




Complete Genome Sequence of *Agrococcus* sp. Strain SGAir0287, Isolated from Tropical Air Collected in Singapore

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ABSTRACT *Agrococcus* sp. strain SGAir0287 was isolated from tropical air samples collected in Singapore. Assembled using single-molecule real-time (SMRT) sequencing and MiSeq reads, the genome consists of one circular chromosome of 3,084,767 bp. The entire genome has 2,870 protein-coding genes, 45 tRNAs, and 3 rRNAs.

Agrococcus species are Gram-positive, nonmotile bacteria classified in the *Actinobacteria* phylum (1). *Agrococcus jenensis* and its strains 2002-39/1^T and ST54 were initially identified as *Agromyces* spp. but were later assigned to the new genus *Agrococcus* based on their 16S rRNA gene sequence and lack of a mycelial phase (2). Strain 2002-39/1^T was isolated from a frozen compost soil, and strain ST54 was isolated from a sandstone building surface. Several new species of *Agrococcus* have been reported since and were found in various environments such as medieval paintings (3), air (4), cheese (5), seaweed (6), soil from cold deserts (7), forests (8), and coal mines (9). *Agrococci* have an irregular coccoid morphology with characteristic menaquinones and 2,4-diaminobutyric acid cell walls (1). They thrive on tryptic soy agar (TSA; Sigma-Aldrich, USA) at 30°C, with colonies appearing yellow when cultured in light and white when cultured in darkness (1).

Agrococcus sp. strain SGAir0287 was isolated from air collected indoors in Singapore (global position system coordinates, 130.048N, 103.791E) using the BioStage single-stage impactor with a QuickTake 30 pump at 28.3 liters/min for 3 min (SKC, Inc., USA). The air was impacted onto Reasoner's 2A agar (Becton Dickinson, USA), and colonies were further isolated onto TSA plates at 30°C. Genomic DNA was purified using the Wizard genomic DNA purification kit (Promega, USA) according to the manufacturer's instructions. Library preparation was performed with the SMRTbell template prep kit 1.0 (Pacific Biosciences, USA), followed by single-molecule real-time (SMRT) sequencing on the PacBio RS II platform. Short reads were generated on a MiSeq platform (Illumina, USA) with a 300-bp paired-end run, using whole-genome shotgun libraries constructed with the TruSeq Nano DNA library preparation kit.

A total of 122,093 long reads were used for *de novo* assembly with the Hierarchical Genome Assembly Process version 3 (10). It was then polished in Quiver (10) and error corrected using Pilon version 1.16 (11), including a total of 814,959 MiSeq paired-end reads. The final genome assembly provided one circular contig with 3,084,767 bp (263.16-fold coverage) with a G+C content of 72.99%.

Taxonomic identification was performed using average nucleotide identity (ANI) and 16S rRNA identification, resulting in assignment to the genus *Agrococcus*. ANI was conducted with the microbial species identifier (MiSI) method (12) and had 80.0% identity to *Agrococcus lahaulensis* DSM 17612, with an alignment fraction of 17%. The

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16S rRNA gene analysis using Barrnap version 0.7 (13) and BLASTn (14) against the SILVA database (15) resulted in a match with 98.2% identity to *Agrococcus terreus*.

The genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline version 4.2 (16). A total of 2,963 genes were predicted, as follows: 2,870 protein-coding genes; 3 rRNA gene operons, including 5S, 16S, and 23S rRNA; 45 tRNA genes; 3 noncoding RNA genes; and 42 pseudogenes. Functional classification of genes was performed with Rapid Annotations using Subsystems Technology (RAST) (17–19). The annotation results showed that the genome possesses Gram-positive cell wall components but no motility-related genes. It also has menaquinone biosynthesis genes that are characteristic of *Agrococcus* spp. Interestingly, alkanesulfonate assimilation genes were also predicted in its genome. Alkanesulfonate is commonly used in paints, which may explain the presence of *Agrococcus* spp. in paintings (3) and in air near wall paintings in Virgilkapelle (Vienna, Austria) (4).

Data availability. The complete genome sequence of *Agrococcus* sp. strain SGAir0287 has been deposited in DDBJ/EMBL/GenBank under the accession number [CP027942](https://doi.org/10.1099/CP027942), and its corresponding Sequence Read Archive (SRA) numbers are [SRR8894903](https://doi.org/10.1099/SRR8894903) and [SRR8894904](https://doi.org/10.1099/SRR8894904).

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REFERENCES

- Kämpfer P, Busse HJ. 2015. *Agrococcus*. In Whitman WB (ed), *Bergey's manual of systematics of archaea and bacteria*. Wiley & Sons, Hoboken, NJ.
- Groth I, Schumann P, Weiss N, Martin K, Rainey FA. 1996. *Agrococcus jenensis* gen. nov., sp. nov., a new genus of *Actinomycetes* with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* 46:234–239. <https://doi.org/10.1099/00207713-46-1-234>.
- Wieser M, Schumann P, Martin K, Altenburger P, Burghardt J, Lubitz W, Busse HJ. 1999. *Agrococcus citreus* sp. nov., isolated from a medieval wall painting of the chapel of Castle Herberstein (Austria). *Int J Syst Bacteriol* 49:1165–1170. <https://doi.org/10.1099/00207713-49-3-1165>.
- Zlamala C, Schumann P, Kämpfer P, Rosselló-Mora R, Lubitz W, Busse H-J. 2002. *Agrococcus baldri* sp. nov., isolated from the air in the “Virgilkapelle” in Vienna. *Int J Syst Evol Microbiol* 52:1211–1216. <https://doi.org/10.1099/00207713-52-4-1211>.
- Bora N, Vancanneyt M, Gelsomino R, Swings J, Brennan N, Cogan TM, Larpin S, Desmasures N, Lechner FE, Kroppenstedt RM, Ward AC, Goodfellow M. 2007. *Agrococcus casei* sp. nov., isolated from the surfaces of smear-ripened cheeses. *Int J Syst Evol Microbiol* 57:92–97. <https://doi.org/10.1099/ijs.0.64270-0>.
- Lee SD. 2008. *Agrococcus jejuensis* sp. nov., isolated from dried seaweed. *Int J Syst Evol Microbiol* 58:2297–2300. <https://doi.org/10.1099/ijs.0.65731-0>.
- Mayilraj S, Suresh K, Schumann P, Kroppenstedt RM, Saini HS. 2006. *Agrococcus lahaulensis* sp. nov., isolated from a cold desert of the Indian Himalayas. *Int J Syst Evol Microbiol* 56:1807–1810. <https://doi.org/10.1099/ijs.0.64247-0>.
- Zhang J-Y, Liu X-Y, Liu S-J. 2010. *Agrococcus terreus* sp. nov. and *Microcococcus terreus* sp. nov., isolated from forest soil. *Int J Syst Evol Microbiol* 60:1897–1903. <https://doi.org/10.1099/ijs.0.013235-0>.
- Dhanjal S, Kaur I, Korpole S, Schumann P, Cameotra SS, Pukall R, Klenk HP, Mayilraj S. 2011. *Agrococcus carbonis* sp. nov., isolated from soil of a coal mine. *Int J Syst Evol Microbiol* 61:1253–1258. <https://doi.org/10.1099/ijs.0.024745-0>.
- Chin C-S, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569. <https://doi.org/10.1038/nmeth.2474>.
- Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 9:e112963. <https://doi.org/10.1371/journal.pone.0112963>.
- Varghese NJ, Mukherjee S, Ivanova N, Konstantinidis KT, Mavrommatis K, Kyrpides NC, Pati A. 2015. Microbial species delineation using whole genome sequences. *Nucleic Acids Res* 43:6761–6771. <https://doi.org/10.1093/nar/gkv657>.
- Seemann T. 2013. Barrnap 0.7: rapid ribosomal RNA prediction.
- Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402. <https://doi.org/10.1093/nar/25.17.3389>.
- Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2012. The SILVA ribosomal RNA gene database project: improved data processing and Web-based tools. *Nucleic Acids Res* 41:D590–D596. <https://doi.org/10.1093/nar/gks1219>.
- Tatusova T, DiCuccio M, Badretdin A, Chetverin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <https://doi.org/10.1186/1471-2164-9-75>.
- Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* 42:D206–D214. <https://doi.org/10.1093/nar/gkt1226>.
- Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Sci Rep* 5:8365. <https://doi.org/10.1038/srep08365>.