1 **Functional characterization of sucrose phosphorylase and *scrR*, a regulator of**
2 **sucrose metabolism in *Lactobacillus reuteri***
3
4 Januana S. Teixeira[1], Reihaneh Abdi[1,2], Marcia Shu-Wei Su[1,3], Clarissa Schwab[1,4], and
5 Michael G. Gänzle [1§]
6
7 [1]University of Alberta, Department of Agricultural, Food and Nutritional Science,
8 Edmonton, Canada
9 [2]Isfahan University of Technology, Department of Food Science, College of Agriculture,
10 Isfahan, Iran
11 [3]Present address, Pennsylvania State University, Department of Biology, University Park,
12 PA, U.S.A.
13 [4]Present address, University of Vienna, Department of Genetics in Ecology, Vienna,
14 Austria
15
16 [§]Corresponding author
17 4-10 Ag/For Centre
18 Edmonton, AB, T6G 2P5
19 Canada
20
21 e-mail, mgaenzle@ualberta.ca
22 phone, + 1 780 492 0774
23 fax, + 1 780 492 4265
24

Abstract

Lactobacillus reuteri harbors alternative enzymes for sucrose metabolism, sucrose phosphorylase, fructansucrases, and glucansucrases. Sucrose phosphorylase and fructansucrases additionally contribute to raffinose metabolism. Glucansucrases and fructansucrases produce exopolysaccharides as alternative to sucrose hydrolysis. *L. reuteri* LTH5448 expresses a levansucrase (*ftfA*) and sucrose phosphorylase (*scrP*), both are inducible by sucrose. This study determined the contribution of *scrP* to sucrose and raffinose metabolism in *L. reuteri* LTH5448, and elucidated the role of *scrR* in regulation sucrose metabolism. Disruption of *scrP* and *scrR* was achieved by double crossover mutagenesis. *L. reuteri* LTH5448, LTH5448Δ*scrP* and LTH5448Δ*scrR* were characterized with respect to growth and metabolite formation with glucose, sucrose, or raffinose as sole carbon source. Inactivation of *scrR* led to constitutive transcription of *scrP* and *ftfA,* demonstrating that *scrR* is negative regulator. *L. reuteri* LTH5448 and the LTH5448Δ*scrP* or LTH5448Δ*scrR* mutant strains did not differ with respect to glucose, sucrose or raffinose utilization. However, *L. reuteri* LTH5448Δ*scrP* produced more levan, indicating that the lack of sucrose phosphorylase is compensated by an increased metabolic flux through levansucrase. In conclusion, the presence of alternate pathways for sucrose and raffinose metabolism and their regulation indicate that these substrates, which are abundant in plants, are preferred carbohydrate sources for *L. reuteri*.

45 Key words: sucrose, sucrose phosphorylase, lactobacillus, *Lactobacillus reuteri,*
46 sourdough
47
48 **List of abbreviations**
49 LAB, lactic acid bacteria
50 GI, gastrointestinal
51 PTS, phosphotransferase system
52 ScrP, sucrose phosphorylase
53 ScrR, sucrose regulator
54 LevS, levansucrase
55 FtfA, fructosyltransferase A
56 ScrP, sucrose phosphorylase
57 MRS, deMan-Rogosa-Sharpe medium
58 LB, Luria-Bertani
59 HPLC, high performance liquid chromatography
60 HPAEC-PAD, high performance anion exchange chromatography with integrated pulsed
61 amperometric detection
62 qPCR, quantitative real time polymerase chain reaction
63 FOS, fructo-oligosaccharides
64

65 ## 1. Introduction

66 *Lactobacillus reuteri* is a stable member of sourdough microbiota (Gänzle and Vogel,
67 2002; De Vuyst and Neysens, 2005) that occurs predominantly in industrial sourdough
68 fermentations prepared for the production of baking improvers (Brandt, 2007) and in
69 cereal fermentation in tropical climates (Vogel et al., 1999). Sucrose is the most abundant
70 carbon source in ungerminated cereal grains. Wheat and rye grains contain more than
71 0.6% and 1.2% sucrose, respectively, and sucrose is commonly used in bread formulas.
72 Raffinose is additionally present in concentrations of 0.1 – 0.4% (Belitz et al., 2004). The
73 ability of *L. reuteri* to metabolize sucrose and raffinose as sole carbon sources contributes
74 to its ecological fitness in food fermentations, and impacts the quality of fermented cereal
75 products (Figure 1, Tieking et al., 2005; Schwab et al., 2007; Teixeira et al., 2012).

76 Lactobacilli harbour a multitude of enzymes and transport systems involved in sucrose
77 metabolism (Kaplan and Hutkins, 2003; Barrangou et al., 2006; van Hijum et al., 2006;
78 Goh et al., 2006; Saulnier et al., 2007). Sucrose metabolism is mediated by β-
79 fructofuranosidases (SacA or BfrA) catalyzing hydrolysis of sucrose or sucrose-
80 phosphate (Saulnier et al., 2007; Nakai et al., 2012; for review, see Gänzle and Follador,
81 2012). The intracellular sucrose phosphorylase (ScrP) or extracellular glucansucrases and
82 fructansucrases complement or substitute β-fructofuranosidases in few *Lactobacillus* spp.
83 (van Hijum et al., 2006; Gänzle and Follador, 2012). Fructansucrases but not
84 glucansucrases also use raffinose, stachyose, and verbascose as substrates (van Hijum et
85 al., 2006; Teixeira et al., 2012).

86 The expression of sucrose PTS systems is regulated by operon-specific (local)
87 transcriptional regulators, in conjunction with the catabolite control protein A, CcpA, a

88 transcriptional regulator of the LacI/GalR family (Reid and Abratt, 2005, Andersson et
89 al., 2005 Monedero et al., 2007, Francke et al., 2008). In *L. plantarum, L. paracasei* and
90 *L. acidophilus*, sucrose metabolic genes are induced by sucrose or short chain fructo-
91 oligosaccharides. MrmR / SacR were identified as putative local regulators of the sucrose
92 catabolic operons on the basis of sequence similarities to other regulatory proteins (Goh
93 et al., 2006; Saulnier et al., 2007). Taken together, these mechanisms mediate repression
94 of carbon catabolite operons in the presence of more favourable carbon sources, and
95 expression in the presence of the substrates.
96 Genome sequence data of more than 10 strains of *L. reuteri* in combination with
97 functional analysis of sucrose metabolic enzymes demonstrate that sucrose utilization in
98 this species is mediated by extracellular glucansucrases or fructansucrases and sucrose
99 phosphorylase. Sucrose PTS systems or intracellular β-fructofuranosidases are absent
100 (Schwab et al., 2007; Frese et al., 2011; Teixeira et al, 2012, Gänzle and Follador, 2012).
101 The expression of sucrose phosphorylase and levansucrase in *L. reuteri* is induced by
102 sucrose but the expression of glucansucrases is not influenced by the carbon source
103 (Schwab et al., 2006; Årsköld et al., 2007). The regulation of sucrose catabolism in *L.*
104 *reuteri* thus differs fundamentally from *L. plantarum* and *L. acidophilus*, the two species
105 of lactobacilli for which experimental data is available (for review, see Gänzle and
106 Follador, 2012). It was the aim of this study to characterize the regulation of sucrose
107 metabolism in *L. reuteri*. The study employed *L. reuteri* LTH5448, a sourdough isolate
108 harbouring a sucrose phosphorylase (*scrP*) and a levansucrase (*ftfA*) (Schwab et al., 2007;
109 Teixeira et al., 2012). The role of the putative sucrose regulator *scrR* was elucidated by

110 quantification of the expression of sucrose metabolic genes in a *scrR* mutant and the wild
111 type strain.

112 **2. Materials and methods**

113 **2.1 Strains, media and growth conditions.**

114 Bacterial strains and plasmids used in this study are shown in Table 1. *Escherichia coli*
115 JM109 (Promega, Nepean, Canada) was cultured in Luria-Bertani (LB) broth at 37°C. *L.*
116 *reuteri* strains were anaerobically cultivated at 37 °C in modified deMan-Rogosa-Sharpe
117 medium (mMRS) (Stolz et al., 1995) or MRS containing 20 g L^{-1} sucrose (sucMRS); 20 g
118 L^{-1} raffinose (rafMRS); 20 g L^{-1} fructo-oligosaccharides (fosMRS); and 20 g L^{-1} each of
119 glucose and sucrose (glusucMRS) as carbon sources. Fructo-oligosaccharides were
120 obtained from Orafti (Tienen, Belgium) with a degree of polymerization of 3 – 8. HPLC
121 analysis verified that glucose, fructose and sucrose were essentially absent (data not
122 shown). Other carbohydrates were purchased with 99% purity from Sigma (Oakville,
123 Canada).

124 The production of oligosaccharides and exopolysaccharides was also monitored in MRS
125 containing 100 g L^{-1} sucrose (suc100MRS). Ampicillin (100 mg L^{-1}) or erythromycin
126 (500 mg L^{-1}) was added to LB for selecting antibiotic-resistant *E. coli*. Erythromycin (10
127 mg L^{-1}) was added to mMRS medium to select erythromycin-resistant *L. reuteri.*

128 **2.2 General molecular methods.**

129 Genomic DNA was isolated from overnight cultures grown in mMRS using DNeasy
130 Blood & Tissue kit (Qiagen, Mississauga, Canada). DNA was amplified by polymerase
131 chain reaction (PCR) using Taq DNA polymerase and dNTPs from Invitrogen
132 (Burlington, Canada). Primer design for sequencing *scrR*, *scrP*, and the flanking regions

133 of the genes was based on the genome sequence of *L. reuteri* 100-23. Primers were
134 purchased from Integrated DNA Technologies (Coralville, USA) and are listed in Table
135 2. PCR products were visualized after electrophoretic separation on agarose gels. DNA
136 sequencing was performed by Macrogen (MacrogenUSA, Rockville, USA).

137 **2.3 Phylogenetic analysis of *scrR*.**

138 Sucrose regulators and CcpA were aligned using CLUSTALW implemented in BioEdit.
139 Phylogenetic analysis of ScrR sequences was performed using MEGA (Tamura et al.,
140 2011) and the maximum likelihood method applying the Jones-Taylor-Thornton (JTT)
141 substitution model. Bootstrap support was calculated for 100 replicates.

142 **2.4 Generation and verification of *L. reuteri* LTH5448Δ*scrP* and LTH5448Δ*scrR***
143 **mutants.**

144 Sequencing of *scrP* and *scrR* genes in *L. reuteri* LTH548 was achieved with primers
145 specific for the corresponding loci in the genome sequenced strain *L. reuteri* 100-23
146 (Gene bank Accession number AAPZ00000000.2, primers listed in Table 2). In-frame
147 truncation of *scrP* and *scrR* genes was achieved with the temperature-sensitive shuttle
148 vector pJRS233 (Su et al., 2011). In brief, flanking fragments of the target genes were
149 amplified from genomic DNA of *L. reuteri* LTH5448 by PCR (primers are listed in Table
150 2). The amplicons were ligated into pGEMTeasy vector (Promega) to generate pScrP-A,
151 pScrP-B, pScrR-A, and pScrR-B (Table 1). The flanking fragments of *scrP* and *scrR*
152 from these plasmids were digested with the respective restriction enzymes (New England
153 Biolabs, Pickering, Canada), purified, and ligated into pGEMTeasy to create pScrP-AB
154 and pScrR-AB. The co-ligated DNA fragments in pScrP-AB and pScrR-AB were cut
155 with the respective restriction enzymes, and ligated into pJRS233 using T4 DNA ligase

156 (Epicentre, Markham, Canada). The resulting plasmids pKO-*scrP*-AB and pKO-*scrR*-AB
157 were electrotransformed into competent *L. reuteri* LTH5448 cells suspended in water
158 with 30% (v/v) polyethylene glycol (MW 3350; J.T. Baker Chemical, Phillipsburg, NJ).
159 Transformants were grown in mMRS broth containing erythromycin at 42°C for 80
160 generations to select for single crossover mutants. *L. reuteri* with pKO-*scrP*-AB and
161 pKO-*scrR*-AB integrated into chromosome were cured by culturing in mMRS broth at
162 37°C for 100 generations. Erythromycin-sensitive double crossover mutants were
163 identified by replica plating on mMRS and mMRS-erythromycin agar. The in-frame
164 deletion of *scrP* and *scrR* in *L. reuteri* LTH5448Δ*scrP* and *L. reuteri* LTH5448Δ*scrR*,
165 respectively, was confirmed by PCR. DNA sequencing was conducted to verify the
166 deletion region using sequencing primers (Table 2).

167 **2.5 Isolation of mRNA and synthesis of cDNA libraries.**

168 RNA was isolated from exponentially growing cells (OD_{600} of 0.5) of *L. reuteri*
169 LTH5448 and its mutant LTH5448Δ*scrR* in mMRS, gluMRS, sucMRS, rafMRS or
170 glusucMRS. Two volumes of RNAprotect (Qiagen) were added, and cells were harvested
171 by centrifugation at 5000 × *g*. Cells were resuspended in 1 mL TRIzol (Invitrogen), and
172 disrupted using a bead beater (Biospec products, Bartlesville, USA) and 0.1 mm zirconia-
173 silica beads. The supernatant was transferred to 1.5 mL Eppendorf tubes for RNA
174 isolation according to TRIzol manufacturer's manual. Reagents and enzymes for DNase I
175 treatment and reverse transcription were purchased from Promega (Madison, USA) and
176 used according to the manufacturer's instructions. Transcription of *scrP*, *scrR,* and *ftfA* in
177 wild type *L. reuteri* LTH5448 grown in mMRS and sucMRS was analysed by PCR using
178 the primers shown in Table 2.

179 **2.6 Quantification of transcriptional levels of the *scrP*, *scrR* and *ftfA* genes in**
180 **response to glucose, sucrose, and raffinose.**

181 Quantitative analysis of the expression of *scrP*, *scrR,* and *ftfA* in *L. reuteri* LRH5448 and
182 LTH5448Δ*scrR* was carried out using cDNA as a template in quantitative PCR (qPCR)
183 reactions. DNase-I treated mRNA preparations were used as negative control;
184 chromosomal DNA was used as positive control. Amplifications were carried out in a
185 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) with primers
186 listed in Table 2. Melting curve analysis and determination of amplicon size by agarose
187 gel electrophoresis verified the specific amplification of the appropriate cDNA.
188 Expression levels of *scrP*, *scrR* and *ftfA* in cultures grown in mMRS, gluMRS, rafMRS
189 or glusucMRS were calculated by the $\Delta\Delta C_T$ method according to Pfaffl (2001). The
190 phosphoketolase gene (*pho)* was used as housekeeping gene. Maltose and / or sucrose
191 rather than glucose are preferred carbon sources for *L. reuteri* (Stolz et al., 1993; Schwab
192 et al., 2007); therefore, cultures grown in sucMRS were used as reference conditions.
193 Gene expression was quantified in duplicate from cDNA libraries obtained from three
194 (*scrP* and *ftfA*) or two (*scrR*) independent experiments.

195 **2.7 Determination of growth kinetics and metabolite formation.**

196 Growth of *L. reuteri* LTH5448, LTH5448Δ*scrP*, and LTH5448Δ*scrR* was assessed in
197 mMRS, sucMRS, rafMRS or fosMRS. Media were inoculated with 1% of washed
198 overnight cultures, incubated at 37°C, and growth was monitored by changes in the
199 optical density (OD) at 600 nm over 24 hours. Samples were taken for metabolite
200 analysis by HPLC as described (Teixeira et al., 2012). Data shown are mean values for

201 duplicate (*L. reuteri* LTH5448 and LTH5448Δ*scrP*) or triplicate (LTH5448Δ*scrR*)
202 independent experiments.
203 Oligosaccharide and levan formation by *L. reuteri* LTH5448 and LTH5448Δ*scrP* was
204 assessed in suc100MRS. Media were inoculated with overnight cultures and incubated at
205 37°C. Samples were taken for analysis by HPAEC-PAD and size exclusion
206 chromatography as described (Galle et al., 2010). The amount of high molecular weight
207 levan (relative molecular weight of more than 10^5) in suc100MRS was calculated using
208 purified levan from *L. reuteri* LTH5448 purified by ethanol precipitation, dialysis, and
209 phenol-chlorofom extraction (Wang et al., 2010) as standards. Levan with intermediate
210 molecular weight (10^4 – 10^5) was not quantified in culture supernatants due to
211 interference of media components. Data shown are mean values for duplicate (FOS) or
212 triplicate (levan) independent experiments.
213 Polysaccharides from culture and supernatant were hydrolyzed with 2M H_2SO_4 at 80°C
214 for 2 hour in order to differentiate bound levan from levan released into the culture
215 medium. The monosaccharides obtained from acid hydrolysis were quantified with an
216 Aminex 87H column as described (Schwab et al., 2007). To assess levan hydrolysis by *L.*
217 *reuteri* LTH5448 and LTH5448ΔftfA, cells from overnight cultures were washed with
218 50 mmol L^{-1} phosphate buffer (pH 6.5) and incubated with 5 g L^{-1} levan for 24h. Samples
219 were taken periodically and levan was quantified by size exclusion chromatography as
220 described above. The incubation in phosphate buffer avoids interference with media
221 components, and thus allows quantification of levan with intermediate molecular weight
222 (molecular weight range 10^4 -> 10^6).
223 **2.8 Nucleotide Accession numbers.**

224 The nucleotide sequences of *scrP* and *scrR* in *L. reuteri* LTH5448, and the sequences of
225 truncated *scrP* and *scrR* in *L. reuteri* LTH5448Δ*scrP* and *L. reuteri* LTH5448Δ*scrR* are
226 deposited in GenBank (accession number KC539460, KC539461, KC539462, and
227 KC539463, respectively.

228 **3. Results**

229 **3.1 Identification of genes coding for sucrose phosphorylase and a putative sucrose**
230 **dependent regulator in *L. reuteri* LTH5448.**

231 A partial sequence of *scrP* coding for sucrose phosphorylase was previously identified in
232 *L. reuteri* LTH5448 (Schwab et al., 2007). Sequencing of this gene and its flanking
233 regions confirmed the presence of a sucrose phosphorylase in *L. reuteri* LTH5448. The
234 gene sequence and the genetic organization (Figure 2A) are homologous to *L. reuteri*
235 100-23.

236 Bioinformatic analysis of the genome of *L. reuteri* 100-23 identified the putative sucrose
237 regulator *scrR*. The *scrR* gene was flanked by divergently oriented muramidase and a
238 hypothetical protein (Figure 2B) and located distant from *scrP* or *ftfA*. An *scrR* gene
239 (length 942 base pairs) was amplified by PCR in *L. reuteri* LTH5448. In *L. reuteri*
240 LTH5448, the coding region of *scrR* and *scrP* and their flanking sequences were highly
241 homologous to *L. reuteri* 100-23. The prediction of putative regulator ScrR encodes 314
242 amino acid residues. The gene is present in all sequenced genomes of *L. reuteri* strains
243 with 96-98% homology, and is highly homologous to sucrose regulators of *Streptococcus*
244 *mutans* (ScrR, U 46902) (48% identity in 307 aa) and *L. acidophilus* NCFM (MsmR,
245 68% identity in 141 aa). ScrR of *L. reuteri* clustered with sucrose regulators of other
246 lactic acid bacteria and bifidobacteria in a cluster distant from the global regulator CcpA

247 (Figure 3). The putative sucrose regulator ScrR possesses the N-terminal helix-turn helix
248 (HTH) DNA binding domain of the LacI-GalR-type transcriptional regulators, and the
249 core unit with a motive indicative of periplasmic binding proteins and the sugar binding
250 domain of the LacI family. Qualitative analysis of cDNA libraries of *L. reuteri*
251 LTH54448 indicated that *scrR* is expressed in *L. reuteri* LTH5448 (data not shown).

252 **3.2 Generation of *scrR* and *scrP* deletion mutants of *L. reuteri* LTH5448**

253 To identify the regulatory role of *scrR* in transcription of *scrP* and *ftfA,* and the impact of
254 *scrP* on sucrose utilization, double crossover mutants of *L. reuteri* LTH5448 were
255 generated. The truncation in *scrP* of *L. reuteri* LTH5448Δ*scrP* was confirmed by PCR
256 with the primers *pho*-KO1b-F-*Xho*I and *pho*-KO4b-R-*Pst*I (Table 2). PCR amplified the
257 expected amplicons of 3.2 kb and 1.8 kb with DNA from *L. reuteri* LTH5448 and
258 LTH5448Δ*scrP*, respectively, as template. A deletion in *scrR* of *L. reuteri*
259 LTH5448Δ*scrR* was verified by PCR with the primers *reg*-KO1a-F-*XhoI* and *reg*-KO4b-
260 R-*HindIII* (Table 2), yielding amplicons of 3.3 kb and 2.6 kb for *L. reuteri* LTH5448 and
261 LTH5448Δ*scrR*, respectively. DNA sequencing verified the truncation of *scrP* and *scrR*
262 in *L. reuteri* LTH5448Δ*scrP* and LTH5448Δ*scrR*, respectively.

263 **3.3 Expression of *scrP* and *scrR* in *L. reuteri* LTH5448 and LTH5448Δ*scrR.***

264 The transcription of *scrP, scrR*, and *ftfA* in wild type *L. reuteri* and its LTH5448Δ*scrR*
265 mutant in response to glucose, sucrose, and raffinose was quantified relatively to its
266 expression in sucMRS (Table 3 and data not shown). The wild type strain showed
267 comparable expression of *scrR* when grown with or without sucrose (data not shown).
268 *ScrP* was expressed in presence of sucrose and raffinose, but was not repressed by
269 glucose. In contrast to the wild type strain, the LTH5448Δ*scrR* mutant strain showed

270 expression of *scrP* when grown without sucrose (Table 3). The levanuscrase FtfA was
271 also highly expressed in the presence of sucrose but expression levels were much lower
272 when sucrose was absent. Comparable to the expression of *scrP,* expression of *ftfA* in *L.*
273 *reuteri* LTH5448 was not repressed by glucose and deletion of *scrR* resulted in
274 constitutive expression of the enzyme (Table 3). These data demonstrate that *scrR* codes
275 for a transcriptional regulator of the LacI/GalR-family that mediates repression of sucrose
276 metabolic genes in the absence of sucrose.

277 **3.4 Growth kinetics and carbohydrate metabolism in mMRS**

278 In order to determine the contribution of *scrP* and *scrR* to sucrose metabolism, growth
279 and carbohydrate metabolism of *L. reuteri* LTH5448 and its mutants were compared in
280 media with different carbon sources. There were no differences in the growth of *L.*
281 *reuteri* LTH5448, LTH5448Δ*scrP* and LTH5448Δ*scrR* during growth in mMRS,
282 sucMRS or rafMRS (Figure 4). Growth and metabolism of *L. reuteri* LTH5448 and
283 LTH5448Δ*scrR* were also compared in gluMRS. The results confirmed that disruption of
284 *scrR* does not result in a phenotype that is detectable by metabolite analysis (data not
285 shown). Samples obtained at 24 hours of fermentation showed that all strains completely
286 metabolized the sugars in all three media (data not shown), formed comparable amounts
287 of lactate, acetate, and ethanol, and reduced comparable amounts of fructose to mannitol
288 (Table 4). These similarities can be explained by the fact that the expression of sucrose
289 metabolic genes is not different between *L. reuteri* LTH5448 and LTH5448Δ*scrR* when
290 the substrate is present. In the absence of the substrate, the expression of sucrose
291 metabolic genes in *L. reuteri* LTH5448Δ*scrR* does not influence sucrose metabolism. In
292 *L. reuteri* LTH5448Δ*scrP*, FtfA activity compensates for the absence of ScrP. In

293 fosMRS, all strains grew poorly and did not produce considerable amounts of organic
294 acids (data not shown).

295 **3.5 Synthesis of levan and fructo-oligosaccharides**

296 *L. reuteri* LTH5448 and LTH5448Δ*scrP* produced FOS and levan during growth in
297 suc100MRS. Wild type and LTH5448Δ*scrP* produced four different FOSs (FOS_1-FOS_4)
298 (Figure 5). Maximum levan levels were 4.6 g L^{-1} in *L. reuteri* LTH5448 and 6.7 g L^{-1} in
299 LTH5448Δ*scrP* after 6 and 8 hours of fermentation, respectively (Figure 5B). Levan
300 concentration increased during the exponential phase of growth followed by an apparent
301 decrease during subsequent incubation. The quantification of total fructans after
302 hydrolysis of levan and FOS with H_2SO_4 indicated that this decrease is attributable to
303 partial hydrolysis of levan to polysaccharides with intermediate molecular weight that
304 were not accounted by the analytical tools employed. Indeed, inubation of *L. reuteri*
305 LTH5448 or LTH5448Δ*ftfA* with purified levan in phosphate buffer resulted in a reduced
306 molecular weight of levan but not in hydrolysis to fructose or FOS, excluding that FtfA
307 was responsible for the decrease in levan concentration during stationary growth phase
308 (data not shown).

309 **4. Discussion**

310 A multitude of enzymes, transport systems, and regulatory elements mediate sucrose
311 metabolism in lactobacilli (van Hijum et al., 2006; Goh et al., 2006; Saulnier et al., 2007).
312 Metabolic pathways for the metabolism of sucrose and fructo-oligosaccharides partially
313 overlap (Kaplan and Hutkins, 2003; Goh et al., 2006; Saulnier et al., 2007; Gänzle and
314 Follador, 2012). Transcriptome analysis in *L. acidophilus* suggested that metabolism of
315 sucrose but not metabolism of FOS in *L. acidophilus* is likely mediated by a sucrose PTS

316 system (Barrangou et al 2006). Functional analysis of sucrose metabolism in *L.*
317 *plantarum* and *L. paracasei* demonstrated that transport and hydrolysis of sucrose as well
318 as short chain FOS are mediated by phosphotransferase systems and associated
319 (phospho)-β-fructofuranosidases (Saulnier et al., 2007; Goh et al., 2006).

320 In *L. reuteri* LTH5448, sucrose metabolism occurs via two alternative enzymes, the
321 extracellular levansucrase FtfA and the intracellular sucrose phosphorylase ScrP. Sucrose
322 phosphorylase is inducible by sucrose and raffinose in *L. reuteri, L. acidophilus* and
323 *Bifidobacterium animalis* (Trindade et al., 2003; Barrangou et al., 2006, Schwab et al.,
324 2007; Teixeira et al., 2012) but does not recognize FOS or raffinose as substrate
325 (Kawasaki et al., 1996; van der Broek et al., 2004). The induction of ScrP by raffinose is
326 thus likely mediated by the intracellular release of sucrose from raffinose by intracellular
327 α-galactosidase activity (Yoon and Hwang, 2008; Teixeira et al., 2012).

328 Sucrose and raffinose utilization by *L. reuteri* LTH5448 was not impaired by deletion of
329 sucrose phosphorylase as ScrP and FtfA are equally suited to support rapid turnover of
330 sucrose (this study, Schwab et al., 2007). Remarkably, *L. reuteri* LTH5448Δ*scrP*
331 produced about 25% more levan, confirming that the lack of sucrose phosphorylase is
332 compensated by increased turnover by levansucrase as sole sucrose converting enzyme.
333 Formation of 3.3 g levan / kg by *L. reuteri* during growth in sourdough improved bread
334 volume, and delayed staling (Galle et al., 2012); the use of sucrose phosphorylase-
335 negative strains may thus be beneficial in applications aiming at a high yield of polymeric
336 fructan.

337 This study identified a putative sucrose regulator ScrR of *L. reuteri* with homology to
338 regulatory proteins of the GalR-LacI family including MsmR and SacR. Structurally

339 related ScrR regulate the (phospho)-β-fructofuranosidases ScrA and enzymeII^{suc} of
340 sucrose PTS systems in *S. mutans, P. pentosaceus, L. lactis* (Figure 3) and sucrose
341 phosphorylases in *Bifidobacterium lactis* (Gering and Brückner, 1996; Luesink et al.,
342 1999; Hiratsuka et al., 1998; Trinidade et al., 2003). In contrast to *L. plantarum* and *L.*
343 *acidophilus*, genes coding for sucrose metabolic enzymes and their regulation in *L.*
344 *reuteri* are not encoded on a single operon but are on three distinct genetic loci.
345 Moreover, sucrose metabolic enzymes in *L. reuteri* do not support catabolism fructo-
346 oligosaccharides other than sucrose but contribute to metabolism of raffinose-family
347 oligosaccharides (Teixeira et al., 2012; this study).
348 Inactivation of *scrR* in *L. reuteri* LTH5448 resulted in constitutive transcription of *ftfA*
349 and *scrP* when the strain was grown in the presence of media containing glucose,
350 indicating that *scrR* is a transcriptional repressor similar to *scrR* of *L. lactis, S. mutans,*
351 and *Staphylococcus xylosus* (Gering and Brückner, 1996; Luesink et al., 1999; Hiratsuka
352 et al., 1998). Regulation of gene expression by *scrR* in *L. reuteri* is thus substrate
353 controlled, comparable to the function of *scrR* of *B. animals* (Trindade et al., 2003) and *S.*
354 *mutans, msmR* and *scrR* of *L. acidophilus* and *L. plantarum,* respectively. Moreover, *scrR*
355 in *L. reuteri* regulated the expression of *ftfA*. Regulation of fructansucrases by *scrR* was
356 not observed in past studies on transcriptional regulators of sucrose metabolism in lactic
357 acid bacteria. In *L. sanfranciscensis*, levansucrase is the only enzyme capable of sucrose
358 hydrolysis and is constitutively expressed (Tieking et al., 2005). In addition to its
359 contribution to sucrose and raffinose metabolism in *L. reuteri*, levansucrase also provides
360 protection against environmental stressors and its expression is induced by heat stress and
361 sublethal concentrations of proton-ionophores (Schwab et al., 2006). The finding that *ftfA*

362 is regulated by *scrR* adds to our understanding of the multiple roles of levansucrases in
363 the ecology of *L. reuteri* (Gänzle and Schwab, 2009).
364 In conclusion, *L. reuteri* takes a very different approach to utilize sucrose and to regulate
365 sucrose metabolic genes when compared to its closely related organisms. Lactobacilli
366 have adapted to nutrient rich habitats by reduction of the genome size (Makarova and
367 Koonin, 2007). Among lactobacilli, *L. plantarum* has retained a relatively large genome
368 size and the ability to metabolise a large spectrum of carbohydrates, and is found in
369 diverse habitats (Makarova and Koonin, 2007; Siezen and van Hylckama Vlieg, 2011).
370 Glucose is metabolized preferentially over maltose or sucrose, reflecting carbon
371 catabolite repression (Andersson et al., 2005; Saulnier et al., 2007). *L. reuteri* underwent
372 a more extensive reductive evolution to adapt to specific vertebrate hosts (Walter, 2008;
373 Frese et al., 2011). In these narrow ecological nieches, particularly the upper intestine of
374 (grain-feeding) animals, sucrose and raffinose are major carbohydrate sources. This study
375 demonstrates that sucrose metabolism in *L. reuteri* is not subject to repression by glucose.
376 *L. reuteri* apparently has abandoned regulatory circuits to repress sucrose metabolic
377 enzymes in presence of glucose. This preferential metabolism of sucrose contributes to
378 the ecological fitness of *L. reuteri* (Sims et al., 2011; Walter et al., 2008). Differences in
379 the regulation of sucrose metabolism between *L. reuteri* and other lactobacilli thus reflect
380 the ecological adaptation of *L. reuteri.*
381 Sourdough isolates of *L. reuteri* do not represent a separate, extra-intestinal evolutionary
382 lineage of *L. reuteri* but are of intestinal origin (Su et al. 2012). The concurrent
383 expression of sucrose (and maltose) metabolic enzymes matches the carbohydrate
384 availability in cereal fermentations, and accounts for the simultaneous utilization of

385 carbohydrates during growth in sourdough (this study, Gänzle et al., 2007). The
386 simultaneous utilization of maltose and sucrose also determines the formation of the
387 exopolysaccharides reuteran and levan as well as acetate, and thus has a substantial
388 impact on bread quality (Tieking et al., 2003; Kaditzky and Vogel, 2008; Galle et al.,
389 2012).

390 **Acknowledgements**

391 We are grateful to Xiaoyan Chen for providing levan purified from *L. reuteri* LTH5448
392 for use as external standard. The NSERC Discovery grant program is acknowledged for
393 funding. M. Gänzle acknowledges support from the Canada Research Chairs program.

394 **References**

395 Andersson, U., Molenaar, D., Radström, P., & de Vos, W.M. (2005). Unity in
396 organisation and regulation of catabolic operons in *Lactobacillus plantarum, Lactococcus*
397 *lactis*, and *Listeria monocytogenes*. *Systematic and Applied Microbiology,* 28, 187-195.

398 Årsköld, E., Svennson, M., Grage, H., Roos, S., Rådström, P., & van Niel, E.W.J. (2007).
399 Environmental influences on exopolysaccharide formation in *Lactobacillus reuteri*
400 ATCC55730. *International Journal of Food Microbiology,* 116, 159-167.

401 Barrangou, R., Azcarate-Peril, M.A., Duong, T., Conners, S.B., Kelly, R.M., &
402 Klaenhammer, T.R. (2006). Global analysis of carbohydrate utilization by *Lactobacillus*
403 *acidophilus* using cDNA microarrays. *Proceedings of the National Academy of Science*
404 (USA), 103, 3816-3821.

405 Belitz, H.D., Grosch, W., & Schieberle, P. (2004). Food Chemistry (3rd ed). Berlin:
406 Springer (Chapter 15).

407 Brandt, M.J. (2007). Sourdough products for convenient use in baking. *Food*
408 *Microbiology*, 24, 161-164.
409 Daniel, R.A., Haiech, J., Denizot, F., & Errington, J. (1997). Isolation and
410 characterization of the lacA gene encoding beta-galactosidase in *Bacillus subtilis* and a
411 regulator gene lacR. *Journal of Bacteriology,* 179, 5636 – 5638.
412 De Vuyst, L.D. & Neysens, P. (2005). The sourdough microflora: biodiversity and
413 metabolic interations. *Trends in Food Science & Technology*, 16, 43-56.
414 Francke, C., Kerkhoven, R., Wels, M., & Siezen, R. (2008). A generic approach to
415 identify transcription factor specific operator motifs; inferences for LacI-family mediated
416 regulation in *Lactobacillus plantarum* WCFSI. *BMC Genomics,* 9, 145.
417 Frese, S.A., Benson, A.K., Tannock, G.W., Loach, D.M., Kim, J., Zhang, M., Oh, P.L.,
418 Heng, N.C., Patil, P.B., Juge, N., Mackenzie, D.A., Pearson, B.M., Lapidus, A., Dalin, E.,
419 Tice, H., Goltsman, E., Land, M., Hauser, L., Ivanova, N., Kyrpides, N.C., & Walter, J.
420 (2011). The evolution of host specialization in the vertebrate gut symbiont *Lactobacillus*
421 *reuteri*. *PLoS Genetics*, 7, e1001314.
422 Gänzle, M.G., Vermeulen, N., & Vogel, R.F. (2007). Carbohydrate, peptide and lipid
423 metabolism of lactobacilli in sourdough. *Food Microbiology,* 24, 128-138.
424 Gänzle, M. & Schwab, C. (2009). Ecology of exopolysaccharide formation by lactic acid
425 bacteria: sucrose utilization, stress tolerance, and biofilm formation. In M. Ullrich (ed),
426 *Bacterial polysaccharides* (pp. 263-278). Norfolk: Caister Academic Press.
427 Gänzle, M.G., & Follador, R. (2012). Metabolism of oligosaccharides in lactobacilli: a
428 review. *Frontiers in Microbiology,* 3, 340.

429 Galle, S., Schwab, C., Arendt, E., & Gänzle, M.G. (2010). Exopolysaccharide-forming
430 *Weissella* strains as starter cultures for sorghum and wheat sourdoughs. *Journal of*
431 *Agricultural and Food Chemistry*, 58, 5834–5841.
432 Galle, S., Schwab, C., Dal Bello, F., Coffey, A., Gänzle, M., & Arendt, E. (2012).
433 Influence of in situ synthesised exopolysaccharides on the quality of gluten-free sorghum
434 sourdough bread. *International Journal of Food Microbiology*, 155, 105-112.
435 Gering, M., & Brückner, R. (1996). Transcriptional regulation of the sucrase gene of
436 *Staphylococcus xylosus* by the Repressor ScrR. *Journal of Bacteriology,* 178, 462–469.
437 Goh, Y.J., Zhang, C., Benson, A.K., Schlegel, V., Lee, J.H., & Hutkins, R.W. (2006).
438 Identification of a putative operon involved in fructooligosaccharide utilization by
439 *Lactobacillus paracasei*. *Applied and Environmental Microbiology*, 72, 7518-7550.
440 Hiratsuka, K., Wang, B., Sato, Y., & Kuramitsu, H. (1998). Regulation of sucrose-6-
441 phosphate hydrolase activity in *Streptococcus mutans*: Characterization of the *scrR* Gene.
442 *Infection and Immunity,* 66, 3736–3743.
443 Kaditzsky, S., Seitter, M., Hertel, C., & Vogel, R.F. (2008). Performance of *Lactobacillus*
444 *sanfranciscensis* TMW 1.392 and its levansucrase deletion mutant in wheat dough and
445 comparison of their impact on bread quality. *European Food Research and Technology,*
446 227, 433-442.
447 Kaplan, H., & Hutkins, R.W. (2003). Metabolism of fructooligosaccharides by
448 *Lactobacillus paracasei* 1195. *Applied and Environmental Microbiolog,* 69, 2217–2222.
449 Kawasaki, H., Nakamura, N., Ohmori, M., and Sakai, T. (1996). Cloning and expression
450 in *Escherichia coli* of sucrose phosphorylase gene from *Leuconostoc mesenteroides* No.
451 165. *Bioscience Biotechnology and Biochemistry,* 60, 322-324.

452 Luesink, E.J., Marugg, J.D., Kuipers O.P., & de Vos, W.M. (1999). Characterization of
453 the divergent *sacBK* and *sacAR* operons, involved in sucrose utilization by *Lactococcus*
454 *lactis*. *Journal of Bacteriology,* 181, 1924–1926.
455 Makarova, K.S. & Koonin, E.V. (2007). Evolutionary genomics of lactic acid bacteria.
456 *Journal of Bacteriology*, 189, 1199-1208.
457 Monedero, V., Mazé, A., Boël, G., Zúñiga, M., Beaufils, S., Hartke, A., & Deutscher, J.
458 (2007). The phosphotransferase system of *Lactobacillus casei*: regulation of carbon
459 metabolism and connection to cold shock response. *Journal of Molecular Microbiology*
460 *and Biotechnology*, 12, 20-32.
461 Nakai, H., Svensson, B., & Ohtsubo, K. (2012). Substrate recognition by two members of
462 glycoside hydrolase family 32 involved in fructo-oligosaccharide metabolism in
463 *Lactobacillus acidophilus* NCFM. Bulletin of the Faculty of Agriculture, Niigata
464 University, 64, 115-126.
465 Perez-Casal, J., Price, J.A., Maguin, E., & Scott, J.R. (1993). An M protein with a single
466 C repeat prevents phagocytosis of *Streptococcus pyogenes*: use of a temperature-sensitive
467 shuttle vector to deliver homologous sequences to the chromosome of *S. pyogenes*.
468 *Molecular Microbiology*, 8, 809-819.
469 Pfaffl, M.W. (2001). A new mathematical model for relative quantification in real-time
470 RT-PCR. *Nucleic Acids Research, 29*, e45.
471 Reid, S.J., & Abratt, V.R. (2005). Sucrose utilisation in bacteria: genetic organisation and
472 regulation. *Applied Microbiology and Biotechnolology,* 67, 312-321.

473 Saulnier, D.M.A., Molenaar, D., de Vos, W.M., Gibson, G.R., & Kolida, S. (2007).
474 Identification of prebiotic fructooligosaccharide metabolism in *Lactobacillus plantarum*
475 WCFS1 through microarrays. *Applied and Environmental Microbiology* 73, 1753-1765.
476 Schwab, C., & Gänzle, M.G. (2006). Effect of membrane lateral pressure on the
477 expression of fructosyltransferases in *Lactobacillus reuteri. Systematic and Applied*
478 *Microbiology*, 29, 89-99.
479 Schwab, C., Walter, J., Tannock, G.W., Vogel, R.F., & Gänzle, M.G. (2007). Sucrose
480 utilization and impact of sucrose on glycosyltransferase expression in *Lactobacillus*
481 *reuteri*. *Systematic and Applied Microbiology*, 30, 433-443.
482 Siezen, R.J. & van Hulckama Vlieg, J.E.T. (2011). Genomic diversity and versatility of
483 *Lactobacillus plantarum*, a natural metabolic engineer. *Microbial Cell Factories*,
484 10(Suppl. 1), S3.
485 Sims, I.M., Frese, S.A., Walter, J., Loach, D., Wilson, M., Appleyard, K., Eason, J.,
486 Livingston, M., Baird, M., Cook, G., & Tannock, G.W. (2011). Structure and functions of
487 exopolysaccharide produced by gut commensal *Lactobacillus reuteri* 100-23. *ISME*
488 *Journal*, 5:1115-1124.
489 Stolz, P., Böcker, G., Vogel, R. F., & Hammes, W. P. (1993). Utilization of maltose and
490 glucose by lactobacilli isolated from sourdough. *FEMS Microbiology Letters,* 109, 237-
491 242.
492 Stolz, P., Böcker, G., Hammes, W. P., & Vogel, R. F. (1995). Utilization of electron
493 acceptors by lactobacilli isolated from sourdough. *Zeitschrift für Lebensmittel-*
494 *Untersuchung und-Forschung,* 201, 91-96.

495 Su, M.S.W., Schlicht, S., & Gänzle, M.G. (2011). Contribution of glutamate
496 decarboxylase in *Lactobacillus reuteri* to acid resistance and persistence in sourdough
497 fermentation. *Microbial Cell Factories,* 10(Suppl 1), S8.
498 Su, M.S.W., Oh, P.L., Walter, J., & Gänzle, M.G. (2012). Phylogenetic, genetic, and
499 physiological analysis of sourdough isolates of *Lactobacillus reuteri*: food fermenting
500 strains are of intestinal origin. *Applied and Environmental Microbiology,* 78, 6777-6780.
501 Tamura, K., Peterson, D., Peterson, N., Stecher, G., & Kumar, S. (2011) MEGA5:
502 molecular evolutionary genetics analysis using maximum likelihood, evolutionary
503 distance and maximum parsimony methods. *Molecular Biolology and Evolution,* 28,
504 2731 – 2739.
505 Teixeira, J.S., McNeill, V., & Gänzle, M.G. (2012). Levansucrase and sucrose
506 phoshorylase contribute to raffinose, stachyose, and verbascose metabolism by
507 lactobacilli. *Food Microbiology,* 31, 278 - 284.
508 Tieking, M., Ehrmann, M.A., Vogel, R.F., & Gänzle, M.G. (2005). Molecular and
509 functional characterization of a levansucrase from *Lactobacillus sanfranciscensis*.
510 *Applied Microbiology and Biotechnology,* 66, 655-663.
511 Trindade, M.I., Abratt, V.R., & Reid, S.J. (2003). Induction of sucrose utilization genes
512 from *Bifidobacterium lactis* by sucrose and raffinose. *Applied and Environmental*
513 *Microbiology,* 69, 24–32.
514 Van der Broek, L.A.M., van Boxtel, E.L., Kievit, R.P., Verhoef, R., Beldman, G., &
515 Voragen, A.G.J. (2004). Physico-chemical and transglucosylation properties of
516 recombinant sucrose phosphorylase from *Bifidobacterium adolescentis* DSM20083.
517 *Applied Microbiology and Biotechnology,* 65, 219–227.

518 van Hijum, S. A., Kralj, S., Ozimek, L. K., Dijkhuizen, L., & van Geel-Schutten, I. G.
519 (2006). Structure-function relationships of glucansucrase and fructansucrase enzymes
520 from lactic acid bacteria. *Microbiolgy and Molecular Biology Reviews,* 70, 157-176.
521 Vogel, R.F., Knorr, R., Müller, M.R.A., Steudel, U., Gänzle, M.G., & Ehrmann, M.A.
522 (1999). Non-dairy lactic fermentations: The cereal world. *Antonie van Leeuwenhoek,* 76,
523 403-411.
524 Walter, J. (2008). Ecological role of lactobacilli in the gastrointestinal tract: implications
525 for fundamental and biomedical research. *Applied and Environmental Microbiolology,*
526 74, 4985-4996.
527 Walter, J., Schwab, C., Gänzle, M., & Tannock, G.W. (2008). Glucosyltransferase (GtfA)
528 and Inulosucrase (Inu) of *Lactobacillus reuteri* TMW1.106 contribute to cell aggregation,
529 *in vitro* biofilm formation and colonization of the mouse gastrointestinal tract.
530 *Microbiology* 154, 72-80.
531 Wang, Y., Gänzle, M.G., & Schwab, C. (2010). EPS synthesized by *Lactobacillus reuteri*
532 decreases binding ability of enterotoxigenic *Escherichia coli* to porcine erythrocytes.
533 *Applied and Environmental Microbiolology,* 76, 4863-4866.
534 Yoon, M.Y. & Hwang, H.-J. (2008). Reduction of soybean oligosaccharides and
535 properties of α-D-galactosidase from *Lactobacillus curvatus* R08 and *Leuconostoc*
536 *mesenteriodes* JK55. *Food Microbiology*, 25, 815-823.
537

538 **Figure legends.**

539 **Figure 1.** Sucrose and raffinose utilization in *L. reuteri* LTH5448 (modified from 540 Teixeira et al., 2012). FtfA, levansucrase, α-Gal, α-galactosidase, SucP, sucrose 541 phorphorylase. The scheme does not indicate the transport proteins involved in 542 metabolism because transport proteins with specificity for sucrose, melibiose, or raffinose 543 are not annotated in *L. reuteri* genomes, and have not been characterized on the 544 biochemical level.

545 **Figure 2.** Gene loci encoding sucrose phosphorylase (*scrP*) (**Panel A)** and its putative 546 regulator (*scrR*) (**Panel B**) in *L. reuteri* 100-23 (Gene bank Accession number 547 AAPZ00000000.2). The lines below represent the homologous sequences in *L. reuteri* 548 LTH5448. PCR primers used for the generation of LTH5448Δ*scrR* and LTH5448Δ*scrP* 549 mutants are indicated by arrows.

550 **Figure 3.** Phylogenetic analysis of sucrose specific regulator ScrR and related 551 transcriptional factors in the LacI/GalR family. The distance tree was constructed using 552 the maximum likelihood method. Bootstrap values were calculated with 100 replicates.

553 **Figure 4.** Growth of *L. reuteri* LTH5448 (●), *L. reuteri* LTH5448Δ*scrP* (○) and 554 *L. reuteri* LTH5448Δ*scrR* (▼) with maltose, glucose and fructose (mMRS); sucrose 555 (sucMRS); or raffinose (rafMRS) as substrate. Data shown are mean values for duplicate 556 (*L. reuteri* LTH5448 and LTH5448Δ*scrP*) and triplicate (LTH5448Δ*scrR*) independent 557 experiments.

558 **Figure 5.** Formation of fructooligosaccharides and levan during growth of *L. reuteri* 559 LTH5448 and *L. reuteri* LTH5448Δ*scrP* in presence of 100 g L^{-1} sucrose. **Panel A.** FOS 560 formation was quantified after 24 hours of incubation. **Panel B.** Levan concentration in

561 the culture supernatant of *L. reuteri* LTH5448 (●) and *L. reuteri* LTH5448Δ*scrP* (○).
562 Data are shown as means ± standard deviation of three independent replicates. Significant
563 differences ($P<0.001$) between the levan concentrations in *L. reuteri* LTH5448 and
564 LTH5448Δ*scrP* were determined by Student's *t*-test and are indicates by an asterisk.

565 **Table 1.** Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Source or reference
	Strains	
E. coli JM109	Cloning host for pGEMTeasy- and pJRS233-derivative plasmids	Promega
L. reuteri LTH5448	Sourdough isolate	Schwab *et al.* 2007
L. reuteri LTH5448Δ*scrP*	Wild type strain derivative with a deletion in *scrP*	This study
L. reuteri LTH5448Δ*scrR*	Wild type strain derivative with a deletion in *scrR*	This study
	Plasmids	
pGEMTeasy	Cloning vector used in *E. coli*; 3.0 kb; Amp^r	Promega
pScrP-A	pGEMTeasy containing a 0.9 kb sequence upstream of *scrP*; 3.9 kb; Amp^r	This study
pScrP-B	pGEMTeasy containing a 0.9 kb sequence downstream of *scrP*; 3.9 kb; Amp^r	This study
pScrP-AB	pGEMTeasy containing the upstream and downstream sequences of *scrP*; 4.8 kb; Amp^r	This study
pScrR-A	pGEMTeasy containing a 1.2 kb sequence upstream of *scrR*; 4.2 kb; Amp^r	This study
pScrR-B	pGEMTeasy containing a 1.2 kb sequence downstream of *scrR*; 4.2 kb; Amp^r	This study
pScrR-AB	pGEMTeasy containing the upstream and downstream sequences of *scrR*; 5.4 kb; Amp^r	This study
pJRS233	Shuttle vector used in the hosts *E. coli* and *L. reuteri*; 6.0 kb; Erm^r	Perez-Casal *et al.* 1993
pKO-*scrP*-AB	pJRS233 containing 1.8 kb of the flanking sequences of *scrP*; 7.8 kb; Erm^r	This study
pKO-*scrR*-AB	pJRS233 containing 2.4 kb of the flanking sequences of *scrR*; 8.4 kb; Erm^r	This study

Amr^r, Erm^r, ampicillin and erythromycin resistance, respectively

566 **Table 2.** Oligonucleotide primers used in this study

Gene	Primer	Sequence (5' – 3')[a]	Application
scrP	*pho*-KO1b-F-*Xho*I	CCG*CTGGA*GGTATGAAGGTACAACC	Insert primer
	pho-KO2-R-*Xba*I	GC*TCTAGA*TTAGTCAGAGTAAGTAATTAAC	Insert primer
	pho-KO3-F-*Xba*I	GC*TCTAGA*TTCACTATTACAGCTAACGGCGAA	Insert primer
	pho-KO4a-R-*Pst*I	AA*CTGCAG*GCCTGGGAATTTCTACCC	Insert primer
	pho-1b-F	GTATGAAGGTACAACC	Sequencing
	pho-4a-R	GCTTGGGAATTTCTACCC	Sequencing
	SucPho F	CTGGTAAGAACCGTCCAAC[b)]	qPCR
	SucPho R	CAGTTAAGATATCCTTAGCAT[b)]	qPCR
scrR	*reg*-KO1a-F-*Xho*I	CCG*CTCGAG*TAATTCCAAATAGGTTATG	Insert primer
	reg-KO2-R-*BamH*I	CG*GGATCC*TCATTGAGCAACATCTTTTAAC	Insert primer
	reg-KO3-F-*BamH*I	CG*GGATCC*TAAACTTGTTCCGGTTGA	Insert primer
	reg-KO4b-R-*Hind*III	CCC*AAGCTT*CTTCCAATGGTCAAAT	Insert primer
	reg-5-F	CCGTCGATTGTTCAAATATG	Sequencing
	reg-6-R	CTGTTGAACGAATTCACG	Sequencing
	reg-9-F	TGGGGATAGAGGAATATCATC	qPCR
	reg-10-R	CGATTGATGGTAGGTGAAAC	qPCR
pho	Phoket V	GTAACCTTCAAGGAATCC[b)]	qPCR
	Phoket R	CGTCTTTACGCATTCCTTG[b)]	qPCR
ftfA	Leureu1001 V	GAATGGCTATCAACTTGTG[b)]	qPCR
	Leureu1001 R	CTTCTACTTGCGGGTTC [b)]	qPCR

567 [a] Restriction enzyme sites are indicated in italic form

568 [b)] Primers from Schwab et al., 2007; other primers were designed in this study

569

570 **Table 3.** Sucrose phosphorylase and levansucrase expression in *L. reuteri* LTH5448 and
571 LTH5448Δ*scrR*. Data are shown as means ± standard deviation of triplicate independent
572 experiments.

L. reuteri **strain**	**Target gene**	**Sugar composition of media**			
		Glucose, Maltose, Fructose	**Glucose**	**Raffinose**	**Glucose, Sucrose**
		Log_{10} [relative gene expression]			
LTH5448	***scrP***	-2.17 ± 0.45	-2.76 ± 0.29	0.18 ± 0.47	0.11 ± 0.36
LTH5448Δ*scrR*		0.02 ± 0.23	0.58 ± 0.21	0.41 ± 0.23	0.36 ± 0.86
LTH5448	***ftfA***	-1.87 ± 0.27	-1.61 ± 1.12	0.64 ± 0.45	0.31 ± 0.58
LTH5448Δ*scrR*		-0.67 ± 0.53	-0.20 ± 0.77	-0.17 ± 0.29	-0.29 ± 0.50

573 Gene expression was calculated by the $\Delta\Delta C_T$ method using wild type cells grown in
574 presence of sucrose as reference conditions; the phosphoketolase gene *pho* was used as
575 endogenous control.

576

577 **Table 4.** Formation of organic acids of *L. reuteri* LTH5448 and LTH5448Δ*scrP* mutant

578 during growth in mMRS with maltose, glucose and fructose; sucrose (sucMRS); or

579 raffinose (rafMRS) as substrate. Data are shown as means ± standard deviation of

580 duplicate independent experiments.

Medium	***L. reuteri* strain**	**Metabolites [mmol L^{-1}]**			
		Mannitol	**Lactate**	**Acetate**	**Ethanol**
mMRS	**LTH5448**	18 ± 0	77 ± 4	10 ± 1	58 ± 6
	Δ*scrP*	19 ± 1	76 ± 4	10 ± 1	53 ± 7
sucMRS	**LTH5448**	14 ± 0	26 ± 0	11 ± 0	10 ± 2
	Δ*scrP*	14 ± 1	25 ± 2	10 ± 2	10 ± 1
rafMRS	**LTH5448**	15 ± 0	61 ± 3	10 ± 2	49 ± 1
	Δ*scrP*	18 ± 2	62 ± 1	10 ± 1	46 ± 4

581

582

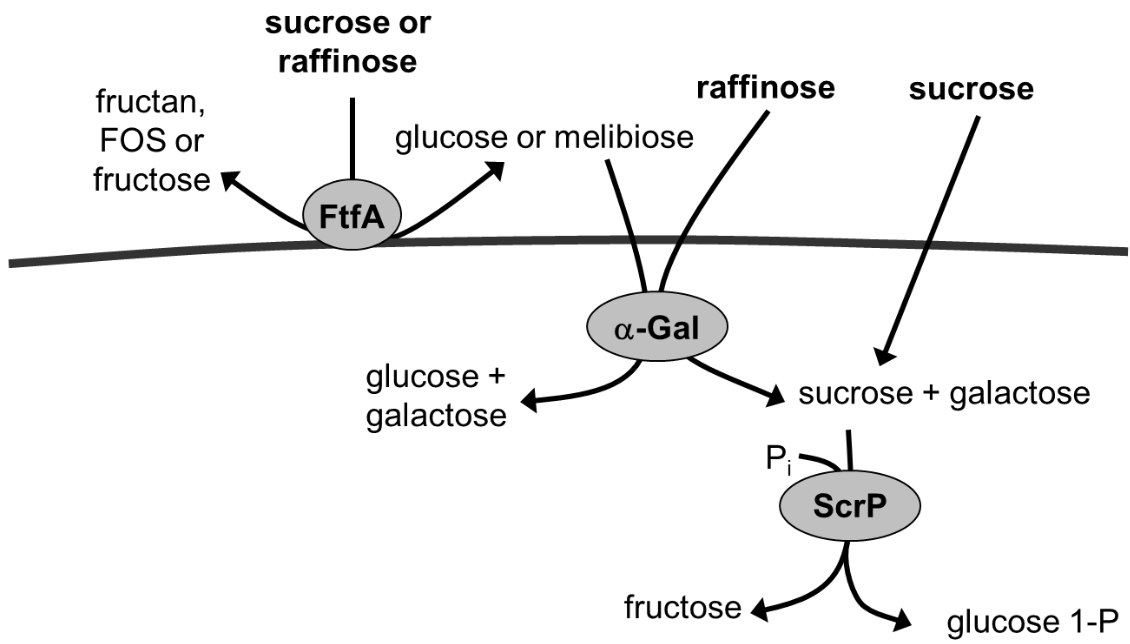
583 **Figure 1. Teixeira et al.**

584

585

586

587



588 **Figure 2. Teixeira et al.**

589

590

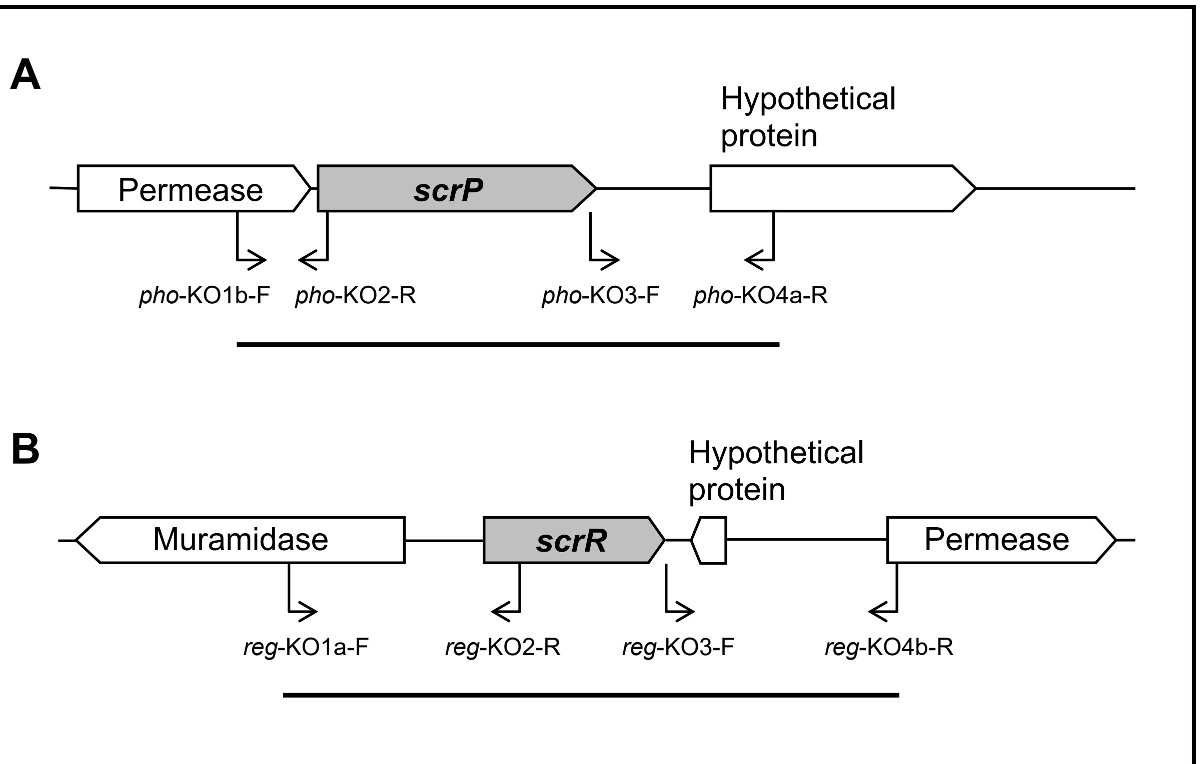
591

592

593

594

595



596

597

598

599 **Figure 3. Teixeira et al.**

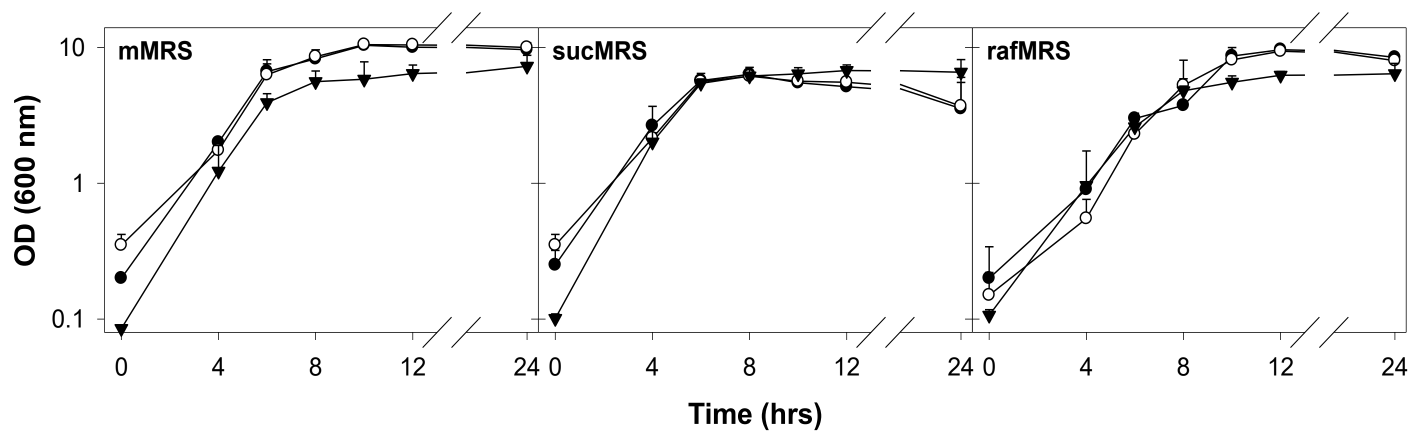
600

601

602

603

604 **Figure 4. Teixeira et al.**



609

610

611

612