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Analysis of σ^{54}-dependent genes in *Enterococcus faecalis*: a mannose PTS permease (EII^{Man}) is involved in sensitivity to a bacteriocin, mesentericin Y105

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The σ^{54} RNA polymerase subunit has a prominent role in susceptibility of *Listeria monocytogenes* and *Enterococcus faecalis* to mesentericin Y105, a class IIa bacteriocin. Consequently, σ^{54}-dependent genes as well as specific activators also required for expression of these genes were sought. Five putative σ^{54}-associated activators were detected in the genome of *E. faecalis* V583, and all but one could activate the transcription of permease genes belonging to sugar phosphotransferase systems (PTSs). Interestingly, these activators display a helicase signature not yet reported in this activator family, which could explain the ATP-dependent mechanism of DNA unwinding preceding the start of transcription. To find which activator is linked to susceptibility of *E. faecalis* to mesentericin Y105, their respective genes were subsequently interrupted. Among them, only *mptR* gene interruption led to a resistance phenotype. Immediately downstream from *mptR*, a putative σ^{54}-dependent operon was found to encode a mannose PTS permease, namely EII_t^{Man}. Moreover, in liquid culture, glucose and mannose induced the sensitivity of *E. faecalis* to mesentericin Y105. Since sugars have previously been reported to induce PTS permease expression, it appears that EII_t^{Man} expression, presumably induced in the presence of glucose and mannose, leads to an enhanced sensitivity of *E. faecalis* to the bacteriocin. Additional information was gained from knockouts within the permease operon. Interruption of the distal *mptD* gene, which encodes the IID subunit of EII_t^{Man}, strikingly led to resistance to mesentericin Y105. Moreover, MptD appears to be a peculiar membrane subunit, bearing an additional domain compared to most known IID subunits. According to these results, EII_t^{Man} is clearly involved in susceptibility to mesentericin Y105 and could even be its receptor at the *E. faecalis* surface. Finally, it is hypothesized that MptD could be responsible for the targeting specificity, via an interaction between its additional domain and mesentericin Y105.

Keywords: antagonism, subclass IIa, phosphotransferase, sugar, helicase

INTRODUCTION

Lactic acid bacteria are largely used in food fermentation and have been subjected to numerous studies, leading to the description of many antibacterial peptides, named bacteriocins (Jack *et al*., 1995; Klaenhammer, 1993). Consequently, the latter or their producing strains are of great interest in food protection. For example, nisin (Breukink & de Kruijff, 1999), a bacteriocin produced by *Lactococcus lactis*, has been used as a food preservative since 1957. Nisin has a broad spectrum of inhibition against several foodborne Gram-positive pathogens, e.g. *Listeria monocytogenes* and *Clostridium botulinum*. It belongs to the class I bacteriocins, termed lantibiotics, which are post-translationally modified peptides. Among other families, subclass IIa bacteriocins deserve special interest as they share an anti-*Listeria*

Abbreviations: 2DG, 2-deoxyglucose; PTS, phosphotransferase system.

activity and have a conserved structural motif (YGNGVXC) (for a review see Ennahar *et al*., 2000). They were reported to act by permeabilizing the membrane of susceptible bacteria (Abee, 1995), although their molecular mode of action is largely unknown. For example, the need of a protein receptor at the bacterial surface is still debated. We focused on mesentericin Y105, a subclass IIa bacteriocin, and we looked for its mode of action at the molecular level (Héchard *et al*., 1992; Maftah *et al*., 1993). We found that the σ^{54} transcription factor is involved in sensitivity of *L. monocytogenes* (Robichon *et al*., 1997) and *Enterococcus faecalis* (Dalet *et al*., 2001) to mesentericin Y105, suggesting that σ^{54} directs the expression of an essential protein for sensitivity. σ factors are subunits of bacterial RNA polymerase holoenzymes responsible for recognition of promoters. Among the σ family, σ^{54} is a unique factor (Merrick, 1993). It recognizes particular promoters having a consensus sequence localized at position $-24/-12$ from the transcription start site, and requires activator proteins (Buck *et al*., 2000) to initiate transcription. These activators, referred to as σ^{54}-associated activators, share a conserved central domain, already used to identify new protein members of this family. The first σ^{54} factor described in Gram-positive bacteria is encoded by *sigL* in *Bacillus subtilis* (Débarbouillé *et al*., 1991b). It directs, in association with the activator LevR, transcription of the *lev* operon encoding a permease of the phosphotransferase system (PTS) and the levanase enzyme (Débarbouillé *et al*., 1991a). Interestingly, two PTS permeases were recently connected with resistance to subclass IIa bacteriocins of *L. monocytogenes* spontaneous mutants. In the first study, a mannose IIAB PTS component was absent in a leucocin-A-resistant strain (Ramnath *et al*., 2000), while in the second, a β-glucoside PTS permease was shown to be overexpressed in a pediocin-PA1-resistant strain (Gravesen *et al*., 2000). The PTS transfers a phosphoryl group from phosphoenolpyruvate to sugar via EI, Hpr and EII enzymes (Postma *et al*., 1993; Saier & Reizer, 1994). The latter, EII, is a PTS permease involved in transport and concomitant phosphorylation of sugars. EII is composed of three subunits: IIA and IIB, which are cytoplasmic subunits involved in phosphorylation, and IIC, a membrane protein responsible for sugar transport. An additional membrane subunit, IID, is specifically found within the PTS permease of the mannose family.

Interestingly, most of the subclass IIa bacteriocins were described to be also active against another pathogenic species, *E. faecalis*, in which the σ^{54} factor is involved in sensitivity (Dalet *et al*., 2001). Consequently, σ^{54}-associated activators and σ^{54}-dependent genes were sought and analysed. Knockout of these different genes was then achieved to study their involvement in *E. faecalis* sensitivity to mesentericin Y105.

METHODS

Bacterial strains and growth conditions. *E. faecalis* V583 or JH2-2 and its derivatives were grown at 37 °C in brain-heart infusion (BHI) or in Luria–Bertani (LB) media supplemented or not with sugar. *Escherichia coli* XL-1 Blue, used for molecular cloning, was grown at 37 °C in LB medium with vigorous shaking. Erythromycin (5 μg ml^{-1}), kanamycin (50 μg ml^{-1}) or ampicillin (100 μg ml^{-1}) was added, as needed. *Leuconostoc mesenteroides* Y105, which produced mesentericin Y105, was grown in Man-Rogosa-Sharp (MRS) medium at 30 °C.

DNA manipulations and gene interruption. Molecular cloning and DNA manipulations were performed as described previously (Sambrook *et al*., 1989). Restriction and modification enzymes purchased from 'Life technologies' were used as recommended by the manufacturer. Internal gene fragments, used for knockout experiments, were amplified by PCR with the following primers bearing a *Hin*dIII site: *mpoR* primers, GTCAAGCTTCTGGCTATACCCG and TTTTAAGCTTCGCCCATCCGTG; *mptR* primers, GTAAAGCTTTGAATCAACTGGTTCG and CAGAAAGCTTCCATCTGCTTCATC; *mphR* primers, TCAAAGCTTCCAAGAAATGATCGATG and TGGCAAAGCTTAACCGACACG; *lpoR* primers, AGTAAGCTTCGGACAAGTCAGCG and TCCAAGCTTAATGGCAAAAGCAGATG; *mptB* primers, CGTTAAGCTTGAATTGATGATCG and AATAAGCTTGGCTGTCTGCTGC; *mptD* primers, AGCTGAAGCTTGGCGTTCAAC and AGAACAAGCTTTGTAACCAAACTC. The resulting PCR fragments were digested with *Hin*dIII and ligated at the same site in pUCB300 (Frère *et al*., 1993), a non-replicative plasmid bearing an erythromycin-resistance gene. It gave rise to the pEF17 ('*mpoR*'), pEF16 ('*mptR*'), pEF23 ('*mphR*'), pEF5 ('*lpoR*'), pEF18 ('*mptB*') and pEF19 ('*mptD*') plasmids. They were then used to transform *E. faecalis* JH2-2 as described by Wyckoff *et al*. (1991) to achieve independent gene knockout by homologous recombination with the *E. faecalis* JH2-2 chromosome. The resulting interrupted mutants (erythromycin-resistant) from each experiment were analysed by Southern blotting of chromosomal DNA (Sambrook *et al*., 1989), previously digested by *Hin*dIII. The DNA probes used for hybridization were synthesized by random priming from the PCR fragments described above.

2-Deoxyglucose-resistant strain selection. *E. faecalis* JH2-2 was grown on LB agar plates supplemented with fructose at 2 g l^{-1}, as a carbon source, and 2-deoxyglucose (2DG), a non-metabolizable analogue of glucose, at 10 mM. The resulting colonies, corresponding to the growth of 2DG-resistant mutants, were isolated twice on the same medium. 2-DG is a toxic molecule that enters bacteria via a PTS permease of the mannose family (Bond *et al*., 1999). Consequently, this permease is not usually expressed in 2DG-resistant mutants.

Bacteriocin purification and assays. Mesentericin Y105, produced by *Leuconostoc mesenteroides* Y105, was purified as previously described (Guyonnet *et al*., 2000). *E. faecalis* susceptibility was assayed by spot on lawn or microtitre plate tests. The former was achieved by overlaying a BHI agar plate (1·5 %) with a BHI agar lawn (0·7 %) inoculated at 1 % with an *E. faecalis* culture. Purified mesentericin Y105 (5 μl) was spotted on the lawn and the plate was then incubated overnight at 37 °C before inhibition zones were noted. The microtitre plate assay was carried out by inoculating 200 μl BHI or LB supplemented with various sugars at 2 g l^{-1}. Bacterial growth was monitored by measurement of the OD_{620} and 5 μl purified mesentericin Y105 was added when the OD_{620} reached 0·1.

DNA sequencing. Cycle sequencing was achieved with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer) and analysed with the ABI Prism 310 genetic analyser. Sequence data from *E. faecalis* V583 were

obtained from the Institute for Genomic Research through the website at http://www.tigr.org/.

RESULTS

Computer analysis of the σ^{54}-dependent genes

Neither σ^{54}-associated activators nor σ^{54}-dependent genes have been reported so far in *E. faecalis*. We first looked for σ^{54}-associated activator genes within the *E. faecalis* V583 genome, taking advantage of two main features: one, the activator genes are often found upstream from the σ^{54}-dependent genes they control; and two, the activator proteins have a highly conserved central domain, used for their identification (Studholme & Buck, 2000). Interestingly, shotgun sequencing of *E. faecalis* V583 has been completed and closure of gaps was under way, providing sufficient information for our study.

Thus, we first used an amino acid sequence of the central domain to screen the *E. faecalis* V583 genome. BLAST results displayed five high scores (> 45% identities), corresponding to five ORFs, named *mpoR*, *mptR*, *mphR*, *lpoR* and *xpoR*. These genes potentially encode σ^{54}-associated activators of 937, 961, 923, 901 and 403 amino acids, respectively. The latter, *xpoR*, is interrupted by a sequence which is identical to the transposon Tn*4001* from *Staphylococcus aureus* (Byrne *et al*., 1989). Interestingly, all these activators share highest identities, about 30%, with an unusual member of the σ^{54}-associated activator family, LevR of *B. subtilis* (Débarbouillé *et al*., 1991a). Besides the classical motifs usually described in σ^{54}-associated activators, the central domain of all these activators displayed a DEAH motif, according to Prosite (Fig. 1), usually found in helicases. Such similarities with helicases have already been looked for in activators to explain their involvement in DNA conformational changes (Buck *et al*., 2000), but none have been found. We suggest that, owing to the presence of the DEAH motifs, these activators could be directly responsible for ATP-dependent DNA unwinding, allowing initiation of transcription. In addition, we found degenerate DEAH motifs in all the other σ^{54}-associated activators so far described in the database (data not shown), indicating that they could also bear a helicase activity.

We then looked, downstream of the activator genes, for the −24/−12 consensus sequence (TTGGCACNNN-NNTTGCT), identifying a promoter recognized by σ^{54}. A σ^{54} promoter was found just downstream of every activator gene, except the truncated *xpoR*, therefore preceding a putative σ^{54}-dependent operon. Four ORFs were found in each operon downstream of *mpoR*, *mptR* and *mphR* (Fig. 2). These ORFs putatively encode proteins that display highest similarities with PTS permeases (EII) of the mannose family, i.e. IIA^{Man}, IIB^{Man}, IIC^{Man} or IID^{Man} subunits (a fused $IIAB^{Man}$ subunit is encoded by *mptA* in the *mpt* operon). Finally, downstream of *lpoR*, two σ^{54} promoter sequences were found, respectively followed by three ORFs that putatively encode proteins with highest similarities with PTS permease of the lactose family, i.e. IIA^{Lac}, IIB^{Lac} and IIC^{Lac} subunits. Surprisingly, all these σ^{54}-dependent operons probably encode a PTS permease, while to date only one σ^{54}-dependent PTS permease has been described (Débarbouillé *et al*., 1991a). This is a fructose permease encoded by the *lev* operon and controlled by the LevR activator in *B. subtilis*.

Role of σ^{54}-associated activators in sensitivity to mesentericin Y105

E. faecalis V583 and JH2-2 were both sensitive to mesentericin Y105. However, further experiments were performed only with JH2-2, since our previous studies on σ^{54} involvement were conducted with the latter strain. Each activator gene, i.e. *mpoR*, *mptR*, *mphR* and *lpoR*, was knocked-out in *E. faecalis* JH2-2 to assess its involvement in mesentericin Y105 sensitivity. To this aim, an internal fragment of each gene was amplified by PCR from JH2-2 genomic DNA and cloned in pUCB300. The resulting plasmids were independently used to transform *E. faecalis* JH2-2 and led to homologous

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XpoR     IGSQGSAKKAIEQCKAAMLYPPLGMPLLIHGASGVGKSFLAKLIYEYLKNEQIIGLEK 133
MptR     IGSAGSMKTPVEQAKAAILYPPKGLNCLITGPTGSGKTYFAHAMFQFAKLNQIVAKEK 180
LpoR     VGANDSLKVSIQQAKAAILYPPRGLHTIIFGKTGTGKSLFAECMYRFAIDSKTLDEDA 140
MpoR     IGFDGSLADAIKKLKAAILYPPNGLNILLTGESGVGKTLIAEQLHQFYQVK--MNQEV 173
MphR     IGYRGSLAQTIEQLKMAALYPGGGLPVLITGESGTGKSFLASLYYQFCLSKELLDDSA 164
         :*  .*   .::: * * ***  *:  :: * :* **: :*   ..:   .  :  .

XpoR    -FYTFNCADYANNPELLSSILFGHTKGAFTGAESEKQGLLAQANN***SVLFLDEVHR***LSNEN 192
MptR    EFVVFNCADYAHNPELLMSHLFGYVEGAFTGATKAKEGIIDEADG***SILFLDEVHR***LPPEG 240
LpoR    PFVSFNCADYAQNPQLLFGHIFGVKKGAYTGANEDSPGLMAKADG***GILFLDEIHR***LPPEG 200
MpoR    PFIYFNCAEYFNNPELLTSHLFGYKKGSFTGAVNDQKGLVELADG***GFLFLDEVHR***LTSEG 233
MphR    PFVTVNCAQYANNPELLTSQLFGHLKGAFTGADSDKIGAFQSAEG***GVLFLDEVHR***LSPEG 224
         *  .***:* :**:** . :**  :*::*** . . * .  *:...*****:***. *.
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Fig. 1. Alignment of part of the conserved central domain found within putative σ^{54}-associated activators of *E. faecalis* V583 (ClustalW program). The putative ATP/GTP-binding sites (Prosite PDOC00017) are underlined and the putative DEAH-box subfamily ATP-dependent helicase signatures (Prosite PDOC00039) are in bold and in italics. Identical residues in all sequences in the alignment are indicated by an asterisk (*); conserved substitutions are indicated by a colon (:) and semi-conserved substitutions are indicated by a stop (.).

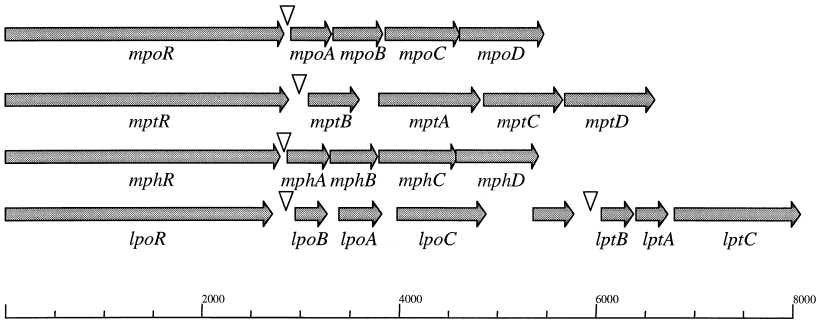

Fig. 2. Organization of σ^{54}-associated activator genes and σ^{54}-dependent PTS operons. The inverted triangles represent the putative −24/−12 promoters.

Table 1. Sensitivity of *E. faecalis* strains to purified mesentericin Y105, assessed by a spot on lawn assay

Strain	Diameter of inhibition zone (mm)
E. faecalis V583 (WT)	11
E. faecalis JH2-2 (WT)	12
JH1 (*rpoN*)	0
JH3 (*lpoR*)	12
JH6 (2DG-resistant)	0
JH7 (*mptR*)	0
JH8 (*mpoR*)	12
JH9 (*mptB*)	0
JH10 (*mptD*)	0
JH11 (*mphR*)	12

recombination in the chromosome. Knockout of *mpoR*, *mptR*, *mphR* and *lpoR*, verified by both PCR and Southern blotting (data not shown), led to the JH8, JH7, JH11 and JH3 strains, respectively.

The sensitivity of these knockout strains was then tested by a spot on lawn assay with purified mesentericin Y105 (Table 1). *E. faecalis* JH3, JH8 and JH11 remained sensitive to mesentericin Y105, similarly to the wild-type JH2-2 strain. In contrast, the JH7 strain (knockout of *mptR*) became fully resistant to purified mesentericin Y105, as was the previously described JH1 strain (σ^{54}-deficient mutant) (Dalet *et al*., 2001). Consequently, MptR is the only *E. faecalis* σ^{54}-associated activator presumably involved in sensitivity to mesentericin Y105. This suggests that MptR and σ^{54} control the expression of proteins involved in *E. faecalis* sensitivity to mesentericin Y105.

Role of EII_t^{Man} in sensitivity to mesentericin Y105

σ^{54}-associated activator genes were often found just upstream of the operon controlled by the activator. Downstream of *mptR*, four ORFs flanked by two hairpin loops with a calculated free energy of −84 and −109·2 kJ, respectively, probably compose an operon, named *mpt*. As described above, these ORFs putatively encode four proteins that display highest similarities with PTS permease components of the mannose family. They were consequently named MptB (170 aa), MptA (342 aa), MptC (267 aa) and MptD (303 aa). Knockout of *mptB* and *mptD*, the proximal and the distal genes of the *mpt* operon, was achieved independently by homologous recombination, leading to the JH9 and JH10 mutants, respectively. These knockout mutants were tested for sensitivity to mesentericin Y105. They were both resistant to mesentericin Y105, as were MptR- and σ^{54}-deficient mutants (Table 1), which supports the operon *mpt* being involved in sensitivity and being controlled by MptR and σ^{54}. Moreover, since knockout of *mptD*, the distal gene of the operon, led to resistance, the corresponding MptD protein should play a crucial role in sensitivity. In this respect, the resistance of JH9 (knockout of *mptB*) was probably due to the lack of *mptD* expression through a polar effect. Interestingly, MptD displays an additional domain compared to the other IID subunits found within the *E. faecalis* genome (Fig. 3) and to the other IID subunits reported in the GenBank database, except ManN of *Streptococcus salivarius*. Moreover, according to structure prediction with the TMpred program, this additional domain could be located within an extracellular loop. We thus hypothesize that this motif could be involved in mesentericin Y105 targeting of the MptD subunit, partly explaining the narrow spectrum of mesentericin Y105 activity.

Finally, *E. faecalis* 2DG-resistant strains were isolated on selective medium. Among them, six clones (JH6-1 to JH6-6) were tested for sensitivity to mesentericin Y105 by a spot on lawn assay. All of them were resistant to mesentericin Y105 (Table 1). This result emphasizes that an EII^{Man} of *E. faecalis*, possibly EII_t^{Man}, is actually involved in sensitivity to mesentericin Y105, because 2DG-resistant strains are known to be defective in EII^{Man} expression (Veyrat *et al*., 1994).

Sugar effects on *E. faecalis* JH2-2 sensitivity to mesentericin Y105

Since *E. faecalis* JH2-2 sensitivity to mesentericin Y105 seems to be linked to a specific PTS mannose permease, the effect of various sugars on sensitivity was tested. Indeed, expression of PTS permeases is specifically induced by the transported sugar (Saier & Reizer, 1994). *E. faecalis* JH2-2 was grown in LB medium supplemented independently with the following sugars at

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MpoD   218 SPMT--------------------------------FKVTGGATIVLQDIL 236
MptD   205 LPIVSQVKLDKGAYIEWDKLPAGGEGMHKAFEQVNQGLALSPTKVTTLQDNL 256
MphD   213 IPIT--------------------------------ISGSGKNAVTVQNIF 231
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Fig. 3. Alignment of putative extracellular loops of IID subunits of σ^{54}-dependent mannose PTS permeases. The additional domain of MptD is in bold.

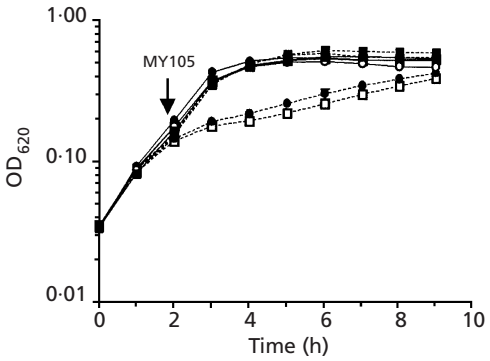

Fig. 4. Effect of several sugars on *E. faecalis* JH2-2 sensitivity to mesentericin Y105. *E. faecalis* was grown in LB supplemented with cellobiose (■), fructose (○), glucose (●) or mannose (□) at 2 g l^{-1}. The dotted lines represent growth curves in the presence of mesentericin Y105 (addition is indicated by an arrow) and the unbroken lines represent growth curves in the absence of mesentericin Y105.

2 g l^{-1}: fructose, cellobiose, glucose and mannose. Fig. 4 shows that the sensitivity of *E. faecalis* was highly increased in the presence of glucose or mannose, compared to cellobiose or fructose. *E. faecalis* was also weakly sensitive to mesentericin Y105 in LB medium without any added sugar (data not shown). This suggests that glucose and mannose activate the expression of a protein involved in sensitivity to mesentericin Y105, probably the EII_t^{Man} PTS permease.

DISCUSSION

We had previously shown that the σ^{54} factor of *E. faecalis* is involved in sensitivity to mesentericin Y105 and various subclass IIa bacteriocins (Dalet *et al*., 2001). Consequently, we studied the σ^{54} regulon, via the characterization of five σ^{54}-associated activators and several σ^{54}-dependent genes. Although previous sequence analysis of σ^{54}-associated activators did not reveal any similarity with helicases (Buck *et al*., 2000), we found that in *E. faecalis* all of them bear a helicase motif (DEAH box). This helicase motif, together with the nucleotide binding site, could be involved in ATP-dependent DNA unwinding that promotes initiation of transcription.

According to the acquired resistance phenotype displayed by knockout mutants, MptR appears as the only one, among the five activators, to be involved in sensitivity to mesentericin Y105. MptR probably activates the transcription, together with σ^{54}, of the flanking downstream operon, which encodes a mannose PTS permease, EII_t^{Man}. In addition, knockout within this operon also led to resistance, which supports EII_t^{Man} being involved in resistance and being dependent on MptR. We have also recently shown that knockout within an operon encoding a σ^{54}-dependent PTS permease of the mannose family leads to *L. monocytogenes* resistance (K. Dalet, Y. Cenatiempo, P. Cossart, The European Listeria Genome Consortium and Y. Héchard, unpublished results). Moreover, the IIAB subunit of a mannose PTS permease was shown to be absent in a spontaneous mutant of *L. monocytogenes* resistant to leucocin A (Ramnath *et al*., 2000), favouring the lack of EII^{Man} expression. Another spontaneous mutant of *L. monocytogenes*, resistant to pediocin PA-1 (a subclass IIa bacteriocin), has been reported by others to overexpress a PTS permease from the β-glucoside family (Gravesen *et al*., 2000). However, the same group found out that knockout of the β-glucoside operon did not modify the sensitivity to pediocin PA-1 and that this PTS is also overexpressed in our *L. monocytogenes rpoN* mutant, lacking σ^{54} (A. L. Gravesen, personal communication). These results on both *E. faecalis* and *L. monocytogenes* point towards an essential role of an EII^{Man} permease in sensitivity to mesentericin Y105 and related subclass IIa bacteriocins. Our current hypothesis is that EII^{Man} is probably a receptor for these bacteriocins. Finally, we showed that the presence of glucose and mannose in the culture medium greatly increases *E. faecalis* sensitivity to mesentericin Y105. Since PTS permease expression has been reported to be specifically induced by transported sugars, we hypothesize that glucose and mannose induce the expression of EII_t^{Man}, thereby leading to an increase in the number of potential protein receptors for mesentericin Y105.

Further data favour the above hypotheses and focus on a particular component of the permease. Knockout of *mptD*, the distal gene of this operon, led to resistance and thus the corresponding MptD subunit seems essential in *E. faecalis* sensitivity to mesentericin Y105. In *L. monocytogenes*, knockout or in-frame deletion of a gene, encoding a IID^{Man} subunit, also led to resistance (K. Dalet, Y. Cenatiempo, P. Cossart, The European Listeria Genome Consortium and Y. Héchard, unpublished results). Moreover, this IID^{Man} subunit of *L. monocytogenes* harbours an additional motif, as found here in *E. faecalis* MptD, and the above-mentioned in-frame deletion removed this domain. Thus, in these two organisms, the IID subunit of the EII^{Man} involved in sensitivity bears an additional motif (a putative external loop) not found within the other IID subunits described, except ManN of *S. salivarius*. The additional domain of *E. faecalis* shares 64% similarity with that of *L. monocytogenes* and only 39% with that of *S. salivarius*. Moreover, IID subunits are integral membrane proteins and could therefore interact directly with mesentericin

Y105. Accordingly, we propose that an EII^{Man} is a receptor for various subclass IIa bacteriocins in *L. monocytogenes* and *E. faecalis* and that its IID^{Man} component plays a central role in bacteriocin action, probably by a direct protein–protein interaction involving the additional domain.

Whether an EII^{Man} is also implicated in sensitivity to subclass IIa bacteriocins when present in other bacteria is being analysed. Direct protein–protein interaction between subclass IIa bacteriocins and the permease will be further investigated, together with the implication of the additional domain found in their IID components.

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