Kribbella catacumbae sp. nov. and *Kribbella sancticallisti* sp. nov., isolated from whitish-grey patinas in the catacombs of St Callistus in Rome, Italy

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Several nocardioform actinomycetes were isolated from tufaceous surfaces with whitish-grey patinas in the catacombs of St Callistus in Rome, Italy. The morphology of the isolates and their chemotaxonomic characteristics such as LL-diaminopimelic acid in the cell-wall peptidoglycan, the major menaquinone of MK-9(H₄), phosphatidylinositol, phosphatidylcholine, phosphatidylglycerol and diphosphatidylglycerol as the major polar lipids, as well as complex cellular fatty acid patterns with anteiso-C_{15:0}, iso-C_{16:0} and iso-C_{15:0} as predominating components, were in agreement with their classification as members of the genus *Kribbella* by 16S rRNA gene sequence analysis. The isolates fell into two clusters as revealed by their ribosomal intergenic spacer, RiboPrint and cellular fatty acid patterns and by their MALDI-TOF mass spectra. The two clusters were represented by the strains BC631^T and BC633^T which shared 97.9% 16S rRNA gene sequence similarity. Strain BC631^T represented a cluster of yellow pigmented strains and was a phylogenetic neighbour of *Kribbella koreensis* DSM 17837^T (gene sequence similarity 98.0%), while strain BC633^T was related to *Kribbella flavida* DSM 17836^T and *Kribbella karoensis* DSM 17344^T (gene sequence similarities of 98.8% and 98.6%, respectively). Strains BC631^T and BC633^T could be differentiated from each other and from their closest phylogenetic neighbours by phenotypic characteristics and DNA–DNA relatedness values far below 70%. It is concluded that the two new strains represent two novel species, for which the names *Kribbella catacumbae* sp. nov. (type strain BC631^T=DSM 19601^T=JCM 14968^T) and *Kribbella sancticallisti* sp. nov. (type strain BC633^T=DSM 19602^T=JCM 14969^T) are proposed.

Hypogean environments such as catacombs support the growth of different bacteria which often give rise to green, whitish-grey or black biological patinas and biodeterioration (Urzì *et al.*, 2003). These sites are rich sources for the

Abbreviations: LL-Dpm, LL-2,6-diaminopimelic acid; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; RISA, ribosomal intergenic spacer analysis; SEM, scanning electron microscopy.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of the strains BC627 to BC635 are AM778571–AM778579, respectively.

Supplementary figures showing SEM images of cells of strains BC633^T and BC631^T, a Euclidean distance dendrogram based on the fatty acid profiles of members of the genera *Kribbella*, a gel of the ITS PCR products of strains of the genus *Kribbella*, a dendrogram based on *Pvu*II RiboPrint patterns and a dendrogram generated by BioTyper software based on MALDI-TOF MS spectra are available with the online version of this paper.

isolation of novel bacterial taxa as demonstrated by several recent publications (Groth *et al.*, 2005, 2006; Jurado *et al.*, 2005a, b). Among chemo-organotrophic bacteria, Gram-positive bacteria and especially actinobacteria (Stackebrandt *et al.*, 1997) are the most common organisms, being isolated as high percentages (>70%) of the total of cultivable bacteria (Groth & Saiz Jimenez, 1999; Urzì *et al.*, 2003).

In the course of an isolation program as part of the EC project CATS (<http://www2.bio.uniroma2.it/biologia/laboratori/lab-botanica/Algae/CATS.htm>), several strains were found among the new isolates which could be affiliated to the genus *Kribbella*.

The genus *Kribbella* belongs to the family *Nocardioideaceae*. It was established by Park *et al.* (1999) to accommodate nocardioform actinobacteria that contain LL-2,6-diaminopimelic acid (LL-Dpm) in the cell wall. Since then, 13 novel

species of *Kribbella* have been described: *Kribbella flavida*, *Kribbella sandramycini* (Park *et al.*, 1999), *Kribbella solani* and *Kribbella jejuensis* (Song *et al.*, 2004), *Kribbella antibiotica* (Li *et al.*, 2004), *Kribbella lupini* (Trujillo *et al.*, 2006), *Kribbella yunnanensis* and *Kribbella alba* (Li *et al.*, 2006), and *Kribbella karoonensis* and *Kribbella swartbergensis* (Kirby *et al.*, 2006). *Kribbella koreensis*, described first as *Hongia koreensis* by Lee *et al.* (2000), was transferred to the genus *Kribbella* by Sohn *et al.* (2003). The geographical distribution of the strains is wide as they have been isolated in Korea, China, South Africa and Spain, mostly from soil, but also associated with plants (*K. solani* and *K. lupini*). Recently, two further species of the genus *Kribbella* were isolated from soil, *Kribbella aluminosa* from a medieval mine in Germany (Carlssohn *et al.*, 2007) and *Kribbella hippodromi* (Everest & Meyers, 2008) from a racecourse soil in South Africa.

We describe two additional novel species of the genus *Kribbella* that were isolated from deteriorated surfaces in the St. Callistus Catacombs in Rome, Italy. Nine strains were isolated from two different sites in the same room (Ocean Cubicle, CSC13), designated CSC13a and CSC13b, and both characterized by a tufaceous substrate and whitish-grey patina.

Samples taken from the altered surfaces were suspended (1:10) in physiological solution (0.9 % NaCl in distilled water) with 0.001 % of Tween 80. Further decimal dilutions were prepared and 1 ml of each dilution was included in the BRII medium solidified by agar (Urzi *et al.*, 2001).

After 21 days incubation at 28 °C, bacterial colonies were randomly picked from Petri dishes and then transferred for isolation to tryptic soy agar (TSA; Oxoid). Nine strains were initially characterized by macro- and micro-morphological studies, Gram staining and catalase and oxidase tests. The strains were maintained on yeast extract agar (ISP 2 medium) as well as on Luedemann medium (Luedemann, 1968) as modified by Urzi *et al.* (2001) without the addition of CaCO₃ at pH 7.

Strains BC627, BC628, BC629, BC630, BC631^T and BC632 (cluster A) originated from isolation site CSC13a, while strains BC633^T, BC634 and BC635 (cluster B) were isolated from site CSC13b.

Colony morphology was determined on Luedemann medium after 7–14 days incubation at 28 °C. Cell morphology was determined by epifluorescent microscopy by using the fluorescent dye acridine orange (0.1 mg ml⁻¹) with a DMR HCS microscope (Leica) with a mercury lamp (HBO 50 W; Osram). For scanning electron microscopy (SEM) analysis, a 1 cm² sample of bacterial colonies growing on Luedemann medium was cut, fixed in glutaraldehyde buffer for 2 h, dehydrated with a series of ethanol solutions of increasing concentration, critical-point dried with CO₂ and sputter coated with gold before examination under SEM (S420; Cambridge).

All strains were characterized as having irregular colonies (5–12 mm in diameter), with a crateriform profile and

rough surface. Micromorphology was filamentous, no rod formation was observed and hyphae had the tendency to penetrate into the agar (see Supplementary Fig. S1a and b in IJSEM Online). The colour of the colonies, pigment production and the size of hyphae enabled the colonies to be clustered in two separate groups. In fact, colonies of cluster A produced a yellow pigment that became darker as the colonies aged (unlike other strains of the genus *Kribbella*). Members of cluster A produced abundant melanin in ISP 7 medium and the mean diameter of the hyphae was 0.36 µm. Strains of cluster B formed creamy coloured colonies; melanin production in ISP 7 medium was weak and the diameter of hyphae was 0.51 µm.

Physiological characterization of the novel strains was conducted by using selected tests performed as described by Williams *et al.* (1989). ISP media were prepared according to Shirling & Gottlieb (1966). Luedemann medium with or without addition of CaCO₃ was prepared as modified by Urzi *et al.* (2001). All physiological tests were carried out at 28 °C and results were recorded after 7, 14, 21 and 30 days. Utilization of carbon sources was tested as described by Pridham & Gottlieb (1948) using carbon sources at a concentration of 1 %, except for sodium citrate which was tested at 0.1 % as suggested by Williams *et al.* (1983). Hydrolysis of starch, DNA, tyrosine and casein was tested as described by Sneath *et al.* (1986). Esterase and lipase activities were determined as described by Anderson (1939) and Sierra (1957), respectively. Growth at different temperatures, pH and NaCl concentrations was examined on Luedemann medium without CaCO₃ addition. Aerobic versus anaerobic metabolism was tested in VL agar medium as described by Tiecco (1975). Susceptibility to antibiotics (cephaloridine 10 µg ml⁻¹, chloramphenicol 50 µg ml⁻¹, gentamicin 3 µg ml⁻¹, neomycin 1.5 µg ml⁻¹, oleandomycin 15 µg ml⁻¹, penicillin G 10 U ml⁻¹, rifampicin 4 µg ml⁻¹, streptomycin 3–10 µg ml⁻¹, tetracycline 1.5–30 µg ml⁻¹ and vancomycin 1.5–10 µg ml⁻¹) was tested on Mueller–Hinton Agar (Difco) following the standardized antibiogram method of Bauer *et al.* (1966).

The results are shown in Table 1 and the characteristics of the two strains designated to represent clusters A and B are compared with those of the type strains of other species of the genus *Kribbella*.

The main menaquinone was determined by HPLC (Groth *et al.*, 1996) after extraction as described by Collins *et al.* (1977). The isomer of diaminopimelic acid was determined by TLC of whole-cell hydrolysates (4 M HCl, 16 h at 100 °C) using cellulose plates (Merck) according to the method of Rhuland *et al.* (1955). All nine isolates from the catacombs contained LL-diaminopimelic acid (LL-Dpm) as the diagnostic diamino acid of the peptidoglycan and tetrahydrogenated menaquinones with nine isoprenoid units [MK-9(H₄)].

Polar lipids were analysed according to the method described by Minnikin *et al.* (1979). The polar lipid

Table 1. Comparison of phenotypic characteristics between strains BC631^T, BC633^T and the other recognized species of the genus *Kribbella*

Taxa: 1, strain BC631^T; 2, strain BC633^T; 3, *K. flavida* KACC 20248^T (data from Park *et al.*, 1999); 4, *K. sandramycini* KACC 20249^T (Park *et al.*, 1999); 5, *K. koreensis* KACC 20250^T (Lee *et al.*, 2000); 6, *K. solani* DSA1^T (Song *et al.*, 2004); 7, *K. jejuensis* HD9^T (Song *et al.*, 2004); 8, *K. lupini* LU14^T (Trujillo *et al.*, 2006); 9, *K. antibiotica* YIM 31530^T (Li *et al.*, 2004); 10, *K. yunnanensis* YIM 30006^T (Li *et al.*, 2006); 11, *K. alba* YIM 31075^T (Li *et al.*, 2006); 12, *K. swartbergensis* HMC25^T (Kirby *et al.*, 2006); 13, *K. karoensis* Q41^T Kirby *et al.* (2006); 14, *K. aluminosa* HKI 0478^T (Carlssohn *et al.*, 2007); 15, *K. hippodromi* S1.4^T (Everest & Meyers, 2008). ++, Good growth; +, growth; w, weak growth; –, no growth; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Utilization as sole carbon source:															
(+)-L-Arabinose	+	+	–	w	+	+	+	+	+	+	+	w	w	+	+
(–)-D-Fructose	+	+	+	+	+	ND	ND	ND	+	+	+	+	+	+	+
(+)-D-Galactose	+	+	–	+	+	–	+	+	+	–	+	ND	ND	ND	+
Glycerol	+	+	+	+	+	ND	ND	ND	+	+	+	+	+	ND	+
Lactose	+	+	–	+	+	+	+	ND	+	+	+	w	+	ND	–
(+)-D-Mannose	+	+	–	+	+	+	–	+	+	+	+	w	+	ND	+
myo-Inositol	–	–	+	+	+	–	–	ND	+	+	ND	w	+	+	++
(+)-D-Raffinose	w	+	+	+	+	+	+	+	+	+	+	w	+	+	+
(+)-L-Rhamnose	+	+	–	+	+	+	+	+	+	+	+	w	+	+	+
D-Sorbitol	w	–	+	–	+	ND	ND	+	+	+	+	+	w	ND	ND
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	ND	+	+
Trisodium citrate	–	–	+	–	+	ND	ND	–	+	+	–	w	w	ND	w
(+)-D-Xylose	+	+	–	+	+	+	+	ND	+	+	+	w	+	+	–
Nitrate reduction	–	+	+	+	–	–	–	–	–	–	–	–	+	–	+
Degradation of:															
Casein	+	+	+	+	+	–	w	+	+	+	–	+	+	+	+
Hypoxanthine	+	+	–	–	+	–	–	ND	+	ND	ND	+	+	+	+
Tween 80	+	+	+	+	–	+	–	–	ND	ND	ND	+	w	+	+
L-Tyrosine	+	+	ND	ND	+	–	–	–	ND	ND	ND	+	+	ND	+
Urea	–	–	+	+	+	–	+	–	ND	ND	ND	–	+	+	–
Hydrolysis of starch	–	–	–	w	+	–	–	–	+	+	+	+	+	ND	w
Gelatin liquefaction	+	+	–	+	+	ND	+	ND	+	–	+	ND	+	ND	ND
Melanin production	+	w	+	–	+	ND	ND	ND	+	w	+	ND	ND	ND	ND
Growth at temperature (°C):															
10	w	w	ND	ND	ND	–	ND	ND	ND	ND	ND	ND	ND	–	ND
15	+	+	–	–	+	–	–	+	ND	ND	ND	ND	ND	–	ND
20	+	+	+	+	+	–	ND	+	ND	ND	ND	+	+	+	+
30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
37	–	+	+	+	+	–	+	+	–	ND	ND	+	+	+	+
45	–	–	–	–	–	–	–	–	–	ND	ND	+	–	–	–
Growth at pH:															
5	+	+	+	+	–	–	–	–	–	ND	ND	w	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	–	–	+	ND	ND	ND	+	+	–	+
10	w	+	ND	ND	ND	–	–	–	ND	ND	ND	ND	ND	–	ND
Growth at NaCl (% w/v):															
2	+	+	–	+	+	+	–	ND	–	ND	ND	+	++	+	+
3	w	+	–	+	–	ND	–	+	–	ND	ND	+	w	ND	+
5	–	w	–	–	–	ND	–	+	–	ND	ND	–	–	–	+
Growth in aerobic conditions	+	+	+	+	+	+	+	ND	+	+	+	++	++	+	+
Growth in anaerobic conditions	–	–	–	w	w	+	w	ND	–	ND	ND	–	–	ND	–
Antibiotic resistance (µg ml ^{–1}):															
Chloramphenicol (50)	–	–	–	+	–	+	+	ND	–	ND	ND	–	–	ND	ND
Cephaloridine (10)	+	+	+	–	+	+	–	ND	+	ND	ND	+	–	ND	ND
Gentamicin SO ₄ (3)	–	+	ND	ND	–	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Neomycin SO ₄ (1.5)	+	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Streptomycin SO ₄ (10)	+	–	+	+	+	+	+	ND	+	ND	ND	+	+	ND	ND

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Tobramycin SO ₄ (1.5)	—	—	ND	ND	—§	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Vancomycin HCl (10)	+	—	+§	++§	++§	++	++	ND	+	ND	ND	—	—	ND	ND

*Data from Song *et al.* (2004).†Data from Trujillo *et al.* (2006).‡Data from Li *et al.* (2004).§Data from Kirby *et al.* (2006).||Data Carlsohn *et al.* (2007).

pattern contained phosphatidylinositol, phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol and several unknown phospho- and glycolipids in minor amounts.

Fatty acid methyl esters were obtained from cells grown on TSA at 28 °C for 24 h as described by Stead *et al.* (1992). The GC analysis was performed by using the Sherlock Microbial Identification System (MIDI) according to the instructions given in the operating manual. The MIDI dendrogram constructed on the basis of the cellular fatty acid profiles (see Supplementary Fig. S2 in IJSEM Online) confirmed that the catacomb isolates fell into two clusters. The fatty acid profiles of the six strains of cluster A were characterized by the predominating components 13-methyltetradecanoic acid (i-C_{15:0}; 20.5–28.7%), 12-methyltetradecanoic acid (ai-C_{15:0}; 22.4–28.3%) and 15-methylhexadecanoic acid (i-C_{17:1ω9c}; 9.9–10.6%), while the three strains of cluster B contained 14-methylpentadecanoic acid (i-C_{16:0}; 24.0–27.6%), 12-methyltetradecanoic acid (ai-C_{15:0}; 19.7–20.8%) and 13-methyltetradecanoic acid (i-C_{15:0}; 9.1–10.8%) as major fatty acids.

Ribosomal intergenic spacer analysis (RISA) was carried out to cluster species belonging to the same phylogenetic group (Borin *et al.*, 1997; Gürtler & Stanisich, 1996). Genomic DNA was extracted as described by Rainey *et al.* (1996) after growth of the strains in Luedemann medium for 7 days at 28 °C. Amplification of the internal transcribed spacer was performed as described by Gürtler (1993) using F1492 (5'-AAGTCGTAACAAGGTAGCCG-3') and R23 (5'-GATGCTCGCGTCCACTGTGC-3') primers. The PCR products were separated in a 2% agarose gel. RISA bands, stained with ethidium bromide, were examined by the use of the Kodak Digital Science 1d 2.0 software and the analysis was carried out on the basis of the number of bands and their sizes as compared to a 50 bp ladder marker (Invitrogen).

RISA analysis showed the novel *Kribbella* strains clustered in two groups in agreement with the isolation site. Strains of cluster A (BC627, BC628, BC629, BC630, BC631^T and BC632) showed a band of 510 bp, while strains of cluster B (BC633^T, BC634 and BC635) showed a band of 490 bp (see Supplementary Fig. S3 in IJSEM Online).

Genomic DNA extraction and PCR amplification of the 16S rRNA gene sequence were performed as described by Rainey *et al.* (1996). About 1400 bp of the gene sequences of the strains were obtained at BMR-Genomics using universal primers F27, R1549 (Lee *et al.*, 2000) and COM1 (Schwieger & Tebbe, 1998). Sequences were compared against those already deposited in the EMBL-EBI database (<http://www.ebi.ac.uk/fasta33/nucleotide.html>) and aligned using CLUSTAL W (Thompson *et al.*, 1994) against those of members of the family *Nocardioideaceae*. A phylogenetic tree (Fig. 1) was constructed by using PHYLIP version 3.6 (Felsenstein, 1993). The stability of the relationships was evaluated by performing bootstrap analysis of the neighbour-joining dataset based on 1000 resamplings (Saitou & Nei, 1987).

The phylogenetic analysis confirmed that the novel strains clustered in two groups. Strains of cluster A (BC627, BC628, BC629, BC630, BC631^T and BC632) shared gene sequence similarities of 98.8–99.9% with each other and of 97.9–98.9% with strains of cluster B. The strains of cluster B, BC633^T, BC634 and BC635, shared a similarity of 99.8–99.9% with each other. The similarity of the strain BC631^T with *K. koreensis* DSM 17837^T was 98.0%. Strain BC633^T had a similarity of 98.8% with *K. flavida* DSM 17836^T and 98.6% with *K. karoensis* DSM 17344^T.

DNA–DNA hybridization was thus necessary to examine the species status of the isolated strains. DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out in 2 × standard saline citrate buffer supplemented with 10% (v/v) formamide at 70 °C as described by De Ley *et al.* (1970) with consideration of the modifications proposed by Huß *et al.* (1983). The measurement was performed by using a model Cary 100 Bio UV/Vis-spectrophotometer equipped with a Peltier-thermostated 6 × 6 multicell changer and a temperature controller with an *in situ* temperature probe (Varian). Strain BC631^T showed only 31.5% DNA–DNA relatedness with *K. koreensis* DSM 17837^T and thus the two strains belong to different species (Wayne *et al.*, 1987). DNA–DNA hybridization of strain BC633^T against *K. flavida*

Fig. 1. 16S rRNA gene sequence dendrogram displaying the relatedness of the new isolates of the genus *Kribbella* to the other type strains of the family *Nocardioidaceae*. *Streptosporangium roseum* DSM 43021^T was used as the outgroup. Bar, 0.01 substitutions per nucleotide position.

DSM 17836^T and *K. karoensis* DSM 17344^T revealed even lower DNA–DNA relatedness values (12.8 and 22.8%, respectively). Thus, strain BC633^T does not belong to either *K. flavida* or to *K. karoensis*. DNA–DNA relatedness between strains BC631^T and BC633^T was 29.4%, indicating that the two strains represent two distinct genomospecies of the genus *Kribbella*.

The genomic homogeneity of the two *Kribbella* clusters was examined in comparison with *K. flavida* DSM 17836^T, *K. koreensis* DSM 17837^T and *K. karoensis* DSM 17344^T by RiboPrinting and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS. Automated RiboPrinting of the isolates was accomplished by using the RiboPrinter system (DuPont Qualicon) and *PvuII* as the restriction enzyme for cutting genomic DNA (Bruce, 1996; Stackebrandt *et al.*, 2002). RiboPrint patterns were evaluated by using the BioNumerics software package (version 3.00, Applied Maths).

Sample preparation for MALDI-TOF analysis was carried out according to the OS-extraction protocol of Bruker Daltonics. Around 10 mg biomass from agar cultures was suspended in 100 µl water by careful mixing. Then 100 µl of 5% trifluoroacetic acid in acetonitrile was added to the suspension. Immediately after centrifugation, the supernatant was removed and aliquots of 1.5 µl were placed on

each spot of a stainless steel target plate. After air drying, 1.5 µl matrix solution (saturated solution of α -cyano-hydroxy-cinnamic acid in 50% aqueous acetonitrile supplemented with 2.5% trifluoroacetic acid) per spot was applied. MALDI-TOF mass spectrometry was conducted using a Microflex L20 MS (Bruker Daltonics) equipped with a N₂ laser. All spectra were recorded in linear, positive ion mode. The acceleration voltage was 20 kV. Spectra were collected as a sum of 500 shots across a spot. A mass range of 2000–20 000 *m/z* was used for analysis. MALDI-TOF mass spectra were compared by using the BioTyper software package (version 1.1, Bruker Daltonics).

The results of RiboPrint analyses (see Supplementary Fig. S4) and MALDI-TOF MS analysis (see Supplementary Fig. S5) of protein extracts were in agreement with our other investigations and showed that the novel strains isolated from the catacombs were clearly separated from the phylogenetically closest species *K. flavida*, *K. karoensis* and *K. koreensis*. Both methods confirmed that the catacomb isolates fell into two different clusters A and B with the only exception that the RiboPrint pattern of strain BC633^T was split from cluster B as its bands differed from those of strains BC634 and BC635 in intensity and position.

For the first time, strains belonging to the genus *Kribbella* have been isolated from deteriorated stone surfaces. The strains were characterized by their ability to penetrate through the substrate and by their capacity to precipitate CaCO_3 crystals. It is worth mentioning that the novel isolates belonging to cluster A differed from previously described members of the genus *Kribbella* by the formation of yellow pigmented colonies.

Based on phylogenetic, genotypic and phenotypic analyses, we suggest that the new isolates represent two novel species of the genus *Kribbella* for which the names *Kribbella catacumbae* sp. nov. and *K. sancticallisti* sp. nov. are proposed.

Description of *Kribbella catacumbae* sp. nov.

Kribbella catacumbae (cat.a'cum.bae. L. gen. n. *catacumbae* of a catacomb, isolated from a Roman catacomb).

Gram-positive. Aerobic. Forms irregular colonies (diameter 5–10 mm), with lobate margin, crateriform profile and rough surface. Aerial mycelium is white and the vegetative hyphae are pale yellow/orange–yellow, becoming dark orange in old colonies on Luedemann medium. Hyphae (diameter 0.36 μm) are extensively branched and penetrate into the agar. No diffusible pigments are produced on glycerol-asparagine agar (ISP 5). Melanin pigment is produced on tyrosine agar (ISP 7), but not on peptone-yeast extract-iron agar (ISP 6). Nitrate is not reduced. Catalase- and oxidase-positive. Urease-negative, utilizes *N*-acetylglucosamine, (+)-L-arabinose, (–)-D-fructose, (+)-D-galactose, (+)-D-glucose, glycerol, lactose, (+)-D-maltose, (–)-D-mannitol, (+)-D-mannose, (+)-L-rhamnose, sucrose and (+)-D-xylose as sole carbon sources, with weak growth on (+)-D-raffinose and sorbitol after 21 days. Grows at 10–30 °C and at pH 5–10. Aesculin, arbutin, casein, gelatin, tyrosine and Tweens 20, 40, 60 and 80 are hydrolysed, with weak DNA hydrolysis and hypoxanthine and elastin decomposition. Grows in the presence of 3 % NaCl. Sensitive to 0.01 % lysozyme. Grows in the presence of cephaloridine (10 $\mu\text{g ml}^{-1}$), streptomycin sulphate (10 $\mu\text{g ml}^{-1}$) and vancomycin HCl (10 $\mu\text{g ml}^{-1}$). The cell wall contains LL-Dpm. The main menaquinone is MK-9(H_4). Major fatty acids are i-C_{15:0}, ai-C_{15:0} and i-C_{17:1}ω9c.

The type strain, BC631^T (=DSM 19601^T=JCM 14968^T), was isolated from the Catacomb of St Callistus in Rome, Italy. Other strains are BC627, BC628, BC629, BC630 and BC632, isolated from the same site, CSC13a.

Description of *Kribbella sancticallisti* sp. nov.

Kribbella sancticallisti (san.cti.call.i'sti. L. n. *Sanctus Callistus* Saint Callistus; N.L. gen. n. *sancticallisti* of Saint Callistus, isolated from the Saint Callistus Roman catacombs).

Gram-positive. Aerobic. Forms irregular colonies (diameter 5–10 mm), with lobate margin, crateriform profile

and rough surface. Aerial mycelium is white and the vegetative hyphae are cream on Luedemann medium. Hyphae (diameter 0.51 μm) are often branched. No diffusible pigments are produced on glycerol-asparagine agar (ISP 5). Weak melanin production on tyrosine agar (ISP 7), no melanin production on peptone-yeast extract-iron agar (ISP 6). Nitrate is reduced. Catalase- and oxidase-positive. Urease-negative. Utilizes *N*-acetylglucosamine, L-arabinose, (–)-D-fructose, (+)-D-galactose, glycerol, gluconate, lactose, DL-malate, (+)-D-maltose, (–)-D-mannitol, D(+)-mannose, (+)-D-raffinose, (+)-L-rhamnose, sucrose and (+)-D-xylose. Grows at 10–37 °C and at pH 5–10. Aesculin, casein, gelatin, tyrosine, DNA and Tweens 20, 40, 60, 80 are hydrolysed, with weak arbutin hydrolysis. Hypoxanthine and elastin are decomposed, with weak xanthine decomposition. Grows at a concentration of up to 5 % NaCl. Grows in the presence of cephaloridine (10 $\mu\text{g ml}^{-1}$), gentamicin (3 $\mu\text{g ml}^{-1}$), oleandomycin (15 $\mu\text{g ml}^{-1}$), penicillin G (10 U ml^{-1}), and rifampicin (4 $\mu\text{g ml}^{-1}$). The cell wall contains LL-Dpm. The main menaquinone is MK-9(H_4). Major fatty acids are i-C_{16:0}, ai-C_{15:0} and i-C_{15:0}.

The type strain, BC633^T (=DSM 19602^T=JCM 14969^T), was isolated from tufacean surfaces with grey–whitish alterations in the Roman catacombs of St Callistus (Rome, Italy), site CSC13b. Other strains are BC634 and BC635, isolated from the same site CSC13b.

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