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FRUCTOSE 6-PHOSPHATE PHOSPHOKETOLASE ACTIVITY IN WILD-TYPE STRAINS OF *Lactobacillus*, ISOLATED FROM THE INTESTINAL TRACT OF PIGS

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Phosphoketolases are key enzymes of the phosphoketolase pathway of heterofermentative lactic acid bacteria, which include lactobacilli. In heterofermentative lactobacilli xylulose 5-phosphate phosphoketolase (X5PPK) is the main enzyme of the phosphoketolase pathway. However, activity of fructose 6-phosphate phosphoketolase (F6PPK) has always been considered absent in lactic acid bacteria. In this study, the F6PPK activity was detected in 24 porcine wild-type strains of *Lactobacillus reuteri* and *Lactobacillus mucosae*, but not in the *Lactobacillus salivarius* or in *L. reuteri* ATCC strains. The activity of F6PPK increased after treatment of the culture at low-pH and diminished after porcine bile-salts stress conditions in wild-type strains of *L. reuteri*. Colorimetric quantification at 505 nm allowed to differentiate between microbial strains with low activity and without the activity of F6PPK. Additionally, activity of F6PPK and the X5PPK gene expression levels were evaluated by real time PCR, under stress and nonstress conditions, in 3 *L. reuteri* strains. Although an exact correlation, between enzyme activity and gene expression was not obtained, it remains possible that the *xpk* gene codes for a phosphoketolase with dual substrate, at least in the analyzed strains of *L. reuteri*.

Bacteria belonging to the genus *Lactobacillus* are common inhabitants of the gastrointestinal tracts of vertebrate animals and have received considerable attention due to their putative health-promoting properties when they are ingested as probiotics. Although they comprise only a minor part of the bacterial community in human feces and animals, such as pigs, chickens, mice, and rats, lactobacilli are the predominant bacteria in the proximal regions of the gut [1]. Since low pH, bile acids and increasing osmolality are encountered by bacteria during gastrointestinal tract transit, gene expression in these conditions is likely to code for different proteins, including carbon and energy sources, which enable *Lactobacillus* to compete and survive better in hostile environment conditions [2]. Several strains of *Lactobacillus* also appear to be metabolically active *in vivo* in the intestine [3], however, the factors that allow lactobacilli to become established and persist in the gastrointestinal tract are unknown [1, 3].

Phosphoketolases are key enzymes of the phosphoketolase pathway of heterofermentative and facultative heterofermentative lactic acid bacteria, and of the fructose 6-phosphate shunt of bifidobacteria. Phosphoketolases catalyze an irreversible thiamine diphosphate dependent phosphorolytic reaction splitting d-xylulose-5-phosphate (X5PPK, EC 4.1.2.9) or d-fructose-6-phosphate (F6PPK, EC 4.1.2.22) in the presence of inorganic phosphate [4]. Bifidobacteria have two distinct types of F6PPK. One is specific for fructose 6-phosphate; the other is less stringent and is able to utilize d-xylulose-5-phosphate as an alternative substrate. The dual-specific X5P/F6PPK is encoded by the gene *xfp*, first described in *Bifidobacterium animalis* subsp. *lactis* [5].

In heterofermentative lactobacilli, X5PPK is the central enzyme of the phosphoketolase pathway. This enzyme is encoded by the gene *xpk* [6], and is the only phosphoketolase activity expected. Moreover, for many years it has been stated that "the most direct and reliable characteristic for the assignment of Gram-positive rod shaped bacteria to the genus *Bifidobacterium* is that based on the demonstration of F6PPK in cellular extracts" [7]. This taxonomic criterion is still used for a rapid identification of the *Bifidobacterium* genus [8] and to demonstrate that novel strains are members of the genus *Bifidobacterium* [9]. Nevertheless, it has been demonstrated that other related genera: *Gardnerella*, also posesses the activity of this enzyme [10].

Four years ago, publications of protein sequences in the GenBank database (http://www.ncbi.nlm.nih.gov/Genbank/index.html) were reporting the presence of F6PPK in *Lactobacillus reuteri* (accession number: ZP_01273782). However, recently this sequence was removed "because it has been superseded by a new assembly of the genome" (http://www.ncbi.nlm.nih.gov/protein/92088824). Nevertheless, to our knowledge,

no other genus belonging to lactic acid bacteria, that colonizes the gastrointestinal tract of vertebrates, has been associated with F6PPK activity.

The aim of the present study was to evaluate the presence of F6PPK, under stress and nonstress conditions, in wild-type strains of *L. reuteri* and *L. salivarius*, isolated from the intestinal tract of pigs. Moreover, in 3 wild-type strains of *L. reuteri*, the expression level of the xylulose 5-phosphate phosphoketolase gene, was evaluated by correlation search between the F6PPK activity and *xpk* gene expression under stress and nonstress conditions.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The bacterial strains, including 39 strains *Lactobacillus* and 6 strains of *Bifidobacterium,* used in this study are listed in Table 1. The wild-type strains were isolated from the gastrointestinal tract of healthy post-weaning and slaughtered adult pigs, and characterized by polyphasic taxonomy in a previous work [11]. Wild-type *Lactobacillus* and *L. reuteri* ATCC 53608 strains were grown at 37°C in 5% CO_2 atmosphere in MRS (Man, Rogosa & Sharpe) broth (Difco, USA). *Lactobacillus salivarius* subsp. *salivarius* ATCC 29602 and *L. salivarius* subsp. *salicinus* ATCC 11742 strains were grown at 37°C in MRS broth under aerobic conditions. *Bifidobacterium* strains were grown anaerobically at 37°C in MRS broth, (pH 6.0) supplemented with 0.05% (w/v) cysteine hydrochloride (Sigma, USA). All strains were conserved in MRS broth with glycerol (20% v/v) at −20°C.

The F6PPK activity. The detection of F6PPK activity was carried out as previously described [12]. All reagents were obtained from Sigma (USA). The ability of the bacterial strains to express F6PPK, after stress conditions, was evaluated as follows: all the wild-type strains were grown overnight in MRS broth at 37°C as described previously. After incubation, culture media were distributed into 3 aliquots. One aliquot was kept at 37°C in 5% CO_2 and used as control. The other two aliquots were centrifuged to precipitate the cell pack (5 min at 2000 g). One of the pellets was suspended in MRS broth, pH 3.0 (low pH), and the other one was suspended in MRS broth with 0.5% (w/v) porcine bile salts. Both samples were incubated for 1 h at 37°C in aerobic conditions and used with control sample for the detection of F6PPK activity. Subsequently, they were centrifuged to clarify the bacterial suspension (5 min at 2600 g) and absorbance was measured at 505 nm spectrophotometrically. Each procedure was carried out by duplicate.

Absorption spectra. Some samples obtained from different strains of lactobacilli and bifidobacteria were selected for absorption spectra measurement. Ultraviolet/visible difference spectra with tandem trays were carried out in an Ultrospec 400 (Pharmacia Biotech, UK). Difference spectrum was obtained by subtracting the contribution of the blank control (all the reagents used in the detection of F6PPK activity, except the microorganism) spectrum.

Table 1. Bacterial strains used in this study

Species	Code	Source
B. breve	ATCC 15700, CECT 4839	Human
B. infantis	ATCC 15697	Human
B. longum	CECT 4503	Human
B. angulatum	CECT 5775	Human
B. lactis	Bb12	Lactic product
L. reuteri	ATCC 53608, 2, 30, 32, 107, 119, 124, 169, 676, 703, 1415, 1447, 1703, 1704, 1705, 1709, 1715, 1717, 1722, 1723, 1725, 1726, 1729,	Pig
	L6D14	Piglet
L. salivarius	L5I22, L6D6, L6YD6, L7Y17, L7Y18, L7Y20, L7Y21, L7Y24, L7Y28, L8YD6, L8YD15, L817	Piglet
L. mucosae	L7Y23	Piglet
L. salivarius subsp. *salivarius*	ATCC 29602, ATCC 11742	Human
L. salivarius subsp. *salicinus*	ATCC 11742	

Protein quantification. In order to obtain an adjusted value for enzymatic activity, protein measurement was performed in an aliquot of the selected samples after cetyl trimethyl ammonium bromide (CTAB) treatment as previously described [12]. Total proteins were obtained by a trichloroacetic acid precipitation procedure, as previously described [13]. Total protein in the crude cell extract was quantified with the Bradford protein assay, and the quotient OD_{505}/total protein was obtained for the adjusted value of phosphoketolase activity.

Statistic analysis. Differences between species were evaluated by two samples t-tests. Differences between stressing and not stressing conditions were evaluated by paired t-tests. All statistics were performed in a standard computerized statistical program (NCSS statistical software, USA). A probability of $p < 0.05$ in two tailed test was used as the criterion for statistical significance.

Bacterial strains and culture conditions for *xpk* gene expression. Wild type *L. reuteri* strains 2,676 and 1704 were grown at 37°C in 5% CO_2 atmosphere in MRS broth for 10–11 h. After incubation, culture media were divided in 4 aliquots. 3 aliquots were used to make the control, low-pH and bile-salts samples as it was described in chapter "The F6PPK activity". The fourth aliquot after centrifugation and the low pH treatment was centrifuged again, suspended in MRS broth with 0.5% (w/v) porcine bile salts and incubated

for 1 h at 37°C in aerobic conditions. After incubation, all the samples: control, low-pH, bile-salts and low-pH+bile-salts were used for RNA isolation. The same stress procedures were used to evaluate the F6PPK activity. After incubation, all the samples and one positive control (*B. breve* strain), were used for the detection of F6PPK as mentioned above. Each procedure was carried out by triplicate.

Real time PCR (RT-PCR). Total RNA was isolated from all the treatments of the three wild-type strains of *L. reuteri* using RNAprotect bacteria reagent (Qiagen, Germany) and RNeasy mini kit (Qiagen, USA), according to manufacturer's protocols. Total RNA was quantified spectrophotometrically and the integrity was evaluated in a 1.2% agarose gel (Sigma, USA).

The primers for the xylulose 5-phosphate phosphoketolase gene XPK3F (5'-TTGATGCTTACTGGCGTG-3') and XPK4R (5'-AACTTGACCACCGTGACCT-3') were designed according to *xpk* sequence from *L. reuteri* strain ATCC 55730 (GenBank accession number DQ466581). 16S rRNA was used as internal control for normalization of expression [14] and the primers LR16S1F (5'-TTTGGCTATCACTCTGGGA-3') and LR16S2R (5'-CCGAAACCCTTCTTCACTC-3') were designed from a 180 to 200 bp consensus sequence obtained from the three wild-type strains as mentioned above. All primers were designed using DNAMAN software package (Lynnon, Canada). *In silico* comparisons and PCR amplification products confirmed that the 16S rRNA and X5PPK primers sets were specific for *L. reuteri* and would not hybridize to other *Lactobacillus* species neither to other related genera (data not shown). All primers were purchased from Sigma-Genosys (Sigma, USA) and evaluated by conventional PCR with genomic DNA, the resulting PCR products were purified using GFX columns (GE Healthcare, USA) and sequenced at the Genomic Analysis and Technology Core at the University of Arizona (USA). Sequences were compared to the data available at GenBank using BLAST software [15], available at NCBI: http://www.ncbi.nlm.nih.gov/BLAST/.

All the RNA samples were treated with DNase I (Sigma, USA) to eliminate any remaining genomic DNA contamination prior to cDNA synthesis. Reverse transcription was carried out using 100 ng of total RNA and the specific reverse primers with QuantiTect reverse transcription kit (Qiagen, Germany), according to manufacturer's instructions. Negative controls (all the reagents except reverse transcriptase) were prepared for each treatment and each strain. The resulting cDNA samples were stored at −20°C until use.

RT-PCR amplification was performed in 96 well plates on an iQ5 RT-PCR detection system (Bio-Rad, USA), using SYBR green for the product detection. Each well contained SYBR green master mix (Bio-Rad, USA), 200 nM 16S rRNA or 800 nM X5PPK of each primer and cDNA synthesized from 100 ng of total RNA as template. Negative controls: NTC (no template control) and NPC (no prime control) and reverse transcription negative controls were included for each treatment and each strain. PCR amplification was initiated at 95°C for 5 min, followed by 40 cycles of 95°C for 30 s, 60°C for 35 s and 72°C for 30 s. PCR specificity and product detection were evaluated by examining the dissociation curves of the PCR products. These melting curve profiles were generated by heating the samples from 60°C to 94.5°C for 30 s.

RT-PCR data analysis. For RT-PCR, all the samples and their respective negative controls were analyzed by triplicate and the averages of the C_T values were used for further analysis. Real time PCR amplification efficiencies were determined for each primer pair by standard curves generated by plotting the starting amplicon concentrations against the observed C_T values in serial ten-fold dilutions. The slope of the calibration curve was used to determine the reaction efficiency as $E = 10$ (−1/slope) and final comparisons were done by double delta C_T analysis, and the mean, standard deviation (SD) and coefficient of variation (CV) were then determined from the triplicate samples at each time point [16].

RESULTS AND DISCUSSION

F6PPK activity. The F6PPK test is considered the standard phenotypic assay in the identification of bacteria belonging to *Bifidobacterium* genus [7, 8] and to demonstrate that novel strains are members of this genus [9]. In this work, all the bifidobacteria strains showed a typical positive reaction, with colors from red to purplish, in the F6PPK assay. In contrast, all wild-type *L. salivarius* strains, *L. reuteri* ATCC 53608, *L. salivarius* subsp. *salivarius* ATCC 29602 and *L. salivarius* subsp. *salicinus* ATCC 11742 revealed a typical negative reaction to the test, with a pale yellow color.

However, the colors obtained for wild-type *L. reuteri* strains were from orange to red, and were clearly distinguishable from the typical yellow color observed in the negative controls and the *L. salivarius* strains, although not as intense as the *Bifidobacterium* strains. Furthermore, the authors of the original technique for detection of acyl phosphates, considered that "depending on the concentration of acyl phosphate the color shade will be from orange-brown to purplish brown" [17]. In fact, the appearance of a red to purple color is considered as positive, and a yellow color is considered a negative result of the test [12, 18]. In addition, some bifidobacterial strains do not have considerable phosphoketolase activity [18]. Differences between the absorbance values of wild-type *L. reuteri* and *L. salivarius* strains were clearly observed (Fig. 1). Whereas the mean absorbance for *L. reuteri* strains was 0.212 ± 0.046, the mean absorbance for *L. salivarius* was 0.092 ± 0.022 in a two sample t-test analysis. Taking into account these results, it was considered that the *L. reuteri* strains were positive for the test and the

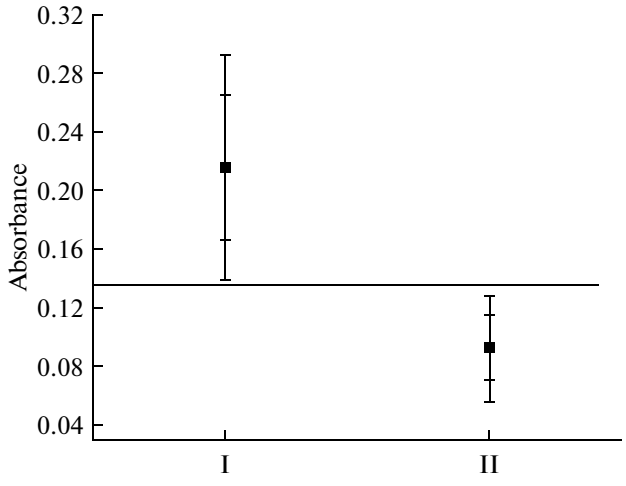

Fig. 1. F6PPK activity in 35 wild-type strains of lactobacilli. Representation of statistic values: mean, standard deviation and interquartile range, obtained for 23 wild-type strains of *L. reuteri* (I) and 12 wild-type strains of *L. salivarius* (II) in F6PPK activity.

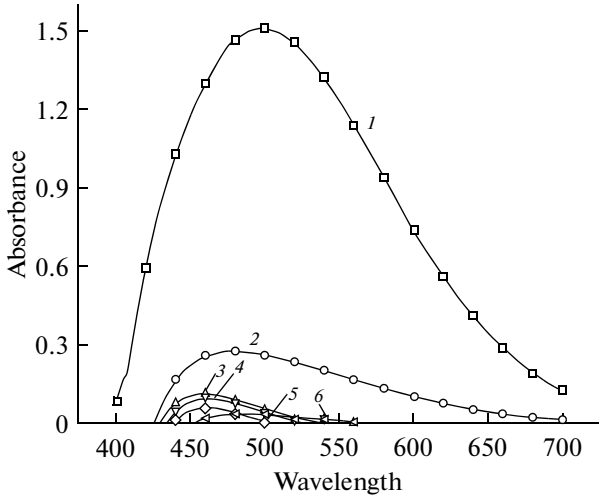

Fig. 2. Absorption spectra obtained from the F6PPK assay in the selected *Lactobacillus* and *Bifidobacterium* strains. *1* – *B. infantis* ATCC 15697; *2* – *L. reuteri* 676; *3* – *L. salivarius* subsp. *salivarius* ATCC 29602; *4* – *L. salivarius* subsp. *salicinum* ATCC 11742; *5* – *L. salivarius* L7Y18; *6* – *L. reuteri* ATCC 53608.

L. salivarius strains were negative. The value of 0.260 obtained for the *L. mucosae* strain revealed F6PPK activity, similar to that observed in the *L. reuteri* strains.

Absorption spectra. The absorption spectra were quite different between strains. In Fig. 2, absorption spectra of selected strains are demonstrated. It could be seen that *Bifidobacterium infantis* ATCC 15697 displayed a characteristic increase in absorption between 480 and 505 nm, Although *L. reuteri* strains did not shown an identical pattern to those obtained for bifidobacteria, it is possible to detect the same behavior between 479 and 490 nm. However, as mentioned above, it has been noted that some bifidobacterial strains do not have considerable phosphoketolase activity, but could be differentiated from other *Streptococcus* and *Lactobacillus* strains that did not lead to color development in the test [18]. Moreover, absorption spectra of *L. salivarius*, as it could be seen in *L. salivarius*, strain L7Y18 spectrum, showed a maximum absorption between 455 and 465 nm, values that correspond to the yellow color observed in the test, and at 505 nm it is possible to differentiate from *L. reuteri* (Fig. 2).

The only wild-type strain of *L. mucosae* evaluated, exhibited the F6PPK activity. This species has been previously isolated from pig's intestine and differentiated from other related species, but it is closely related to *L. reuteri* [19]. A possible explanation of our results is that the *L. reuteri* and *L. mucosae* phosphoketolases can utilize both substrates: xylulose 5-phosphate and fructose 6-phosphate, as it had been demonstrated for the bifidobacteria [5, 20] and *Leuconostoc oenos* phosphoketolases [21]. Another possible explanation for this low activity of F6PPK observed in *L. reuteri* wild-type strains could be that fructose 6-phosphate is not splitted as efficiently as the enzyme's original substrate: xylulose 5-phosphate.

Adjusted F6PPK activity. Selected strains were assayed to obtain adjusted F6PPK activity (Table 2). The absorption spectra were quite different between strains and although it could not be possible to obtain specific activity, since the enzyme was not purified, it was pos-

Table 2. Comparison between λ_{max} and adjusted F6PPK activity in selected strains of lactobacilli and *B. infantis*

Strain	λ_{max}, nm	$OD_{\lambda max}$	OD_{505}	Protein, μg/ml	Adjusted activity*
L. reuteri 676	481	0.273	0.252	55	0.0046
L. salivarius L7Y18	463	0.058	0	305	–
L. reuteri ATCC 53608	489	0.035	0.032	165	1.9×10^{-4}
L. salivarius subsp. *salivarius* ATCC 29602	462	0.111	0.045	634	7.09×10^{-5}
L. salivarius subsp. *salicinus* ATCC 11742	462	0.094	0.032	954	3.35×10^{-5}
B. infantis ATCC 15697	501	1.511	1.507	308	0.0048

* Adjusted the F6PPK activity according to protein concentration in the crude extract.

Table 3. Comparison of the F6PPK activity obtained for 23 wild-type strains of *L. reuteri* under stress and nonstress conditions

Treatment	Group	OD_{505}	*P**
Low pH	Control	0.215 ± 0.05	0.000004
	Low pH	0.298 ± 0.06	
Bile salts	Control	0.215 ± 0.05	0.00003
	Bile salts	0.163 ± 0.07	

* Two tailed paired t-tests.

sible to obtain adjusted phosphoketolase activity, according to protein concentration in the crude extract. In Table 2 it could be appreciated differences in λ_{max} and OD_{505} between all strains. However, when activity is adjusted to total protein content in crude extracts, activity of F6PPK is almost identical in *L. reuteri* strain 676 and *B. infantis* ATCC 15697.

In this work, wild-type strains of *L. salivarius* did not show F6PPK activity. These results are logical since this species is considered as obligate homofermentative, so it does not have phosphoketolase activity [22]. However, the complete genomic sequence of *L. salivarius* strain UCC118 has been obtained, and this strain contains genes that codify for enzymes of the phosphoketolase pathway. Although *L. salivarius* is currently regarded as homofermentative, the authors, based on these results, recommended that it should be grouped among facultative heterofermentative lactobacilli [23]. It could be that *L. salivarius* phosphoketolase is specific for xylulose 5-phosphate, that the expression of the enzyme requires some stimulation or that this enzyme is not functional, since the presence of the gene for phosphoketolase does not guarantee its expression, as observed previously in a great number of organisms [4].

F6PPK activity under stress conditions. After stress conditions with low pH and bile salts, the F6PPK activity remains negative in *L. salivarius* strains. A slight, and statistically significant, increase in absorbance values was detected after incubation with bile salts. As well, an increase in the mean from 0.092 in control to 0.112 after bile-salts treatment was detected, but these results remained negative. Another interesting feature evidenced in this work is that in *L. reuteri*, the phosphoketolase activity increased after exposure to low-pH stress and decreased after treatment by bile salts, as it could be seen in Table 3. It has been demonstrated that phosphoketolase expression increased in response to the combination of the lactic acid stress and a lower growth rate in *Lactobacillus plantarum* [24], and that the expression of proteins participating in the sugar metabolism is generally rather variable [25].

Since bile salts are toxic for bacterial cells, the autochthonous gastrointestinal microbiota must have developed strategies to prevent damage caused by bile salts [26]. When wild-type strains of *L. reuteri* were stressed with porcine bile salts, the F6PPK activity diminished. These results do not agree with previous studies in *Bifidobacterium* strains. Sánchez and colleagues [27] found that *B. longum* NCIMB 8809 strain increases the F6PPK activity, after exposure to bile salts. Although some *L. reuteri* strains possess the bile salt hydrolase activity, and this property is utilized in the selection of potentially probiotic organisms [28], some studies had demonstrated that free bile acids are toxic to lactobacilli [29], and this condition could be the responsible for the low F6PPK activity detected in the bile-salts stress conditions.

F6PPK and *xpk* gene expression. Three wild-type strains of *L. reuteri* were selected for simultaneous evaluation of the F6PPK activity and mRNA levels of *xpk* gene after exposure to low pH, bile salts and low pH+bile salts. After these stress conditions the activity of F6PPK was characterized by high variability and distinct patterns in each strain. Although a great variability could be observed in Table 4, tendencies observed in the mean values of each treatment and controls were maintained in the replicas of each procedure. Using the $2^{-\Delta\Delta C_T}$ method [16], the data are presented as the fold change in mRNA levels as normalized to an endogenous reference gene (16S rRNA) and relative to the untreated control. Stress conditions influenced the *xpkA* gene expression in all strains. Low pH had more influence in strains 32 and 676 as shown in Table 4. However, low pH did not influence the mRNA levels of *xpk* in strain 1704.

In *L. lactis* subsp. *cremoris* MG 1363 the changes in glycolytic enzyme concentrations, under acid stress conditions, did not correlate directly with modifications in transcript concentrations because acceleration of glycolysis was regulated by both, an increase in the concentrations of glycolytic enzymes (hierarchical regulation) and the specific modulation of enzyme activities (metabolic regulation) [30]. Moreover, it has been documented that *L. reuteri* ATCC 55730, while growing in an optimal medium and when all the enzymes of both pathways are active, prefers the phosphoketolase functioning over the Embden-Meyerhoff pathway [2].

The F6PPK activity after consecutive stress with low pH and bile salts resulted in an apparent lower activity of the enzyme with similar results to those obtained after bile stress in strains 32 and 1704, but in strain 676 the result resembled more those obtained after low pH treatment. The low-pH+bile-salts stress showed similar results to those observed in bile-salts stress in strains 32 and 676. Whereas in the first strain the expression was almost the same with the treatment as in the control, in strain 676 the treatment with low-pH+bile-salts generated the highest levels of expression observed in this work. The increase of phosphoketolase gene expression in strain 1704 after low pH- bile salts treatment was notable since this was the only

Table 4. F6PPK activity and *xpk* expression, under stress and nonstress conditions in wild-type strains of *L. reuteri*

Strain	Treatment	F6PPK	*xpk* gene expression	
		OD_{505}	$2^{-\Delta\Delta C_T}$	CV*
32	Control	0.272 ± 0.107		
	Low pH	0.350 ± 0.112	2.10 ± 0.204	9.728
	Bile salts	0.185 ± 0.060	1.44 ± 0.092	6.405
	Low pH+ bile salts	0.178 ± 0.078	1.04 ± 0.098	9.450
676	Control	0.212 ± 0.091		
	Low pH	0.189 ± 0.065	3.93 ± 0.271	6.909
	Bile salts	0.099 ± 0.069	2.41 ± 0.523	21.712
	Low pH+ bile salts	0.179 ± 0.111	3.33 ± 0.384	11.542
1704	Control	0.194 ± 0.110		
	Low pH	0.242 ± 0.067	1.00 ± 0.074	7.445
	Bile salts	0.144 ± 0.065	0.67 ± 0.061	9.101
	Low pH+ bile salts	0.110 ± 0.074	1.84 ± 0.153	8.308

* CV – coefficient of variation.

treatment that really affected positively expression in this strain.

It is known that tolerance to some lethal treatments can be triggered by preexposure to sublethal pretreatments [31]. In *B. longum* NCIMB 8809, resistance level of bile-resistant derivatives was dependent on the external pH, decreasing at neutral values and increasing in acidic environments [26]. General stress proteins are induced by bile in agreement with the cross-protection against bile after thermal or detergent pretreatment that has been observed in several bacteria including *Listeria monocytogenes* [32], and *Bifidobacterium adolescentis* [33]. For example, *L. monocytogenes* LO28 cells are exquisitely sensitive to unconjugated bile acids, but prior adaptation to sublethal levels of bile acids or heterologous stresses, such as acid, heat, salt, or SDS, significantly enhanced bile resistance. This adaptation response was independent of protein synthesis, and in the cases of the bile and SDS adaptation, occurred in seconds [32].

Although the double delta C_T, results were not too high, variations in gene expression were detected in this study. In *L. plantarum* WCFS1 strain, the relative expression levels of the *in-vivo* inducible genes increased up to 350-fold in the mouse intestine compared to levels observed for the *L. plantarum* WCFS1 cells grown in a rich laboratory medium [3]. Moreover, several genes displayed intestinal compartment–specific (small intestine versus colon) activities, confirming that this strain displayed specific and differential responses at various sites along the mammalian intestine. Furthermore, comparisons of transcript levels found in stationary- and exponential-phase of the *L. plantarum* cells revealed that many genes were more highly expressed in the stationary-phase cells fed to mice [3].

It is noteworthy that two genes and some insert sequences identified in *Lactobacillus reuteri* LTH5531 during type II sourdough fermentation were not present in the genome of *L. reuteri* ATCC 55730, which is a human isolate. As the available sequence is estimated to cover 90 to 95% of the complete genome, it was suggested that these genes or sequences may be located in the unsequenced regions or may not be present in the genome of the bacteria obtained from humans [34]. This situation must call the attention to some intriguing aspects related to the presence of specific genes, required, utilized or present only in some strains of the same species, but that colonize different hosts like pig and human.

In some cases, RNA levels may not correlate directly to the levels of the proteins produced by the cell, since post-transcriptional regulation occurs. RT-PCR data are totally uninformative about protein activity [35], but genome-wide transcript analyses using DNA microarrays will provide opportunities for even more comprehensive and integrative views of bacterial activities occurring within the intestinal tract [3].

In conclusion, activity of F6PPK was detected in porcine wild-type strains of *L. reuteri*, but not in wild-type *L. salivarius* or in ATCC *L. reuteri* and *L. salivarius* strains. This activity seems to be influenced by stress conditions in wild-type strains of *L. reuteri* and colorimetric quantification at 505 nm could differentiate between strains with low activity and strains without activity of phosphoketolase. Furthermore, low pH and porcine bile stresses influenced the X5PPK gene expression. Although an exact correlation, between enzyme activity and gene expression could not be ob-

tained, it remains possible that the *xpk* gene encodes for a phosphoketolase with dual substrate, at least in these *L. reuteri* strains.

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