Functional analysis of circular and linear bacteriocins of Gram-positive bacteria
Kemperman, Robèr Antoine

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2005

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Chapter 5


Robèr A. Kemperman, Jan Kok and Oscar P. Kuipers
ABSTRACT

The genes for three novel putative circular bacteriocins were identified by screening genome databases for translation products with homology to CirC, a protein involved in circularin A production by *Clostridium beijerinckii* ATCC 25752. The gene region of three CirC homologues showed an organization similar to that of the circularin A coding region. One bacteriocin homologue was encoded in the chromosomes of *Staphylococcus aureus* (strains Mu50, N315, MW2 and 467), one in the chromosome of *Geobacillus stearothermophilus* DSM13240 and the third in the chromosome of *Oenococcus oeni* PSU-1. In close proximity, genes characteristic for circular peptide biosynthesis and immunity were identified in the chromosomes of the various strains. The immunity proteins lack primary sequence homology, but show structural homology even towards immunity proteins of non-circular bacteriocins, indicating a possible common mechanism conferring immunity. The presence of a conserved box in the CirC homologues is discussed with respect to the putative function of the proteins. Additionally, we propose that two other bacteriocins, boticin B and acidocin B, are circular molecules based on a re-evaluation of previously published data.

INTRODUCTION

Bacteriocins are small anti-microbial peptides produced by a large variety of bacteria. Some bacteriocins have a limited activity range, killing or inhibiting only a few related strains, but increasingly more bacteriocins are being discovered that have a wider range of activity.

Bacteriocins can be classified in different groups (23), one of which encompasses the recently added circular head-to-tail ligated bacteriocins (Class V) (22). So far, four circular bacteriocins, i.e. circularin A, enterocin AS-48 (Bac21), gassericin A and butyrivibriocin AR10 have been described (16, 19, 22, 27, 33). The loci encoding circularin A (21) and enterocin AS-48 (27, 33) have a similar make-up. Enterocin AS-48 shows structural homology to mammalian NK-lysin, an anti-bacterial peptide produced by T- and natural killer (NK) cells (13). Gassericin A is nearly identical to acidocin B except for two amino acid residues in the leader and a single amino acid change in the mature part of the protein (19, 26). It has been reported that microcin J25, produced by *Escherichia coli*, is also a circular bacteriocin (6, 7). Recently, Rosengren et al. (31) have shown that circularization of microcin J25 is not accomplished via head-to-tail peptide bond formation.
Bacteria protect themselves against their own bacteriocin by producing an immunity protein, a dedicated protein with no other function, the gene of which is usually located within the bacteriocin-coding region. Immunity proteins differ widely with respect to size, ranging from rather small in the case of the circularin A immunity protein CirE (49 amino acids) to medium-sized for that of pediocin PA-1 immunity protein PedB (112 amino acids) (34). In some cases the bacteriocin producing cell is protected by the ABC-type transporter also involved in bacteriocin secretion, like is the case for LmrB (567 amino acids) involved in conferring resistance to the lactococcal bacteriocins LsBA and LsBB (12).

A blocked N-terminus during amino acid sequencing of bacteriocins is often considered as an indication that the peptide is a lantibiotic rather than a circular bacteriocin. However, sequencing of internal peptide fragments is required to distinguish between both possibilities and to unambiguously determine the peptide ligation site of a circular bacteriocin (20, 22).

Here, we identify regions encoding novel, putative circular bacteriocins in the genomes of the Gram-positive bacteria *Staphylococcus aureus*, *Oenococcus oeni* PSU-1 and *Geobacillus stearothermophilus* DSM 13240, bacteria not known to produce circular bacteriocins, by screening for orthologs of processing enzymes rather than of bacteriocins. Structural homology exists among immunity proteins of circular bacteriocins as well as those of unrelated bacteriocins. Furthermore, we postulate that the published bacteriocins boticin B and acidocin B, which have been described in literature but not recognized as such, are in fact circular bacteriocins. In conclusion, we indicate that circular bacteriocins are more common than currently recognized.

**MATERIAL AND METHODS**

**Computational methods.**

Protein sequence alignments were done using the ClustalW program made available by the EMBL-EBI (European Bioinformatics Institute) [http://www.ebi.ac.uk/clustalw]. Homology comparisons were performed using the basic logical alignment tool (BLAST release 2.2.9) as described by Altschul et al. (1). BLAST searches were performed against the NCBI non-redundant protein database and the NCBI microbial genomes database [http://www.ncbi.nlm.nih.gov/BLAST/]. Homologies with conserved domains from the Pfam database [http://www.sanger.ac.uk/Software/Pfam/] (3) were also identified using BLAST searches. Putative transmembrane helices were identified using the TMHMM2.0 program [http://www.cbs.dtu.dk/services/TMHMM-2.0/] (24). Dyad symmetries, Open Reading Frames (ORFs) and isoelectric points (pI) were determined using the program Clonemanager 4 (SEcentral; Scientific & Educational software, 600 Pinner Weald Way Ste 202, Cary, USA [http://www.scied.com/sescat.htm]).

Unpublished nucleotide sequences of finished and unfinished bacterial genomes were obtained from the following sources. The *Geobacillus stearothermophilus* Genome Sequencing Project was funded by the NSF EPSCoR (Experimental Program to Stimulate Competitive Research), Grant #EPS-9550478 and the
unpublished data can be found at the website of the University of Oklahoma's Advanced Center for Genome Technology [http://www.genome.ou.edu/]. Preliminary nucleotide sequence data on the chromosome of *Staphylococcus aureus* was obtained from The Institute for Genomic Research [http://www.tigr.org], sequencing was accomplished with support from NIAID/MGRI (National Institute of Allergy and Infectious Diseases/ Merck Genome Research Institute). The nucleotide sequence data of the *S. aureus* 252 and *S. aureus* 467 chromosomes were produced by the *S. aureus* Sequencing Group at the Sanger Institute and can be obtained from [ftp://ftp.sanger.ac.uk/pub/pathogens/sa/]. *Oenococcus oeni* PSU-1 sequence data was produced by the US Department of Energy Joint Genome Institute [http://www.jgi.doe.gov/].

**RESULTS**

**A common motif in CirC homologues.**

The region minimally required for production of the circular bacteriocin circularin A by *Clostridium beijerinckii* ATCC25752 consists of five genes (cirABCDE). Screening protein and genome databases for homologues to circularin A only resulted in a single hit on the chromosome of *Geobacillus stearothermophilus* DSM 13240. We examined whether other proteins involved in circularin A production and immunity could be used to identify novel circular bacteriocin-coding regions. Of the possible proteins, CirB, part of the ABC-transporter CirBD, is too indiscriminant as it contains no conserved domains and does not have any homologue in a standard BLAST search against the non-redundant database to serve this purpose. CirD is too general as it contains an ATP binding domain and ATP binding domains are present in a wide variety of protein families. CirE, the circularin A immunity protein is too small, resulting in underestimation in genome annotations and a smaller chance to identify homologues in BLAST searches to use to identify novel bacteriocin coding regions. CirC, most likely the circularization protein in the production of circularin A, has a right size, meaning that it will be included in genome annotation procedures, and CirC contains a characteristic domain (DUF95; as defined in the Pfam database). Members of the DUF95 family have several predicted transmembrane regions but have not been further characterized. Some members of this family are annotated as "Stage II sporulation protein M related", which is probably based on a weak homology to proteins with a similar annotation. A common motif, N-x(12,13)-[G]-x(6,7)-[LIF]-x(2)-[NT]-x(2,3)-[IL]-G-x(16-20)-PH-[AFG]-[IFV]-[IFP]-E-x(40-47)-[VI]-x(3)-[ILM]-[TE], can be identified in CirC homologues (Fig. 1) and in proteins encoded by the genomes of a number of archaea (Fig. 2). The CirC homologues encoded in circular bacteriocin coding regions can be subdivided based on their phylogeny (Fig. 2) into two groups with mutual high homology.
Figure 1: CirC homologues. Homologues (putatively) involved in bacteriocin synthesis are shown. For better alignment, two groups were created A) accessory proteins of circularin-A-like proteins B) accessory proteins of gassericin-A-like proteins. CirC is included in both groups to illustrate mutual homology. C) conserved motif. Identical residues are indicated by an asterisk. (:) and (:.) are conserved and semi-conserved amino acid substitutions according to the Cus{}tal{}W grouping of amino acids, respectively. Gaps are introduced in the sequence to maximize alignment.
Figure 2: Phylogram of CirC homologues. Distances in the tree branch lengths are proportional to the amount of estimated evolutionary change relative to a common ancestor. For bacteria protein names are given and for archea species names.

**Novel (putative) bacteriocin coding regions.**

CirC was used in a Blast search against both the non-redundant and the microbial genomes database available at [http://www.ncbi.nlm.nih.gov/BLAST/]. Hits were examined to select those proteins of which the size was comparable to that of CirC (177 amino acids). The regions surrounding the genes of the CirC homologues were examined for homology with genes close to the \( \text{cirC} \) locus. The *in silico* studies did not identify any bacteriocin encoding sequences in the regions surrounding genes encoding archaeal homologues of CirC. However, four putative Class-V-bacteriocin-coding regions in the chromosomes of *Staphylococcus aureus* Mu50, N315, MW2, 467 (2, 25), *Oenococcus oeni* PSU-1 (NZ_AABJ02000001.1), *Butyrivibrio fibrisolvens* AR10 (AF076529) (16) and on the mega-plasmid pBt0xis of *Bacillus thuringiensis* subsp. *israelensis* (5) (Fig. 3) encoding the putative bacteriocins Sav0200, Ooen02000382, AR10, PBt136 were identified, respectively. The fifth region encoding a putative Class V bacteriocin is present in the genome of *Geobacillus stearothermophilus* DSM13240. In the latter case the presence of a bacteriocin encoding region is less clear as the putative structural gene is located on another fragment as the CirC homologue. Interestingly, the *S. aureus* strains MW2 and N315 are resistant against methicilline, a semi-synthetic penicillin derivative, (MRSA) while *S. aureus* 467 is methicilline susceptible (MSSA) (11). *S. aureus* Mu50 is also vancominic resistant (VRSA). Other *S. aureus* strains that are either MRSA (*S. aureus* 252 and COL) (11) or MSSA (*S. aureus* NCTC 8325) do not have a copy of this putative bacteriocin gene cluster. The bacteriocin coding regions of the *S. aureus* strains are almost identical except for a 357-bp in-frame deletion in sav0197 on the chromosomes of strains MW2 and 467 but not on those of strains N315 and Mu50 (Fig. 3). Furthermore, a single base pair deletion in the MW2 sequence (Fig. 3) results in premature truncation of Sav0199, a protein that is homologous to BacI involved in enterocin AS-48 production.
The Blast search identified homology between CirC and BviE (Fig. 1), the gene of which is linked to the structural gene of the bacteriocin butyrivibriocin AR10 produced by \textit{B. fibrisolvens} AR10 (17). The homology between CirC and BviE (this study), the presence of a blocked N-terminus and the homology of butyrivibriocin AR10 to gassericin A (acidocin B) (Fig. 2), a Class V bacteriocin produced by \textit{Lactobacillus gasseri} LG39, support the notion that butyrivibriocin AR10 likely is a circular bacteriocin (18). This supposition has been confirmed by Kalmokoff et al. (16). The loci of the gassericin A and butyrivibriocin AR10 structural genes are slightly different. The CirB homologues of both \textit{B. fibrisolvens} (BviB; 218 amino acids) and \textit{L. gasseri} (GaaI; 162 amino acids) are less than half the size of CirB (581 amino acids) and contain only 5 and 4 TMSs, respectively. In addition, the arrangement of the genes in the two loci is different from that of the genes in the \textit{C. beijerinkii}, \textit{E. faecalis}, \textit{S. aureus} and pBtoxis loci. This also applies for the \textit{O. oeni} PSU-1 coding region but it does encode a CirB homologue with 12 predicted TMSs.

**Similarities among circular bacteriocins.**

The (putative) bacteriocins show limited mutual amino acid sequence similarity (Fig. 4), but their predicted chemical, physical and structural homology is quite large (Table 1). The putative bacteriocins PBT136, Ooen02000382 and Sav02000 can be classified as circularin-A-like, based on their homology to circularin A, the fact that they all have a C-terminal aromatic residue in the precursor peptide and all have a predicted pl between 9.6 and 12.4 (Fig 4, Table1). Butyrivibriocin AR10 is gassericin-A-like, based on homology to gassericin A, the fact that is carries a C-terminal alanine in the precursor peptide and, like gassericin A, has a mildly acidic pl (4.8 and 6.4, respectively; Fig 4, Table1). This grouping matches with the division that could be made among the CirC homologues (see Fig 2). Like circularin A and enterocin AS-48, butyrivibriocin AR10 is more resistant to proteinase digestion than non-circular proteins (15, 17, 22).

<table>
<thead>
<tr>
<th>Table 1: Features of (putative) circular bacteriocins</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name</strong></td>
</tr>
<tr>
<td>CirA</td>
</tr>
<tr>
<td>AS-48</td>
</tr>
<tr>
<td>Ooen02000382</td>
</tr>
<tr>
<td>Pbt136</td>
</tr>
<tr>
<td>Sav02000</td>
</tr>
<tr>
<td>GsteoA</td>
</tr>
<tr>
<td>AR10</td>
</tr>
<tr>
<td>GaaA</td>
</tr>
</tbody>
</table>

Table 1: Features of (putative) circular bacteriocins. Features are calculated based on linear, unprocessed proteins. Protein size is expressed in amino acids (AA). Between brackets the (putative) protein size after processing is depicted. For \textit{G. stearothermophilus} this number is omitted as a clear prediction of the ligation site could not be made. The pl is based on the linear unprocessed protein. TMS: Number of putative transmembrane segments of the processed linear molecule.
C. beijerinckii ATCC 25752

E. faecalis S-48

S. aureus Mu50

B. thuringiensis subsp. israelensis plasmid pBtoxis

G. stearothermophilus DSM13240

O. oeni PSU-1

L. gasseri LG39

B. fibrisolvens AR10

Figure 3: Overview of (putative) circular bacteriocin loci. Structural genes are underlined. Homologous protein products putatively involved in bacteriocin production are shaded identically. Genomic features are indicated by the following symbols. ▼, a deletion in S. aureus MW2 and 467; ▼, a truncation in S. aureus MW2; ○,Dyad symmetries.

The circularin A, enterocin AS-48, Sav0200 and PBt136 loci have a common make up (Fig.3). They consist of a putative structural gene followed by a strong stem-loop structure (ΔG ranging form -6.8 to -20.7 kcals/mol) and four or five genes encoding, respectively, a small putative immunity protein, an ATP-binding protein, a protein consisting of 10 or 11 predicted transmembrane segments (TMS; probably forming an ABC-transporter together with the ATP binding protein) and a CirC homologue. Either upstream or downstream of this region three additional genes, likely involved in enhancing bacteriocin production, are located.
Figure 4: Homology between (putative) Class V bacteriocins: A). Circularin-A-like proteins. B). Gassericin-A-like proteins. Black arrow heads indicate proven head-to-tail ligation sites. Grey arrowheads indicate head-to-tail ligation sites predicted based upon the homology. Identical residues are indicated by an asterisk. (:) and (.) : conserved and semi-conserved amino acid substitutions according to the CustalW grouping of amino acids, respectively. Gaps are introduced in the sequence to maximize alignment. The amino acid sequences matching the 5 α-helices of the enterocin AS-48 structure are boxed. The fifth, C-terminal, α-helix is continued over the ligat ion site, indicated by a small arrow attached to the box.

The predicted bacteriocin of *G. stearothermophilus* is not included in either category as it has a C-terminal aromatic residue, while the unprocessed protein has a mildly acidic pI. It will therefore not be included in further analysis. Based on amino acid sequence alignments, a prediction of the cleavage and ligation sites the precursor peptides can be made (Fig. 4). For Sav0200, PBT136 and Ooen02000382 the predicted cleavage site is proposed to be between the 5th and 6th amino acid N-terminal of the first conserved glycine residue of the mature, non-circularized peptide, as both circularin A and AS-48 are cleaved and circularized at that position. For Sav0200 cleavage between the 3rd and 4th amino acid N-terminal of the first conserved glycine residue of the mature, non-circularized peptide, as both circularin A and AS-48 are cleaved and circularized at that position. For Sav0200 cleavage between the 3rd and 4th amino acid N-terminal of the conserved glycine is a good alternative. If this is the case, cleavage of the three-amino-acid-leader peptide would, like for CirA, yield a peptide of 69 amino acids. As butyrivibriocin AR10 and gassericin A (acidocin B) show a higher degree of overall homology
compared to the circularin A like bacteriocins the predicted cleavage site in the former is most likely as indicated in Fig 4. This cleavage site and a circular nature of the resulting mature bacteriocin are in agreement with the size of the purified peptide and the amino acid sequences of internal peptides of butyrivibriocin AR10 (16, 17).

**Sequence similarities between immunity proteins.**

The immunity proteins belonging to circularin A and enterocin AS-48, CirE and AS-48D1, respectively, have been functionally identified (21, 27, 33). Due to their small sizes, protein homology searches will not easily identify homologues of these two proteins in protein or translated genome databases. When manually forcing an alignment, still little amino acid sequence similarity can be identified. However, similarities exist in general structural features, such as predicted protein size (49-108 amino acids), pl (>9.5) and the number of predicted transmembrane segments (two in most cases) (Table 2). By screening for these features in translation products of genes in the direct vicinity of the genes of the CirC homologues, we were able to pin-point the putative bacteriocin immunity genes in the loci described here (Table 2). In those cases in which the genome sequence was not complete yet, the ORFs specifying the putative immunity proteins were identified using the Clonemanager program. The aforementioned structural features are also present in the immunity proteins of unrelated bacteriocins like Pep5, epicidin 280, lactocin S and divergicin A (Table 2) (14, 29, 30, 32, 36).

Attempt to produce the putative bacteriocin Sav0200 by cloning the structural gene and its cognate immunity protein in a host expressing the circularin A secretion machinery failed, as well as attempts to functionally express larger parts of the

<table>
<thead>
<tr>
<th>Name</th>
<th>Source</th>
<th>Size (AA)</th>
<th>pI</th>
<th>TMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CirE</td>
<td>C. beijerinckii ATCC 25752</td>
<td>49</td>
<td>10.6</td>
<td>2</td>
</tr>
<tr>
<td>AS-48D1</td>
<td>E. faecalis S-48</td>
<td>56</td>
<td>10.5</td>
<td>2</td>
</tr>
<tr>
<td>PB1140</td>
<td>B. thuringiensis subsp. israelensis</td>
<td>93</td>
<td>9.5</td>
<td>3</td>
</tr>
<tr>
<td>Sav0204</td>
<td>S. aureus Mu50, MW2, N315 and 467</td>
<td>58</td>
<td>10.1</td>
<td>2</td>
</tr>
<tr>
<td>Ooen02000384.2</td>
<td>O. oeni PSU-1</td>
<td>108</td>
<td>10.8</td>
<td>2</td>
</tr>
<tr>
<td>BviC</td>
<td>B. fibriosolvens AR10</td>
<td>53</td>
<td>9.7</td>
<td>2</td>
</tr>
<tr>
<td>Gaa-orf2</td>
<td>L. gasseri LG39</td>
<td>53</td>
<td>11.5</td>
<td>2</td>
</tr>
<tr>
<td>PepI</td>
<td>Streptococcus epidermidis 5</td>
<td>69</td>
<td>10.1</td>
<td>2</td>
</tr>
<tr>
<td>Ecil</td>
<td>S. epidermidis BN 280</td>
<td>62</td>
<td>9.9</td>
<td>2</td>
</tr>
<tr>
<td>LasJ</td>
<td>Lactobacillus sakei L45</td>
<td>57</td>
<td>10.3</td>
<td>2</td>
</tr>
<tr>
<td>divergicin A immunity</td>
<td>Carnobacterium divergens</td>
<td>56</td>
<td>10.2</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2: Features of several (putative) immunity proteins. Protein size is expressed in amino acids (AA). *) TMS: Number of putative transmembrane segments.
sav0200 gene cluster. The proof of antimicrobial activity of the putative circular peptides described still needs to be given and is a subject of attention.

**DISCUSSION**

This study identifies novel (putative) circular bacteriocins in the rapidly growing nucleotide sequence databases. Sav0200, circularin A and PBt136 share 58%, 72% and 65% homology to enterocin AS-48, respectively. The 3-D structure of enterocin AS-48 is a tightly folded peptide consisting of five $\alpha$-helices. Enterocin AS-48 has structural resemblance to NK-lysin, an antibacterial and tumorlytic peptide of T- and natural killer (NK) cells (13). Whether the proteins responsible for bacteriocin secretion and/or immunity could, based on the structural homology, help the producer bacteria to evade NK-lysin action is an open question. If so, the bacteriocin gene clusters identified here could be virulence factors, enhancing the ability of S. aureus strains encoding the enterocin AS-48 homologue Sav0200, to infect the human body.

Genes encoding AS-48FGH homologues, which are involved in enterocin AS-48 secretion and immunity (8), are present in the cirA, PBt136, sav0200 and ooen02000382 loci. AS-48FGH and their homologues belong to the DUF214 family of proteins (8, 21), which includes the LolCDE system of *Escherichia coli* that is involved in secretion of lipid-modified proteins from the outer leaflet of the cell membrane (37). Bacteriocins and lipid-modified proteins have a strong affinity for bacterial membranes and it is thus tempting to speculate that the DUF214 family of proteins encoded in the cirA, PBt136, sav0200 and ooen02000382 loci are involved in removing the respective bacteriocins from the bacterial cytoplasmic membrane. Interestingly, homologues of AS-48FGH are also specified by pRJ9, a plasmid from S. aureus A53, involved in production of the non-circular, leaderless bacteriocin aureocin A53 (28).

A common motif is present in all CirC-like proteins suggesting a common function in the biosynthesis of circular bacteriocins. As CirB and CirD, together constituting an

![Figure 5: Realignment of Boticin B chymotryptic peptide fragments to the unprocessed peptide. Lower case: alignment according to Dineen at al. (9). Upper case: alignment assuming that boticin B is a circular bacteriocin; a line is introduced to illustrate to connection between the C-terminal valine and the N-terminal leucine of a precursor peptide. Arrows indicate chymotryptic cleavage sites. The amino acid sequence of the unprocessed form of boticin B is from Dineen et al. (9).](image)
ABC-transporter and essential for bacteriocin production, are likely to be involved in the bacteriocin secretion, it is presumed that this motif in CirC has an essential role in bacteriocin circularization. The fact that CirC contains a motif specific for circular bacteriocins makes it possible to use this protein to identify novel bacteriocin coding regions in genome sequences. Subsequent screening of the genome sequences surrounding the gene encoding the CirC homologues for, respectively, genes encoding an ATP-binding protein, an small peptide with a high pI (a putative immunity protein) and a bacteriocin precursor makes identification of a bacteriocin coding region possible. The presence of a gene encoding a CirC homologue (BviE) and a gene encoding an ATP-binding protein in the vicinity of the structural bacteriocin gene for butyrivibriocin AR10 is therefore an additional indication that it is indeed a circular bacteriocin like already recognized by Kalmokoff et al. (16). However, internal amino acid sequencing covering the ligation point is still essential to confirm the circular nature and identify the point of circularization of this and other (putative) circular bacteriocins (Sav0200, Pbt136, GsteoA and Ooen02000382). For microcin J25, previously identified as a circular peptide, this has been omitted, leading to misinterpretation of the 3-D structure of the bacteriocin, which is now believed to be a lassoed-tail (4, 31, 35).

Identifying bacteriocin immunity proteins via protein homology studies is rather difficult as even immunity proteins of bacteriocins of the same type often have little sequence similarities. However, based on predicted structural features it is possible to recognize bacteriocin immunity proteins. Immunity proteins linked to circular bacteriocins can be grouped with those conferring immunity to non-circular bacteriocins, suggesting that a common immunity mechanism exists. This immunity mechanism could be either blockage of pore formation e.g. by direct binding of the bacteriocin or receptor shielding as the (predicted) immunity proteins of circular bacteriocins are small and lack discriminating features. Resistance conferred via active transport of the bacteriocins from the cell seems less likely as that would normally require a larger ATP-binding protein/transporter such as LmrB in the case of LsbA and LsbB resistance (12).

Sometimes bacteriocins might not directly be identified as being circular, like boticin B, a bacteriocin produced by Clostridium botulinum strain 213B (9). Boticin B It shows peculiarities of circular bacteriocins namely a blocked N-terminus and resistance to proteases (10). A possible gene encoding a CirC homologue could not be identified in the vicinity of the bacteriocin structural gene due to insufficient sequence data, but a reinterpretation of the internal amino acid sequencing results indicates a circular nature for boticin B. Presuming boticin B to be circular, would explain the odd N-terminal sequence of a chymotryptic peptide fragment with an unexpected valine, as depicted in Fig. 5 (9). Furthermore, the mass of a circular form of boticin B (4007 Da)
would match the measured mass of 4003 Da better than the proposed linear peptide (4025 Da).

In conclusion, circular antimicrobial peptides are more widespread in nature than is currently apparent from available literature. Determination of their ecological role, host range, mode of action, the identity of the proteins involved in circularization and the circularization mechanism itself, warrant further research of these proteins. Moreover, we provide a method for the rapid identification of circular bacteriocin encoding genes in forth coming genome sequences, aiding the correct annotation of these genes.

REFERENCES


