Multiple Genome Sequences of Heteropolysaccharide-Forming Acetic Acid Bacteria

Julia U. Brandt, Frank Jakob, Andreas J. Geissler, Jürgen Behr, Rudi F. Vogel
Technische Universität München, Lehrstuhl für Technische Mikrobiologie, Freising, Germany; Technische Universität München, Bavarian Biomolecular Mass Spectrometry Center, Freising, Germany

ABSTRACT We report here the complete genome sequences of the acetic acid bacteria (AAB) Acetobacter aceti TMW 2.1153, A. persici TMW 2.1084, and Neoasaia chiangmaiensis NBRC 101099, which secrete biotechnologically relevant heteropolysaccharides (HePSs) into their environments. Upon genome sequencing of these AAB strains, the corresponding HePS biosynthesis pathways were identified.

Acetic acid bacteria (AAB) are Gram-negative obligate aerobes belonging to the Alphaproteobacteria subdivision. They are well known to produce large amounts of exopolysaccharides (EPS)—either homopolysaccharides (HoPSs) such as levans (1–3) and cellulose (4, 5), or heteropolysaccharides (HePSs) such as acetan (6) and gluconacetan (7). HePSs, in particular, have unique properties, since their complex, mostly branched structures are responsible for drastic viscosity increases of aqueous solutions. The food industry is taking increasing advantage of the unique rheological properties of bacterial HePSs. We identified three potential HePS-forming AAB, Acetobacter aceti TMW 2.1153, A. persici TMW 2.1084, and Neoasaia chiangmaiensis NBRC 101099, on sucrose-deficient media. To gain insights into the HePS biosynthesis we sequenced the complete genomes of the identified HePS-producing AAB for further identification of specific HePS biosynthesis clusters.

A. aceti TMW 2.1153 and A. persici TMW 2.1084 were isolated from water kefir in our laboratory in Freising, Germany, and N. chiangmaiensis NBRC 101099 was isolated from a Thai red ginger flower at the National Institute of Technology and Evaluation Biological Resource Center, Japan (8). High-molecular-weight DNA was purified from modified sodium-gluconate (NaG) medium liquid cultures using the Genomic-tip 100/G kit (Qiagen, Hilden, Germany), as described previously (9). Single-molecule real-time (SMRT) sequencing (PacBio RS II) was carried out at GATC (Konstanz, Germany) (10). A single library was prepared for each of the three strains, and an insert size of 8 to 12 kb was selected for library creation, resulting in at least 200 Mb of raw data from 1 SMRT cell (1 × 120-min movies), applying P4-C2 chemistry. The generated sequences were assembled with SMRT Analysis version 2.2.0.p2 using the Hierarchical Genome Assembly Process version 3 (HGAP3) (11). Initial open reading frame predictions and annotations were accomplished automatically using the NCBI Prokaryotic Genome Annotation Pipeline and Rapid Annotations using Subsystems Technology (RAST), a SEED-based, prokaryotic genome annotation service (12–14).

The genomes were assembled to one circularized chromosome with overall chromosome sizes ranging from 3.23 Mb for A. persici TMW 2.1084 to 3.72 Mb for A. aceti TMW 2.1153 and G+C contents of 56.83 to 61.50%. A. persici TMW 2.1084 harbors an additional plasmid comprising 526,169 bp. The detailed characteristic data, sequencing statistics, genome information, and accession numbers are given in Table 1.
The genome of \textit{A. aceti} TMW 2.1153 harbors three complete rRNA operons (5S, 16S, and 23S) and 52 tRNA genes. \textit{A. persici} TMW 2.1084 encodes 62 tRNAs, and \textit{N. chiangmaiensis} NBRC 101099 encodes 54 tRNAs; both harbor four complete rRNA operons. Among the identified genes, HePS-forming gene clusters could be detected, including \textit{pol} genes of the \textit{polABCDE} cluster associated with pellicle formation (15). Furthermore, in \textit{A. aceti} TMW 2.1153, \textit{gum}-like genes, similar to genes in the \textit{gum} cluster of \textit{Xanthomonas campestris} (16, 17), could be identified. Their accessibility will allow a better investigation of AAB-derived HePSs and the connected biosynthesis, based on specific HePS clusters.

\textbf{Accession number(s).} The three complete genomes have been deposited in DDBJ/EMBL/GenBank under the accession numbers given in Table 1.

\section*{ACKNOWLEDGMENTS}

This work was supported by the German Research Foundation (DFG) and the Technical University of Munich (TUM) in the framework of the Open Access Publishing Program and the German Federal Ministry for Economic Affairs and Energy via the German Federation of Industrial Research Associations (AiF) (FEI), project number AiF 18071 N.

None of the funding sources had any influence on the study design; the collection, analysis, and interpretation of the data; the writing of the report; or the decision to submit the article for publication.

\section*{REFERENCES}

1. Jakob F. 2014. Novel fructans from acetic acid bacteria. PhD dissertation. Technische Universität München, Freising, Germany.
2. Jakob F, Meißner D, Vogel RF. 2012. Comparison of novel GH 68 levan-sucrases of levan-overproducing Gluconobacter species. Acetic Acid Bacteria 12. https://doi.org/10.4081/aab.2012.e2.
3. Jakob F, Paff A, NovaNa-Carballal R, Rübsam H, Becker T, Vogel RF. 2013. Structural analysis of fructans produced by acetic acid bacteria reveals a relation to hydrocolloid function. Carbohydr Polym 92:1234–1242. https://doi.org/10.1016/j.carbpol.2012.10.054.
4. Chawla PR, Bajaj IB, Sur evade SA, Singhal RS. 2009. Microbial cellulose: fermentative production and applications. Food Technol Biotechnol 47:107–124.
5. Raspor P, Goranov D. 2008. Biotechnological applications of acetic acid bacteria. Crit Rev Biotechnol 28:101–124. https://doi.org/10.1080/0738850802046749.
6. Jansson PE, Lindberg J, Wimalasiri KMS, Dankert MA. 1993. Structural studies of acetan, an exopolysaccharide elaborated by \textit{Acetobacter xylinus}. Carbohydr Polym 24:303–310. https://doi.org/10.1016/0144-8617(93)80079-T.
7. Kornmann H, Duboc P, Marion I, von Stockar UV. 2003. Influence of nutritional factors on the nature, yield, and composition of exopolysaccharides produced by \textit{Gluconacetobacter xylinus} I-2281. Appl Environ Microbiol 69:6091–6098. https://doi.org/10.1128/AEM.69.10.6091-6098.2003.
8. Yukphan P, Malimas T, Potatcharen W, Tanasupawat S, Tanticharoen M, Yamada Y. 2005. \textit{Neocasia chiangmaiensis} gen. nov., sp. nov., a novel osmotolerant acetic acid bacterium in the \textit{\alpha}-proteobacteria. J Gen Appl Microbiol 51:301–311. https://doi.org/10.2323/jgam.51.301.
9. Brandt JU, Jakob F, Behr J, Geissler AJ, Vogel RF. 2016. Dissection of exopolysaccharide biosynthesis in \textit{Kozakia ballensis}. Microb Cell Fact 15:170. https://doi.org/10.1186/s12934-016-0572-x.
10. Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P, Bettman B, Bibillo A, Bjornson K, Chaudhuri B, Christians F, Cicero R, Clark S, Dalal R, Dewinter A, Dixon J, Foquet M, Gaertner A, Hardenbol P, Heiner C, Hester K, Holden D, Kears G, Kong X, Kuse R, Lacroix Y, Lin S, Lundquist P, Ma C, Marks P, Maxham M, Murphy D, Park I, Pham T, Phillips M, Roy J, Sebra R, Shen G, Sorensen J, Tomanay A, Travers K, Trulson M, Viecelli J, Wegener J, Wu D, Yang A, Zaccarin D. 2009. Real-time DNA sequencing from single polymerase molecules. Science 323:133–138. https://doi.org/10.1126/science.1162986.
11. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korfach J. 2013. Non-hybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563–569. https://doi.org/10.1038/nmeth.2474.
12. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Diz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The \textit{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.
13. Angiolli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G, Kodira CD, Kyripides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O. 2008. Toward an online repository of standard operating procedures
(SOPs) for (meta)genomic annotation. OMICS 12:137–141. https://doi.org/10.1089/omi.2008.0017.

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.

Deeraksa A, Moonmangmee S, Toyama H, Yamada M, Adachi O, Matsushita K. 2005. Characterization and spontaneous mutation of a novel gene, polE, involved in pellicle formation in Acetobacter tropicalis SKU1100. Microbiology 151:4111–4120. https://doi.org/10.1099/mic.0.28350-0.

Becker A, Katzen F, Pühler A, Ielpi L. 1998. Xanthan gum biosynthesis and application: a biochemical/genetic perspective. Appl Microbiol Biotechnol 50:145–152. https://doi.org/10.1007/s002530051269.

Katzen F, Ferreiro DU, Oddo CG, Ielmini MV, Becker A, Pühler A, Ielpi L. 1998. Xanthomonas campestris pv. campestris gum mutants: effects on xanthan biosynthesis and plant virulence. J Bacteriol 180:1607–1617.