Short communication: Homologous expression of recombinant and native thurincin H in an engineered natural producer

G. Wang, D. C. Manns, G. K. P. Guron, J. J. Churey, and R. W. Worobo¹

Department of Food Science, New York State Agricultural Experiment Station, Cornell University, 630 W North Street, Geneva 14456

ABSTRACT

The *Bacillus* bacteriocin thurincin H exhibits a wide inhibitory spectrum of activity against various foodborne pathogens, such as *Listeria monocytogenes*, and dairy spoilage bacteria, especially different *Bacillus* species commonly existing in dairy products. Previously, we constructed 3 plasmids to express native thurincin H homologously in an engineered natural producer, *Bacillus thuringiensis* SF361thnH[−]. This host is deficient in thurincin H production because of an in-frame deletion of structural genes *thnA1*, *thnA2*, and *thnA3* from the chromosome of the natural producer *B. thuringiensis* SF361. The previously constructed expression vectors were constructed by cloning the native thurincin H promoter, 3 (or 1) copies of structural genes, and the native (or Cry protein) terminator into an *Escherichia coli*–*B. thuringiensis* shuttle vector pHT315. In this study, 3 corresponding expression vectors (pGW134, pGW135, and pGW136) were constructed to express recombinant thurincin H-His6 in the same host, in which a 6-histidine tag was fused to the C terminus of each structural gene. The resulting low level of bacteriocin production indicated that the His tag might negatively interfere with subsequent posttranslational modification or exportation processes after the thurincin H-His6 prepeptide was translated. Additionally, in order to overexpress native thurincin H, 2 additional plasmids (pGW137 and pGW138) were constructed, consisting of the sporulation-dependent Cry protein dual promoter BtI and BtII, the *thnA1* structural gene, and the thurincin H native or Cry protein terminator. However, production was low on Luria broth plates and absent on sporulation plates. It is possible that the resulting thurincin H prepeptide was not correctly modified or exported to the extracellular environment, due to the undesired biochemical and physiological changes during the sporulation phase.

Key words: *Bacillus* bacteriocin, histidine tag, heterologous expression, Cry protein promoter, Cry protein terminator

Short Communication

Bacteriocins are ribosomally synthesized peptides or proteins produced by bacteria that exhibit antimicrobial activity against other bacteria mostly within the same species (narrow spectrum) or sometimes across different genera (broad spectrum; Cotter et al., 2005). Bacteriocins produced by gram-positive bacteria, most commonly lactic acid bacteria, have been widely applied in the food industry because of their effectiveness against various foodborne pathogens and spoilage microorganisms (Settanni and Corsetti, 2008). However, *Bacillus* spp. have gained emerging research interest because they are considered rich producers of different types of antibiotics, antifungal substances, and antimicrobial proteins or peptides, usually with a much broader inhibition spectrum than lactic acid bacteria bacteriocins (Stein, 2005; Abriouel et al., 2011).

Thurincin H is a 31-AA bacteriocin produced by food-grade *Bacillus thuringiensis* SF361, which was originally isolated from domestic honey in the United States (Lee et al., 2009). It exhibits inhibitory activity against different foodborne pathogens and spoilage bacteria, such as *Listeria monocytogenes*, as well as *Micrococcus* and *Bacillus* spp., which are common pathogens and spoilage microorganisms in various dairy products (Lee et al., 2009; Konosonoka et al., 2012). Using bacteriocins as natural preservatives in dairy products is an emerging trend because of consumer concerns about chemical and irradiation preservation methods. The development of heterologous or homologous bacteriocin expression systems has several advantages over using the natural host system. Heterologous or homologous expression systems might be able to provide increased production levels, use easy and fast purification tags, and have controllable gene expression and broadened inhibitory spectrum (O'Connor et al., 2007).

Thurincin H contains 4 pairs of unique sulfur to α -carbon thioether bonds catalyzed by the putative radical S-adenosylmethionine superfamily enzyme (ThnB) present to maintain the hairpin structure with

Received April 1, 2014.

Accepted April 15, 2014.

¹Corresponding author: rww8@cornell.edu

a helical backbone (Sit et al., 2011). As elucidated by bioinformatics studies, 3 identical thurincin H prepeptides were first translated from 3 tandem thurincin H precursor genes (*thnA1*, *thnA2*, and *thnA3*), existing in an 8.14-kb *thnP–thnI* gene cluster. The other genes in the same gene cluster are putatively responsible for thurincin H prepeptide processing, regulation, modification, exportation, and producer self-immunity (Lee et al., 2009).

For heterologous expression of those extensively modified bacteriocins, such as lantibiotics, closely related species are usually used as the expression hosts, wherein the natural systems are still available to modify certain unique bonds (Rodríguez et al., 2003). In this study, we constructed 5 plasmids based on the *Escherichia coli*–*B. thuringiensis* shuttle vector pHT315, aiming to express sufficient C terminal His-tagged thurincin H (thurincin H-His6), or overexpress native thurincin H using a previously constructed *B. thuringiensis* SF361thnH[−] as an expression host.

Both *E. coli* and *Bacillus* strains were cultivated in trypticase soy broth (TSB) or on trypticase soy agar (TSA; Difco/Becton Dickinson Co., Sparks, MD) at 37°C. General molecular cloning methods used in this study were performed as previously described by Sambrook and Russell (2001). Strains, primers, and plasmids are detailed in Table 1. Generally, to construct a recombinant plasmid based on pHT315, an insertion fragment was amplified using a 1-step or 2-step PCR using PrimeSTAR Max DNA Polymerase (R045, Takara, Dalian, China) in a total volume of 50 µL. The PCR conditions included a template denaturation step at 98°C (1 min) followed by 30 cycles of denaturing at 98°C (10 s), annealing at 55°C (10 s), and polymerization at 72°C (60 s), with a final hold at 72°C (7 min). Transformants of *E. coli* were selected on TSA (ampicillin, 100 µg/mL), and *Bacillus* transformants were selected on TSA (erythromycin, 25 µg/mL). Recombinant plasmids were purified from *E. coli* using QIAprep Spin Miniprep Kit (Qiagen, Hilden, Germany) and passed through *E. coli* K12 ER2925 to produce nonmethylated plasmids as preparation for *B. thuringiensis* transformation (Macaluso and Mettus, 1991). Recombinant plasmids were transformed to *B. thuringiensis* strains by electroporation as previously described (Lereclus et al., 1989).

A fragment containing the native promoter, *thnA1his6*, *thnA2his6*, *thnA3his6*, and the native terminator (noted as P_{nat}*thnA1his6thnA2his6thnA3his6*T_{nat}) was synthesized (Figure 1A) by GenScript (Piscataway, NJ) and cloned into pHT315, resulting in the plasmid pGW134. Gene synthesis was used to produce the fragment, because 18-bp additions of DNA sequences encoding the 6-histidine tag after each of the 3 tandem

structural genes by PCR is very difficult. Using primers TH21 and TH22, the fragment containing P_{nat}*thnA1his6* was amplified from *B. thuringiensis* SF361 genomic DNA, purified, and used as an amplification template. Using this template, a second fragment P_{nat}*thnA1his6*T_{nat} was amplified with primers TH21 and TH23 (Figure 1B), and cloned into pHT315, resulting in the plasmid pGW135. Using primers TH24 and TH25, a fragment containing P_{nat}*thnA1* was amplified from previously constructed pGW132, purified, and used as an amplification template. Using this template, a fragment P_{nat}*thnA1his6*T_{cry} was amplified with primers TH24 and TH27 (Figure 1C) and cloned into pHT315, resulting in the plasmid pGW136. The bacteriocin production of *B. thuringiensis* SF361thnH[−] carrying the 3 plasmids was determined by a modified deferred antagonism assay (Birri et al., 2010) against the indicator strain *Bacillus cereus* F4552. However, inhibition zones formed by those constructs were significantly lower than with the wild-type producer (Figure 2). In earlier work, several similar expression vectors were constructed to express native thurincin H. Two of those native thurincin H expression vectors, corresponding to pGW134 and pGW136, displayed inhibitory activity similar to that of the wild-type producer (Wang et al., 2014). The only difference between the corresponding vector and pGW134 and pGW136 was the absence of the 18-bp DNA sequence coding the 6 histidines. These results indicated that the extracellular thurincin H-His6 was either produced at a lower level or had a lower antimicrobial activity due to the His tag. Attempts were also made to produce and purify thurincin-His6 in TSB. A 1% inoculation of *B. thuringiensis* SF361thnH[−]pGW135 overnight culture in 150 mL of TSB was incubated at 37°C for 16 h with shaking at 225 rpm. The supernatant was collected by centrifugation (10,000 × *g*, 20 min, 4°C). Crude protein extract was precipitated by 40 to 65% saturated ammonium sulfate solution, resuspended in 15 mL of lysis-equilibration-wash (LEW) buffer (50 mM NaH₂PO₄, 0.3 M NaCl, pH 7.5), and purified by using the High Specific PrepEase Histidine-tagged Protein Purification Kit (USB, Cleveland, OH) according to the standard protocol. Based on an imidazole gradient concentration test, an optimal elution buffer (50 mM NaH₂PO₄, 0.3 M NaCl, 0.25 M imidazole, pH 8.0) was chosen to elute thurincin H-His6 from the nickel column. The 0.25 M imidazole buffer did not inhibit *B. cereus* F4552 (data not shown). Low bacteriocin activity against *B. cereus* F4552 was detected in both the supernatant and the 0.25 M imidazole eluate, using a spot-on-lawn method (Fujita et al., 2007). Directly loading supernatant to the nickel column led to very low binding efficacy.

To determine if the His tag on thurincin H-His6 interfered with the natural production of mature thurincin

Table 1. Strains, plasmids, and primers used in this study

Item	Property	Reference
Strain		
<i>Escherichia coli</i> DH5 α	Cloning host, produced methylated plasmid	New England Biolabs (Ipswich, MA)
<i>E. coli</i> K12 ER2925	<i>Dam dcm</i> , produced demethylated plasmid	New England Biolabs
<i>Bacillus thuringiensis</i> SF361	Thurincin H producer strain, carrying the putative thurincin H gene cluster	Lee et al., 2009
<i>B. thuringiensis</i> SF361thnH ⁻	Thurincin H producer strain with in-frame deletion of <i>thnA1</i> , <i>thnA2</i> , and <i>thnA3</i> genes	Wang et al., 2014
<i>Bacillus cereus</i> F4552	Indicator strain, sensitive to thurincin H	Lee et al., 2009
Plasmid		
pHT315	<i>E. coli</i> - <i>B. thuringiensis</i> shuttle vector, 6.5 kb, Ery ^R Amp ^R	Arantes and Lereclus, 1991
pGW132	<i>thnA1</i> gene with native promoter (P _{nat}) and native terminator (T _{nat}), cloned in pHT315	Wang et al., 2014
pGW134	<i>thnA1his6</i> , <i>thnA2his6</i> and <i>thnA3his6</i> genes with P _{nat} and T _{nat} , cloned in pHT315	This study
pGW135	<i>thnA1his6</i> gene with P _{nat} and T _{nat} , cloned in pHT315	This study
pGW136	<i>thnA1his6</i> gene with P _{nat} and Cry protein terminator (T _{cry}), cloned in pHT315	This study
pGW137	<i>thnA1</i> gene with BtI&II dual promoter (P _{BtI&II}) and T _{cry} , cloned in pHT315	This study
pGW138	<i>thnA1</i> gene with P _{BtI&II} and T _{cry} , cloned in pHT315	This study
Primer	Sequence	
TH21	gatgatctgcagaatgcacaccatacacaccatacacagtta	This study
TH22	ttagtggtggtggtggtggtggtgcttcagtagtagccctgt	This study
TH23	cagaagcttaatttataggacttattcttaacataactgtttgtcattagaataagtcctaatttaattagtggtggtggtggtg	This study
TH24	gatggactgcaggtaaatatattgtcacaaaatattaaag aaacacacacaa aatgtttg	This study
TH25	gcttgtagtagtagccctgt	This study
TH27	gatggagaattctagtaaaacggacatcactccgtttcaatggaggtgatgtccgttttatttaatttcaccataaacactctcacatttttaatttt agtgggtggtggtggtggtggtgcttgtagtagtagccctgt	This study
DTC3	ctgcaaggcgattaaagtgggtaac	Wang et al., 2014
DTC4	cggataacaatttcacacaggaaacagcta	Wang et al., 2014
TH28	gatggactgcagtagttgcattttgtgcatttttcataagatgagtcatatgttttaattgtagtaatgaaaaacagtattatcataatgaatt ggtatcttaataaaaagagatggaggttaacttatggaaacaccagtagtacaaccaagg	This study
TH29	gatggtgaattcgtttttagtttatgtattacaaaaatcccatactcgtttc	This study
TH30	gatggtgaattctagtaaaacggacatcactccgtttcaatggaggtgatgtccgttttatttaatttcaccataaacactctcacatttttaattttta	This study

Figure 1. Insertion fragment construction scheme in different expression vectors. P_{nat} = native promoter; rbs = ribosomal binding site; P_{BtI&II} = BtI and BtII dual promoter; T_{nat} = native terminator; T_{cry} = Cry terminator.

H, pGW135 was subsequently transformed to wild-type *B. thuringiensis* SF361. The bacteriocin activity of *B. thuringiensis* SF361pGW135 showed lower antimicrobial activity compared with the wild-type producer (Figure 3), even though pGW135 contains 15 additional copies of thurincin H-His6 precursor structural genes (Arantes and Lereclus, 1991). This indicated that the thurincin H-His6 prepeptide had been translated from plasmid pGW135 and negatively interfered with one or more steps of regulation, modification, or exportation along the native thurincin H production pathway. Although many bacteriocins fused with a His tag have been reported to retain high activity, such as bacteriocin 51 (Yamashita et al., 2011) and enterocin P (Herranz and Driessen, 2005), a His tag could impair the expression or activity of some enzymes (Freydank et al., 2008; Sabaty et al., 2013) or result in a change in the crystal structure (Loschi et al., 2004). In the case of thurincin

H, the low production of thurincin H-His6 and native thurincin H production could be explained by several factors. The addition of 6 hydrophilic His residues to the small (31-AA) hydrophobic thurincin H (Sit et al., 2011) might drastically alter the hydrophobicity of thurincin H-His6 prepeptide from the native thurincin H. The formation of the unique sulfur to α -carbon thioether bonds catalyzed by S-adenosylmethionine enzyme might also be affected, because the hydrophilic His tag might interfere with the hydrophobic patch surrounding the whole hairpin structure. In some bacteriocin expression systems such as piscicolin 126 (Gibbs et al., 2004), His tags are fused to the N terminus and cleaved off before being used for subsequent research. However, in this system, the His tag could not be added to the N-terminus of the thurincin H precursor peptide because the 9-AA leader peptide will eventually be cleaved off the thurincin H prepeptides. It is also not

Figure 2. Inhibitory activities of natural producer *Bacillus thuringiensis* SF361 and *B. thuringiensis* SF361thnH⁻ carrying different thurincin H-His6 expression vectors against *B. cereus* F4552. (A) Natural producer *B. thuringiensis* SF361 (+++++); (B) *B. thuringiensis* SF361thnH⁻ pGW134 (+); (C) *B. thuringiensis* SF361thnH⁻ pGW135; and (D) *B. thuringiensis* SF361thnH⁻ pGW136 (+). Images in this figure represent multiple independent experiments with reproducible results. Antibacterial activity of the producer strain was graded from – (no activity) to +++++ (highest activity).

Figure 3. Inhibitory activities of natural producer *Bacillus thuringiensis* SF361 and *B. thuringiensis* SF361 carrying the thurincin H-His6 expression vector pGW135 against *B. cereus* F4552. (A) *B. thuringiensis* SF361 (+++++); and (B) *B. thuringiensis* SF361pGW135 (+). Images in this figure represent multiple independent experiments with reproducible results. Antibacterial activity of the producer strain was graded from – (no activity) to +++++ (highest activity).

an ideal strategy to add the 18-bp His coding sequence between the C terminus of the leader peptide and the N terminus of the 31-AA mature thurincin H peptide, because the fragment of insertion has a high probability of affecting the cleavage of the leader peptide and the S-adenosylmethionine posttranslational modification.

Two additional plasmids carrying *thnA1* under the Cry protein overlapping dual BtI and BtII promoters were constructed in an attempt to overexpress native thurincin H. A template fragment was amplified from pGW132 using DTC3 and DTC4 as primers. Using this fragment as a template, a first fragment containing the BtI and BtII dual promoters ($P_{BtI\&II}$), *thnA1*, and the native terminator (T_{nat}) was amplified with primers TH28 and TH29 (Figure 1D), and cloned into pHT315, resulting in plasmid pGW137. A second fragment containing the BtI and BtII promoters ($P_{BtI\&II}$), *thnA1*, and the Cry terminator (T_{cry}) was amplified with primers TH28 and TH30 (Figure 1E) and cloned into pHT315, resulting in pGW138. The 2 constructed plasmids were separately transformed to *B. thuringiensis* SF361 $thnH^-$. The antimicrobial activity of the resulting transformants was detected by the modified deferred antagonism assay as described above, except that to test the effect of different media on the expression, the transformants were spotted on Luria broth (LB) LB and SPO 8 (Pol et al., 2001) plates and incubated for 72 h at room temperature (22°C) before being overlaid with *B. cereus* F4552. On LB plates, *B. thuringiensis* SF361 $thnH^-$ carrying pGW137 and pGW138 exhibited smaller inhibition zones compared with that of the wild-type producer (Figure 4, colonies A, B, C). On SPO 8 plates, *B. thuringiensis* SF361 $thnH^-$ carrying pGW137 and pGW138 did not exhibit any inhibition zones (Figure 4, colonies E, F). However, the wild-type producer still exhibited an inhibition

zone on this sporulation medium (Figure 4, colony D). The rationale of *B. thuringiensis* species producing high yields of Cry proteins was adapted in the last 2 plasmid constructs (pGW137 and pGW138). The high expression of Cry proteins in *B. thuringiensis* is partly the result of the strong overlapping promoters (BtI and BtII) and stable mRNA from the strong terminator (Agaisse and Lereclus, 1995). Several proteins such as chitinase (Hu et al., 2009) have been heterologously expressed in *B. thuringiensis* strains using the overlapping promoter and terminator based on pHT315. The same strategy was attempted in our research to produce thurincin H at higher levels. However, a decrease in thurincin H production was observed under these experimental conditions. This might be because the production of the active thurincin H not only depends on the yield of thurincin H prepeptide, but also relies heavily on the cooperation of the other components related to regulation, modification, and exportation. In addition, the overlapping promoter is turned on once sporulation starts, but changes in cellular morphology, biochemistry, and physiology are also induced in the cell (Errington, 1993). These changes might negatively affect other steps in the thurincin H modification and exportation pathways. To our knowledge, this is the first time the Cry protein promoter and terminator were adapted to express a bacteriocin in *B. thuringiensis* or other bacterial hosts. Our research indicates that in homologous expression of extensively modified bacteriocins, such as thurincin H, the processes of post-translational modification, regulation, and exportation need to be taken into consideration in addition to the expression level of the structural genes.

Technically, 2-step PCR was used to construct the plasmids in order to reduce nonspecific binding because some of the primers used in this study were longer than usual (up to 156 bp). The *B. thuringiensis* SF361 genomic DNA contains 3 tandem structural genes that additionally enhance nonspecific binding and nonspecific amplification of fragments. We first amplified a short core fragment to serve as a template DNA in PCR and ensure sequence accuracy in the final products.

Besides providing a PCR method to accurately fuse long promoter and terminator to short piece of structural genes, our research underscores the risk of using a His-tag system as a means for purification in heterologously expressed bacteriocins, especially extensively modified bacteriocins, such as thurincin H, because the His tag may negatively interfere with subsequent modification or exportation steps. A Cry protein promoter and terminator should also be carefully considered because changes occurring during sporulation might exert a negative effect on the function of other components in the pathway. In future research, a xylose inducible

Figure 4. Inhibitory activities of natural producer *Bacillus thuringiensis* SF361 and *B. thuringiensis* SF361thnH⁻ carrying different thurincin H-His6 expression vectors with Cry protein promoters and the native (or Cry) terminator against *Bacillus cereus* F4552. (A) *B. thuringiensis* SF361 on Luria broth (LB) agar; (B) *B. thuringiensis* SF361thnH⁻ pGW137 on LB agar; (C) *B. thuringiensis* SF361thnH⁻ pGW138 on LB agar; (D) *B. thuringiensis* SF361 on SPO 8 (Pol et al., 2001) agar; (E) *B. thuringiensis* SF361thnH⁻ pGW137 on SPO8 agar; and (F) *B. thuringiensis* SF361thnH⁻ pGW138 on SPO8 agar. Images in this figure represent multiple independent experiments with reproducible results.

expression system will be constructed to control the expression time and level of the structural genes to improve and better control expression of thurincin H.

ACKNOWLEDGMENT

This research was supported by USDA-CSREES (Washington, DC; project no. 2008-51110-0688).

REFERENCES

- Abriouel, H., C. M. Franz, N. Ben Omar, and A. Galvez. 2011. Diversity and applications of *Bacillus* bacteriocins. *FEMS Microbiol. Rev.* 35:201–232.
- Agaisse, H., and D. Lereclus. 1995. How does *Bacillus thuringiensis* produce so much insecticidal crystal protein? *J. Bacteriol.* 177:6027–6032.
- Arantes, O., and D. Lereclus. 1991. Construction of cloning vectors for *Bacillus thuringiensis*. *Gene* 108:115–119.
- Birri, D. J., D. A. Brede, T. Forberg, H. Holo, and I. F. Nes. 2010. Molecular and genetic characterization of a novel bacteriocin locus in *Enterococcus avium* isolates from infants. *Appl. Environ. Microbiol.* 76:483–492.
- Cotter, P. D., C. Hill, and R. P. Ross. 2005. Bacteriocins: Developing innate immunity for food. *Nat. Rev. Microbiol.* 3:777–788.
- Errington, J. 1993. *Bacillus subtilis* sporulation: Regulation of gene expression and control of morphogenesis. *Microbiol. Rev.* 57:1–33.
- Freydank, A. C., W. Brandt, and B. Dräger. 2008. Protein structure modeling indicates hexahistidine-tag interference with enzyme activity. *Proteins* 72:173–183.
- Fujita, K., S. Ichimasa, T. Zendo, S. Koga, F. Yoneyama, J. Nakayama, and K. Sonomoto. 2007. Structural analysis and characterization of lactacin Q, a novel bacteriocin belonging to a new family of unmodified bacteriocins of gram-positive bacteria. *Appl. Environ. Microbiol.* 73:2871–2877.
- Gibbs, G. M., B. E. Davidson, and A. J. Hillier. 2004. Novel expression system for large-scale production and purification of recombinant class IIa bacteriocins and its application to piscicolin 126. *Appl. Environ. Microbiol.* 70:3292–3297.
- Herranz, C., and A. J. Driessen. 2005. Sec-mediated secretion of bacteriocin enterocin P by *Lactococcus lactis*. *Appl. Environ. Microbiol.* 71:1959–1963.
- Hu, S. B., P. Liu, X. Z. Ding, L. Yan, Y. J. Sun, Y. M. Zhang, W. P. Li, and L. Q. Xia. 2009. Efficient constitutive expression of chitinase in the mother cell of *Bacillus thuringiensis* and its potential to enhance the toxicity of CryIAc protoxin. *Appl. Microbiol. Biotechnol.* 82:1157–1167.
- Konosonoka, I. H., A. Jemeljanovs, B. Osmane, D. Ikauniece, and G. Gulbe. 2012. Incidence of *Listeria* spp. in dairy sows feed and raw milk in Latvia. *ISRN Vet. Sci.* 2012:435187.
- Lee, H., J. J. Churey, and R. W. Worobo. 2009. Biosynthesis and transcriptional analysis of thurincin H, a tandem repeated bacteriocin genetic locus, produced by *Bacillus thuringiensis* SF361. *FEMS Microbiol. Lett.* 299:205–213.
- Lereclus, D., O. Arantes, J. Chauvaux, and M. Lecadet. 1989. Transformation and expression of a cloned delta-endotoxin gene in *Bacillus thuringiensis*. *FEMS Microbiol. Lett.* 51:211–217.

- Loschi, L., S. J. Brokx, T. L. Hills, G. Zhang, M. G. Bertero, A. L. Lovering, J. H. Weiner, and N. C. Strynadka. 2004. Structural and biochemical identification of a novel bacterial oxidoreductase. *J. Biol. Chem.* 279:50391–50400.
- Macaluso, A., and A. M. Mettus. 1991. Efficient transformation of *Bacillus thuringiensis* requires nonmethylated plasmid DNA. *J. Bacteriol.* 173:1353–1356.
- O'Connor, E., E. Barrett, G. Fitzgerald, C. Hill, C. Stanton, and R. P. Ross. 2007. Production of vitamins, exopolysaccharides and bacteriocins by probiotic bacteria. Chapter 8. In *Probiotic Dairy Products*. A. Tamime, ed. Blackwell Science Ltd., Oxford, UK. 10.1002/9780470995785.ch8.
- Pol, I. E., W. G. van Arendonk, H. C. Mastwijk, J. Krommer, E. J. Smid, and R. Moezelaar. 2001. Sensitivities of germinating spores and carvacrol-adapted vegetative cells and spores of *Bacillus cereus* to nisin and pulsed-electric-field treatment. *Appl. Environ. Microbiol.* 67:1693–1699.
- Rodríguez, J. M., M. I. Martínez, N. Horn, and H. M. Dodd. 2003. Heterologous production of bacteriocins by lactic acid bacteria. *Int. J. Food Microbiol.* 80:101–116.
- Sabaty, M., S. Grosse, G. Adryanczyk, S. Boiry, F. Biaso, P. Arnoux, and D. Pignol. 2013. Detrimental effect of the 6 His C-terminal tag on YedY enzymatic activity and influence of the TAT signal sequence on YedY synthesis. *BMC Biochem.* 14:28.
- Sambrook, J., and D. W. Russell. 2001. *Molecular Cloning: A Laboratory Manual*. 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Settanni, L., and A. Corsetti. 2008. Application of bacteriocins in vegetable food preservation. *Int. J. Food Microbiol.* 121:123–138.
- Sit, C. S., M. J. van Belkum, R. T. McKay, R. W. Worobo, and J. C. Vederas. 2011. The 3D solution structure of thurincin H, a bacteriocin with four sulfur to alpha-carbon crosslinks. *Angew. Chem. Int. Ed. Engl.* 50:8718–8721.
- Stein, T. 2005. *Bacillus subtilis* antibiotics: Structures, syntheses and specific functions. *Mol. Microbiol.* 56:845–857.
- Wang, G., D. C. Manns, J. J. Churey, and R. W. Worobo. 2014. Homologous expression system development and the systematic site-directed mutagenesis analysis of thurincin H, a bacteriocin produced by *Bacillus thuringiensis* SF361. *Appl. Environ. Microbiol.* <http://dx.doi.org/10.1128/AEM.00433-14>.
- Yamashita, H., H. Tomita, T. Inoue, and Y. Ike. 2011. Genetic organization and mode of action of a novel bacteriocin, bacteriocin 51: Determinant of VanA-type vancomycin-resistant *Enterococcus faecium*. *Antimicrob. Agents Chemother.* 55:4352–4360.