AGRICULTURE AND FOOD DEVELOPMENT AUTHORITY

This article is provided by the author(s) and Teagasc T-Stór in accordance with publisher policies.

Please cite the published version.

The correct citation is available in the T-Stór record for this article.

NOTICE: This is the author's version of a work that was accepted for publication in Current Opinion in Biotechnology. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in Current Opinion in Biotechnology, volume 24(2), (April 2013). DOI 10.1016/j.copbio.2012.12.003

This item is made available to you under the Creative Commons Attribution-Non commercial-No Derivatives 3.0 License.

10 **Strategies to improve the bacteriocin protection provided by lactic acid bacteria**

11 Eileen F. O' Shea^{1, 3}, Paul D. Cotter^{1, 2}, R. Paul Ross^{1, 2} and Colin Hill^{2, 3}

12

13 ¹Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland.

14 ²Alimentary Pharmabiotic Centre, Cork, Ireland.

15 ³Department of Microbiology, University College Cork, Ireland.

16

17 * Corresponding author:

18 Paul Ross,

19 Teagasc Food Research Centre,

20 Moorepark,

21 Fermoy,

22 Co. Cork,

23 Ireland.

24 Email: paul.ross@teagasc.ie

25 Tel: +353 (0)25 42229

26 Fax: +353 (0)25 42340

27 **Abstract**

28 Lactic acid bacteria (LAB) produce a wide variety of antimicrobial peptides
29 (bacteriocins) which contribute to the safety and preservation of fermented foods.
30 This review discusses strategies that have or could be employed to further enhance the
31 commercial application of bacteriocins and/or bacteriocin-producing LAB for food
32 use.

Introduction

Bacteriocin production is a desirable trait among LAB from the perspective of controlling microbial populations in fermented foods in order to extend product shelf-life and safety. Bacteriocins produced by LAB are a diverse group of ribosomally-synthesized antimicrobial peptides which may be divided into two main groups i.e. class I peptides, which contain post-translational modifications, and class II, or unmodified, peptides [1]. Broad spectrum bacteriocins, such as nisin (class I), inhibit Gram positive food-borne pathogens and spoilage microbes and, when combined with additional hurdles, Gram negative targets [2]. Narrow spectrum bacteriocins can also be of value, for example, lactococcin A (class II) has a lytic effect on sensitive lactococci which, through the release of key enzymes, can accelerate cheese ripening and enhance the development of important organoleptic properties [3]. Bacteriocins may be introduced into a food *via in situ* production by bacterial starter or adjunct strains in fermented foods, by the addition of purified or semi-purified preparations (e.g. nisin containing powders such as Nisaplin) or as an ingredient based on a fermentate of a bacteriocin producing strain (such as ALTA2431 which contains pediocin PA1). However, the commercial application of specific bacteriocins can be hindered by low or inconsistent production levels, high production costs, a non-ideal antimicrobial spectrum and potency, the risk of the emergence of resistance and the poor/lack of growth of some producing strains in particular foods. This review discusses some of the strategies developed to overcome such limiting factors.

Influence of growth parameters

Many studies have been dedicated to optimising bacteriocin production by manipulating growth media composition, temperature or pH [4-6]. Investigations of

alternative carbon, nitrogen and mineral sources have successfully led to increased bacteriocin yields or more cost effective production [7-9]. Another strategy has been the inclusion of additional stimuli. In the case of *Lactobacillus plantarum* NC8, a starter strain used in Spanish-style green olive fermentations, and *Leuconostoc citreum* GJ7, a kimchi isolate, this occurs through the addition of specific adjunct strains that induce bacteriocin-production [10,11].

Use of conjugation to transfer a bacteriocin producing phenotype

Conjugation provides a natural mechanism by which genes can be transferred from one LAB to another while maintaining the food grade status of the recipient strain. As many bacteriocins are plasmid encoded, this approach has been widely exploited to disseminate bacteriocin-producing phenotypes. This is exemplified by the generation of over 30 food grade *Lactococcus* strains that produce the broad spectrum two-component class I bacteriocin lacticin 3147 through the transfer of pMRC01, a conjugative plasmid which contains the corresponding genetic determinants [12]. Although the presence of pMRC01 may impose an additional metabolic burden on LAB, which can increase cell permeability and autolysis, it does not impact on the acidification capacity of the strain [13]. In addition to the transfer of the bacteriocin producing phenotype itself, bacteriocin production (and the associated bacteriocin immunity phenotype) can also serve as a food grade selectable marker when transferring additional, plasmid-linked, industrially relevant traits. Indeed, while conjugal transfer of pMRC01 to the lacticin 481 producing host *Lactococcus lactis* DPC5552 generated a co-producing transconjugant which exhibited synergistic activity [14], both bacteriocins have served as effective food grade selectable markers for the transfer of bacteriophage resistance phenotypes to important commercial dairy

83 starter strains, thereby reducing phage sensitivity [15,16]. It should also be noted that
84 the conjugal transfer of plasmids encoding other traits, such as lactose utilisation and
85 protease activity, can facilitate increased bacteriocin production by adapting the
86 producing strain for better growth in food environments. Indeed, Garcia-Parra et al,
87 reported a 40-fold increase in nisin production by one such transconjugant in milk
88 [17]. The genetic determinants for nisin are also located on a conjugative transposon
89 enabling its food-grade transfer to other LAB [18], and the nisin resistance phenotype
90 has also been widely exploited as a selection marker for food grade improvement of
91 starter strains [19,20].

92 Strategies have also been adapted to mobilize bacteriocin-encoding non-conjugative
93 plasmids for the improvement of starter strains in a food grade manner [21,22]. Such
94 plasmids, unable to mediate their own transfer, require the sequence of the origin of
95 transfer (*oriT*) and *mobA* gene, while the genes encoding other conjugal functions are
96 supplied *in trans* from a conjugative plasmid or sex factor. *L. lactis* IFPL359
97 transconjugants generated in this way to harbour the lactacin 3147-encoding non-
98 conjugative plasmid pBaC105 successfully accelerated proteolysis and development
99 of sensory characteristics of semi-hard goat cheese [21].

100 Aside from the ‘donor’ strain from which these plasmids and transposons are being
101 mobilised, it should also be noted the genetic composition of recipient strains can
102 ultimately influence the success of the conjugative approach. Indeed, *L. lactis* subsp
103 *lactis* IL1403 is frequently selected for studies of lactococcin A (LcnA; class II)-type
104 bacteriocins as this strain contains chromosomal analogues of the genes involved in
105 LcnA secretion and maturation (i.e. *lcnC* and *lcnD*). Indeed, the extent of bacteriocin
106 production by this strain following the conjugal transfer of pS140, a plasmid
107 harbouring the genetic determinants for a lactococcin A-like bacteriocin, was greater

than that of other lactococcal transconjugants, presumably as a consequence of the additional copies of *lcnCD* already present [23].

Subcloning and expression of bacteriocin genes or gene clusters

Subcloning and expression of bacteriocin genes and gene clusters has also been applied as a means of conferring a bacteriocin positive phenotype on LAB strains or to facilitate over-production in a strain that is already a natural bacteriocin producer. Indeed, a particularly effective strategy employed for the overproduction of various class I bacteriocins has been the introduction of additional copies of biosynthesis-associated genes to an existing bacteriocin-producing host. This has led to greater yields of nisin [24,25] and of the individual lactacin 3147 component peptides, Ltn α and Ltn β , as well as improved yields of bioengineered lactacin 3147 variants [26]. Such studies have also established that the introduction of additional copies of immunity (self-protection) genes can be important to overcome self-toxicity-associated limitations when overproducing these peptides.

The heterologous production of class II bacteriocins by LAB is dependant on several factors such as the host strain, the expression and secretion systems employed, plasmid stability and copy number and the presence of the cognate bacteriocin immunity genes. While expression systems employing constitutive promoters and inducible promoters (such as the nisin-inducible promoter, (P_{nisA} , of the NIsin Controlled gene Expression (NICE) system [27]) have both been highly exploited, inducible systems have in general been more successful. Regardless of promoter, the strategies involved have varied from cloning of the entire, intact bacteriocin biosynthetic gene cluster [28,29] to the creation of gene fusions (to facilitate efficient bacteriocin transport) through the exploitation of bacteriocin leader or secretion

signals [30-37]. Yeast based platforms have also shown considerable promise. These may be useful for the large-scale production of bacteriocins or for yeast based fermentations [38-41].

Although these recombination techniques can facilitate increased levels of bacteriocin production and activity, and the construction of improved multi-bacteriocin producing strains, they remain genetically modified organisms (GMO) which may limit their application in the wider Food Industry.

Bioengineering of bacteriocin peptides

There have been a number of instances in which bioengineering of bacteriocin structural genes (through manipulation of the gene in a natural producer or in a strain which produces the bacteriocin heterologously) has been employed with a view to expanding or altering the associated antimicrobial spectrum. This strategy initially evolved from a desire to gain a better appreciation of the importance of specific residues or domains within these peptides, i.e. to assess the negative consequences of mutating these regions. However, this approach has evolved such that strains with greater antimicrobial potency have resulted which can potentially provide for the better control of spoilage or pathogenic microbes.

Bioengineering-based strategies were first applied to LAB producers of class I bacteriocins, with the targeting of nisin [42-44] being of greatest relevance to this review. Subsequent, manipulations of nisin were crucial with respect to elucidating the mechanism of action of the peptide [45-48]. From these, and more recent studies, some bioengineered nisins are notable by virtue of possessing enhanced antimicrobial activity against at least one, albeit non-pathogenic, Gram positive target [49-52]. Other bioengineered derivatives of nisin have been identified which more effectively

inhibit one or more pathogenic targets. The majority of these are derivatives in which residues within a central, 3-amino acid, stretch known as the ‘hinge’ region have been altered. Here, examples include nisins N20K and M21K [53], nisin M21V [54-56], nisin K22T (figure 1), [55] and nisin N20P [54]. Recently, nisin peptides in which serine 29 has been altered have drawn attention by virtue of exhibiting enhanced activity against both Gram positive and Gram negative pathogens [57]. It should also be noted that there have also been instances in which the nisin structural gene has been altered to facilitate the production of other natural variants of nisin (nisin Z, F and Q; [58]) or in a manner that has resulted in peptides which exhibit enhanced diffusion through complex matrices [59]. Other class I bacteriocin producing LAB, i.e. the producers of lacticin 3147 and lacticin 481, have also been the focus of bioengineering-based strategies. In these cases, the outcomes have been of greater importance from a fundamental science, rather than applied, perspective [60-66], with only one example of a partial enhancement having been described to date [64]. Aside from the lantibiotics, as derivatives of the unmodified class II bacteriocins can be generated both synthetically [67,68] or through heterologous expression [69-77] with relative ease, there are relatively few examples of instances in which LAB producers of the class II bacteriocins have themselves been engineered. However, the potential exists to reconstitute production of some of the more interesting derivatives in the original host strain should the need or desire arise.

It is important to note that while all bioengineering based strategies are valid if the aim is to create bacteriocins for fundamental analyses or applications by the pharmaceutical industry, the application of bioengineered bacteriocin peptides as food preservatives is a bigger obstacle in some jurisdictions. Indeed, many of the strategies employed to produce the engineered bacteriocins described above involve approaches

that result in the producer needing to be described as a GMO. However, alternatives exist. Indeed, self cloning of non-pathogenic microorganisms is not considered to lead to a GMO as long as containment of the organism is guaranteed (directive 90/219/EC). Accordingly, the temporary introduction of plasmids, the deletion of specific DNA sequences, or introduction of DNA from another micro-organism belonging to the same species fall within the definition of self-cloning. Thus, subtle alterations to bacteriocin structural genes (such as the changing of single codons) made using food grade strategies [78] fall outside the remit of the Contained Use legislation and therefore are not regulated as GMOs.

Conclusions

There are various methods available to improve the bacteriocin-mediated protection provided by food grade LAB. While genetic manipulation by recombinant and bioengineering based approaches offer great promise, only strains which have been modified through non-recombinant approaches can be directly added to food. In addition to the further improvement of existing strains, advances with respect to high throughput screening strategies are likely to result in the identification of novel antimicrobials with considerable potential for food applications. Few naturally occurring multi-bacteriocin producing LAB have been identified [79-83]. However, ongoing developments in traditional microbiological, mass spectrometric, molecular and bioinformatic screening techniques [84,85] has led to the isolation [86-94] and characterisation of several novel bacteriocins [95-98] reported this year alone which may find applications in food. Regardless of the strain and bacteriocin in question, it is fair to say that the application of bacteriocin producing LAB, alone or in combination with additional antimicrobial hurdles [2], continues to be a relatively

underutilised strategy that, through various enhancements such as those described here, could be more widely applied by the food industry.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Des Field for providing original figures. This work was funded by the Food Institutional Research Measure of the Department of Agriculture, Fisheries and Food (grant no. 04R and DC) and the Alimentary Pharmabiotic Centre (which is funded by the Science Foundation of Ireland [SFI] through the Irish Government's National Development Plan). We and the work were supported by SFI (grant no. 07/CE/B1368).

References

1. Rea MC, Ross RP, Cotter PD, Hill C: **Classification of bacteriocins from Gram-positive bacteria** In *Prokaryotic Antimicrobial Peptides*. Edited by Drider D, Rebuffat S. New York: Springer; 2011:29-53.
 2. Mills S, Stanton C, Hill C, Ross RP: **New developments and applications of bacteriocins and peptides in foods**. *Annu Rev Food Sci Technol* 2011, **2**:299-329.
- This is a comprehensive review on the use and potential of bacteriocins and peptides in food systems.
3. Morgan S, Ross RP, Hill C: **Bacteriolytic activity caused by the presence of a novel lactococcal plasmid encoding lactococcins A, B, and M**. *Appl Environ Microbiol* 1995, **61**:2995-3001.
 4. Kanmani P, Kumar RS, Yuvaraj N, Paari KA, Pattukumar V, Arul V: **Application of response surface methodology in the optimisation of a growth medium for enhanced natural preservative bacteriocin production by a probiotic bacterium**. *Nat Prod Res* 2012, **26**:1539-1543.
 5. Papagianni M: **Effects of dissolved oxygen and pH levels on weissellin A production by *Weissella paramesenteroides* DX in fermentation**. *Bioprocess Biosyst Eng* 2012, **35**:1035-1041.
 6. Ben Belgacem Z, Rehaïem A, Fajardo Bernardez P, Manai M, Pastrana Castro L: **Interactive effects of pH and temperature on the bacteriocin stability by response surface analysis**. *Mikrobiologiia* 2012, **81**:214-219.
 7. Kanmani P, Kumar RS, Yuvaraj N, Paari KA, Pattukumar V, Arul V: **Design and optimization of fermentation medium for enhanced bacteriocin**

- 242 **production by probiotic bacterium *Enterococcus faecium* MC13. Prep**
243 *Biochem Biotechnol* 2011, **41**:40-52.
- 244 8. Furuta Y, Maruoka N, Nakamura A, Omori T, Sonomoto K: **Utilization of**
245 **fermented barley extract obtained from a by-product of barley shochu for**
246 **nisin production. *J Biosci Bioeng* 2008, **106**:393-397.**
- 247 9. Gonzalez-Toledo SY, Dominguez-Dominguez J, Garcia-Almendarez BE, Prado-
248 Barragan LA, Regalado-Gonzalez C: **Optimization of nisin production by**
249 ***Lactococcus lactis* UQ2 using supplemented whey as alternative culture**
250 **medium. *J Food Sci* 2010, **75**:M347-353.**
- 251 10. Ruiz-Barba JL, Caballero-Guerrero B, Maldonado-Barragan A, Jimenez-Diaz R:
252 **Coculture with specific bacteria enhances survival of *Lactobacillus***
253 ***plantarum* NC8, an autoinducer-regulated bacteriocin producer, in olive**
254 **fermentations. *Food Microbiol* 2010, **27**:413-417.**
- 255 11. Chang JY, Lee HJ, Chang HC: **Identification of the agent from *Lactobacillus***
256 ***plantarum* KFRI464 that enhances bacteriocin production by *Leuconostoc***
257 ***citreum* GJ7. *J Appl Microbiol* 2007, **103**:2504-2515.**
- 258 12. Trotter M, McAuliffe OE, Fitzgerald GF, Hill C, Ross RP, Coffey A: **Variable**
259 **bacteriocin production in the commercial starter *Lactococcus lactis***
260 **DPC4275 is linked to the formation of the cointegrate plasmid pMRC02.**
261 *Appl Environ Microbiol* 2004, **70**:34-42.
- 262 13. Fallico V, McAuliffe O, Fitzgerald GF, Hill C, Ross RP: **The presence of**
263 **pMRC01 promotes greater cell permeability and autolysis in lactococcal**
264 **starter cultures. *Int J Food Microbiol* 2009, **133**:217-224.**

- 265 14. O'Sullivan L, Ryan MP, Ross RP, Hill C: **Generation of food-grade lactococcal**
266 **starters which produce the lantibiotics lacticin 3147 and lacticin 481.** *Appl*
267 *Environ Microbiol* 2003, **69**:3681-3685.
- 268 15. Mills S, Coffey A, O'Sullivan L, Stokes D, Hill C, Fitzgerald GF, Ross RP: **Use of**
269 **lacticin 481 to facilitate delivery of the bacteriophage resistance plasmid,**
270 **pCBG104 to cheese starters.** *J Appl Microbiol* 2002, **92**:238-246.
- 271 16. Coakley M, Fitzgerald G, Ros RP: **Application and evaluation of the phage**
272 **resistance- and bacteriocin-encoding plasmid pMRC01 for the**
273 **improvement of dairy starter cultures.** *Appl Environ Microbiol* 1997,
274 **63**:1434-1440.
- 275 17. Garcia-Parra M, Campelo A, Garci-Aalmendarez B, Regalado C, Rodriguez A,
276 Martinez B: **Enhancement of nisin production in milk by conjugal transfer**
277 **of the protease-lactose plasmid pLP712 to the wild strain *Lactococcus***
278 ***lactis* UQ2.** *International Journal of Dairy Technology* 2010, **63**:523-529.
- 279 18. Horn N, Swindell S, Dodd H, Gasson M: **Nisin biosynthesis genes are encoded**
280 **by a novel conjugative transposon.** *Mol Gen Genet* 1991, **228**:129-135.
- 281 19. Meijer W, van de Bunt B, Twigt M, de Jonge B, Smit G, Hugenholtz J: **Lysis of**
282 ***Lactococcus lactis* subsp. *cremoris* SK110 and its nisin-immune**
283 **transconjugant in relation to flavor development in cheese.** *Appl Environ*
284 *Microbiol* 1998, **64**:1950-1953.
- 285 20. Harrington A, Hill C: **Construction of a bacteriophage-resistant derivative of**
286 ***Lactococcus lactis* subsp. *lactis* 425A by using the conjugal plasmid**
287 **pNP40.** *Appl Environ Microbiol* 1991, **57**:3405-3409.

- 288 21. Martinez-Cuesta MC, Requena T, Pelaez C: **Use of a bacteriocin-producing**
289 **transconjugant as starter in acceleration of cheese ripening.** *Int J Food*
290 *Microbiol* 2001, **70**:79-88.
- 291 22. Hickey RM, Twomey DP, Ross RP, Hill C: **Exploitation of plasmid pMRC01 to**
292 **direct transfer of mobilizable plasmids into commercial lactococcal**
293 **starter strains.** *Appl Environ Microbiol* 2001, **67**:2853-2858.
- 294 23. Kojic M, Strahinic I, Topisirovic L: **Proteinase PI and lactococcin A genes are**
295 **located on the largest plasmid in *Lactococcus lactis* subsp. *lactis* bv.**
296 ***diacetylactis* S50.** *Can J Microbiol* 2005, **51**:305-314.
- 297 24. Cheigh CI, Park H, Choi HJ, Pyun YR: **Enhanced nisin production by**
298 **increasing genes involved in nisin Z biosynthesis in *Lactococcus lactis***
299 **subsp. *lactis* A164.** *Biotechnol Lett* 2005, **27**:155-160.
- 300 25. Heinzmann S, Entian KD, Stein T: **Engineering *Bacillus subtilis* ATCC 6633 for**
301 **improved production of the lantibiotic subtilin.** *Appl Microbiol Biotechnol*
302 2006, **69**:532-536.
- 303 26. Cotter PD, Draper LA, Lawton EM, McAuliffe O, Hill C, Ross RP:
304 **Overproduction of wild-type and bioengineered derivatives of the**
305 **lantibiotic lactacin 3147.** *Appl Environ Microbiol* 2006, **72**:4492-4496.
- 306 27. de Ruyter PG, Kuipers OP, de Vos WM: **Controlled gene expression systems**
307 **for *Lactococcus lactis* with the food-grade inducer nisin.** *Appl Environ*
308 *Microbiol* 1996, **62**:3662-3667.
- 309 28. Reviriego C, Fernandez L, Rodriguez JM: **A food-grade system for production**
310 **of pediocin PA-1 in nisin-producing and non-nisin-producing *Lactococcus***
311 ***lactis* strains: application to inhibit *Listeria* growth in a cheese model**
312 **system.** *J Food Prot* 2007, **70**:2512-2517.

- 313 29. Renye JA, Jr., Somkuti GA: **Nisin-induced expression of pediocin in dairy**
314 **lactic acid bacteria.** *J Appl Microbiol* 2010, **108**:2142-2151.
- 315 30. Fernandez A, Horn N, Gasson MJ, Dodd HM, Rodriguez JM: **High-level**
316 **coproduction of the bacteriocins nisin A and lactococcin A by *Lactococcus***
317 ***lactis*.** *J Dairy Res* 2004, **71**:216-221.
- 318 31. Arques JL, Rodriguez JM, Gasson MJ, Horn N: **Immunity gene pedB enhances**
319 **production of pediocin PA-1 in naturally resistant *Lactococcus lactis***
320 **strains.** *J Dairy Sci* 2008, **91**:2591-2594.
- 321 32. Moon GS, Pyun YR, Park MS, Ji GE, Kim WJ: **Secretion of recombinant**
322 **pediocin PA-1 by *Bifidobacterium longum*, using the signal sequence for**
323 **bifidobacterial alpha-amylase.** *Appl Environ Microbiol* 2005, **71**:5630-5632.
- 324 33. Eom JE, Moon SK, Moon GS: **Heterologous production of pediocin PA-1 in**
325 ***Lactobacillus reuteri*.** *J Microbiol Biotechnol* 2010, **20**:1215-1218.
- 326 34. Gutierrez J, Larsen R, Cintas LM, Kok J, Hernandez PE: **High-level heterologous**
327 **production and functional expression of the sec-dependent enterocin P**
328 **from *Enterococcus faecium* P13 in *Lactococcus lactis*.** *Appl Microbiol*
329 *Biotechnol* 2006, **72**:41-51.
- 330 35. Borrero J, Jimenez JJ, Gutiez L, Herranz C, Cintas LM, Hernandez PE: **Protein**
331 **expression vector and secretion signal peptide optimization to drive the**
332 **production, secretion, and functional expression of the bacteriocin**
333 **enterocin A in lactic acid bacteria.** *J Biotechnol* 2011, **156**:76-86.
- 334 36. Sanchez J, Borrero J, Gomez-Sala B, Basanta A, Herranz C, Cintas LM,
335 Hernandez PE: **Cloning and heterologous production of Hiracin JM79, a**
336 **Sec-dependent bacteriocin produced by *Enterococcus hirae* DCH5, in**

337 **lactic acid bacteria and *Pichia pastoris*. *Appl Environ Microbiol* 2008,**
338 **74:2471-2479.**

339 37. Du L, Somkuti GA, Renye JA, Jr.: **Molecular analysis of the bacteriocin-**
340 **encoding plasmid pDGL1 from *Enterococcus durans* and genetic**
341 **characterization of the durancin GL locus. *Microbiology* 2012, 158:1523-**
342 **1532.**

343 38. Gutierrez J, Criado R, Martin M, Herranz C, Cintas LM, Hernandez PE:
344 **Production of enterocin P, an antilisterial pediocin-like bacteriocin from**
345 ***Enterococcus faecium* P13, in *Pichia pastoris*. *Antimicrob Agents Chemother***
346 **2005, 49:3004-3008.**

347 39. Basanta A, Gomez-Sala B, Sanchez J, Diep DB, Herranz C, Hernandez PE, Cintas
348 LM: **Use of the yeast *Pichia pastoris* as an expression host for secretion of**
349 **enterocin L50, a leaderless two-peptide (L50A and L50B) bacteriocin**
350 **from *Enterococcus faecium* L50. *Appl Environ Microbiol* 2010, 76:3314-**
351 **3324.**

352 40. Basanta A, Herranz C, Gutierrez J, Criado R, Hernandez PE, Cintas LM:
353 **Development of bacteriocinogenic strains of *Saccharomyces cerevisiae***
354 **heterologously expressing and secreting the leaderless enterocin L50**
355 **peptides L50A and L50B from *Enterococcus faecium* L50. *Appl Environ***
356 ***Microbiol* 2009, 75:2382-2392.**

357 41. Borrero J, Kunze G, Jimenez JJ, Boer E, Gutiez L, Herranz C, Cintas LM,
358 * Hernandez PE: **Cloning, Production, and Functional Expression of the**
359 **Bacteriocin Enterocin A, Produced by *Enterococcus faecium* T136, by the**
360 **Yeasts *Pichia pastoris*, *Kluyveromyces lactis*, *Hansenula polymorpha*, and**
361 ***Arxula adeninivorans*. *Appl Environ Microbiol* 2012, 78:5956-5961.**

362 This study generated yeast-based systems capable of producing significantly larger
 363 amounts of enterocin A with higher antimicrobial activity than both the parent strain
 364 and previously generated overproducing recombinant LAB hosts.

365 42. Kuipers OP, Rollema HS, Yap WM, Boot HJ, Siezen RJ, de Vos WM:
 366 **Engineering dehydrated amino acid residues in the antimicrobial peptide**
 367 **nisin.** *J Biol Chem* 1992, **267**:24340-24346.

368 43. Dodd HM, Horn N, Giffard CJ, Gasson MJ: **A gene replacement strategy for**
 369 **engineering nisin.** *Microbiology* 1996, **142 (Pt 1)**:47-55.

370 44. Dodd HM, Horn N, Gasson MJ: **A cassette vector for protein engineering the**
 371 **lantibiotic nisin.** *Gene* 1995, **162**:163-164.

372 45. Breukink E, van Kraaij C, Demel RA, Siezen RJ, Kuipers OP, de Kruijff B: **The**
 373 **C-terminal region of nisin is responsible for the initial interaction of nisin**
 374 **with the target membrane.** *Biochemistry* 1997, **36**:6968-6976.

375 46. Breukink E, Wiedemann I, van Kraaij C, Kuipers OP, Sahl H, de Kruijff B: **Use of**
 376 **the cell wall precursor lipid II by a pore-forming peptide antibiotic.**
 377 *Science* 1999, **286**:2361-2364.

378 47. Van Kraaij C, Breukink E, Rollema HS, Siezen RJ, Demel RA, De Kruijff B,
 379 Kuipers OP: **Influence of charge differences in the C-terminal part of nisin**
 380 **on antimicrobial activity and signaling capacity.** *Eur J Biochem* 1997,
 381 **247**:114-120.

382 48. Wiedemann I, Breukink E, van Kraaij C, Kuipers OP, Bierbaum G, de Kruijff B,
 383 Sahl HG: **Specific binding of nisin to the peptidoglycan precursor lipid II**
 384 **combines pore formation and inhibition of cell wall biosynthesis for**
 385 **potent antibiotic activity.** *J Biol Chem* 2001, **276**:1772-1779.

386 49. Kuipers OP, Bierbaum G, Ottenwalder B, Dodd HM, Horn N, Metzger J, Kupke
387 T, Gnau V, Bongers R, van den Bogaard P, et al.: **Protein engineering of**
388 **lantibiotics**. *Antonie Van Leeuwenhoek* 1996, **69**:161-169.

389 50. Cotter PD, Hill C, Ross RP: **Bacterial lantibiotics: strategies to improve**
390 **therapeutic potential**. *Curr Protein Pept Sci* 2005, **6**:61-75.

391 51. Lubelski J, Rink R, Khusainov R, Moll GN, Kuipers OP: **Biosynthesis,**
392 **immunity, regulation, mode of action and engineering of the model**
393 **lantibiotic nisin**. *Cell Mol Life Sci* 2008, **65**:455-476.

394 52. Rink R, Wierenga J, Kuipers A, Kluskens LD, Driessen AJ, Kuipers OP, Moll
395 GN: **Dissection and modulation of the four distinct activities of nisin by**
396 **mutagenesis of rings A and B and by C-terminal truncation**. *Appl Environ*
397 *Microbiol* 2007, **73**:5809-5816.

398 53. Yuan J, Zhang ZZ, Chen XZ, Yang W, Huan LD: **Site-directed mutagenesis of**
399 **the hinge region of nisinZ and properties of nisinZ mutants**. *Appl*
400 *Microbiol Biotechnol* 2004, **64**:806-815.

401 54. Field D, O'Connor PM, Cotter PD, Hill C, Ross RP: **The generation of nisin**
402 **variants with enhanced activity against specific Gram-positive pathogens**.
403 *Mol Microbiol* 2008, **69**:218-230.

404 55. Field D, Quigley L, O'Connor PM, Rea MC, Daly KM, Cotter PD, Hill C, Ross
405 ** RP: **Studies with bioengineered Nisin peptides highlight the broad-**
406 **spectrum potency of Nisin V**. *Microbial biotechnology* 2010, **3**:479-496.

407 This study demonstrates that the enhanced potency of nisin derivatives is maintained
408 within a complex food matrix.

409 56. Carroll J, Field D, O'Connor PM, Cotter PD, Coffey A, Hill C, Ross RP,
410 O'Mahony J: **Gene encoded antimicrobial peptides, a template for the**
411 **design of novel anti-mycobacterial drugs.** *Bioeng Bugs* 2010, **1**:408-412.

412 57. Field D, Begley M, O'Connor PM, Daly KM, Hugenholtz F, Cotter PD, Hill C,
413 ** Ross RP: **Bioengineered nisin A derivatives with enhanced activity against**
414 **both Gram positive and Gram negative pathogens.** *PLoS One* 2012,
415 **7**:e46884.

416 The first study to generate lantibiotic derivatives with enhanced activity against both
417 Gram positive and Gram negative pathogens.

418 58. Piper C, Hill C, Cotter PD, Ross RP: **Bioengineering of a Nisin A-producing**
419 ***Lactococcus lactis* to create isogenic strains producing the natural**
420 **variants Nisin F, Q and Z.** *Microb Biotechnol* 2011, **4**:375-382.

421 59. Rouse S, Field D, Daly KM, O'Connor PM, Cotter PD, Hill C, Ross RP:
422 * **Bioengineered nisin derivatives with enhanced activity in complex**
423 **matrices.** *Microb Biotechnol* 2012, **5**:501-508.

424 This study demonstrated the enhanced ability of a nisin derivative to control *Listeria*
425 *monocytogenes* in a carrageenan-containing food.

426 60. Hindre T, Didelot S, Le Pennec JP, Haras D, Dufour A, Vallee-Rehel K:
427 **Bacteriocin detection from whole bacteria by matrix-assisted laser**
428 **desorption ionization-time of flight mass spectrometry.** *Appl Environ*
429 *Microbiol* 2003, **69**:1051-1058.

430 61. Cotter PD, Deegan LH, Lawton EM, Draper LA, O'Connor PM, Hill C, Ross RP:
431 **Complete alanine scanning of the two-component lantibiotic lacticin 3147:**
432 **generating a blueprint for rational drug design.** *Mol Microbiol* 2006,
433 **62**:735-747.

- 434 62. Cotter PD, O'Connor PM, Draper LA, Lawton EM, Deegan LH, Hill C, Ross RP:
435 **Posttranslational conversion of L-serines to D-alanines is vital for optimal**
436 **production and activity of the lantibiotic lacticin 3147.** *Proc Natl Acad Sci*
437 *U S A* 2005, **102**:18584-18589.
- 438 63. Field D, Collins B, Cotter PD, Hill C, Ross RP: **A system for the random**
439 **mutagenesis of the two-peptide lantibiotic lacticin 3147: analysis of**
440 **mutants producing reduced antibacterial activities.** *J Mol Microbiol*
441 *Biotechnol* 2007, **13**:226-234.
- 442 64. Deegan LH, Suda S, Lawton EM, Draper LA, Hugenholtz F, Peschel A, Hill C,
443 * Cotter PD, Ross RP: **Manipulation of charged residues within the two-**
444 **peptide lantibiotic lacticin 3147.** *Microb Biotechnol* 2010, **3**:222-234.
- 445 The first report of the generation of a derivative of a lacticin 3147 peptide which
446 exhibits enhanced specific activity.
- 447 65. Suda S, Westerbeek A, O'Connor PM, Ross RP, Hill C, Cotter PD: **Effect of**
448 **bioengineering lacticin 3147 lanthionine bridges on specific activity and**
449 **resistance to heat and proteases.** *Chem Biol* 2010, **17**:1151-1160.
- 450 66. Suda S, Hill C, Cotter PD, Ross RP: **Investigating the importance of charged**
451 **residues in lantibiotics.** *Bioeng Bugs* 2010, **1**:345-351.
- 452 67. Fimland G, Blingsmo OR, Sletten K, Jung G, Nes IF, Nissen-Meyer J: **New**
453 **biologically active hybrid bacteriocins constructed by combining regions**
454 **from various pediocin-like bacteriocins: the C-terminal region is**
455 **important for determining specificity.** *Appl Environ Microbiol* 1996,
456 **62**:3313-3318.

- 457 68. O'Shea EF, O'Connor PM, Cotter PD, Ross RP, Hill C: **Synthesis of trypsin-**
458 **resistant variants of the *Listeria*-active bacteriocin salivaricin P.** *Appl*
459 *Environ Microbiol* 2010, **76**:5356-5362.
- 460 69. Fimland G, Pirneskoski J, Kaewsrichan J, Jutila A, Kristiansen PE, Kinnunen PK,
461 Nissen-Meyer J: **Mutational analysis and membrane-interactions of the**
462 **beta-sheet-like N-terminal domain of the pediocin-like antimicrobial**
463 **peptide sakacin P.** *Biochim Biophys Acta* 2006, **1764**:1132-1140.
- 464 70. Fimland G, Eijsink VG, Nissen-Meyer J: **Mutational analysis of the role of**
465 **tryptophan residues in an antimicrobial peptide.** *Biochemistry* 2002,
466 **41**:9508-9515.
- 467 71. Johnsen L, Fimland G, Eijsink V, Nissen-Meyer J: **Engineering increased**
468 **stability in the antimicrobial peptide pediocin PA-1.** *Appl Environ*
469 *Microbiol* 2000, **66**:4798-4802.
- 470 72. Johnsen L, Fimland G, Nissen-Meyer J: **The C-terminal domain of pediocin-**
471 **like antimicrobial peptides (class IIa bacteriocins) is involved in specific**
472 **recognition of the C-terminal part of cognate immunity proteins and in**
473 **determining the antimicrobial spectrum.** *J Biol Chem* 2005, **280**:9243-
474 9250.
- 475 73. Kazazic M, Nissen-Meyer J, Fimland G: **Mutational analysis of the role of**
476 **charged residues in target-cell binding, potency and specificity of the**
477 **pediocin-like bacteriocin sakacin P.** *Microbiology* 2002, **148**:2019-2027.
- 478 74. Haugen HS, Fimland G, Nissen-Meyer J: **Mutational analysis of residues in the**
479 **helical region of the class IIa bacteriocin pediocin PA-1.** *Appl Environ*
480 *Microbiol* 2011, **77**:1966-1972.

- 481 75. Haugen HS, Kristiansen PE, Fimland G, Nissen-Meyer J: **Mutational analysis of**
482 **the class IIa bacteriocin curvacin A and its orientation in target cell**
483 **membranes.** *Appl Environ Microbiol* 2008, **74**:6766-6773.
- 484 76. Tominaga T, Hatakeyama Y: **Determination of essential and variable residues**
485 **in pediocin PA-1 by NNK scanning.** *Appl Environ Microbiol* 2006, **72**:1141-
486 1147.
- 487 77. Tominaga T, Hatakeyama Y: **Development of innovative pediocin PA-1 by**
488 **DNA shuffling among class IIa bacteriocins.** *Appl Environ Microbiol* 2007,
489 **73**:5292-5299.
- 490 78. Cotter PD, Hill C, Ross RP: **A food-grade approach for functional analysis and**
491 **modification of native plasmids in *Lactococcus lactis*.** *Appl Environ*
492 *Microbiol* 2003, **69**:702-706.
- 493 79. Diep DB, Straume D, Kjos M, Torres C, Nes IF: **An overview of the mosaic**
494 **bacteriocin pln loci from *Lactobacillus plantarum*.** *Peptides* 2009, **30**:1562-
495 1574.
- 496 80. Cintas LM, Casaus P, Herranz C, Havarstein LS, Holo H, Hernandez PE, Nes IF:
497 **Biochemical and genetic evidence that *Enterococcus faecium* L50**
498 **produces enterocins L50A and L50B, the sec-dependent enterocin P, and**
499 **a novel bacteriocin secreted without an N-terminal extension termed**
500 **enterocin Q.** *J Bacteriol* 2000, **182**:6806-6814.
- 501 81. Bravo D, Rodriguez E, Medina M: **Nisin and lacticin 481 coproduction by**
502 ***Lactococcus lactis* strains isolated from raw ewes' milk.** *J Dairy Sci* 2009,
503 **92**:4805-4811.
- 504 82. O'Shea EF, O'Connor PM, Raftis EJ, O'Toole PW, Stanton C, Cotter PD, Ross
505 RP, Hill C: **Production of multiple bacteriocins from a single locus by**

506 gastrointestinal strains of *Lactobacillus salivarius*. *J Bacteriol* 2011,
507 **193**:6973-6982.

508 83. Wescombe PA, Burton JP, Cadieux PA, Klesse NA, Hyink O, Heng NC, Chilcott
509 CN, Reid G, Tagg JR: **Megaplasמידs encode differing combinations of**
510 **lantibiotics in *Streptococcus salivarius***. *Antonie Van Leeuwenhoek* 2006,
511 **90**:269-280.

512 84. Zendo T, Nakayama J, Fujita K, Sonomoto K: **Bacteriocin detection by liquid**
513 **chromatography/mass spectrometry for rapid identification**. *J Appl*
514 *Microbiol* 2008, **104**:499-507.

515 85. Marsh AJ, Hill C, Ross RP, Cotter PD: **Strategies to identify modified**
516 **ribosomally synthesized antimicrobials**. In *Antimicrobial drug discovery:*
517 *emerging strategies*. Edited by Tegos A, Mylonakis E: CABI; 2012:166-186.

518 86. Cosentino S, Fadda ME, Deplano M, Melis R, Pomata R, Pisano MB:
519 **Antilisterial activity of nisin-like bacteriocin-producing *Lactococcus lactis***
520 **subsp. lactis isolated from traditional Sardinian dairy products**. *J Biomed*
521 *Biotechnol* 2012, **2012**:376428.

522 87. Chahad OB, El Bour M, Calo-Mata P, Boudabous A, Barros-Velazquez J:
523 **Discovery of novel biopreservation agents with inhibitory effects on**
524 **growth of food-borne pathogens and their application to seafood**
525 **products**. *Res Microbiol* 2012, **163**:44-54.

526 88. Kumari A, Akkoc N, Akcelik M: **Purification and partial characterization of**
527 **bacteriocin produced by *Lactococcus lactis* ssp. *lactis* LL171**. *World J*
528 *Microbiol Biotechnol* 2012, **28**:1647-1655.

- 529 89. Perin LM, Moraes PM, Silva A, Jr., Nero LA: **Lantibiotics biosynthesis genes**
530 **and bacteriocinogenic activity of *Lactobacillus* spp. isolated from raw**
531 **milk and cheese.** *Folia Microbiol (Praha)* 2012, **57**:183-190.
- 532 90. Digaitiene A, Hansen AS, Juodeikiene G, Eidukonyte D, Josephsen J: **Lactic acid**
533 **bacteria isolated from rye sourdoughs produce bacteriocin-like inhibitory**
534 **substances active against *Bacillus subtilis* and fungi.** *J Appl Microbiol* 2012,
535 **112**:732-742.
- 536 91. Kaktcham Pierre M, Zambou Ngoufack F, Abbasi A, Anwar F, Syed Abid A,
537 Sieladie Djomne V, Tchouanguép Mbiapo F: **Characterisation of a**
538 **bacteriocin produced by *Lactobacillus plantarum* Lp6SH isolated from**
539 **"Sha'a", a maize-based traditionally fermented beverage from**
540 **Cameroon.** *International Journal of Biology* 2012, **4**:149-158.
- 541 92. Ravi Sankar N, Deepthi Priyanka v, Srinivas Reddy P, Rajanikanth P, Kiran
542 Kumar V, Indira M: **Purification and characterization of bacteriocin**
543 **produced by *Lactobacillus plantarum* isolated from cow milk.** *International*
544 *Journal of Microbiological Research* 2012, **3**:133-137.
- 545 93. Sifour M, Tayeb I, Ouled Haddar H, Namous H, Aissaoui S: **Producton and**
546 **characterization of bacteriocin of *Lactobacillus plantarum* F12 with**
547 **inhibitory activity against *Listeria monocytogenes*.** *The Online Journal of*
548 *Science and Technology* 2012, **2**:55-61.
- 549 94. K. N, Saenbagam D, Senthilkumar B, Udhayashree N, Gurusamy R:
550 **Characterization of bacteriocin producing lactic acid bacteria and its**
551 **application as a food preservative.** *African Journal of Microbiology*
552 *Research* 2012, **6**:1138-1146.

- 553 95. Tosukhowong A, Zendo T, Visessanguan W, Roytrakul S, Pumpuang L,
554 Jaresitthikunchai J, Sonomoto K: **Garvieacin Q, a novel class II bacteriocin**
555 **from *Lactococcus garvieae* BCC 43578. *Appl Environ Microbiol* 2012,**
556 **78:1619-1623.**
- 557 96. Sawa N, Wilaipun P, Kinoshita S, Zendo T, Leelawatcharamas V, Nakayama J,
558 Sonomoto K: **Isolation and characterization of enterocin W, a novel two-**
559 **peptide lantibiotic produced by *Enterococcus faecalis* NKR-4-1. *Appl***
560 ***Environ Microbiol* 2012, 78:900-903.**
- 561 97. Masuda Y, Zendo T, Sawa N, Perez RH, Nakayama J, Sonomoto K:
562 **Characterization and identification of weissellicin Y and weissellicin M,**
563 **novel bacteriocins produced by *Weissella hellenica* QU 13. *J Appl***
564 ***Microbiol* 2012, 112:99-108.**
- 565 98. Birri DJ, Brede DA, Nes IF: **Salivaricin D, a novel intrinsically trypsin-**
566 **resistant lantibiotic from *Streptococcus salivarius* 5M6c isolated from a**
567 **healthy infant. *Appl Environ Microbiol* 2012, 78:402-410.**

Figure 1. Enhanced activity of bioengineered *L. lactis* producing nisin derivatives mutated within the ‘hinge’ region, K22T and K22S, against *Streptococcus agalactiae* ATCC13813 as compared with the respective nisin A producing controls (as adapted from Field et al., (2008)).