Multiple Genome Sequences of Exopolysaccharide-Producing, Brewery-Associated Lactobacillus brevis Strains

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ABSTRACT Lactobacillus brevis represents one of the most relevant beer-spoiling bacteria. Besides strains causing turbidity and off flavors upon growth and metabolite formation, this species also comprises strains that produce exopolysaccharides (EPSs), which increase the viscosity of beer. Here, we report the complete genome sequences of three EPS-producing, brewery-associated L. brevis strains.

Beer represents a microbiologically stable beverage, as it combines a low pH and nutrient availability with the presence of hop compounds, ethanol, carbon dioxide, and anaerobility (1). Nevertheless, certain lactobacilli are still able to grow in beer and spoil it. The resulting spoilage manifests as acidity, turbidity, off flavor, or increased viscosity. The latter is attributed to exopolysaccharides (EPSs) derived from members of the family Lactobacillaceae (2). To gain insights into this EPS synthesis, we sequenced the complete genomes of three brewery-associated Lactobacillus brevis strains.

L. brevis TMW 1.2108 and L. brevis TMW 1.2111 were isolated from wheat beer, and L. brevis TMW 1.2113 was isolated from a brewery surface. The Genomic-tip 100/G kit (Qiagen, Hilden, Germany) was used to isolate high-molecular-weight DNA from MRS liquid cultures. Single-molecule real-time (SMRT) sequencing (PacBio RS II) was performed at GATC (Konstanz, Germany) (3). For each of the three strains, a single library was prepared by selecting an insert size of 8 to 12 kb, resulting in at least 200 Mb of raw data from one 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 (HGAP) version 3 (4). The genome was completed by manual curation according to PacBio instructions and annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) and the Rapid Annotations using Subsystems Technology (RAST) server (5–7).

Table 1 summarizes the characteristics, sequencing statistics, genome information, and accession numbers for each strain. The chromosome sizes range from 2.54 to 2.57 Mb, with G+C contents of 45.8% to 45.9%. The strains harbor 4 to 8 plasmids with G+C contents between 41.3% and 41.9% and sizes between 130.5 and 352.0 kb. The chromosomes encode five complete rRNA operons and 66 to 69 tRNAs.

All strains possess a plasmid-encoded glycosyltransferase-2 (gtf-2), which has been described as a key enzyme for EPS synthesis of slimy, wine-spoiling members of the family Lactobacillaceae (8–10). Comparison of the gtf-2 gene between the wine spoilers Pediococcus parvulus IOEB8801 (GenBank accession no. AF196967), P. damnosus 2.6 (GenBank accession no. AY999683), and L. diolivorans G77 (GenBank accession no. AY999684) and the beer spoilers reported here reveals sequence identities of 99%. This shows the gtf-2 gene to be species-independent and highly conserved and might indicate a common origin.

The availability of these L. brevis genome sequences will allow a better understanding of EPS synthesis and its contribution to the spoilage of beer.

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The three complete *L. brevis* genomes have been deposited in DDBJ/EMBL/GenBank under the accession numbers given in Table 1.

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### REFERENCES


### TABLE 1  Strain characteristics, sequencing statistics, genome information, and accession numbers

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>BioSample no.</th>
<th>Accession no.</th>
<th>Coverage (X)c</th>
<th>Size (Mb)</th>
<th>No. of contigsd</th>
<th>G+C content (%)</th>
<th>No. of CDSse</th>
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<td>CP019734 to CP019742</td>
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<tr>
<td><em>L. brevis</em> TMW 1.2113 Brewery surface</td>
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<td>CP019750 to CP019754</td>
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<td>2.67</td>
<td>8</td>
<td>45.74</td>
<td>2,357</td>
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</tr>
</tbody>
</table>

aAll BioSamples are part of BioProject no. PRJNA331253.
bAccession numbers are listed for all contigs of each whole genome (as a range).
cAverage coverage of HGAP assembly.
dIn the chromosomes plus plasmids and partial plasmids.
eCDSs, number of coding sequences (total) based on NCBI PGAP.