH adjusted to 2.7 with H₂SO₄], which had previously been autoclaved (final pH was approximately 4).

PCR amplification and sequencing of the 16S rRNA gene of the novel strain and phylogenetic analysis were performed as described by Tamura & Hatano (2001). Distances (distance options according to the Kimura two-parameter model) were calculated and clustering was performed by using the neighbour-joining and maximum-parsimony

methods; bootstrap values based on 1000 replications were used for this purpose. 16S rRNA gene sequence analysis revealed that strain Aac-30^T formed a monophyletic cluster with *C. acidiphila* (Fig. 1). The level of similarity between strain Aac-30^T and the type strain of *C. acidiphila* was 99.4 %.

Analyses of whole-cell sugar patterns, cell-wall amino acids, menaquinones, the acyl type of the peptidoglycan, mycolic acid and DNA base composition were performed as described by Tamura *et al.* (1994). Processing and analysis of fatty acids as methyl esters were performed by using the protocol of the MIDI Sherlock Microbial Identification System (Sasser, 1990). Freeze-dried cells for chemotaxonomic analyses were obtained from a culture grown in yeast extract–glucose broth (1.0 % yeast extract, 1.0 % D-glucose; pH adjusted to 5.5 with H₂SO₄) on a rotary shaker at 28 °C. The predominant menaquinones of strain Aac-30^T were MK-9(H₆) and MK-9(H₈); MK-9(H₄) was present as a minor component. The strain contained LL-A₂pm, glycine, alanine and glutamic acid as cell-wall amino acids, and ribose, mannose, arabinose and glucose as whole-cell sugars. The fatty acid profile of strain Aac-30^T comprised iso-C_{16:0} (47.9 %), anteiso-C_{17:0} (22.2 %), 9 methyl C_{16:0} (4.7 %), iso-C_{17:0} (4.7 %), anteiso-C_{17:1} (4.4 %), iso-C_{15:0} (3.8 %), anteiso-C_{15:0} (3.2 %), C_{16:0} (3.1 %), iso-C_{16:1} (2.0 %), C_{16:1}ω9c (1.9 %), C_{17:0} cyclo (1.5 %) and 10 methyl C_{17:0} (1.2 %). Significant differences in the fatty acid profiles of strains Aac-30^T and *C. acidiphila*

Abbreviation: LL-A₂pm, LL-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Aac-30^T is AB180773.

A table detailing the cellular fatty acid profiles of strain Aac-30^T and *Catenulispora acidiphila* is available with the online version of this paper.

Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequences of strain Aac-30^T and members of the suborder *Catenulisporineae*. The tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987). The 16S rRNA gene sequence of *Streptomyces ambofaciens* ATCC 23877^T was used as the outgroup. Numbers at branch points are confidence limits (expressed as percentages) estimated from a bootstrap analysis of 1000 replicates. Bar, 0.01 K_{nuc} .

NBRC 102108^T were found only for the proportions of iso-C_{16:0} and anteiso-C_{17:0} (Supplementary Table S1 available in IJSEM Online). As diagnostic phospholipids, phosphatidylglycerol and phosphatidylinositol were detected, but phosphatidylcholine, phosphatidylethanolamine and some other phospholipids containing glucosamine were not detected (phospholipid type PI *sensu* Lechevalier *et al.*, 1977). The DNA G + C content of strain Aac-30^T was 69.1 mol%.

Morphological characteristics were determined with the use of scanning electron microscopy as described by Tamura *et al.* (1994). Strain Aac-30^T formed a rectiflexible or straight spore chain consisting of rod-shaped spores with a diameter of 0.7–1.5 µm. Determination of cultural and physiological characteristics was performed as described by Gordon *et al.* (1974), Seino *et al.* (1985), Shirling & Gottlieb (1966) and Yokota *et al.* (1993), and by using the API ZYM system (bioMérieux). Detailed results of these analyses are provided in the species description below and in Tables 1 and 2. Strain Aac-30^T formed red colonies that developed on most of the acidic media tested. The colour of the aerial mycelia and spore chains ranged from white to pink on International *Streptomyces* Project (ISP) media 2, 4, 5 and 7 in which the pH was adjusted to approximately 5. This strain grew on media in which the

pH was adjusted to 4–6.5 but did not grow on medium with pH adjusted to 7. On acidified ISP medium 9 (Shirling & Gottlieb, 1966), good growth was observed in the presence of D-glucose, L-arabinose, D-xylose, fructose, sucrose and raffinose; no visible colonies were observed in the presence of inositol or rhamnose, and scant growth was observed in the presence of mannitol. Positive reactions were found for starch hydrolysis, esterase, trypsin and β-glucuronidase. The cultural characteristics of strain Aac-30^T were clearly different from those of *C. acidiphila* (Table 2).

The microplate hybridization method developed by Ezaki *et al.* (1988, 1989) was applied to determine DNA relatedness. The level of DNA–DNA relatedness between strain Aac-30^T and *C. acidiphila* NBRC 102108^T was below 26 %. Strain Aac-30^T was also distinguishable from *C. acidiphila* based on several phenotypic properties (Table 2). Therefore, on the basis of morphological, physiological and chemotaxonomic data, DNA–DNA hybridization results and phylogenetic distinctiveness, strain Aac-30^T is considered to represent a novel species of the genus *Catenulispora*, for which the name *Catenulispora rubra* sp. nov. is proposed.

Description of *Catenulispora rubra* sp. nov.

Catenulispora rubra (ru'bra. L. fem. adj. *rubra* red).

Gram-positive, non-acid-fast aerobic actinomycete that forms extensively branched substrate hyphae. The vegetative hypha is finely branched and does not fragment. Monopodially or dichotomously branching aerial mycelia develop sparsely. The aerial mycelium contains spore chains arranged in a hook-like or flexuous manner. Spores (0.7–1.5 µm in diameter) are rod-shaped, smooth-surfaced and non-motile. Contains mannose, arabinose and glucose. Colonies are red-pigmented. The optimum temperature range for growth is 20–30 °C, and optimum pH is 5. D-Glucose, raffinose, L-arabinose, D-xylose, sucrose and fructose, but not inositol or rhamnose, are used as sole carbon sources. Positive for alkaline phosphatase, esterase, leucine aminopeptidase, valine aminopeptidase, acid phosphatase, phosphohydrolase, α-glucosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and trypsin, but negative for lipase, cystine aminopeptidase,

Table 1. Cultural characteristics of strain Aac-30^T

The diffusible pigment was not produced in all media tested. Colour codes in parentheses correspond to those given by Rayner (1970).

Medium	Growth	Reverse colour	Aerial mass, colour
Yeast extract–malt extract agar (ISP-2)	Good	Strong red (2)	Moderate, white
Oatmeal agar (ISP-3)	Weak	Pale yellow (45)	Absent
Inorganic salts–starch agar (ISP-4)	Good	Strong red (2) to dark purplish red (82)	Good, moderate to dark purplish pink (55)
Glycerol–asparagine agar (ISP-5)	Good	Strong red (2) to dark purplish red (82)	Good, moderate yellowish pink (58)
Peptone–yeast extract agar (ISP-6)	Absent	–	–
Tyrosine agar (ISP-7)	Good	Strong red (2) to dark purplish red (82)	Good, pink

Table 2. Differential characteristics between strain Aac-30^T and *Catenulispora acidiphila*

Taxa: 1, strain Aac-30^T (data from this study); 2, *C. acidiphila* (data from Busti *et al.*, 2006 and this study). Both strains utilize D-glucose, L-arabinose, D-xylose and D-fructose, but not *myo*-inositol or L-rhamnose. Both strains are positive for alkaline phosphatase, leucine aminopeptidase, valine aminopeptidase, acid phosphatase, phosphohydrolase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase and α -mannosidase but negative for lipase, cystine aminopeptidase, α -galactosidase and α -fucosidase. +, Positive; –, negative.

Characteristic	1	2
Utilization of sucrose	+	–
Esterase	+	–
Trypsin	+	–
β -Glucuronidase	+	–
Gelatin hydrolysis	–	+
Reverse colour on ISP-2, ISP-4, ISP-5 and ISP-7	Red	Brown to orange
Diffusible pigment in ISP-2, ISP-3, ISP-4, ISP-5 and ISP-7	–	Brown

α -galactosidase and α -fucosidase. Hydrolyses starch and aesculin, but not gelatin or urea. The predominant menaquinones are MK-9(H₆) and MK-9(H₈). The cellular fatty acids consist of iso-branched, anteiso-branched, saturated and unsaturated fatty acids with iso-C_{16:0} and anteiso-C_{17:0} as major components. The cell wall contains LL-A₂pm and glycine (peptidoglycan type A3 γ' *sensu* Schleifer & Kandler, 1972). The DNA G+C content is 69.1 mol%.

The type strain, Aac-30^T (=NBRC 101179^T=DSM 44948^T), was isolated from forest soil in Oodate, Japan.

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