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Kytococcus aerolatus sp. nov., isolated from indoor air in a room colonized with moulds☆

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Abstract

A Gram-positive, coccoid bacterial isolate (02-St-019/1^T), forming beige pigmented colonies was obtained from an indoor air sample. Based on 16S rRNA gene sequence similarity studies it was determined that this isolate 02-St-019/1^T belonged to the genus *Kytococcus*, showing sequence similarties of 98.6% to *Kytococcus schroeteri* DSM 13884^T and 98.3% to *Kytococcus sedentarius* DSM 20547^T, respectively. The diagnostic diaminoacid of the peptidoglycan was lysine, cell wall sugars were ribose and xylose. The major menaquinones detected were MK-7 and MK-8. The polar lipid profile consisted of the major phospholipids diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine and phosphatidylinositol mannoside. Fatty acid patterns were composed of major amounts of the iso- and anteiso-branched fatty acids anteiso $C_{17:0}$, iso $C_{15:0}$ and iso $C_{17:0}$ and unsaturated fatty acids ($C_{17:1}$ ω8c, iso $C_{17:1}$ ω9c, and $C_{17:1}$ ω8c) with smaller amounts of the straight-chain fatty acids $C_{15:0}$, $C_{16:0}$ and $C_{17:0}$. The results of DNA–DNA hybridizations and physiological and biochemical tests clearly allowed a genotypic and phenotypic differentiation of strain 02-St-019/1^T from the two described *Kytococcus* species. On the basis of these results a novel species to be named *Kytococcus aerolatus* sp. nov., is proposed, with the type strain 02-St-019/1^T (= DSM 22179^T = CCM 7639^T).

The genus *Kytococcus*, described by Stackebrandt et al. [18] comprises at present only the two species *Kytococcus sedentarius* [18] and *Kytococcus schroeteri* [1]. The genus forms a separate lineage in the 16S rRNA sequence based tree and can also be differentiated from the most closely related genera by chemotaxonomic properties [18]. Strain 02-St-019/1^T was enriched and recovered from the air in a room with walls colonized with moulds. Microorganisms were collected by filtration of 100 l of air through a gelatine filter. This filter was placed on an agar plate containing ISP-3 medium [16] and incubated at 28 °C. After 2 weeks the strain 02-St-019/1 was isolated from the plate and maintained on medium 79 [11].

Morphological properties, Gram-staining and cell morphology were observed microscopically as described

☆The EMBL accession number for the 16S rRNA gene sequence of strain 02-St-019/1^T is FM992368.

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previously [7]. Isolation of the DNA was performed with a commercial DNA extraction kit (GenElute™ Plant Genomic DNA Kit, Sigma) after disruption of cells by an 1 min bead-beating step with 1 g of 0.1 ∅ Zirconia beads at maximum speed.

Multiple sequence alignment and analysis of the data were performed using the software package Molecular Evolutionary Genetics Analysis (MEGA) version 4 [17]. Genetic distance calculations (distance options according to the Kimura-2 model, 17) and clustering with the neighbor-joining method (Fig. 1) was performed by using bootstrap values based on 1000 replications. The 16S rRNA gene sequence of strain 02-St-019/1^T was a continuous stretch of 1348 bp and was deposited in EMBL, under the accession number FM992368.

Sequence similarity calculations after a neighbor-joining analysis showed, that the most similar sequences to that of strain 02-St-019/1^T belonged to *Kytococcus schroeteri* DSM 13884^T (98.6%) and *K. sedentarius* DSM 20547^T (98.3%).

Bacterial biomass for chemotaxonomic investigations of the isolates was prepared by cultivating strain 02-St-019/1 for 24–48 h in shake flasks in liquid organic medium M79 at 180 rpm at 28 °C, except for fatty acid analyses cells were grown on tryptic soy agar (TS agar, [7]). Standard HPLC and TLC procedures were used to determine whole-organism sugars [2,12], quinone system [4] and polar lipids [3,10]. Fatty acid analysis was performed according to Kämpfer & Kroppenstedt [6]. Peptidoglycan was obtained after disintegration of cells with glass beads in a Vibrogen cell mill (Johanna Otto GmbH, D-72408 Bodelshausen, Germany) and subsequent trypsin digestion [15]. The elucidation of the peptidoglycan structure was carried out as described elsewhere [13,14] with the modification that TLC on cellulose was applied instead of paper chromatography. Quantitative analysis of amino acids was performed after derivatization by gas chromatography and gas chromatography/mass spectrometry (320-MS Quadrupole GC/MS, Varian) [5,9].

The following amino acids were detected in the total hydrolysate of the peptidoglycan (4 N HCl, 100 °C, 16 h): alanine:glycine:glutamic acid:lysine = 1.6:0.1:2.5:1.0 (molar ratio). Dinitrophenylation according to Schleifer [13] revealed glutamic acid as *N*-terminus of the interpeptide bridge. The partial hydrolysate (4 N HCl, 100 °C, 0.75 h) of the peptidoglycan contained in addition to the amino acids the peptides L-Ala-D-Glu and L-Lys-D-Ala. The traces of glycine may result from minor amounts of contaminating proteins in the

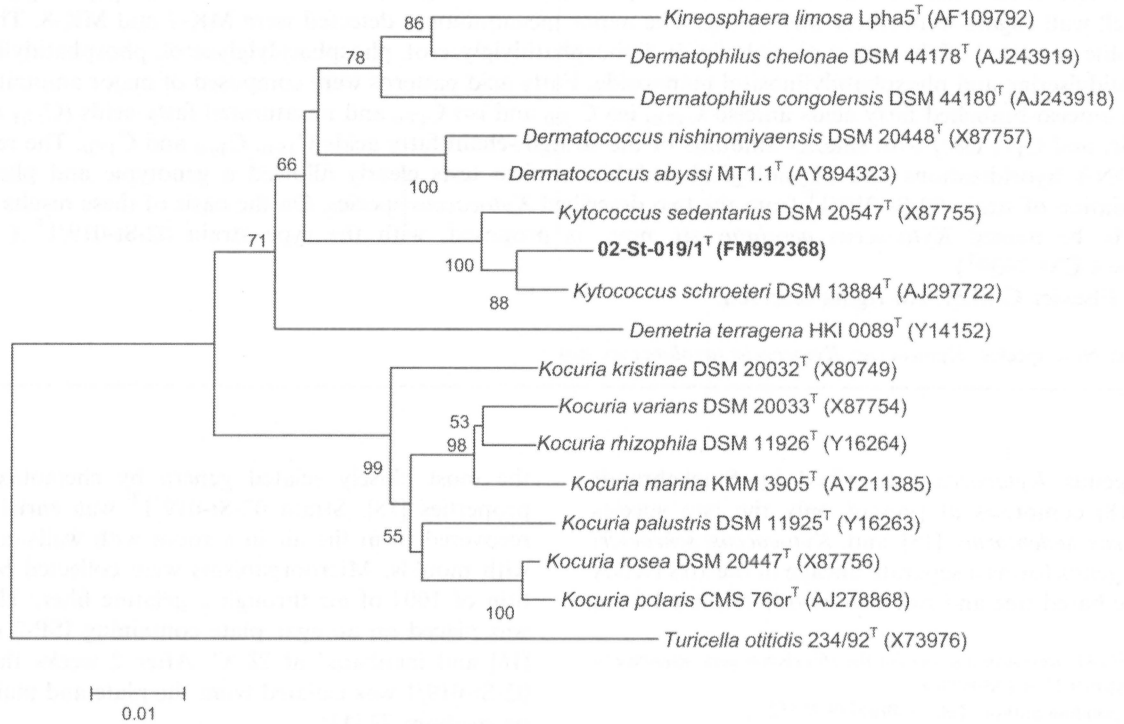

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the European Molecular Biology Laboratory data library (accession numbers are given in brackets). Multiple alignement, distances (distance options according to the Kimura-2 model) and clustering with the neighbor-joining method were performed by using the software packages Molecular Evolutionary Genetics Analysis (MEGA) version 4 (Tamura et al. [17]). Bootstrap values based on 1000 replications are listed as percentages at the branching points.

peptidoglycan preparations. From these data the peptidoglycan type A4α L-Lys-D-Glu-D-Glu (A11.43 according to http://www.dsmz.de/microorganisms/main.php?content_id=35) can be concluded which is in agreement with the affiliation of strain 02-St-019/1 to the genus *Kytococcus*.

The whole-organism hydrolysate contained the sugars ribose and xylose. In difference to the already described *Kytococcus* species strain 02-St-019/1^T exhibited a quinone system with the predominant menaquinones MK-7 (64%), MK-8 (28%) and minor amounts of MK-6 (3%) whereas the isoprenoid quinones of *K. schroeteri* were represented by approximately equivalent amounts of MK-8, MK-7 and minor amounts of MK-9 (peak area ratio 43:36:1, [1]). The polar lipid profile was rather complex consisting of eight components (Fig. 2). In accordance with the genus description major lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. Additionally four unknown lipids – two phospholipids, one glycolipid and one ninhydrin-positive lipid, phosphatidylserine and phosphatidylinositol mannoside were detected as well. Although unknown lipids were found in *K. sedentarius*, phosphatidylserine and phosphatedylinositol mannoside were not detected.

The fatty acid profile of strain 02-St-019/1^T was similar to those of the other closely related species *K. schroeteri* and *K. sedentarius*, but more similar to that of *K. schroeteri* [1]. The fatty acid profile was dominated by iso- and anteiso-branched fatty acids anteiso $C_{17:0}$, iso $C_{15:0}$ and iso $C_{17:0}$, and unsaturated fatty acids ($C_{17:1}$ ω8c, iso $C_{17:1}$ ω9c, and $C_{17:1}$ ω8c), which were also present in *K. schroeteri* and *K. sedentarius* (Table 1 and [1]).

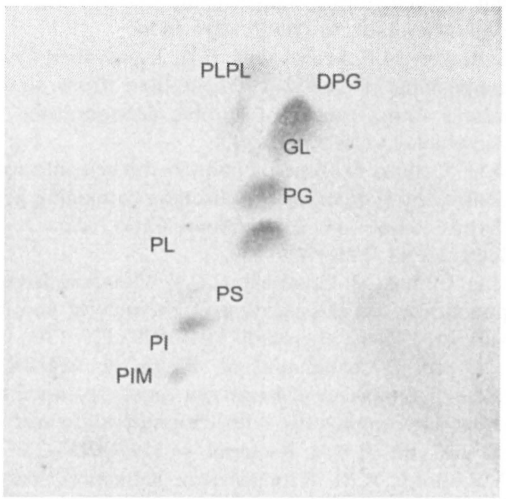

Fig. 2. Two-dimensional TLC of polar lipid extracts from strain 02-Be-019^T, stained with molybdatophosphoric acid. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; PIM, phosphatidylinositol mannoside; GL, unknown glycolipid; PL, unknown phospholipid.

Results of the comparative physiological characterization using always identical test conditions are given in Table 2 and the species description were obtained with methods as described previously [8]. Only few carbon sources were utilized by strain 02-St-019/1^T.

DNA–DNA hybridisation experiments were performed with 02-St-019/1^T and the type strains of *K. schroeteri* DSM 13884^T, and *K. sedentarius* DSM 20547^T using the method described by Ziemke et al. [19], with minor variation in the nick translation step, where 2 µg of DNA was labelled during a 3 h incubation at 15 °C. Strain 02-St-019/1^T showed a relatively low DNA–DNA similarity to *K. schroeteri* DSM 13884^T of 40%, (reciprocal 45%), and to *K. sedentarius* DSM 20547^T 20.8% (reciprocal 23.6%). The observed physiological and chemotaxonomic differences between these type strains (Tables 1 and 2) clearly indicate that strain 02-St-019/1^T represents a novel species for which the name *Kytococcus aerolatus* sp. nov is proposed.

Table 1. Fatty acid composition of strains 1, 02-St-019/1^T; 2, *K. sedentarius* DSM 20547^T; 3, *K. schroeteri* DSM 13884^T.

	1	2	3
Saturated			
C15:0	3.9	2.3	1.1
C16:0	4.5	1.0	0.7
C17:0	3.0	9.6	
Unsaturated			
C15:1 ω6c	6.6	1.9	0.7
Iso C17:1 ω9c	12.1	17.1	23.7
Anteiso C17:1 ω9c	3.2	3.3	3.3
C17:1 ω8c	11.2	16.4	0.9
C17:1 ω6c	1.9	1.8	
Branched-chain fatty acid			
iso-C11:0			0.5
iso-C13:0			1.0
iso-C15:0	4.8	2.7	29.7
anteiso-C15:0	1.7	2.5	4.4
iso-C16:0	3.5	2.9	3.0
iso-C17:0	7.3	14.5	16.7
anteiso-C17:0	18.8	22.3	10.6
10-methyl C17:0		0.6	
C18:1 Iso H			1.0
Summed features 1*			**1.0**
Summed features 3*	**17.5**	**2.1**	**1.0**
Summed features 4*			**0.6**

Data for all strains from this study. All strains were grown on TS agar for 48 h at 28 °C.

*Summed feature 1: iso-C15:1 H and/or C13:0 3-OH.Summed feature 3: C16:1 ω7c and/or iso-C15:0 2-OH.Summed feature 4: iso-C17:1 I and/or anteiso-C17:1 B.

Table 2. Physiological test results for strains 1, 02-St-019/1^T; 2, *K. sedentarius* DSM 20547^T; 3, *K. schroeteri* DSM 13884^T.

Tests[$]	1	2	3
Hydrolysis of:			
pNP-α-D-glucopyranoside	–	+	–
Bis-pNP-phosphate	–	+	+
pNP-phenyl-phosphonate	–	+	+
pNP-phosphoryl-choline	–	+	–
L-Alanine-pNA	–	(+)	+
Assimilation of:			
D-Fructose	–	(+)	–
D-Glucose	–	+	+
D-Mannose	–	(+)	–
D-Maltose	–	+	(+)
Sucrose	–	+	(+)
D-Trehalose	–	+	+
Propionate	–	(+)	+
Glutarate	–	–	+
DL-3-Hydroxybutyrate	–	–	+
Itaconate	–	–	–
L-Malate	–	(+)	–
Oxoglutarate	–	–	+
L-Aspartate	–	+	+
L-Histidine	–	+	–
L-Leucine	–	–	+
L-Ornithine	–	–	+
L-Phenylalanine	–	–	–
L-Proline	–	+	+

Data for all strains from this study. None of the strains produced acid from the following carbohydrates: Glucose, Lactose, Sucrose, D-Mannitol, Dulcitol, Salicin, Adonitol, Inositol, Sorbitol, L-arabinose, Raffinose, Rhamnose, Maltose, D-Xylose, Trehalose, Cellobiose, Methyl-D-glucoside, Erythritol, Melibiose, D-Arabitol, D-Mannose. The following chromogenic substrates were not hydrolysed: Esculin, oNP-ß-D-galactopyranoside, pNP-ß-D-glucuronide, pNP-ß-D-glucopyranoside, pNP-ß-D-xylopyranoside, 2-Deoxythymidine-5'-pNP-phosphate, L-Glutamate-gamma-3-carboxy-pNA, L-Proline-pNA. None of the strains utilized the following compounds as sole sources of carbon: N-Acetyl-D-galactosamine, N-Acetyl-D-glucosamine, L-Arabinose, p-Arbutin, D-Cellobiose, D-Galactose, Gluconate, alpha-D-Melibiose, L-Rhamnose, D-Ribose, Salicin, D-Xylose, Adonitol, i-Inositol, Maltitol, D-Mannitol, D-Sorbitol, Putrescine, *cis*-Aconitate, *trans*-Aconitate, Adipate, 4-Aminobutyrate, Azelate, Citrate, Mesaconate, Suberate, L-Alanine, ß-Alanine, L-phenylalanine, L-Serine, L-Tryptophane, 3-Hydroxybenzoate, 4-Hydroxybenzoate and Phenylacetate. All strains utilized acetate, fumarate, DL-lactate and pyruvate (for strain 02-St-019/1^T only weak positive results were observed).

Description of *Kytococcus aerolatus* sp. nov.

Kytococcus aerolatus (ae.ro.la'tus. Gr. n. aer air; L. part. adj. latus carried; N.L. masc. part. adj. aerolatus airborne).

Coccoid cells, about 1.3 µm in diameter. Gram-positive, oxidase-positive, catalase-positive showing an oxidative metabolism. Beige pigmented colonies are formed on nutrient agar. Good growth occurs after 24 h of incubation on tryptone soy agar, R2A agar and nutrient agar at 25–30 °C. The peptidoglycan type is A4α (L-Lys-D-Glu-D-Glu). The quinone system of 02-St-019/1^T is composed of MK-7 and MK-8. The polar lipid profile consists of the major lipids diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine and phosphatedylinositol mannoside. Major fatty acids are iso- and anteiso-branched fatty acids anteiso $C_{17:0}$, iso $C_{15:0}$ and iso $C_{17:0}$ and unsaturated fatty acids ($C_{17:1}$ ω8c, iso $C_{17:1}$ ω9c, and $C_{17:1}$ ω8c) with smaller amounts of the straight-chain fatty acids $C_{15:0}$, $C_{16:0}$ and $C_{17:0}$. Carbon source utilizations (including differentiating characters using always identical conditions) are indicated in Table 1.

Isolated in Stuttgart, Germany, sampled from the air in a house with walls colonized with moulds. Type strain is 02-St-019/1^T (= DSM 22179^T = CCM 7639^T).

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