

Draft Genome Sequence of Saccharopolyspora rectivirgula

B. M. Fredrik Pettersson,^a P. R. Krishna Behra,^a Satyam Manduva,^a Sarbashis Das,^b Santanu Dasgupta,^a Alok Bhattacharya,^c Leif A. Kirsebom^a

Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden^a; School of Computational and Integrative Sciences, Jawaharlal Nehru University, New Delhi, India^b; Schools of Life Sciences, Jawaharlal Nehru University, New Delhi, India^c

We have sequenced the genome of *Saccharopolyspora rectivirgula*, the causative agent of farmer's lung disease. The draft genome consists of 182 contigs totaling 3,977,051 bp, with a GC content of 68.9%.

Received 25 November 2013 Accepted 2 December 2013 Published 9 January 2014

Citation Pettersson BMF, Behra PRK, Manduva S, Das S, Dasgupta S, Bhattacharya A, Kirsebom LA. 2014. Draft genome sequence of Saccharopolyspora rectivirgula. Genome Announc. 2(1):e01117-13. doi:10.1128/genomeA.01117-13.

Copyright © 2014 Pettersson et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to Leif A. Kirsebom, Leif.Kirsebom@icm.uu.se.

Saccharopolyspora rectivirgula is a Gram-positive thermophilic sporulating actinomycete (1) that causes farmer's lung disease (FLD), a type of hypersensitivity pneumonitis (2). FLD can develop into a chronic disease and lead to irreversible lung damage and even death (3). S. rectivirgula is often found in high concentrations in the air in barns where wet hay is stored (4). Wet hay may reach temperatures of 55 to 60°C, which promotes growth of S. rectivirgula (5). Hay can generate dust containing S. rectivirgula, which can lead to FLD after inhalation.

Only 1 to 15% of farmers exposed to *S. rectivirgula* develop FLD (6–8). There seems to be a genetic component protecting against the hyperreactive allergic response that is associated with FLD (6, 9, 10). Interestingly, smokers seem to have a lower incidence of the disease (2, 11). By use of a mouse model, it was shown that nicotine reduced the allergic response and lung damage (11). In contrast, other studies have shown that the allergic response gets stronger after a recent viral infection (12, 13).

The only efficient long-term treatment of FLD is removal of the antigen (6, 9). For the individual, this may have large economic and social consequences. Knowing the genome sequence of *S. rectivirgula* may provide a better understanding of the cause of the hyperreactive allergic reaction, which can be used to develop better tools to monitor and detect this working environmental hazard and lead to new methods of treatment.

The *S. rectivirgula* type strain DSM 43747 was obtained from and grown under conditions recommended by the Deutsche Sammlung von Mikroorganismen und Zellkulturen in Germany. Genomic DNA was isolated by lysis using bead beating in equal volumes DNAzol (Invitrogen) and Tris-EDTA (TE) buffer (10 mM Tris [pH 7.9] and 1 mM EDTA) using conditions previously described (14), and the pellet was resuspended in TE buffer. The DNA was treated with RNase A and proteinase K according to standard protocols. Whole-genome sequencing of *S. rectivirgula* was performed at the SNP&SEQ Technology Platform of Uppsala University on a HiSeq2000 (Illumina) platform. A total of 9.3 million paired-end reads were generated, with an average read length of 100 nucleotides. Assembly of the reads was done using the A5 assembly pipeline (15). The reads were assembled into 182 contigs making up a total genome size of 3,977,051 bp with a GC

content of 68.9% and an N_{50} value of 52,597. The average coverage was $469\times$. The contigs were annotated using the RAST server (16). This analysis resulted in 3,840 predicted protein-coding genes, 50 tRNA genes, and 2 ribosomal RNA operons. Interestingly, the *S. rectivirgula* genome is considerably smaller than those of the previously sequenced *Saccharopolyspora erythraea* (8.21 Mb) (17) and *Saccharopolyspora spinosa* (8.58 Mb) (18). By use of PanOCT v 1.9 (19), it was predicted that only 1,951 genes are shared among all three genomes and 1,467 genes are present only in *S. rectivirgula*.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited in DDBJ/EMBL/GenBank under the accession no. AYJW00000000. The version described in this paper is the first version, AYJW01000000.

ACKNOWLEDGMENTS

This work was funded by the Swedish Research Council (M), SIDA/ SAREC, the Swedish Research Council-SIDA, and the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS). A. Bhattacharya acknowledges the Department of Biotechnology, India, and we thank the Council of Scientific and Industrial Research, India, for financial support to S. Das. Sequencing was performed by the SNP&SEQ Technology Platform in Uppsala. The platform is part of Science for Life Laboratory at Uppsala University and supported as a national infrastructure by the Swedish Research Council. The computations were performed on resources provided by SNIC through Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX) under Project b2011072.

REFERENCES

- 1. Korn-Wendisch F, Kempf A, Grund E, Kroppenstedt RM, Kutzner HJ. 1989. Transfer of *Faenia rectivirgula* Kurup and Agre 1983 to the genus *Saccharopolyspora* Lacey and Goodfellow 1975, elevation of *Saccharopolyspora hirsuta* subsp. *taberi* Labeda 1987 to species level, and emended description of the genus *Saccharopolyspora*. Int. J. Syst. Bacteriol. 39: 430–441. http://dx.doi.org/10.1099/00207713-39-4-430.
- Cormier Y. 2007. Respiratory health and farming: an essay. Can. Respir. J. 14:419 – 422.
- 3. Seal RM, Hapke EJ, Thomas GO, Meek JC, Hayes M. 1968. The pathology of the acute and chronic stages of farmer's lung. Thorax 23: 469–489. http://dx.doi.org/10.1136/thx.23.5.469.
- 4. Blais Lecours P, Veillette M, Marsolais D, Duchaine C. 2012. Charac-

- terization of bioaerosols from dairy barns: reconstructing the puzzle of occupational respiratory diseases by using molecular approaches. Appl. Environ. Microbiol. 78:3242–3248. http://dx.doi.org/10.1128/AEM.07661-11.
- Cross T, Maciver AM, Lacey J. 1968. The thermophilic actinomycetes in mouldy hay: *Micropolyspora faeni* sp. nov. J. Gen. Microbiol. 50:351–359. http://dx.doi.org/10.1099/00221287-50-3-351.
- Agache IO, Rogozea L. 2013. Management of hypersensivity pneumonitis. Clin. Transl. Allergy. 3:5. http://dx.doi.org/10.1186/2045-7022-3-5.
- Wolff CH. 2011. Innate immunity and the pathogenicity of inhaled microbial particles. Int. J. Biol Sci. 7:261–268. http://dx.doi.org/10.7150/ijbs.7.261.
- 8. Lacasse Y, Cormier Y. 2006. Hypersensitivity pneumonitis. Orphanet J. Rare Dis. 1:25. http://dx.doi.org/10.1186/1750-1172-1-25.
- Selman M, Lacasse Y, Pardo A, Cormier Y. 2010. Hypersensitivity pneumonitis caused by fungi. Proc. Am. Thorac. Soc. 7:229–236. http://dx.doi.org/10.1513/pats.200906-041AL.
- McSharry C, Anderson K, Bourke SJ, Boyd G. 2002. Takes your breath away—the immunology of allergic alveolitis. Clin. Exp. Immunol. 128: 3–9. http://dx.doi.org/10.1046/j.1365-2249.2002.01849.x.
- Blanchet MR, Israël-Assayag E, Cormier Y. 2004. Inhibitory effect of nicotine on experimental hypersensitivity pneumonitis in vivo and in vitro. Am. J. Respir. Crit. Care Med. 169:903–909. http://dx.doi.org/10.11 64/rccm.200210-1154OC.
- Cormier Y, Samson N, Israël-Assayag E. 1996. Viral infection enhances the response to Saccharopolyspora rectivirgula in mice prechallenged with this farmer's lung antigen. Lung 174:399–407.
- Gudmundsson G, Monick MM, Hunninghake GW. 1999. Viral infection modulates expression of hypersensitivity pneumonitis. J. Immunol. 162: 7397–7401.

- 14. Pettersson BM, Nitharwal RG, Das S, Behra KP, Benedik E, Arasu UT, Islam NM, Dasgupta S, Bhattacharya A, Kirsebom LA. 2013. Identification and expression of stressosomal proteins in *Mycobacterium marinum* under various growth and stress conditions. FEMS Microbiol. Lett. 342:98–105. http://dx.doi.org/10.1111/1574-6968.12118.
- Tritt A, Eisen JA, Facciotti MT, Darling AE. 2012. An integrated pipeline for *de novo* assembly of microbial genomes. PLoS One 7:e42304. http://dx.doi.org/10.1371/journal.pone.0042304.
- 16. 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. http://dx.doi.org/10.1186/1471-2164-9-75.
- Oliynyk M, Samborskyy M, Lester JB, Mironenko T, Scott N, Dickens S, Haydock SF, Leadlay PF. 2007. Complete genome sequence of the erythromycin-producing bacterium Saccharopolyspora erythraea NRRL23338. Nat. Biotechnol. 25:447–453. http://dx.doi.org/10.1038/nbt 1297
- Pan Y, Yang X, Li J, Zhang R, Hu Y, Zhou Y, Wang J, Zhu B. 2011. Genome sequence of the spinosyns-producing bacterium Saccharopolyspora spinosa NRRL 18395. J. Bacteriol. 193:3150-3151. http://dx.doi.org/10.1128/JB.00344-11.
- 19. Fouts DE, Brinkac L, Beck E, Inman J, Sutton G. 2012. Panoct: automated clustering of orthologs using conserved gene neighborhood for pan-genomic analysis of bacterial strains and closely related species. Nucleic Acids Res. 40:e172. http://dx.doi.org/10.1093/nar/gks757.