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1

**Revised manuscript JB00878-16**

2

***Enterococcus faecalis* uses a PTS permease and a host colonization-related**

3

**ABC transporter for maltodextrin uptake**

4

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29

30 Keywords: Enterococci, maltodextrin, phosphotransferase system, ABC transporter, host  
31 colonization

32 Running title: Maltodextrin uptake by *Enterococcus faecalis*

33 **ABSTRACT**

34

35 Maltodextrin is a mixture of maltooligosaccharides, which are produced by the  
36 degradation of starch or glycogen. They are mostly composed of  $\alpha$ -1,4- and some  $\alpha$ -1,6-  
37 linked glucose residues. Genes presumed to code for the *Enterococcus faecalis*  
38 maltodextrin transporter were induced during enterococcal infection. We therefore  
39 carried out a detailed study of maltodextrin transport in this organism. Depending on  
40 their length (3 to 7 glucose residues), *E. faecalis* takes up maltodextrins either via MalT,  
41 a maltose-specific permease of the phosphoenolpyruvate (PEP):carbohydrate  
42 phosphotransferase system (PTS), or the ATP binding cassette (ABC) transporter  
43 MdxEFG/MsmX. Maltotriose, the smallest maltodextrin, is primarily transported by the  
44 PTS permease. A *malT* mutant therefore exhibits significantly reduced growth on  
45 maltose and maltotriose. The residual uptake of the trisaccharide is catalyzed by the  
46 ABC transporter, because a *malT/mdxF* double mutant no longer grows on maltotriose.  
47 The trisaccharide arrives as maltotriose-6''-P in the cell. MapP, which dephosphorylates  
48 maltose-6''-P, also releases P<sub>i</sub> from maltotriose-6''-P. Maltotetraose and longer  
49 maltodextrins are mainly (or exclusively) taken up via the ABC transporter, because  
50 inactivation of the membrane protein MdxF prevents growth on maltotetraose and  
51 longer maltodextrins up to at least maltoheptaose. *E. faecalis* also utilizes panose and  
52 isopanose and we show for the first time that in contrast to maltotriose its two isomers  
53 are primarily transported via the ABC transporter. We confirm that maltodextrin  
54 utilization via MdxEFG/MsmX affects the colonization capacity of *E. faecalis*, because  
55 inactivation of *mdxF* significantly reduced enterococcal colonization and/or survival in  
56 kidneys and liver of mice after intraperitoneal infection.

57

58

59 **IMPORTANCE** Infections by enterococci, major health-care associated pathogens, are  
60 difficult to treat due to their increasing resistance to clinically relevant antibiotics and new  
61 strategies are urgently needed. A largely unexplored aspect is how these pathogens proliferate  
62 and which substrates they use in order to grow inside infected hosts. The use of maltodextrins  
63 as carbon and energy source was studied in *Enterococcus faecalis* and linked to its virulence.  
64 Our results demonstrate that *E. faecalis* can efficiently use glycogen degradation products.  
65 We show that depending on the length of the maltodextrins one of two different transporters is  
66 used: The maltose-PTS transporter MalT or the MdxEFG/MsmX ABC transporter. The latter  
67 takes up longer maltodextrins as well as complex molecules such as panose and isopanose.

68

69 **INTRODUCTION**

70

71 Maltose, maltotriose and higher maltodextrins represent an important carbon and  
72 energy source for numerous bacteria, including several human pathogens, such as  
73 *Streptococcus pyogenes* (1), *Streptococcus pneumoniae* (2), *Listeria monocytogenes* (3) and  
74 *Neisseria meningitidis* (4). Maltose and maltodextrins are produced by the degradation of  
75 starch or glycogen and are therefore abundant in decaying plants and in the intestine and oral  
76 cavity of humans and many animals. Maltose catabolism in *Enterococcus faecalis* requires the  
77 proteins encoded by the five genes *malP*, *pgcM*, *malM*, *malT*, and *mapP* (5–7). The first three  
78 genes are organized in an operon and they encode a maltose phosphorylase, a  $\beta$ -  
79 phosphoglucomutase and an aldose-1-epimerase, respectively. The *malT* gene encodes a  
80 maltose-specific permease belonging to the glucose family of phosphoenolpyruvate  
81 (PEP):carbohydrate phosphotransferase system (PTS) transporters; it has the domain order  
82 EIICBA<sup>Mal</sup> (6, 7). The *malT* gene is located upstream from *malP* but oriented in opposite  
83 direction. MalT and the two general cytoplasmic PTS components EI and HPr form a  
84 phosphorylation cascade in order to catalyze the uptake and phosphorylation of maltose. EI  
85 autophosphorylates at a conserved histidine by using PEP as phosphoryl donor (8). The  
86 phosphoryl group is then transferred via His-15 in HPr (9) and presumably His-645 in the  
87 EIIA domain (10) to Cys-492 in the EIIB domain of MalT. In the last step, the P~Cys-EIIB<sup>Mal</sup>  
88 domain donates its phosphoryl group to a maltose molecule bound to the membrane-spanning  
89 EIIC<sup>Mal</sup> domain. Phosphorylation at the 6'-position of the disaccharide probably lowers its  
90 affinity for the EIIC<sup>Mal</sup> domain and maltose-6'-P is released into the cytoplasm (11).

91 *E. faecalis* uses MapP, the gene of which is located just downstream from *malT*, to  
92 dephosphorylate maltose-6'-P formed during PTS-catalyzed transport (7). Subsequently,

93 MalP phosphorylates intracellular maltose to  $\alpha$ -D-glucose and glucose-1-P. The  $\beta$ -  
94 phosphoglucomutase PgcM transforms glucose-1-P into glucose-6-P (7).

95 In addition to the PTS permease MalT, enterococci and streptococci contain an ATP  
96 binding cassette (ABC) transporter, which takes up maltooligosaccharides (2, 12, 13) and in  
97 some organisms also maltose (14, 15). In *E. faecalis*, the residual maltose uptake activity  
98 observed for the *malT* mutant was proposed to be mediated by the ABC transporter (6, 7). The  
99 maximum chain length of maltodextrins taken up via ABC transporters varies between 8 and  
100 10 (16). The maltodextrin-specific ABC transporters are composed of the substrate binding  
101 protein MdxE and the two integral membrane proteins MdxF and MdxG. Their genes are  
102 organized in an operon (Fig. 1). The MdxEFG transporter is completed by an ATP binding  
103 protein either called MalK or MsmX. MsmX serves as ATP hydrolase for other ABC  
104 transport systems (17) and in contrast to *malK* its gene is usually not associated with the  
105 *mdxEFG* operon. Interestingly, the *E. faecalis* genes presumed to encode MdxEFG and  
106 MsmX were recently reported to be strongly induced during *E. faecalis* infection using a  
107 mouse peritonitis model (18).

108 While most enterococcus and streptococcus species seem to primarily use the PTS  
109 permease MalT and the maltose-6'-P phosphatase MapP for the uptake and first catabolic step  
110 of maltose, the transport of maltotriose and maltotetraose, the two smallest  
111 maltooligosaccharides, seems to vary from one species to another. For example, *S. pyogenes*  
112 takes up maltose, maltotriose and higher maltooligosaccharides mainly via an efficient ABC  
113 transport system. A mutant defective in MalE grew slowly on maltose and maltotriose (1, 19).  
114 Nevertheless, slow uptake of these two compounds is catalyzed by MalT. In contrast to *S.*  
115 *pyogenes*, the *S. mutans* PTS permease MalT (SMU.2046c) was reported to efficiently  
116 transport maltose and maltotriose and to also contribute to maltotetraose uptake. Deletion of  
117 *malT* prevented utilization of maltose, strongly diminished maltotriose consumption and

118 significantly slowed growth on maltotetraose (20). Finally, *S. pneumoniae* takes up maltose  
119 primarily via the PTS permease MalT (SP0758), while maltotriose is transported with similar  
120 efficiency by MalT and the ABC transporter MalXCD. Maltooligosaccharides are almost  
121 exclusively taken up by the ABC transporter. The residual maltose uptake observed for the  
122 *malT* mutant is not catalyzed by the ABC transporter (2).

123         The variations in maltotriose and maltotetraose uptake in the three described  
124 streptococci, which nevertheless use the same set of transport enzymes, prompted us to carry  
125 out a detailed study of maltooligosaccharide transport in *E. faecalis*. We found that  
126 maltotriose is mainly taken up via the enterococcal PTS permease MalT and that maltotriose-  
127 6''-P is also dephosphorylated by the phosphatase MapP. In contrast, maltotetraose and  
128 higher maltodextrins are mainly taken up via the ABC transporter MdxEFG, which also  
129 catalyzes the transport of panose and isopanose, two maltotriose isomers formed during  
130 glycogen degradation. Interestingly, genes encoding enzymes involved in maltodextrin  
131 metabolism were reported to be strongly expressed during mice soft tissue infection with *S.*  
132 *pyogenes* (21). A similar observation was recently made during intraperitoneal infection of  
133 mice with *E. faecalis* (18), suggesting that maltodextrin metabolism might be important for *E.*  
134 *faecalis* virulence. We here confirm this hypothesis by demonstrating that deletion of the  
135 ABC transporter, but not of the PTS permease MalT, significantly reduced mice organ  
136 colonisation of *E. faecalis*.

137

138 **MATERIALS AND METHODS**

139

140 **Bacterial strains and growth conditions.** The *E. faecalis* wild-type strain used in this study  
141 is a maltotetraose-positive clone isolated from our stock of strain JH2-2, which is also called  
142 TX4000. The *malT* and *mdxF* mutants used in this study were derived from the maltotetraose-  
143 positive clone (Table 1). *E. faecalis* strains were routinely grown at 37°C without shaking in  
144 100 ml sealed bottles filled with 20 to 50 ml of Luria-Bertani (LB) medium (Difco, New  
145 Jersey, USA) containing 0.5% w/v glucose. Growth studies were carried out with carbon-  
146 depleted M17MOPS (M17cc) medium, which was prepared as previously described (22). It  
147 was supplemented with 0.3% w/v of either glucose, maltose, maltotriose, maltotetraose,  
148 maltopentaose, maltohexaose, maltoheptaose, panose or isopanose. Erythromycin,  
149 chloramphenicol and tetracycline were added when appropriate at concentrations of 150 µg  
150 ml<sup>-1</sup>, 10 µg ml<sup>-1</sup> and 5 µg ml<sup>-1</sup>, respectively.

151 *E. coli* strains NM522 and Top10F' used for protein purification and cloning  
152 experiments, respectively (Table 1), were grown aerobically by gyratory shaking at 250 rpm  
153 in LB medium at 37°C and transformed by electroporation with a Gene Pulser apparatus (Bio-  
154 Rad Laboratories). Growth was followed by measuring the absorption at 600 nm in a  
155 Novaspec II spectrophotometer. When appropriate, erythromycin was added at a final  
156 concentration of 150 µg ml<sup>-1</sup>.

157 The *Lactococcus lactis* strain IL1403 was used as host during plasmid construction for  
158 complementation experiments and was grown in M17 medium at 30°C.

159

160 **General molecular methods.** Restriction endonucleases, shrimp alkaline phosphatase and T4  
161 DNA ligase were obtained from Promega and used according to the manufacturers'  
162 instructions. PCR experiments were performed with the thermocycler "Master Cycler

163 Gradient” (Eppendorf) using GoTaq or Phusion DNA polymerase (Promega). The primers  
164 used for the PCR experiments are listed in Table 2. If needed, PCR products were purified  
165 using the Nucleospin® Extract II kit (Macherey-Nagel). *E. coli* was transformed by  
166 electroporation with the Gene Pulser Apparatus (Bio-Rad Laboratories), as described in (23)  
167 and *E. faecalis* or *L. lactis* as described in (24). Plasmids were extracted from *E. coli* by using  
168 the Nucleospin® Plasmid kit (Macherey-Nagel). RNA extraction was performed as previously  
169 described (25). The 5' end of the mRNA corresponding to the *mdxEFG* operon (Fig. 1) was  
170 mapped on a 5'RACE (Rapid Amplification of cDNA Ends) PCR product obtained by using  
171 the 3'/5' RACE kit (ThermoFisher) and the primer race1\_41965 (Table 2) for the reverse  
172 transcriptase reaction and primer race2\_41965 for the PCR after poly A tailing. The PCR  
173 product was subsequently purified and sequenced with the primer race3\_41965.

174 For the complementation of the *E. faecalis mdxF* mutant the wild-type *mdxF* gene was  
175 cloned into vector pAGent (26). In this plasmid, genes inserted at the multiple cloning site are  
176 expressed from the agmatine-inducible *aguB* promoter in the presence of 40 mM agmatine,  
177 because the plasmid contains also the gene for the repressor AguR of the *aguBDAC* operon.  
178 The *mdxF* gene and the plasmid were amplified with the primer pairs  
179 pAGent\_66\_forkit/pAGent\_66\_revkit and RPV\_AGent\_RBS/FRV\_AGent\_Term,  
180 respectively (Table 2). The assembly of the two PCR fragments was achieved with the  
181 NEBuilder HiFi DNA Assembly Cloning Kit (NewEngland Biolabs). *L. lactis* strain IL1403  
182 was transformed with the resulting plasmid (named pAGent:66c) and subsequently plated  
183 onto GM17 medium with chloramphenicol at a final concentration of 5  $\mu\text{g ml}^{-1}$ . The correct  
184 sequence of the inserted DNA fragment was confirmed for one clone and its plasmid was  
185 subsequently used to complement the *E. faecalis mdxF* mutant. For control experiments,  
186 empty pAGent was electroporated into the wild-type strain and the *mdxF* mutant.

187 For Quantitative real-time PCR (qRT-PCR) experiments, total RNA was extracted  
188 from maltotetraose-negative and -positive strains by using the Direct-zol™ RNA MiniPrep  
189 Kit (Zymo research). The strains were grown for 4 h in M17cc medium containing either  
190 0.5% w/v of maltodextrin or 0.5% w/v maltodextrin and 0.5% w/v glucose. The isolated RNA  
191 was spectrophotometrically quantified and 2 µg of the total RNA was subsequently utilized  
192 for reverse transcription with the QuantiTect® Reverse Transcription kit (Qiagen). qRT-PCR  
193 was subsequently carried out with SYBR Green fluorescence using the Cfx96 Real Time PCR  
194 Machine (Bio Rad). The primers used for the qRT-PCR experiments are EF1344R and  
195 EF1344L for the *mdxF* gene and EF1345R and EF1345L for the *mdxE* gene (Table 2). Raw  
196 data were converted into expression data by the absolute quantification method using the  
197 standard curve and then normalized using the housekeeping 5S RNA gene as reference. The  
198 mean values and standard deviations were calculated from at least 3 independent experiments.  
199

200 **Construction of *E. faecalis maltT* and *mdxF* mutants.** *E. faecalis* mutants were constructed  
201 by introducing two stop codons at the beginning of each ORF in order to produce short  
202 truncated proteins. For the *maltT* mutant, two PCR products of 1000 bp were amplified by  
203 using chromosomal DNA as template and the two primer pairs pMAD-41760-for/Mut-41760-  
204 rev and pMAD-41760-rev/Mut-41760-for, respectively (Table 2). The 5' ends of Mut-41760-  
205 rev and Mut-41760-for contain *EcoRI* restriction sites. After amplification, both PCR  
206 products were therefore cut with *EcoRI* and ligated. A second PCR was performed using the  
207 reaction mixture of the ligation as DNA template and primers pMAD-41760-for / pMAD-  
208 41760-rev. The amplified 2 kb fragment was cloned into pMAD cut with *BamHI* and *NcoI*  
209 (27) and after purification from *E. coli* Top10F' the resulting plasmid was used to transform a  
210 maltotetraose-positive *E. faecalis* JH2-2 strain according to a previously described protocol  
211 (7). After electroporation, cells were plated on GM17 agar medium containing 50 µg/ml

212 erythromycin and 2.35  $\mu$ M X-Gal and incubated at 37°C. The presence of the plasmid in blue  
213 clones resistant to the antibiotic was verified by PCR using oligonucleotide Verif\_41760 and  
214 a plasmid-based primer. To allow the second crossing over, integrants were first grown for 6  
215 hours at 30°C and subsequently overnight at 42°C. After 2 or 3 cycles of temperature shifting,  
216 cells were plated on solid GM17 medium containing X-Gal but no antibiotics and incubated at  
217 37°C. In several white clones the presence of the introduced *EcoRI* site in the PCR amplified  
218 *malT* gene was confirmed by restriction with this enzyme and the presence of the stop codons  
219 was confirmed by DNA sequencing. The same strategy was applied for the construction of the  
220 *mdxF* mutant, except that we used primer pairs pMAD-41966-for/Mut-41966-rev and pMAD-  
221 41966-rev/Mut-41966-for (Table 2). The two Mut primers contain *NheI* and *SpeI* restriction  
222 sites, two isoschizomeric enzymes. The correct sequence in the mutant was confirmed by  
223 PCR amplification using a specific primer of the modified sequence Verif-mut-41966 and  
224 subsequent DNA sequencing.

225

226 **Growth studies with wild-type and mutant strains.** The media used for the growth studies  
227 were inoculated with overnight cultures, which were started from the glycerol stocks kept at -  
228 20°C. Each overnight culture was washed once with one volume of physiological solution and  
229 the final ODs at 600 nm were adjusted to 2.0. A 96-well plate was filled with 200  $\mu$ l of  
230 M17MOPS medium supplemented with different sugars per well and inoculated with 2  $\mu$ l of  
231 the washed overnight culture. Three drops of paraffin oil were added on top of the medium to  
232 avoid desiccation and to create anaerobic conditions. A Microplate Reader 680 (BioRad  
233 Laboratories) was used to follow growth at 37°C. The absorption at 595 nm was measured  
234 every 30 min over a total period of 12 to 16 hours. Growth studies were carried out at least  
235 three times and always provided very similar results.

236

237 **Purification of His-tagged *E. faecalis* maltose-6'-P phosphatase.** The maltose-6'-P  
238 phosphatase of the *E. faecalis* strain JH2-2 is encoded by the *mapP* (*EFT41761*) gene. The  
239 enzyme was overexpressed in *E. coli* strain NM522 from the His-tag expression vector  
240 pQE30 and purified as previously described (7). The enzyme was used for dephosphorylation  
241 assays of maltotriose-6''-P and maltotetraose-6'''-P as described below.

242

243 **Synthesis of maltotriose-6''-P and maltotetraose-6'''-P.** Maltotriose and maltotetraose  
244 phosphorylated at the O-6 position of the glucose moiety at their non-reducing end were  
245 prepared enzymatically via the  $\alpha$ -glucoside specific PTS present in palatinose-grown cells of  
246 *Klebsiella pneumoniae* (28). This PTS corresponds to the  $\alpha$ -glucoside-specific EIICB PTS  
247 component with the protein id KFJ74572.1, which is encoded by the *aglB* gene present on  
248 chromosome 1 of the *Klebsiella pneumoniae* type strain ATCC 13883 (29). Phosphorylated  
249 derivatives of the  $\alpha$ -linked oligosaccharides were isolated by ethanol and  $Ba^{2+}$  precipitation,  
250 and further purified by ion-exchange and paper chromatography. Structures and product  
251 purity were confirmed by thin-layer chromatography, mass spectrometry and NMR  
252 spectroscopy.

253

254 **Mass spectrometric analysis of phosphorylated maltooligosaccharides after MapP**  
255 **treatment.** Solutions containing maltotriose-6''-P or maltotetraose-6'''-P at a concentration of  
256 2 M were prepared with 20 mM ammonium bicarbonate. Aliquots (5  $\mu$ l) of these solutions  
257 were added to either 40  $\mu$ l of purified MapP (0.8 mg ml<sup>-1</sup>, dialyzed against 20 mM ammonium  
258 bicarbonate) or to 40  $\mu$ l of 20 mM ammonium bicarbonate and incubated for 2 h at 37°C. The  
259 samples were subsequently lyophilized and rehydrated with 10  $\mu$ l of water. Aliquots of 1  $\mu$ l  
260 were mixed with 9  $\mu$ l of a sugar matrix solution and 1  $\mu$ l was spotted onto the MALDI steel  
261 plate. The sugar matrix was freshly prepared and contained 100  $\mu$ g/ml 2,5-dihydroxybenzoic

262 acid dissolved in a mixture of H<sub>2</sub>O/acetonitrile/N,N-dimethylaniline (1:1:0.02 vol/vol). The  
263 samples were analyzed by MALDI-TOF MS (Voyager DE super STR, AB SCIEX) by  
264 irradiating them with a nitrogen laser (337 nm, 10 Hz) integrated in this instrument and by  
265 recording mass spectra in the reflectron mode using a delay extraction time of 120 ns and a  
266 m/z mass range between 200 and 1000 Da.

267

268 **Determination of the phosphatase activity of MapP with the malachite green assay.** To

269 follow the MapP-catalyzed dephosphorylation of maltose-6'-P, maltotriose-6''-P and  
270 maltotetraose-6'''-P over various time periods we used the malachite green assay (30).

271 Dephosphorylation experiments were carried out in 650 µl assay mixtures containing 50 mM

272 Tris/HCl, pH 7.4, 5 mM MgCl<sub>2</sub> and 2 or 4 mM of the phosphorylated maltooligosaccharides.

273 The reaction was started by adding MapP and the samples were incubated for various time

274 periods at 37°C. The amount of MapP used in the assay was 40 µg, which allowed the

275 detection of phosphate after a few minutes incubation. Aliquots of 100 µl were withdrawn

276 after 0, 1.5, 3, 6, 10 and 20 min incubation and immediately mixed with 25 µl of the

277 malachite green reagent (30). The samples were kept for 20 min at ambient temperature

278 before 375 µl of water was added and the OD<sub>630</sub> determined. In Table 3, the specific activity

279 (µmol per min and mg protein) determined for the MapP-catalyzed dephosphorylation of the

280 various phospho-compounds is also expressed relative to the activity measured with maltose-

281 6'-P, which was set to 100%.

282

283 **Colonization assay of mouse organs.** The maltooligosaccharide-positive *E. faecalis* JH2-2

284 clone as well as the *mdxF*, *maltT* and *tpx* mutants derived from it were grown in GM17 broth

285 at 37°C. The *tpx* mutant was used as a negative control. The *tpx* gene encodes a

286 thiolperoxidase involved in stress response and its inactivation has previously been shown to

287 lower the survival of *E. faecalis* in an LD50 mouse infection model (31). All animal  
288 experiments were performed at the platform of biological resources (CURB;  
289 <http://icore.unicaen.fr/plateformes/curb/curb-272372.kjsp>) at the University of Caen in  
290 accordance with the French legislation on biomedical experimentation (Ethic authorization  
291 B14118015 and N/01-09-12/20/09-15) and the European Communities Council Directive  
292 from 22 september 2010 (2010/63/UE). Before being used, mice were housed at  $21 \pm 0.5^\circ\text{C}$ ,  
293 in Perspex home cages with free access to food and water.

294 *E. faecalis* wild-type and mutant strains were harvested at mid-log phase and  
295 resuspended in physiological solution to reach a density of  $\approx 8 \times 10^8$  CFU/ml. Male and female  
296 6- to 8 weeks old Swiss white mice (CURB, France) were infected intraperitoneally with 1 ml  
297 of the bacterial suspensions. The experiments were conducted with 5 mice per group. After 24  
298 h of infection, mice were anesthetized by inhalation of 5% isoflurane in a mixture of 30% O<sub>2</sub>  
299 and 70% N<sub>2</sub>O before euthanasia. A peritoneal wash was performed with 5 ml of cold  
300 physiological solution and the resulting fluid was collected in sterile tubes and placed on ice  
301 for 30 min. The abdomen was subsequently opened and kidneys, liver, and spleen were  
302 removed and placed on ice for 30 min. The organs were crushed using pestle and mortar,  
303 transferred into sterile tubes and resuspended in 3 volumes of cold physiological solution. The  
304 suspensions were then homogenized by vortexing twice for 30 sec. The peritoneal fluid was  
305 centrifuged (10 min at 4000 g and 4°C) and the bacterial pellet was subsequently resuspended  
306 in 1 ml of cold physiological solution. Serial dilutions of all samples were plated onto GM17  
307 agar in order to determine the CFU numbers. In order to determine whether there is a  
308 significant difference between two groups of mice we used the Mann-Whitney test in the  
309 GraphPad Prism software (GraphPad Software, San Diego, CA). A *P*-value < 0.05 was  
310 considered to be significant. All experiments were carried out twice.

311

312 **RESULTS AND DISCUSSION**

313

314 *E. faecalis* transports maltotriose mainly via the PTS permease MalT. In order to  
315 determine to which extent the ABC and the PTS transport systems of *E. faecalis* contribute to  
316 the uptake of maltotriose, the smallest maltodextrin, we carried out growth studies with  
317 mutants devoid either of the maltose-specific PTS permease MalT or the ABC transport  
318 protein MdxF. We observed that the wild-type strain grew with similar efficiency on  
319 maltotriose, maltose and glucose. In contrast, the *malT* mutant grew significantly slower on  
320 maltose and maltotriose (Fig. 2). These results therefore suggested that *E. faecalis* transports  
321 and phosphorylates the smallest maltodextrin mainly via the PTS permease MalT, which has  
322 previously been shown to transport and phosphorylate maltose (6, 7). Indeed, a mutant deleted  
323 for *mdxF*, which encodes one of the transmembrane components of the ABC transporter,  
324 exhibited a growth rate on maltotriose similar to that of the wild-type strain (compare Fig. 2  
325 and Fig. 3). We also constructed a *malT/mdxF* double mutant and tested its growth on the  
326 three above-mentioned carbon sources. The double mutant grew normally on glucose, but was  
327 no longer able to grow on maltotriose (Fig. 3), indicating that the ABC transporter  
328 MdxEFG/MsmX is responsible for the residual growth on the trisaccharide observed for the  
329 *malT* mutant (Fig. 2). In contrast, the slow growth on maltose observed for the *malT* mutant  
330 (Fig. 2) was not altered in the *malT/mdxF* double mutant (Fig. 3). It therefore seems that an  
331 unknown carbohydrate transporter, possibly an ABC transporter or a PTS permease, is  
332 responsible for the residual growth on maltose observed for the *malT* mutant. An identical  
333 observation was made for *S. pneumoniae*, where similar to the *malT* single mutant the  
334 *malT/mdxE* double mutant was also able to slowly grow on maltose (2). But in contrast to *S.*  
335 *pneumoniae*, which takes up maltotriose equally well by MalT and the ABC transporter, *E.*

336 *faecalis* transports the trisaccharide primarily by the PTS and only slowly by  
337 MdxEFG/MsmX.

338

339 ***E. faecalis* transports maltotetraose and higher maltodextrins mainly via the ABC**  
340 **transporter MdxEFG/MsmX.** We also tested whether *E. faecalis* transports longer  
341 maltooligosaccharides ranging from maltotetraose to maltoheptaose as well as the mixture of  
342 oligosaccharides present in a commercially available maltodextrin via the PTS permease  
343 MalT or the ABC transporter MdxEFG/MsmX. Growth studies revealed that the wild-type  
344 strain JH2-2 is able to utilize maltotetraose (Fig. 2) and maltopentaose, maltohexaose and  
345 maltoheptaose (Fig. 5) as well as maltodextrin (data not shown). However, growth on  
346 maltotetraose and longer maltooligosaccharides was significantly slower than growth on  
347 glucose, maltose or maltotriose. Compared to JH2-2, the *mdxF* mutant grew significantly  
348 slower on maltotetraose (Fig. 3), maltodextrin (data not shown) and maltopentaose (Fig. 5)  
349 and did not grow at all on maltohexaose and maltoheptaose (Fig. 5). In contrast, the wild-type  
350 strain and the *malT* mutant grew with identical efficiency on the above carbon sources (data  
351 not shown). These results establish that *E. faecalis* takes up maltooligosaccharides composed  
352 of more than three glucose residues mainly or exclusively via MdxEFG/MsmX.

353 We also complemented the *mdxF* mutant with the *mdxF* wild-type allele cloned into  
354 plasmid pAGEnt, in which the inserted genes are expressed from the agmatine-inducible *aguB*  
355 promoter (26). Only slight maltotetraose utilization was observed for the *mdxF* mutant  
356 transformed with empty pAGEnt (Fig. 6). In contrast, when the *mdxF* mutant was transformed  
357 with pAGEnt carrying the *mdxF* gene, growth on maltotetraose was restored to half the level  
358 observed for the wild-type strain.

359

360 *E. faecalis* MapP dephosphorylates both, maltotriose-6''-P and maltotetraose-6'''-P.  
361 Because the PTS phosphorylates its oligosaccharide substrates at the 6-position of the  
362 glycosyl residue at the non-reducing end, maltotriose arrives as maltotriose-6''-P in the cell.  
363 Similarly, maltose enters the cytoplasm as maltose-6'-P and due to the lack of a 6-P- $\alpha$ -  
364 glucosidase in enterococci, it is intracellularly dephosphorylated to maltose by the enzyme  
365 MapP (6, 7). To investigate whether MapP dephosphorylates the phosphorylated  
366 trisaccharide, we synthesized maltotriose-6''-P as described in Materials and Methods and  
367 carried out mass spectrometry and malachite green assays. Mass spectrometry revealed that  
368 MapP indeed dephosphorylates maltotriose-6''-P. The synthesized maltotriose-6''-P sample  
369 produced a major peak corresponding to maltotriose-6''-P carrying one Na<sup>+</sup> ion (607.14 m/z),  
370 a minor peak with the mass of maltotriose-6''-P coordinated with two Na<sup>+</sup> ions (629.12 m/z),  
371 and a very small peak corresponding to unphosphorylated maltotriose carrying one Na<sup>+</sup>  
372 (527.16 m/z) (Fig. 4A). After incubation for 2 h at 37°C with MapP, the peaks corresponding  
373 to maltotriose-6''-P had disappeared and a strong peak with the m/z value of  
374 unphosphorylated maltotriose carrying one Na<sup>+</sup> became visible (Fig. 4B).

375 By using the semiquantitative malachite green assay, which detects inorganic  
376 phosphate (30), we were able to confirm that MapP efficiently dephosphorylates maltotriose-  
377 6''-P. The maximum reaction rate was about 80% of that observed with maltose-6'-P (Table  
378 3). It is therefore likely that after its uptake and phosphorylation by the PTS, maltotriose-6''-P  
379 is dephosphorylated to intracellular maltotriose. Indeed, an enzyme catalyzing the hydrolysis  
380 of unphosphorylated maltotriose to maltose and glucose has been identified (P. Joyet, N.  
381 Sauvageot, A. Hartke and J. Deutscher, unpublished results).

382 Because MalT of *S. mutans* had been suggested to catalyze slow transport of  
383 maltotetraose, we hypothesised that the slow growth of the *E. faecalis* *mdxF* mutant on  
384 maltotetraose (Fig. 3) and maltopentaose (Fig. 5) might be due to their slow uptake by MalT.

385 In fact, only when the *malT* gene was also deleted, the resulting double mutant had  
386 completely lost its ability to grow on maltotetraose (Fig. 3). It was therefore likely that similar  
387 to maltose-6'-P and maltotriose-6''-P, maltotetraose-6'''-P produced during slow PTS-  
388 catalyzed uptake is also dephosphorylated by MapP in order to allow its further catabolism.  
389 We therefore synthesized also maltotetraose-6'''-P and tested its dephosphorylation by MapP.  
390 Mass spectrometry revealed that similar as observed with maltotriose-6''-P, MapP also  
391 completely dephosphorylated maltotetraose-6'''-P to maltotetraose carrying one Na<sup>+</sup>. After  
392 incubation for 2 h at 37°C with MapP, the two peaks corresponding to maltotetraose-6'''-P  
393 and maltotetraose-6'''-P carrying one Na<sup>+</sup> ion disappeared and a strong peak with the mass of  
394 unphosphorylated maltotetraose coordinated with one Na<sup>+</sup> became visible (data not shown).  
395 According to the malachite green assay, dephosphorylation of maltotetraose-6'''-P was  
396 slightly slower than dephosphorylation of maltose-6'-P, but as fast as dephosphorylation of  
397 maltotriose-6''-P (Table 3). These results support the concept that maltotetraose is not only  
398 efficiently taken up by the maltodextrin transporter MdxEFG/MsmX, but also taken up slowly  
399 by the PTS permease MalT. In addition, the fast dephosphorylation of maltotetraose-6'''-P by  
400 MapP indicates that the rate-limiting step of the observed slow maltotetraose utilization in the  
401 *mdxF* mutant must be the MalT-catalyzed transport and phosphorylation of maltotetraose. The  
402 slow utilization of maltopentaose by the *mdxF* mutant (Fig. 5) might also be mediated by  
403 MalT. Maltotetraose (20) and maltopentaose are the longest oligosaccharides suggested to be  
404 transported by the PTS.

405

406 **A spontaneous mutation in the *mdxEFG* promoter also prevents the utilization of longer**  
407 **maltooligosaccharides.** When carrying out control experiments with *E. faecalis* JH2-2-  
408 derived mutants affected in genes not related to maltose and maltodextrin metabolism, we  
409 noticed that most of them did not grow on maltotetraose and higher maltooligosaccharides

410 (data not shown). By carrying out a more extensive study we observed that only 12.5% of the  
411 bacteria in our stock of *E. faecalis* JH2-2 were able to grow on maltotetraose. We isolated  
412 several maltotetraose-positive clones, grew them for about 100 generations in glucose-  
413 containing M17 medium and found that the maltotetraose-positive phenotype was stable. We  
414 subsequently sequenced the entire *mdxEFG* operon and its upstream region in several  
415 maltotetraose-positive and -negative clones and found that the only difference between them  
416 was a single base modification in the intergenic region between the *mdxE* and the *EFT4\_1964*  
417 gene (Fig. 1). We determined the transcription start site of the *mdxEFG* operon by RACE  
418 PCR and found that the -10 promoter region of the *mdxEFG* operon was changed from  
419 CATAATT in maltotetraose-utilizing strains to CATAAGG in strains not able to grow on  
420 maltodextrin (Fig. 1). The T for G exchange probably strongly decreases the expression of the  
421 *mdxEFG* operon and thus prevents growth on maltotetraose and higher maltooligosaccharides.  
422 qRT-PCR revealed indeed that growth on maltodextrin caused 400- to 1000-fold stronger  
423 induction of the *mdxE* and *mdxF* genes in the maltotetraose-positive strain compared to the  
424 maltotetraose-negative strain (Fig. 7). In the presence of glucose, the strong induction of  
425 *mdxE* and *mdxF* by maltodextrin was not significantly altered in the maltotetraose-positive  
426 strain, indicating that the *mdxEFG* operon is not submitted to catabolite repression (Fig. 7).  
427 To determine whether the capacity of enterococci to grow on maltooligosaccharides is rather  
428 the norm or an exception, we tested 21 different *E. faecalis* strains and found that they were  
429 all able to grow on maltotetraose (data not shown). Only the TX4000 (JH2-2) clone used for  
430 genome sequencing (see NCBI WebSite:  
431 [http://www.ncbi.nlm.nih.gov/genome/808?genome\\_assembly\\_id=168551](http://www.ncbi.nlm.nih.gov/genome/808?genome_assembly_id=168551)) has the CATAAG  
432 -10 promoter sequence and should therefore also fail to grow on maltotetraose and higher  
433 maltooligosaccharides. A comparison with the genome sequences of different *E. faecalis*  
434 strains revealed that all contain an *mdxEFG* -10 promoter sequence identical to that of the

435 maltotetraose-positive JH2-2 strain. Finally, it is important to mention that all mutants used in  
436 this study were derived from a maltotetraose-positive JH2-2 clone.

437

438 ***E. faecalis* transports panose and isopanose via the ABC transporter MdxEFG/MsmX.**

439 Maltodextrins produced from branched chain polysaccharides, such as starch and glycogen,  
440 usually contain linkage isomers of maltotriose, such as panose and isopanose. Panose is also  
441 produced in large amounts during the degradation of pullulan by the enzyme neopullulanase  
442 ( $\alpha$ -amylase family 13) (EC 3.2.1.135) (32). Maltotriose and the maltotriose isomers panose  
443 and isopanose differ in the linkages of the three glucose residues. In maltotriose, the three  
444 glucose residues are linked with  $\alpha$ -1,4 glycosidic bonds. However, in panose, the first and  
445 second glucose residue (from the non-reducing end) are linked with an  $\alpha$ -1,6 glycosidic bond.

446 In isopanose, the  $\alpha$ -1,6 linkage is located between the two glucose residues at the reducing  
447 end (33). The *E. faecalis* strain V583 has been shown to grow on panose (34) and several  
448 other bacteria were reported to utilize both panose and isopanose (35–37). We found that  
449 maltotetraose-positive *E. faecalis* JH2-2 can also grow on both maltotriose isomers (Fig. 8).  
450 However, in contrast to maltotriose, its two linkage isomers are not taken up by the PTS  
451 permease MalT, but are transported by the MdxEFG/MsmX ABC transporter. While the *malt*  
452 mutant grew with similar efficiency as the wild-type strain on panose and isopanose, the  
453 *mdxF* mutant was not able to utilize the two trisaccharides (Fig. 8A), indicating that the ABC  
454 transporter has a broad substrate specificity, which is not limited to  $\alpha$ -1,4 glycosidic linear  
455 forms of polysaccharides. Therefore we propose that the function of this ABC transporter is  
456 dedicated to recover the products of degradation from starch or, in a context of infection, from  
457 glycogen.

458 No growth was obtained with isomaltotriose, an  $\alpha$ -1,6-linked trisaccharide (data not  
459 shown). The main difference between maltotriose, panose or isopanose and isomaltotriose is

460 that the latter contains two consecutive  $\alpha$ -1,6 linkages. Molecules with this structure might  
461 not be recognized by the *E. faecalis* ABC transporter. Complementation of the *mdxF* mutant  
462 with the *mdxF* wild-type allele under control of the agmatine-inducible *aguB* promoter  
463 restored growth on panose and isopanose to about half the level observed for the wild-type  
464 strain (Fig. 8B).

465 *E. faecalis* strain JH2-2 was not able to grow on  $\beta$ -cyclodextrin ( $\alpha$ -1,4-linked cyclic  
466 dextrin composed of seven glucose residues) and grew only very slowly on  $\alpha$ -cyclodextrin  
467 (composed of six glucose residues). The slow transport of  $\alpha$ -cyclodextrin seems to be  
468 catalyzed by the ABC transporter, because only the *malF* but not the *malT* mutant had lost the  
469 capacity to slowly grow on the cyclic maltohexaose (data not shown).

470

471 **The *mdxF* mutant exhibits reduced colonization of mice organs.** An increased expression  
472 of maltodextrin genes was observed during soft tissue infection with *S. pyogenes* (21).  
473 Similarly, a transcriptome study with cells of the *E. faecalis* strain V19 (a plasmid-cured  
474 derivative of strain V583) isolated from the peritoneum of infected mice had revealed that  
475 both the *mdxEFG* operon and the *msmX* gene are strongly expressed during infection. In  
476 contrast, the transcription level of the *malT* gene was not increased (18). These results  
477 suggested that the maltodextrin-specific ABC transporter might play a role in *E. faecalis*  
478 virulence. We therefore tested whether deletion of the *mdxF* gene would affect survival of *E.*  
479 *faecalis* in a mouse organ colonization model. A mutant deleted for the *tpx* gene, which  
480 encodes a thiolperoxidase that plays a role in enterococcal virulence (31), and the *malT*  
481 mutant served as positive and negative control strains, respectively. Twenty-four hours after  
482 intraperitoneal injection the bacterial load was determined in the peritoneum, kidneys, liver  
483 and spleen as described in Materials and Methods. No significant differences were observed  
484 between the wild-type strain and the *malT* mutant in the three organs and the peritoneum (Fig.

485 9A). In contrast, colonization of kidneys and liver by the *tpx* mutant was less efficient than by  
486 the wild-type strain (Fig. 9B), which confirmed its previously suggested implication in  
487 enterococcal virulence using an LD<sub>50</sub> mouse model (31). Interestingly, compared to the wild-  
488 type strain the *mdxF* mutant also exhibited significantly lower CFU numbers in kidneys  
489 (almost 5-fold lower) and in liver (16-fold lower) (Fig. 9C).

490 The link between carbohydrate utilization and virulence has been studied in pathogens  
491 like group A streptococci (GAS) or more recently in *S. suis* using transcriptome analyses or  
492 mouse oropharynx colonization experiments (1, 38). The combined results demonstrate that  
493 maltodextrin acquisition is likely to be a key factor in the ability of GAS to successfully infect  
494 the oropharynx. Our results extend the importance of maltodextrin metabolism to deep tissue  
495 infections. Indeed, the colonization of murine organs, especially the liver, by *E. faecalis* was  
496 less efficient in a mutant deficient in maltodextrin transport. The greatest impact on  
497 colonization was observed in the liver. This is of special interest as this organ is the storage  
498 site for glycogen, which can be degraded to maltodextrins. Glycogen released from host  
499 tissues may serve as substrate for growth of *E. faecalis*. However, cultures realized on  
500 glycogen or starch as substrates showed that *E. faecalis* JH2-2 does not grow on these  
501 polysaccharides, which is in agreement with the absence of genes related to glycogen  
502 degradation or encoding amylases in the genome of enterococci. Hence, the step between  
503 glycogen release and the formation of maltodextrins metabolizable by *E. faecalis* remains  
504 unclear. In conclusion, we demonstrate that *E. faecalis* uses the same enzymes (MalT, MapP)  
505 for efficient transport and the first catabolic step of maltose and maltotriose. MapP can also  
506 dephosphorylate maltotetraose-6'''P, suggesting that the enzyme recognizes primarily the  
507 phosphorylated glucose residue at the non-reducing end of maltooligosaccharides. While the  
508 transport system responsible for the residual uptake of maltose in the *maltT* mutant remains  
509 unknown, maltotriose was found to be also slowly taken up by the MdxEFG/MsmX ABC

510 transporter (Fig. 10). The ABC transporter functions as the major uptake system not only for  
511 maltotetraose and longer maltooligosaccharides, but also for the maltotriose linkage isomers  
512 panose and isopanose. *E. faecalis* strain JH2-2 is not able to utilize cyclic maltodextrins. The  
513 PTS-catalyzed transport of maltose and maltotriose was found to be more efficient than the  
514 MdxEFG/MsmX-catalyzed uptake of maltotetraose and longer maltooligosaccharides.  
515 However, the utilization of maltotriose by the *malt* mutant via the ABC transporter was less  
516 efficient than that of maltotetraose, but was similarly efficient as the utilization of the two  
517 trisaccharides panose and isopanose by the wild-type strain. In conclusion, *E. faecalis*  
518 transports maltotriose and maltotetraose, which are usually present in maltodextrin, by  
519 mechanisms and transporters which strongly resemble those reported for *S. mutans* (20), but  
520 which exhibit significant differences from those reported for other streptococci, such as *S.*  
521 *pyogenes* (19) and *S. pneumoniae* (2). To the best of our knowledge, the transport of the  
522 maltotriose linkage isomers panose and isopanose, which are usually also present in small  
523 amounts in maltodextrin, has so far not been studied in bacteria. In *E. faecalis*, they are  
524 exclusively transported by the MdxEFG/MsmX ABC transporter.

525           Interestingly, while deletion of the PTS permease had no effect on *E. faecalis*  
526 virulence, inactivation of the membrane-spanning MalF significantly reduced the colonisation  
527 ability of the pathogen in kidneys and liver in a mouse infection model. With the liver and to a  
528 lesser extent the kidney being the place for storage and breakdown of glycogen, it is not  
529 surprising that inactivation of the maltodextrin ABC transporter affects the fitness of the  
530 pathogen in these organs.

531

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536

537

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550

551

552 **CONFLICTS OF INTEREST**

553 The authors declare no conflicts of interest.

554

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- 673
- 674
- 675

676 TABLE 1 Bacterial strains and plasmids used in this study.

677

678

Bacterial stains	Relevant Characteristics	Source or reference
<u>Enterococcus faecalis</u>		
JH 2-2	Fus <sup>R</sup> , Rif <sup>R</sup> , plasmid free, maltotetraose-positive wild-type strain	(39)
<i>mdxF</i> mutant	JH2-2 carrying stop codons at the beginning of the <i>mdxF</i> gene.	This study
<i>maltT</i> mutant	JH2-2 carrying stop codons at the beginning of the <i>maltT</i> gene.	This study
JH2-2 pAGEnt	JH2-2 harboring empty pAGEnt	This study
<i>mdxF</i> + pAGEnt	<i>mdxF</i> mutant harboring empty pAGEnt	This study
<i>mdxF</i> + pAGEnt:66c	<i>mdxF</i> mutant harboring pAGEnt with the <i>mdxF</i> gene under control of the agmatine-inducible <i>aguB</i> promoter	This study
<u>Lactococcus lactis</u>		
IL1403	Wild-type strain	(40)
<u>Escherichia coli</u>		
Top10F'	<i>F</i> '[ <i>lacIq</i> Tn10 (Tet <sup>R</sup> ) <i>mcrA</i> Δ( <i>mrr</i> - <i>hsdRMS</i> - <i>mcrBC</i> ) φ80 <i>lacZ</i> Δ <i>M15</i> Δ <i>lacX74</i> <i>recA</i> 1 <i>araD139</i> Δ( <i>ara</i> - <i>leu</i> ) 7697 <i>galU</i> <i>galK</i> <i>rpsL</i> ( <i>Str</i> <sup>R</sup> ) <i>endA1</i> <i>nupG</i>	Invitrogen
NM522[pQE30-mapP]	<i>supE</i> <i>thi-1</i> D( <i>lac-proAB</i> ) D( <i>mcrB</i> - <i>hsdSM</i> ) (rk- mk-)[F' <i>proAB</i> <i>lacIqZ</i> DGM15] [pQE30-mapP]	(7)
Plasmid s		
pMAD	Origin pE194ts, <i>Erm</i> <sup>R</sup> , <i>Amp</i> <sup>R</sup> , <i>bgab</i>	(27)
pAGEnt	modified pNZ8048 plasmid, <i>Cm</i> <sup>R</sup> , contains <i>aguR</i> <i>PaguB</i> (promoter inducible with agmatine)	(26)
pAGEnt:66c	pAGEnt plasmid with the <i>mdxF</i> gene under control of the agmatine-inducible <i>aguB</i> promoter	This study

679

680

681

682 **TABLE 2** Primers used in this study.

683

Primers	Sequence (5' to 3') <sup>a</sup>	Orientation <sup>b</sup>
pMAD_41760_for	CCGGCCATGGAATGAAAAAATGTTAGTTTG	+
pMAD_41760_rev	GGTGGATCCTAGTTGATTAATAACTAC	-
mut41760_for	CGTTAGAATTCTAGTTTATGTTTGCTG	+
mut41760_rev	ACATGAATTCTTACGGCTCTGTCACAC	-
Verif_41760	CGCCGGAAGCTAGTAAATCTTC	-
pMAD_41966_for	GTCAGTGGATCCTGCACCGTAGAAGTTAG	-
pMAD_41966_rev	AAAATCGGATCCAATTTGGCGAAGATGGC	+
mut41966_for	AGGAACACTAGTAGCCTAACAGCAAGGATTAG	+
mut41966_rev	GGGTCGCTAGCGTTCATAGCATCATTAATGCG	-
Verif_mut_41966	TGTTAGGTCACTAGCGTTCA	-
pAGEnt_66_forkit	TTTTAGGAGGAACACATCATGTTCAAGAAAAAGAAAGC	+
pAGEnt_66_revkit	AAGCAACACGTGCTGTAATTTTACGCCTCCTTAAATGAATTC	-
RPV_AGEnt_RBS	CATGATGTGTTCTCCTAAAAG	
FRV_AGEnt_Term	AATTACAGCACGTGTTGCTTT	
race1_41965	TCTTTTCAAATCGGCGAC	-
race2_41965	CCCATAATTTACGTCACCAGCTG	-
race3_41965	TTCGTTTTTCTGCGCCACC	-
EF1344R	TCATTAATGCGTGGAACCA	-
EF1344L	AACTTCGCCAACAAGCAGT	+
EF1345R	ATGCCTTCCTTCTGGTCCT	+
EF1345L	CCATTGCCAAAATCAATGGT	-

684

685 <sup>a</sup>Underlined sequences correspond to restriction sites and bold letters indicate stop codons.686 <sup>b</sup>The + means oriented in and the - oriented against the direction of transcription.

687

688 **TABLE 3** Specific activity of MapP determined with the malachite green assay in the  
 689 presence of 2 or 4 mM of its substrates maltose-6'-P, maltotriose-6''-P, and maltotetraose-  
 690 6'''-P. The mean values together with the standard deviations from three independent  
 691 experiments are presented.  
 692

Substrate	Specific activity (2 mM) $\mu\text{mol min}^{-1} \text{mg protein}^{-1}$	Specific activity (4 mM) $\mu\text{mol min}^{-1} \text{mg protein}^{-1}$
Maltose-6'-P	$161 \pm 15$ (100%)	$173 \pm 28$ (100%)
Maltotriose-6''-P	$123 \pm 21$ (76%)	$141 \pm 22$ (81%)
Maltotetraose-6'''-P	$133 \pm 17$ (82%)	$139 \pm 18$ (80%)

693

694 **FIGURE LEGENDS**

695

696 **FIG 1** The *E. faecalis* JH2-2 chromosomal region containing the *mdxEFG* and the upstream  
697 operon, which both encode genes required for maltodextrin utilization. (A) Presented are the  
698 three genes of the *mdxEFG* operon and the three upstream genes. They encode the two  
699 membrane components of the ABC transporter and the periplasmic binding protein and two  
700  $\alpha$ -glucosidases and a glucosyl transferase, respectively (P. Joyet, N. Sauvageot, A. Hartke and  
701 J. Deutscher, unpublished results). The two operons are transcribed from two divergent  
702 promoters (small arrows). (B) A major part of our JH2-2 stock carries a mutation in the  
703 intergenic region leading to the replacement of the T in the sixth position of the -10 promoter  
704 region with a G (dashed box). The -10 promoter region of the *mdxEFG* operon is underlined,  
705 the transcription initiation point is marked with a small arrow and the ribosome binding site is  
706 written in italics. The start codon of *mdxE* is written in bold letters. The T to G mutation  
707 prevents the utilization of maltotetraose and longer maltooligosaccharides as well as of  
708 panose and isopanose. The *E. faecalis* strain V583 and all other strains, for which the genome  
709 has been completely sequenced, have the CATAAT -10 promoter sequence for the *mdxEFG*  
710 operon. However, these strains lack the T directly following the -10 promoter in JH2-2.

711

712 **FIG 2** Growth studies with the maltotetraose-positive *E. faecalis* strain JH2-2 (filled symbols)  
713 and the *malT* mutant derived from it (open symbols). Strains were grown in M17cc medium  
714 supplemented with 0.3% of either glucose (◆), maltose (■), maltotriose (▲) or maltotetraose  
715 (●). Growth studies were carried out as described in Materials and Methods.

716

717 **FIG 3** Growth studies with the *E. faecalis mdxF* mutant (filled symbols) and the *malT/mdxF*  
718 double mutant (open symbols). The two strains were grown in M17cc medium supplemented  
719 with 0.3% of either glucose (◆), maltose (■), maltotriose (▲) or maltotetraose (●)

720

721 **FIG 4** MapP-catalyzed dephosphorylation of maltotriose-6''-P as evidenced by mass  
722 spectrometry. Dephosphorylation assays of maltotriose-6''-P with *E. faecalis* MapP and  
723 subsequent analysis by mass spectrometry were carried out as described in Materials and  
724 Methods. (A) Untreated maltotriose-6''-P: The weak peak with a mass  $m/z$  of 527.16  
725 corresponds to unphosphorylated maltotriose containing one  $\text{Na}^+$  ion. The two strongest  
726 peaks at 604.14 and 629.12 correspond to maltotriose-6''-P with one  $[(\text{M}+\text{H})+\text{Na}^+]$  or two  
727  $[(\text{M}+\text{H})+2\text{Na}^+]$   $\text{Na}^+$  ions, respectively. (B) MapP-treated maltotriose-6''-P: The two strong  
728 peaks in (A) completely disappeared after treatment with MapP and a very strong single peak  
729 appeared at 527.19, which corresponds to maltotriose carrying one  $\text{Na}^+$  adduct.

730

731 **FIG 5** Growth studies with the maltotetraose-positive *E. faecalis* JH2-2 strain (filled symbols)  
732 and the *mdxF* mutant derived from it (open symbols). Cells were grown in M17cc medium  
733 supplemented with 0,3% (w/v) of either maltopentaose (■), maltohexaose (◆) or  
734 maltoheptaose (●).

735

736 **FIG 6** Growth studies with the complemented *E. faecalis mdxF* mutant. The maltotetraose-  
737 positive *E. faecalis* strain JH2-2 with the empty plasmid pAGent (▲) and the *mdxF* mutant  
738 transformed with either the empty plasmid pAGent (◆) or pAGent carrying the *mdxF* allele  
739 (pAGent:66c) (■) were grown in M17cc medium supplemented with 0.3% of maltotetraose.

740

741 **FIG 7** Relative transcription of the *mdxE* (black bars) and *mdxF* genes (white bars) in  
742 maltotetraose-negative (Tetra<sup>-</sup>) and maltotetraose-positive (Tetra<sup>+</sup>) JH2-2 clones. The strains  
743 were grown in M17cc medium containing either 0.5% maltodextrin or 0.5% maltodextrin plus  
744 0.5% glucose. The mean values calculated from three independent experiments are presented.  
745 Standard deviations are indicated with vertical bars.

746

747 **FIG 8** Growth studies with *E. faecalis* strains on panose and isopanose. (A) Growth was  
748 followed for the maltotetraose-positive JH2-2 strain (■), the *mdxF* (▲) and the *malT* (◆)  
749 mutants in M17cc medium supplemented with 0.3% (w/v) of either panose (open symbols) or  
750 isopanose (filled symbols). (B) The maltotetraose-positive strain JH2-2 (■) and the *mdxF*  
751 mutant derived from it (◆) were transformed with empty plasmid pADGEnt. The *mdxF*  
752 mutant was also transformed with pADGEnt:66c, which contains the *mdxF* gene under  
753 control of the agmatine-inducible *aguD* promoter (▲). The three strains were grown in M17cc  
754 medium supplemented with 0,3% (w/v) of either panose (open symbols) or isopanose (filled  
755 symbols).

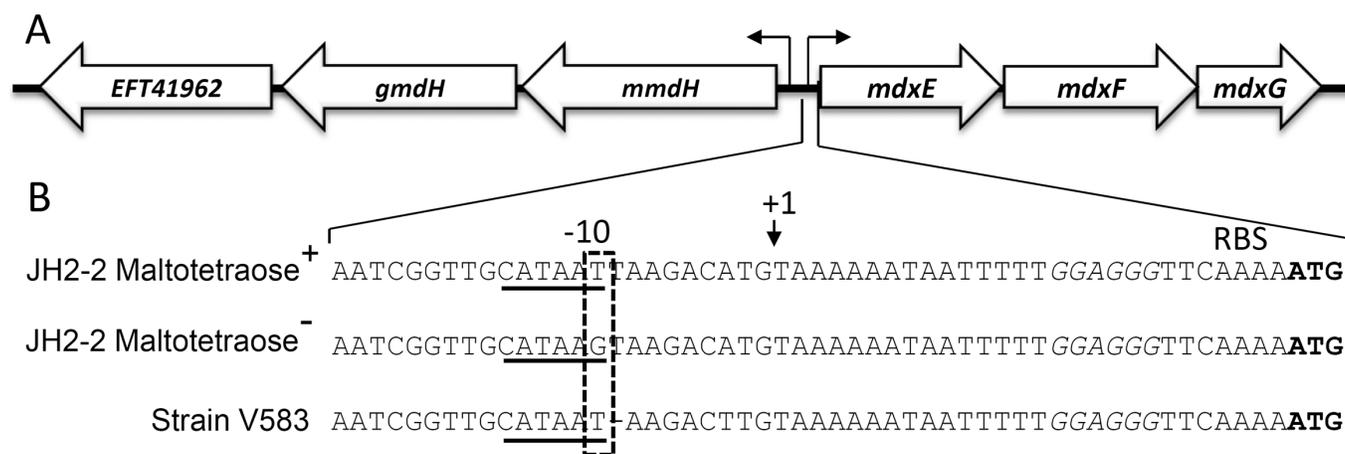
756

757 **FIG 9** Colonization of mouse organs by various *E. faecalis* strains. Standardized inocula of  
758  $8 \times 10^8$  CFU of the maltotetraose-positive *E. faecalis* strain JH2-2 and either (A) the *malT*, (B)  
759 the *tpx* and (C) the *mdxF* mutants derived from it were intraperitoneally injected into Swiss  
760 white mice. After 24 h (*malT* and *mdxF* mutant) or 72 h (*tpx* mutant) the survival rate was  
761 determined for each strain by bacterial counts in kidneys, liver and spleen (CFU/g tissue) and  
762 in the peritoneum (CFU/ml washing fluid). The experiments were carried out with 5 mice per  
763 group. The experiment with the *mdxF* mutant was performed twice and the results obtained  
764 with the ten mice are shown (C). The numbers obtained for each individual mouse (dots) are  
765 presented as  $\log_{10}$ . The results are expressed in CFU/g (for the organs) and as CFU/ml (for the

766 peritoneal wash); mean values are presented as black bars. To compare the results obtained  
767 for two different groups we used the Mann-Whitney test. The calculated *P* values of <0.05 (\*)  
768 and <0.005 (\*\*) (grey horizontal bars) reflect the overall difference of the CFU values  
769 determined in kidney or liver, respectively, between the groups of mice infected with the  
770 *mdxF* mutant and the ones infected with the wild-type strain.

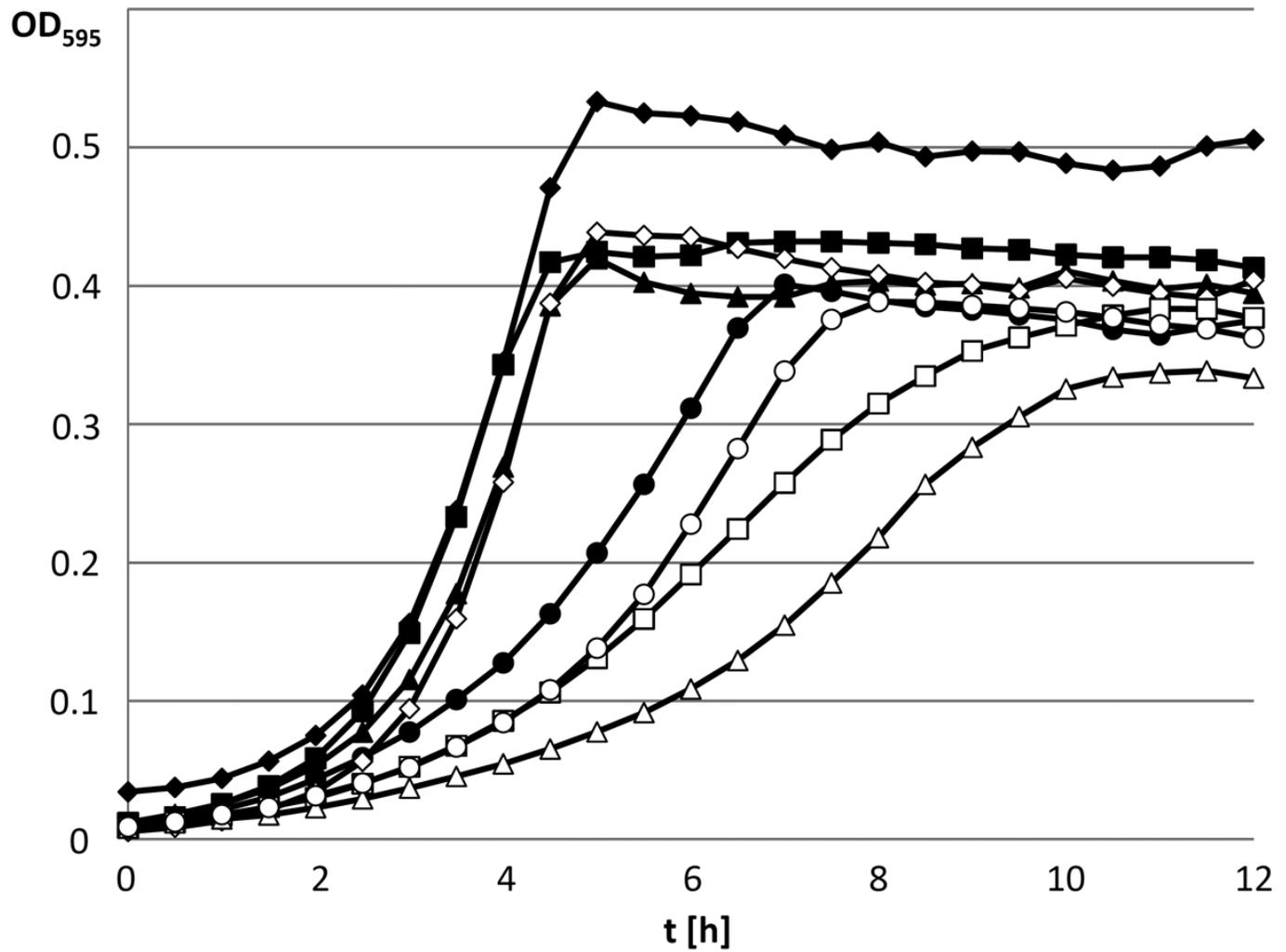
771

772 **FIG 10** Schematic presentation of the *E. faecalis* transport systems catalyzing the uptake of  
773 maltose, maltotriose, maltotetraose and the maltotriose linkage isomers panose and isopanose.  
774 While maltose and maltotriose are primarily transported by the PTS permease MalT,  
775 maltotetraose and longer maltooligosaccharides as well as panose and isopanose are taken up  
776 by the ABC transporter MdxEFG/MsmX. When substrates or arrows are in grey, this  
777 indicates that they are transported with low efficiency. This applies for the transport of  
778 maltotriose by the ABC transporter MdxEFG/MsmX and the uptake of maltose by a yet  
779 unknown transport protein.



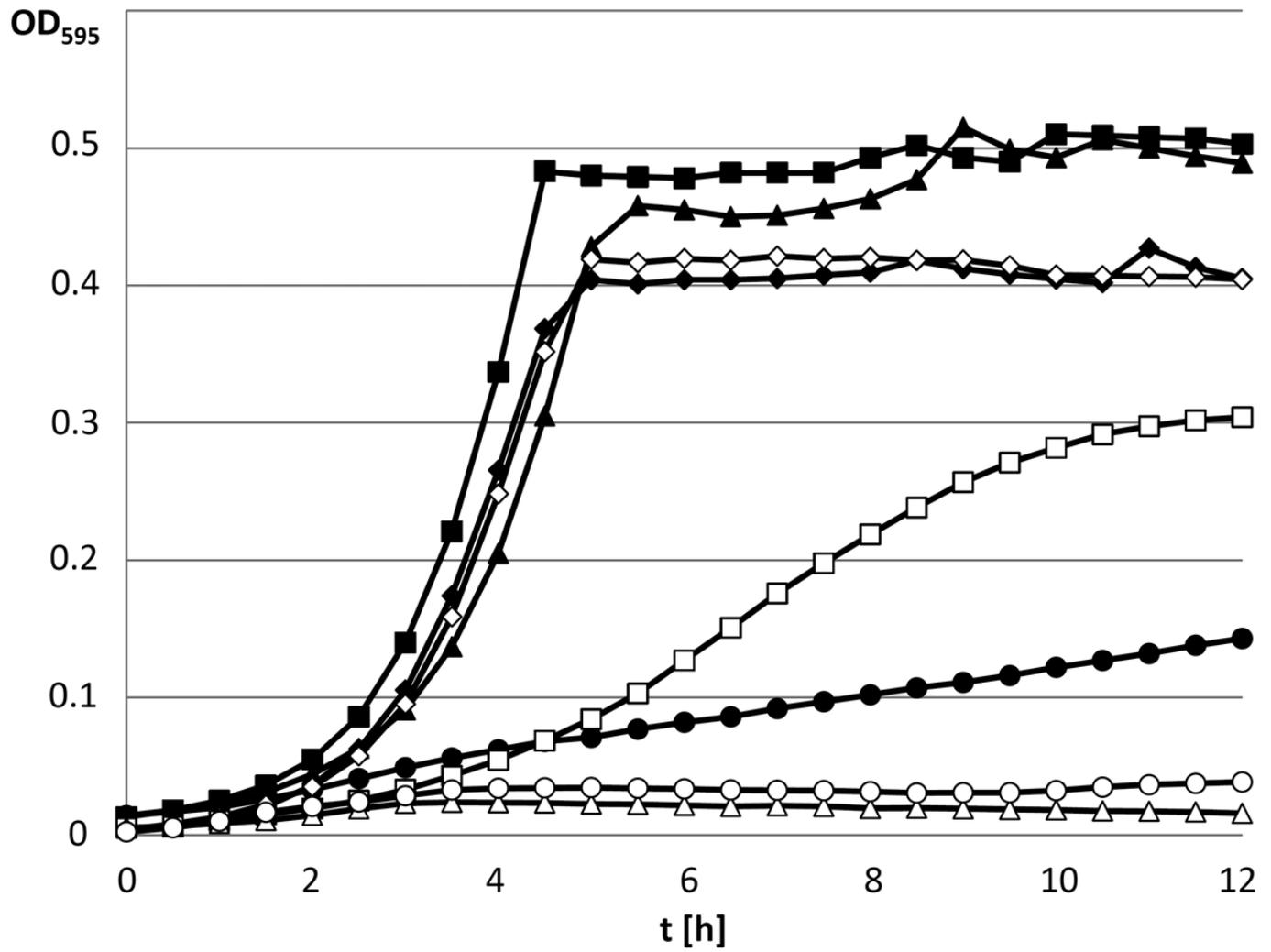
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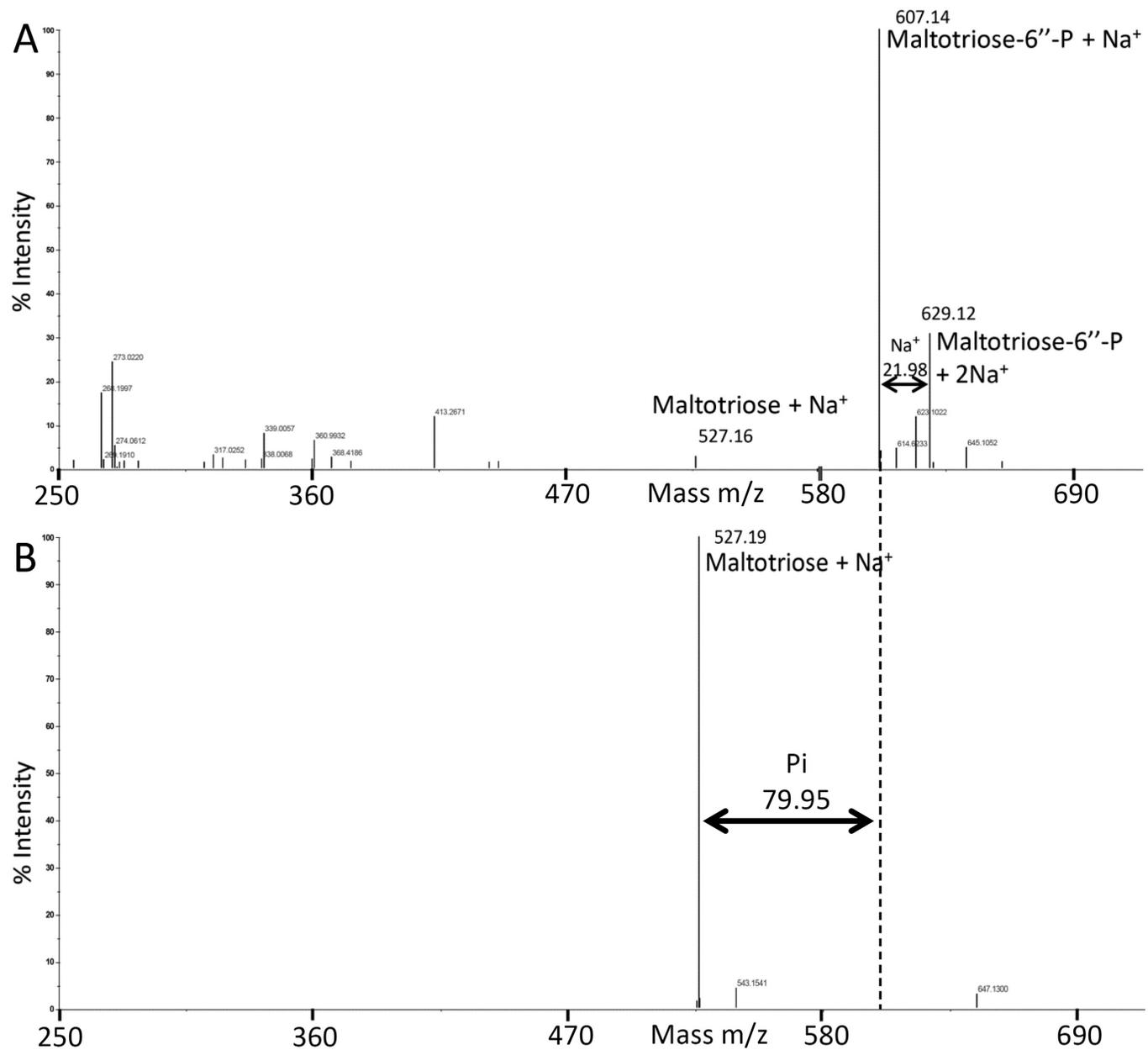
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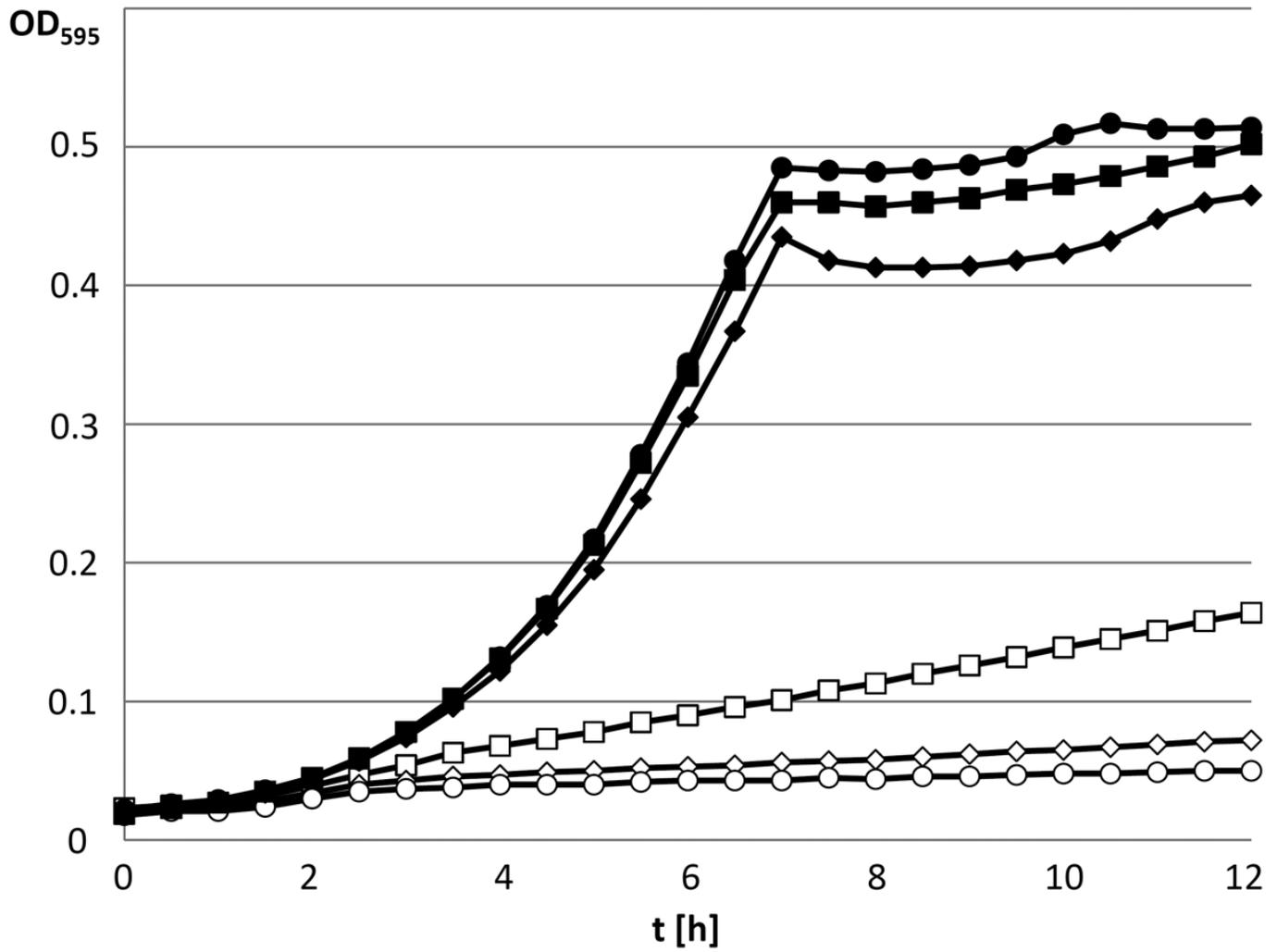
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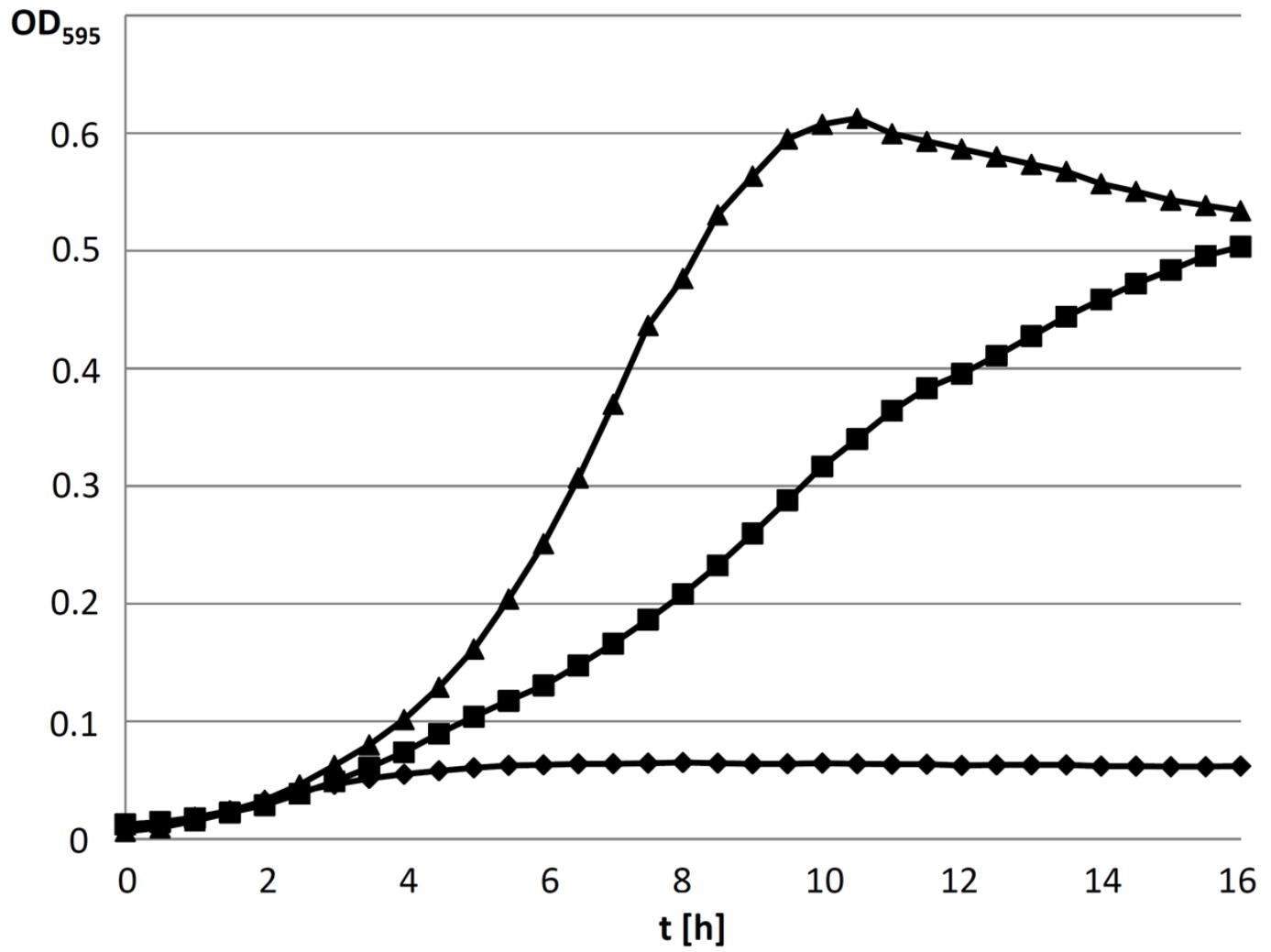
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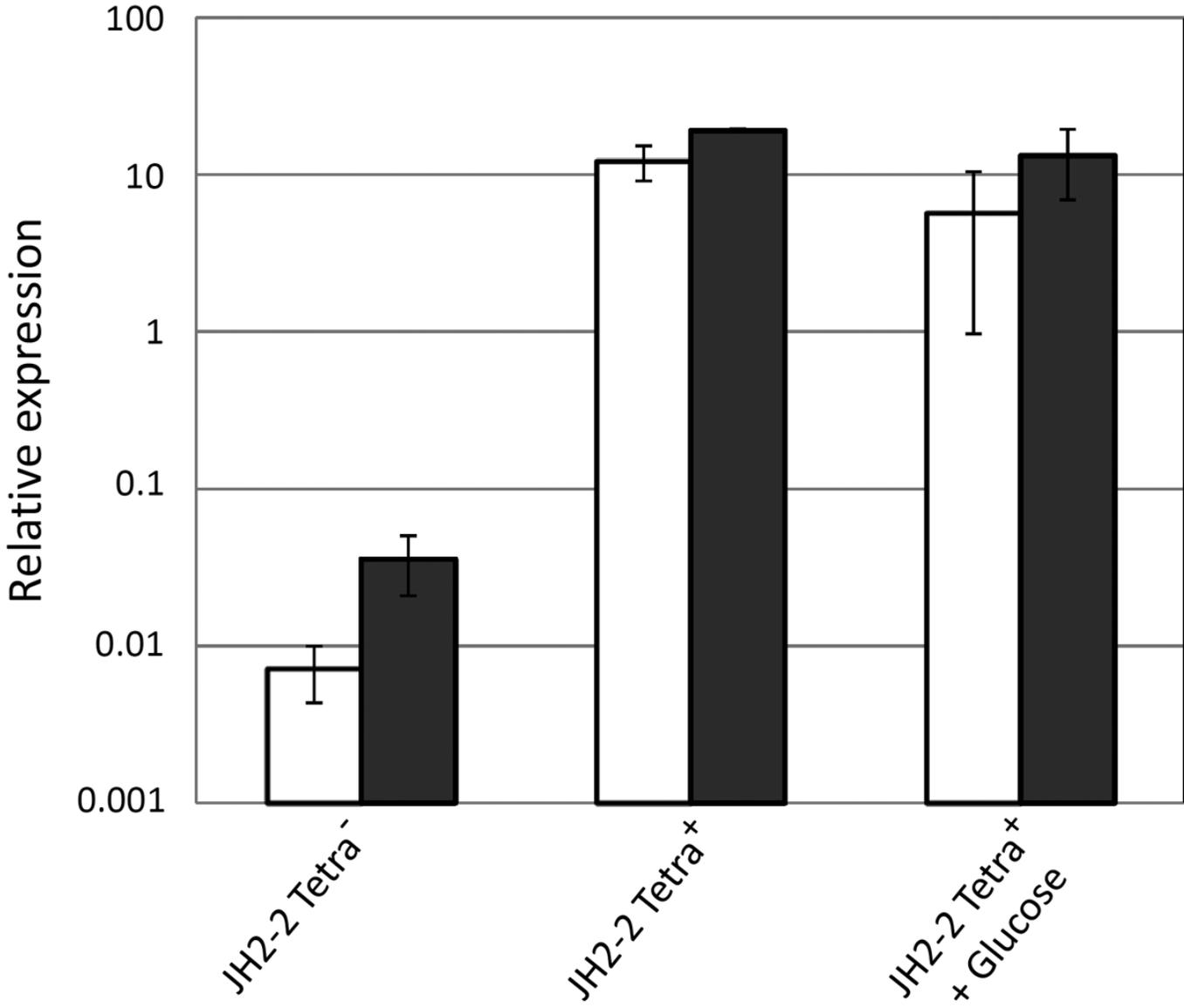
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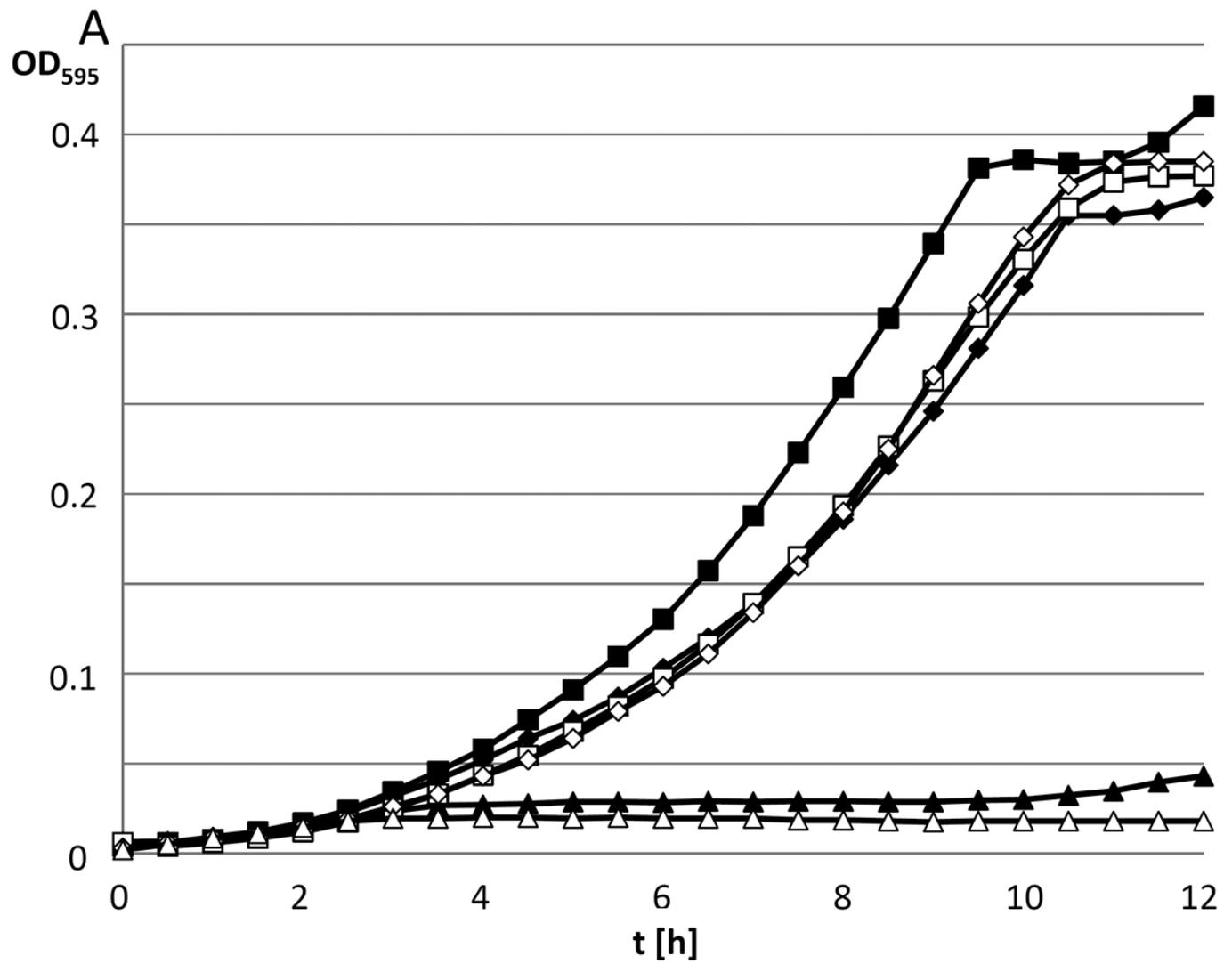


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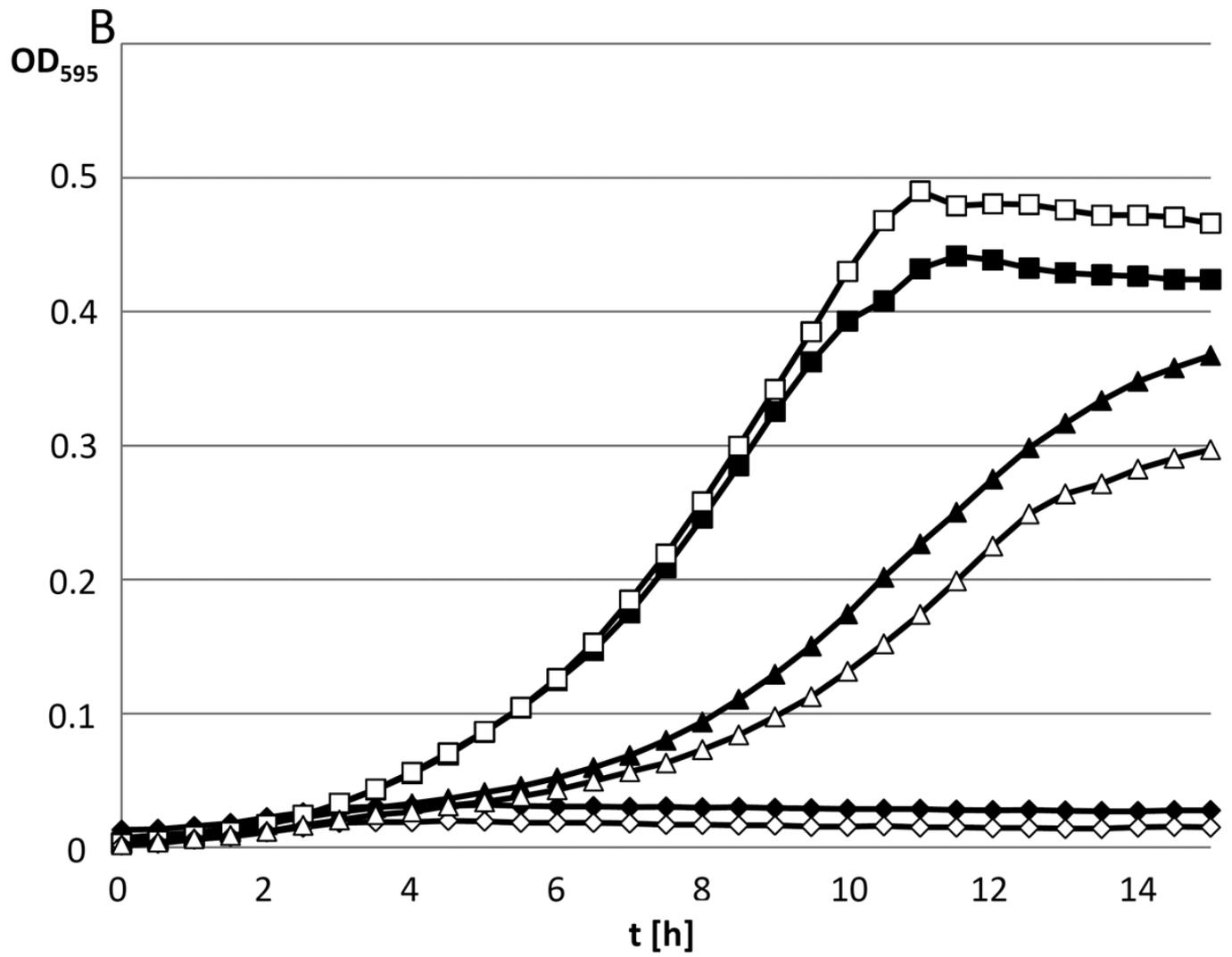


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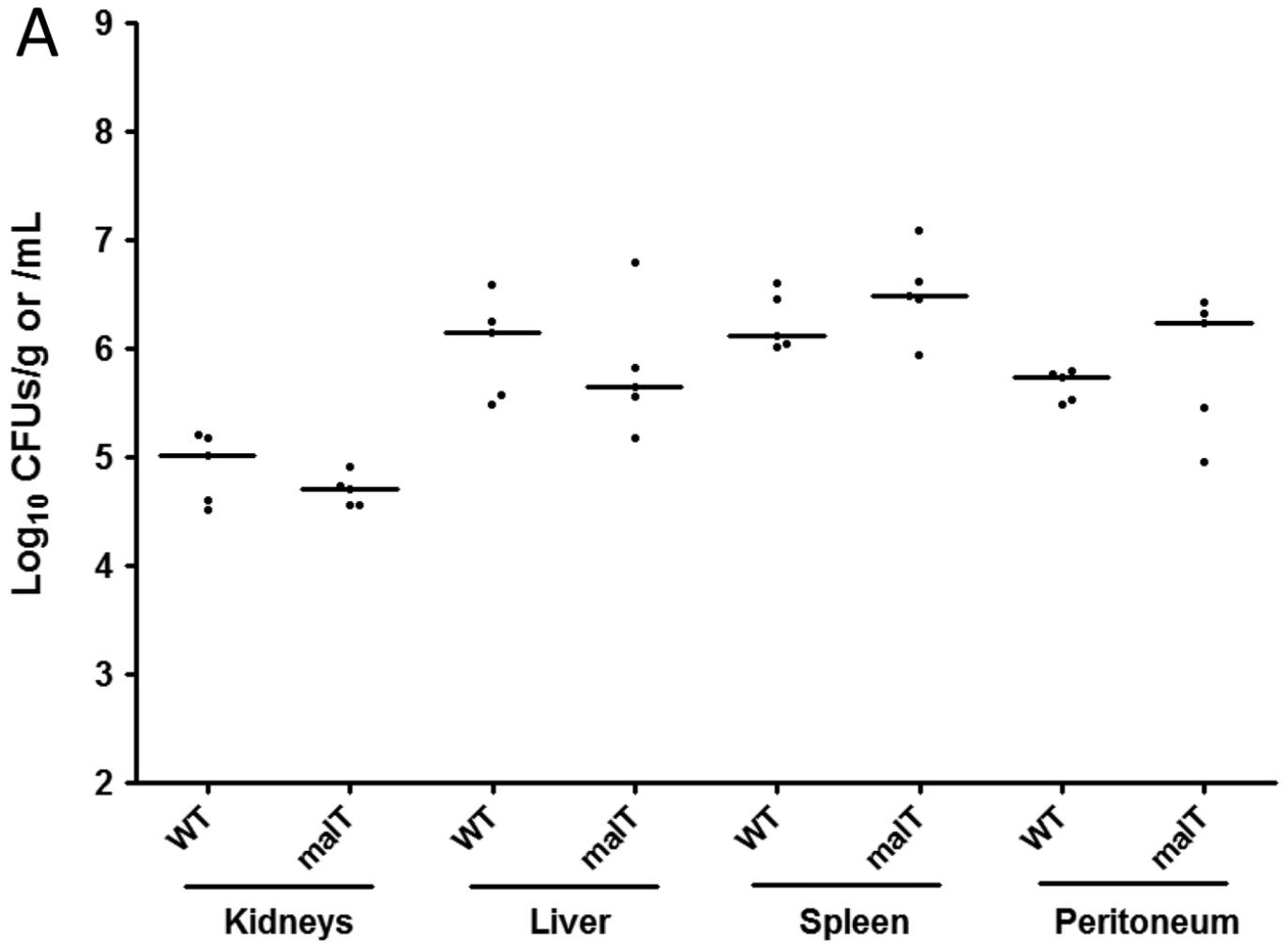
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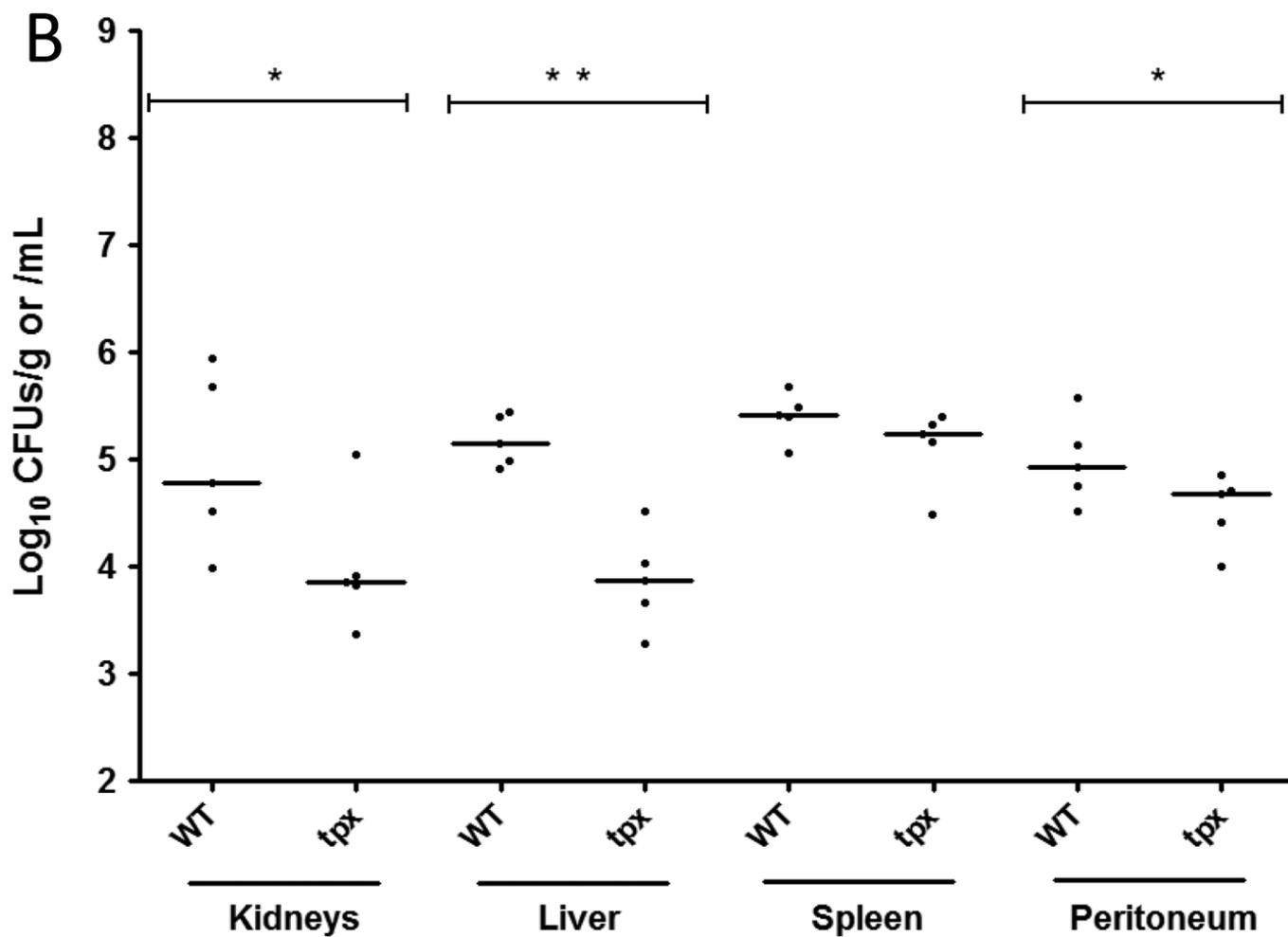
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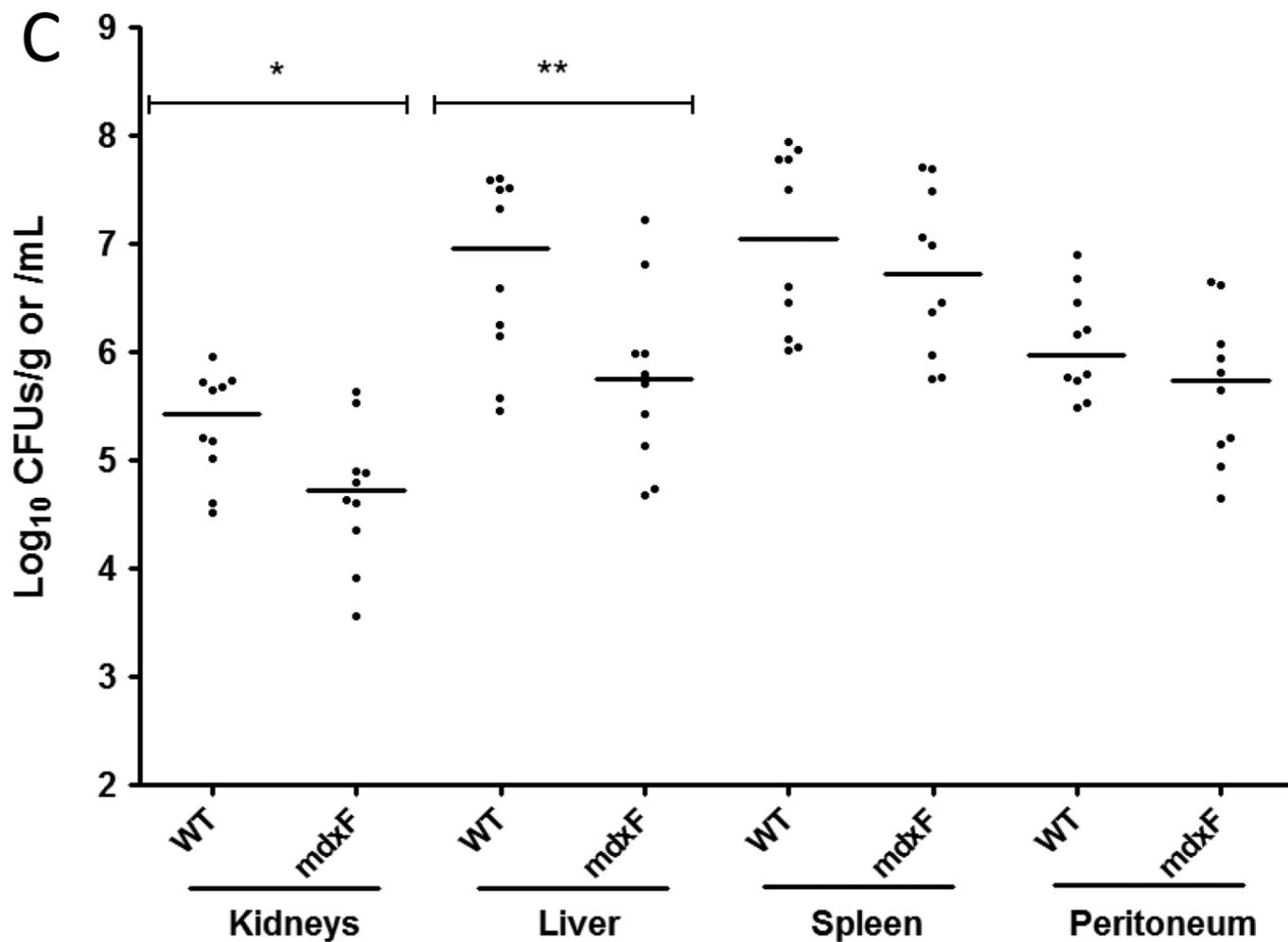
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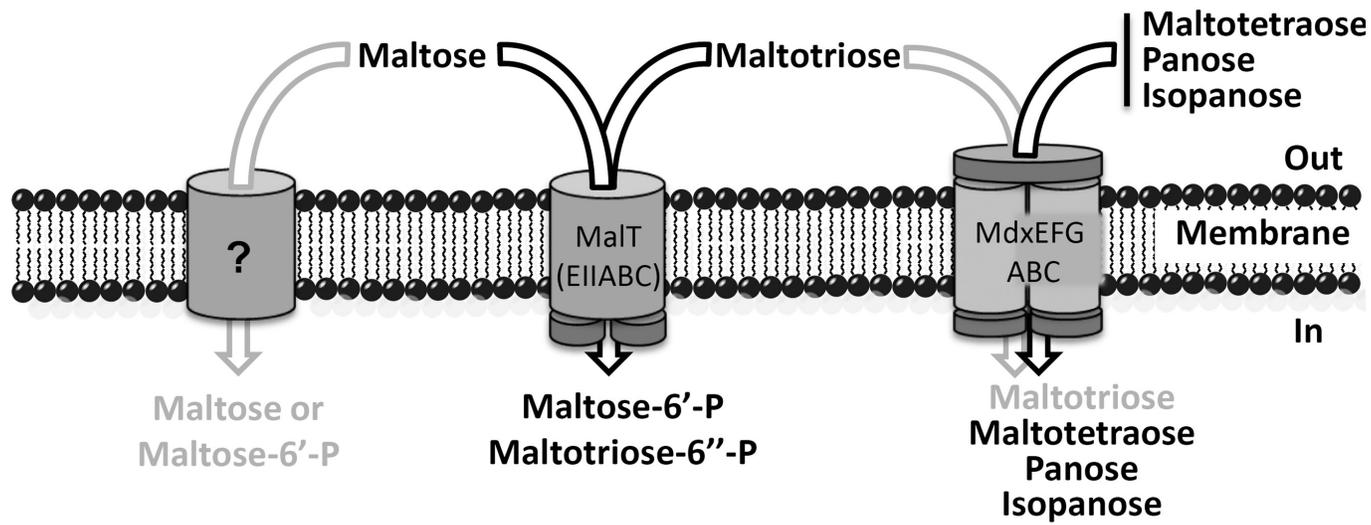
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