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MicroCommentary

An 'Upp'-turn in bacteriocin receptor identification

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Summary

Bacteriocins are gene encoded, bacterially produced antimicrobial peptides that have been the focus of considerable scientific interest but which are relatively underutilized by the food, veterinary and medical industries. One means via which the latter issue can be overcome is through a better understanding of how these peptides work or, more specifically, the identification of bacteriocin receptors and the subsequent application of such information to enhance the potency, and commercial value, of bacteriocins. For a time since the identification of lipid II and subunits of the mannose phosphotransferase system as receptors for several class I (modified) and class II (unmodified) bacteriocins, respectively, there were relatively few developments in this area. However, a number of recent studies have addressed this issue, resulting in the identification of a maltose ABC transporter and metallopeptidase as the targets for the garvicin ML (class IIc) and LsbB (class IId) bacteriocins, respectively, and, most recently, the identification of UppP as the receptor for lactococcin G and enterocin 1071 (both class IIb). In addition to these exciting discoveries, the development, and further application, of new strategies to facilitate receptor identification has the potential to lead to even further breakthroughs in bacteriocin research.

Bacteriocins are small, bacterially produced, ribosomally synthesized peptides that are active against other bacteria and against which the producer has a specific immunity mechanism (Cotter *et al.*, 2005). Bacteriocins are generally subdivided into those which are translationally modified (class I) and unmodified or minimally modified (i.e. cyclic) peptides (class II) (Rea *et al.*, 2011; Cotter *et al.*, 2013). These peptides are studied from a fundamental science perspective and also with a view to their use for food (preservatives), veterinary and/or medical (alternatives to antibiotics) applications (Cotter *et al.*, 2005; 2013; Hassan *et al.*, 2012). However, with a few exceptions, such as the widely used nisin (Delves-Broughton *et al.*, 1996), bacteriocins are more known for their potential, rather than actual, commercial value. Despite this, the increasing consumer demand for natural food preservatives, such as bacteriocins produced by lactic acid bacteria, and the continuing emergence of antibiotic-resistant bacteria, in the face of declining numbers of new antibiotics (Braine, 2011), make the application of bacteriocins an ever-more attractive proposition. With respect to the control of antibiotic-resistant bacteria, there is a particular desire for antimicrobials that target sites that differ from those at which currently utilized antibiotics act, thereby making cross-resistance unlikely and facilitating the use of combinations of different antimicrobials to better control pathogens. Thus information relating to the receptors to which bacteriocins bind is potentially of great significance.

Unfortunately, until recently, relatively little has been known about the receptors to which bacteriocins that target Gram-positive bacteria bind, with two major exceptions, i.e. lipid II and subunits of the mannose phosphotransferase (PTS) system (Fig. 1). Lipid II, and related cell wall precursors, has been identified as the receptor, as well as target, for several class I bacteriocins from the lantibiotic subgroup (Martin and Breukink, 2007; Muller *et al.*, 2012; Munch *et al.*, 2014) as well as for the class II bacteriocin, lactococcin 972 (Martinez *et al.*, 2008). Lipid II is an essential cell wall precursor consisting of the bactoprenol carrier lipid (C55-P), which is linked to the peptidoglycan building block N acetyl-muramyl-pentapeptide-N-acetylglucosamine (MurNAc-pp-GlcNAc) via a pyrophosphate bridge. Despite quite an amount of information suggesting the role of components of the mannose PTS system as a receptor for some bacteriocins (Ramnath *et al.*, 2000; 2004; Gravesen *et al.*, 2002; Hechard and Sahl, 2002), it was not until 2007 that this was firmly established as a receptor for the class IIa (i.e. pediocin-like), and some class IId (lactococcin A and lactococcin B; linear, non-pediocin-like), bacteriocins (Diep *et al.*, 2007; Kjos *et al.*, 2011). It should also be noted that

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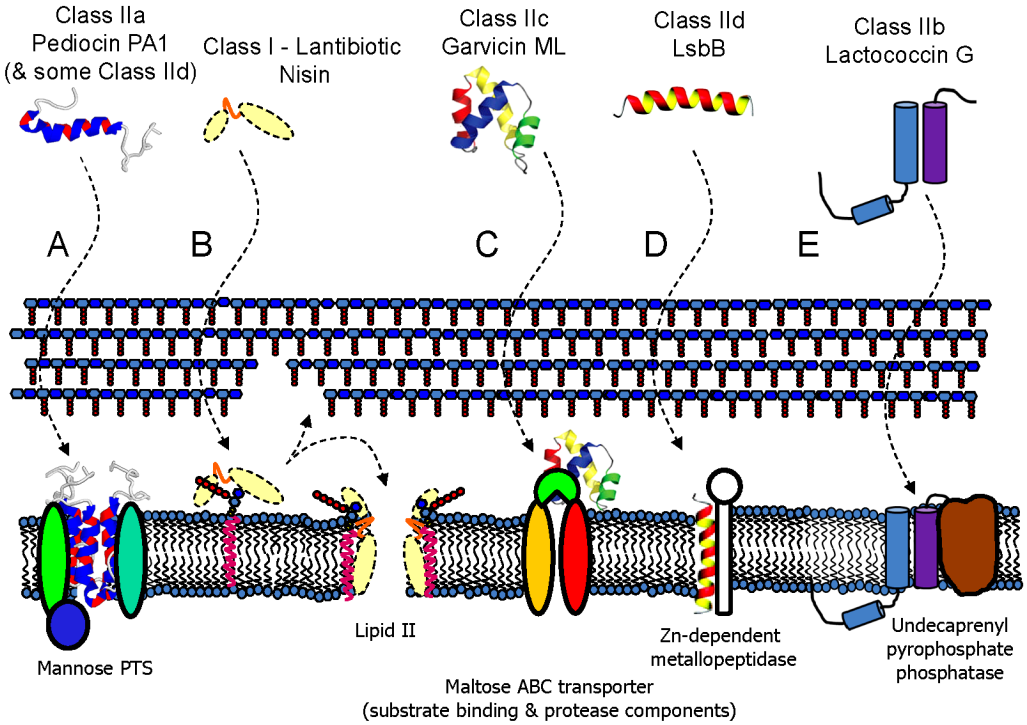

Fig. 1. Proposed model for different bacteriocin systems that target Gram-positive bacteria, i.e. (A) class IIa and some class IId bacteriocins (involving components of the Mannose PTS system); (B) class I (lantibiotics) and lactococcin 972 (involving lipid II and related peptidoglycan precursors); (C) the class IIc bacteriocin, garvicin ML (involving the maltose ABC transporter); (D) the class IId bacteriocin, LsbB (involving a Zn-dependent metallopeptidase) and (E) lactococcin G-like class IIb bacteriocins (involving UppP).

while other class I peptides, such as the thiopeptides and bottromycins (which have only recently been recognized as bacteriocins), control Gram-positive bacteria by targeting translation, the mechanism via which they enter target cells has not been determined. In light of this information, it is particularly notable that several new bacteriocin receptors have been identified in Gram-positive targets.

The first of these relatively recent developments occurred in 2012 when Gabrielsen *et al.* (2012) reported the identification of the maltose ABC transporter in *Lactococcus lactis* as the receptor for the circular (class IIc) bacteriocin, garvicin ML, produced by *Lactococcus garvieae* DCC43. This was achieved following observations that multiple garvicin ML-resistant mutants of *L. lactis* IL1403 were unable to grow with either starch or maltose as a carbohydrate source. This was ultimately traced to the deletion, most likely facilitated by genes encoding IS*981*l elements, of a 13.5 kb region involved in carbohydrate metabolism from these mutants. Within the region that was absent from the garvicin ML-resistant mutants, the only genes associated with membrane located proteins were *malEFG*, i.e. those encoding the maltose ABC transporter (Fig. 1). Crucially, reintroduction of these genes into the garvicin ML-resistant mutants restored a garvicin ML-sensitive phenotype (Gabrielsen *et al.*, 2012). These investigations do not reveal whether targeting of the maltose ABC transporter is responsible for the activity of garvicin ML, perhaps by inducing the release of intracellular constituents via the permease component of the transporter, or if the maltose transporter simply acts as a docking molecule that facilitates subsequent activities by the bacteriocin. Nevertheless, the importance of the transporter in determining the sensitivity of the strain to this bacteriocin is without doubt. It is as yet unclear, however, if this observation will be extended to other class IIc bacteriocins. Although garvicin ML peptide shares only limited amino acid similarity with carnocyclin A (30% identity) and AS-48 (28% identity) (Borrero *et al.*, 2011), many of the class IIc peptides are thought to share a common structural motif (Martin-Visscher *et al.*, 2009).

In another instance, a Zn-dependent metallopeptidase was found to be responsible for sensitivity to the class IId bacteriocin LsbB (Uzelac *et al.*, 2013). This was achieved by generating a cosmid library of the LsbB-sensitive strain *L. lactis* BGMN1-596 and expressing this library in LsbB-resistant derivatives of BGMN1-596. Subcloning of a 40 kb insert that restored sensitivity revealed a 1.9 kb fragment, containing the Zn-dependent membrane bound metallopeptidase-encoding *yvjB*, which was sufficient to confer the LsbB-sensitive phenotype. This initial break-

through was followed by further investigations which confirmed the role of the metallopeptidase in bacteriocin activity (Fig. 1). These included genome sequencing, which revealed the presence of *yvjB* mutations in the LsbB-resistant strains, the resistance-inducing targeted disruption of *yvjB* in BGMN1-596 and IL1403 and the conferring of a LsbB-sensitive phenotype on naturally resistant *Lactobacillus casei* and *Enterococcus faecalis* through the heterologous expression of *yvjB* in these backgrounds (Uzelac *et al*., 2013). LsbB does not resemble other known bacteriocins and so it is not clear if other bacteriocins exist that target this metallopeptidase.

Most recently, Kjos *et al*. have extended our knowledge of bacteriocin receptors by, for the first time, identifying what appears to be a receptor for a class IIb (unmodified, two-peptide) bacteriocin, lactococcin G (Kjos *et al*., 2014) (Fig. 1). This was elegantly achieved through the creation of 12 lactococcin G-resistant derivatives of *L. lactis* (strains IL1403 and MG1363) and subsequent genome sequencing of these mutants. In all cases, a mutation was found in or near *uppP* (also known as *bacA*), encoding an undecaprenyl pyrophosphate phosphatase. The importance of this gene was confirmed through heterologous expression in *Streptococcus pneumoniae*, to confer a bacteriocin-sensitive phenotype on an otherwise insensitive strain. This pattern of resistance and induced sensitivity was also apparent when the related class IIb bacteriocin, enterocin 1071 (Table 1), was employed (Kjos *et al*., 2014). It is also highly likely that sensitivity to lactococcin Q (Zendo *et al*., 2006), which even more closely resembles lactococcin G (Table 1), is also dependent on the presence of UppP. UppP is a membrane spanning protein (Fig. 1) that has a role in the dephosphorylation of undecaprenyl pyrophosphate to undecaprenyl phosphate (Bickford and Nick, 2013). Despite UppP being a relatively highly conserved protein, lactococcin G targets lactococci specifically thus suggesting that there are specific regions that are exclusive to the lactococcal UppP that are recognized by this bacteriocin. It should be noted that other class IIb peptides do not closely resemble lactococcin G, lactococcin Q and enterocin 1071 and, thus, it is unlikely that these also function in an UppP dependent manner.

While the identification of the importance of UppP with respect to the activity of lactococcin G and enterocin 1071 is important, it may be that the further evolution of genome-sequencing-based strategies to identify bacteriocin targets will be of even greater significance in the field of bacteriocin, and antimicrobial, research. While, in the case of Uzelac *et al*., genome sequencing of bacteriocin-resistant mutants was employed as a means of confirming the involvement of *yvjB* in the activity of LsbB (Uzelac *et al*., 2013), Kjos *et al*. (2014) use this approach as the key first step *en route* to establishing the significance of

Table 1. Sequence alignments of the related two peptide class II bacteriocins, lactococcin G, lactococcin Q and enterocin 1071.

Bacteriocin	Peptide	Amino acid sequence
Lactococcin G	LcnG-α	G T W D D I G Q G I G R V A Y W V G K A M G N M S D V N Q A S R I N R K K K H
Lactococcin Q	LcnQ-a	S I W G D I G Q G V G K A A Y W V G K A M G N M S D V N Q A S R I N R K K K H
Enterocin 1071	Ent-α	E S V F S K I G N A V G P A A Y W I L K G L G N M S D V N Q A D R I N R K K – H
Lactococcin G	LcnG-β	K K W G W L A W V D P A Y E F I K G F G K G A I K E G N K D K W K N I
Lactococcin Q	LcnQ-β	K K W G W L A W V E P A G E F L K G F G K G A I K E G N K D K W K N I
Enterocin 1071	Ent-β	G P G K W L P W L Q P A Y D F V T G L A K G I G K E G N K N K W K N V

Sequences that are identical in all three bacteriocins are shaded in black. Those that are conserved in two are shaded in grey.

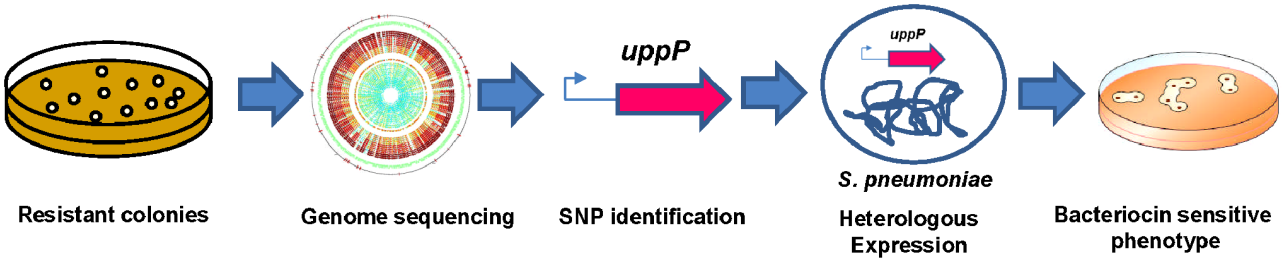

Fig. 2. Strategy taken to identify the lactococcin G receptor. Following the isolation of bacteriocin-resistant mutants, genome sequencing revealed that all contained mutations in or around the *uppP* gene. Heterologous expression of this gene in a (lactococcin G resistant) *S. pneumoniae* strain induced a lactococcin G-sensitive phenotype.

UppP with respect to lactococcin G activity (Fig. 2). As a consequence of highlighting the success of this strategy, coupled with the decreasing cost of DNA sequencing means, it would seem likely that many researchers will take a similar approach to better understand the mechanism of action of bacteriocins, and other antimicrobials, with which they work. Given the very heterogeneous structures of bacteriocins it is likely that there are many such receptors that have yet to be identified.

While the identification of new bacteriocin receptors and the further elucidation of mechanisms of action are worthwhile goals, one might ask if these advances in our understanding of fundamental bacteriocin biology can lead to an increased number of commercial bacteriocin-related applications. The answer is clearly yes. First, having a clearer understanding of the mechanism of action of specific bacteriocins is of considerable value with respect to attracting interest from the pharmaceutical industry in that it represents an important piece of information in the 'dossier' for any new antimicrobial. As is apparent in the examples above, it also frequently highlights that the receptors being targeted are distinct from those targeted by existing antibiotics and, thus, that cross resistance is not an issue and that these bacteriocins could be used in combination with other antimicrobials. It is also likely that, in the longer term, identification of bacteriocin receptors will lead to further structure-based investigations to better understand specific bacteriocin-receptor interactions. This information can be exploited to engineer bacteriocins, create hybrid-antimicrobials or design new peptides that will have a broader target range or which exhibit potent narrow spectrum activity against specific pathogens.

References

Bickford, J.S., and Nick, H.S. (2013) Conservation of the PTEN catalytic motif in the bacterial undecaprenyl pyrophosphate phosphatase, BacA/UppP. *Microbiology* **159:** 2444–2455.

Borrero, J., Brede, D.A., Skaugen, M., Diep, D.B., Herranz, C., Nes, I.F., *et al.* (2011) Characterization of garvicin ML, a novel circular bacteriocin produced by *Lactococcus garvieae* DCC43, isolated from mallard ducks (*Anas platyrhynchos*). *Appl Environ Microbiol* **77:** 369–373.

Braine, T. (2011) Race against time to develop new antibiotics. *Bull World Health Organ* **89:** 88–89.

Cotter, P.D., Hill, C., and Ross, R.P. (2005) Bacteriocins: developing innate immunity for food. *Nat Rev Microbiol* **3:** 777–788.

Cotter, P.D., Ross, R.P., and Hill, C. (2013) Bacteriocins – a viable alternative to antibiotics? *Nat Rev Microbiol* **11:** 95–105.

Delves-Broughton, J., Blackburn, P., Evans, R.J., and Hugenholtz, J. (1996) Applications of the bacteriocin, nisin. *Antonie Van Leeuwenhoek* **69:** 193–202.

Diep, D.B., Skaugen, M., Salehian, Z., Holo, H., and Nes, I.F. (2007) Common mechanisms of target cell recognition and immunity for class II bacteriocins. *Proc Natl Acad Sci USA* **104:** 2384–2389.

Gabrielsen, C., Brede, D.A., Hernandez, P.E., Nes, I.F., and Diep, D.B. (2012) The maltose ABC transporter in *Lactococcus lactis* facilitates high-level sensitivity to the circular bacteriocin garvicin ML. *Antimicrob Agents Chemother* **56:** 2908–2915.

Gravesen, A., Ramnath, M., Rechinger, K.B., Andersen, N., Jansch, L., Hechard, Y., *et al.* (2002) High-level resistance to class IIa bacteriocins is associated with one general mechanism in *Listeria monocytogenes*. *Microbiology* **148:** 2361–2369.

Hassan, M., Kjos, M., Nes, I.F., Diep, D.B., and Lotfipour, F. (2012) Natural antimicrobial peptides from bacteria: characteristics and potential applications to fight against antibiotic resistance. *J Appl Microbiol* **113:** 723–736.

Hechard, Y., and Sahl, H.G. (2002) Mode of action of modified and unmodified bacteriocins from Gram-positive bacteria. *Biochimie* **84:** 545–557.

Kjos, M., Borrero, J., Opsata, M., Birri, D.J., Holo, H., Cintas, L.M., *et al.* (2011) Target recognition, resistance, immunity and genome mining of class II bacteriocins from Gram-positive bacteria. *Microbiology* **157:** 3256–3267.

Kjos, M., Oppegård, C., Diep, D.B., Nes, I.F., Veening, J.-W., Nissen-Meyer, J., and Kristensen, T. (2014) Sensitivity to the two-peptide bacteriocin lactococcin G is dependent on UppP, an enzyme involved in cell-wall synthesis. *Mol Microbiol* **92:** 1177–1187.

Martin, N.I., and Breukink, E. (2007) Expanding role of lipid II as a target for lantibiotics. *Future Microbiol* **2:** 513–525.

Martin-Visscher, L.A., Gong, X., Duszyk, M., and Vederas, J.C. (2009) The three-dimensional structure of carnocyclin A reveals that many circular bacteriocins share a common structural motif. *J Biol Chem* **284:** 28674–28681.

Martinez, B., Bottiger, T., Schneider, T., Rodriguez, A., Sahl, H.G., and Wiedemann, I. (2008) Specific interaction of the unmodified bacteriocin Lactococcin 972 with the cell wall precursor lipid II. *Appl Environ Microbiol* **74:** 4666–4670.

Muller, A., Ulm, H., Reder-Christ, K., Sahl, H.G., and Schneider, T. (2012) Interaction of type A lantibiotics with undecaprenol-bound cell envelope precursors. *Microb Drug Resist* **18:** 261–270.

Munch, D., Muller, A., Schneider, T., Kohl, B., Wenzel, M., Bandow, J.E., *et al*. (2014) The lantibiotic NAI-107 binds to bactoprenol bound cell wall precursors and impairs membrane functions. *J Biol Chem* **289:** 12063–12076.

Ramnath, M., Beukes, M., Tamura, K., and Hastings, J.W. (2000) Absence of a putative mannose-specific phosphotransferase system enzyme IIAB component in a leucocin A-resistant strain of *Listeria monocytogenes*, as shown by two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Appl Environ Microbiol* **66:** 3098–3101.

Ramnath, M., Arous, S., Gravesen, A., Hastings, J.W., and Hechard, Y. (2004) Expression of *mptC* of *Listeria monocytogenes* induces sensitivity to class IIa bacteriocins in *Lactococcus lactis*. *Microbiology* **150:** 2663–2668.

Rea, M.C., Ross, R.P., Cotter, P.D., and Hill, C. (2011) Classification of bacteriocins from Gram-positive bacteria. In *Prokaryotic Antimicrobial Peptides*. Drider, D., and Rebuffat, S. (eds). New York: Springer, pp. 29–53.

Uzelac, G., Kojic, M., Lozo, J., Aleksandrzak-Piekarczyk, T., Gabrielsen, C., Kristensen, T., *et al*. (2013) A Zn-dependent metallopeptidase is responsible for sensitivity to LsbB, a class II leaderless bacteriocin of *Lactococcus lactis* subsp. *lactis* BGMN1-5. *J Bacteriol* **195:** 5614–5621.

Zendo, T., Koga, S., Shigeri, Y., Nakayama, J., and Sonomoto, K. (2006) Lactococcin Q, a novel two-peptide bacteriocin produced by *Lactococcus lactis* QU 4. *Appl Environ Microbiol* **72:** 3383–3389.