Characterization of the amylovorin locus of \textit{Lactobacillus amylovorus} DCE 471, producer of a bacteriocin active against \textit{Pseudomonas aeruginosa}, in combination with colistin and pyocins

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bacteriocin; amylovorin; colistin; pyocins; \textit{Pseudomonas aeruginosa}.

Abstract
\textit{Lactobacillus amylovorus} DCE 471 produces amylovorin L, a bacteriocin with an antibacterial activity against some strains of the \textit{Lactobacillus} lineage. Based on the sequence of one active peptide, a gene encoding active amylovorin L was cloned and sequenced. Genome walking allowed us to sequence a larger fragment of 7577 bp of genomic DNA, with 12 predicted ORFs. The previously characterized amylovorin L peptide-encoding gene is preceded by another gene encoding a small polypeptide with a typical bacteriocin-processing double-glycine site, suggesting that amylovorin L is a two-component class IIb bacteriocin (amylovorin L\textsubscript{a}/\textsubscript{b}). L\textsubscript{a} and L\textsubscript{b} show the highest similarity to gassericin T from \textit{Lactobacillus gasseri} SBT2055 and BlpN from \textit{Streptococcus pneumoniae} R6, respectively, and to LafA and LafX, which form the lactacin F bacteriocin of \textit{Lactobacillus johnsonii} NCC 533. As for other lactic acid bacteria bacteriocins, amylovorin L showed no activity against the Gram-negative opportunistic pathogen \textit{Pseudomonas aeruginosa} on its own, but showed synergistic inhibitory activity when used in combination with the peptide antibiotic colistin, and, remarkably, with the \textit{P. aeruginosa} soluble bacteriocins, pyocins S1 and S2.

Introduction
Bacteriocins are antibacterial peptides or proteins produced by bacteria to kill other bacteria, often closely related to the producer strain (Tagg \textit{et al.}, 1976). In Gram-positive bacteria, bacteriocins are generally small peptides of 3–10 kDa (with the exception of the protein bacteriocins of class III), which are produced ribosomally (Nes \textit{et al.}, 1996; Héchard & Sahl, 2002). The small peptide lactic acid bacteria (LAB) bacteriocins belong to two major classes. Class I contains the modified lantibiotics, because they undergo post-translational modifications, resulting in the formation of thioether amino acids or lanthionines (Héchard & Sahl, 2002). Class II are unmodified, cationic, hydrophobic, and heat-stable peptides of 20–60 amino acids (Nes & Holo, 2000; Héchard & Sahl, 2002), and two subclasses can be distinguished: subclass IIa and subclass IIb (Nes & Holo, 2000). Subclass IIa bacteriocins are characterized by a conserved sequence motif at the N-terminal of the mature peptide, YGNGV(X)C(X)\textsubscript{4}C(X)V(X)\textsubscript{4}A (Drider \textit{et al.}, 2006).
Subclass IIb are called two-peptide bacteriocins, because two separate peptides are necessary for their action (Garneau \textit{et al.}, 2002). LAB bacteriocins kill sensitive bacteria by inserting themselves into the cytoplasmic membrane, resulting in pore formation, membrane permeabilization, and leakage of essential molecules (Moll \textit{et al.}, 1999; Héchard & Sahl, 2002). Previous work demonstrated the production of small, strongly hydrophobic, and heat-stable bacteriocins by two phenotypically and genotypically different strains of the cereal fermentation bacterium \textit{Lactobacillus amylovorus}, a member of the \textit{Lactobacillus acidophilus} DNA homology group (De Vuyst \textit{et al.}, 1996; Contreras \textit{et al.}, 1997; Callewaert \textit{et al.}, 1999). Lactobin A is produced by \textit{L. amylovorus} LMG P-13139 (Contreras \textit{et al.}, 1997), while amylovorin L is produced by \textit{L. amylovorus} DCE 471 (De Vuyst \textit{et al.}, 1996; Callewaert \textit{et al.}, 1999). The structural genes for lactobin A and amylovorin L are in fact identical (De Vuyst \textit{et al.}, 2004).
LAB bacteriocins have no effect on Gram-negative bacteria because of their outer membrane (OM), which confers
high intrinsic resistance to a large range of toxic compounds (Hancock, 1997). The smallest bacteriocins are 3 kDa, making them too large to reach the cytoplasmic membrane via the OM porins (Stiles & Hastings, 1991; Klaenhammer, 1993). *Pseudomonas aeruginosa* is a Gram-negative opportunistic pathogen known for its high level of resistance against different antibiotics (Mesaros et al., 2007). *Pseudomonas aeruginosa* is, however, susceptible to polymyxins, a group of polypeptide cationic antibiotics, including the drug colistin (polymyxin E), often used as the last resort because of its toxicity (Li et al., 2007). These cationic molecules compete and displace Ca$^{2+}$ and Mg$^{2+}$ ions, which stabilize the lipopolysaccharide molecules of the OM of Gram-negative bacteria. This destabilization results in leakage of the cell contents, cell lysis, and death (Hancock & Chapple, 1999). Pyocins are large (c. 60 kDa) bacteriocins produced by *P. aeruginosa* that kill related strains (Michel-Briand & Baysse, 2002).

In this work, we describe the amylovorin L genomic locus, which reveals the presence of other putative bacteriocin genes and/or other peptides with a double-glycine motif as well as a dedicated ATP-binding cassette (ABC) transporter and accessory protein. Further, we demonstrate that although *P. aeruginosa* is not susceptible to amylovorin L, this LAB bacteriocin can decrease the minimal inhibitory concentration (MIC) of colistin and acts synergistically with pyocins.

### Materials and methods

#### Bacterial strains and media

All bacterial strains used throughout this study are presented in Table 1. *Lactobacillus amylovorus* DCE 471 (LMG P-13139) was used as a source of amylovorin L and *Lactobacillus delbrueckii* ssp. *bulgaricus* LMG 6901<sup>T</sup> as a bacteriocin-sensitive indicator strain. Before isolation experiments, cultures were propagated twice in MRS (Oxoid, Basingstoke, UK) at 37 °C for 12 h, and 1% (v/v) was used as a transfer inoculum. *Pseudomonas aeruginosa* strains (ATCC 27853, PA01, PA14, 59.20, O:9) were grown at 37 °C in rich Luria Broth (LB) medium or iron-poor Casamino acid (CAA) medium in a New Brunswick Innova<sup>TM</sup> 4000 shaker at 200 r.p.m. All strains were stored at −80 °C in the appropriate cultivation medium with glycerol at a final concentration of 25%, v/v.

#### Southern blot and hybridization

DNA isolation from *L. amylovorus* DCE 471 was performed as described by Gevers et al. (2001). Genomic DNA (20 μg) was digested with 25 U of the restriction enzymes BamHI, ClaI, EcoRI, EcoRV, HindIII, NsiI, SalI, SpeI, SspI, and XhoI (Fermentas GMBH, St. Leon-Rot, Germany) or with a combination of NsiI and one of the following: BamHI, ClaI, EcoRV, HindIII, Sall, Sphi, and Xhol (Fermentas GMBH, St. Leon-Rot, Germany) or with a combination of NsiI and one of the following: BamHI, ClaI, EcoRV, HindIII, Sall, Sphi, and Xhol (Fermentas GMBH, St. Leon-Rot, Germany). The restricted DNA was separated using electrophoresis in a 0.8% (w/v) agarose gel and transferred to a nylon hybridization membrane (Hybond-H<sup>1</sup>, GE Healthcare). Two probes (Fig. 1) were amplified by PCR using Taq polymerase (Roche). Probe A, which corresponds to the complete sequence of the amyL gene (NCBI, AJ301625), was amplified by primers Fw-amyL-A (5′-GTAGGGATTTAAGATGAAACAATTG-3′) and Rv-amyL-AB (5′-CTTTACGAACATAACCCGCCAAGCC-3′). For probe B, Fw-amyL-B (5′-GCTTTGGGATGCGCTGTCCC-3′) and Rv-amyL-AB were used. The probes were labeled with

#### Table 1. Strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Characteristics</th>
<th>Reference or culture collection* number</th>
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</thead>
<tbody>
<tr>
<td><strong>Strains</strong></td>
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<tr>
<td><em>Lactobacillus</em></td>
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<tr>
<td><em>L. amylovorus</em> DCE 471</td>
<td>Amylovorin producer</td>
<td>LMG P-13139</td>
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<tr>
<td><em>L. delbrueckii</em> ssp. <em>bulgaricus</em></td>
<td>Amylovorin-sensitive strain</td>
<td>LMG 6901&lt;sup&gt;T&lt;/sup&gt;</td>
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<tr>
<td><em>P. aeruginosa</em></td>
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<tr>
<td>PA01</td>
<td>Wild type</td>
<td>ATCC 15692</td>
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<tr>
<td>O:9</td>
<td>Wild-type, pyocin Sa-sensitive indicator strain</td>
<td>Denayer et al. (2007)</td>
</tr>
<tr>
<td>S9.20</td>
<td>Wild type</td>
<td>De Chial et al. (2003)</td>
</tr>
<tr>
<td>PA14</td>
<td>Wild type</td>
<td>Rahme et al. (1995)</td>
</tr>
<tr>
<td>ATCC27853</td>
<td>Wild type</td>
<td>ATCC</td>
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<td><strong>E. coli</strong></td>
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<td>DH5α</td>
<td>supE44ΔlacU169 (Φ80lacZΔM15 recA</td>
<td>Hanahan (1983)</td>
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<tr>
<td></td>
<td>hsdR17 recA1 endA1 gyrA96 thi-1 relA1)</td>
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<tr>
<td><strong>Plasmids</strong></td>
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<td>Promega</td>
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<tr>
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<td>Pyocin S1 gene-containing plasmid</td>
<td>Denayer et al. (2007)</td>
</tr>
<tr>
<td>pYMP51</td>
<td>Pyocin S2 gene-containing plasmid</td>
<td>Denayer et al. (2007)</td>
</tr>
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</table>

*LMG, Belgian collection of microorganisms.
ATCC, American type tissue collection.

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digoxigenin-dUTP, using the DIG DNA labeling kit (Roche Diagnostics, Vilvoorde, Belgium). A chemiluminescent substrate (CDP-Star, Roche) was used for the detection. Light emission was recorded on a luminescent imager (GeneGnome, Syngene, Cambridge, UK).

Subcloning of upstream and downstream amylL regions

Total DNA was digested with either NsiI or SalI. Fragments with the same size as the hybridizing bands were excised, cleaned using the GenElute Gel extraction kit (Sigma-Aldrich NV, Bornem, Belgium), and subcloned into a pBluescript II KS(1/1/C0) plasmid that was digested with either PstI or SalI and subsequently dephosphorylated with Shrimp Alkaline Phosphatase (Fermentas).

Hybridization and sequence analysis

Colonies were lifted to Hybond-H membranes and hybridization with the probes was performed according to the manufacturer’s instructions (Roche). The complete sequences of the inserts from the positive clones were obtained by primer walking of both strands. The sequences were analyzed in silico using Kodon (Applied Maths, Sint-Martens-Latem, Belgium), EMBOSS (Rice et al., 2000) at EBI (http://www.ebi.ac.uk/EMBOSS/), and by manual inspection. Protein sequences were compared with public sequences in the EMBL and GenBank databases using BLAST (Altschul et al., 1997) (http://www.ebi.ac.uk/blastall/). Protein domains and motifs were identified using InterProScan (Zdobnov & Apweiler, 2001). Protein sequences were aligned using CLUSTALW (Thompson et al., 1994).

Real-time PCR analysis

Total RNA extraction was carried out using the RNeasy mini kit of Qiagen (the Netherlands).

First-strand cDNA was generated from 200 and 500 ng RNA from cultures after 6 and 8 h of growth, respectively, in a reaction (20 µL final volume) using random hexamer primers and RevertAid™ H Minus M-MuLV RT (Fermentas). PCR reactions were prepared in a 25-µL volume containing the SYBR Green PCR Master Mix (Invitrogen, Belgium), 0.5 µL cDNA, and 150 nM each forward and reverse primer. Amplification cycles were carried out in an iCycler IQ (Bio-Rad Laboratories) using the following protocol: 50 °C for 2 min, 95 °C for 8 min and 30 s, 40 cycles at 95 °C for 15 s, and 60 °C for 60 s, followed by melting curve analysis. Forward and reverse primers are listed in supplementary Table S1.

Partial purification of amylovorin L bacteriocin and activity test

Amylovorin L was isolated from L. amylovorus DCE 471 (LMG P-13139) and bacteriocidal activity was tested using L. delbrueckii ssp. bulgaricus LMG 6901T, as described by De Vuyst et al. (1996). Briefly, the total volume of the final growth medium was 2 L and the Lactobacillus deMan Rosa Sharpe (MRS) was adjusted to pH 6.5 for bacteriocin extraction. Amylovorin L was concentrated using ammonium sulfate precipitation, followed by an extraction with chloroform and methanol (2:1, v/v). Bacteriocin activity was measured using an agar spot test on overlays prepared by propagating fresh cultures to an OD600 nm of 0.6 and adding 100 µL of culture to 3.5 mL overlay agar (7 g L−1). Plates were incubated overnight at 37 °C. Activity was defined as the reciprocal of the highest dilution demonstrating complete
inhibition of the indicator lawn and was expressed in activity units (AU) mL⁻¹ per culture medium.

**Colistin and pyocin activity test**

To check the sensitivity of *P. aeruginosa* strains for pyocins S1 and S2, 10 µL pyocin lysate and dilutions thereof (see Results) were spotted on a bacterial cell layer containing 5 x 10⁶ cells mL⁻¹. Pyocins were partially purified from *Escherichia coli* DH5α bearing plasmids pYMSS11 or pYMPS1 for pyocins S1 and S2, respectively (Denayer et al., 2007). For colistin activity testing, the same setup was used.

**Growth characteristics**

Growth parameters were measured using a Bioscreen Apparatus (Life technologies), with the following parameters: shaking for 20 s every 1 min; reading every 30 min; temperature of 37 °C; and culture volume of 300 µL. A fresh culture of PAO1 (OD₆₀₀nm = 0.5) was diluted 5000 times. Growth was measured under different conditions: in the presence of different concentrations of colistin and in combination with one of the bacteriocins at a fixed concentration of 800 AU mL⁻¹. Growth was measured under different conditions: in the presence of different concentrations of colistin and in combination with one of the bacteriocins at a fixed concentration of 800 AU mL⁻¹. Each condition was realized in triplicate and repeated two times. All media were filter-sterilized before use.

**Results and discussion**

**Southern blot analysis and cloning of the amyL locus**

Hybridization with probe A (Fig. 1a) yielded two bands of 5.6 and 4.8 kb for DNA digested with NsiI, whereas probe B hybridized only with the 5.6-kb band (data not shown), suggesting that the 4.8-kb fragment contains the region upstream of *amyL*. However, only one clone was obtained corresponding to the 5.6-kb fragment. For SalI-restricted DNA, a 5.5-kb band was detected and cloned. This fragment contained 1750 bp upstream of *amyL*.

**Sequence analysis and in silico homology searches**

A 7577-bp region from *L. amylovorus* DCE 471 was sequenced and 12 ORFs were detected (Fig. 1b). ORF 1, transcribed in the opposite orientation, encodes a protein with a very high similarity to a transposase from *Lactobacillus crispatus* (99% identity, Interpro accession Q5SVS3). ORF2 encodes a protein of 70 amino acids, with two potential transmembrane domains, showing no significant similarity to any known protein, but with a bacteriocin-like leader peptide with a double-glycine motif. ORF3 encodes an 80-amino-acid polypeptide with 46% identity to the α subunit of the class IIb bacteriocin gassericin (Q9XDR7) and 47% identity to the LfA subunit of the bacteriocin lactacin F (P24022), suggesting that it could be a structural component of the amylovorin L bacteriocin. Gassericin Tα and β have been isolated from *L. gasseri* SBT 2055 and their genes were sequenced (Kawai et al., 2000). Likewise, *lafA* and *lafX* encode the two subunits of lactacin F in *Lactobacillus johnsonii* NCC 533 (Muriana & Klaenhammer, 1991; Pridmore et al., 2004). ORF4 encodes the 65-amino-acid amylovorin L bacteriocin precursor (P80696), which shows the highest similarity (55% identity, 63% similarity) to another bacteriocin, BlpN (Q2ES34), produced by *Streptococcus pneumoniae* (De Saizieu et al., 2000). It also shows similarity (40% identity, 62% similarity) to the product of the *laf* gene from *L. johnsonii* NCC 533 (Muriana & Klaenhammer, 1991; Pridmore et al., 2004). ORF5 encodes an unknown protein of 98 amino acids with three predicted transmembrane domains. ORF6 encodes a small protein of 16 amino acids with a high level of similarity (43% identity, 56% similarity) to a putative bacteriocin from *L. acidophilus* NCFM encoded by the gene LBA1797 (Q5FI73) (Altermann et al., 2005). It also shows 41% identity and 54% similarity to SakTα, one of the two components of sakacin T, a two-peptide bacteriocin produced by *Lactobacillus sakei* 5 (Vaughan et al., 2003). ORF7 encodes an 84-residue polypeptide, also with a potential double-glycine leader peptide. Interestingly, the highest similarity (59% identity, 69% similarity) is also found to LBA1797. ORF8 encodes a larger protein of 143 amino acids predicted to have both a double-glycine leader peptide and two transmembrane helices. ORF9 encodes a large protein of 700 amino acids predicted to be an ABC transporter. It shows 93% identity to the product of the LBA1796 gene from *L. acidophilus* NCFM (Q5FI74) (Altermann et al., 2005) and to the lactacin F transporter from *L. johnsonii* NCC 533 (Q74KH8) (Pridmore et al., 2004). ORF10 (195 amino acids) encodes a protein with high similarity to the so-called bacteriocin auxiliary transporters (Drider et al., 2006). It shows a high degree of similarity to the product of the LBA1794 gene (81% identity, 93% similarity) (Q5FI75) from *L. acidophilus* NCFM, and 56% identity and 74% similarities to the lactacin F transporter auxiliary protein from *L. johnsonii* NCC 533 (Q74KH7). ORF11 encodes a smaller protein of 119 amino acids, rich in charged amino acids, which shows 63% identity and 73% similarity to the product of the LBA1793 gene from *L. johnsonii* NCC 533 (Q5FI76). ORF12 encodes a short polypeptide of 65 amino acids with a predicted double-glycine leader peptide. The highest similarity (76% identity and 96% similarity) is to the product of the gene LBA1792 from *L. johnsonii* NCC 533 (Q5FI77). In a previous work (Callewaert et al., 1999), only one active peptide could be fully sequenced, which was named amylovorin L; nevertheless, two bands with antibacterial activity were detected after separation of the peptides on a polyacrylamide gel. The
result presented in this work shows that lactobin A amylovorin L belongs to the subclass IIb bacteriocins, as they lack the consensus sequence found in subclass IIa bacteriocins (Drider et al., 2006), and because of their putative molecular organization. Furthermore, in the same article, the authors show that two bands with antibacterial activities could be detected after separation of the peptides on a polyacrylamide gel. However, they could only obtain the peptide sequence from one peak in fast protein liquid chromatography (FPLC).

**Real-time PCR transcription analysis**

mRNA was extracted from 6- and 8-h-grown cultures of *L. amylovorus* and reverse transcribed into cDNA, using primers corresponding to ORFs 2–8. The results are shown in Table 2. Transcripts corresponding to all ORFs were detected as soon as after 6 h of growth and cotranscription of ORFs 3–4, and ORFs 6–8 could be clearly observed, confirming their organization in an operon. This means that ORFs 3 and 4, corresponding to amylA and amylB, are cotranscribed, and the two putative two-component bacteriocin peptides that are the predicted products of ORFs 6 and 7. Again, the fact that the two genes amylA and amylB are cotranscribed suggests that amylovorin L is a class IIb bacteriocin. It has been proposed that in two-peptide bacteriocins the partner peptide induces the formation of an α-helix in the killing peptide, facilitating its insertion into the membrane of the target bacterium and the formation of a pore (Garneau et al., 2002). Interestingly, amylovorin Lα is similar to gassericin Tα but its partner, amylovorin Lβ, is more similar to an *S. pneumoniae* two-peptide bacteriocin component, BlpN (De Saizieu et al., 2000). Amylovorin Lα and Lβ are also similar to LafA and LafX of the bacteriocin lactacin F from *L. johnsonii* NCC 533 (Frémaux et al., 1993; Abee et al., 1994; Allison et al., 1995; Pridmore et al., 2004). The molecular organization of this locus indicates a mosaic structure of the amylovorin L bacteriocin genes. Also, the products of ORF6 and ORF7 probably also form a two-component bacteriocin. Interestingly, both peptides are very similar (results not shown) and are also similar to the product of gene LBA1797 from *L. acidophilus* NCFM (Altermann et al., 2005). It is unusual that the two peptides of a subclass IIb bacteriocin are highly similar, as in the case of the peptides encoded by ORF6 and ORF7 (Garneau et al., 2002). Analysis of the genes cluster also suggests that *L. amylovorus* DCE 471 has the capacity to produce other bacteriocins, a hypothesis strongly supported by our transcription analysis data, which show that all four genes corresponding to bacteriocins are expressed.

**Sensitivity to colistin and pyocins**

Four strains of *P. aeruginosa* were tested for their sensitivity to colistin alone or in combination with amylovorin L. All strains were found to be sensitive to colistin, and, as expected, no strain was sensitive to amylovorin L. Both via the plate assay (results not shown) and bioscreen growth analysis (Fig. 2), a reduction of the minimal killing activity of colistin by a factor of two could be observed, depending on the strain. As shown in Fig. 2a for strain PA14, the growth was completely inhibited at a 2.5 mg L⁻¹ concentration of colistin in the medium and this occurred for the duration of

| Table 2. Ct value (threshold cycle) to detect cotranscription of ORFs (the list of primers can be found in supplementary Table S1) |
|------------------|------------------|
|                  | 6 h              | 8 h              |
| ORF-2-3          | 21.7             | 21.8             |
| ORF-3-4          | 16.3             | 15.6             |
| ORF-4-5          | 30.1             | 27.1             |
| ORF-5-6          | 32.6             | 32.1             |
| ORF-6-7          | 25.8             | 19               |
| ORF-7-8          | 16.4             | 15.5             |
| ORF-8-9          | 25.9             | 25.3             |

Values above 20 were taken as the cut-off; lower values indicate cotranscription.

**Fig. 2.** Growth of *Pseudomonas aeruginosa* PA14 in the presence of 0.08 mg L⁻¹ (●), 0.31 mg L⁻¹ (○), 0.63 mg L⁻¹ (△), 1.25 mg L⁻¹ (▲), and 2.5 mg L⁻¹ (■) of colistin in the absence (a) or in the presence (b) of 800 AU of amylovorin L (in this case, the 2.5 mg L⁻¹ curve is not shown).
the experiment (80 h). Already a 0.31 mg L\(^{-1}\) concentration of colistin caused a prolongation of the lag phase (Fig. 2a). In the presence of 800 U of amylovorin L, the lag phase was further increased and total growth inhibition occurred already at 1.25 mg L\(^{-1}\) of colistin (Fig. 2b). For strains PAO1 and 59.20, similar results were observed, but not for strain ATCC 2753, which showed a higher sensitivity to colistin (growth inhibition at 0.63 mg L\(^{-1}\)), but for which no synergistic effect was observed (results not shown). We suggest that colistin destabilizes the OM, facilitating the penetration of the amylovorin L. A similar effect was described for the combination of the fish antimicrobial peptide pleurocidin and the LAB bacteriocins pediocin PA-1, sakacin P, and curvacin A against \textit{E. coli} (Lüders \textit{et al.}, 2003).

As shown in Fig. 3, addition of amylovorin L to both pyocins S1 and S2 strongly enhanced their killing potential. For pyocin S1, a clear killing zone could be observed at a dilution 1/8, when the pyocin was mixed with amylovorin (1/2 for the pyocin alone), while for pyocin S2 the killing was still evident at a dilution of 1/64 (1/8 for the pyocin alone). The combination of the membrane-destructing agent EDTA and amylovorin L had no effect on the growth of \textit{P. aeruginosa} (results not shown). S1 and S2 pyocins recognize different receptors at the level of the OM of sensitive \textit{P. aeruginosa} strains (Michel-Briand & Bayse, 2002; Denayer \textit{et al.}, 2007). By attaching to the receptors, pyocins could destabilize the OM, while the pore-forming activity of the lactic acid bacterium bacteriocin could facilitate their translocation.

In conclusion, this work demonstrates a remarkable increase in the bactericidal activity of Gram-negative bacteriocins and in colistin by the addition of an otherwise, in itself inactive, Gram-positive bacteriocin, providing new perspectives for the treatment of infections.

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Authors’ contribution
M.R.F.M. and B.B. contributed equally to this work.

Statement
Sequence accession number: AM403502.

References


Supplementary material

The following supplementary material for this article is available online:

Table S1. List of primers used for the quantitative real-time PCR experiments.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1574-6968.2008.01275.x (this link will take you to the article abstract).

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