



Genome Sequences of Three Species of *Hanseniaspora* Isolated from Spontaneous Wine Fermentations

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Members of the genus *Hanseniaspora* represent a significant proportion of the normal flora of grape berries and play a significant role in wine fermentation. Here, we present genome sequences for three species of *Hanseniaspora*, *H. opuntiae*, *H. osmophila*, and *H. uvarum*, which were isolated from spontaneous Chardonnay wine fermentation.

Received 21 September 2016 Accepted 30 September 2016 Published 17 November 2016

Citation Sternes PR, Lee D, Kutyna DR, Borneman AR. 2016. Genome sequences of three species of *Hanseniaspora* isolated from spontaneous wine fermentations. Genome Announc 4(6):e01287-16. doi:10.1128/genomeA.01287-16.

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Members of the genus *Hanseniaspora* represent a significant proportion of the normal flora of grape berries and play a significant role in wine fermentation (1). Besides a role in grape wine fermentation, *Hanseniaspora* species have been used as starter cultures in the fermentation of fruit wines, ciders, and spirits (2–5) and cocoa (6, 7).

One isolate of each of three species of *Hanseniaspora*, AWRI3578 (*H. opuntiae*), AWRI3579 (*H. osmophila*), and AWRI3580 (*H. uvarum*), were obtained from spontaneously fermenting Chardonnay grape must (Adelaide Hills, South Australia, Australia) in 2014. Each isolate was identified to the species level by sequence identity analysis of a fragment of the rDNA internal transcribed spacer (ITS) region (8) against the QIIME UNITE database (ver6_dynamic_s_10.09.2014), with results corroborated against the NCBI nr database using Blast (AWRI3579, 99% identity with *H. osmophila* CBS 313^T; AWRI3578, 98% identity with *H. opuntiae* CBS 8873^T; AWRI3580, 100% identity with *H. uvarum* CBS 314^T).

Sequencing was performed using a combination of Illumina Nextera mate-pair (2- to 5-kb and 6- to 12-kb size selected) and TruSeq PCR-free sequencing libraries that were prepared from purified DNA and run using 2×300 bp MiSeq chemistry (Ramaciotti Centre for Functional Genomics, Australia). Sequences for each isolate were assembled using MIRA (version 4.0.2 [http://sourceforge.net/projects/mira-assembler/]) with the resultant contigs (in .ace format) manually refined using SeqManPro (DNAStar, USA).

The genomes of statistics of AWRI3578 (H. opuntiae) and

AWRI3580 (*H. uvarum*) were very similar (Table 1), while AWRI3579 (*H. osmophila*) produced a far larger but also more fragmented assembly at the contig level; however, the contigs were readily connected by scaffolding to produce a similar number of scaffolds as the other two species.

Augustus annotation (9) predicted 4,176, 4,061, and 4,660 proteins for AWRI3578, AWRI3580, and AWRI3579, respectively. Of these, 3,391, 3,410, and 4,187 proteins could be assigned to OrthoMCL clusters (10, 11). Both the size and predicted protein content of the AWRI3578 and AWWRI3580 genomes are similar to that of *H. valbyensis* (http://genome.jgi.doe.gov/Hanva1_1/Hanva1_1.home.html), while the AWRI3579 genome assembly was similar in size and coding potential to that of *H. vinae* (12). The differences observed in genome size and coding potential are consistent with phylogenies produced by both concatenating 2,045 orthologous proteins predicted in this work from the five species, and from 26S rRNA gene (13), which position *H. opuntiae*, *H. uvarum*, and *H. valbyensis* as a distinct clade and *H. valbyensis* and *H. osmophila* as a separate sister group.

Accession number(s). These whole-genome shotgun projects have been deposited in DDBJ/ENA/GenBank under the accession numbers provided in Table 1. The versions described in this paper are the first versions.

ACKNOWLEDGMENTS

Special thanks to Louisa Rosa and Alana Seabrook of Yalumba and Alison Soden of Treasury Wine Estates for supplying must and wild fermentation

 TABLE 1 Genome assembly statistics

Strain	Presumptive species (ITS sequence)	No. of contigs	No. of scaffolds	Assembly size (Mb)	Contig N_{50} (kb)	Accession no.
AWRI3578	Hanseniaspora opuntiae	67	18	8.83	636	LPNL00000000
AWRI3579	Hanseniaspora osmophila	899	17	11.37	8	LPNM0000000
AWRI3580	Hanseniaspora uvarum	44	18	8.81	739	LPNN00000000

samples. The Australian Wine Research Institute is a member of the Wine Innovation Cluster in Adelaide.

FUNDING INFORMATION

This work was supported by Australian grape growers and winemakers through their investment body, Wine Australia, with matching funds from the Australian government and was partially funded by the UNSW Science Leveraging Fund.

REFERENCES

- 1. Jolly NP, Varela C, Pretorius IS. 2014. Not your ordinary yeast: non-*Saccharomyces* yeasts in wine production uncovered. FEMS Yeast Res 14: 215–237. http://dx.doi.org/10.1111/1567-1364.12111.
- Mingorance-Cazorla L, Clemente-Jiménez JM, Martínez-Rodríguez S, Heras-Vázquez FJL, Rodríguez-Vico F. 2003. Contribution of different natural yeasts to the aroma of two alcoholic beverages. World J Microbiol Biotechnol 19:297–304. http://dx.doi.org/10.1023/A:1023662409828.
- De Arruda Moura Pietrowski G, dos Santos CM, Sauer E, Wosiacki G, Nogueira A. 2012. Influence of fermentation with *Hanseniaspora* sp. yeast on the volatile profile of fermented apple. J Agric Food Chem 60: 9815–9821. http://dx.doi.org/10.1021/jf302290k.
- Rodríguez Madrera R, Pando Bedriñana R, García Hevia A, Arce MB, Suárez Valles B. 2013. Production of spirits from dry apple pomace and selected yeasts. Food Bioprod Process 91:623–631. http://dx.doi.org/ 10.1016/j.fbp.2013.04.005.
- Xu Y, Zhao GA, Wang LP. 2006. Controlled formation of volatile components in cider making using a combination of *Saccharomyces cerevisiae* and *Hanseniaspora valbyensis* yeast species. J Ind Microbiol Biotechnol 33:192–196. http://dx.doi.org/10.1007/s10295-005-0051-6.
- 6. Moreira IMDV, Miguel MGDCP, Duarte WF, Dias DR, Schwan RF. 2013. Microbial succession and the dynamics of metabolites and sug-

ars during the fermentation of three different cocoa (*Theobroma cacao* L.) hybrids. Food Res Int 54:9–17. http://dx.doi.org/10.1016/ j.foodres.2013.06.001.

- Batista NN, Ramos CL, Dias DR, Pinheiro AC, Schwan RF. 2016. The impact of yeast starter cultures on the microbial communities and volatile compounds in cocoa fermentation and the resulting sensory attributes of chocolate. J Food Sci Technol 53:1101–1110. http://dx.doi.org/10.1007/ s13197-015-2132-5.
- Bokulich NA, Mills DA. 2013. Improved selection of internal transcribed spacer-specific primers enables quantitative, ultra-high-throughput profiling of fungal communities. Appl Environ Microbiol 79:2519–2526. http://dx.doi.org/10.1128/AEM.03870-12.
- Stanke M, Morgenstern B. 2005. Augustus: a Web server for gene prediction in eukaryotes that allows user-defined constraints. Nucleic Acids Res 33:W465–W467. http://dx.doi.org/10.1093/nar/gki458.
- Fischer S, Brunk BP, Chen F, Gao X, Harb OS, Iodice JB, Shanmugam D, Roos DS, Stoeckert CJ. 2011. Using OrthoMCL to assign proteins to OrthoMCL-DB groups or to cluster proteomes into new ortholog groups. Curr Protoc Bioinformatics 6:1–19. http://dx.doi.org/10.1002/ 0471250953.bi0612s35.
- Li L, Stoeckert CJ, Roos DS. 2003. OrthoMCL: identification of ortholog groups for eukaryotic genomes. Genome Res 13:2178–2189. http:// dx.doi.org/10.1101/gr.1224503.
- Giorello FM, Berná L, Greif G, Camesasca L, Salzman V, Medina K, Robello C, Gaggero C, Aguilar PS, Carrau F. 2014. Genome sequence of the native apiculate wine yeast *Hanseniaspora vineae* T02/19AF. Genome Announc 2(3):e00530-14. http://dx.doi.org/10.1128/genomeA.00530-14.
- Cadez N, Poot GA, Raspor P, Smith MT. 2003. Hanseniaspora meyeri sp. nov., Hanseniaspora clermontiae sp. nov., Hanseniaspora lachancei sp. nov. and Hanseniaspora opuntiae sp. nov., novel apiculate yeast species. Int J Syst Evol Microbiol 53:1671–1680. http://dx.doi.org/ 10.1099/ijs.0.02618-0.