

# The Carbohydrate Metabolism Signature of *Lactococcus lactis* Strain A12 Reveals Its Sourdough Ecosystem Origin

Delphine Passerini,<sup>a,b,c,d,e</sup> Michèle Coddeville,<sup>a,c</sup> Pascal Le Bourgeois,<sup>a,c</sup> Pascal Loubière,<sup>b,d,e</sup> Paul Ritzenthaler,<sup>a,c</sup> Catherine Fontagné-Faucher,<sup>a,f</sup> Marie-Line Daveran-Mingot,<sup>a,c</sup> Muriel Coccain-Bousquet<sup>b,d,e</sup>

Université de Toulouse, Université Paul Sabatier, Toulouse, France<sup>a</sup>; Université de Toulouse, INSA, UPS, INP, LISBP, Toulouse, France<sup>b</sup>; CNRS, Laboratoire de Microbiologie et de Génétique Moléculaires, Toulouse, France<sup>c</sup>; INRA, UMR792 Ingénierie des Systèmes Biologiques et des Procédés, Toulouse, France<sup>d</sup>; CNRS, UMR5504, Toulouse, France<sup>e</sup>; IUT Paul Sabatier, EA 4565, Laboratoire de Biotechnologies Alimentaire et Environnementale, Auch, France<sup>f</sup>

***Lactococcus lactis* subsp. *lactis* strain A12 was isolated from sourdough. Combined genomic, transcriptomic, and phenotypic analyses were performed to understand its survival capacity in the complex sourdough ecosystem and its role in the microbial community. The genome sequence comparison of strain A12 with strain IL1403 (a derivative of an industrial dairy strain) revealed 78 strain-specific regions representing 23% of the total genome size. Most of the strain-specific genes were involved in carbohydrate metabolism and are potentially required for its persistence in sourdough. Phenotype microarray, growth tests, and analysis of glycoside hydrolase content showed that strain A12 fermented plant-derived carbohydrates, such as arabinose and  $\alpha$ -galactosides. Strain A12 exhibited specific growth rates on raffinose that were as high as they were on glucose and was able to release sucrose and galactose outside the cell, providing soluble carbohydrates for sourdough microflora. Transcriptomic analysis identified genes specifically induced during growth on raffinose and arabinose and reveals an alternative pathway for raffinose assimilation to that used by other lactococci.**

*Lactococcus lactis* is a mesophilic Gram-positive species, related to *Streptococcaceae* (1), which is subdivided into two subspecies: *lactis* and *cremoris*. Lactococci have been extensively studied for their role in dairy products manufacturing, but the subspecies *lactis* is able to colonize various environments other than milk, such as plant and animal material (2–5).

*L. lactis* subsp. *lactis* is occasionally found among the sourdough microflora used in traditional Italian (6), Portuguese (7), French (8), and Mexican (9) bread-making. Sourdough is a mixture of flour and water that is fermented by a heterogeneous population of lactic acid bacteria (LAB) and yeast and is used for the production of bread and sweet leavened baked goods. The LAB microflora is 100 times more abundant than yeast (10, 11) and is very diverse. LAB detected in sourdough are often obligate heterofermentative lactobacilli (*Lactobacillus brevis*, *Lactobacillus reuteri*, or *Lactobacillus sanfranciscensis*). They can also include less prevalent facultative heterofermentative and obligate homofermentative genera such as *Lactobacillus*, *Pediococcus*, *Streptococcus*, *Enterococcus*, and *Lactococcus* spp. or obligate heterofermentative genera such as *Leuconostoc* and *Weissella* spp. (10). Analysis of bacterial community dynamics revealed that *L. lactis* is found at the start of back-slopped fermentation and can predominate in some cases (12). Its functional role remains unknown. Sourdough biodiversity and species stability are influenced by various determinants including the cereal type used (flour composition), fermentation process (temperature, pH, redox potential, dough hydration, additional ingredients, or back-slopping methods), and metabolic activities resulting from the cooperation and competitiveness of microbiota (13–17). Studies on the most frequent LAB-yeast associations (*Lactobacillus sanfranciscensis* and *Kazachstania exigua* [syn. *Saccharomyces exigua*] or *Saccharomyces cerevisiae*) or LAB-LAB associations (*Lactobacillus sanfranciscensis* and *Lactobacillus plantarum*) reveal trophic and energetic relationships (16, 18, 19); this complex ecosystem has a very diverse set of metabolic activities. Sourdough is expected to enhance properties of end

product by developing its sensory qualities (aroma, taste, and texture), increasing the shelf-life, enhancing preservation and food safety by inhibiting pathogens or fungi, improving its nutritive value, and removing undesired compounds (10).

This study aims to understand the genetic features involved for adaptation and persistence of *L. lactis* in sourdough (an atypical niche for this species), by characterizing the genetic and metabolic properties of a strain isolated from traditional French sourdough (8). Strain A12 belongs to the group of environmental strains; these are more genetically diverse than domesticated/dairy strains (5). We investigate here the specific features of strain A12 that are relevant in a sourdough ecosystem by comparing its genome content and phenotype to the laboratory strain IL1403 (20). Strain IL1403 is a derivative of a dairy strain (21) which has been extensively studied at the physiological level (22, 23). The genes specifically activated by strain A12 on raffinose and arabinose, two sugars relevant for sourdough fermentation, were investigated by transcriptional analysis.

## MATERIALS AND METHODS

**Bacterial strains.** *L. lactis* subsp. *lactis* strain LBAE A12, isolated from a traditional French wheat sourdough (8), belongs to the LBAE-UPS (Auch, France) culture collection and has been previously described in the Genoferm collection (5).

Received 7 May 2013 Accepted 7 July 2013

Published ahead of print 19 July 2013

Address correspondence to Marie-Line Daveran-Mingot, marie-line.mingot@ibcg.biotoul.fr.

M.-L.D.-M. and M.C.-B. contributed equally to this article.

Supplemental material for this article may be found at <http://dx.doi.org/10.1128/AEM.01560-13>.

Copyright © 2013, American Society for Microbiology. All Rights Reserved.  
doi:10.1128/AEM.01560-13

**A12 genome sequencing, annotation, and comparison.** A draft genome was obtained by a whole-genome DNA shotgun strategy with 454/ Roche GS FLX pyrosequencing (Eurofins MWG Operon, France). We obtained 147 contigs (228,605 reads, 2,727,544 bp, mean coverage sequencing depth of 18 X) using the GS De Novo Assembler software from Newbler package (454 Life Sciences); the 42 contigs of >1 kb were deposited in public databases. Automatic annotation of DNA sequences was performed using the Agml platform (INRA Jouy-en-Josas, France) (24), and specific genes were manually checked. Genes are described according to 1 of 15 functional categories (defined by Bolotin et al. [20]), designated as follows: amino acid biosynthesis (AMI), biosynthesis of cofactors, prosthetic groups, and carriers (COF), cell envelope (ENV), cellular processes (CEL), central intermediary metabolism (INT), energy metabolism (NRJ), fatty acid and phospholipid metabolism (FAT), purines, pyrimidines, nucleosides, and nucleotides (PUR), regulatory functions (REG), replication (REP), transcription (TRS), translation (TRD), transport and binding proteins (TSP), other categories (OTH), and unknown (UNK). Determination of strain-specific DNA regions was performed using Pan-seq v2.0 software (25) with default options. All A12-specific regions were individually compared to the IL1403 chromosome by local homology searches (FastScan method) using clone Manager 9.2 (Sci-Ed Software, Cary, NC). Glycoside hydrolases (GH) of sequenced Lactococci genomes listed in the CAZy database (<http://www.cazy.org/>) (26) allowed us to detect putative active carbohydrate enzymes (CAZymes) in strain A12 using NCBI-BLASTP. We used the InterPro collection of protein signature databases to classify new GH, which were found in strain A12 by automatic annotation. To detect possible sequence errors due to pyrosequencing, the nucleotide sequence of the *LL2138* central region was verified by Sanger sequencing.

**Global phenotypic analysis.** The Phenotype Microarray (Biolog, Hayward, CA) was used for global phenotypic analysis of strains IL1403 and A12 (27). Two replicates of each plate, PM1 and PM2A (carbon substrates), and one replicate for PM9 (osmotic sensitivity), PM10 (pH), and PM13 (oxidizing agents) were inoculated from isolated colonies on BUG B medium. We added 100  $\mu$ l of cell suspension at 81% of transmittance to each well according to the manufacturer's instructions. Microplates were incubated at 33°C in Omnilog plate reader, which recorded the Dye G opacity, for 72 h. The data were analyzed using OmniLog Phenotype MicroArray software (release 1.2). The substrate was considered as being specifically metabolized by the strain A12 when the corresponding height value of the color-coded kinetic graphs exceeded that of the strain IL1403, with a threshold of 80.

**Effect of oxygen on growth.** The specific growth rates ( $\mu_{\max}$ ) under aerobic and anaerobic conditions were measured (four replicates). For anaerobic conditions, strains were grown at 30°C in M17 medium (Merck, Germany) supplemented with glucose (10 g/liter; GM17) in tubes under an  $N_2$  atmosphere. For aerobic conditions, cultures were grown with the same medium, under an air gas-phase in 200-ml Erlenmeyer flasks at 30°C and 200 rpm. The optical density at 580 nm ( $OD_{580}$ ) was recorded approximately every 30 min to estimate the maximal growth rate, which was calculated from the slope of the exponential growth phase.

**Growth tests on different carbon sources.** Carbon source utilization by strains A12 and IL1403 was tested by inoculating strains in 10 ml of the chemically defined medium (CDM) (28) supplemented with one carbon source (at 2 g/liter), followed by incubation for 24 h. At least two biological replicates were performed for each condition. We tested 28 carbon sources belonging to the following groups: monosaccharides such as aldohexoses (glucose and galactose), cetohexose (fructose), aldopentoses (*L*-arabinose and *D*-xylose) and aldohexoses (rhamnose), disaccharides (saccharose, *D*-melibiose, gentiobiose, *D*-cellobiose, *D*-maltose, tréhalose, palatinose, and lactose), trisaccharides (*D*-raffinose, maltotriose, and melizitose), tetrasaccharides (stachyose), polysaccharides (dextran, pectin, xylan, hydroxyl-cellulose, inuline, and amidon), acid sugars (*D*-glucuronate, *D*-galacturonate, and *D*-gluconate), and aryl-monosaccharides

(amygdalin). We measured growth by recording the  $OD_{580}$  with a Spectronic spectrophotometer (Milter Roy).

**Hydrolase activity.** Enzymatic activities of A12 and IL1403 strains were determined using insoluble chromogenic polysaccharides: AZCL- $\beta$ -glucan, AZCL-xylan, AZCL-amylose, AZCL-arabinoxylan, AZCL-galactan, AZCL-rhamnogalacturonan, or cellulose-AZUR (Megazyme, Bray, Ireland). Each strain was incubated in M17 medium supplemented with glucose (0.5%) and an insoluble AZCL-polysaccharide (0.1%). After incubation at 30°C for 48 h, polysaccharide digestion by endohydrolases was detected by complete pellet solubilization. To test arabinofuranosidase activity, bacterial strains were plated on GM17 agar containing the substrate 5-bromo-4-chloro-3-indolyl- $\alpha$ -*L*-arabinofuranoside (*X*-ara; 100  $\mu$ M; Carbosynth, United Kingdom). Enzymatic activity was indicated by blue colonies.

**Fermentation culture and metabolic analyses.** Strain A12 was grown under a nitrogen atmosphere in a 500-ml fermentor (Sartorius, Germany) for 24 h, at 30°C and agitated at 150 rpm. The automatic addition of KOH (10 N) maintained a pH of 6.6. CDM (28) was supplemented with either 10 g of glucose, 10 g of arabinose, 10 g of fructose, 10 g of galactose, or 9 g of raffinose/liter (equivalent to 330 mmol C/liter for each). Two independent replicates were carried out for each growth condition.

Spectrophotometric measurements at 580 nm (Hitachi U1100; 1 U of absorbance is equivalent to 0.3 g of biomass liter<sup>-1</sup> for *L. lactis*) were taken to estimate bacterial growth. Raffinose, glucose, galactose, fructose, arabinose, lactate, acetate, formate, and ethanol concentrations were measured by high-pressure liquid chromatography (Hewlett-Packard 1050; Waters 717 autosampler; Bio-Rad Microguard; H+ column, Bio-Rad HPX87H; Perkin-Elmer LC90Bio UV detector; Hewlett-Packard 1047A refractometer). High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was used to study the breakdown of raffinose. A 4- by 250-mm Dionex Carbo-pack PA100 was used for separation. A gradient of sodium acetate (in 150 mM NaOH) was applied at a flow rate of 1 ml/min: from 0 to 300 mM in 30 min, from 300 to 450 mM in 1 s, from 450 to 0 mM in 5 min, and then at 0 mM for 10 min.

The maintenance energy coefficient (*m*) corresponds to the specific rate of sugar consumption when the growth rate is zero. This was calculated during the deceleration phase from the specific substrate consumption rate *q* (mmol C/gX/h, where gX is grams of biomass) as a function of the growth rate ( $\mu$ ), a linear relationship, as described previously (29).

**Transcriptomic analysis.** A nylon array, containing 1,948 PCR fragments (Eurogentec) that correspond to 84% of the IL1403 annotated open reading frame (ORF) (accession number NC\_002662), was supplemented with 433 PCR amplicons (ClinEurodiag, Belgium) corresponding to A12-specific genes. Partial prophage, transposases, and coding DNA sequences (CDS) smaller than 200 bp were omitted from the macroarray. Membrane spotting and analytical support were provided by the GeT platform (Genopole, Toulouse, France).

The equivalent of 3 mg of dried cells was harvested during the early exponential growth phase ( $OD_{580} = 1$ ) of the fermentation process for transcriptional analysis. Cell lysis, RNA extraction, and hybridization with radiolabeled cDNA were carried out as previously described (23). Membranes were exposed to a phosphorimager screen for 5 days and scanned with a phosphorimager (Storm 860; Molecular Dynamics).

Hybridization signals were quantified from four replicates (two biological and two technical replicates), assigned to gene names, and statistically treated with Biplot software (GeT platform). The local background was removed, and the signals were normalized to the mean membrane intensity. The expression ratios for each sugar pairing were calculated; they were estimated as statistically different from 1 when the false discovery rate was under 10% (as previously described [22]) (see Table S1 in the supplemental material).

**Nucleotide sequence accession numbers.** The GenBank accession numbers assigned to the *L. lactis* strain A12 nucleotide sequence are CBLU010000001 to CBLU010000042.

## RESULTS

**The dispensable genome of strain A12 reveals a particular adaptation for sugar metabolism.** A draft sequence of the *L. lactis* subsp. *lactis* A12 genome was established by shotgun pyrosequencing (Roche/454). Of the 147 contigs generated, the 42 that were larger than 1 kbp (227,901 reads, 2,700,005 bp) were retained for further analysis. A12 strain-specific DNA regions, thought to contain genes required for adaptation to sourdough ecosystem, were determined by comparison to the chromosome of strain IL1403. These specific regions represent nearly 630 kb (23% of total A12 genetic content) and are distributed in 78 DNA fragments ranging from 513 bp to 57 kbp. Comparison with the chromosome and plasmids of strain KF147, a nondairy strain isolated from mung bean sprouts (30), revealed nearly 560 kbp of A12-specific regions distributed in 100 fragments ranging from 507 bp to 57 kbp in size. This suggests high diversity of genomic content among *L. lactis* subsp. *lactis*, even between strains from plant origin. The functional categories of the specific genes were manually classified according to the method of Bolotin et al. (20): 40% of A12-specific CDS encoded unknown functions and 27% encoded protein related to prophages. The remaining CDS corresponded mostly to adaptive functions such as transporters (36 CDS: 22 involved in sugars transport), sugar energetic metabolism (35 CDS), cell envelope constitution (33 CDS), and regulation (17 CDS). A large proportion of genes identified are involved in sugar metabolism and sugar transport; analysis of specific glycoside hydrolases (GH) was therefore performed. GH, listed in the CAZy (Carbohydrate-Active Enzyme) database (26), catalyze the hydrolysis of the glycoside bond between two or more carbohydrates or between a carbohydrate and a noncarbohydrate moiety. All 32 GH coded in the IL1403 genome were also found in the A12 genome. However, the A12 genome encoded eight additional GH: LL1981 (family GH 1), SucP (GH 13), XylS (GH 31), Aga (GH 36), LL2113 (GH 65), LL1819 (GH 65), LL498 (GH 113), and LL2114 (GH 101). In contrast to other GH, the genetic sequence for LL2114 was not found in the genomes of the two nondairy strains KF147 (30) and IO-1 (31; data not shown). The GH 101 family contains enzymes with an endo- $\alpha$ -N-acetylgalactosaminidase activity and therefore represents a new activity in *L. lactis* subsp. *lactis*.

The large set of strain-specific genes found in *L. lactis* subsp. *lactis* suggests substantial potential for adaptation in a complex ecosystem. The genome of strain A12 contained genetic sequences relating to more extensive variation in carbohydrate metabolism than that of strain IL1403. The functionality of these genes was examined by phenotypic analysis.

**Strain A12 is able to metabolize plant-derived carbohydrates.** The global phenotype of the strains A12 and IL1403 was investigated using Phenotype Microarray (Biolog). A phenotypic profile comparison of these two strains enabled us to determine specific capacities of strain A12 possibly related to its sourdough survival. The Biolog method measures the reduced coenzyme NADH production capacity during sugar fermentation, but this is not necessarily correlated with biomass production (27). Carbon source utilization was the most apparent phenotypic difference between the two strains (see Table S1 in the supplemental material); we detected only minor differences in responses to osmotic sensitivity, pH variation, and oxidizing agent sensitivity. Growth tests were carried out in liquid CDM supplemented with various sugars. We tested 28 carbon sources displaying diverse levels of

TABLE 1 Growth rates ( $\mu_{\max}$ ) of strains IL1403 and A12 on different carbon sources under anaerobic conditions

Substrate	Family	Mean growth rate ( $\mu_{\max}$ [h <sup>-1</sup> ]) $\pm$ SD <sup>a</sup>	
		IL1403	A12
Glucose	Aldohexose	0.63 $\pm$ 0.07	0.34 $\pm$ 0.04
Galactose	Aldohexose	–	0.13 $\pm$ 0.03
Fructose	Cetohexose	0.35 $\pm$ 0.04	0.12 $\pm$ 0.04
L-Arabinose	Aldohexose	–	0.18 $\pm$ 0.09
D-Xylose	Aldohexose	–	–
Rhamnose	Aldohexose	–	–
Sucrose	Disaccharide	–	0.06 $\pm$ 0.04
D-Melibiose	Disaccharide	–	0.36 $\pm$ 0.06
Gentiobiose	Disaccharide	0.03 $\pm$ 0.01	0.30 $\pm$ 0.03
D-Cellobiose	Disaccharide	0.54 $\pm$ 0.03	0.40 $\pm$ 0.07
D-Maltose	Disaccharide	0.35 $\pm$ 0.05	0.09 $\pm$ 0.06
Trehalose	Disaccharide	0.66 $\pm$ 0.02	0.17 $\pm$ 0.00
Palatinose	Disaccharide	–	–
Lactose	Disaccharide	–	–
D-Raffinose	Trisaccharide	–	0.37 $\pm$ 0.06
Maltotriose	Trisaccharide	0.36 $\pm$ 0.01	0.26 $\pm$ 0.06
Melizitose	Trisaccharide	–	–
Stachyose	Tetrasaccharide	–	0.30 $\pm$ 0.08
Dextran	Polysaccharide	–	–
Pectin	Polyoside	–	–
Xylan	Polyoside	–	–
Hydroxycellulose	Polysaccharide	–	–
Inulin	Polysaccharide	–	–
Starch	Polysaccharide	–	–
D-Glucuronate	Sugar acid	–	–
D-Galacturonate	Sugar acid	–	–
D-Gluconate	Sugar acid	0.06 $\pm$ 0.03	0.05 $\pm$ 0.03
Amygdalin	Aryl-disaccharide	–	–

<sup>a</sup> Two to four replicates were performed for each growth test. –, no growth.

complexity (monosaccharides, disaccharides, trisaccharides, tetrasaccharides, and polysaccharides) (Table 1). The effect of oxygen on IL1403 and A12 growth was investigated to optimize culture conditions. Strain IL1403 exhibited similar maximum specific growth rates ( $\mu_{\max}$ ) under anaerobic and aerobic conditions; strain A12 had a 40% higher  $\mu_{\max}$  under anaerobic conditions (data not shown). All physiological studies were therefore performed in anaerobic conditions, i.e., conditions that also prevail during sourdough fermentation.

Neither strain A12 nor IL1403 were able to grow on D-xylose, rhamnose, palatinose, lactose, melizitose, and complex polysaccharide carbon sources (Table 1). A lack of growth on complex sugars by strain A12 correlated with a lack of enzymes responsible for the cleavage of the commercial chromogenic polysaccharides:  $\beta$ -glucans, xylans, amylose, arabinoxylan, and cellulose (Table 2). Strain A12 exhibited a growth rate lower than that of strain IL1403 for other sugars (glucose, fructose, D-cellobiose, D-maltose, trehalose, and maltotriose). The cellobiose metabolism was efficient since the strain A12 displayed the highest growth rate on this plant derivative substrate (Table 1). Only strain A12 was able to grow on galactose, L-arabinose, sucrose, D-melibiose, gentiobiose, D-raffinose, and stachyose (strain IL1403 could grow at a very low  $\mu_{\max}$  on gentiobiose). These carbon sources are plant-derived carbohydrates: gentiobiose is the glucose moiety of the amygdalin found in some vegetables (32), L-arabinose and D-galactose are hemicellulose constituents of plant cell walls (33), and D-raffinose, stachyose, and the related disaccharide melibiose are



TABLE 2 Enzymatic activities of strains IL1403 and A12

Substrate	Detected enzyme	Enzymatic activity <sup>a</sup>	
		IL1403	A12
AZCL-β-glucan	β-Glucanase	–	–
AZCL-xylan	Endo-xylanase	–	–
AZCL-amylose	α-Amylase	–	–
AZCL-arabinoxylan	Endo-xylanase	–	–
Cellulose-AZUR	Cellulase	–	–
AZCL-galactan	Endo-1,4-β-galactanase	–	+
AZCL-rhamnogalacturonan	Rhamnogalacturonan hydrolase	–	+
X-ara	Arabinofuranosidase	–	–

<sup>a</sup> +, hydrolysis of a chromogenic polysaccharide by a related enzyme; –, no activity.

important soluble α-galactosides in plant tissues (34, 35). Strain A12 cleaved chromogenic galactan and rhamnogalacturonan, showing the presence of an active galactanase that can hydrolyze α-galactoside bonds (Table 2).

**Selective advantage of strain A12 for growth on arabinose and raffinose.** We assessed the biomass and fermentation end products of strain A12, when grown on various plant sugars, to investigate the potential selective advantage of this strain during plant sugar fermentation. Cultures were performed in a bioreactor where pH, agitation, and anaerobic atmosphere were controlled. Five sugars were selected: a plant pentose (arabinose), a complex sugar belonging to the α-galactoside family (raffinose), and three related monosaccharides (galactose, glucose, and fructose). The physiological characteristics of strain A12 differed according to the carbon source used (Table 3). The metabolism of glucose and fructose was homolactic; lactate represented the major fermentation end product (>90%). Deviation from homolactic to mixed acid fermentation was observed on galactose, raffinose, and, in particular, on arabinose (where 33.3% ± 0.5% of carbon flux was routed toward formate, acetate, and ethanol production). This was twice as great as when raffinose (16.08% ± 0.03%) or galactose (18.53% ± 2.24%) were used as the carbon source. Such a metabolic shift has been previously associated with low growth rates, either when sugar is metabolized slowly (e.g., galactose) or in sugar-limited chemostat cultures, and would be mainly controlled by the NADH/NAD<sup>+</sup> ratio (36, 37). Our results were consistent with this notion for galactose and arabinose carbon sources, where strain A12 exhibited lower μ<sub>max</sub> values (0.18 ± 0.00 h<sup>-1</sup> and 0.28 ± 0.01 h<sup>-1</sup>, respectively, versus 0.53 ± 0.01 h<sup>-1</sup>

for glucose) (Table 3). However, strain A12 had a high μ<sub>max</sub> on raffinose (0.46 ± 0.01 h<sup>-1</sup>), as well as favoring a mixed acid pathway, suggesting an original metabolic pathway control in this strain. The molar ratios between lactate and acetate, a relevant parameter for aromatic quality of bread, were 1.4 on arabinose, 5 on galactose, and 6.5 on raffinose. These values were similar to the optimal values found in rye, spelt, and wheat sourdough (ratios between 1.5 and 4) (38, 39).

Physiological maintenance, corresponding to the minimum substrate necessary to maintain cell survival, was estimated for strain A12 on each substrate (these were estimated using the instantaneous growth rates and measures of sugar consumption during the decelerating phase [see Materials and Methods]). On glucose, fructose, and galactose, maintenance coefficients were ~30 mmol C/gX/h, corresponding to the value calculated for IL1403 cultivated on glucose (22). On plant-derived carbohydrates, the maintenance coefficient significantly decreased to 19 mmol C/gX/h (R<sup>2</sup> = 0.92) on arabinose and 6 mmol C/gX/h (R<sup>2</sup> = 0.97) on raffinose. This suggests that strain A12 is metabolically efficient on arabinose and raffinose: it needs little energy to ensure its maintenance and may use the majority of carbon provided to produce biomass. The cleavage of raffinose (α-D-galactosyl-α-D-glucosyl-β-D-fructose) into individual units can occur in two ways: α-1,6 linkage hydrolysis (generating sucrose and galactose) or β-1,2 linkage hydrolysis (generating melibiose and fructose). We used high-pressure liquid chromatography, with culture supernatants, to monitor the concentrations of raffinose, fructose, galactose, glucose, melibiose, and sucrose in culture supernatants. Raffinose was consumed from the beginning of fermentation and had a maximal specific rate of consumption of 163.6 ± 27.9 mmol C/gX/h (as high as the glucose assimilation rate; Table 3). Sucrose and galactose were accumulated transiently during growth, but there was no trace of fructose, glucose, or melibiose in the supernatant (Fig. 1A). Galactose excretion has been observed during lactose catabolism of *Lactococcus lactis* subsp. *cremoris* (40) and other LAB (41, 42). Here, galactose and sucrose were excreted after 2 and 3 h of fermentation, respectively. Their concentrations reached a maximum of 7 mM (galactose) and 4 mM (sucrose) after 8 to 9 h of growth and then fell until reaching total depletion at 24 h. The amount of released sucrose and galactose was proportional to that of metabolized raffinose, but lower than the theoretical maximum, which would correspond to the absence of bacterial consumption (Fig. 1B). The slopes of linear correlations showed that, in a medium containing raffinose, ca. 50% of galactose and 70% of sucrose were directly used by cell. This suggests

TABLE 3 Growth characteristics of strain A12 on different sugars

Substrate	Structure	Mean μ <sub>max</sub> (h <sup>-1</sup> ) ± SD	Maximum rate sugar of consumption (mmol C/gX/h)	Mean concn (mM) ± SD of fermentation end products at 24 h				Mean % carbon routed through the mixed acid pathway at 24 h (%) ± SD <sup>a</sup>	Estimated maintenance energy coefficient <sup>b</sup> (mmol C/gX/h)
				Lactate	Formate	Acetate	Ethanol		
Glucose	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	0.53 ± 0.01	128.4 ± 32.1	79.9 ± 1.9	7.8 ± 1.3	4.3 ± 0.9	8.3 ± 0.7	7.3 ± 0.1	24.9
Fructose	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	0.17 ± 0.00	44.6 ± 0.2	86.6 ± 0.8	6.3 ± 0.6	3.9 ± 0.1	7.1 ± 0.2	6.0 ± 0.0	37.0
Galactose	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	0.18 ± 0.00	34.7 ± 2.7	59.2 ± 5.7	15.4 ± 0.8	12.4 ± 0.6	14.5 ± 2.1	18.5 ± 2.2	32.0
Arabinose	C <sub>5</sub> H <sub>10</sub> O <sub>5</sub>	0.28 ± 0.01	73.6 ± 0.3	52.0 ± 0.6	10.6 ± 0.9	36.7 ± 0.6	15.3 ± 1.2	33.31 ± 0.5	19.4
Raffinose	C <sub>18</sub> H <sub>32</sub> O <sub>16</sub>	0.46 ± 0.01	163.6 ± 27.9	52.2 ± 2.7	10.4 ± 4.0	7.6 ± 2.0	12.4 ± 3.0	16.1 ± 0.0	5.7

<sup>a</sup> Calculated according to the following formula: 3([acetate] + [ethanol])/([lactate] + 3([acetate] + [ethanol])).

<sup>b</sup> Determined as described in Materials and Methods.

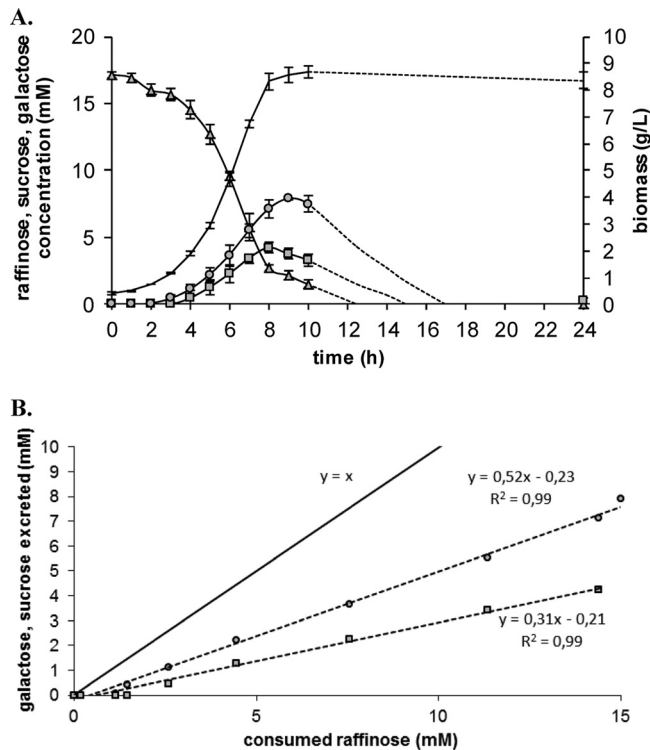


FIG 1 Raffinose metabolism by *L. lactis* subsp. *lactis* strain A12. (A) Kinetics of carbohydrates quantified in supernatant culture; (B) sucrose and galactose excreted, as a function of the raffinose metabolized. The line  $y = x$  represents the theoretical maximum of excreted sugar without any consumption of sugar. Data for raffinose ( $\Delta$ ), galactose ( $\circ$ ), and sucrose ( $\square$ ) are shown. No melibiose, fructose, or glucose was detected.

that sucrose was more efficiently used through raffinose metabolism ( $\mu_{\max} = 0.46 \text{ h}^{-1}$ ) than alone ( $\mu_{\max} = 0.06 \text{ h}^{-1}$ , Table 2). Intracellular sucrose is well metabolized, but not when it is provided as the sole carbon source; sucrose transport was probably the limiting step for sucrose metabolism by strain A12. Indeed, the described gene for sucrose transport (*scrA*) is lacking in the A12 genome.

**Few genes displayed a specific expression variation related to arabinose and raffinose metabolism in strain A12.** The global transcriptional response of strain A12 was investigated during the early exponential growth phase, in the conditions described above, to identify genes involved in arabinose and raffinose metabolism and to analyze their expression levels.

In order to highlight the first steps of the sugar pathway or an original pathway in strain A12, the transcriptomic responses on arabinose and raffinose were compared to those on the three simple sugars, galactose, glucose and fructose (Fig. 2). This allowed us to exclude genes whose expression was modified by fermentation type (homolactic or mixed) or by growth rate variation (see Table S2 in the supplemental data). Analysis of the transcriptomic responses of strain IL1403, in continuous cultures at different growth rates, revealed that nearly 700 genes displayed variation in their expression (22). Our comparison method detected only a small number of differentially expressed, sugar-specific genes: three genes were identified during growth on raffinose (*LL2138*, *aga*, and *sucP*), and nine were detected during growth on arabinose (*araR*, *araT*, *araB*, *araA*, *araD*, *ptk*, *xylX*, *xylT*, and *arcB*). The method also detected genes specific to glucose, fructose, and ga-

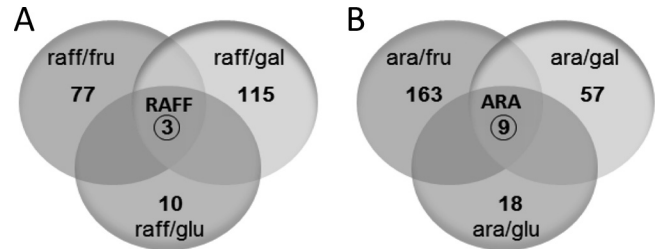


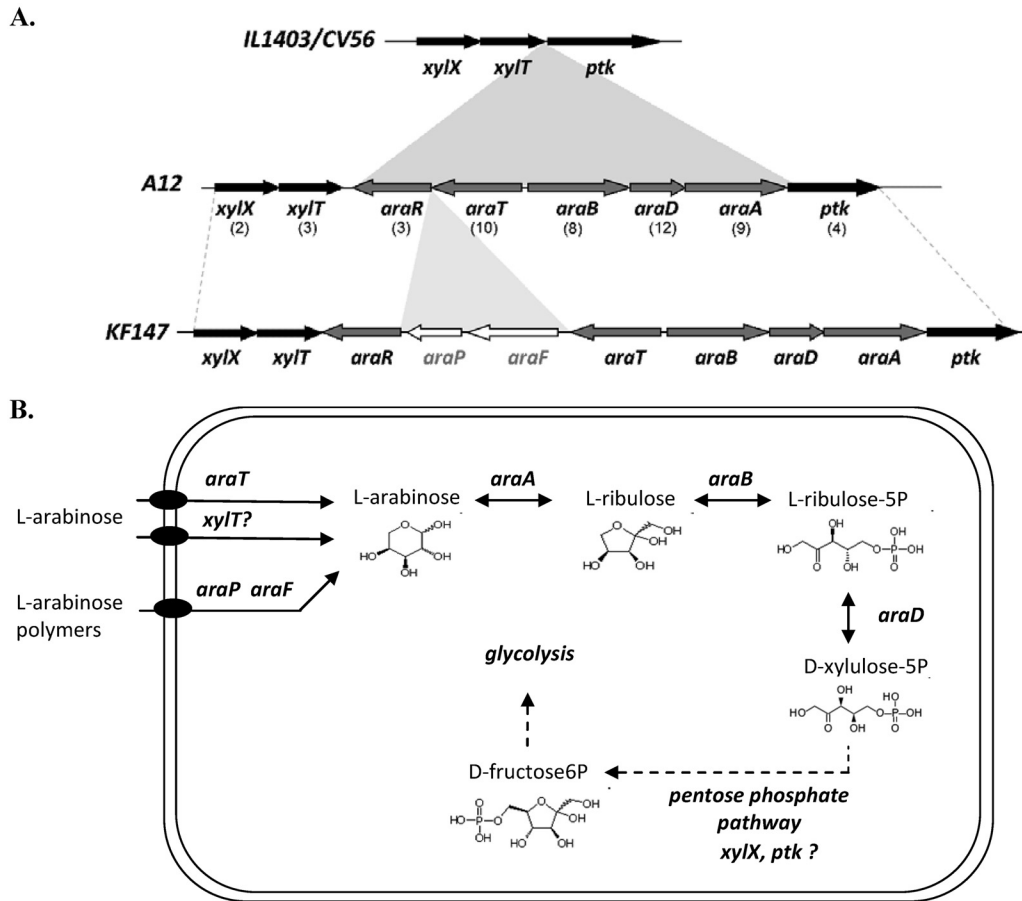
FIG 2 Venn diagrams representing the number of strain A12 genes with variable expression in different sugar substrates: combined comparison of raffinose (raff) (A) or arabinose (ara) (B) with the three simple sugars (glucose [glu], fructose [fru], and galactose [gal]), highlighting raffinose- and arabinose-specific genes. The variation of gene expression was considered significant when the false discovery rate was  $<10\%$ .

lactose metabolism (1, 15, and 24 genes, respectively [see Table S3 in the supplemental material]).

Raffinose metabolism was investigated using pairwise comparisons of the transcriptomic response on this substrate and that of other simple carbon substrates (Fig. 2). In addition to the raffinose-specific activation of *LL2138*, *sucP*, and *aga* genes, strain A12 induced a substantial number of other genes. The genes *galK*, *galM*, *galT*, *lacZ*, *yugA*, and *yugB*, specific to galactose metabolism, were upregulated on raffinose compared to glucose or fructose. The *gal* genes encode enzymes of the Leloir pathway, which facilitate the conversion of galactose into glucose-1-phosphate further routed toward glycolytic pathway (43). No genes involved in the tagatose-6-phosphate pathway, the alternative pathway for galactose conversion, were induced; the plasmid-borne lactose operon (involved in the first steps of the tagatose-6-phosphate pathway) is not found in strain A12 (5). Expression of *fruC* was greater on raffinose than on glucose or galactose. This gene, originally identified as the tagatose-6-phosphate kinase encoding *lacC*, has been re-annotated as the 1-phosphofructokinase *fruC* and is involved in fructose metabolism (44). Increased expression of genes specifically related to galactose and fructose pathways is indicative of the metabolism of both raffinose subunits: this result is supported by the physiological data.

**An original gene cluster encoding raffinose metabolism in strain A12.** We investigated the genetic organization of induced genes when strain A12 was cultivated on arabinose and raffinose. The genes *araR*, *araT*, *araB*, *araA*, and *araD*, induced when strain A12 was cultivated on arabinose, corresponded to a genomic island previously detected by comparative genome hybridization in another *L. lactis* subsp. *lactis* strain isolated from plant material (45). The *ara* gene cluster had a different organization compared to that found in strain KF147 but was determined to be functional by transcriptomic analysis. It is likely that *ptk*, *xylX*, and *xylT* (found in the core genome of *L. lactis* and on either side of the arabinose cluster when it was present), were involved in arabinose metabolism (Fig. 3).

When strain A12 was cultivated on raffinose, *LL2138*, *aga*, and *sucP* were specifically induced. These genes are clustered upstream of ORF *LL2157* that encodes a putative transcriptional regulator of the AraC family (Fig. 4). The *aga* and *LL2138* genes were expressed on raffinose at rates 10 times greater than on glucose or fructose and five times greater than on galactose (see Table S3 in the supplemental material). *sucP* was expressed approximately four times more on raffinose than on the other sugars. The activation of *aga*

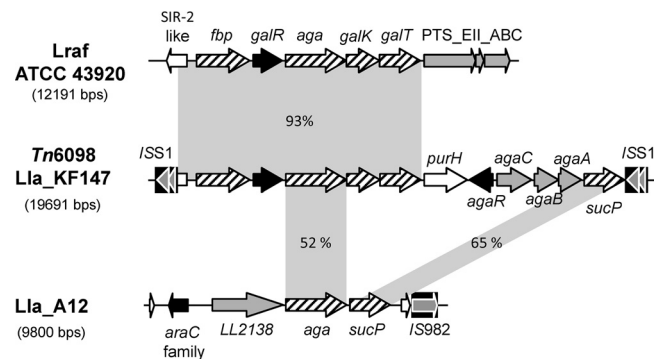


**FIG 3** Arabinose metabolism by *L. lactis* subsp. *lactis*. (A) Localization and genetic organization of the arabinose operon in sequenced genomes; (B) diagram of the related metabolic pathway. The numbers in brackets correspond to the average ratio values obtained by transcriptomic analysis for arabinose versus that of simple sugars. *xylX*, acetyltransferase; *xylT*, D-xylose proton-symporter; *araA*, L-arabinose isomerase; *araD*, L-ribulose-5-phosphate 4-epimerase; *araB*, L-ribulokinase; *araT*, arabinose-proton symporter; *araF*,  $\alpha$ -N-arabinofuranosidase; *araP*, disaccharide permease; *araR*, GntR family arabinose operon repressor; *ptk*, phosphoketolase.

and *sucP* is correlated with the cleavage of raffinose in our physiological study. The *aga* gene encodes an  $\alpha$ -galactosidase (EC 3.2.1.22) of 725 amino acids that belongs to the family GH 36 and is responsible for the hydrolysis of the  $\alpha$ -1,6-galactosyl bond

(characteristic of the  $\alpha$ -galactoside family). No signal peptide was observed in the *aga* gene sequence of strain A12, suggesting that Aga is probably an intracellular protein. The *sucP* gene encodes a 489-amino-acid GH with an  $\alpha$ -amylase domain that belongs to the family GH13. This sucrose phosphorylase (EC 2.4.1.7) is known to cleave sucrose into D-fructose and  $\alpha$ -D-glucose-1-phosphate. The SucP protein is relatively well conserved in LAB and has a protein similarity to *Leuconostoc mesenteroides*, *Streptococcus mutans*, and other *L. lactis* strains that is >80%.

*Lactococcus* genes relating to raffinose metabolism have only been previously described for the strains KF147 and *Lactococcus raffinolactis* ATCC 43920 (data not shown). A comparison of the raffinose gene clusters found in the three strains (A12, KF147, and *Lactococcus raffinolactis* ATCC 43920) suggests that the ability of strain A12 to use raffinose is associated with genes of xenologous origin (Fig. 4). The location of the raffinose gene cluster differs for strain A12: it is located on a plasmid in strain A12 but on the chromosome in strains KF147 (30, 45, 46) and ATCC 43920 (47), close to the *galKT* genes cluster involved in the galactose metabolism. This was confirmed by hybridization of the PFGE fingerprint using LL2138 as a probe (data not shown). There are also differences in the nucleotide sequences of *aga* genes: those in KF147 and ATCC 43920 strains are 93% identical, but the A12 *aga* gene sequence shares only 52% identity with those in the other strains



**FIG 4** Comparison of the organization of genes involved in raffinose metabolism in *Lactococcus raffinolactis* ATCC 43920, *Lactococcus lactis* subsp. *lactis* KF147, and *Lactococcus lactis* subsp. *lactis* A12. Arrows correspond to the different genes: sugar catabolism genes are indicated by hatched arrows, sugar transporter genes are represented in gray, regulatory genes are represented in black, and strain-specific or unknown genes are represented in white. Numbers indicate the percent identities of nucleotide sequences of common CDS identified.

(Fig. 4). The characteristic motifs of family GH36 are present in all strains, but some amino acids were found to be specific to strain A12 (see Fig. S1 in the supplemental material). The *sucP* genes of strains A12 and KF147 share only 65% identity, a value substantially less than that observed among *L. lactis* subsp. *lactis* strains (98% [data not shown]) or between *cremoris* and *lactis* subspecies (88% [data not shown]). Strain A12 also differed in that it contains neither the putative raffinose ABC transporter (encoded by *agaA*, *agaB*, and *agaC* in KF147 strain) nor the putative PTS system (encoded by *orf2*, *orf3*, and *orf4* in the ATCC 43920 strain). It was found to contain a particular transporter encoded by the *LL2138* gene. This gene encodes a 856-amino-acid protein, corresponding to the translational fusion of permease and kinase domains. The first 450 N-terminal amino acids display 61% identity (78% similarity) with a glycoside-pentoside-hexuronide (GPH): cation symporter of *Leuconostoc mesenteroides*, and the 406 C-terminal amino acids display 54% identity (70% similarity) to a galactokinase of *Lactococcus garviae*. Such fusion has not been described before and could indicate a previously undescribed raffinose transporter.

## DISCUSSION

Sourdough constitutes an ecological niche that is highly suitable for lactic acid bacteria (LAB), particularly *Lactobacillus*. In some cases, other LAB such as *Pediococcus*, *Leuconostoc*, or *Lactococcus* are found in traditional sourdough (18), but their functional role remains elusive. Here, *L. lactis* subsp. *lactis* strain A12 (isolated from French traditional sourdough) was genetically, physiologically, and metabolically characterized. We investigated its role and adaptation in sourdough, the unusual ecosystem in which it is found.

Genome comparison with the sequenced *L. lactis* subsp. *lactis* dairy strain IL1403 revealed that 23% of the A12 genome corresponds to strain-specific genes. Their annotation revealed that more than half of these genes coded proteins for unknown function or phages proteins and that others encoded proteins mainly involved in sugar metabolic pathways. Survival in sourdough is therefore likely to be achieved by its adaptations for metabolizing carbohydrates. The specific ability of strain A12 to metabolize plant sugars suggests that it is an autochthonous strain, brought into sourdough fermentation on the raw materials.

Our work suggests that trophic relationships in the sourdough ecosystem are managed by microbial interactions; microbial relationships related to maltose have been extensively described in sourdough (16, 18, 19). Here, we show new possible trophic interaction types. Strain A12 showed a slow fermentation rate of the sugars fructose, sucrose, and maltose, which are, along with glucose, the main soluble carbohydrate sources of sourdough, but a much higher fermentation rate of raffinose and arabinose. These two carbon sources are, at best, weakly metabolized by most lactobacilli and yeasts (6), revealing a competitive advantage for strain A12 in utilizing these sourdough carbon sources. Strain A12 was able to grow on some plant sugars (such as raffinose, melibiose, and gentiobiose) with growth rates similar to those observed on glucose. Its metabolism on raffinose and arabinose was particularly efficient, requiring between two and four times less energy for its own physiological maintenance than on fructose, glucose, and galactose. During raffinose fermentation by strain A12, by-products of sucrose and galactose were released into the medium and were thus available for other microorganisms to use. As well as trophic cooperation with other organisms, properties of strain

A12 can influence the final product. Strain A12 produces non-negligible amounts of diacetyl with a very low carbon requirement (48) which may enhance the flavor of bread. Like other *L. lactis* strains isolated from sourdough, strain A12 also produces bacteriocins that differ from nisin and lactococcin (49; data not shown). A putative bacteriocin gene, similar to *LLKF2208* in strain KF147, was detected in the A12 genome (89% sequence identity [data not shown]). Due to its capacity to ferment raffinose and other  $\alpha$ -galactosides such as stachyose and melibiose, strain A12 may enhance the digestibility of plant-based foods, such as legumes, which are particularly rich in  $\alpha$ -galactosides. These nondigestible oligosaccharides contribute to flatulence and other gastrointestinal disorders when they are fermented by intestinal gas-producing microorganisms in mammals lacking  $\alpha$ -galactosidase (50). Engineered strains of *L. lactis* that express a heterologous  $\alpha$ -galactosidase were tested for their ability to improve the digestibility of soymilk in rodents (51). Therefore, the strain A12 could act as a “digestibility enhancer” and may deliver a useful probiotic effect.

The unusual plasmid location of the raffinose gene cluster in strain A12 could facilitate the transfer of the  $\alpha$ -galactosidase gene (or the complete cluster) to other strains. This plasmid would be especially useful if it was conjugative, as is the case for the sole raffinose megaplasmid described in *Enterococcus faecium* (52). To investigate this further, isolation of the plasmid and characterization of its size and genetic content is required. Combined genetic, physiological, and transcriptomic analysis allowed the A12 raffinose pathway to be partly elucidated; this metabolic pathway has not previously been described in *L. lactis* or other LAB. We have identified an original transport system, encoded by *LL2138*, which contains both permease and kinase domains. This represents a new type of fusion for permease transport, although various combinations of two or three domains fusion were previously reported in the phosphotransferase system (PTS). These include fusion between PTS component proteins or between PTS and non-PTS proteins (for example, EIIA<sup>Glc</sup> and Na<sup>+</sup>/melibiose symporter protein in *Lactobacillus* subsp.) (53). Such a fusion could be related to a decrease in energetic requirements for cells; in the case of *LL2138*, it may contribute to the low maintenance cost observed during growth on raffinose. Transcriptomic analysis revealed the simultaneous metabolism of glucose, fructose, and galactose (by the Leloir pathway); this was confirmed by the transient accumulation of sucrose and galactose during raffinose metabolism. We showed activity of the raffinose pathway and hydrolysis of  $\alpha$ -1,6 linkage via the sucrose pathway. Further investigation is required to completely elucidate raffinose catabolism and demonstrate whether strain A12 has  $\beta$ -1,2 hydrolysis activity.

These findings constitute a preliminary study of specific carbohydrate metabolism probably involved in the survival of the strain A12 in sourdough and provide a basis for further exploration of this metabolism in such ecosystems. More generally, an integrated approach of functional genomics can help to explain the adaptation of a strain to a complex ecosystem and be used to assess its ecological impact.

## REFERENCES

1. Teuber M, Geis A. 2006. The genus *Lactococcus*, p 205–228. In Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (ed), *The prokaryotes*. Springer, New York, NY.
2. Gutiérrez-Méndez N, Rodríguez-Figueroa JC, González-Córdova AF, Nevárez-Moorillón GV, Rivera-Chavira B, Vallejo-Cordoba B. 2010.



- Phenotypic and genotypic characteristics of *Lactococcus lactis* strains isolated from different ecosystems. *Can. J. Microbiol.* 56:432–439.
3. Klijn N, Weerkamp AH, de Vos WM. 1995. Detection and characterization of lactose-utilizing *Lactococcus* spp. in natural ecosystems. *Appl. Environ. Microbiol.* 61:788–792.
  4. Nomura M, Kobayashi M, Narita T, Kimoto-Nira H, Okamoto T. 2006. Phenotypic and molecular characterization of *Lactococcus lactis* from milk and plants. *J. Appl. Microbiol.* 101:396–405.
  5. Passerini D, Beltramo C, Coddeville M, Quentin Y, Ritzenthaler P, Daveran-Mingot M-L, Le Bourgeois P. 2010. Genes but not genomes reveal bacterial domestication of *Lactococcus lactis*. *PLoS One* 5:e15306. doi:10.1371/journal.pone.0015306.
  6. Corsetti A, Lavermicocca P, Morea M, Baruzzi F, Tosti N, Gobetti M. 2001. Phenotypic and molecular identification and clustering of lactic acid bacteria and yeasts from wheat (species *Triticum durum* and *Triticum aestivum*) sourdoughs of Southern Italy. *Int. J. Food Microbiol.* 64:95–104.
  7. Rocha JM, Malcata FX. 1999. On the microbiological profile of traditional Portuguese sourdough. *J. Food Prot.* 62:1416–1429.
  8. Robert H, Gabriel V, Fontagné-Faucher C. 2009. Biodiversity of lactic acid bacteria in French wheat sourdough as determined by molecular characterization using species-specific PCR. *Int. J. Food Microbiol.* 135:53–59.
  9. Escalante A, Wachter C, Farrés A. 2001. Lactic acid bacterial diversity in the traditional Mexican fermented dough pozol as determined by 16S rDNA sequence analysis. *Int. J. Food Microbiol.* 64:21–31.
  10. De Vuyst L, Vrancken G, Ravys F, Rimaux T, Weckx S. 2009. Biodiversity, ecological determinants, and metabolic exploitation of sourdough microbiota. *Food Microbiol.* 26:666–675.
  11. Lattanzi A, Minervini F, Di Cagno R, Diviccaro A, Antonielli L, Cardinali G, Cappelle S, De Angelis M, Gobetti M. 2013. The lactic acid bacteria and yeast microbiota of eighteen sourdoughs used for the manufacture of traditional Italian sweet leavened baked goods. *Int. J. Food Microbiol.* 163:71–79.
  12. Weckx S, Van der Meulen R, Allemeersch J, Huys G, Vandamme P, Van Hummelen P, De Vuyst L. 2010. Community dynamics of bacteria in sourdough fermentations as revealed by their metatranscriptome. *Appl. Environ. Microbiol.* 76:5402–5408.
  13. Brandt MJ, Hammes WP, Gänzle MG. 2004. Effects of process parameters on growth and metabolism of *Lactobacillus sanfranciscensis* and *Candida humilis* during rye sourdough fermentation. *Eur. Food Res. Technol.* 218:333–338.
  14. Gänzle MG, Ehmann M, Hammes WP. 1998. Modeling of growth of *Lactobacillus sanfranciscensis* and *Candida milleri* in response to process parameters of sourdough fermentation. *Appl. Environ. Microbiol.* 64:2616.
  15. Hammes WP, Brandt MJ, Francis KL, Rosenheim J, Seitter MFH, Vogelmann SA. 2005. Microbial ecology of cereal fermentations. *Trends Food Sci. Technol.* 16:4–11.
  16. Vogelmann SA, Hertel C. 2011. Impact of ecological factors on the stability of microbial associations in sourdough fermentation. *Food Microbiol.* 28:583–589.
  17. Vrancken G, Rimaux T, Weckx S, Leroy F, De Vuyst L. 2011. Influence of temperature and backslipping time on the microbiota of a type I propagated laboratory wheat sourdough fermentation. *Appl. Environ. Microbiol.* 77:2716–2726.
  18. De Vuyst L, Neysens P. 2005. The sourdough microflora: biodiversity and metabolic interactions. *Trends Food Sci. Technol.* 16:43–56.
  19. Gobetti M, De Angelis M, Corsetti A, Di Cagno R. 2005. Biochemistry and physiology of sourdough lactic acid bacteria. *Trends Food Sci. Technol.* 16:57–69.
  20. Bolotin A, Wincker P, Mauger S, Jaillon O, Malarne K, Weissenbach J, Ehrlich SD, Sorokin A. 2001. The complete genome sequence of the lactic acid bacterium *Lactococcus lactis* subsp. *lactis* IL1403. *Genome Res.* 11:731–753.
  21. Chopin A, Chopin MC, Moillo-Batt A, Langella P. 1984. Two plasmid-determined restriction and modification systems in *Streptococcus lactis*. *Plasmid* 11:260–263.
  22. Dressaire C, Redon E, Milhem H, Besse P, Loubiere P, Coccain-Bousquet M. 2008. Growth rate regulated genes and their wide involvement in the *Lactococcus lactis* stress responses. *BMC Genomics* 9:343. doi:10.1186/1471-2164-9-343.
  23. Redon E, Loubière P, Coccain-Bousquet M. 2005. Role of mRNA stability during genome-wide adaptation of *Lactococcus lactis* to carbon starvation. *J. Biol. Chem.* 280:36380–36385.
  24. Bryson K. 2006. AGMIAL: implementing an annotation strategy for prokaryote genomes as a distributed system. *Nucleic Acids Res.* 34:3533–3545.
  25. Laing C, Buchanan C, Taboada EN, Zhang Y, Kropinski A, Villegas A, Thomas JE, Gannon VP. 2010. Pan-genome sequence analysis using Panseq: an online tool for the rapid analysis of core and accessory genomic regions. *BMC Bioinformatics* 11:461. doi:10.1186/1471-2105-11-461.
  26. Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B. 2009. The Carbohydrate-Active EnZymes database (CAZY): an expert resource for glycogenomics. *Nucleic Acids Res.* 37:D233–D238.
  27. Bochner BR. 2009. Global phenotypic characterization of bacteria. *FEMS Microbiol. Rev.* 33:191–205.
  28. Poolman B, Konings WN. 1988. Relation of growth of *Streptococcus lactis* and *Streptococcus cremoris* to amino acid transport. *J. Bacteriol.* 170:700.
  29. Tempest DW, Neijssel OM. 1984. The status of YATP and maintenance energy as biologically interpretable phenomena. *Annu. Rev. Microbiol.* 38:459–486.
  30. Siezen RJ, Bayjanov J, Renckens B, Wels M, van Hijum SAFT, Molenaar D, van Hylckama Vlieg JET. 2010. Complete genome sequence of *Lactococcus lactis* subsp. *lactis* KF147, a plant-associated lactic acid bacterium. *J. Bacteriol.* 192:2649–2650.
  31. Kato H, Shiwa Y, Oshima K, Machii M, Araya-Kojima T, Zendo T, Shimizu-Kadota M, Hattori M, Sonomoto K, Yoshikawa H. 2012. Complete genome sequence of *Lactococcus lactis* IO-1, a lactic acid bacterium that utilizes xylose and produces high levels of L-lactic acid. *J. Bacteriol.* 194:2102–2103.
  32. Dumville J, Fry S. 2003. Gentiobiose: a novel oligosaccharin in ripening tomato fruit. *Planta* 216:484–495.
  33. Zykowska A, Thibault J-F, Ralet M-C. 2007. Organization of pectic arabinan and galactan side chains in association with cellulose microfibrils in primary cell walls and related models envisaged. *J. Exp. Bot.* 58:1795–1802.
  34. Trugo LC, Farah A, Cabral L. 1995. Oligosaccharide distribution in Brazilian soya bean cultivars. *Food Chem.* 52:385–387.
  35. Gänzle MG, Follador R. 2012. Metabolism of oligosaccharides and starch in lactobacilli: a review. *Front. Microbiol.* 3:340.
  36. Garrigues C, Loubiere P, Lindley ND, Coccain-Bousquet M. 1997. Control of the shift from homolactic acid to mixed-acid fermentation in *Lactococcus lactis*: predominant role of the NADH/NAD<sup>+</sup> ratio. *J. Bacteriol.* 179:5282–5287.
  37. Thomas TD, Turner KW, Crow VL. 1980. Galactose fermentation by *Streptococcus lactis* and *Streptococcus cremoris*: pathways, products, and regulation. *J. Bacteriol.* 144:672–682.
  38. Coda R, Nionelli L, Rizzello CG, De Angelis M, Tossut P, Gobetti M. 2010. Spelt and emmer flours: characterization of the lactic acid bacteria microbiota and selection of mixed starters for bread making. *J. Appl. Microbiol.* 108:925–935.
  39. Corsetti A, Settanni L. 2007. Lactobacilli in sourdough fermentation. *Food Res. Int.* 40:539–558.
  40. Benthin S, Nielsen J, Villadsen J. 1994. Galactose expulsion during lactose metabolism in *Lactococcus lactis* subsp. *cremoris* FD1 due to dephosphorylation of intracellular galactose 6-phosphate. *Appl. Environ. Microbiol.* 60:1254–1259.
  41. Hickey MW, Hillier AJ, Jago GR. 1986. Transport and metabolism of lactose, glucose, and galactose in homofermentative lactobacilli. *Appl. Environ. Microbiol.* 51:825–831.
  42. Hutkins RW, Ponne C. 1991. Lactose uptake driven by galactose efflux in *Streptococcus thermophilus*: evidence for a galactose-lactose antiporter. *Appl. Environ. Microbiol.* 57:941–944.
  43. Grossiord BP, Luesink EJ, Vaughan EE, Arnaud A, de Vos WM. 2003. Characterization, expression, and mutation of the *Lactococcus lactis* galP-MKTE genes, involved in galactose utilization via the Leloir pathway. *J. Bacteriol.* 185:870–878.
  44. Barrière C, Veiga-da-Cunha M, Pons N, Guédon E, van Hijum SAFT, Kok J, Kuipers OP, Ehrlich DS, Renault P. 2005. Fructose utilization in *Lactococcus lactis* as a model for low-GC gram-positive bacteria: its regulator, signal, and DNA-binding site. *J. Bacteriol.* 187:3752–3761.
  45. Siezen RJ, Bayjanov JR, Felis GE, van der Sijde MR, Starrenburg M, Molenaar D, Wels M, van Hijum SAFT, van Hylckama Vlieg JET. 2011. Genome-scale diversity and niche adaptation analysis of *Lactococcus lactis*



- by comparative genome hybridization using multi-strain arrays. *Microb. Biotechnol.* 4:383–402.
46. Machielsen R, Siezen RJ, van Hijum SAFT, van Hylckama Vlieg JET. 2011. Molecular description and industrial potential of Tn6098 conjugative transfer conferring alpha-galactoside metabolism in *Lactococcus lactis*. *Appl. Environ. Microbiol.* 77:555–563.
  47. Boucher I, Vadeboncoeur C, Moineau S. 2003. Characterization of genes involved in the metabolism of  $\alpha$ -galactosides by *Lactococcus raffinolactis*. *Appl. Environ. Microbiol.* 69:4049–4056.
  48. Passerini D, Laroute V, Coddeville M, Le Bourgeois P, Loubière P, Ritzenthaler P, Cocaign-Bousquet M, Daveran-Mingot M-L. 2013. New insights into *Lactococcus lactis* diacetyl- and acetoin-producing strains isolated from diverse origins. *Int. J. Food Microbiol.* 160:329–336.
  49. Corsetti A, Settanni L, van Sinderen D. 2004. Characterization of bacteriocin-like inhibitory substances (BLIS) from sourdough lactic acid bacteria and evaluation of their in vitro and in situ activity. *J. Appl. Microbiol.* 96:521–534.
  50. Quigley ME, Hudson GJ, Englyst HN. 1999. Determination of resistant short-chain carbohydrates (non-digestible oligosaccharides) using gas-liquid chromatography. *Food Chem.* 65:381–390.
  51. LeBlanc JG, Silvestroni A, Connes C, Juillard V, de Giori GS, Piard J-C, Sesma F. 2004. Reduction of non-digestible oligosaccharides in soymilk: application of engineered lactic acid bacteria that produce alpha-galactosidase. *Genet. Mol. Res.* 3:432–440.
  52. Zhang X, Vrijenhoek JEP, Bonten MJM, Willems RJJ, van Schaik W. 2011. A genetic element present on megaplasms allows *Enterococcus faecium* to use raffinose as carbon source. *Environ. Microbiol.* 13:518–528.
  53. Barabote RD, Saier MH, Jr. 2005. Comparative genomic analyses of the bacterial phosphotransferase system. *Microbiol. Mol. Biol. Rev.* 69:608–634.