A σ^{54} -dependent PTS permease of the mannose family is responsible for sensitivity of *Listeria monocytogenes* to mesentericin Y105

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Sensitivity of *Listeria monocytogenes* to the bacteriocin mesentericin Y105 was previously shown to be dependent on the σ^{54} subunit of the RNA polymerase. This points towards expression of particular σ^{54} -dependent genes. The present study describes first, ManR, a new σ^{54} -associated activator, and second, EII^{Man}, a new σ^{54} -dependent PTS permease of the mannose family, both involved in sensitivity to mesentericin Y105, since interruption of their corresponding genes led to resistance of *L. monocytogenes* EGDe. EII^{Man} is likely composed of three subunits encoded by the *mpt* operon (*mptA*, *mptC* and *mptD* genes). Interruption of either the proximal (*mptA*) or distal (*mptD*) gene led to resistance, supporting results obtained in *Enterococcus faecalis*. Accordingly, such PTS permeases of the mannose family should be involved in sensitivity of different target strains to mesentericin Y105. In *L. monocytogenes*, expression of the *mpt* operon is shown to be controlled by σ^{54} and ManR and to be induced by both glucose and mannose. The latter result indicates that these sugars are transported by the EII^{Man} permease. Moreover, these sugars correlatively induce sensitivity of *L. monocytogenes* to mesentericin Y105, strongly favouring the primary role of EII^{Man}. MptD, a membrane subunit of EII^{Man}, presents an additional domain compared to most IID^{Man} subunits described in data banks. An in-frame deletion of this domain in *mptD* led to resistance of *L. monocytogenes*, showing its connection with sensitivity and suggesting that it could be directly involved in the recognition of the target cell by mesentericin Y105. Taken together, the results of this work demonstrate that EII^{Man} is prominent in sensitivity to mesentericin Y105 and could be a receptor for subclass IIa bacteriocins.

Keywords: bacteriocin, receptor, helicase, transport, sugar

INTRODUCTION

Bacteriocins are antibacterial proteinaceous compounds produced by bacteria. In Gram-positive bacteria, they are divided into four classes (Klaenhammer, 1993). Within the class II, constituted by non-modified peptides produced mainly by lactic acid bacteria, bacteriocins of the subclass IIa, such as mesentericin Y105 (Héchard *et*

al., 1992), are of particular interest (for a review see Ennahar *et al.*, 2000). They are active against the foodborne pathogen *Listeria monocytogenes* and share a similar primary structure, with a conserved N-terminal motif (YGNGV). Subclass IIa bacteriocins induce membrane permeabilization (Abee, 1995; Maftah *et al.*, 1993) of sensitive strains, but their target specificity and their molecular mode of action remain elusive. Whether

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Abbreviation: PTS, phosphotransferase system.

a receptor or a docking molecule is necessary for activity of subclass IIa bacteriocins is still debated and, to date, such molecules have not been reported. We previously hypothesized that the transcriptional factor σ^{54} (encoded by *rpoN*) could direct expression of a receptor since *rpoN* mutants of *L. monocytogenes* (Robichon *et al.*, 1997) and *Enterococcus faecalis* (Dalet *et al.*, 2000) are resistant to mesentericin Y105 and related subclass IIa bacteriocins. σ factors are subunits of the RNA polymerase holoenzyme involved in the initial step of transcription. Among them, σ^{54} is unique since it targets conserved -24/-12 promoter sequences and requires an activator protein for transcription initiation, referred to as σ^{54} -associated activator (Morett & Segovia, 1993; Shingler, 1996). σ^{54} -associated activators are classically composed of three distinct domains respectively involved in signal reception, transcriptional activation and DNA binding. The central domain, which directs the transcriptional activation via σ^{54} interaction, is the most highly conserved and clearly identifies these activators. To date, neither σ^{54} -regulated operons nor activator genes have been described in *L. monocytogenes* and, consequently, a direct link could not be established between σ^{54} and sensitivity of *L. monocytogenes* to subclass IIa bacteriocins. Recently, two bacteriocin-resistant spontaneous mutants of *L. monocytogenes* have been linked to phosphotransferase systems (PTSs). In the first mutant, resistant to leucocin A, a two-dimensional SDS-PAGE protein analysis revealed absence of a IIAB subunit of a PTS permease (Ramnath *et al.*, 2000). In the second mutant, resistant to pediocin PA-1, a β -glucoside PTS permease was described to be overexpressed (Gravesen *et al.*, 2000). PTSs are involved in both phosphorylation and transport of sugars (Postma *et al.*, 1993; Saier & Reizer, 1994). A phosphate moiety is transferred from phosphoenolpyruvate (PEP) to the transported sugar via the PTS proteins EI, HPr and EII. The latter is a complex permease, made of three or four subunits which can be fused: IIA and IIB are cytoplasmic subunits responsible for phosphorylation, whereas IIC is an integral membrane subunit involved in sugar transport. A IID membrane subunit, associated with IIC, is found specifically in permeases of the mannose family. Interestingly, σ^{54} has been described to be involved in PTS expression. Finally, we recently described that interruption of genes encoding a σ^{54} -associated activator (MptR) and a σ^{54} -dependent PTS permease of the mannose family (termed EII_t^{Man}) led to resistance of *E. faecalis* to mesentericin Y105.

To find a link between σ^{54} and sensitivity of *L. monocytogenes* to mesentericin Y105, we searched for σ^{54} -associated activators and σ^{54} -dependent genes in the *L. monocytogenes* EGDe genome. We found an activator and a PTS permease of the mannose family that are required for sensitivity of *L. monocytogenes* to mesentericin Y105.

METHODS

Bacterial strains and growth conditions. *L. monocytogenes* EGDe and its derivatives were grown at 37 °C in brain heart

infusion (BHI) or in Luria-Bertani (LB) medium supplemented or not with various sugars that support *L. monocytogenes* growth (i.e. glucose, mannose, fructose and cellobiose). *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) or ampicillin (100 µg ml⁻¹) was added, as needed. *Leuconostoc mesenteroides* Y105, which produces the bacteriocin mesentericin Y105, was grown in MRS medium at 30 °C.

DNA manipulations and gene interruption. Molecular cloning and DNA manipulations were performed as described by Sambrook *et al.* (1989). Restriction and modification enzymes purchased from Life Technologies were used as recommended by the manufacturer. DNA fragments, used for gene interruption experiments, were amplified by PCR using *Taq* polymerase and specific primers bearing a *Hind*III site, as follows: *manR* primers, 5'-CTGCCAAGCTTGGGAAGA-ACG-3' and 5'-CATCATCTTCCAAAGCTTGATCC-3'; *mptA* primers, 5'-GCTGAAGCTTTTTTGCAGTCCG-3' and 5'-GATGAGAAAGCTTCAATCAACATTGG-3'; *mptD* primers, 5'-CATCTCCAAAGCTTGGGGTAAC-3' and 5'-GGCGCAAGCTTGATATTTACCC-3'. A 1559 bp *Hind*III fragment, corresponding to the Tn917-*lac/rpoN* junction of pRT758 (Robichon *et al.*, 1997), was used for *rpoN* interruption. The latter DNA fragment and the PCR products were digested with *Hind*III and ligated at the same site in the erythromycin-resistant pHV1248ΔTn10 plasmid (Petit *et al.*, 1990), giving rise to plasmids pLMK50 ('*rpoN*'), pLMK51 ('*manR*'), pLMK54 ('*mptA*') and pLMK55 ('*mptD*'). These plasmids were used to create independent knockouts in *rpoN*, *manR*, *mptA* and *mptD* by homologous recombination with the *L. monocytogenes* EGDe chromosome, as previously described (Kocks *et al.*, 1992). Several mutants from each transformation were analysed by Southern blotting of chromosomal DNA digested by *Hind*III and hybridized with probe labelled by random priming from the PCR products described above (Sambrook *et al.*, 1989).

Allelic exchange of *mptD*. Allelic exchange of *mptD* was achieved by integration-excision of a plasmid bearing a deleted fragment of *mptD*. This fragment was obtained by PCR as follows. One gene fragment from each side of the additional domain we wanted to delete was amplified with the following primers: Del1 (5'-ATGAAGCTTTTCAAGGGGTAAAGTTGG-3') and Del2 (5'-GCAAGGTTGTTACTTTAATTTTCGACCTTCATCAAGTTTAACCTTTCG-3') together and Del3 (5'-CGAAAGTTAACTTGATGAAGGTGCGGAAATTAAGTAACAACCTTGC-3') and Del4 (5'-ACGTAAGCTTTAAGTCCAGTATACGC-3') together. The resulting PCR products, overlapping by 25 bp, were then used in a PCR reaction, leading to a 84 bp deleted fragment of *mptD* (Δ₆₅₅₋₇₃₈*mptD*). This fragment was first cloned in pGEM-T (Promega), confirmed by sequencing and then subcloned as a *Hind*III fragment in pHV1248ΔTn10. The resulting plasmid, pLMY2, was used to achieve integration in *mptD* by homologous recombination. Excision events were then screened by their erythromycin sensitivity. Finally, erythromycin-sensitive clones were tested for the presence of the required deletion by PCR with primers Del1 and Del4 and sequencing. This gave rise to the strain EGY2 (EGDe-Δ₆₅₅₋₇₃₈*mptD*).

Bacteriocin purification and assays. Mesentericin Y105 was purified as reported (Guyonnet *et al.*, 2000). Nisin, a class I bacteriocin, was purchased from Sigma. *L. monocytogenes* sensitivity was assayed by spot-on-lawn or microtitre plate tests. The former was achieved by overlaying a BHI agar (1.5%) plate with a BHI agar lawn (0.7%) previously inoculated with 1% *L. monocytogenes*. Purified bacteriocin

was then spotted on the lawn, the plate was incubated overnight at 37 °C and zones of inhibition were recorded. The microtitre plate tests were conducted as follows. Bacteria were grown overnight in LB medium and inoculated in 1 ml fresh LB medium to a final OD₆₂₀ between 0.01 and 0.03. The culture was supplemented or not with either fructose, glucose, mannose or cellobiose at 2 g l⁻¹. Four aliquots of 200 µl from each sample were then distributed in a microtitre plate. Plates were incubated at 37 °C with agitation at 120 r.p.m. and bacterial growth was monitored by measurement of the OD₆₂₀. Purified mesentericin Y105 (100 ng) was added after 2 h, when the culture had reached an OD₆₂₀ between 0.05 and 0.1.

Transcription analysis. *L. monocytogenes* EGDe and its derivatives were grown in 3 ml LB medium supplemented or not with glucose, mannose, fructose or cellobiose (2 g l⁻¹) to an OD₆₀₀ of 0.6. The bacterial pellets collected by centrifugation were resuspended in 100 µl lysis buffer (10 mg lysozyme ml⁻¹, 10 mM Tris, 1 mM EDTA, pH 7.5) and incubated for 1 h at 37 °C. Total RNA was then extracted with the RNAwiz reagent (Ambion) and treated as recommended. The RNA pellets were finally resuspended in 50 µl diethylpyrocarbonate-treated water containing 20 U RNase-free DNase I. Slot-blot hybridization was performed as described by Sambrook *et al.* (1989). A ³²P-labelled *mptD* probe was prepared by random priming from the PCR product obtained with the *mptD* primers described above. Radioactivity was measured with an Instant Imager apparatus (Packard).

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.

RESULTS

σ^{54} -associated activators and σ^{54} -dependent genes in the EGDe genome

We used the central domain of σ^{54} -associated activators to screen the *L. monocytogenes* EGDe genome. Three ORFs, encoding putative σ^{54} -associated activators, were found in BLAST searches. One ORF has similarity (35 % identity) with activators of the NifA/NtrC family whereas the two others display similarity (31 and 38 % identity) with activators of the LevR family. Inactivation of each activator gene was performed; only one, named *manR*, was further studied since, in contrast to the others (data not shown), its inactivation led to resistance of *L. monocytogenes* EGDe to mesentericin Y105 (see below). The *manR* gene encodes a putative 938 aa protein (GenBank accession number AF397144), which displays highest similarity with LevR of *Bacillus subtilis* (38 % identity) (Débarbouillé *et al.*, 1991) and MptR of *E. faecalis* (40 % identity) (Hécharde *et al.*, 2001). According to Prosite, ManR has classical motifs found in other σ^{54} -associated activators: an ATP/GTP-binding site motif A (position 144–151) and a σ^{54} -interaction domain (position 209–224). Interestingly, ManR also bears a 10 aa sequence (position 217–226, see Fig. 1) integrally matching the classical DEAH motif, usually found in helicases (Luking *et al.*, 1998). Because helicase activity could explain the energy coupling of DNA unwinding in σ^{54} -dependent transcription, such simi-

Consensus pattern of the DEAH motif (Prosit PDOC00039):

[GSAH]-X-[LIVMF] (3)-D-E-[ALIV]-H-[NECR]

ManR (<i>Listeria monocytogenes</i>)	: GILFLDEIHR
LevR (<i>Bacillus subtilis</i>)	: GILFMDEIHR
NifA (<i>Alcaligenes faecalis</i>)	: GTLFLDEIGe
NifA (<i>Azotobacter vinelandii</i>)	: GTLFLDEIGe
NifA (<i>Enterobacter agglomerans</i>)	: GTLFLDEIGe
NifA (<i>Klebsiella pneumoniae</i>)	: GTLFLDEIGe
NifA (<i>Klebsiella oxytoca</i>)	: GTLFLDEIGe
NtrC (<i>Aquifex aeolicus</i>)	: GTLFLDEIGe
XylR (<i>Pseudomonas putida</i>)	: GTIFLDEViE
HoxA (<i>Alcaligenes eutrophus</i>)	: GTIFLDEIGd
RocR (<i>Bacillus subtilis</i>)	: GTLLLDEIns

Fig. 1. Alignment of helicase DEAH domains found in several σ^{54} -associated activators. Only the residues displayed in lower-case characters deviate from the consensus pattern.

Fig. 2. Genetic organization of the *mpt* operon. The -24/-12 denotes a σ^{54} promoter. The black rectangle represents the DNA fragment deleted in $\Delta_{655-738}$ *mptD*. T1 and T2 indicate putative transcription terminators.

larities between σ^{54} -associated activators and helicases were searched for but not yet found (Buck *et al.*, 2000). In addition, we found a DEAH motif, differing in only one or two positions (see Fig. 1) in other σ^{54} -associated activators. These observations suggest that ManR and other σ^{54} -associated activators could harbour both an ATPase and a helicase activity, allowing initiation of transcription. Finally, ManR has two PTS regulation domains (PRD-I and PRD-II in positions 501–566 and 863–929, respectively), described in LevR of *B. subtilis* to be involved in regulation of its activity by PTS components.

LevR controls the expression of the *lev* operon encoding a PTS permease of the mannose family (i.e. with a IID subunit). Consequently, we searched, in the *L. monocytogenes* EGDe genome, for similar operons harbouring a consensus -24/-12 promoter sequence. Two operons, *mpo* (mannose permease one) and *mpt* (mannose permease two), encoding putative mannose PTS permeases were found and subsequently interrupted. The *mpo* operon is located just downstream of *manR* whilst *mpt* was found elsewhere on the chromosome. In contrast to interruption of the *mpo* operon (data not shown), knockout of the *mpt* operon led to full resistance of *L. monocytogenes* to mesentericin Y105 (see below). The *mpt* operon (GenBank accession number AF397145) is delineated by two hairpin loops, T1 and T2, with a calculated free energy of -18 and -16 kcal mol⁻¹ (75.3 and 66.9 kJ mol⁻¹), respectively. The *mpt* operon is composed of three ORFs, *mptA*, *mptC* and *mptD* (Fig. 2). They putatively encode the proteins MptA (321 aa), MptC (268 aa) and MptD (303

MptD-Lmo	201-VNIQFAPIISKVKLDEGAYIDWSHL PQGAQGIKTAL EQQA GLALSEIKVTTLQNNLDNL-260
MptD-Efa	201-VSIKFLPIVSQVKLDKGAYIEWDKLPAGGEGMHKAFEQVNOGLALSPTKVTTLQDNLDQL-260
IID ^{Man} -Ssa	201-VSIVFTVNLPGKVLKSGAYIEWPKGNVSGDQLKTLGQVNDKLSFDKIQVDTLQKQLDSL-260
IID ^{Sor} -Lca	209-TTINVPLVVSSTTV-----NGKTTVTTLQNLIDQF-238
IID ^{Fru} -Bsu	201-TTINIPIVVSRIKD-E-----SGKVDVQTVQNVLDLSI-231
IID ^{Man} -Eco	211-THVNIPLVVSRTD-Q-----TGKEHVTTVQTILDQL-241
IID ^{NAG} -Eco	193-VHINVVTSEIDNT-----HSVALQQDFFDKV-219

Fig. 3. Partial alignment of IID subunits of PTS permeases. *Listeria monocytogenes* (Lmo), *Enterococcus faecalis* (Efa), *Streptococcus salivarius* (Ssa), *Lactobacillus casei* (Lca), *Bacillus subtilis* (Bsu) or *Escherichia coli* (Eco). Residues in bold characters are those of the additional domain deleted in mutant $\Delta_{219-246}$ IID2^{Man}.

aa), which share high similarities with subunits of PTS permeases of the mannose family (i.e. IIAB^{Man}, IIC^{Man}, IID^{Man}, respectively). MptA, MptC and MptD thus compose a putative PTS permease, named EII_t^{Man}.

ManR and EII_t^{Man} are involved in sensitivity to mesentericin Y105

rpoN, *manR*, *mptA* and *mptD* were interrupted by recombination between plasmids pLMK50, pLMK51, pLMK54 and pLMK55, respectively, and the chromosome of *L. monocytogenes* EGDe. The corresponding derivative strains were named EGK50, EGK51, EGK54 and EGK55. Southern blots with specific homologous probes verified gene interruption in these derivative strains (data not shown). Sensitivity of *L. monocytogenes* EGDe and its derivatives to purified mesentericin Y105 was then assessed by a spot-on-lawn assay. As expected, *L. monocytogenes* EGDe was sensitive down to 0.5 ng mesentericin Y105. By comparison, the mutant strains EGK50, EGK51, EGK54 and EGK55 (interrupted in *rpoN*, *manR*, *mptA*, *mptD*, respectively) were fully resistant, even to 250 ng mesentericin Y105. Accordingly, the mutations increased the resistance at least 500-fold. All these mutants remained sensitive to nisin, a class I bacteriocin, underlying a specificity in resistance to mesentericin Y105. These results indicate that σ^{54} , ManR and EII_t^{Man} are involved in sensitivity of *L. monocytogenes* EGDe to mesentericin Y105, as previously shown for σ^{54} of *L. monocytogenes* L028 (Robichon *et al.*, 1997).

MptD plays a particular role in sensitivity

The distal gene of the *mpt* operon, *mptD*, seems to play a particular role in sensitivity since its interruption led to resistance. Interestingly, MptD bears an additional domain compared to three other IID^{Man} subunits found in the *L. monocytogenes* EGDe genome and to most of IID^{Man} subunits described in the literature except *E. faecalis* (Hécharde *et al.*, 2001) and *Streptococcus salivarius* (Lortie *et al.*, 2000) or found in GenBank (Fig. 3). Among 22 bacterial sequenced genomes (finished or unfinished) found to possess at least one orthologue of IID^{Man}, only several Gram-positive bacteria (i.e. *E. faecalis*, *Streptococcus* spp. (5 examples), *Lactococcus lactis* and *Clostridium acetobutylicum*) have a IID^{Man}

Fig. 4. Expression of the *mpt* operon assayed by slot-blot analysis. *L. monocytogenes* EGDe was grown in LB medium supplemented or not with cellobiose (Cel), fructose (Fru), glucose (Glu) or mannose (Man). Radioactivity is expressed in c.p.m. per mm².

with an additional domain. Interestingly, these Gram-positive bacteria have sometimes been described to be sensitive to subclass IIa bacteriocins. The mutant strain EGY2 has an 84 bp in-frame deletion of *mptD* ($\Delta_{655-738}$ *mptD*), created by allelic exchange (see Methods). It likely encodes the $\Delta_{219-246}$ MptD protein with a 28 aa deletion in the additional domain (bold characters in Fig. 3). Strain EGY2 was tested for sensitivity to mesentericin Y105, as described above. It was fully resistant, indicating that the presence of the additional domain is of primary importance for sensitivity.

Expression of the *mpt* operon is controlled by ManR and glucose or mannose

Slot-blot analysis was first performed with total RNA of *L. monocytogenes* EGDe grown in LB medium supplemented or not with either cellobiose, fructose, glucose or mannose at 2 g l⁻¹. Expression of the *mpt* operon was followed with the *mpt* probe. Fig. 4 shows that expression of the *mpt* operon is highly induced in the presence of glucose or mannose. This indicates that the EII_t^{Man} permease, putatively encoded by the *mpt* operon, is responsible for glucose and mannose transport.

Fig. 5. Growth curves of *L. monocytogenes* EGDe in LB medium supplemented with various sugars: (a) cellobiose (●), fructose (□), glucose (■) or mannose (○) at 2 g l⁻¹; (b) mannose 0.125 g l⁻¹ (○), 0.5 g l⁻¹ (□), 2 g l⁻¹ (●). Bacterial growth without (unbroken line) or with mesentericin Y105 (dotted line) was followed by measurement of optical density at 620 nm. The arrow indicates the addition of 0.5 ng mesentericin Y105 μl⁻¹.

Slot-blot analysis, using the same *mptD* probe, was performed with total RNA of *L. monocytogenes* EGK50 and EGK51 grown in LB medium supplemented with mannose at 2 g l⁻¹. The *mpt* operon was not expressed in these derivative strains (data not shown), showing that σ^{54} and ManR are responsible for its expression.

Mannose and glucose influence sensitivity of *L. monocytogenes* to mesentericin Y105

L. monocytogenes EGDe was grown in LB medium supplemented with glucose, mannose, fructose or cellobiose at 2 g l⁻¹. Fig. 5(a) shows that, in the absence of mesentericin Y105, no significant difference in *L. monocytogenes* EGDe growth curves could be observed, whereas the presence of mesentericin Y105 affected *L. monocytogenes* growth in a medium supplemented with mannose or glucose but not with cellobiose or fructose. These results show that glucose and mannose specifically induce sensitivity of *L. monocytogenes* to mesentericin Y105.

To see whether mannose or glucose influences the sensitivity in a dose-dependent manner, *L. monocytogenes* EGDe was grown in LB medium supplemented with mannose at various concentrations (2, 0.5 and

0.125 g l⁻¹, respectively). Fig. 5(b) shows that, in the absence of mesentericin Y105, growth of *L. monocytogenes* increases along with mannose concentration whereas, in the presence of mesentericin Y105, growth inhibition increases along with mannose concentration. These results show that mannose (and glucose, data not shown) has a dose-dependent effect on *L. monocytogenes* sensitivity to mesentericin Y105. It underlines that the level of sensitivity is tightly linked to sugar availability, suggesting that mannose or glucose directly causes expression of a molecule responsible for sensitivity to mesentericin Y105.

DISCUSSION

Mesentericin Y105 and other subclass IIa bacteriocins have been described to permeabilize the membrane of target strains. However, the main unanswered question is whether or not these bacteriocins need a docking molecule or receptor, as described for nisin, a pore-forming bacteriocin (Breukink *et al.*, 1999). Since σ^{54} has been described to direct sensitivity of *L. monocytogenes* and *E. faecalis* to subclass IIa bacteriocins, we thus hypothesized that it could be responsible for the expression of such a receptor.

We demonstrate here that the σ^{54} regulon of *L. monocytogenes* is clearly involved in sensitivity to mesentericin Y105, bringing experimental support to assessments arising from our earlier work on *E. faecalis* (Hécharde *et al.*, 2001). Interruption of either *rpoN*, *manR*, *mptA* or *mptD* (encoding, respectively, σ^{54} , a σ^{54} -associated activator and two subunits of the EII^{Man} permease) led to resistance of *L. monocytogenes* EGDe. EII^{Man}, a PTS permease of the mannose family, is encoded by the *mpt* operon. This operon, which bears a -24/-12 promoter, was not expressed in the *rpoN* and *manR* mutants, showing that its expression is positively controlled by σ^{54} and ManR. In *E. faecalis*, the *mpt* operon also bears a putative -24/-12 promoter although it was not experimentally demonstrated to be regulated by MptR or σ^{54} (Hécharde *et al.*, 2001). The localization of the *mpt* operon immediately downstream from *manR* suggests that it could also be controlled by ManR and preliminary results indicate a possible cross-regulation between the *mpt* and *mpt* operons. Accordingly, ManR likely controls these two operons whereas MptR of *E. faecalis* presumably controls expression of *mpt* only. Relationships between these operons would not be surprising according to the known regulation of carbohydrate metabolism.

The presence of glucose or mannose induced sensitivity of *L. monocytogenes* and *E. faecalis* to mesentericin Y105. These sugars also induced expression of the *mpt* operon of *L. monocytogenes* (not shown in *E. faecalis*), indicating that EII^{Man} transports glucose and mannose in accordance with previous observations showing a specific inducible effect of the transported sugar on PTS permease expression (Postma *et al.*, 1993). These correlated results suggest that the level of EII^{Man} expression is

directly linked to sensitivity of *L. monocytogenes* to mesentericin Y105.

Since mesentericin Y105 and related subclass IIa bacteriocins have a narrow spectrum of inhibition, we are wondering about the specificity of the target strains. The IID^{Man} membrane subunit of EII_t^{Man}, MptD, contains an additional domain compared to most other IID^{Man} proteins. The mutant EGY2, which putatively encodes a truncated MptD protein, i.e. lacking 28 aa in this additional domain, became resistant to mesentericin Y105. Assuming that the truncated MptD protein is expressed and remains functional, it strongly suggests a primary role of this domain in the sensitivity of *L. monocytogenes*. The introduction of point mutations in the additional domain constitutes the next step of our work to confirm the role of this domain and to identify the implicated amino acids as well as their possible interaction.

The involvement of EII_t^{Man} in sensitivity to subclass IIa bacteriocins is emphasized by the report of a spontaneous mutant resistant to leucocin A, a subclass IIa bacteriocin (Ramnath *et al.*, 2000). The authors clearly showed the absence of expression of a IIAB^{Man} PTS component in this mutant. Moreover, the N-terminal sequence of the protein shares high identity (17 of 20 residues) with the MptA protein described here. In addition, Gravesen *et al.* (2000) recently reported the overexpression of a β -glucoside PTS in a spontaneous *L. monocytogenes* mutant resistant to pediocin PA-1, another subclass IIa bacteriocin. This pediocin PA-1 resistant mutant was then shown to be defective in EII_t^{Man} expression (Y. Héchard, unpublished results) and the β -glucoside PTS was shown to be overexpressed in our *L. monocytogenes* LUT758 mutant lacking σ^{54} (A. L. Gravesen, personal communication). In *S. salivarius*, mutants that lack the synthesis of IIAB_L^{Man} (similar to MptA) are derepressed for several genes, such as the β -galactosidase gene (Gauthier *et al.*, 1990). We thus speculate that overexpression of the β -glucoside PTS is a consequence of the lack of EII_t^{Man} expression. Taken together, these results are evidence that EII_t^{Man} is a key component for sensitivity of *L. monocytogenes* to different subclass IIa bacteriocins.

In conclusion, we propose that EII_t^{Man} could either influence the expression of an unknown molecule involved in sensitivity or, via its IIC^{Man}–IID^{Man} membrane complex, be a docking molecule or a receptor for mesentericin Y105 and other subclass IIa bacteriocins. Since deletion of the additional domain of MptD led to resistance, we propose that this domain could directly interact with bacteriocins or that its deletion could change the structure of the permease, leading to a lower affinity for the bacteriocins. Finally, a IIC^{Man}–IID^{Man} complex has already been described to facilitate penetration of phage lambda DNA across the inner membrane of *Escherichia coli* (Esquinas-Rychen & Erni, 2001). It would be interesting to see whether both phage and bacteriocin could interact with cells via a similar mechanism.

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