Kribbella albertanoniae sp. nov., isolated from a Roman catacomb, and emended description of the genus Kribbella

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A novel actinobacterium, strain BC640T, was isolated from a biofilm sample collected in 2009 in the Saint Callistus Roman catacombs. Analysis of the 16S rRNA gene sequence showed that the strain belonged to the genus Kribbella. Phylogenetic analysis using the 16S rRNA gene and concatenated gyrB, rpoB, relA, recA and atpD gene sequences showed that strain BC640T was most closely related to the type strains of Kribbella yunnanensis and Kribbella sandramycini. Based on gyrB genetic distance analysis, strain BC640T was shown to be distinct from all Kribbella type strains. DNA–DNA hybridization experiments confirmed that strain BC640T represents a genomic species distinct from its closest phylogenetic relatives, K. yunnanensis DSM 15499T (53.5 ± 7.8 % DNA relatedness) and K. sandramycini DSM 15626T (33.5 ± 5.0 %). Physiological comparisons further showed that strain BC640T is phenotypically distinct from the type strains of K. yunnanensis and K. sandramycini. Strain BC640T (=DSM 26744T=NRRL B-24917T) is thus presented as the type strain of a novel species, for which the name Kribbella albertanoniae sp. nov. is proposed.

The genus Kribbella was proposed by Park et al. (1999) and contains nocardiform actinobacteria with L-L-diaminopimelic acid in the cell-wall peptidoglycan. The most recent development in the study of the genus is the publication of a multilocus sequence analysis (MLSA) method that can be used to assess whether an environmental Kribbella isolate belongs to a novel species, without the need for DNA–DNA hybridization (DDH) experiments (Curtis & Meyers, 2012). The MLSA study extended previous phylogenetic analysis of Kribbella type strains using the gyrB gene alone (Kirby et al., 2010). With the description of Kribbella amoyensis (Xu et al., 2012) and Kribbella endophytica (Kaewkla & Franco, 2013), there are 18 Kribbella species with validly published names at the time of writing (Euzéby, 2012). Here, we describe the characterization of a novel member of the genus, isolated from the same site as the previously described species Kribbella catacumba and K. sancticallisti in the Saint Callistus catacomb in Rome (Urzi et al., 2008).

Strain BC640T was isolated from site CSC13 from a dark-green biofilm by using the adhesive tape (Fungi Tape DID) technique (Urzi & De Leo, 2001). Growth of colonies (quantified as the number of c.f.u. per cm² of adhesive tape) was carried out on R2A medium (Reasoner & Geldreich, 1985) at 28 °C for 15 days. Ten to 20 colonies were selected randomly, preliminarily characterized after transferring to tryptic soy agar (TSA; BBL) and subsequently maintained on yeast extract-malt extract agar [International Streptomyces Project (ISP) medium 2] (Shirling & Gottlieb, 1966).

Genomic DNA was extracted as described by Everest & Meyers (2008). The 16S rRNA gene was amplified as described by Cook & Meyers (2003), the gyrB gene as described by Kirby et al. (2010) and the atpD, recA, relA and rpoB genes as described by Curtis & Meyers (2012). Approximately 500 ng template DNA was used in the PCR amplification of the 16S rRNA and gyrB genes, with 1 μg DNA being used for amplification of the atpD, recA, relA and rpoB genes. PCR products were purified using an MSB Spin PCRapace kit (Invitrogen) and sequenced (Macrogen). Sequence analysis was performed using DNAMAN version...
5.2.9 (Lynnon BioSoft). MEGA version 5.05 (Tamura et al., 2011) was used to conduct phylogenetic analyses and to calculate the gyrB and concatenated-gene genetic distances using Kimura’s two-parameter model (Kimura, 1980). Phylogenetic trees were reconstructed using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Takahashi & Nei, 2000) and neighbour-joining (Saitou & Nei, 1987) methods.

All ISP media were prepared according to Shirling & Gottlieb (1966). Morphological properties were determined on ISP 2 and inorganic salts-starch agar (ISP 4). Physiological tests were carried out as described by Williams et al. (1989). All plates were incubated at 30 °C, for the recommended periods, unless stated otherwise. Carbon-source utilization was tested according to the methods of Shirling & Gottlieb (1966), with all carbon sources being filter-sterilized and tested at final concentrations of 1 % (w/v), with the exception of the sodium salts, which were tested at 0.1 % (w/v). Nitrogen-source utilization was performed according to Williams et al. (1989). All nitrogen sources were filter-sterilized and tested at final concentrations of 0.1 % (w/v). NaCl tolerance was determined on ISP 2 medium incubated for 14 days. Growth at pH 4.3, 7 and 10 and at 20, 30, 37 and 45 °C was assessed on Bennett’s medium (Atlas, 2004), incubated for 14 days.

Analysis of the isomer of diaminopimelic acid and the whole-cell sugar pattern was conducted as described by Hasegawa et al. (1983). For the whole-cell sugar analysis, the solvent system used was ethyl acetate/pyridine/distilled water (100 : 35 : 25, by vol.). The phospholipid pattern was determined as described by Komaga & Suzuki (1987) and Minnikin et al. (1984) using p-anisaldehyde, α-naphthol, ninhydrin and molybdenum blue reagents. Analysis of the respiratory quinones was carried out by the Identification Service of the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). All these chemotaxonomic analyses were performed on freeze-dried cells of a culture of strain BC640T grown in ISP 2 broth, with moderate shaking, at 30 °C for 3 days. Fatty acid analysis was performed as a service by the BCCM/LMG Culture Collection, according to the recommendations of the commercial identification system MIDI (Microbial Identification System, Inc.) [MIDI Sherlock

![Phylogenetic tree](image)

**Fig. 1.** 16S rRNA gene phylogenetic tree showing the position of strain BC640T within the genus Kribbella. The tree was reconstructed using the maximum-likelihood method based on 1208 bp sequences. Values at nodes are percentage bootstrap values from 1000 replications (only values above 70 % are shown); asterisks (*) indicate clades that were conserved in the maximum-likelihood, neighbour-joining and maximum-parsimony trees. Accession numbers are indicated in parentheses. Bar, 1 substitution per 100 nucleotide positions. *Microlunatus phosphovorus* NM-1T was used as an outgroup.
DDH analysis was performed as a service by the BCCM/LMG Culture Collection. DNA was isolated using a modification of the method of Gevers et al. (2001) using a combination of lysozyme, mutanolysin and lysostaphin for cell lysis. DDH was performed in the presence of 50 % formamide at 51 °C, according to a modification of the method described by Ezaki et al. (1989).

A query against the EzTaxon-e server (Kim et al., 2012) with 1465 bp of 16S rRNA gene sequence indicated that strain BC640T belonged to the genus Kribbella and was most closely related to Kribbella sandramycini ATCC 39419\(^\top\) (99.18 % sequence similarity over 1455 bp, by local alignment in DNAMAN) and Kribbella yunnanensis YIM 30006\(^\top\) (99.11 %, 1464 bp, DNAMAN). This association was confirmed by reconstructing a 16S rRNA gene maximum-likelihood phylogenetic tree in which BC640\(^\top\) grouped with K. yunnanensis and K. sandramycini with moderate bootstrap support (82 %) (Fig. 1). This association was also observed in the neighbour-joining and maximum-parsimony phylogenetic trees. Phylogenetic analysis based on the concatenated gyrB–rpoB–recA–relA–atpD gene sequences strongly supported the association of strain BC640\(^\top\) with the type strain of K. yunnanensis (bootstrap value of 99 % in the maximum-likelihood tree). Kribbella antibiotica DSM 15501\(^\top\) (98.57 % 16S rRNA gene sequence similarity over 1464 bp in DNAMAN) was also closely associated with these two strains, but the type strain of K. sandramycini was not associated with strain BC640\(^\top\) in the concatenated gyrB–rpoB–recA–relA–atpD gene tree (Fig. 2). The association of K. antibiotica, strain BC640\(^\top\) and K. yunnanensis was also observed in the neighbour-joining and maximum-parsimony phylogenetic trees, and was strongly supported by a bootstrap value of 91 % in the maximum-likelihood tree.

Genetic distances were calculated between strain BC640\(^\top\) and all Kribbella type strains to assess the likelihood of this strain representing a novel genomic species. The gyrB-based genetic distances ranged from 0.05 to 0.101, with those between strain BC640\(^\top\) and the type strains of K. yunnanensis, K. sandramycini and K. antibiotica being 0.05, 0.101 and 0.085, respectively. These values are all above the 0.04 gyrB genetic distance threshold proposed to represent novel species in the genus (Kirby et al., 2010). The concatenated five-gene genetic distances ranged from 0.031 to 0.109. The values between strain BC640\(^\top\) and the type strains of K. yunnanensis, K. sandramycini and K. antibiotica were 0.031, 0.066 and 0.051, respectively. The proposed threshold for this concatenated gene, above which strains can be assumed to belong to distinct genomic species, is 0.04 (Curtis & Meyers, 2012). As the concatenated-gene genetic distance between strain BC640\(^\top\) and K. yunnanensis DSM 15499\(^\top\) was <0.04, DDH analysis was required in
DDH experiments revealed that strain BC640<sup>T</sup> shared 33.5 ± 5.0% DNA relatedness with <i>K. sandramycini</i> DSM 1562<sup>T</sup> and 53.5 ± 7.8% with <i>K. yunnanensis</i> DSM 15499<sup>T</sup>. Strain BC640<sup>T</sup> thus represents a separate genomic species when the threshold value of 70% DNA relatedness by DDH is used to delineate bacterial species (Wayne et al., 1987). DDH analysis between strain BC640<sup>T</sup> and the type strain of <i>K. antibiotica</i> was not performed, as the genetic distances between these strains were above the thresholds for distinguishing genomic species using both the <i>gyrB</i> and concatenated-gene sequences.

Physiological characterization of strain BC640<sup>T</sup> showed that it was phenotypically distinct from the type strains of <i>K. antibiotica</i>, <i>K. sandramycini</i> and <i>K. yunnanensis</i>. The results of the phenotypic characterization of strain BC640<sup>T</sup> are presented in Table 1 and in the species description. Chemotaxonomic analysis of strain BC640<sup>T</sup> showed that it had chemotaxonomic characteristics that are consistent with membership of the genus <i>Kribbella</i>: L-1-diaminopimelic acid as the diagnostic diamino acid in the peptidoglycan, a phospholipid pattern characterized by the presence of phosphatidylcholine (and the absence of phosphatidylethanolamine), MK-9(H<sub>4</sub>) as the major menaquinone (70%), with minor amounts of MK-7(H<sub>4</sub>) (4%), MK-9(H<sub>6</sub>) (4%), MK-9 (2%) and MK-9(H<sub>2</sub>) (2%), and anteiso-C<sub>15:0</sub> as the predominant fatty acid.

**Emended description of the genus <i>Kribbella</i> Park et al. 1999 emend. Sohn et al. 2003**

The genus description is as given by Park et al. (1999) and emended by Sohn et al. (2003) with the following changes. The polar lipid pattern contains phosphatidylcholine; most strains also contain phosphatidylglycerol. There are no diagnostic sugars present in the whole-cell sugar pattern. The DNA G+C content ranges from 66 to 77 mol%. Oxidase and urease activities are variable.

**Description of <i>Kribbella albertanoniae</i> sp. nov.**

<i>Kribbella albertanoniae</i> (al.ber.ta.no.9.ni.æ. N.L. fem. gen. n. <i>albertanoniae</i> of Albertano, named after Professor Patrizia Albertano).

Gram-stain-positive, catalase-positive, oxidase-negative, non-motile actinobacterium. Colonies appear convoluted with irregular edges on most media. Vegetative mycelium appears cream to orange in colour, with the pigmentation

### Table 1. Phenotypic characteristics that allow differentiation of strain BC640<sup>T</sup> from type strains of closely related <i>Kribbella</i> species

Reference strains: 1, <i>K. yunnanensis</i> DSM 15499<sup>T</sup>; 2, <i>K. sandramycini</i> DSM 1562<sup>T</sup>; 3, <i>K. antibiotica</i> DSM 15501<sup>T</sup>. All data were determined in this study. Supporting and conflicting (in parentheses) data from other studies are indicated. +++, Strongly positive; +, positive; +w, weakly positive; −, negative.

<table>
<thead>
<tr>
<th>Test</th>
<th>BC640&lt;sup&gt;T&lt;/sup&gt;</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tr>
<td>Nitrate reduction</td>
<td>−</td>
<td>(+)</td>
<td>−</td>
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<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;S production</td>
<td>+</td>
<td>(−)</td>
<td>(+)</td>
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<td>Degradation of:</td>
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<td>Allantoin</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>Gelatin</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Guanine</td>
<td>+w</td>
<td>+w</td>
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<tr>
<td>Hypoxanthine</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Urea</td>
<td>−</td>
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<td>Xylan</td>
<td>+</td>
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<td>Hydrolysis of starch</td>
<td>+w</td>
<td>+</td>
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<td>Growth at:</td>
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<td>37 °C</td>
<td>+w</td>
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<td>−</td>
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<td>pH 4.3</td>
<td>+w</td>
<td>+w</td>
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<td>pH 10</td>
<td>+w</td>
<td>+</td>
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<td>Utilization as sole carbon source</td>
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<td>(+)-L-Arabinose</td>
<td>+</td>
<td>+ (+)</td>
<td>− (+w)</td>
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<tr>
<td>Inulin</td>
<td>−</td>
<td>+</td>
<td>+ w (+)</td>
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<tr>
<td>Lactose</td>
<td>+</td>
<td>+ (+)</td>
<td>+ (+)</td>
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<td>(+)-D-Mannose</td>
<td>+</td>
<td>+ (+)</td>
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<tr>
<td>Sodium acetate</td>
<td>+w</td>
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<td>Sodium citrate</td>
<td>+w</td>
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<td>Utilization as sole nitrogen source</td>
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<td>L-Phenylalanine</td>
<td>+</td>
<td>−</td>
<td>+w</td>
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*Data taken from: a, Kaewkla & Franco (2013); b, Li et al. (2004); c, Li et al. (2006); d, Park et al. (1999); e, Trujillo et al. (2006).*
being temperature-dependent (orange pigment not produced at 20 °C, but produced at 28 °C and above). Hyphae are highly branched and fragment in both liquid and agar cultures. Aerial mycelium appears white on ISP 4. A yellow diffusible pigment is produced on ISP 5. Melanin is not produced on peptone-yeast extract-iron agar (ISP 6) or tyrosine agar (ISP 7). Nitrate is not reduced to nitrite. Produces H2S. Utilizes adonitol, (+)-L-arabinose, (+)-cellobiose, (-)-D-fructose, (+)-D-glucose, myo-inositol, lactose, (+)-D-mannose, (-)-D-mannitol, melibiose, raffinose, rhamnose, sucrose and (+)-D-xylene as sole carbon sources, with weak growth on salicin, sodium acetate and sodium citrate. Unable to utilize inulin as a sole carbon source. Utilizes L-arginine, L-asparagine, L-histidine, 4-hydroxy-L-proline, L-phenylalanine, potassium nitrate, L-serine, L-threonine and L-valine as sole nitrogen sources, with weak growth on DL-α-amino-n-butyric acid, L-cysteine and L-methionine. Grows at 20 and 30 °C and grows weakly at 37 °C, but does not grow at 45 °C. Grows optimally at pH 7 and weakly at pH 4.3 and 10. Starch is hydrolysed weakly. Adenine, aesculin, casein, gelatin, hypoxanthine, Tween 80, L-tyrosine and xylan are degraded. Butatin and guanine are degraded weakly. Allantoin, urea and xanthine are not degraded. Grows in the presence of 2% (w/v) NaCl, not at 5% (w/v) NaCl. The cell-wall peptidoglycan are not degraded. Grows in the presence of 2% (w/v) NaCl, guanine are degraded weakly. Allantoin, urea and xanthine are highly branched and fragment in both liquid and agar cultures. Aerial mycelium appears white on ISP 4. A yellow diffusible pigment is produced on ISP 5. Melanin is not produced on peptone-yeast extract-iron agar (ISP 6) or tyrosine agar (ISP 7). Nitrate is not reduced to nitrite. Produces H2S. Utilizes adonitol, (+)-L-arabinose, (+)-cellobiose, (-)-D-fructose, (+)-D-glucose, myo-inositol, lactose, (+)-D-mannose, (-)-D-mannitol, melibiose, raffinose, rhamnose, sucrose and (+)-D-xylene as sole carbon sources, with weak growth on salicin, sodium acetate and sodium citrate. Unable to utilize inulin as a sole carbon source. Utilizes L-arginine, L-asparagine, L-histidine, 4-hydroxy-L-proline, L-phenylalanine, potassium nitrate, L-serine, L-threonine and L-valine as sole nitrogen sources, with weak growth on DL-α-amino-n-butyric acid, L-cysteine and L-methionine. Grows at 20 and 30 °C and grows weakly at 37 °C, but does not grow at 45 °C. Grows optimally at pH 7 and weakly at pH 4.3 and 10. Starch is hydrolysed weakly. Adenine, aesculin, casein, gelatin, hypoxanthine, Tween 80, L-tyrosine and xylan are degraded. Butatin and guanine are degraded weakly. Allantoin, urea and xanthine are not degraded. Grows in the presence of 2% (w/v) NaCl, not at 5% (w/v) NaCl. The cell-wall peptidoglycan contains L-diaminopimelic acid and glycine (chemotype I; Lechevalier & Lechevalier, 1970). Glucose, ribose and an unidentified pentose are present in the whole-cell sugar hydrolysate. The polar lipid profile includes diphostidylglycerol, phosphatidylcholine, phosphatidylglycerol, phosphatidylglycerol, phosphatidylglycerol, eight unidentified phospholipids, four unidentified aminolipids, five unidentified glycolipids, three unidentified aminoglycolipids and two unidentified phosphoglycolipids (Fig. S1). The major menaquinone is MK-9(H₄), with minor amounts of MK-7(H₂), MK-9(H₄), MK-9 and MK-9(H₂). The major fatty acids (present in proportions >10% of total fatty acids) are anteiso-C₁₅:₀, iso-C₁₅:₀ and iso-C₁₆:₀. The full fatty acid profile of the type strain is shown in Table S2.

The type strain, BC640T (=DSM 26744T=NRRL B-24917T), was isolated from site CSC13 of the Saint Callistus catacomb in Rome.

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