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Metabolic capabilities of Lactococcus lactis

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CHAPTER 3. DRAFT GENOME SEQUENCES OF THREE AMINO ACID-SECRETING *LACTOCOCCUS LACTIS* STRAINS

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ABSTRACT

Three *Lactococcus lactis* strains with the ability to secrete various amino acids (leucine, isoleucine, methionine, valine, glutamic acid and histidine) were sequenced in order to identify the mechanisms involved in the secretion. Amino acids contribute to flavor formation. Therefore, bacterial strains with this ability are relevant for the food industry.

ANNOUNCEMENT

Bacteria secrete several compounds during growth as well as in stationary phase. Some of these compounds are relevant in food industry, for instance the large-scale production of amino acids that find application as feed additives, flavor-promoting compounds or as ingredients in pharmaceuticals (Krämer, 1994; Hirasawa and Shimizu, 2016; Ma et al., 2017). Moreover, the relationship between amino acids and flavor formation has been studied extensively in lactic acid bacteria (LAB) used in dairy fermentations in order to understand and improve the organoleptic properties of dairy products (Juillard et al., 1995; Niven et al., 1998). In particular, *Lactococcus lactis* is widely used as a starter culture for the manufacture of buttermilk, quark and a wide variety of cheeses (Laroute et al., 2017). Its proteolytic system provides the cells with essential amino acids from casein (Savijoki et al., 2006). The amino acids, obtained from casein degradation, are either flavor compounds or flavor precursors (Ayad et al., 1999; Smit et al., 2005).

In this work, we report three amino acid-secreting *L. lactis* strains from the laboratory collection of the Molecular Genetics department at the University of Groningen (Groningen, The Netherlands; J.A. Hernandez-Valdes, Chapter 4). The strains were originally isolated from dairy environments. The *L. lactis* C17 strain was obtained from the NIZO collection, the *L. lactis* NCDO176 strain

was obtained from the DSMZ collection, and the *L. lactis* WW4 strain was obtained from the MolGen collection. A single colony growing on a LM17-agar plate of each strain was selected and grown as standing culture in 5 mL of M17 broth, supplemented with 0.5 % (w/v) lactose (LM17), and incubated overnight at 30 °C. Cells from the three cultures were collected by centrifugation at 10 000 rpm for 3 min in a Microfuge 16 centrifuge (Beckman Coulter, Woerden, The Netherlands). Genomic DNA was isolated with a GenElute bacterial genome DNA kit (Sigma-Aldrich, Munich, Germany) according to the manufacturer's instructions.

The genomes of the lactococal strains were paired-end sequenced at the Beijing Genomics Institute (BGI, Copenhagen N, Denmark) on a BGISEQ-500 platform. A total of 5 million paired-end reads (150 bp) were generated. FastQC version 0.11.5 (Andrews et al., 2015) was used to examine the quality of the reads, and low-quality reads were removed with Trimmomatic version 0.38 (Bolger et al., 2014). Subsequently, SPAdes version 3.11.1 (Nurk et al., 2013) was used with default parameters to perform a *de novo* paired-end assembly on each genome, resulting in the draft genome sequences. The coverages of the three sequenced genomes all exceeded 150X. The characteristics of the assemblies and genome features obtained are described in Table 1. Taxonomic assignment of reads was performed with Kraken 2.0.7 (Wood and Salzberg, 2014). The Rapid Annotations using Subsystems Technology (RAST) server (Aziz et al., 2008) and Prokka (Seemann, 2014) were used to annotate the genomes. The further analysis of the genomes in order to discover the mechanisms underlying amino acid secretion by these bacteria, is underway.

Table 1. Genome features and accession numbers of the three *Lactococcus lactis* strains.

Strain	Genome size (bp)	G+C content (%)	Number of coding sequences	Number of contigs	GenBank accesion number	SRA accesion number
Lactococcus lactis subsp. *lactis* C17	2,552,877	35.0	2717	130	WJUK 00000000	SRR10 203129
Lactococcus lactis subsp. *lactis* NCDO176	2,445,329	35.1	2579	120	WJUL 00000000	SRR10 203130
Lactococcus lactis subsp. *lactis* WW4	2,553,867	34.9	2716	132	WJUM 00000000	SRR10 203131

DATA AVAILABILITY

The genome sequences of the three *Lactococcus lactis* strains have been deposited in GenBank under the accession numbers listed in Table 1. The raw reads were submitted to the Sequence Read Archive under the accession numbers listed in Table 1.

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