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Genome Sequence of a Food Spoilage Lactic Acid Bacterium, *Leuconostoc gasicomitatum* LMG 18811^T, in Association with Specific Spoilage Reactions$^{\nabla}$†

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***Leuconostoc gasicomitatum* is a psychrotrophic lactic acid bacterium causing spoilage of cold-stored, modified-atmosphere-packaged (MAP), nutrient-rich foods. Its role has been verified by challenge tests in gas and slime formation, development of pungent acidic and buttery off odors, and greening of beef. MAP meats have especially been prone to *L. gasicomitatum* spoilage. In addition, spoilage of vacuum-packaged vegetable sausages and marinated herring has been reported. The genomic sequencing project of *L. gasicomitatum* LMG 18811^T was prompted by a need to understand the growth and spoilage potentials of *L. gasicomitatum*, to study its phylogeny, and to be able to knock out and overexpress the genes. Comparative genomic analysis was done within *L. gasicomitatum* LMG 18811^T and the three fully assembled *Leuconostoc* genomes (those of *Leuconostoc mesenteroides*, *Leuconostoc citreum*, and *Leuconostoc kimchii*) available. The genome of *L. gasicomitatum* LMG 18811^T is plasmid-free and contains a 1,954,080-bp circular chromosome with an average GC content of 36.7%. It includes genes for the phosphoketolase pathway and alternative pathways for pyruvate utilization. As interesting features associated with the growth and spoilage potential, LMG 18811^T possesses utilization strategies for ribose, external nucleotides, nucleosides, and nucleobases and it has a functional electron transport chain requiring only externally supplied heme for respiration. In respect of the documented specific spoilage reactions, the pathways/genes associated with a buttery off odor, meat greening, and slime formation were recognized. Unexpectedly, genes associated with platelet binding and collagen adhesion were detected, but their functionality and role in food spoilage and processing environment contamination need further study.**

Industrially manufactured food must have a reasonably long shelf life due to the production chain, involving logistics and retail sale before the domestic storage and consumption of a product. CO_2 in modified-atmosphere packaging (MAP) and refrigerated temperatures are two main extrinsic hurdles used by the food industry. They create negative selective pressure to aerobic Gram-negative spoilage bacteria. Under these circumstances, psychrotrophic, i.e., cold-tolerant, lactic acid bacteria (LAB) prevail in nutrient-rich foods, such as meat (4, 18, 21). Compared to the aerobic Gram-negative spoilage bacteria, the stationary growth phase associated with the production of sensory changes is reached more slowly by spoilage LAB. In addition, the end products of LAB carbohydrate fermentation are not sensed as unpleasant, as in protein or amino acid degradation. Therefore, the shift from aerobic Gram-negative bacteria to LAB is preferred.

Despite the generally moderate role of psychrotrophic LAB in spoilage, they are still spoilage organisms. The growth rate of psychrotrophic LAB can usually be predicted and the shelf life can be estimated with adequate accuracy. However, some psychrotrophic LAB may cause considerable hardship to the food industry. *Leuconostoc gasicomitatum* (3) is a LAB that was first encountered causing a spoilage problem of MAP, tomato-marinated, raw broiler meat strips in 1997. The packages already showed clear bulging due to CO_2 formation in 5 days, even though the manufacturer-defined shelf life was expected to be 14 days. Since the first spoilage problem, *L. gasicomitatum* has been shown to form slime and CO_2 in acetic acid-preserved herring (24), cause greening and off odor to value-added MAP, raw beef steaks (42, 45), and cause slimy spoilage and bulging of cooked vegetable sausages packaged under vacuum (45). In addition, this species has been documented to prevail in MAP, marinated broiler meat strips (38) and minced meat (29). Table 1 shows reported *L. gasicomitatum* spoilage with descriptive spoilage characteristics verified in food challenge tests. However, none of these have yet been confirmed through expression or other types of molecular analyses in food *in situ*.

The genomic sequencing project of *L. gasicomitatum* LMG 18811^T was prompted by a need to understand the growth and spoilage potentials of *L. gasicomitatum*, to study its phylogeny, and to be able to knock out and overexpress genes. This is the first complete genome sequence of a psychrotrophic food spoilage LAB, and it is presented in this study with particular emphasis on the food spoilage capabilities. Comparative genomic analysis was also carried out with the three other

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Leuconostoc species with completely assembled genomes, *L. citreum* KM20 (20), *L. mesenteroides* ATCC 8293^T (25), and *L. kimchii* IMSNU11154 (30). *L. gasicomitatum* diverges from the three other leuconostocs, while it has not been used as a starter culture in food fermentations.

MATERIALS AND METHODS

Origin, culturing, and maintenance of LMG 18811^T. *L. gasicomitatum* LMG 18811^T originates from a study identifying a spoilage LAB population associated with the rapid gaseous spoilage of MAP, marinated broiler meat (3). In a follow-up study (44) dealing with isolates recovered from foods from 1997 to 2007, strain LMG 18811^T was shown to belong to genotype 4, which has repeatedly been detected in the products of two large-scale poultry processing plants.

L. mesenteroides type strain DSM 20343^T (ATCC 8293^T) was also used in the growth test. Unless otherwise stated, cultures were grown aerobically in MRS broth (Difco Laboratories, Detroit, MI) or anaerobically on MRS agar (Oxoid, Basingstoke, United Kingdom) at 25°C and were maintained at −72°C in MRS broth.

Phenotypic analyses and growth in hemin-containing medium. Table 1 shows the main carbon sources associated with the foods affected by *L. gasicomitatum* spoilage. To depict the variety of food-associated carbon and nitrogen sources in more detail and complement the previous analyses (3), phenotype MicroArrays PM1 to PM20 were purchased from Biolog Inc. (Hayward, CA). Table S1 in the supplemental material lists the most relevant substrates for this study.

L. gasicomitatum LMG 18811^T and *L. mesenteroides* DSM 20343^T (ATCC 8293^T) were grown (three experimental replicates) in MRS broth with and without hemin (in dimethyl sulfoxide [DMSO]; Sigma-Aldrich) supplementation (2 μg/ml). In medium without hemin, an equivalent amount of DMSO was added. Growth was tested under aerobic, anaerobic, and high-oxygen MAP (20% CO_2 and 80% O_2) atmospheres. Cultures grown under aerobic and high-oxygen MAP atmospheres were shaken at 250 rpm, and cultures grown under anaerobic conditions were left stationary. All cultures were grown at 25°C for 48 h, and growth was measured as the optical density (OD) at 600 nm. Microscopy was done to check cell size and culture density.

DNA isolation, sequencing, and assembly. DNA was isolated using a modified (2) method of Pitcher et al. (33), and the genomic DNA was mechanically sheared with a needle. Fosmid libraries were constructed in Copy Control pCC1FOS vector (Epicentre). Plasmid libraries of several insert sizes (2, 4, 6, and 10 kb) were constructed in the pZEro 2 vector (Invitrogen). Fosmid ends and plasmids were sequenced using an ABI 3730 sequencer and BigDye chemistry (Applied Biosystems). In total, 41,549 reads (ca. 8 times) were obtained. Contig order and gaps were filled by PCR from genomic DNA and direct sequencing of the fragments, linker PCR, and using *in vitro* Mu transposition of appropriate clones. Genome sequences were quality checked using the Phred program and assembled with the Phrap program, followed by editing by the Gap4 program in the Staden package (36).

Prediction of genes and annotation. The completed *L. gasicomitatum* sequence was annotated using the Manatee program (http://manatee.sourceforge.net). Open reading frame (ORF) sequences were determined using the EasyGene (28) and Glimmer (11) programs, and the predicted ORFs were also manually reviewed and alterations were made when appropriate on the basis of the presence of potential ribosomal binding sites, sequence alignments, and available literature data. Details about bioinformatic annotations are presented in File S1 in the supplemental material.

RESULTS

Genome and general aspects related to growth in food. Table 2 shows the main properties of the genome of *L. gasicomitatum* LMG 18811^T (GenBank accession no. FN822744). The genome is plasmid free and contains a 1,954,080-bp circular chromosome with an average GC content of 36.7%. Two prophages which are not located within the operons of interest in respect to spoilage reactions were detected. Figure 1 shows the genome map of *L. gasicomitatum* LMG 18811^T colored according to the Automated Resource Classifier (ARC) classification based on the gene annotation. The proteome is presented

TABLE 1. Reported *Leuconostoc gasicomitatum* spoilage with spoilage characteristics and challenge test outcomes

Type of food	Food product	Package type	Spoilage characteristics			Challenge outcome[a]	Reference
			Sensory defects	Main carbohydrate(s)	Spoilage-associated end products		
Fresh meat	Marinated broiler meat	Modified atmosphere (80% CO_2, 20% N_2)	Gaseous spoilage, pungent off odor	Glucose from marinade, meat-derived glucose and ribose	CO_2, acetic acid	Not challenged	Björkroth et al. (3)
	Moisture enhanced and marinated beef	Modified atmosphere (70% O_2, 30% CO_2)	Greening, buttery off odor	Glucose from marinade, meat-derived glucose and ribose	H_2O_2, diacetyl	Beef challenged, manifested greening and buttery off odor	Vihavainen and Björkroth (42)
	Marinated and unprocessed beef, pork, and broiler	Modified atmosphere (0-70% O_2, 20-80% CO_2, 20-80% N_2)	Some packages with buttery and other off odors	Glucose from marinade, meat-derived glucose and ribose	Diacetyl, other volatiles	Not challenged	Vihavainen and Björkroth (44)
Fish product	Acetic acid-preserved herring	Plastic container	Slime, gaseous spoilage	Sucrose from marinade	Dextran, CO_2	Herring challenged, showed gas formation verified to be CO_2	Lyhs et al. (24)
Other	Cooked vegetable sausages	Vacuum	Slime, gaseous spoilage and sour off odor	Glucose, sucrose	Dextran, CO_2, lactic acid, acetic acid	Vegetable sausages challenged, showed gas and slime formation	Vihavainen et al. (45)

[a] In a challenge test, the food in question was spiked with *L. gasicomitatum* in a controlled experiment published in the indicated reference.

TABLE 2. Main properties of the genome of *Leuconostoc gasicomitatum* 18811^T

Main property	Value
Genome size (bp)	1,954,080
No. of protein-encoding genes	1,913
No. (%) of genes with a functional annotation[a]	1,566 (81)
No. of putative pseudogenes	12
No. of plasmids	0
No. of rRNA operons	4
No. of tRNA genes	67 (+1 pseudo)
No. of tmRNAs	1
% GC content	37
% coding efficiency	87
Avg gene size (bp)	890

[a] Genes not containing the word "hypothetical" in the annotation.

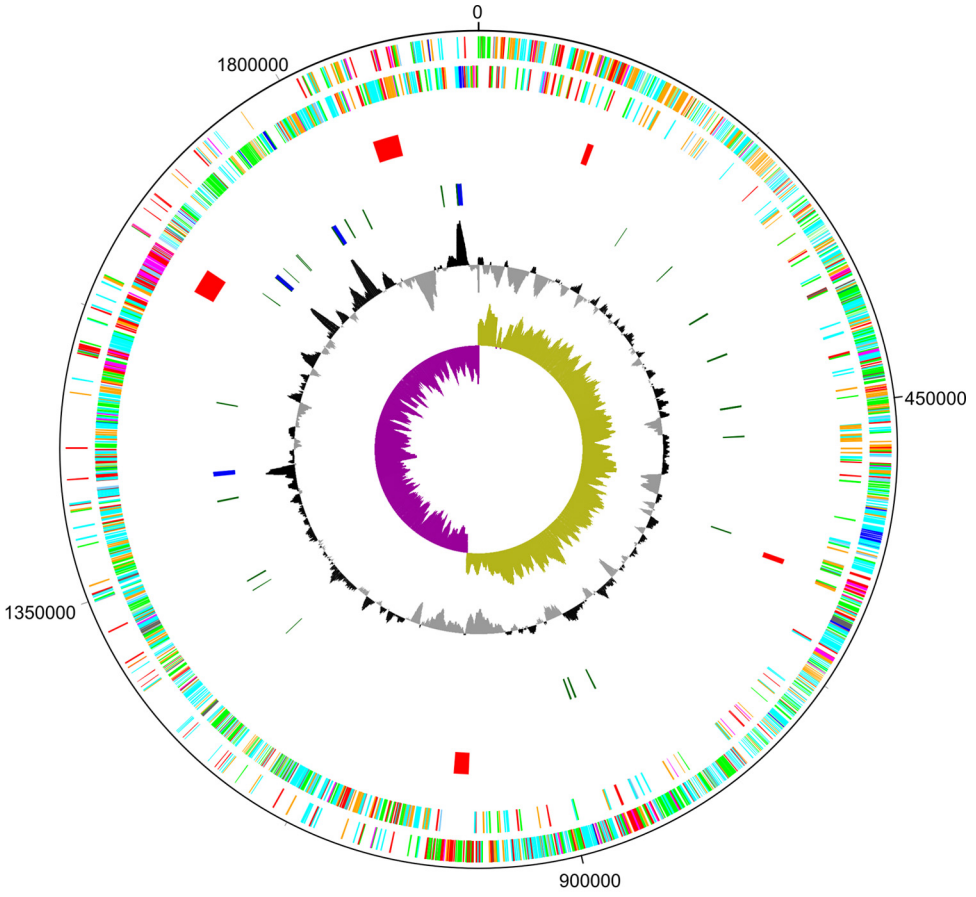
according to clusters of orthologous groups categories in Table S2 in the supplemental material.

Figure 2 shows a Venn diagram comparing the genome of *L. gasicomitatum* LMG 18811^T to the three other publicly available *Leuconostoc* genomes: those of *L. citreum* KM20 (20), *L. mesenteroides* ATCC 8293^T (25), and *L. kimchii* IMSNU11154 (30). The unique genes for each of the four species are listed in Table S3 in the supplemental material. Like the other leuconostocs, LMG 18811^T has a wide set of genes involved in the uptake of sugars, citrate, and amino acids. The genome includes the genes for the phosphoketolase pathway and three alternative pathways for pyruvate utilization by lactate dehydrogenase, pyruvate dehydrogenase, and α-acetolactate synthase. Compared to *L. mesenteroides* ATCC 8293^T, it has fewer pathways involved in the biosynthesis of amino acids, vitamins, and cofactors (see Table S4 in the supplemental material). Pyruvate utilization is a hub for many of the spoilage reactions, and Table 3 summarizes the enzymes and coding genes attributed to the formation of spoilage compounds.

Utilization of ribose. The majority of LAB does not utilize ribose, which is a pentose of interest in plant- and meat-derived foods. Within the genus *Leuconostoc*, the species *L. carnosum*, *L. gasicomitatum*, *L. gelidum*, *L. inhae*, and *L. kimchii*, which belong to the same 16S rRNA gene-based phylogenetic branch, can utilize ribose (14). Most ribose-utilizing LAB transport it via an H^+ symporter, e.g., RbsU of *L. sakei* (37). *L. gasicomitatum* LMG 18811^T and *L. kimchii* IMSNU11154 have the ribose ABC transporter RbsDACB (46) (LEGAS_0026 to LEGAS_0029 in *L. gasicomitatum*) generally more common in the bacterial domain. In the genomes of *L. mesenteroides* ATCC 8293^T and *L. citreum* KM20, genes do not exist either for the ribose ABC transporter or for the RbsU symporter. In the pentose phosphate pathway, the enzyme ribose 5-phosphate isomerase A (RpiA, EC 5.3.1.6) plays an important role in the branching between the pentose phosphate pathway and the nucleotide pathways. All four sequenced *Leuconostoc* genomes contain multiple *rpiA* genes (LEGAS_0278, LEGAS_0031, and LEGAS_1232 in *L. gasicomitatum*), but the function and expression of them are unknown.

Salvage and utilization of nucleotides, nucleosides, and nucleobases. All four fully assembled *Leuconostoc* genomes show the ability for *de novo* synthesis of purines and pyrimidines. They do not have the genes encoding nucleotide phosphorylases or phosphopentomutase (*deoB*) for nucleotide salvage, nor do they have deoxyriboaldolase (*deoC*) for degradation of the pentose moiety of the nucleosides. They do, however, have *ntd* (LEGAS_0737), encoding *N*-deoxyribosyltransferase, which catalyzes the exchange reaction of the nucleobase of deoxyribonucleoside to salvage the deoxyribose moiety and convert between the different deoxyribonucleosides (8). *L. gasicomitatum* LMG 18811^T can use external nucleotides and nucleosides as both a carbon and an energy source; and the amino group of nucleotides and free nucleobases can be utilized as a nitrogen source (see Table S1 in the supplemental material), or they can be salvaged and rescued for nucleotide synthesis. Both guanosine and xanthine, but not adenosine or cytidine, can be used as nitrogen sources, whereas inosine, uridine, and adenosine, but not thymidine, can be utilized as carbon sources (see Table S1 in the supplemental material). 2′-Deoxyadenosine and 2-deoxyribose can also be utilized as carbon sources (see Table S1 in the supplemental material), although it is not clear how. The *deoQKPX* genes, for the uptake and utilization of 2-deoxyribose in other species (9), are not found in *Leuconostoc* genomes. Instead, genes for nucleotidases dephosphorylating the nucleotides to the corresponding nucleoside exist (*L. gasicomitatum* LEGAS_1431 and LEGAS_0848), as do genes for a nucleoside permease, *nupC* (LEGAS_0024), and *rnsACD*, encoding a nucleoside ABC transporter (LEGAS_1844 to LEGAS_1847) with two separate nucleoside-binding subunits, *rnsB1* and *rnsB2* (LEGAS_1460 and LEGAS_1179), enabling transportation of nucleosides into the cell. Genes for three transporters of nucleobases exist: a uracil transporter, *pyrP* (LEGAS_1771), and two guanine/hypoxanthine transporters, *pbuG1* and *pbuG2* (LEGAS_1320 and LEGAS_0450). Ribonucleosides can also be hydrolased by the ribonucleoside hydrolases (*rihA1*, *rihA2*, *rihB*, and *rihC*, corresponding to LEGAS_0022, LEGAS_1534, LEGAS_0456, and LEGAS_0023, respectively), to generate free nucleobases and ribose. The ribose formed can subsequently be fed into the pentose phosphate pathway. Figure 3 shows salvage and catabolic pathways for nucleosides in *L. gasicomitatum* LMG 18811^T.

Citrate metabolism and buttery off odor. *L. gasicomitatum* 18811^T can utilize citrate. It has a *citIMCDEFGXRP* citrate locus (LEGAS_0211 to LEGAS_0219), which encodes the enzymes necessary for the uptake and conversion of citrate to pyruvate. The genes for the diacetyl/acetoin pathway are present. This pathway consumes pyruvate, forming α-acetolactate by the catabolic α-acetolactate synthase (LEGAS_0526). Under aerobic circumstances, α-acetolactate may be decarboxylated to acetoin, either via diacetyl, by a nonenzymatic decarboxylative oxidation followed by an NAD(P)H-dependent reduction to acetoin by diacetyl reductase (LEGAS_0209, LEGAS_1299), or directly to acetoin by acetolactate decarboxylase (LEGAS_1346) (Fig. 1). Acetoin and diacetyl can also be formed from the amino acid aspartate in the presence of α-ketoglutarate (22), but leuconostocs do not have the glutamate dehydrogenase, which can convert glutamate to α-ketoglutarate as in a few other LAB species (41). Instead, *L. gasicomitatum* 18811^T has transporters for both aspartate (LEGAS_1791) and α-ketoglutarate (LEGAS_1138), and in the presence of α-ketoglutarate, the aspartate aminotransferase (LEGAS_1168) may convert aspartate to oxaloacetate and glutamate. The oxaloacetate can enter the diacetyl/acetoin pathway via the last



Color	ARC Classification	No. of Genes
	Cell Division	20
	Hypothetical Proteins	352
	Information (Replication, Transcription & Translation)	362
	Translocation and Secretion	21
	Metabolism	630
	General Function	75
	Stress	18
	Signal and Communication	22
	Unclassified	91
	Cell Wall, Cell Membrane and Transport	322

FIG. 1. Genome map of *Leuconostoc gasicomitatum* LMG 18811^T with the genes colored according to the ARC classification on the basis of the gene annotation. The two outer rings denote genes on the forward and reverse strands, respectively. The following four rings inwards represent the positions of complete and partial prophages (red), the positions of rRNA (blue) and tRNA (green) genes, percent GC plot (gray), and GC skew ([G − C]/[G + C]), respectively.

enzyme of the citrate pathway oxaloacetate decarboxylase (LEGAS_0212). Acetoin can in turn be converted to 2,3-butanediol by 2,3-butanediol dehydrogenase (LEGAS_1018).

Enhanced growth when heme and O_2 are available and the genes associated with the electron transport chain. All four *Leuconostoc* genomes possess genes encoding cytochrome *bd* terminal oxidase and for synthesizing menaquinone. However, unlike *L. mesenteroides* DSM 20343^T, *L. gasicomitatum* LMG 18811^T has a functional electron transport requiring only externally supplied heme for respiration. The presence of heme increased the produced biomass by ~50% under aerobic and high-oxygen MAP cultivation, while the addition of heme had no effect on anaerobically growing cells (Fig. 4). Cell size did not alter between the experiments. The nonfunctional cytochromes of *L. mesenteroides* ATCC 8293^T were also reported by Brooijmans et al. (5). The *L. gasicomitatum* LMG 18811^T genome encodes a cytochrome *bd* terminal oxidase (LEGAS_1333 and LEGAS_1334), an NADH dehydrogenase (LEGAS_1702), and the entity related to the ability to synthesize menaquinone in eight enzymatic steps

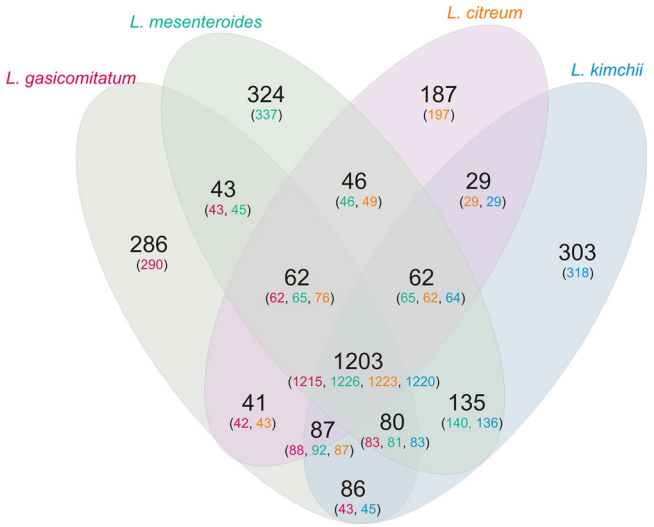


FIG. 2. Venn diagram showing the distribution of orthologous relationships of genes between four *Leuconostoc* species.

(LEGAS_0426, LEGAS_0912 to LEGAS_0915, LEGAS_1879 and LEGAS_1880, and LEGAS_1895).

Genes associated with H_2O_2 production and meat greening. Of the enzymes known to generate hydrogen peroxide in LAB, *L. gasicomitatum* LMG 18811^T has only the genes for pyruvate oxidase (*poxB*, LEGAS_1053), NADH oxidase (*nox*, LEGAS_0926), and two unknown NADH:flavin oxidoreductase/NADH oxidases (LEGAS_0056 and LEGAS_1753). The NADH oxidases likely produce water and not hydrogen peroxide as an end product. For protection against peroxide, a capability for three different peroxidases, thioredoxin peroxidase (*tpx*, LEGAS_0306), glutathione peroxidase (*bsaA*, LEGAS_1017), and heme-containing Dyp-type peroxidase (LEGAS_1694), exists, whereas no potential for catalase production was detected.

Genes encoding EPSs, adhesion, and mucus binding. *L. gasicomitatum* LMG 18811^T has genes for two dextransucrases: *epsA* (LEGAS_0699) is part of a large exopolysaccharide (EPS) cluster, while *dsrA* (LEGAS_1012) is located as a single gene in the chromosome. Three genes encoding proteins containing putative LPXTG anchors were detected. LEGAS_0414 encodes a putative mucus binding protein with unknown function. Orthologs were also detected in the genomes of *L. mesenteroides* and *L. citreum* (plasmid encoded). LEGAS_0537 encodes Srr-2, a serine-rich protein. The serine-rich domain shows homology to the platelet-binding protein GspB of streptococci (34), but since it is missing a large portion of the nonrepeat region, the function cannot be predicted. The genes *secY2*, *asp1* to *asp3*, *secA2*, *nss*, and *gftAB* were located in the same locus with LEGAS_0537. They all are required for the secretion and glycosylation of Srr-2 (39). LEGAS_1063 is part of an intercellular adhesion locus (*ica*) ABC, where it encodes the putative collagen adhesion protein IcaC. The *ica* locus has been shown to be required for biofilm formation in *Staphylococcus aureus* (10) and among LAB is otherwise found in only a few *Lactococcus* species (35). No orthologs of LEGAS_0537 or LEGAS_1063 are found in other *Leuconostoc* genomes (see Table S3 in the supplemental material).

DISCUSSION

An unexpected story related to a novel food spoilage organism in Finland has been seen over the last 14 years. The first incident was considered to be related to the use of a specific tomato-based marinade (3), but the following years have shown that this species is persisting (44) and causing spoilage in many types of cold-stored MAP foods of several manufacturers in Finland.

An ability to grow well on MAP meat with no added carbohydrates (29, 44) is interesting, while this species is not able to obtain energy from proteinaceous substrates, lactate, or fatty acids. It has the genes required for energetic catabolism of nucleosides, and it also grows well on adenosine and inosine. Nucleosides, particularly inosine, are abundant in meat, and if glucose is exhausted, they provide an alternative source of energy. Differing from *Lactobacillus sakei* 23K, which is considered a meat ecosystem-adapted LAB (6, 7), *L. gasicomitatum* LMG 18811^T cannot release amino acids from meat proteins or utilize arginine as an energy source. Bearing food safety aspects in mind, the genome analysis confirms the high-pressure liquid chromatography determinations (27) that the meat-derived amino acids are not decarboxylated as biogenic

TABLE 3. Enzymes and coding genes in genome of *L. gasicomitatum* LMG 18811^T involved in formation of spoilage compounds

Spoilage compound	Enzyme	Gene(s)	Locus tag
Acetate	Acetate kinase	*ackA1*, *ackA2*	LEGAS_1085 LEGAS_1559
	Citrate lyase complex	*citCDEF*	LEGAS_0213 to LEGAS_0216
	N-Acetylglucosamine-6-phosphate deacetylase	*nagA*	LEGAS_0472
CO_2	Acetolactate decarboxylase	*alsD*	LEGAS_1346
	Oxaloacetate decarboxylase	*citM*	LEGAS_0212
	6-Phosphogluconate dehydrogenase	*gnd1*, *gnd2*	LEGAS_1343 LEGAS_0931
	Pyruvate dehydrogenase complex	*pdhABCD*	LEGAS_1381 to LEGAS_1378
	Pyruvate oxidase	*poxB*	LEGAS_1053
Diacetyl	Acetolactate synthase	*alsS*	LEGAS_0526
H_2O_2	Pyruvate oxidase	*poxB*	LEGAS_1053
Slime	Dextransucrase	*dsrA*	LEGAS_1012
	Protein cluster related to formation of an EPS with unknown structure	*eps* gene cluster	LEGAS_0699 to LEGAS_0710

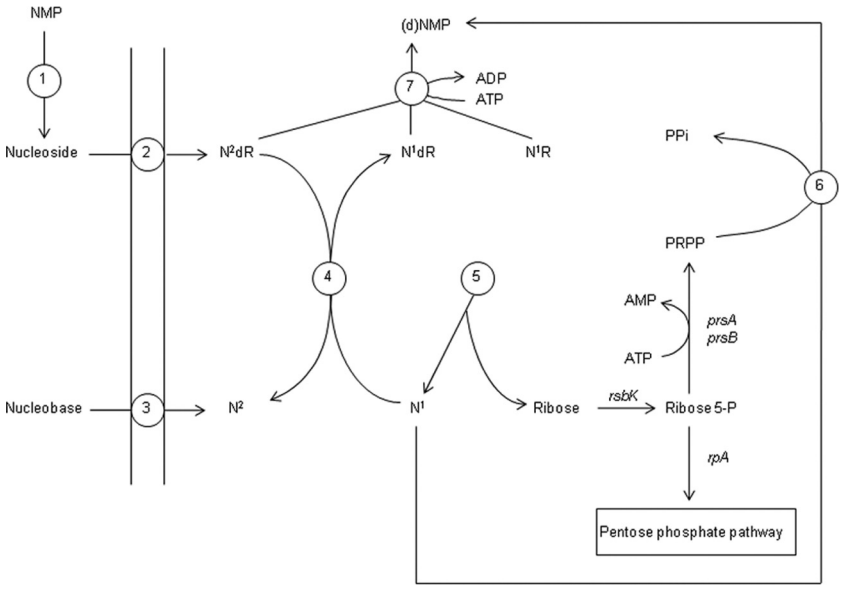


FIG. 3. Salvage and catabolic pathways for nucleosides in *L. gasicomitatum*. External nucleotides are dephosphorylated by extracellular nucleotidases (step 1) (LEGAS_1431, LEGAS_0848) and are then transported into the cell by a nucleoside permease or nucleoside ABC transporter (step 2) (LEGAS_0024, LEGAS_1844–1846, LEGAS_1460, LEGAS_1179); there are also separate nucleobase transporters (step 3) (LEGAS_1771, LEGAS_1320, LEGAS_0450). Nucleosides entering the cell can be directly phosphorylated by the corresponding kinase (step 7) (LEGAS_0367, LEGAS_0687, LEGAS_0374, LEGAS_0393, LEGAS_0602, LEGAS_1125, LEGAS_1345, LEGAS_1390, LEGAS_1662, LEGAS_1710), or deoxyribonucleosides can first exchange nucleobases by a *N*-deoxyribosyltransferase (step 4) (LEGAS_0737), ribonucleosides can be cleaved to free nucleobase and ribose by ribonucleoside hydrolases (step 5) (LEGAS_0022, LEGAS_1534, LEGAS_0456, LEGAS_0023), and the free nucleobase can be salvaged either by the *N*-deoxyribosyltransferase (step 4) or by one of the phosphoribosyltransferases (step 6) (LEGAS_0433, LEGAS_1197, LEGAS_0720, LEGAS_0874, LEGAS_1261, LEGAS_1902). The ribose formed by the hydrolysis of ribonucleosides can be either salvaged by the phosphoribosyltransferase (step 6) or used as an energy source by feeding it to the pentose phosphate pathway. NMP, nucleoside monophosphate; N^1dR, a deoxyribonucleoside; N^2dR, another deoxyribonucleoside; N^1R, a nucleoside; PRPP, phosphoribosyl pyrophosphate.

amines. Few leuconostocs (17, 32) are capable of forming biogenic amine from tyrosine.

Related to the metabolism of pentoses or citrate, *L. gasicomitatum* LMG 18811^T has the central genes involved in the pyruvate-dissipating routes leading to the formation of acetate and diacetyl. Like the other leuconostocs (1, 47), *L. gasicomitatum* is likely to metabolize pyruvate to acetate or diacetyl when intracellular pyruvate accumulates, for example, when oxygen, citrate, or fructose is available. Presence of oxygen has been reflected in the type of off odor (Table 1). The buttery off

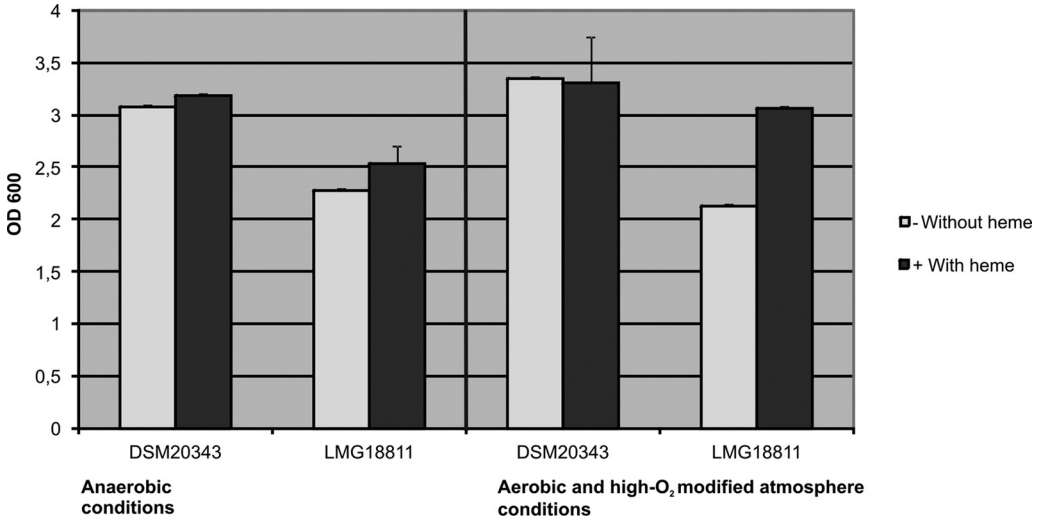


FIG. 4. Growth of *Leuconostoc gasicomitatum* LMG 18811^T and *Leuconostoc mesenteroides* DSM 20343^T in MRS broth with and without hemin supplementation (2 μg/ml) under aerobic and anaerobic conditions. Error bars show the differences obtained between three tests. Under a modified atmosphere containing 20% CO_2 and 80% oxygen, growth was similar to growth under aerobic conditions.

odor marking diacetyl has been associated with products packaged under an oxygen-containing modified atmosphere (MA) or containers with an aerobic atmosphere (24, 42), whereas the pungent acidic odor has occurred in foods packaged under oxygen-deprived atmospheres (3, 45). Notably, some *Lactobacillus* and *Lactococcus* strains can also form diacetyl via catabolism of aspartate (19, 22), an amino acid present in meat. The genes required for aspartate catabolism are present in *L. gasicomitatum* 18811^T, but no gene exists for glutamate dehydrogenase, considered important for the formation of α-ketoglutarate, the amino group acceptor essential for the pathway (40). Instead, *L. gasicomitatum* LMG 18811^T encodes an α-ketoglutarate transporter, suggesting that exogenous α-ketoglutarate may be exploited. However, whether *L. gasicomitatum* LMG 18811^T produces diacetyl via aspartate catabolism and if this occurs in the meat ecosystem must be further studied.

Vihavainen and Björkroth proposed (42) that H_2O_2 produced by an NADH oxidase in *L. gasicomitatum* strains caused green discoloration on beef steaks. Analysis of the genome of *L. gasicomitatum* LMG 18811^T revealed that pyruvate oxidase is the only enzyme with a known ability to generate H_2O_2. Slime formation on vegetable sausages (45) and in a herring product (24) was proposed to be due to sucrose-derived homopolysaccharide dextran, since *L. gasicomitatum* produces slime from sucrose *in vitro* (3). Consistent with this, *L. gasicomitatum* LMG 18811^T encodes a dextransucrase, a cell wall-associated glycosyltransferase catalyzing the formation of dextran from sucrose. In addition, a gene cluster homologous to the heteropolysaccharide EPS gene cluster present in *Streptococcus thermophilus* (26) was detected. Heteropolysaccharide formation in leuconostocs has not been reported. Compared to homopolysaccharides, their biosynthesis is more complex (12, 26), ruling out prediction of the EPS structure, physical properties, and possible role in food spoilage.

Addition of heme to aerated MRS medium increased the biomass formation of *L. gasicomitatum* considerably, whereas addition of CO_2 (20%) to the oxygen-containing atmosphere to mimic the atmosphere used to create the high-oxygen MA for red meats did not limit the biomass increase. Since neither the present study nor that of Brooijmans et al. (5) showed functional respiration in *L. mesenteroides* ATCC 8293^T, we did not anticipate this finding. Heme-induced respiration dramatically alters the phenotype of *Lactococcus lactis*, as it improves not only growth efficiency but also robustness as improved stress resistance (13, 16). No heme uptake transporters have been characterized in any LAB, despite numerous efforts (15, 31). Nevertheless, meat contains heme, and since the CO_2 added in the atmosphere did not have any effect, *L. gasicomitatum* may respire while growing on high-oxygen MAP meats, leading to succession in the spoilage LAB population due to effective growth and improved stress resistance.

L. gasicomitatum has not been detected on skin or mucous membranes of broiler chickens (43) or pigs (23). The precise habitat of this species is not known, but on the basis of its growth temperatures and carbon source utilization (plant-derived pentoses), we have considered it an environmental LAB. Thus, it was interesting to detect unique genes (see Table S3 in the supplemental material) associated with adhesion and platelet binding. For genes encoding the putative mucus binding protein LEGAS_0414, orthologs were detected in the genomes of *L. mesenteroides* and *L. citreum* (plasmid encoded), but none of the other genomes contain orthologs for the putative collagen adhesion protein enabling biofilm formation in staphylococci (10). The collagen binding capabilities might enable better survival in the meat environment. In preliminary analyses, *L. gasicomitatum* strain KG1-16, isolated in the spoiled vegetable sausages (47), did not harbor these genes. The role and expression of these genes in association with meat spoilage will be an interesting target of further studies.

With the help of the genome sequence, many of the spoilage reactions found their rationale. In addition, interesting new hypotheses arose, such as the potential increase of growth and stress resistance capabilities through respiratory capacity in high-oxygen meat products. *L. gasicomitatum* 18811^T provides an interesting model of psychrotrophic spoilage LAB for future studies.

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