Adaptation of *Lactobacillus plantarum* IMDO 130201, a Wheat Sourdough Isolate, to Growth in Wheat Sourdough Simulation Medium at Different pH Values through Differential Gene Expression^{▽†}

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Sourdough is a very competitive and challenging environment for microorganisms. Usually, a stable microbiota composed of lactic acid bacteria (LAB) and yeasts dominates this ecosystem. Although sourdough is rich in carbohydrates, thus providing an ideal environment for microorganisms to grow, its low pH presents a particular challenge. The nature of the adaptation to this low pH was investigated for *Lactobacillus plantarum* IMDO 130201, an isolate from a laboratory wheat sourdough fermentation. Batch fermentations were carried out in wheat sourdough simulation medium, and total RNA was isolated from mid-exponential-growth-phase cultures, followed by differential gene expression analysis using a LAB functional gene microarray. At low pH values, an increased expression of genes involved in peptide and amino acid metabolism was found as well as that of genes involved in plantaricin production and lipoteichoic acid biosynthesis. The results highlight cellular mechanisms that allow *L. plantarum* to function at a low environmental pH.

Lactic acid bacteria (LAB) are known to be able to survive in acid environments (4, 25, 34). Important mechanisms to resist acid conditions include intracellular proton removal through H⁺-ATPase activity, alterations in cell membrane composition, and amino acid conversions (3, 21, 26). *Lactobacillus plantarum*, a facultative heterofermentative LAB species, is mostly associated with plant materials (16) and is hence present in many types of food fermentations, including sauerkraut and other vegetable fermentations, cocoa bean fermentations, and sourdough fermentations (1, 2, 8, 9, 27). As a result, strains of *L. plantarum* have developed a large degree of metabolic flexibility to deal with these challenging environments (5, 29, 30).

In a sourdough environment, for example, two metabolic characteristics provide a competitive advantage to bacterial strains, i.e., the use of maltose as an energy source and the ability to convert arginine into ornithine via citrulline by means of the arginine deiminase (ADI) pathway. Indeed, maltose is taken up by a H⁺-maltose symport system, resulting in a higher uptake at a lower pH, and is intracellularly converted into glucose and glucose-1-phosphate by the enzyme maltose phosphorylase (11, 12, 14, 19). This enzyme uses inorganic phosphate and therefore represents an energy saving, as no ATP has to be expended for carbohydrate phosphorylation (14, 15). Furthermore, the ADI pathway generates two moles of ammonia, contributing to survival in acid stress conditions, and one mole of ATP per mole of arginine. The final product of the ADI pathway, ornithine, is a

desirable product during sourdough fermentation, as it is the precursor of 2-acetyl-1-pyrroline, which gives freshly baked bread crust its characteristic flavor (7, 17).

Because of the presence of *L. plantarum* in many fermented food ecosystems and other environmental and human niches, the genome sequences of *L. plantarum* WCFS1 (a human saliva isolate [20]) and *L. plantarum* JDM1 (a human intestinal tract isolate [43]) have been determined, leading to a more fundamental understanding of its biology (13, 23). A detailed study of the response of *L. plantarum* WCFS1 against acid conditions using a whole-genome microarray has revealed the expression of a hitherto unknown class of lactic acid-responsive proteins with unknown function and activation of the phosphoketolase pathway that leads to the production of fermentation products other than solely lactic acid (28).

The aim of the present study was to indicate genes and gene clusters that are involved in cellular responses related to acid adaptation of *L. plantarum* IMDO 130201, an isolate obtained from a laboratory wheat sourdough fermentation (33), during growth in wheat sourdough simulation medium by using a LAB functional gene microarray (40).

MATERIALS AND METHODS

Bacterial strain and medium. The bacterial strain used throughout this study was *L. plantarum* IMDO 130201, an isolate from a 10-day laboratory wheat sourdough fermentation performed through backslipping (33). The strain was stored at –80°C in wheat sourdough simulation medium (W-SSM) (37) supplemented with 25% (vol/vol) glycerol as a cryoprotectant. W-SSM was also used as the medium to perform simulated wheat sourdough fermentations. W-SSM had the following composition (per liter): wheat peptone, 12 g; granulated yeast extract, 12 g; MgSO₄ · 7H₂O, 0.2 g; MnSO₄ · H₂O, 0.05 g; KH₂PO₄, 4 g; K₂HPO₄, 4 g; Tween 80, 1 ml; and vitamin solution, 1 ml. The vitamin solution had the following composition (per liter): cobalamin, 0.2 g; folic acid, 0.2 g; nicotinamide, 0.2 g; pantothenic acid, 0.2 g; pyridoxal-phosphate, 0.2 g; and thiamine, 0.2 g. The carbohydrate concentrations were as follows (per liter): glucose, 0.5 g; fructose, 0.5 g; maltose, 10.0 g; and sucrose, 2.0 g. All chemicals were obtained from VWR International (Darmstadt,

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Germany). Solid W-SSM was prepared by adding 1.5% (wt/vol) agar (Oxoid Ltd., Basingstoke, United Kingdom) to the broth.

Fermentations. Fermentations were carried out in 10 liters of W-SSM in 15-liter BioStat C fermentors (Sartorius AG/B. Braun Biotech International, Melsungen, Germany) as described previously (37). *In situ* sterilization occurred at 121°C for 20 min. The pH of the medium was adjusted to the desired value prior to sterilization. If necessary, final pH corrections were done in the fermentor prior to inoculation. Fermentations were carried out at pH values of 3.5, 4.0, 4.5, 5.0, and 5.5. The energy source was sterilized separately and added aseptically to the fermentor. The fermentation temperature was kept at 30°C. The pH of the medium was kept constant during fermentation through automatic addition of a 10 M NaOH solution to the fermentation medium. The stirring speed was fixed at 100 rpm to keep the medium homogeneous. Sterile air was continuously blown through the headspace of the fermentor at a rate of 1 liter min⁻¹. Temperature, pH, agitation, and airflow were controlled online (Micro MFCs for Windows NT software; Sartorius AG/B. Braun Biotech International). The inoculum was prepared through three subcultures of 12 h in W-SSM at 30°C in a standard incubator. The first two subcultures were carried out in 10 ml of medium, and the third subculture was carried out in 100 ml. The third subculture was used to inoculate the 10-liter fermentation. During fermentation, samples were regularly withdrawn from the fermentor for determination of cell counts (CFU). Cell counts were obtained by plating 10-fold serial dilutions in saline (0.85% [wt/vol] NaCl solution) on W-SSM agar. Every measurement was performed on three independent samples.

RNA isolation. To obtain total RNA from *L. plantarum* IMDO 130201, 5 ml fermentation medium of a mid-exponential-growth-phase culture was collected in 10 ml RNeasy Protect (Qiagen, Hilden, Germany), mixed, and kept at room temperature for at least 5 min. Subsequently, the sample was centrifuged at 5,000 × g for 15 min and the RNA was isolated from the resulting cell pellet by applying an enzymatic lysis using mutanolysin and lysozyme, after which the RNA was extracted from the resulting mixture by using an RNeasy minikit (Qiagen), following standard instructions and including mechanical disruption of the cells using glass beads, as described previously (40). As the RNA profiles, which were obtained after capillary electrophoresis using the Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA), showed an unexpected peak at the lower size range, possibly due to the presence of small RNA molecules (e.g., tRNA), an additional RNA cleanup step was performed. To remove these small RNA molecules, the Microcon YM-50 (Millipore, Bedford, MA) was used according to the manufacturer's standard instructions. Sampling for RNA isolation was performed in duplicate for each fermentation, resulting in two technical repeat samples for each pH value.

Microarray. A LAB functional gene microarray version 2 was used, which is an updated version of the microarray described previously (40). This updated version contains in total 6,209 oligonucleotides, of which 795 oligonucleotides are related to *L. plantarum*. Of these oligonucleotides, 620 were designed based on *L. plantarum* gene sequences, of which 514 were designed based on gene sequences of *L. plantarum* WCFS1. In addition, 175 oligonucleotides were designed based on gene sequences from other LAB species, but they could hybridize *L. plantarum* gene sequences based on *in silico* comparative sequence analysis. The microarray was spotted on CodeLink activated slides as described previously (40).

Microarray hybridization, experimental design, and microarray data analysis to estimate gene expression levels. The isolated RNA was linearly amplified (aRNA) using a Genisphere SensAmp kit (Genisphere, Hatfield, PA), labeled with Cy3 and Cy5 dyes in a reverse transcription reaction, and hybridized for 16 h using a HS 4800 Pro automated hybridization station (Tecan Systems Inc., San Jose, CA), as described previously (40), except that 130 µl hybridization mixture was used instead of 210 µl.

The labeled aRNA samples were hybridized using a loop design, i.e., two samples were hybridized on the same microarray slide (e.g., sample pH 3.5 and sample pH 4.0, sample pH 4.0 and sample pH 4.5, etc.), each labeled with another fluorescent dye (Cy3 or Cy5), and the loop was closed by hybridizing sample pH 5.5 together with sample pH 3.5. Given that each oligonucleotide was spotted in triplicate on the microarray slides and that each sample was hybridized twice, once with each fluorescent dye, the intensity of each oligonucleotide was measured six times. The intensity of an oligonucleotide was considered to be above the background level if the intensities for at least three out of six spots were above the background level, as described previously (40). Prior to further data analysis, the local background intensity was subtracted from the foreground intensity and a base 2-log transformation was applied. For each oligonucleotide, the average over the three Cy3 and three Cy5 intensities was used. In the case if a missing measurement, its value was imputed with the KNNImpute algorithm (with the number of nearest neighbors to use during imputation K set to 10 and the Euclidean distance as a distance measure), as described previously (32).

TABLE 1. Overview of the number of oligonucleotides per cluster, out of a total of 374 oligonucleotides, and their relatedness to *Lactobacillus plantarum* gene sequences, as well as of the number of metabolic functions for the *L. plantarum*-related oligonucleotides

Cluster	No. of oligonucleotides:			No. of metabolic functions ^a
	Total	Designed based on <i>L. plantarum</i> gene sequences	Designed based on other LAB species gene sequences but able to cross-hybridize with <i>L. plantarum</i>	
1	69	61	4	43
2	57	40	5	24
3	9	1	3	4
4	30	3	5	7
5	102	83	8	48
6	73	60	6	40
7	34	1	5	6

^a See the supplemental material for a description of the metabolic functions.

Oligonucleotides whose intensities were never above the background level were omitted, as they were irrelevant for the analysis.

To assess whether the pH had a significant effect on gene expression levels, a two-step mixed model was applied as described previously (42). In the first step, intensity values were normalized between slides to remove array, dye, and their interaction effects. In the second step, gene-specific models were fitted to assess the effect of the pH. Only those oligonucleotides that had a *P* value of <0.05 and that had an intensity above the background level for at least one pH value (i.e., at least six spots above the background level for the 12 measurements corresponding to one pH value) were retained for hierarchical clustering. Therefore, the hybridization intensity profiles were transformed into Z scores by subtracting the average hybridization intensity of all oligonucleotides from the hybridization intensity of the oligonucleotide considered and dividing that result by the standard deviation of all hybridization intensities. Subsequently, these Z score profiles were hierarchically clustered with the complete linkage option with a distance measure of one minus the Pearson correlation (41).

Metabolite target analysis. The concentration of lactic acid was determined by high-performance liquid chromatography (HPLC) using external standards, with the error represented as the standard deviation of three independent analyses as described previously (24). The concentrations of glucose, fructose, sucrose, maltose, and mannitol were determined by high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD), as described previously (37). Concentrations of arginine, citrulline, and ornithine were determined using a Waters HPLC coupled to a mass spectrometer (MS) as described previously (39). For both HPAEC-PAD and HPLC-MS measurements, quantifications were carried out through a standard addition protocol, with the error represented as the standard deviation of the x-intercept of the resulting calibration curves as described previously (37, 39).

Microarray data accession numbers. The microarray data have been deposited in the NCBI Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo/>) under accession numbers GPL10874 (microarray platform, including detailed annotation) and GSE23945 (hybridization experiments).

RESULTS

Microarray data analysis to estimate gene expression levels.

After normalization for array, dye, and their interaction effects, and fitting of gene-specific models to assess the effect of pH, 374 oligonucleotides that had a hybridization intensity above the background level for at least one pH value displayed a *P* value of <0.05. After hierarchical clustering of these oligonucleotides, seven clusters could be distinguished. An overview of the metabolic functions corresponding to the oligonucleotides present in each expression cluster is provided as supplemental material.

All oligonucleotides of cluster 1 (Table 1) showed positive but decreasing Z scores at the lowest pH values of 3.5 and 4.0,

FIG. 1. Overview of gene expression of genes belonging to clusters 1 to 7. Shown are Z scores for cluster 1 (A), cluster 2 (B), cluster 3 (C), cluster 4 (D), cluster 5 (E), cluster 6 (F), and cluster 7 (G).

indicating that the hybridization intensity was higher than expected given the average hybridization intensity of all oligonucleotides (Fig. 1A). At pH values of 4.5, 5.0, and 5.5, the Z scores became negative, indicating that the hybridization intensity was lower than expected given the average hybridization intensity of all oligonucleotides. Most of the oligonucleotides in this cluster corresponded to genes involved in oligopeptide transport and in amino acid conversions, including the urea cycle genes argininosuccinate lyase and argininosuccinate synthase. Furthermore, this was the only cluster that contained genes involved in bacteriocin production, i.e., *plnJ*, coding for the precursor peptide of a plantaricin related to *L. plantarum* WCFS1; *plnL*, coding for its immunity protein; and *plnC*, the response regulator. Also, some oligonucleotides related to genes involved in carbohydrate and pyruvate metabolism, among which maltose phosphorylase, were found in this cluster. Finally, this cluster contained oligonucleotides related to genes involved in different kinds of stress response, such as subunits for the F_0F_1 -ATPase, important for intracellular pH regulation, and pyruvate oxidase and glutathione reductase, involved in oxidative stress response.

All oligonucleotides of cluster 2 (Table 1) showed negative but increasing Z scores at the lowest pH values of 3.5, 4.0, and 4.5, pointing to a lower-than-expected expression of the corresponding genes, and positive Z scores at pH values of 5.0 and 5.5, pointing to a higher-than-expected expression (Fig. 1B). Most of the genes represented in this cluster were involved in carbohydrate uptake, i.e., transport systems for cellobiose, maltose, mannitol, and mannose, and in carbohydrate and pyruvate metabolism, including maltose phosphorylase. Stress responses included cold, oxidative, and acid stress.

Oligonucleotides representing the gene maltose phosphorylase (EC 2.4.1.8) appeared in cluster 1 as well as in cluster 2, which seems to be contradictory given the opposite expression patterns. Three of the *L. plantarum* WCFS1 genes encoding maltose phosphorylase were present on the microarray (Table 2). The *map1* gene was represented by two oligonucleotides that both had an intensity above the background level and were found in cluster 1 (Table 2). The *map2* gene was represented by two oligonucleotides, of which one had an intensity above the background level and was found in cluster 2. The *map3* gene was represented by three oligonucleotides, of which one

TABLE 2. Overview of the three genes present on the microarray, accession numbers of the corresponding nucleotide and protein sequences, number of amino acids of the enzyme, percent identity with O87772, oligonucleotide identification numbers, and the cluster to which the oligonucleotides belong^a

Gene	EMBL accession no.	TrEMBL accession no.	No. of amino acids	% Identity with O87772	Oligonucleotide identifier	Cluster
<i>map1</i>	CAD62728	Q890I3	906	24	F007005	1
					F009612	1
<i>map2</i>	CAD62851	Q88ZW3	756	58	F006514	NAB
					F009613	2
<i>map3</i>	CAD64151	Q88WB8	748	58	F009615	2
					F009616	NAB
					F009617	NAB

^a The table represents an overview of the three genes that were present on the microarray out of the four genes coding for maltose phosphorylase in *Lactobacillus plantarum* WCFS1, the accession numbers of the corresponding nucleotide (EMBL) and protein (TrEMBL) sequences, the number of amino acids of the enzyme, the percent identity at the protein level with maltose phosphorylase I (*mapA*) from *L. sanfranciscensis* DSM 20451^T (TrEMBL accession no. O87772) determined using BLASTP, the oligonucleotide identification numbers, and the cluster to which the oligonucleotides belong. The *map4* gene was not represented on the microarray. NAB, not above the background level.

had an intensity above the background level and was found in cluster 2 (Table 2). In addition, the expression profile of α -amylase clustered together with the expression profiles of *map2* and *map3* (cluster 2), marked by a lower-than-average expression at low pH values and higher-than-average expression at high pH values.

The oligonucleotides of cluster 3 (Table 1) displayed a lower-than-expected expression at pH 3.5 (Fig. 1C). Among the four oligonucleotides, two corresponded to cellobiose and mannose transport. Although these oligonucleotides were also present in cluster 2, the general behavior was similar, i.e., higher expression values at high pH values.

The oligonucleotides of cluster 4 (Table 1) showed a moderate positive *Z* score for pH values from 3.5 to 4.5 and negative *Z* scores for pH values 5.0 and 5.5 (Fig. 1D). Genes following this profile included genes coding for 6-phosphofructokinase and alcohol dehydrogenase.

The oligonucleotides of cluster 5 (Table 1) displayed a higher-than-expected expression at low and high pH values (Fig. 1E). Many genes in this cluster were involved in carbohydrate and pyruvate metabolism. Also, this cluster contained several peptidase genes and the glutamate dehydrogenase gene. The cluster contained a lot of genes involved in several stress responses, such as those coding for heat shock proteins, pyruvate oxidase, and F₀F₁-ATPase subunits.

Cluster 6 (Table 1) showed in general the same profile as cluster 5, but expression levels at extreme pH values made the difference (Fig. 1F). The higher-than-expected expression at high pH values was more pronounced than at low pH values. Most of the genes in this cluster were involved in carbohydrate and pyruvate metabolism. Also, this cluster contained several genes involved in the production of amino sugars and several stress responses.

Cluster 7 (Table 1) showed the opposite behavior of clusters 5 and 6, i.e., a maximum *Z* score at pH 4.5 and lower-than-expected expression at extreme pH values (Fig. 1G). The genes represented by these oligonucleotides belonged to carbohydrate and pyruvate metabolism and stress response.

Fermentation course and metabolite target analysis. For all pH values tested, *L. plantarum* IMDO 130201 initiated growth without a noticeable lag phase (Fig. 2). The cell counts in-

creased from about 7.0 log CFU ml⁻¹ to about 9.5 log CFU ml⁻¹. All carbohydrates were consumed to depletion during the exponential growth phase, except for maltose at pH 3.5 (Fig. 2E). The sole fermentation product was lactate. No ethanol, acetate, or mannitol was found. The arginine concentration remained constant during the fermentation for all pH values tested, and no free citrulline or ornithine was produced. At pH 4.0, 4.5, 5.0, and 5.5 (Fig. 2A, B, C, and D), maltose, glucose, fructose, and sucrose were converted stoichiometrically into lactate. At pH 3.5 (Fig. 2E), glucose, fructose, and sucrose were converted stoichiometrically into lactate, but maltose was consumed only partially.

DISCUSSION

LAB are well adapted to live in low pH and high lactic acid environments and are therefore key players in fermented food ecosystems (10). In the current study, gene expression of *L. plantarum* IMDO 130201, an isolate from a spontaneous laboratory wheat sourdough fermentation, was measured in W-SSM as a function of pH by using a LAB functional gene microarray (40). Microarray data analysis revealed that 374 oligonucleotides on the microarray, of which 285 were related to *L. plantarum* genes, displayed differential gene expression profiles that could be explained by differences in pH (*P* < 0.05). Hierarchical clustering of these 374 expression profiles resulted in seven clusters. As the goal of the current study was to estimate the global response of *L. plantarum* IMDO 130202 to low pH values, gene expression was not studied more in depth through quantitative reverse transcription PCR (qRT-PCR). Instead, metabolite target analyses were performed in certain cases to validate expression of key metabolic pathways and associated genes involved.

Genes coding for proteins involved in oligopeptide transport and for the urea cycle enzymes argininosuccinate lyase and argininosuccinate synthase indicated the initiation of arginine biosynthesis. The fact that no variation in extracellular arginine concentrations was found indicates that arginine did not need to be supplied extracellularly. Also, no products of the ADI pathway were found. Although this pathway is of importance for LAB to adapt to acid environments (38) and was found in

FIG. 2. Growth (■) and carbohydrate consumption of *Lactobacillus plantarum* IMDO 130201 at a constant pH. Panels: A, pH 5.5; B, pH 5.0; C, pH 4.5; D, pH 4.0; and E, pH 3.5. Shown are results with glucose (◇), fructose (□), sucrose (△), maltose (○), and lactic acid (●).

some strains of *L. plantarum* (22), some genes might be missing in the strain studied, as is the case for *L. plantarum* WCFS1 (20). Also, physiological tests have shown that *L. plantarum* WCFS1 is auxotrophic for arginine, although no apparent genetic reason for this auxotrophy could be found (30). Interestingly, some genes involved in plantaricin production had higher levels of expression at low pH values, indicating that bacteriocin production was activated under acid stress conditions by the *L. plantarum* IMDO 130201 strain.

Only a few genes involved in carbohydrate degradation were highly expressed at low pH values. All other genes involved in carbohydrate and pyruvate metabolism with a pH-dependent expression profile showed a lower-than-expected expression at

low pH values and a higher-than-expected expression at high pH values. Also, the genes involved in carbohydrate uptake by means of phosphotransferase systems showed an identical trend. Most probably, the bacteria were directed toward survival at a low pH by amino acid conversions rather than by relying on growth. According to the results of the metabolite target analyses of carbohydrates and their metabolites, no variations in carbohydrate metabolism were observed. The lack of observable activity by the phosphoketolase pathway might indicate that, although active, the concentration range of the metabolites produced was too low for the detection techniques used. However, even if active, the contribution of this alternative pathway to overall carbohydrate metabolism was insignif-

icant compared to the activity of the Embden-Meyerhof-Parnas pathway.

The enzyme maltose phosphorylase is of particular importance for sourdough-related LAB strains, as it catalyzes the conversion of maltose into glucose and glucose-1-phosphate using inorganic phosphate (14). As maltose is the main carbohydrate source in sourdough fermentations, resulting from the degradation of starch by flour and bacterial amylases, strains able to express the genes coding for α -amylase and maltose phosphorylase have a competitive advantage to grow in this food matrix (10, 14, 15). Sequence comparison of the MapA protein sequence from *Lactobacillus sanfranciscensis* DSM 20451^T with the protein sequences corresponding with the *map1*, *map2*, and *map3* genes of *L. plantarum* WCFS1, showed a large identity between *mapA* and both *map2* and *map3*. Of the most typical sourdough LAB, for which gene sequence information is available, only *L. sanfranciscensis* DSM 20451^T has multiple genes coding for maltose phosphorylase (35). It has been suggested that the two maltose phosphorylase genes display different functions, i.e., maltose phosphorylase I (*mapA*) would be the catabolic enzyme that, together with phosphoglucosyltransferase, is part of one operon structure (12), whereas maltose phosphorylase II, for which no sequence data are available, would be involved in basic maltose metabolism to provide glucose-1-phosphate for cell wall biosynthesis (36). Based on sequence comparison, the four genes coding for maltose phosphorylase that were found in *L. plantarum* WCFS1 might comprise the same two physiological groups, where *map2* and *map3*, found in cluster 2 together with α -amylase, are involved in maltose catabolism, given a sequence identity of 58%. As there is no sequence information for the maltose phosphorylase II gene from *L. sanfranciscensis* DSM 20451^T, no comparison could be made for that gene. Obviously, further functional characterization of these *L. plantarum* genes should be performed to fully unravel their role in cellular functions.

An increase in teichoic acid biosynthesis at a low pH was indicated by an increased expression of the gene coding for poly(glycerol-phosphate)- α -glucosyltransferase, which points to an alteration of the cell wall composition, as a response to the acid environment (6). Although two types of teichoic acid, i.e., a glycerol type and a ribitol type, may be found together in the same species (6), the teichoic acid of *L. plantarum* has been shown to be of the ribitol type (31). This is in contrast with the genome annotation of *L. plantarum* WCFS1, where only the gene coding for poly(glycerol-phosphate)- α -glucosyltransferase has been found (20). Possibly, a high sequence similarity between the two genes may have led to this questionable annotation. Cell wall adaptation to an acid environment has been shown before for a strain of *Lactobacillus reuteri* in sourdough (18).

To conclude, this study demonstrated the adaptation possibilities of *L. plantarum* IMDO 130201 to an acid environment through enhanced amino acid conversion, bacteriocin production, and teichoic acid biosynthesis. A difference in expression of genes coding for maltose phosphorylase correlated with a proposed different physiological role for the corresponding enzymes. The fact that the central metabolism of *L. plantarum* IMDO 130201 seems to be not much influenced by differences

in environmental pH indicates that this strain has sufficient mechanisms to safeguard normal physiological functioning.

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