Rhodococcus Yananensis Sp. Nov., Isolated From Microbial Fermentation Bed Material From a Pig Farm

Yingying Jiang
Yan'an University School of Life Science
Chaochao Zheng
Yan'an University School of Life Science
Tianfei Yu
Yan'an University School of Life Science
Jing Li
Yan'an University School of Life Science
Jiamin Ai
Yan'an University School of Life Science
Maiping Li
: College of Veterinary Science and Animal Husbandry
Xiaodong Liu
Yan'an University School of Life Science https://orcid.org/0000-0002-3434-1706
Zhenshan Deng (zhenshandeng214@163.com)
Yan'an University School of Life Science

Research Article

Keywords: Rhodococcus, Microbial fermentation bed material, Genome, Prokaryotic taxonomy

Posted Date: October 28th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1014229/v1

Abstract

An opaque, pink-colored, gram-positive, aerobic bacteria (designated FBM22-1^T), 0.5 to 1.0 μm in width and 0.5 to 1.5 μm in length, was isolated from microbial fermentation bed material from a pig farm in northwestern China. Optimal growth occurred at 30–37°C, pH 7.0, and 0.5% NaCl (w/v). Phylogenetic analysis based on the 16S rRNA gene sequences revealed that the novel isolate belonged to the family Nocardiaceae of the class Actinomycetia. FBM22-1^T is closely related to *Rhodococcus zopfii* NBRC 100606^T and *Rhodococcus rhodochrous* NBRC 16069^T, with 16S rRNA gene sequence similarity of 97.95% and 97.73%, respectively. The predominant respiratory quinone in FBM22-1^T was ubiquinone MK-8(H_2), and the cellular fatty acids consisted primarily of $C_{16:1}$ω7c/$_{16:1}$ω6c, C1$_{6:0,}$ and $C_{18:0}$ 10-methly1. The major polar lipids were diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine and glycolipids. The G+C content of FBM22-1^T was 68.64 mol%. Based on the phenotypic, phylogenetic, and chemotaxonomic characterization, in combination with low values of digital DNA–DNA hybridization between FBM22-1^T and its closest neighbors, FBM22-1^T represents a novel species of the genus *Rhodococcus*, for which the name *Rhodococcus yananensis s*p. nov. is proposed; the type strain is FBM22-1^T (=KCTC 49502^T = CCTCC 2020275^T).

Introduction

The genus *Rhodococcus* belonging to the phylum Actinobacteria(Alexander 2019), was first described by Zopf (1891). Currently, the genus comprises more than 70 named species (https://www.bacterio.net/rhodococcus.html). Extensive data from polyphasic taxonomic studies have improved classification of members of this genus (Bunch 1998; Goodfellow 2004; Kampfe 2014). The *Rhodococcus* genus is gram-positive, non-motile, nonsporulating, aerobic bacteria, with a high G+C content (61–71%), and a mycolic acid-containing cell wall(Alexande 2019). *Rhodococcus* species are genetically and physiologically diverse, widely distributed in soil, water, and ocean deposits (Hong 2011; Konishi 2014; Shevtsov 2013). Certain *Rhodococcus* species may be pathogens for plants (Kampfe 2014; Yeon 2020) animals (Leonardo 2018), and humans (Takai 1986).

Members of the genus *Rhodococcus* exhibit diverse metabolic activities, including aliphatic and aromatic hydrocarbon degradation (Goodfellow 2004; Jung-Hoon 2000) and storage compound production (Michael 2008; Bunch 1998). The use of purified enzymes and whole-cell biocatalysts is becoming increasingly popular in synthetic organic chemistry. In the present study, a bacterial strain isolated from microbial fermentation bed material was subjected to polyphasic taxonomic analysis and subsequently allocated to the genus *Rhodococcus*.

Materials And Methods

Sample collection and preservation

The fermentation bed material was collected from a pig farm located in Yan'an, Shaanxi Province (36°61'N; 109°46'E) at an altitude of 845.7m. Samples were serially diluted with sterile water and cultured onto peptone yeast extract glucose agar (PYG; peptone 5g, yeast powder 0.2g, glucose 5g, beef extract 3g, sodium chloride 0.5g, magnesium sulfate heptahydrate 1.5g, agar 15–20g, water 1000ml and pH7.2–7.5) at 30℃ under aerobic conditions. Following incubation for 5 days, single colonies were selected and subcultured onto fresh PYG plates. Pure isolates were preserved at -80℃ in 20% glycerol (v/v).

Morphological, physiological, and biochemical analysis

Colony morphology was observed using cells grown on PYG plates at 30℃ for 3 days. Cell morphology was observed using a scanning electron microscope (SEM; JSM-7610F, Japan). The Gram reaction was determined using the bioMérieux Gram Stain Kit (Marcy-l'Étoile, France), according to the manufacturer's instructions. Growth conditions were determined by incubating the isolates on PYG agar for 10 days at varying temperature (4, 10, 15, 20, 25, 28, 30, 35, 37, 40, and 45℃) and pH ranges (pH 3–11 in increments of 1.0 pH units). Growth in various NaCl concentrations (0%, 0.5%, 1%, 2%, 3%, 8%) was evaluated in PYG broth.

The oxidase activity was determined using 1% p-aminodiphenyl-amine-hydrochloride liquor and 1% a-naphthol ethanol liquor. Methyl red and Voges-Prokauer test (MR-VP) were measured using glucose-peptone liquid medium. The catalase activity test was performed by the observation of the formation of bubbles using a commercial dropper catalase reagent (bioMérieux). Tween-20, Tween-40, and Tween-80 hydrolysis, amylase, gelatin, and benzpyrole production; nitrate reduction; denitrification; H_2S production; and carbon and nitrogen source experiments were evaluated according to multiple classification identification. Sensitivity to the following antibiotics was tested on PYG plates using antibiotic discs (Changde Beekman Biotechnology Co, Ltd., Hunan, China): penicillin, ampicillin, ceftriaxone, gentamicin, tetracycline, erythromycin, ciprofloxacin, lincomycin, cotrimoxazole, and chloramphenicol.

Phylogenetic analysis

16S rRNA gene sequence analysis of FBM22-1^T was performed. Genomic DNA was extracted from a pure culture using a PureLink Genomic DNA Mini Kit (Sangon Biotech, Shanghai, China) according to the manufacturer's instructions. The 16S rRNA gene was amplified by PCR with two universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGAC-3')(Qian-Qian 2014). The 16S rRNA gene sequences of FBM22-1^T and the related strains of the genus *Rhodococcus* was trimmed to 1366 bp and aligned using ClustalW. The phylogenetic tree based on the16S rRNA gene sequences was constructed with MEGA7.0 (Kumar 2016) using the neighbor-joining (NJ) (Wei 2008), maximum-likelihood (ML) (Acitas 2021), and maximum-parsimony (MP) algorithms (Adam 2020).

Genome comparison

To support the classification of the strain as a novel species within the genus *Rhodococcus*, the Average Nucleotide Identity (ANI) using BLAST (ANIb) and MUMmer (ANIm) algorithms, was calculated using the

JSpecies Web Server (Richter 2016). Digital DNA–DNA hybridization (dDDH) was calculated in silico by the Genome-to-Genome Distance Calculator webserver version 2.1 (http://ggdc.dsmz.de//) (Bhattacharya 2020) using the BLAST method. Results were based on recommended formula 2 (identities/HSP length), which is independent of genome length and is therefore robust against the use of incomplete draft genomes. Total DNA of the samples was sequenced using Illumina NovaSeq platform (MAGIGENE, Guangdong, China). Genome coding gene prediction was achieved using Glimmer3 (Arthur 2007).

Chemotaxonomic analysis

The chemotaxonomic profile of the isolated actinobacterial strain was investigated to establish whether it has a chemotaxonomic profile typical of members of the genus *Rhodococcus*. Standard procedures were used to determine the chemical constituents of FBM22-1^T. The strain was cultured in PYG liquid medium (30°C, 160 rpm) for 7 days. Following this, the culture was centrifuged (4000 x g) for 10 min and washed twice with sterile water. Polar lipids were methylated and analyzed by two-dimensional thin-layer chromatography (TLC silica Gel 60 F254:25 Aluminum Sheets 20×20), using chloroform-methanol-water (65: 25: 4, v/v/v) as the first solvent and chloroform-methanol-acetic acid-water (40: 6: 7.5: 1, v/v/v/v) as the second. Individual polar lipids were identified by spraying with molybdophosphoric acid and molybdenum blue. Fatty acid composition of the strains was analyzed using the Sherlock Microbial Identification System (MIDI Inc., DE, USA). Menaquinones were extracted as described by Minnikin et al. (Minnikin 1984) and determined using reversed-phase HPLC as described by Collins (Collins 1979).

Results And Discussion

Morphological and physiological characteristics

The bacterial colonies were dry, opaque, light pink. SEM revealed that the cells were short rods, 0.5–1.0 µm in width and 0.5–1.5 µm in length (Fig. S2). These characteristics matched those of the genus *Rhodococcus*. The FBM22-1^T was identified as gram-positive aerobic bacteria. The organism was able to grow at 10–45 °C (optimum, 30–37°C), pH 4.0–10.0 (optimum 7.0). Strain growth rate decreased with an increase in NaCl concentration, with an optimal growth rate at NaCl concentration of 0.5% (Table S2). *R. zopfii* NBRC100606^T is rod shaped with a size range of 1.10–2.0 µm in length, 0.55–0.80 µm in width, and is light pink in color (Rehfuss 2005). On comparison, it was observed that the two strains were similar in color, and FBM22-1^T was shorter and wider than strain *R. zopfii* NBRC100606 ^{T}, presenting a short rod shape. Regarding the physiological characteristics of FBM22-1^T, glycine, L-phenylalanine, tryptophan, methionine, tyrosine, or glutamic acid could be used as sole nitrogen source for the bacteria. Carbon sources included arabinose, maltose, D-sorbitol, dextrin, inositol, sodium acetate, sucrose, D-ribose, galactose, D-xylose and L-rhamnose, and the strain could hydrolyze tween-20 (Table 1). FBM22-1^T produced catalase and weak indole, and did not produce amylase, oxidase, gelatinase, and H_2S. The MR-VP test was negative. Nitrate reduction and denitrification were positive (Table S2). D-sorbitol, inositol, sucrose, and L-rhamnose could not be utilized by *R. zopfii* NBRC 100606^T (Yoshimoto 2004) while

FBM22-1^T could. Tyrosine, D-Sorbitol, D-Glucose, maltose, trehalose, and sucrose acetate could be utilized as carbon sources by both FBM22-1^T and *R. rhodochrous* NRBC 16069^T (Rehfuss 2005). Differential physiological characteristics of strains *R. rhodochrous* DSM 43241^T (=NRBC 16069^T) and *R. biphenylivorans* TG9^T are presented in Table 1.

In terms of the sensitivity to antibiotics test, penicillin, ampicillin, ceftriaxone, and chloramphenicol produced an inhibition zone that exceeded 1.0 cm. Gentamicin, tetracycline, erythromycin, and ciprofloxacin produced a smaller zone of inhibition of 0.5–1.0 cm, and the strain was resistant to lincomycin and cotrimoxazole (Table S2).

Phylogenetic analysis

Comparison of 16S rRNA gene sequences of FBN22-1^T and other members of the genus *Rhodococcus* showed sequence similarities ranging from 96–98%. The results revealed that FBM22-1^T had the closest relationship with *R. zopfii* NBRC 100606^T, with 97.95% similarity, followed by *R. rhodochrous* NBRC 16069^T (97.73%) and *R. biphenylivorans* TG9^T (97.66%). The phylogenetic reconstructions revealed that FBM22-1^T formed a distinct phylogenetic link within the *Rhodococcus* 16S rRNA gene tree (Fig. 1), adjacent to the type strains *R. artemisiae* YIM 65754^T (Guo 2012) and *R. ruber* DSM 43338^T(Tsukamura 1985). The pairwise similarities to the sequences of these three strains were 97.58 and 97.29%, respectively. Although the NJ bootstrap support for the placement of this novel *Rhodococcus* sequence was low, the other two methods showed a similar topology (Fig. S1). It is likely that phylogenetic stability will be achieved when more novel related *Rhodococcus* strains are described and/or whole genome analyses are performed.

Draft genome sequencing of FBM22-1^T (accession number JAIYEP000000000) yielded a genome of 4,250,953 bp in length after assembly, producing 178 scaffolds, and a N50 and N90 value of 49,062 bp and 11,764 bp, respectively. All scaffolds were > 511 bp and the largest was 16,639 bp. A total of 4303 protein coding genes were predicted. FBM22-1^T had one Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) sequence, and the total length was 393 bp. The G+C mol% content of the FBM22-1^T strain was 68.64 mol%, which falls within the range provided for *Rhodococcus* species.

According to the data obtained, the ANIb and ANIm between strains FBM22-1^T and *R. zopfii* NBRC 100606^T were 78.88% and 85.57%, respectively; the ANIb and ANIm between the FBM22-1^T and the other two species (*R. rhodochrous* NBRC 16069^T and *R. biphenylivorans* TG9^T) tested were < 86% (Table S3). The species cut-off value for ANIb was selected as 95–96%(Michael 2009). The dDDH between strains FBM22-1^T and *R. zopfii* NBRC 100606^T was 26.8%. The dDDH comparison of FBM22-1^T with the draft genome of the two strains (*R. rhodochrous* NBRC 16069^T and *R. biphenylivorans* TG9^T) yielded low percentages (< 27%) (Table S3). The species cut-off value for dDDH was 70% (Wayne LG 1987), thus suggesting the FBM22-1^T strain should be considered as a new species of the genus *Rhodococcus*.

Chemotaxonomic characteristics

The major fatty acids present in FBM22-1^T (Table 2; ≥ 10% of the total) were $C_{16:0}$, $C_{16:1}\omega7c/_{16:1}\omega6c$, $C_{18:0}$ 10-methly1, followed by $C_{18:1}\omega9c$, $C_{16:0}$ 10-methly1 (5–10%), and a small amount (< 5%) $C_{14:0}$, $C_{19:1}\omega7c/_{19:1}\omega6c$, $C_{18:0}$, $C_{17:0}$; it had no cis-fatty acids. In *R. zopfii* (NBRC100606^T = DSM 44108^T), *R. rhodochrous* DSM 43241^T, and *R. biphenylivorans* TG9^T the fatty acids were more complex than that in FBM22-1^T. For *R. zopfii*, the cis-fatty acids and major fatty acids (≥ 10% of the total) were $C_{16:0}$ and $C_{18:0}$ 10-methly1, and $C_{18:1\ cis}$ 9. $C_{16:0}$ was a major fatty acid for the four strains. The predominant respiratory quinone of FBM22-1^T was ubiquinone MK-8(H_2). The polar lipids of FBM22-1^T comprised diphosphatidylglycerol (DPG), phosphatidyl choline (PC), phosphatidylethanolamine (PE), glycolipid (GL), and unknown phospholipids (PL1, PL2; Fig. S3).

Based on the above characteristics, FBM22-1^T was classified as a new species of *Rhodococcus* in this study, for which the name *Rhodococcus yananensis* sp. nov. is proposed. We describe the species as follows.

Description of *Rhodococcus yananensis* sp. nov.

Rhodococcus yananensis (yan.an.en'sis. N.L. masc. adj. *yananensis* a city in Shaanxi province of China, from where the type strain was isolated).

FBM22-1^T is a opaque, pink-colored, gram-positive aerobic bacteria. The strain is short rod, 0.5–1.0μm in width and 0.5–1.5 μm in length. FBM22-1^T can use ammonium nitrate, potassium nitrate, glycine, L-phenylalanine, tryptophan, methionine, tyrosine, and glutamic acid as a sole nitrogen source. Sole carbon sources include arabinose, maltose, D-sorbitol, dextrin, inositol, sodium acetate, sucrose, D-ribose, galactose, D-xylose, and L-rhamnose. Optimal growth environment is 30–37℃, pH 7.0, and NaCl (w/v) 0.5%. FBM22-1^T can hydrolyze Tween-20 and produces catalase and indole. The strain is sensitive to penicillin, ampicillin, ceftriaxone, chloramphenicol, gentamicin, tetracycline, erythromycin, and ciprofloxacin. FBM22-1^T has PC, PE, and phosphatidyl methyl ethanolamine. It contains following fatty acids: $C_{16:0}$, $C_{16:1}\omega7c/_{16:1}\omega6c$, $C_{18:0}$10-methly1, $C_{18:1}\omega9c$, $C_{16:0}$10-methly1, $C_{14:0}$, $C_{19:1}\omega7c/_{19:1}\omega6c$, $C_{18:0}$ and $C_{17:0}$. The predominant respiratory quinone is ubiquinone MK-8(H_2). The G+C content is 68.64%.

The type strain FBM22-1^T (=KCTC 49502^T = CCTCC 2020275^T) was isolated from fermented bedding material from a pig farm, located in a small village in Yan'an, Shaanxi Province (36°61*S;* 109°46W) at an altitude of 845.7m. The GenBank accession number for the 16SrRNA gene sequence of FBM22-1^T is OK161026.

Abbreviations

KCTC The Korean Collection for Type Cultures

CCTCC China Typical Culture Preservation Center

ANI Average nucleotide identity

dDDH Digital DNA-DNA hybridization

MEGA Molecular evolutionary genetics analysis

MIDI Microbial identification system

HPLC High-performance liquid chromatography

TLC Thin-layer chromatography

SEM Scanning Electron Microscope

MR-VP Methyl red and Voges-Prokauer test

NJ Neighbor-joining

ML Maximum-likelihood

MP Maximum-parsimony

GL Glycolipid

PL Unknown phospholipids

DPG Diphosphatidylglycerol

PC Phosphatidylcholine

PE Phosphatidylethanolamine

Declarations

Authors' contributions Strain FBM22-1^T was isolated by C-CZ. Material preparation, experimental operation, data collection and analysis were performed by Y-YJ, C-CZ, T-FY, JL, J-MI, and M-PL. The manuscript was written by C-CZ and Y-YJ. Project guidance and critical revision of manuscripts was performed by Z-JD and X-DL. All authors read and approved the final manuscript.

Funding This work was supported by the National Natural Science Foundation of China (Grant No. 32160003), Doctor Support Project of Yan'an University (No. YDBK2019-44), Key Science and Technology Program at County Level of Shaanxi Province (2018XY-14), Agricultural Green Technology Research and Development Integration Program of Shaanxi Provincial Department of Agriculture in 2019 and Special

Data availability The GenBank accession number for the 16S rRNA gene sequence and Whole Genome Shotgun project of strain FBM22-1^T are OK161026 and JAIYEP000000000, respectively.

Conflict of interests The authors declare that there is no conflict of interest.

References

A. Michael Warhurst, Fewson CA (2008) Biatransformations catalyzed by the genus *Rhodococcus*. Crit Rev Biotechnol 114:29-73. doi:10.3109/07388559409079833

Acitas S, Yenilmez, Ismail, Senoglu, Birdal, Kantar Yeliz, Mert (2021) Modified maximum likelihood estimator under the Jones and Faddy's skew;t;-error distribution for censored regression model. Journal of Applied Statistics 48:2136-2151. doi:10.1080/02664763.2020.1786673

Adam GD, Boyle AP (2020) MapGL: inferring evolutionary gain and loss of short genomic sequence features by phylogenetic maximum parsimony. BMC Bioinformatics 21:1-9. doi:10.1186/s12859-020-03742-9

Alexander Steinbüchel Münster, Germany (2019) Biology of Rhodococcus. vol 16.

Arthur LD, Kirsten AB, Edwin CP, LS S (2007) Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23:673–679. doi:10.1093/bioinformatics/btm009

Bhattacharya D, Sergio de los Santos Villalobos, Ruiz VV SJ, Mukherjee J (2020) *Bacillus rugosus* sp. nov. producer of a diketopiperazine antimicrobial, isolated from marine sponge *Spongia officinalis* L. Antonie Van Leeuwenhoek 113:1675-1687. doi:10.1007/s10482-020-01472-9

Bunch AW (1998) Biotransformation of nitriles by rhodococci. Antonie van Leeuwenhoek 74:89–97. doi:10.1023/A:1001760129546

Collins MD, Goodfellow M, Minnikin DE (1979) Isoprenoid quinones in the classification of coryneform and related bacteria. J Gen Microbiol 110:127-136. doi:10.1099/00221287-110-1-127

Goodfellow M, Jones AL, Maldonado LA, J S (2004) *Rhodococcus aetherivorans* sp. nov., A new species that contains methyl t-butyl ether-degrading actinomycetes. Syst Appl Microbiol 27:61-65. doi:10.1078/0723-2020-00254

Guo-Zhen Z, Jie L, Wen-Yong Z, Shou-Zheng T, Li-Xing Zet al. (2012) *Rhodococcus artemisiae* sp. nov., an endophytic actinobacterium isolated from the pharmaceutical plant *Artemisia annua* L. Int J Syst Evol Microbiol 62:900-905. doi:10.1099/ijs.0.031930-0

Hong Mei Sheng HSG, Lin Gui Xue SD, Hu Yuan Feng, Li Zhe An, Chun Li Song (2011) Analysis of the composition and characteristics of culturable endophytic bacteria within subnival plants of the Tianshan iol 62:923-932. doi:10.1007/s00284-010-9800-5

Jung-Hoon Y, Young-Gyun Cho, Seung Bum Kim, Sung Taik Le, Seok-Sung Kan, Yong-Ha Park (2000) *Rhodococcus koreensis* sp. nov., a 2,4-dinitrophenol-degrading bacterium. Int J Syst Evol Microbiol 50:1193–1201. doi:00207713-50-3-1193

Kampfe P, Dott W, Martin K, Glaeser S (2014) *Rhodococcus defluvii* sp. nov., isolated from wastewater of a bioreactor and formal proposal to reclassify "Corynebacterium hoagii" and *Rhodococcus equi* as comb. nov. Int J Syst Evol Microbiol 64:755-761. doi:10.1099/ijs.0.053322-0

Konishi M, Nishi S, Fukuoka T, Kitamoto D, Watsuji T, Y N (2014) Deep-sea *Rhodococcus* sp. BS-15, lacking the phytopathogenic *fas* genes, produces a novel glucotriose lipid biosurfactant. Mar Biotechnol 16:484-493. doi: 10.1007/s10126-014-9568-x

Kumar S, Stecher G, K T (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33. doi:10.1093/molbev/msw054

Leonardo Jose Silva DTS, Diego Bonaldo Genuario, Harold Alexander Vargas Hoyos, Suikinai Nobre Santos, Luiz Henrique Rosa, Tiago Domingues Zucchi, Itamar Soares Melo (2018) *Rhodococcus psychrotolerans* sp. nov., isolated from rhizosphere of Deschampsia antarctica. Antonie Van Leeuwenhoek 111:629-636. doi:10.1007/s10482-017-0983-7

Matthew AS, Russell PH, Staley JT (1994) *Rhodococcus zopfii* sp. nov., a toxicant-degrading bacterium. Int J Syst Evol Microbiol 44:106-110. doi:10.1099/00207713-44-1-106

Michael R, Rosselló-Móra R (2009) Shifting the genomic gold standard for the prokaryotic species definition. Pro Natl Acad Sci U S A 106:19126–19131.

Minnikin DE ODA, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233-241. doi:10.1016/0167-7012(84)90018-6

Qian-Qian L YW, Juan L, Zong-Jun D, Guan-Jun Chen (2014) *Saccharicrinis carchari* sp. nov., isolated from a shark, and emended descriptions of the genus Saccharicrinis and Saccharicrinis fermentans. Int J Syst Evol Microbiol 64:2204-2209. doi:10.1099/ijs.0.061986-0

Rehfuss M, Urban, J (2005) *Rhodococcus phenolicus* sp. nov., a novel bioprocessor isolated actinomycete with the ability to degrade chlorobenzene, dichlorobenzene and phenol as sole carbon sources. Syst Appl Microbiol 28:695-701. doi:10.1016/j.syapm.2005.05.011

Richter M, Rossello-Mora R, Oliver Glockner F, Peplies, J (2016) JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics 32:929-931. doi:10.1093/bioinformatics/btv681

Shevtsov Alexander, Tarlykov P, Zholdybayeva E, Momynkulov D, Sarsenova Aet al. (2013) Draft genome , a crude oil biodegrader. Genome announcements 1:e00846-

13–e00846-13. doi:10.1128/genomea.00846-13

Takai S, Takeuchi T, S T (1986) Isolation of *Rhodococcus (Corynebacterium) equi* and Atypical Mycobacteria from the Lymph Nodes of Healthy Pigs. Jpn J Vet Sci 48:445-448.

Tsukamura M (1985) Priority of *Rhodococcus lentifragmentus* (Kruse 1896; Tsukamura et al. 1975) Tsukamura 1978 comb. nov. over *Rhodococcus ruber* (Kruse 1896) Goodfellow and Alderson 1980: Request for an Opinion. Int J Syst Bacteriol 35:124-125. doi:10.1099/00207713-35-1-124

Wayne LG, Brenner DJ, RR C (1987) Report of the Ad Hoc Committee on reconciliation of approaches to bacterialsSystematics. Int J Syst Evol Microbiol 37:364-464. doi:10.1099/00207713-37-4-463

Wei Zhang, Sun Z (2008) Random local neighbor joining: A new method for reconstructing phylogenetic trees. Mol Phylogenet Evol 47:117–128.

Wilhelm Z (1891) Über die Wurzelbräune der Lupinen, eine neue Pilzkrankheit. Zeitschrift für Pflanzenkrankheiten 1:72-76.

Xiaomei S, Muhammad ZH, Yindong L, Jinxing H, Min W et al. (2015) *Rhodococcus biphenylivorans* sp. nov., a polychlorinated biphenyl-degrading bacterium. Antonie Van Leeuwenhoek 107:55-63. doi:10.1007/s10482-014-0303-4

YeonJeong L, Kong HG, Lee YH, Kim HR, DH P (2020) First Report of *Rhodococcus fascians* Causing Fasciation of Lilies (Lilium longiflorum Thunb.) in South Korea. Plant Disease. doi:10.1094/PDIS-10-20-2288-PDN

Yoshimoto T, agai F, Fujimoto J, Watanabe K, Mizukoshi H et al. (2004) Degradation of Estrogens by *Rhodococcus zopfii* and *Rhodococcus equi* isolates from activated sludge in wastewater treatment plants. Appl Environ Microbiol 70:5283–5289. doi:10.1128/AEM.70.9.5283-5289.2004

Tables

Table 1. Differential physiological characteristics of FBM22-1^T and its closest phylogenetic neighbors.

Strains: 1, *Rhodococcus yananensis* sp. nov. FBM22-1^T (this study); 2, *R. zopfii* DSM 44108^T(Matthew 1994); 3, *R. rhodochrous* DSM 43241^T(Xiaomei 2015); 4, *R. biphenylivorans* TG9^T(Xiaomei 2015). +, Positive; □, negative; ND, no data available.

Characteristics		1	2	3	4
Hydrolysis of	Tween-20	+	ND	ND	+
	Tween-40	+	ND	ND	+
	Tween-80	+	ND	+	+
	Starch	+	+	+	+
	gelatin	+	ND	+	+
Enzyme activities	catalase	+	ND	ND	ND
	oxidase	+	ND	ND	+
Utilization as sole nitrogen source	glycine	+	ND	ND	+
	L-phenylalanine	+	ND	+	+
	leucine	+	ND	ND	ND
	tryptophan	+	ND	ND	+
	threonine	+	ND	ND	+
	methionine	+	ND	ND	+
	cysteine dioxygenase	+	ND	ND	+
	tyrosine	+	ND	ND	+
	glutamic acid	+	ND	ND	+
Utilization as sole carbon source	arabinose	+	ND	+	+
	maltose	+	+	+	+
	D - sorbitol	+	+	+	+
	dextrin	+	ND	ND	ND
	myso-Inositol	+	+	+	+
	sucrose	+	+	+	+
	D - ribose	+		+	+
	galactose	+	+	+	
	D - xylose	+	ND	ND	ND
	L - rhamnose	+	+	+	+

Table 2 Fatty acid composition (%) of ERM22-1^T and the most closely related *Rhodococcus* species.

Strains 1, *Rhodococcus yananensis* sp. nov. FBM22-1^T (this study); 2, *R. zopfii* DSM 44108^T(Jung 2000; Matthew 1994); 3, *R. rhodochrous* DSM43241^T(Jung 2000; Xiaomei 2015) ; 4, *R. biphenylivorans* TG9^T(Xiaomei 2015). Summed feature 4, $C_{15:0}$ iso 2-OH and/or $C_{16:1}$ trans 9; Summed feature 7, $C_{18:1}$ cis 11, $C_{18:1}$ trans 6 and/or $C_{18:1}$ trans 9.

Fatty Acid	1	2	3	4
Saturated				
$C_{14:0}$	3.59	1.70	1.30	2.10
$C_{15:0}$	□	1.00	1.50	0.80
$C_{16:0}$	31.36	32.70	30.50	34.20
$C_{17:0}$	1.71	□	2.90	1.30
$C_{18:0}$	2.49	8.80	1.20	1.60
$C_{19:0}$	□	0.60	2.80	1.70
$C_{20:0}$	□	2.10	5.70	0.60
iso-$C_{15:0}$	□	□	0.40	1.10
iso-$C_{16:0}$	□	□	□	2.10
iso-$C_{17:0}$	□	□	0.20	0.40
anteiso-$C_{15:0}$	□	□	1.00	3.00
Unsaturated:				
C_{18}:1ω7c	□	□	□	2.40
C_{18}:1ω9c	7.30	□	8.00	7.30
C_{16}:1ω9c	□	□	4.80	□
C_{17}:1ω5c	□	□	0.40	0.40
$C_{16:1}$ω7c/$_{16:1}$ω6c	26.52	□	14.50	17.20
$C_{16:0}$ 10-methly1	7.18	0.60	6.00	5.20
$C_{18:0}$ 10-methly1	13.07	13.10	22.90	13.90
$C_{19:1}$ cyclo ω8c	□	□	□	1.50
$C_{19:1}$ w7c/$_{19:1}$ ω6c	2.70	□	0.20	□
$C_{20:4}$ x6,9,12,15c	□	□	0.40	1.60
$C_{16:1}$ cis 9	□	6.60	□	□
	□	1.20	□	□

$C_{18:1}$ cis 9		16.5		
C_{20}; 1:1 cis11		1.70		
Summed feature 4		9.50		
Summed feature 7		1.30		

Figures

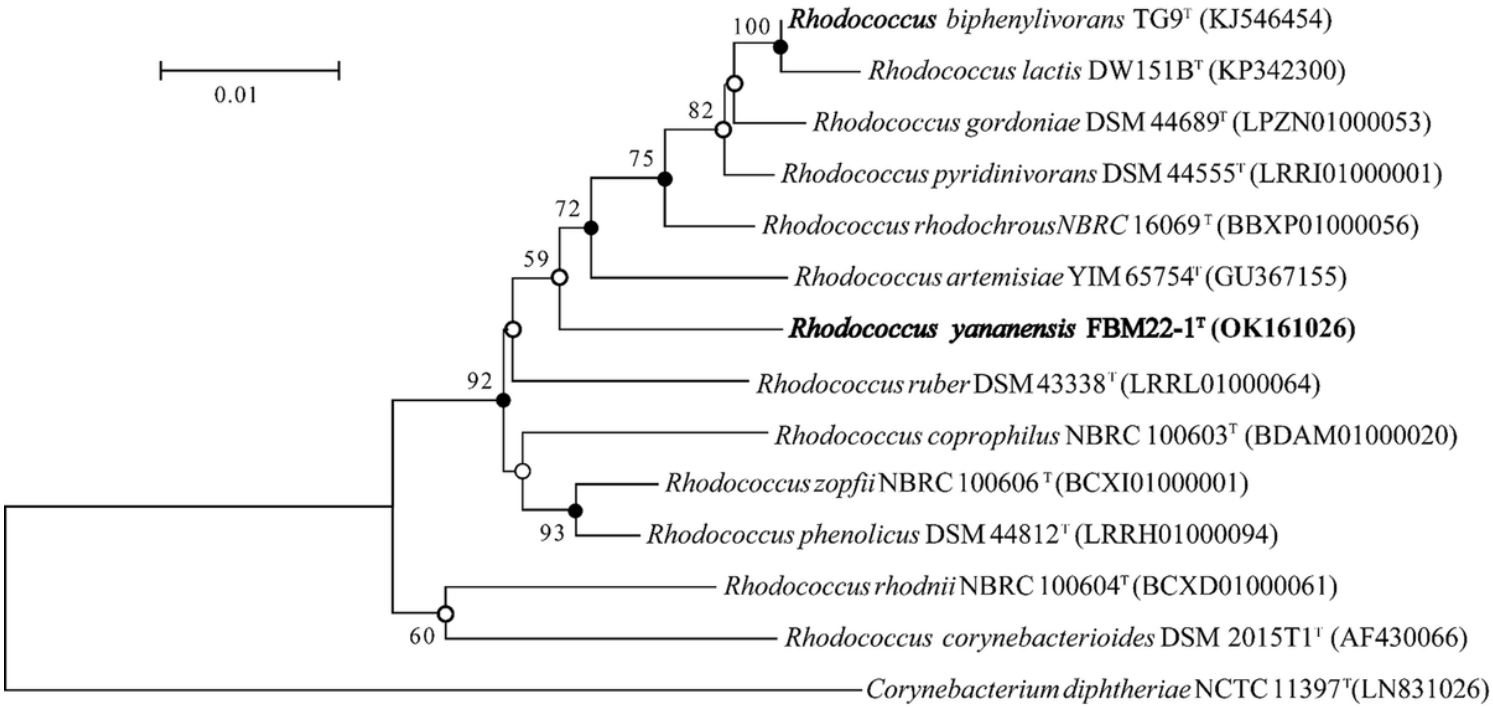

Figure 1

Neighbor-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationship of FBM22-1T and members of the genus Rhodococcus. The sequence of Corynebacterium diphtheriae NCTC 11397T was used as an outgroup. Bootstrap values (percentages based on 1,000 replications) of > 50 % are shown at the branch points. Black circles indicate branches of the tree that were found using the maximum-likelihood and maximum-parsimony tree-making algorithms; white circles indicate branches of the tree only found by the maximum-likelihood or maximum parsimony methods. Bar, 0.01 substitutions per nucleotide position.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Fig.S1.tif
- Fig.S2.tif
- Fig.S3.tif
- supplementaryfigures.docx
- supplementarytables.docx