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Actinocrinis puniceicyclus gen. nov., sp. nov., an actinobacterium isolated from an acidic spring

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Abstract

An aerobic, mildly acidophilic actinobacterium was isolated from the Ochre Beds bog in Kootenay National Park, Canada. Cells of isolate OB1^T were Gram-stain-positive, non-motile, pink- to purple-pigmented filaments. The pH range for growth was pH 3.5–6.5 (optimum pH 5.5), and the temperature range was 13–30°C. The major cellular fatty acids were i-$C_{16:0}$ (28.5 %), i-$C_{15:0}$ (14.6 %) and ai-$C_{15:0}$ (14.3 %), and the major polar lipid was phosphohexose. The major quinone was menaquinone-11 (MK-11), and the peptidoglycan type was A1γ. The DNA G+C content was 70.2 %. Along with growth on complex media including yeast extract, proteose peptone, casamino acids and tryptic soy broth, growth occured on mono- and disaccharides (glucose, sucrose, galactose and xylose) and polysaccharides (starch, gellan, pectin, xylan and alginate). Anaerobic growth was not observed. The cells did not fix atmospheric nitrogen. On the basis of comparative 16S rRNA gene sequence analysis, this isolate belonged to the family *Actinospicaceae*, in the suborder *Catenulisporineae* of the order *Actinomycetales*. The most closely related species was *Actinospica robiniae*. However, the 16S rRNA gene sequence identity to this bacterium was low (92.8 %) and there were several chemotaxonomic differences from this species. We therefore propose a novel genus and species, *Actinocrinis puniceicyclus* gen. nov., sp. nov., with strain OB1^T (=DSM 45618^T=ATCC BAA-2771^T) as the type strain.

Members of the phylum *Actinobacteria* are among the most commonly isolated bacteria from soils [1]. Cultivation-independent studies based on detection of 16S rRNA genes [1, 2] and cultivation-dependent studies [3] have shown them to be prevalent in acidic soils. Cultured actinobacteria include a few extreme acidophiles such as *Acidimicrobium ferrooxidans*, *Ferrimicrobium acidiphilum* and *Ferrithrix thermotolerans* [4]. Mildly acidophilic actinobacteria (pH optima of 4–6) have also been isolated from soils. These include members of the genera *Actinospica*, *Allokutzneria*, *Amycolatopsis*, *Catenulispora*, *Kitasatospora*, *Mycobacterium*, *Nocardia*, *Nonomuraea*, *Rugosimonospora Saccharopolyspora*, *Streptomyces*, *Streptacidiphilus* and *Verrucosispora* [5–11], among others. Many common soil actinobacteria are slow-growing oligotrophs, so the use of dilute, nutrient-poor media and long incubation times is recommended for their cultivation [1, 12]. In this study, we followed such a strategy to isolate a novel actinobacterium from an acidic bog.

The Ochre Beds bog is an iron-rich feature of Kootenay National Park in British Columbia, Canada. Oxidation of iron sulfide minerals dissolved in the source springs produces extremely acid conditions in a natural process similar to acid mine drainage, and deposition of iron oxides leads to the characteristic ochre-colour [13]. We obtained a sample from the oxic, upper 4 cm layer of the Ochre Beds bog in September 2009. The soil had a pH of 3.3 and temperature of 21 °C at the time of sampling and was covered by a shallow pool (4 cm) of water at 30.2 °C.

A loopful of soil was smeared onto plates of the minimal salts medium C10. This was identical to medium FS1V as described by Stott *et al.* [14], except that the pH was adjusted to give a final value of pH 3.9–4.0. Medium was solidified with 15 g gellan l^{-1}, which served as both a solidifying agent and an energy source [14]. After incubation at room temperature for 24 days, purple-pigmented, filamentous colonies were selected from the plates and re-streaked until an isolate

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Abbreviations: 3-OH Dpm, 3-hydroxydiaminopimelic acid; IPL, intact polar lipid; *meso*-Dpm, *meso*-diaminopimelic acid.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Actinocrinis puniceicyclus* OB1^T is FR775984.
Supplementary Information and one supplementary figure are available with the online Supplementary Material.

dubbed OB1^T was obtained. For biochemical testing of the isolate, medium C10 was supplemented with 0.05 % (w/v) yeast extract and adjusted to a near-optimal pH (5.0) to increase the growth rate. This medium was designated C10Y. Alternatively, cells were grown on plates of R2A5 medium, which contained all the components of R2A medium [15], supplemented with 1 ml of trace elements solution 1 [14], adjusted to pH 5.0 and solidified with gellan.

After 15 days of growth at room temperature on C10Y or R2A5 plates, isolate OB1^T appeared as purple, round, convex colonies approximately 0.5 mm in diameter. Colonies were embedded into the gellan medium and could only be removed by digging into the gel with an inoculating loop. Older colonies turned white and produced extensive aerial hyphae. Growth in liquid C10Y medium yielded bright pink or red spherical aggregates, and after 2 weeks soluble pigment was evident in the medium. Aggregates in liquid R2A5 broth were smaller and beige-coloured, suggesting that the bright pigment seen in C10Y may have been a response to low substrate concentrations. The aggregated growth made analysis of optical density impossible; thus, growth was estimated qualitatively for most subsequent tests, or quantitatively via CO_2 production (pH test).

A 14-day-old culture grown in C10Y broth stained Gram-positive. A wet mount (Fig. 1a) showed long filaments of 15–25 µm in length and 0.7–0.9 µm in diameter. Chains of arthrospores similar to those observed in *Actinospica robiniae* [6] were observed. Cells were not motile. For transmission electron microscopy, 21-day-old cells from C10Y broth were used, and samples were prepared as described previously [16]. A thick Gram-positive-type cell wall, nucleolar fibres and inclusion bodies were visible (Fig. 1b).

The pH range for growth of isolate OB1^T was determined in R2A and C10Y broths. Duplicate 100 ml vials containing 40 ml of medium were adjusted to different pH levels between pH 1.0 and 7.5 using H_2SO_4 and NaOH. Vials were inoculated with 0.5 ml of a 13-day-old culture grown in the same medium and then capped gas-tight with butyl rubber stoppers and aluminum crimps. At 5- to 7-day intervals over 3 weeks (R2A) or at 2- to 3-day intervals over 2 weeks (C10Y), 1.0 ml gas samples were removed aseptically from each bottle with a sterile syringe. The CO_2 mixing ratios were determined on a Varian-450 GC equipped with a thermal conductivity detector (oven 40 °C, detector 150 °C, 0.25 m×1/16″×1 mm HayeSep Q 80/100 mesh column). Growth rates were calculated by log-linear curve fitting of CO_2 production versus time in the initial exponential growth phase (generally 5–15 d). At the end of the trials, pH had increased up to 1.1 unit in some vials, so the mean pH between the start and end of the experiment was used in graphing and calculating optima (Fig. 2). The pH optima were pH 5.6 and 5.3 for media C10Y and R2A, respectively (Fig. 2), and the total range observed for growth was pH 3.5–6.5, indicating that isolate OB1^T is an obligate acidophile. Medium C10Y supported a maximum growth rate nearly double that recorded on R2A. Both media contained 5 g yeast extract l^{-1}, so apparently the richer R2A medium contains substances that inhibit growth.

A similar protocol was used to test the temperature range for growth of isolate OB1^T with the exception that growth was recorded as positive or negative as a result of turbidity. Duplicate 100 ml vials with 40 ml of medium R2A5 or C10Y (pH 4.5) were inoculated as in the pH trials, incubated at 5, 10, 13, 25, 30, 37 or 45 °C, and observed after 2 weeks in comparison with a negative control. Growth of isolate OB1^T was visible between 13 and 30 °C, with optimal growth between 20 and 25 °C on C10Y medium. Growth was visible between 20 and 30 °C, with optimal growth at 25 °C on R2A5. No growth was observed at 10 or 37 °C on either medium.

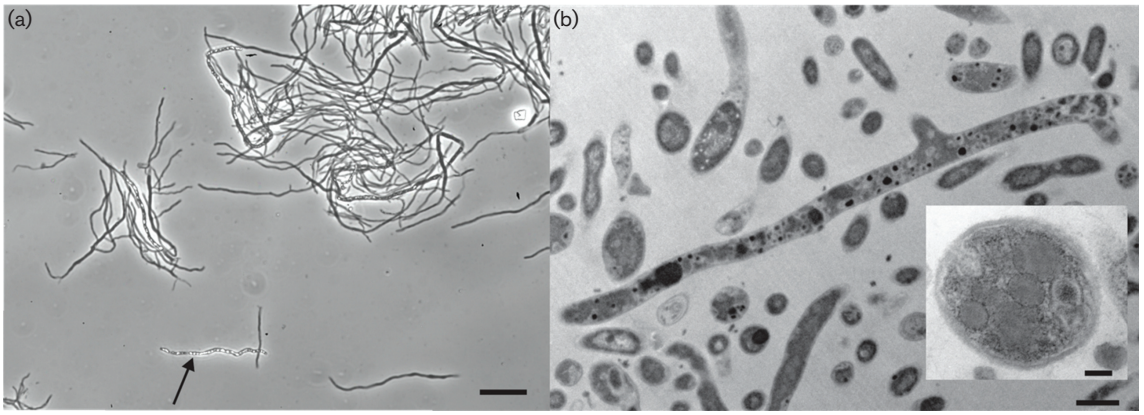

Fig. 1. (a) Wet mount of a 2-week-old culture of strain OB1^T grown on C10Y broth. The arrow at the bottom left illustrates the formation of chains of arthrospores. Bar, 10 µm. (b) Transmission electron micrograph of an ultrathin section of a 4-week-old culture grown on C10Y. Image illustrates a partial longitudinal section of a single filament, surrounded by cross-sections of many others. Bar, 500 nm. The inset shows an enlargement of a cross-section, illustrating the Gram-positive-type cell wall and intracellular inclusion bodies. Bar, 100 nm.

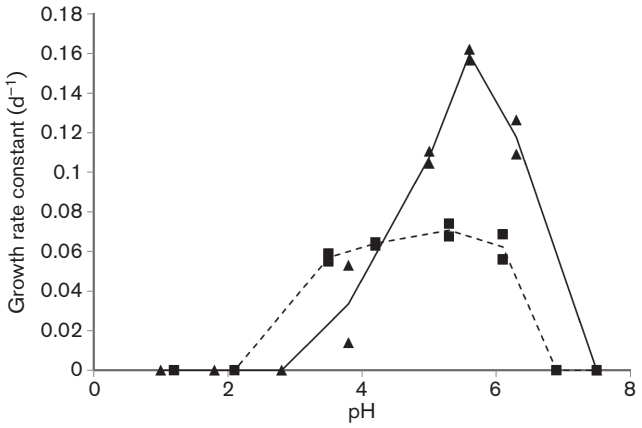

Fig. 2. Growth rate constants of strain OB1^T on media C10Y and R2A adjusted to different pH values, based on logarithmic regression of CO_2 production curves. Duplicate vials were made at each pH level. The x-axis shows the average pH in a vial based on measurements taken at the beginning and the end of the experiment (-▲-, 11 d for C10Y; -■-, 15 d for R2A).

In order to test the range of substrates that isolate OB1^T could use, duplicate 100 ml vials containing 20.0 ml C10 medium (pH 4.5) were supplemented with 0.05 % (w/v) of various sole carbon sources including mono- and polysaccharides, organic acids, alcohols and complex media additives. These were inoculated with 0.5 ml of a 2-week-old culture grown in R2A5 medium. Growth was recorded in comparison with a negative control after 14 d. Growth was assessed visually and was strongest on the complex media tested: yeast extract, nutrient broth, tryptic soy broth (Sigma), casamino acids (Fluka), proteose peptone (Fluka) and tryptone (Fisher Scientific). Growth was also observed on all mono- and dimeric sugars tested (D-glucose, sucrose, galactose, xylose, mannose and mannitol) and on branched polysaccharides (gellan, starch, pectin and xylan), as well as on the linear polysaccharide alginic acid. No growth was observed on agar or microcrystalline cellulose (Avicel). Growth was negative on all organic acids tested (pyruvate, succinate, benzoate, fumarate and acetate, all added as Na salts) and on all alcohols tested (methanol, ethanol and 2-propanol), although the sugar alcohol mannitol supported weak growth. Neither methane (5 %, v/v) nor elemental sulfur supported growth.

Growth in nitrogen-free medium (as an indication of nitrogen fixation) was examined in triplicate 100 ml vials containing 40 ml of 0.05 % (w/v) nitrogen-free C10 medium, with glucose as the sole carbon source. Sterile C-free and N-free medium was used to wash cells of a 2-week-old culture grown in C10Y three times before inoculation. Growth in N-free C10Y medium was not detectable compared with that in medium with added $NaNO_3$ (positive control), indicating that cells were not capable of N-fixation. A PCR targeting the *nifH* gene using primers considered universal to the gene [17, 18] failed to produce any amplification product, supporting the conclusion that strain OB1^T is incapable of N_2-fixation.

The potential for fermentative anaerobic growth in isolate OB1^T was determined in duplicate vials of C10 medium containing 0.05 % glucose. In addition, to test for anaerobic respiration via nitrate reduction, 20 mM $NaNO_3$ was added to duplicate vials of the medium. Vials were capped gas-tight with viton stoppers, and anaerobic conditions were established by evacuating the vials three times (10 min) and refilling with N_2 each time. The inoculum was washed with sterile C-free and N-free medium as above. Results were negative for all trials.

Production of antimicrobial substances was tested using disc diffusion and agar plug assays [19]. The test plates were prepared with 1-day-old cultures of *Escherichia coli* (DH5α) and *Bacillus subtilis* (DB2). Details are given in the Supplementary Information (available in the online Supplementary Material). No antimicrobial activity was detected.

Activities of various enzymes in isolate OB1^T were assayed using the API ZYM system (bioMérieux). Results concurred broadly with those of Brander and Jousimies-Somer [20] who examined the enzymic activity of 63 different strains of actinomycetes using the API ZYM system and determined that they all produced at least some β-galactosidase, leucine arylamidase and *N*-acetyl-β-glucosaminidase. Isolate OB1^T was also positive for these enzymes. As expected from its preference for acid conditions, isolate OB1^T was positive for acid phosphatase. Positive activity was also detected for alkaline phosphatase, valine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase and α-glucosidase, which are consistent observations with those of the related species *Catenulispora subtropica* and *Catenulispora yoronensis,* which belong to the same suborder as species of the genus *Actinospica* and OB1^T: the suborder *Catenulisporineae* [21]. However, unlike isolate OB1^T, these species were also positive for α-mannosidase. Activities of esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase, β-glucuronidase, β-glucosidase and α-fucosidase were negative in strain OB1^T.

Fatty acids were released by acid hydrolysis (1 M HCl in methanol) of cell material harvested from batch cultures grown in C10Y at the late exponential phase and analysed by GC/MS as described by Kulichevskaya *et al.* [22]. The positions of double bonds were determined by dimethyl disulfide derivatization [23]. Analysis of the intact polar lipids (IPLs) was performed by Bligh/Dyer extraction, and analysis by LC/MS as described by Kulichevskaya *et al.* [22]. The predominant fatty acids were i-$C_{16:0}$, i-$C_{15:0}$ and ai-$C_{15:0}$ (Table 1). The major IPL was phosphohexose, with smaller amounts of two unknown IPLs (Table 2). The fatty acid and IPL profiles of OB1^T were similar to those of the related bacteria *Actinospica robiniae* and *Actinospica acidiphila* (Tables 1 and 2).

Table 1. Fatty acid contents (percentage of total) of strain OB1^T in comparison with *Actinospica robiniae* GE134769^T and *Actinospica acidiphila* GE134766^T

Major fatty acids (>5 %) are shown in bold type. Only fatty acids representing more than 1.0 % of the total fatty acids of at least one of the strains are shown. *Actinospica durhamensis* CSCA57^T was not reanalysed here but is reported to have a nearly identical pattern as the other two strains of species of the genus *Actinospica* tested [26].

Fatty acid	OB1^T	GE134769^T	GE134766^T
i-$C_{14:0}$	**6.9**	3.6	4.2
i-$C_{15:0}$	**14.6**	**12.0**	**19.0**
ai-$C_{15:0}$	**14.3**	**11.1**	**14.0**
$C_{15:0}$	0.8	2.5	2.3
i-$C_{16:1\Delta9}$	1.2	1.1	1.0
i-$C_{16:0}$	**28.5**	**34.7**	**30.6**
$C_{16:1\Delta9}$	1.4	0.3	0.3
$C_{16:0}$	3.8	3.1	2.9
i-$C_{17:1\Delta9}$	2.0	3.7	2.8
ai-$C_{17:1\Delta9}$	1.7	1.5	1.2
i-$C_{17:0}$	2.6	1.2	3.2
ai-$C_{17:0}$	**9.5**	**7.6**	**11.6**
$C_{17:1\Delta9}$	1.9	4.7	1.5
$C_{17:0}$	3.8	4.9	2.5
i-$C_{18:1\Delta9}$	1.0	1.1	0.4
$C_{18:1\Delta9}$	1.1	2.8	0.6
$C_{18:1\Delta11}$	1.6	0.6	0.3
$C_{18:0}$	1.2	1.2	0.5
br.$C_{19:1}$	1.1	0.3	0.1

Isoprenoid quinones were extracted from lyophilized cells according to the method of Collins *et al.* [24], and the profile was analysed by HPLC (Shimadzu LC 20A) [25]. Strain OB1^T contained MK-11(H_2), MK-9(H_2), MK-10(H_2) and MK-9(H_6) with the approximate molar ratio of 82 : 11 : 3 : 2. Other members of the family *Actinospicaceae* show primarily MK-8 and/or MK-9 with different patterns of partial hydrogenation [6, 26].

The peptidoglycan of strain OB1^T was isolated by mechanical disruption of cells followed by trypsin digestion as well as treatment with SDS, and the structure was elucidated according to published protocols [27]. Amino acids and peptides in cell-wall hydrolysates were analysed by two-dimensional TLC on cellulose plates by using previously described solvent systems [28]. The molar ratio of the amino acids was determined by GC/MS according to Protocol 10 of Schumann [27]. The analysis of isomers of 2,6-diaminopimelic acid was done by TLC as described by Rhuland *et al.* [29], and the amino acids were detected by spraying with ninhydrin reagent. *meso*-Diaminopimelic acid (Dpm) was detected as the diagnostic diamino acid of the peptidoglycan of strain OB1^T; Ala and Glu were found in addition (Fig. S1). The molar ratio of these amino acids was 0.8 *meso*-Dpm : 2.6 Ala : 1.0 Glu. The occurrence of *meso*-Dpm has been reported only for the peptidoglycan type A1γ and for three variations of peptidoglycan type A4γ (A31.1, A31.2 and A31.3, see www.peptidoglycan-types.info). The 2D-TLC pattern of the partial hydrolysate of the cell wall showed Dpm–D-Ala and L-Ala–D-Glu as the only peptides (Fig. S1). Additional peptides characteristic of peptidoglycan type A4γ variations were not found. The peptide pattern detected and the molar amino acid ratio were in agreement with the peptidoglycan type A1γ. The peptidoglycan composition differed markedly from species of the genus *Actinospica*, which contain 3-hydroxydiaminopimelic acid (3-OH Dpm) as the major diamino acid and

Table 2. Relative abundance and fatty acid composition of IPLs of strain OB1^T in comparison with *Actinospica robiniae* GE134769^T and *Actinospica acidiphila* GE134766^T

Abundance is given relative to the major peak in the LC-MS chromatogram (+++, base peak; ++, 50–100 % of base peak; +, 10–50 % of base peak). Note that the mass spectral response factors for different IPL groups can be quite different. The predominant fatty acid composition is reported as the total number of carbon atoms of the diacyl moieties and the number of double bonds.

IPL*	OB1^T	GE134769^T	GE134766^T
PG		+	
		(33 : 1)	
PE		+	
		(33 : 1)	
Unknown†	++	+	+++
	(31 : 0, 30 : 0, 32 : 0)	(30 : 0, 31 : 0, 32 : 0)	(30 : 0, 31 : 0, 32 : 0)
Phex	+++	+++	++
	(31 : 0, 32 : 0, 30 : 0)	(31 : 0, 32 : 0, 30 : 0)	(31 : 0, 32 : 0, 30 : 0)
Unknown‡	+	+	+
	(31 : 0, 32 : 0, 30 : 0)	(32 : 0, 31 : 0)	(31 : 0, 32 : 0, 30 : 0)

*Listed in order of elution; PG, phosphoglycerol; PE, phosphoethanolamine; Phex, phosphohexose.

†Unknown IPL characterized by M^+- 129.

‡Unknown IPL with high molecular mass.

only traces of *meso*-Dpm [6]. 3-OH Dpm could not be detected in strain OB1^T.

The DNA G+C content of strain OB1^T was determined by sequencing of genomic DNA using the Illumina MiSeq platform. A library with a fragment length of 600 bp was constructed, and the paired-end sequencing was performed using MiSeq reagent kit version 3 with 600 cycles. The reads were assembled with Velvet *de novo* Assembly version 1.0.0 [30]. The draft genome of strain OB1^T consisted of 6 205 455 bases in 1770 contigs, with the estimated genome size of 6.2 Mbps and a DNA G+C content of 70.2 mol%.

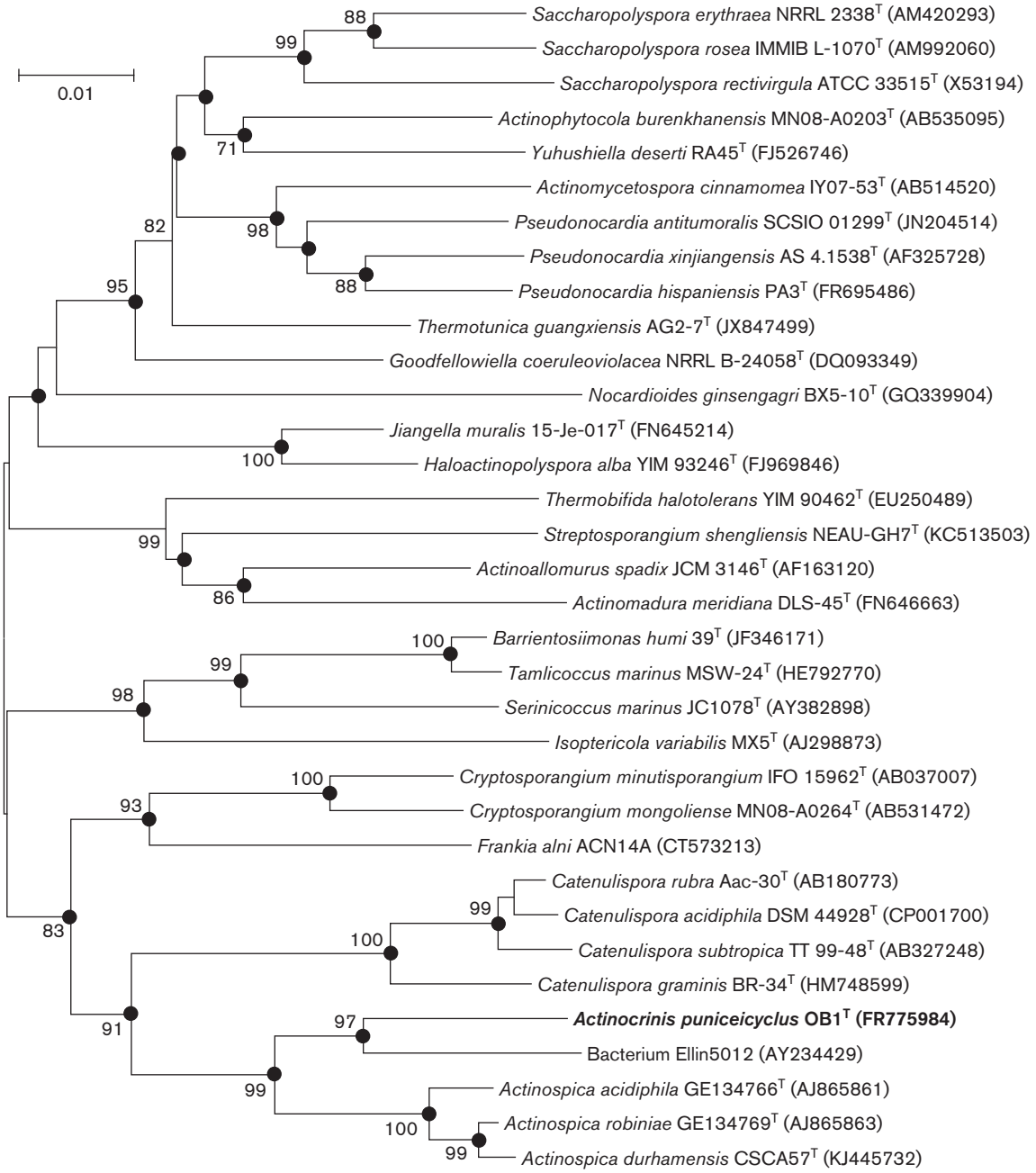

Fig. 3. Phylogenetic tree based on the nearly complete (1433 bp) partial 16S rRNA gene sequence of strain OB1^T, showing its position in comparison with selected members of the phylum *Actinobacteria*. Bar, 0.01 substitutions per nucleotide position. The tree was reconstructed by neighbour-joining with a Jukes–Cantor correction. Bootstrap values (1000 data resamplings) are shown when >70 % for selected nodes showing the relationship of OB1^T to the genera *Actinospica* and *Catenulispora*. Filled circles indicate that the corresponding nodes were also recovered in the maximum-parsimony and maximum-likelihood trees.

PCR amplification of a fragment of the 16S rRNA gene was performed as previously published [16]. The 1433 bp amplicon was purified with the EZ-10 Spin Column PCR Products Purification kit (Bio Basic) and sequenced on an Applied Biosystems 3730 XL (96 capillary) sequencer. There were two large insertions of 14–19 bp that did not align with the sequences of closely related species, one found at *E. coli* base position 208, and the other found at base position 456. According to a BlastN analysis, the most closely related organism to isolate OB1^T with a 16S rRNA gene sequence identity of 92.8 % was bacterium Ellin5012 (GenBank acc. no. AY234429), which was isolated from a pasture of perennial ryegrass (*Lolium perene*) and white clover (*Trifolium repens*) at the Dairy Research Institute, Ellinbank, Victoria, Australia [12]. The closest relative with a validly published name, showing 92.8 % homology, was *Actinospica robiniae* (AJ865863) [6]. These identities increased by up to 2.3 % if the insertions in OB1^T were ignored.

Phylogenetic analysis was carried out as described by Kim *et al.* [31]. The nearly complete 16S rRNA gene sequence was aligned with sequences of closely related strains, and phylogenetic trees for the datasets were inferred from the neighbour-joining, maximum-parsimony and maximum-likelihood methods using MEGA version 6.0 [32, 33]. Distances were calculated based on the neighbour-joining method according to the Jukes–Cantor model. The stability of relationships was assessed by a bootstrap analysis of 1000 datasets (Fig. 3). Phylogenetic analysis placed isolate OB1^T within the family *Actinospicaceae* of the suborder *Catenulisporineae*, in the order *Actinomycetales* of the phylum *Actinobacteria* [34]. The family *Actinospicaceae* is currently composed of three species with validly published names: *Actinospica robiniae* (GenBank acc. no. AJ865863) and *Actinospica acidiphila* (AJ865861) isolated from forest soils in Gerenzano, Italy [6], and *Actinospica durhamensis* CSCA57^T from a spruce forest soil in Durham, England [26].

Compared with the closely related strains of the genus *Actinospica*, strain OB1^T is similar in many aspects. All are non-motile, produce chains of arthrospores at maturity, have similar growth substrates and cannot reduce nitrate. However, chemotaxonomically, OB1^T displays key differences from species of the genus *Actinospica* (Table 3): (i) MK-11 is the major quinone in OB1^T while starins of *Actinospica* lack this quinone, (ii) OB1^T contains *meso*-Dpm but lacks 3-hydroxydiaminopimelic acid present in all species of the genus *Actinospica*, and (iii) all species have distinct IPL compositions. Strain OB1^T differs in several physiological properties (Table 3), including production of pink to purple pigments, and growth at lower pH and temperature. Strain OB1^T tolerates pH values as low as pH 3.5 as opposed to only pH 4.0–4.8 in species of the genus *Actinospica*. Comparing OB1^T with the type strains of species of the genus *Actinospica*: all have i-$C_{16:0}$, i-$C_{15:0}$ and ai-$C_{15:0}$ as the major cellular fatty acids but the relative contents of these differ, and other minor fatty acids differ. These factors combined with the low 16S rRNA gene sequence identity (92.8 % maximum; or 95.0 % ignoring two large insertions) of OB1^T to species of the genus *Actinospica* support the description of strain OB1^T as a representative of a novel genus and species.

Table 3. Major characteristics distinguishing OB1^T from *Actinospica robiniae* GE134769^T, *Actinospica acidiphila* GE134766^T and *Actinospica durhamensis* CSCA57^T

Data for *Actinospica robiniae* and *Actinospica acidiphila* were taken from Cavaletti *et al.* [6] except for the polar lipids, which were reanalysed here. Data for *Actinospica durhamensis* CSCA57^T were from Golinska *et al.* [26]. opt., Optimum; NR, not reported in detail (but see footnotes). Phex, phosphohexose; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phophatidylinositol.

Characteristic	OB1^T	GE134769^T	GE134766^T	CSCA57^T
Colony colour	Purple	Light yellowish, brown	Beige	Variable: white, yellow, yellowish brown, greenish
Soluble pigments	Pink-to-purple	Absent	Absent or greenish	Absent
Growth temperature (°C)	13–30, opt. 20–25	17–33, opt. 22–28	17–33, opt. 28	15–33, opt. 28
Growth pH	3.5–6.5, opt. 5.5	4.8–6.2, opt. 5.5	4.2–6.0, opt. 5.0	4.0–6.0, opt. 5.5
Growth on NaCl	0–1.4 %	1 % not tolerated	1 % tolerated	1 % tolerated, 3 % not tolerated
Casein hydrolysis	Positive	Trace	Trace	Negative
Peptidoglycan amino acids	*meso*-Dpm, Ala, Glu	3-OH Dpm, Gly, Glu, Ala, *meso*-Dpm (trace)	3-OH Dpm, Gly, Glu, Ala *meso*-Dpm (trace)	NR*
Quinones	MK-11(H_2), MK-9(H_2) (82 : 11)	MK-9(H_4), MK-9(H_6), MK-9(H_8) (23 : 40 : 20)	MK-9(H_4), MK-9(H_6), MK-9(H_8) (2 : 25 : 59)	NR†
Polar lipids	Phex	Phex	PG, PE, Phex	DPG, PG, PE, PI
DNA G+C content	70.2 mol%	70.8 mol%	69.2 mol%	68.0 mol%

*Reported to include 3-OH Dpm, *meso*-Dpm [26].

†Reported to consist of di, tetra-, hexa- and octa-hydrogenated menaquinones in the ratio (5 : 22 : 40 : 30) [26].

DESCRIPTION OF *ACTINOCRINIS* GEN. NOV.

Actinocrinis (Ac.ti.no.cri′nis. Gr. n. *aktis -inos* ray; L. masc. n. *crinis* the hair, an object resembling hair; N.L. masc. n. *Actinocrinis* actinomycete with long hair-like filaments).

Gram-stain-positive, filamentous cells, 15–25 µm in length and 1–1.5 µm in diameter. Non-motile. Form chains of spores in final stages of maturation. Major fatty acids are i-$C_{15:0}$, ai-$C_{15:0}$ and i-$C_{16:0}$. Contain phosphohexose as a major IPL. The peptidoglycan type is A1γ based on *meso*-Dpm. Major respiratory quinone is menaquinone-11. Aerobic; do not ferment or reduce nitrate. Do not fix N_2. Form spherical clumps of purple-to-pink-pigmented colonies when grown in suspended media. Acidophiles with pH optimum approximately pH 5.5, range pH 3.5–6.5. Temperature optimum at approximately 25 °C, range 13–30 °C. Phylogenetically most closely related to species of the genus *Actinospica*. Members of the suborder *Catenulisporineae* of the order *Actinomycetales* of the phylum *Actinobacteria*. The G+C content (mol%) of the DNA is 70.2 mol%. The genus contains one species, *Actinocrinis puniceicyclus* OB1^T.

DESCRIPTION OF *ACTINOCRINIS PUNICEICYCLUS* SP. NOV.

Actinocrinis puniceicyclus (pu.ni.ce.i.cy′clus. L. adj. *puniceus* reddish, red, purple-coloured; L. masc. n. *cyclus* a circle; N. L. masc. n. *puniceicyclus* purple circle.).

General characteristics are those given in the genus description. Grows optimally on protein digests and yeast extract. Grows on diverse mono- and polysaccharides, including D-glucose, sucrose, galactose, xylose, mannose, mannitol, gellan, starch, pectin, xylan and alginic acid. Does not grow on organic acids or alcohols. Positive for acid phosphatase, alkaline phosphatase, β-galactosidase, leucine arylamidase, *N*-acetyl-β-glucosaminidase, valine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase and α-glucosidase. Negative for α-mannosidase, esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase, β-glucuronidase, β-glucosidase and α-fucosidase. No antimicrobial activity detected.

The type strain is OB1^T(=DSM 45618^T=ATCC BAA-2771^T), which was isolated from an acidic bog in Kootenay National Park, Canada. The G+C content of the genomic DNA of the type strain is 70.2 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.
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