Kocuria sediminis sp. nov., isolated from a marine sediment sample

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Abstract

A Gram-positive, pinkish-orange pigmented, coccoid strain, FCS-11^T was isolated from a marine sediment sample taken from Kochi fort area, Kerala, India and subjected to polyphasic taxonomic study. The 16S rRNA gene sequence of the strain was determined and the results of 16S rRNA gene sequence analysis showed that the strain FCS-11^T should be assigned to the genus *Kocuria*. The chemotaxonomical data supported this taxonomic placement i.e. menaquinones MK-7(H_2), MK-8(H_2) and MK-9(H_2); major fatty acids anteiso C15:0 and iso-C15:0 and phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG) as major polar lipids. Further phylogenetic analysis of the 16S rRNA gene sequence confirmed that the strain FCS-11^T belonged to the genus *Kocuria* and is closely related to *Kocuria turfanensis* MTCC 10790^T (99.4%) followed by *Kocuria polaris* MTCC 3702^T (98.2%), *Kocuria rosea* MTCC 2522^T (98.2%), *Kocuria flava* MTCC 10971^T (98.2%), *Kocuria aegyptia* MTCC 10791^T (98.0%), *Kocuria himachalensis* MTCC 7020^T (97.5%) and *Kocuria atrinae* MTCC 10972^T (97.1%). However, the DNA-DNA hybridization values obtained between strain FCS-11^T and other related strains were well below the threshold that is required for the proposal of a novel species. The G+C content of the genomic DNA was 60.7 mol%. The phenotypic and genotypic data showed that the strain FCS-11^T merits the recognition as a representative of a novel species of the genus *Kocuria*. It is proposed that the isolate should be classified in the genus *Kocuria* as a novel species, *Kocuria sediminis* sp. nov. The type strain is FCS-11^T (= MTCC 10969^T = JCM 17929^T).

Introduction

The heterogenous nature of the genus *Micrococcus* was reported by Stackebrandt and Woese (1979) on the basis of 16S rRNA analysis as it overlaps with the genus *Arthrobacter*. Re-evaluating this complexi cluster of *Arthrobacter* and *Micrococcus,* Stackebrandt et al. (1995) proposed a new genus *Kocuria* which was clearly demarcated from *Micrococcus*. At present the genus *Kocuria* comprises of 18 species with validly published names (http://www.bacterio.cict.fr/k/kocuria.html) and all these species have been isolated from different environmental sources: *Kocuria rosea, Kocuria varians* and *Kocuria kristinae* (Stackebrandt et al. 1995)*, Kocuria palustris* and *Kocuria rhizophila* (Kovacs et al. 1999)*, Kocuria polaris* (Reddy et al. 2003)*, Kocuria marina* (Kim et al. 2004)*, Kocuria carniphila* (Tvrzova et al. 2005)*, Kocuria aegyptia* (Li et al. 2006)*, Kocuria himachalensis* (Mayilraj et al. 2006)*, Kocuria flava* and *Kocuria turfanensis* (Zhou et al. 2008)*, Kocuria gwangalliensis* (Seo et al. 2009)*, Kocuria halotolerans* (Tang et al. 2009), *Kocuria koreensis* (Park et al. 2010b)*, Kocuria atrinae* (Park et al. 2010a), *Kocuria salsicia* (Yun et al. 2011) and *Kocuria assamensis* (Kaur et al. 2011).

In this present work we report the polyphasic taxonomic description of strain FCS-11^T which was isolated from a marine sediment sample and for which we propose the novel species *Kocuria sediminis sp. nov*.

Materials and methods

Strains, isolation and cultivation of strains

Strain FCS-11^T was isolated from a marine sediment sample taken from Kochi fort area (Latitude: 9° 58' 0" N; Longitude: 76° 14' 0" E), Kerala, India. It was isolated by serially diluting the soil sample and plating samples onto tryptic soy agar medium (TSA, HiMedia, India). Pure colonies were subcultured and maintained on TSA medium at 4ºC and stored at -70ºC as glycerol stocks (10% (v/v) glycerol). The pinkish-orange pigmented strain was selected for further polyphasic study. The reference type strains *K. turfanensis* (MTCC 10790^T), *K. polaris* (MTCC 3702^T), *K. rosea* (MTCC 2522^T), *K. flava* (MTCC 10971^T), *K. aegyptia* (MTCC 10791^T), *K. himachalensis* (MTCC 7020^T) and *K. atrinae* (MTCC 10972^T) were obtained from the Microbial Type Culture Collection & Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India. Reference strains were also cultured on TSA medium.

Morphological, physiological and biochemical characterization

For phenotypic studies, the isolated strain and reference strains were cultivated on TSA medium at 30ºC for 2 days. Gram staining and spore staining were performed by using Gram stain and spore staining kits (HiMedia, India) as per the manufacturer instructions. Colony and cell morphology was determined according to the method of Barrow and Feltham (1993). For anaerobiosis, the cultures were streaked on TSA plates and placed in an anaerobic jar (MART), which was evacuated and flushed with Anoxomat unit (MART) using anaerobic gas mixture consisting of nitrogen (85%), carbon dioxide (10%) and hydrogen (5%). Plates were incubated at 30°C for 5 days. All the biochemical tests were performed at 30ºC. Physiological tests such as growth at different temperatures (15ºC, 20ºC, 25ºC, 30ºC, 37ºC and 42ºC), pH 5, 6, 7, 8, 9, 10, 11 and 12 (adjusting with biological buffers Na_2HPO_4 /NaH_2PO_4 for pH below 8 and Na_2HPO_4/NaOH for pH above 8) were performed using tryptic soy broth (TSB, HiMedia, India) medium. Physiological tests for different NaCl concentrations (0%, 1%, 2%, 3%, 5%, 7%, 9%, 11%, 13% and 15%) were performed by growing the strain on basal TSA supplemented with different concentrations of NaCl. Hydrolysis of gelatin, casein, starch, growth on Simmon's citrate, production of hydrogen sulphide and indole production were performed by Smibert and Krieg (1994); reduction of nitrate was determined by the method of Lanyi (1988). Voges-Proskauer, methyl red, catalase and oxidase (oxidation of tetramethyl-p-phenylenediamine dihydrochloride; sigma) activity tests were performed as described by Barrow and Feltham (1993). Acid production test from various sugars was performed on minimal medium by using the method of Smith et al. (1952) method. Oxidation of various carbon compounds were tested using Biolog GP2 MicroPlates and performed according to the manufacturer's instructions. VITEK® 2-GP cards were used as per the instructions of the manufacturer (bioMérieux).

Chemotaxonomic characterization

For the determination of cellular fatty acids, strain FCS-11^T and the reference strains were grown on TSB medium at 30ºC for 2 days; cellular fatty acids were extracted, methylated and analysed by using Gas Chromatography according to the instructions of the Sherlock Microbial Identification System (MIDI, USA Version 4.0) as described previously (Sasser 1990; Pandey et al. 2002). For chemotaxonomic analysis, freeze dried cell mass was prepared following the growth of strain FCS-11^T and the reference strains in TSB for 3 days at 30ºC. Extraction of polar lipids was carried out based on the modified protocol of Bligh and Dyer (1959). For determination of polar lipids, two-dimensional TLC was run according to the method described by Komagata and Suzuki (1987). Lipid spots were detected by the following spray reagents: molybdatophosphoric acid (5% w/v) in

absolute ethanol for total lipids; molybdenum blue spray reagent (Sigma) for phospholipids; ninhydrin (0.2% w/v) in acetone for aminolipids; and anisaldehyde reagent (Sigma) for detection of glycolipids. Menaquinones were extracted and analysed by methods described by Kroppenstedt and Minnikin (Kroppenstedt 1982; Minnikin et al. 1984). The peptidoglycan structure was examined by using hydrolysates of purified cell walls according to the method of Schleifer (1985). The amino acids and peptides were separated by two-dimensional ascending TLC as described by Schleifer and Kandler (1972) with the modification that TLC on cellulose sheets (Merck 5577) was used instead of paper chromatography.

Determination of 16S rRNA gene sequence, phylogenetic analysis and genomic relatedness

Genomic DNA extraction, amplification and sequencing were performed as described previously (Mayilraj et al. 2006). The complete sequence of the 16S rRNA gene was aligned with those of representative related taxa using the EzTaxon server (http://www.eztaxon.org/; (Chun et al. 2007). The 16S rRNA gene sequence of FCS-11^T and representative closely related species were retrieved from the EzTaxon server and aligned using MEGA version 5.0 (Tamura et al. 2011). Phylogenetic trees were constructed using the neighbour-joining as well as maximum parsimony algorithms and maximum likelihood algorithms. Bootstrap analysis was performed to assess the confidence limits of the branching.

The G+C content of genomic DNA was determined spectrophotometrically (Lambda 35, Perkin Elmer) using thermal denaturation method (Mandel and Marmur 1968). DNA–DNA hybridization was performed each time with freshly isolated genomic DNA and was repeated three times by the membrane filter method (Tourova and Antonov 1988).

Results and discussion

Phenotypic and biochemical characteristics

The colonies of strain FCS-11^T were pinkish orange pigmented, opaque and convex. Based on biochemical reactions, the strain was positive for hydrolysis of starch and negative for methyl red, Voges-Proskauer test, indole production, nitrate reduction, urease test, gelatin liquefaction and hydrogen sulphide production. Growth was observed at temperatures ranging between 25°C to 37°C and optimal at 30°C; the pH range for growth was 6.0 to 10.0 and optimal at pH 8.0. Strain FCS-11^T was able to grow between 0% to 5% NaCl. Strain FCS-11^T was positive for acid production from the sugars arabinose, galactose, dextrose, mannitol, raffinose, salicin, xylose, fructose, lactose,

sorbitol and cellobiose. The strain was positive for the oxidation of various substrates as examined by Biolog GP2 MicroPlate assay: D-cellobiose, D-fructose, α-D-glucose, maltotriose, D-mannitol, D-mannose, D-ribose, D-xylose, α-hydroxybutyric acid, β-hydroxybutyric acid, pyruvic acid methyl ester, succinic acid mono methyl ester, propionic acid. Major distinguishing characteristics are given in Table 1.

Chemotaxonomic characterization

The major fatty acids of the strain were anteiso C15:0 (68.0%) and iso-C15:0 (12.6%). Quantitative differences in cellular fatty acids between strain FCS-11^T and closely related species are given in Table 2. Major polar lipids were found to be phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), three unknown phospholipids (PL) and six unidentified lipids (UK) (Supplementary Figures 1a, 1b and 1c). The major menaquinones detected for the strain FCS-11^T were MK-7(H_2) 26.3%, MK-8(H_2) 31.4% and MK-9(H_2) 42.1%. It was also observed that the strains *K. atrinae* P30^T and *K. salsicia* 104^T had menaquinones MK-7(H_2), MK-8(H_2), MK-9(H_2) and MK-7(H_2), MK-8(H_2) respectively, which are typical of the genus *Kocuria* (Stackebrandt et al. 1995); in contrast Yun et al. (2011) and Park et al. (2010a) had incorrectly reported the presence of MK-7 as the predominant menaquinone in each of these strains. Based on the above observation the MK profile reported for *K. koreensis*, Park et al. (2010b) is considered doubtful and should be re-analysed. These data are important in confirming that the members of the genus *Kocuria* described to date apparently only had menaquinones MK-7(H_2), MK-8(H_2), MK-9(H_2). The diagnostic diamino acid in cell wall hydrolyzates was lysine with peptidoglycan type Lys–Ala_3 (type A3α).

Molecular characterization

Phylogenetic study of the 16S rRNA gene sequence data showed that strain FCS-11^T belonged to the genus *Kocuria*. Sequence analysis revealed that the strain FCS-11^T was most closely related to *K. turfanensis* MTCC 10790^T (99.4% identity), followed by *K. polaris* MTCC 3702^T (98.2%), *K. rosea* MTCC 2522^T (98.2%), *K. flava* MTCC 10971^T (98.2%), *K. aegyptia* MTCC 10791^T (98.0%), *K. himachalensis* MTCC 7020^T (97.5%) and *K. atrinae* MTCC 10972^T (97.1%) at gene sequence similarity levels. The neighbour-joining phylogenetic tree (Fig.1) as well as maximum parsimony algorithms and maximum likelihood algorithms (Supplementary Figures 2a and 2b) very clearly showed that the strain FCS-11^T formed a separate lineage along with the most closely related strain *K. turfanensis*. However, the DNA-DNA relatedness values between the strain FCS-11^T and the other related taxa were found to be 60.32 ± 1.1% for *K. turfanensis*, (followed by 42.38 ± 0.36% for

K. polaris, 38.80 ± 0.20% for *K. rosea*, 34.71 ± 0.75% for *K. flava*, 28.37 ± 0.66% for *K. aegyptia*, 18.88 ± 0.69% for *K. himachalensis* and 33.48 ± 0.36% for *K. atrinae*). These value are all well below the 70% threshold value recommended for the delineation of bacterial species (Wayne et al., 1987). The DNA G+C content of strain FCS-11^T was estimated to be 60.7 (± 0.8) mol%, a value within the range of 60–75 mol% reported for members of the genus *Kocuria* (Stackebrandt et al. 1995; Rainey et al. 1997; Kovacs et al. 1999; Reddy et al. 2003; Kim et al. 2004; Tvrzova et al. 2005; Mayilraj et al. 2006; Li et al. 2006; Zhou et al. 2008; Kaur et al. 2011).

Conclusion

Based on the phenotypic, chemotaxonomic and phylogenetic data, strain FCS-11^T represents a novel species of *Kocuria* and is well differentiated from the closely related taxa *K. turfanensis* (MTCC 10790^T), *K. polaris* (MTCC 3702^T), *K. rosea* (MTCC 2522^T), *K. flava* (MTCC 10971^T), *K. aegyptia* (MTCC 10791^T) *K. himachalensis* (MTCC 7020^T) and *K. atrinae* (MTCC 10972^T). Table 1 and 2 shows the main features that distinguish strain FCS-11^T from these closely related species. Therefore, strain FCS-11^T should be classified as a novel species of the genus *Kocuria* for which the name *Kocuria sediminis* sp. nov. is proposed.

Description of *Kocuria sediminis* sp. nov

Kocuria sediminis (se.di.mi′ nis. L. gen. n. sediminis of sediment, isolated from marine sediment).

The cells are Gram-positive, aerobic, non-spore forming, coccoid, occurring in clusters. Colonies are pinkish-orange, opaque, irregular, convex, entire and 1-2 mm in diameter on tryptic soy agar medium, capable of growing between 25°C and 37°C, at the optimum temperature of 30°C and a pH range from 6.0 to 10.0; can tolerate up to 5.0% NaCl. Strain FCS-11^T shows positive reaction for hydrolysis of starch, negative for casein hydrolysis, urease production, MRVP reaction, hydrogen sulphide production, nitrate reduction. Acid is produced from arabinose, galactose, dextrose, mannitol, raffinose, salicin, xylose, fructose, lactose, sorbitol and cellobiose. Negative for acid production from dulcitol, rhamnose, maltose, *meso*-inositol, adonitol and inulin. Other detailed characteristics are mentioned in Table 1. Major polar lipids are phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), three unknown phospholipids (PL) and six unidentified lipids (UK). The major menaquinones detected for the strain FCS-11^T are MK-7(H_2) 26.3%, MK-8(H_2) 31.4% and MK-9(H_2) 42.1%. The predominant fatty acids are anteiso C15:0 and iso-C15:0. The diagnostic diamino acid in cell wall hydrolyzate was lysine with peptidoglycan type is Lys–Ala_3 (type A3α). The DNA G+C content of the strain is 60.7 mol%. The

type strain, FCS-11^T (=MTCC 10969^T = JCM 17929^T), was isolated from a marine sediment sample collected from Fort Cochin area, coastal Arabian Sea, state of Kerala, India.

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Table 1. Differential phenotypic characteristics of strains 1, FCS-11^T(MTCC 10969^T); 2, *K. turfanensis* (MTCC 10790^T); 3, *K. polaris* (MTCC 3702^T); 4, *K. rosea* (MTCC 2522^T); 5, *K. flava* (MTCC 10971^T) ; 6, *K. aegyptia* (MTCC 10791^T); 7, *K. himachalensis* (MTCC 7020^T); and 8, *K. atrinae* (MTCC 10972^T). All the data from present study. +, Positive; - negative.

All strains were positive for growth at temperatures 25-37^{o}C, pH 6.0 - 10.0, 0-5% NaCl. All are negative for methyl red and Voges-Proskauer tests, indole production, H_2S production, hydrolysis of gelatin, casein and acid production from inulin. In the Biolog GP2 MicroPlate test, all strains are negative for utilization of α-cyclodextrin, glycogen, inulin, mannan, N-acetyl-D-glucosamine, N-acetyl-β-D-mannosamine, amygdalin, D-arabitol, arbutin, L-fucose, gentiobiose, α-methyl-D-galactoside, 3-methyl glucose, α-methyl-D-glucoside, β-methyl-D-glucoside, α-methyl-D-mannoside, sedoheptulosan, stachyose, D-tagatose, xylitol, α-ketoglutaric acid, N-acetyl-L-glutamic acid, L-alaninamide, D-alanine, glycyl-L-glutamic acid, putrescine, 2'-deoxy adenosine, uridine, adenosine-5'-monophosphate, thymidine-5'- monophosphate, uridine-5'- monophosphate, D-fructose-6-phosphate, α-D-glucose-1-phosphate, D-glucose-6-phosphate, DL- α-glycerol phosphate.

Characteristics	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Growth at/on:								
15^{o}C	-	-	+	+	-	-	-	-
42^{o}C	-	-	-	-	+	-	+	-
pH 5.0	-	+	+	-	+	+	+	-
pH11	-	-	+	-	-	+	-	-
7% NaCl	-	+	-	+	+	+	+	-
10% NaCl	-	+	-	-	+	-	-	-
Hydrolysis of starch	+	+	+	+	+	-	-	-
Citrate utilization	-	-	-	-	+	-	-	-
Nitrate reduction	-	+	+	-	+	-	-	+
Catalase	-	+	+	-	+	+	+	+
Oxidase	-	-	+	-	-	-	-	-
Urease	-	-	-	-	+	-	-	-
Acid production from:								
Arabinose	+	-	-	-	-	-	-	-
Galactose	+	+	+	-	-	-	+	+
Dextrose	+	-	-	-	+	+	-	-
Cellobiose	+	-	+	-	-	+	+	+
Mannitol	+	+	-	+	-	-	-	-
Raffinose	+	+	-	-	+	-	-	-
Salicin	+	-	-	-	-	-	-	-

Xylose	+	-	+	+	+	-	-	-
Fructose	+	-	+	-	+	+	-	-
Sucrose	-	-	-	-	+	-	+	+
Lactose	+	-	-	-	+	-	+	+
Sorbitol	+	-	+	+	+	+	+	+
Melibiose	-	+	-	-	+	-	-	-
Trehalose	-	+	-	-	-	+	-	-
Rhamnose	-	-	+	-	-	-	-	-
Mannose	-	-	+	+	-	+	-	-
Adonitol	-	-	-	-	+	-	+	+
Dulcitol	-	-	-	-	-	-	+	+
Maltose	-	-	-	-	+	-	-	-
Inositol	-	-	-	-	+	-	-	-
Utilization of substrate as sole carbon source (using Biolog GP2 MicroPlate) :								
β-Cyclodextrin	-	-	-	-	-	-	-	+
Dextrin	-	-	+	+	+	-	+	-
Tweens 40, 80	-	-	+	+	+	-	-	+
L-Arabinose	+	-	-	-	-	-	-	-
D-Cellobiose	+	-	+	-	-	+	+	+
D-Fructose	+	-	+	-	+	+	-	-
D-Galactose	+	+	+	-	-	-	+	+
D-Galacturonic acid	-	-	-	-	-	-	-	+
D-Gluconic acid	-	-	+	+	-	-	-	-
α-D-Glucose	+	+	+	+	+	+	+	-
meso-inositol	-	-	-	-	+	-	-	-
α-D–Lactose	+	-	-	-	+	-	+	+
Lactulose	-	-	+	+	-	-	-	-
Maltose	-	-	-	-	+	-	-	-
Maltotriose	+	+	+	+	+	-	-	-
D-Mannitol	+	+	-	+	-	-	-	-
D-Mannose	-	-	+	+	-	+	-	-
D-Melezitose	-	-	-	-	-	-	-	+
D-Melibiose	-	+	-	-	+	-	-	-
β–Methyl-D-galactoside	-	-	-	-	-	-	-	+
Palatinose	-	-	+	-	-	-	-	-
D-Psicose	-	+	+	+	+	+	-	-
D-Raffinose	+	+	-	-	+	-	-	-
L-Rhamnose	-	-	+	-	-	-	-	-
D-Ribose	+	+	+	+	+	+	+	-
Salicin	+	-	-	-	-	-	-	-
D-Sorbitol	+	-	+	+	+	+	+	+
Sucrose	-	-	-	-	+	-	+	+
D-Trehalose	-	+	-	-	-	+	-	-

Turanose	-	-	+	+	-	-	-	-
D-Xylose	+	-	+	+	+	-	-	-
Acetic acid	-	-	+	+	+	+	+	-
α-Hydroxybutyric acid	-	-	+	+	+	+	-	-
β-Hydroxybutyric acid	+	+	-	+	+	+	+	-
γ-Hydroxybutyric acid	+	+	+	+	+	-	-	-
p-Hydroxy-phenylacetic acid	-	-	-	-	+	-	+	-
α-Ketovaleric acid	-	-	+	+	-	-	-	-
Lactamide	-	-	+	-	-	-	-	-
D-Lactic acid methyl ester	-	-	+	-	+	-	-	-
L-Lactic acid	-	-	+	+	+	-	-	-
D-Malic acid	-	-	+	-	-	+	-	-
L-Malic acid	-	-	+	-	+	+	-	-
Pyruvic acid methyl ester	+	-	+	+	+	+	+	-
Succinic acid mono methyl ester	+	+	+	-	+	+	-	-
Propionic acid	-	-	+	-	+	-	-	+
Pyruvic acid	+	+	+	+	+	+	+	-
Succinamic acid	-	-	+	-	+	+	-	-
Succinic acid	-	-	+	-	+	+	-	-
L-Alanine	-	-	-	-	-	-	-	+
L-Alanyl glycine	-	-	-	-	-	-	-	+
L-Asparagine	-	-	-	+	+	-	-	-
L-Glutamic acid	-	-	+	+	+	-	-	-
L-Pyroglutamic acid	-	-	+	-	-	-	-	-
L-Serine	-	-	+	+	-	-	-	-
2,3-Butanediol	+	-	-	-	-	-	-	+
Glycerol	+	-	+	-	+	-	-	-
Adenosine	-	-	-	-	+	-	+	-
Inosine	-	-	-	-	-	-	-	+
Thymidine	-	-	-	-	+	-	-	-
Biochemical tests using VITEK® 2-GP card								
α-Glucosidase	-	+	-	-	+	-	-	-
Leucine arylamidase	+	+	+	+	+	+	+	-
α-Galactosidase	-	+	-	-	+	-	-	-
Alanine-arylamidase	+	+	+	-	+	-	+	+
Tyrosine arylamidase	-	-	-	+	-	-	-	-
Ala-Phe-Pro-arylamidase	-	-	-	-	+	-	-	-
Menaquinones	MK7 (H_2) MK8 (H_2) MK9 (H_2)	MK8 (H_2) MK9 (H_2)	MK8 (H_2) MK9 (H_2)	MK7 (H_2) MK8 (H_2) MK9 (H_2)	MK8 (H_2) MK9 (H_2)	MK8 (H_2) MK9 (H_2)	MK7 (H_2) MK8 (H_2) MK9 (H_2)	MK7 (H_2) MK8 (H_2) MK9 (H_2)

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Table 2. Percentage of total cellular fatty acids from strains 1. FCS-11^{T} (MTCC 10969$^{T)}$2. *K. turfanensis* (MTCC 10790^{T}), 3. *K. polaris (*MTCC 3702^{T}), 4. *K. rosea* (MTCC 2522^{T}), 5. *K. flava* (MTCC 10971^{T}), 6. *K. aegyptia* (MTCC 10791 ^{T}), 7. *K. himachalensis* (MTCC 7020^{T}), 8. *K. atrinae* (MTCC 10972^{T}). All data from present study.

Type of fatty acids	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
C12:0	ND	ND	ND	ND	ND	ND	ND	0.7
iso-13:0	0.4	ND	ND	ND	ND	ND	3.0	ND
anteiso C13:0	ND	ND	ND	ND	0.3	ND	0.7	ND
iso-14:0	2.9	3.2	1.0	1.8	2.3	1.9	3.2	1.2
C14:0	1.4	1.8	0.8	1.3	0.7	1.6	1.4	2.9
iso-14:0 3OH	ND	ND	ND	ND	0.5	ND	ND	ND
anteiso C15:1 A	ND	ND	0.5	ND	ND	ND	ND	ND
iso-15:0	12.6	14.2	6.6	5.9	5.2	4.1	26.1	9.8
anteiso C15:0	67.9	66.6	73.1	52.3	65.1	78.6	48.8	67.9
C15:0	ND	ND	ND	ND	ND	ND	ND	0.5
iso-16:1 H	0.6	ND	ND	ND	ND	ND	0.5	ND
iso-16:0	1.4	1.9	1.6	2.4	4.3	1.9	1.8	1.1
C16:1 CIS 9	1.3	2.0	1.7	2.6	ND	1.4	2.4	4.9
C16:0	2.6	1.9	2.3	6.4	3.2	2.7	1.7	2.8
C16:09? METHYL	ND	ND	ND	ND	ND	ND	1.0	0.6
iso-16:0 10 METHYL	ND	ND	ND	ND	2.5	ND	ND	ND
anteiso C17:1 C	1.3	1.7	2.4	1.1	ND	0.8	1.6	2.3
iso-17:1 H	ND	ND	ND	ND	3.0	ND	ND	ND
iso-17:0	ND	ND	1.0	1.0	0.5	ND	0.6	ND
anteiso C 17:0	1.7	1.8	7.2	4.8	9.8	2.6	1.6	2.7
C18:0	1.1	ND	ND	4.4	0.5	0.9	ND	0.9
C18:1 CIS 9	ND	ND	ND	0.6	ND	0.6	ND	ND
C19:0 CYCLO C11-12	ND	ND	ND	ND	ND	0.8	ND	1.0
anteiso C 19:0	ND	ND	ND	9.7	ND	ND	ND	ND
C20:1 CIS 11	2.1	4.5	1.3	4.1	0.5	ND	1.7	ND
Unknown 17.493	2.4	ND	0.4	ND	1.4	1.2	3.2	ND

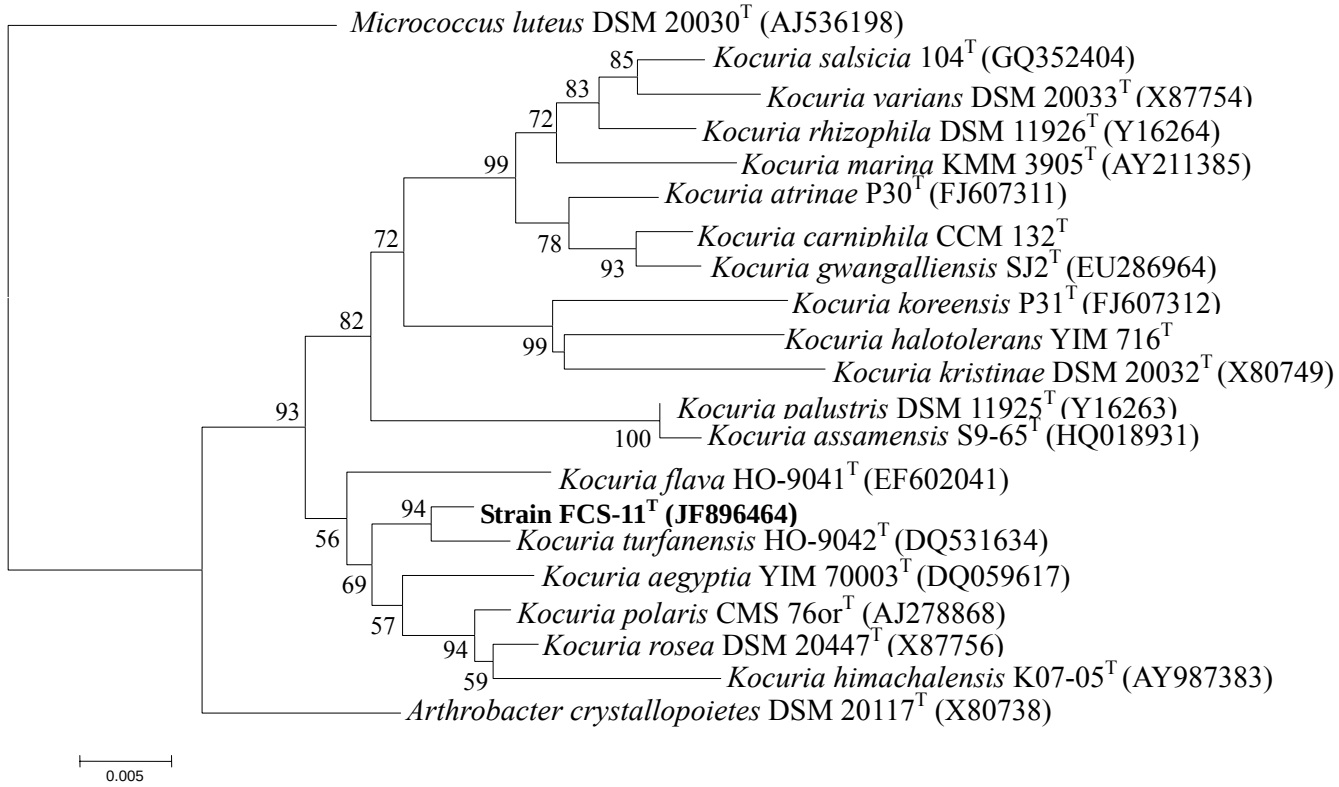
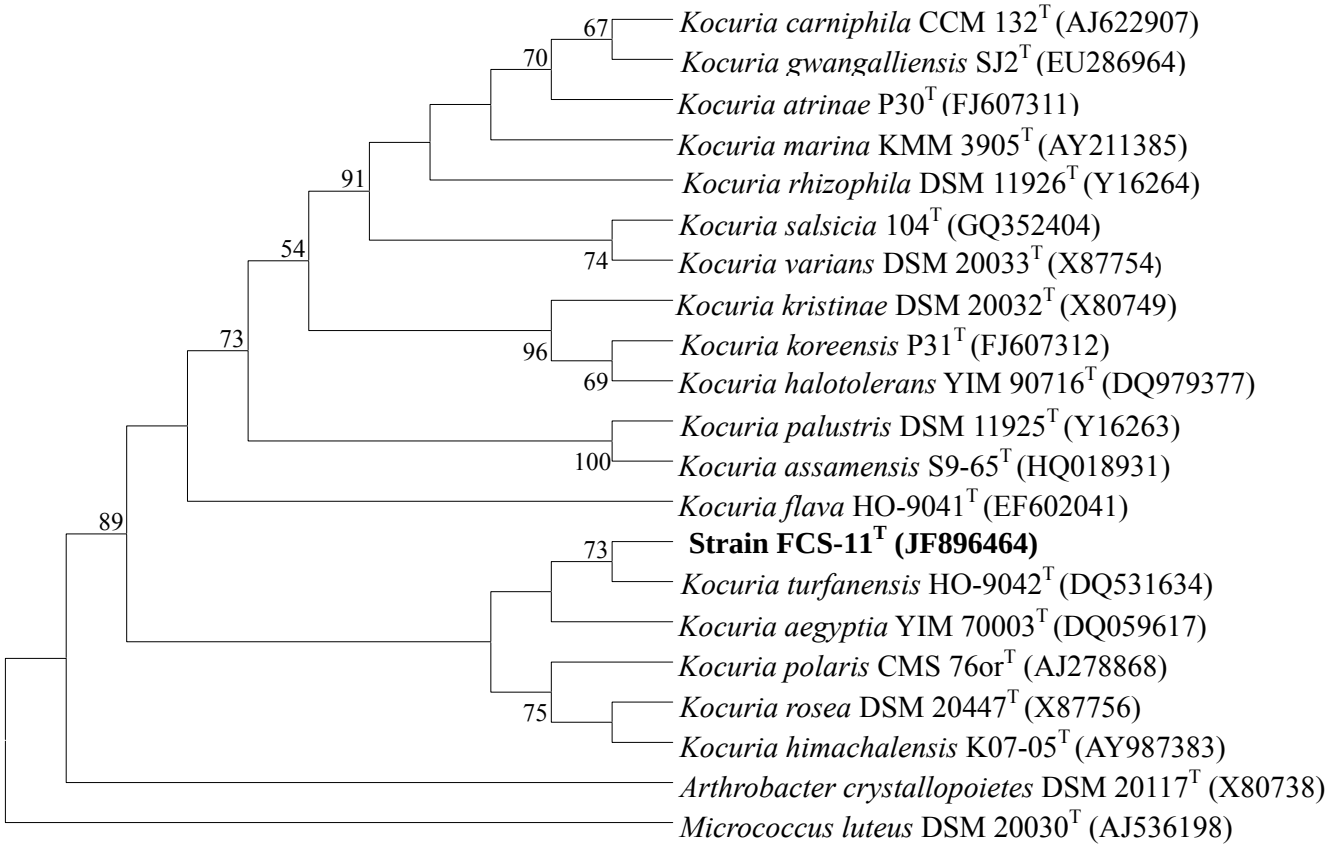
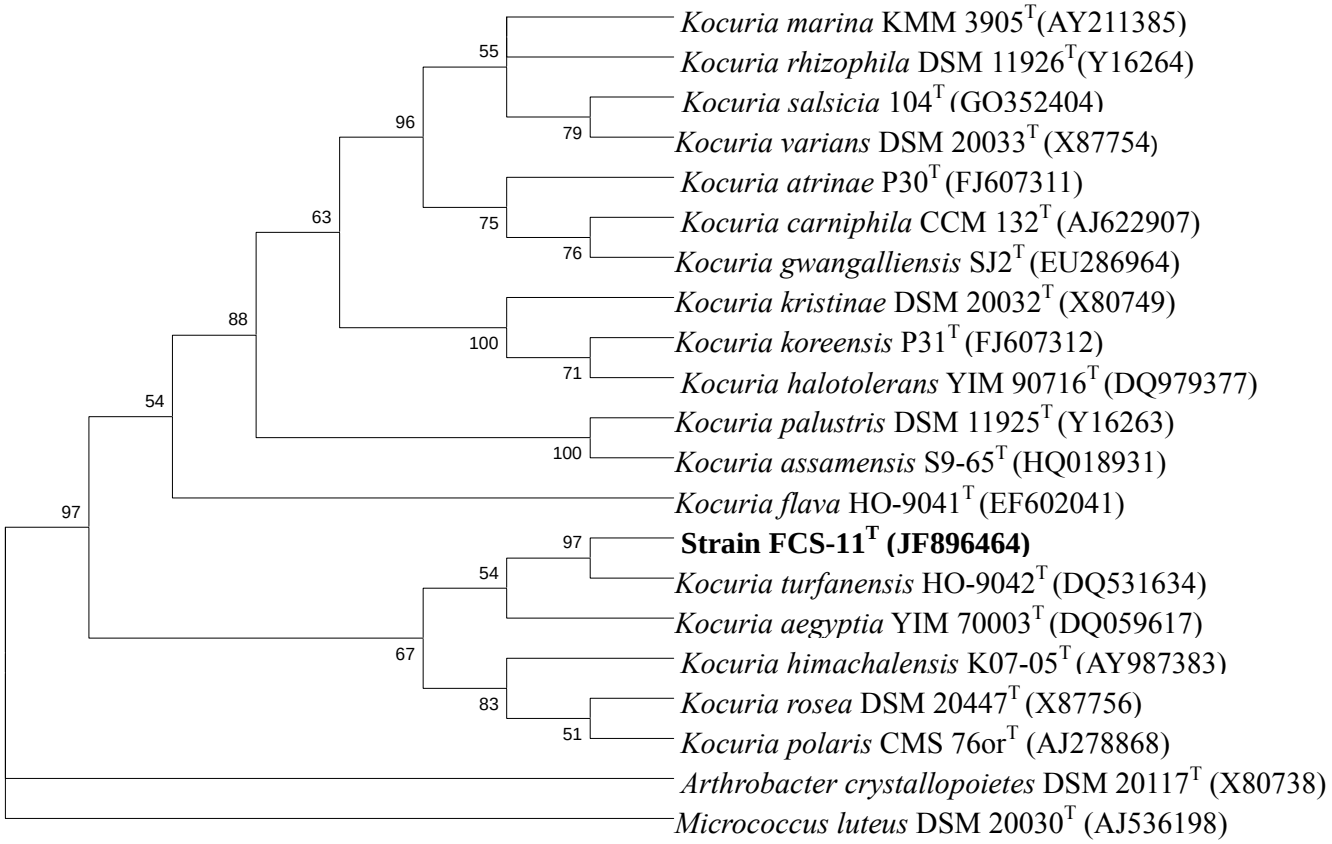


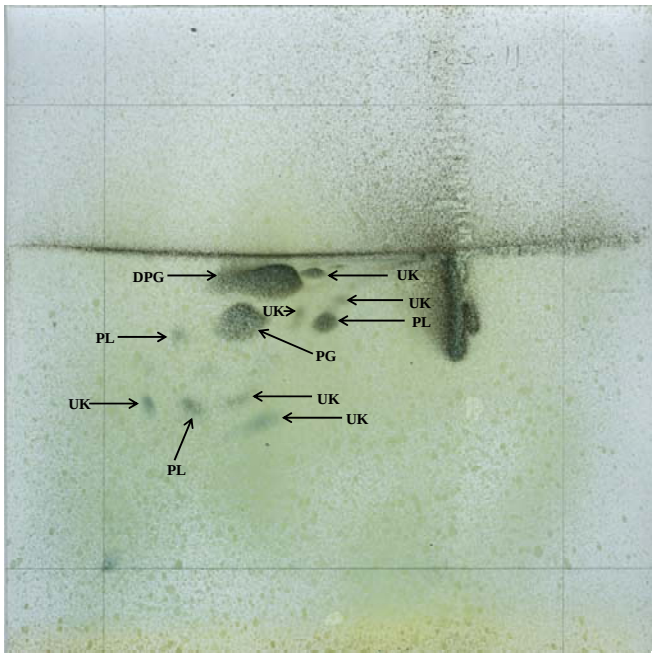
Fig.1. Phylogenetic neighbour-joining tree based on 16S rRNA gene sequences showing the relationship between *Kocuria sediminis* FCS-11^T and related members of the genus *Kocuria.* *Micrococcus luteus* DSM 20030^T (AJ536198) was used as an out-group. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are given at nodes. Bar, 0.005 % sequence variation. GenBank accession numbers are given in parentheses.



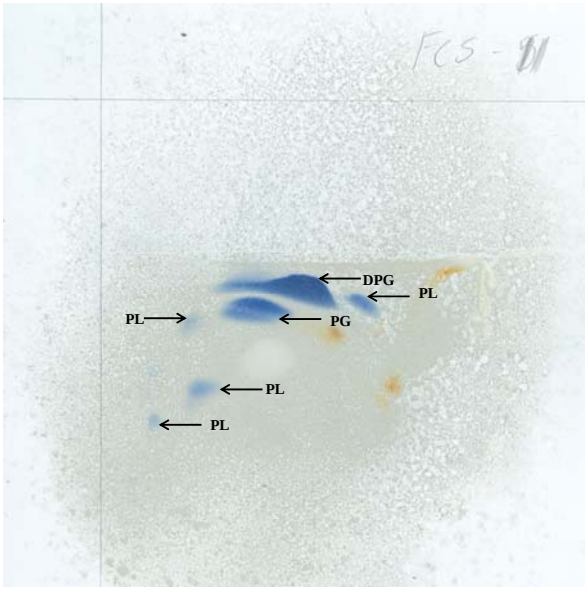
Supplementary Fig 2a. Maximum Parsimony Algorithms Analysis Bootstrap values (expressed as percentage of 100 replications) greater than 50 % are given at the nodes.



Supplementary Fig 2b. Maximum Likelihood Algorithms Analysis Bootstrap values (expressed as percentage of 100 replications) greater than 50 % are given at the nodes.



Supplementary Fig. 1a. Two-dimensional thin layer chromatograms of the total lipids of strain FCS-11^T, detected with molybdatophosphoric acid (5% w/v) in absolute ethanol. Phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), unidentified phospholipid (PL), unknown lipids (UK)



Supplementary Fig. 1b. Two-dimensional thin layer chromatograms of total lipids of strain FCS-11^T, sprayed with molybdenum blue to detect phospholipids. Phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), unidentified phospholipids (PL).

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391 **Supplementary Fig. 1c.** Two-dimensional thin layer chromatograms of total lipids of strain FCS-

392 11^T, sprayed with ninhydrin to detect amine groups. Unknown amines (UKA)

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प्रतिरुप-मानक सूक्ष्मजीव संग्रहण एवं जीन बैंक
MICROBIAL TYPE CULTURE COLLECTION & GENE BANK
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April 28th, 2011

MTCC

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Confirmation of the availability of a strain for the purpose of valid publication of a new name according to the Bacteriological Code

The following information is confidential and serves only to allow the International Journal of Systematic and Evolutionary Microbiology to confirm that the following strain(s) has been deposited and will be available from the MTCC in accordance with the Rules of the Bacteriological Code (1990 revision) as revised by the ICSP at the plenary sessions in Sydney and Paris.

The strain(s) has been deposited in the MTCC under the number(s)

Strain Designation	Accession Number
Kocuria sediminis strain FCS-11^T	MTCC 10969^T

These strains are available in the publicly accessible section of the MTCC and restrictions have not been placed on access to information concerning the presence of this strain in the MTCC. It will be included in published and online catalogues after publication of this number by authors.

This strain has been checked for viability in the MTCC and is stored using one of the standard methods used in the MTCC.

The depositor of this strain has also carried out a "depositor's check" and confirmed the identity of the strain held under this MTCC number.

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