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Bacteriocins of Aquatic Microorganisms and Their Potential Applications in the Seafood Industry

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1. Introduction

Bacteriocins are potent antimicrobial polypeptides and proteins produced by most lineages of Bacteria and, perhaps, by all members of Archaea (O'Connor & Shand, 2002; Riley & Wertz, 2002a, 2002b; Tagg et al., 1976). Although initially the focus of numerous biochemical, evolutionary, and ecological studies, more recently, their potential to serve in human and animal health applications has taken center stage (Gillor et al., 2008). The use of bacteriocins in probiotic applications, as preservatives, and, (most excitingly) as alternatives to classical antibiotics is being broadly explored (Abee et al., 1995; Einarsson & Lauzon, 1995; Gillor & Ghazaryan, 2007; Gillor et al., 2007).

Most bacterial species produce one or more bacteriocins (Cascales et al., 2007). One of the most prolific bacteriocin-producing species is *Pseudomonas aeruginosa*, of which 90% or more of the strains tested produce their own version of bacteriocins, known as pyocins (Govan & Harris, 1985). In contrast, only 15-50% of *Escherichia coli* produce their brand of bacteriocins, known as colicins (Riley & Gordon, 1992). The colicins are exceedingly well characterized proteins, and have been the subject of numerous detailed biochemical, molecular, evolutionary, and ecological analyses (Cascales et al., 2007; Riley et al., 2003; Riley & Gordon, 1999; Riley & Wertz, 2002a, 2002b). Some species of bacteria produce toxins that may exhibit numerous bacteriocin-like features, but have not yet been fully characterized; these toxins are referred to as bacteriocin-like inhibitory substances, or BLIS (Messi et al., 2003; Moro et al., 1997).

In this chapter, we will explore the bacteriocins of aquatic bacteria, particularly those of potential interest in the seafood industry. A short primer of bacteriocin biology is followed by a detailed review of the diversity of bacteriocins described from marine microorganisms. These toxins have received far less attention than bacteriocins produced by terrestrial or human-commensal bacteria, yet they have equivalent potential as antibiotics and even greater promise for use in the creation of probiotic strains for the seafood industry.

2. Bacteriocin basics

Bacteriocins are proteins or short polypeptides, which are generally only toxic to bacteria that are closely related to the producing strain. A typical bacteriocin contains a toxin (bacteriocin)

gene, an immunity gene (which confers resistance to the aforementioned toxin), and a lysis gene, which encodes a protein that aids in toxin release from the producing cell (Chavan & Riley, 2007). Bacteriocins work by binding to and killing only cells with surface receptors that are recognized by that specific bacteriocin (Cascales et al., 2007; Chavan & Riley, 2007). In a microbial community, cells can either be bacteriocinogenic (produce bacteriocin), sensitive, or resistant to each bacteriocin. When all three cell-types are present and are competing for limiting resources, the strain interactions mimic the children's game "rock-paper-scissors" (Kerr et al., 2002). The premise of this game is that paper covers rock, scissors cut paper, and rock breaks scissors, creating a cycle of wins and losses with no one matter dominating as long as all three states are present. The same interaction is observed in microbial communities that employ bacteriocins (Table 1). Only a small percentage of bacteriocinogenic cells will be induced to produce and release bacteriocin. Some sensitive cells are immediately killed by the bacteriocin, while others harbor mutations that confer resistance. These resistant cells rapidly displace the producer cells, due to the cost of bacteriocin production. However, the resistant cells grow more slowly than their sensitive counterparts, because resistance mutations often have a negative effect on fitness (Kerr et al., 2002).

Strain	More Fit Than	Less Fit Than
Bacteriocin-producer	Sensitive	Resistant
Sensitive	Resistant	Bacteriocin-producer
Resistant	Bacteriocin-producer	Sensitive

Table 1. Competition for resources results in a "rock-paper-scissors"-like interaction of microorganisms (adapted from Kerr et al., 2002).

In contrast to traditional antibiotics, which are used in human health applications precisely because of their ability to kill a diversity of bacterial pathogens, bacteriocins generally target only members of their producing species and its closest relatives (although numerous exceptions abound)(Riley et al., 2003; Tagg et al., 1976). Riley et al. (2003) mapped the killing spectrum of bacteriocins from seven enteric species (*Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Citrobacter freundii*, *Hafnia alvei*, and *Serratia plymuthica*) onto the molecular phylogeny of the same species (Fig. 1). This study showed that bacteriocin producers tend to kill strains belonging to their same species. However, there are some exceptions, such as bacteriocins of *E. coli* that inhibit distantly related *H. alvei* (Fig. 1).

2.1 Bacteriocin naming

Bacteriocins were originally named based on the producer species such as colicins produced by *Escherichia coli*, pyocins of *Pseudomonas aeruginosa* (formerly named *pyocyania*), cloacins of *Enterobacter cloacae*, cerecins of *Bacillus cereus*, and pesticins of *Yersinia pestis* (Reeves, 1965). Fredericq (1957) created the first classification, and thus nomenclature, of bacteriocins focusing on the colicins of *E. coli* (Fredericq, 1957). Fredericq grouped colicins into 17 different types (colicins A, B, C, D, E, F, G, H, I, J, K, V, S1, S2, S3, S4, and S5) based on their receptor specificity. These colicins were then further subtyped (colicin E1, E2, and E3, etc.) based on their immunity patterns. In this scheme, all subtypes were recognized by the same receptor, but they possessed different immunity phenotypes (Fredericq, 1957). Later, the addition of the producer strain's name provided further differentiation of bacteriocins produced by strains of the same species (Daw & Falkiner, 1996). This scheme is still used today.

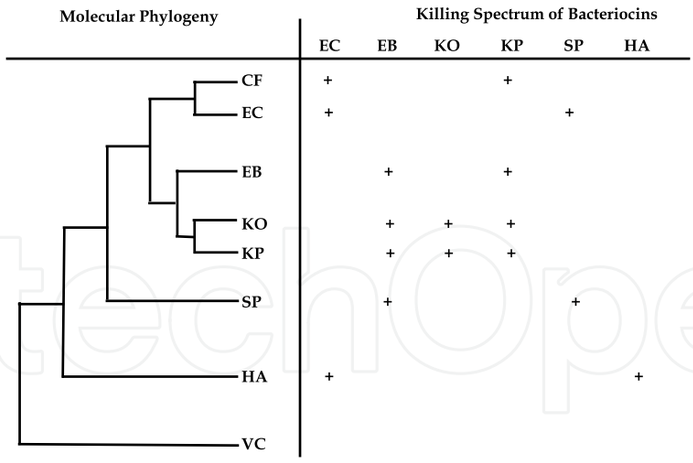

Fig. 1. The breadth of bacteriocin killing in enteric bacteria (adapted from Riley et al., 2003). The bacteriocin killing phenotype of six enteric bacterial species were mapped onto their molecular phylogeny constructed with concatenated sequences of five housekeeping genes and 16s RNA. *Vibrio cholerae* (VC) was used as an outgroup to root the phylogenetic tree. EC, *Escherichia coli*; KP, *Klebsiella pneumoniae*; KO, *Klebsiella oxytoca*; EB, *Enterobacter cloacae*; CF, *Citrobacter freundii*; HA, *Hafnia alvei*; SP, *Serratia plymuthica*.

2.2 Bacteriocin classes

In general, bacteriocins are produced by Bacteria and studied based on the gram designation of their producing species (Gram-negative versus Gram-positive). Additionally, a relatively small number of bacteriocins from Archaeal species have also been characterized. A comprehensive review of bacteriocins from Bacteria and Archaea can be found elsewhere (O'Connor & Shand, 2002; Reeves, 1965; Riley & Gordon, 1999; Riley & Wertz, 2002a, 2002b; Tagg et al., 1976). Below are short descriptions of the bacteriocin classes of Bacteria and Archaea and examples of bacteriocins belonging to each class (Table 2).

	Bacteriocins	Bacteriocin Types /Class	Size (kDa)	Examples	References
Gram-negative Bacteria	Colicins	Pore Formers Nucleases	20-80	Colicins A, B Colicins E2, E3	
	Colicin-like	NA	20-80	S-pyocins Klebicins	Cascales et al., 2007 Michel-Briand & Baysse, 2002
	Phage-tail like	NA	> 80	R and F pyocins	Gillor et al., 2004 Reeves, 1965
	Microcins	Post-translationally modified Unmodified	< 10	Microcin C7 Microcin B17 Colicin V	
Gram-positive Bacteria	Class I	Type A-positively charged and linear Type B-uncharged or negatively charged globular Type C-synergistic	< 5	Nisin Mersacidin Lacticin 3147	Heng et al., 2007 Drider et al., 2006 Field et al., 2007 Maqueda et al., 2004
	Class II	Class IIa-antilisterial Class IIb-synergistic	< 10	Pediocin PA1 Carnobacteriocin B2	
	Class III	Type IIIa-Bacteriolytic enzymes Type IIIb-Nonlytic peptides	> 10	Lysostaphin Helveticin	
	Class IV	Cyclic peptides	< 10	Enterocin AS-48	
Archaea	Halocins	Microhalocins Protein halocins	< 10 > 10	Halocin A4, C8, G1 Halocin H1, H4	Shand et al., 2007 O'Connor & Shand, 2002
	Sulfolobicin	NA	~20	Sulfolobicin	Ellen et al., 2011 Sun et al., 2005

Table 2. Bacteriocins of Bacteria and Archaea

2.2.1 Bacteriocins of Gram-negative bacteria

Bacteriocins of Gram-negative bacteria are categorized into four main classes: colicins, colicin-like bacteriocins, phage-tail like bacteriocins, and microcins (Table 2) (Chavan & Riley, 2007). The colicins, produced by *E. coli*, are the most studied bacteriocins (Cascales et al., 2007). Indeed, they have been used as a model system to study bacteriocin structure, function, and evolution (Cascales et al., 2007; Riley & Gordon, 1999; Riley & Wertz, 2002a, 2002b). In general, colicins are protease sensitive, thermosensitive proteins that vary in size from 25 to 90 kDa (Pugsley & Oudega, 1987). There are two major colicin types based on their mode of killing; pore former and nuclease colicins. Pore former colicins (colicins A, B, E1, Ia, Ib, K, E1, 5) kill sensitive strains by forming pores in the cell membrane. Nuclease colicins (Colicins E2, E3, E4, E5, E6, E7, E8, E9) kill by acting as DNases, RNases, or tRNAses (Gillor et al., 2004). Proteinaceous bacteriocins produced by other Gram-negative species are classified as colicin-like due to the presence of similar structural and functional characteristics (Table 2). Like colicins, they can be nucleases (pyocins S1, S2) and pore formers (pyocin S5) (Michel-Briand & Baysse, 2002). Klebicins of *Klebsiella* species, S-pyocins of *Pseudomonas aeruginosa*, and alveicins of *Hafnia alvei* are among the most studied colicin-like bacteriocins.

Phage-tail like bacteriocins are larger structures that resemble the tails of bacteriophages. Some even argue that they are defective phage particles (Bradley, 1967). R and F pyocins of *P. aeruginosa* are some of the most thoroughly studied phage-tail like bacteriocins (Michel-Briand & Baysse, 2002; Nakayama et al., 2000). They are encoded in a large gene cluster, which spans a DNA region greater than 40 kb (Nakayama et al., 2000). There are 44 open reading frames associated with the R2/F2 phenotypes, which include regulatory, lysis, and toxin genes. The R2 and F2 pyocins show sequence similarity to the tail fiber genes of P2 and lambda phages, respectively (Nakayama et al., 2000). Finally, Gram-negative bacteria produce much smaller (<10 kDa) peptide bacteriocins called microcins. Microcins can be divided into two classes: post-translationally modified (microcins B17, C7, J25, and D93) and unmodified microcins (microcins E492, V, L, H47, and 24) (Table 2). Microcins are chromosomally encoded (Gillor et al., 2004).

2.2.2 Bacteriocins of Gram-positive bacteria

Bacteriocins of Gram-positive bacteria are generally divided into four classes based on size, morphology, physical, and chemical properties (Lee & Kim, 2011). Class I bacteriocins are lantibiotics, which are small peptides (<5 kDa)(Field et al., 2007). They are post-translationally modified, incorporating non-traditional amino acids such as dehydroalanine, dehydrobutyrine, methyl-lanthione, and lantionine (Cleveland et al., 2001). This class is subdivided into Type A, B, and C with the distinction being that members of Type A are positively charged, linear peptides whereas those in Type B are either neutrally or negatively charged rigid globular peptides. Members of Type C require synergistic activity of two peptides to be active. This class includes the well-studied bacteriocins nisin and lacticin (McAuliffe et al., 2001).

Class II bacteriocins are small (<10 kDa), heat-stable peptides that are not post-translationally modified (Heng et al., 2007). Class II is also subdivided into two subgroups. Class IIa are pediocin-like or *Listeria*-active peptides, which contain a conserved N-terminal sequences (YGNGVxCxxxxCxV). Class IIb bacteriocins require the synergistic activity of two peptides to be fully active (Nissen-Meyer et al., 1992). Class III bacteriocins are generally large (>10 kDa),

heat-labile peptides. They are subdivided into two subtypes. Type IIIa are bacteriolysins, which are bacteriolytic enzymes. Lysostaphin is the most studied bacteriocin in this subtype (Schindler & Schuhardt, 1964). Type IIIb are non-lytic bacteriocins. Helveticin J (37 kDa) produced by *Lactobacillus helveticus* belongs to this type (Joerger & Klaenhammer, 1986). Finally, Class IV bacteriocins have unique structural characteristics. The first and last amino acids of these bacteriocins are covalently bonded, thus they have cyclic structures. Enterocin AS-48 produced by *Enterococcus faecalis* subsp. *liquefaciens* S-48 was the first characterized bacteriocin belonging to this class (Maqueda et al., 2004).

2.2.3 Bacteriocins of Archaea

The Archaea also produce unique types of bacteriocin-like antimicrobial compounds, called archaeocins (Shand & Leyva, 2007). They have been much less studied than the bacteriocins of Bacteria. Thus far, two major types of archaeocins have been identified: halocins of halobacteria and sulfolobicins of *Sulfolobus* species. Halocins can be peptides (< 10 kDa) and/or proteins (>10 kDa) (Shand & Leyva, 2007). According to Torreblanca and Meseguer (1994), halocin production is a universal feature of halobacteria. Halocins are located on megaplasmids (or minichromosomes). Halocins H4 and S8 are located on ~300 kbp and ~200 kbp plasmids, respectively (Cheung et al., 1997; Price & Shand, 2000). Their activity is usually detected at the late exponential to early stationary growth phase (Cheung et al., 1997; Price & Shand, 2000). There is not much known about sulfolobicins. Prangishvili et al. (2000) screened sulfolobicin production from *Sulfolobus islandicus* isolated from volcanic vents throughout Iceland. This study predicted that sulfolobicin activity is membrane-associated and is not released from the cell. Sulfolobicins are also associated with membranous vesicles ranging in size from 90 to 180 nm in diameter (Prangishvili et al., 2000). Like many bacteriocins, they are thermostable and sensitive to protease treatment. Their mode of action is still unknown (Ellen et al., 2011).

2.3 Bacteriocin genetics

Bacteriocins can be encoded on chromosomes, plasmids, and other transposable elements. For example, the colicins of *E. coli* and halocin H4 are plasmid-encoded while the pyocins of *P. aeruginosa* are chromosomal (Chavan & Riley, 2007; Cheung et al., 1997; Michel-Briand & Baysse, 2002). Lacticin 481 has been shown to reside on the transposon Tn*5721* (Dufour et al., 2000) and some bacterial species such as *Serratia marcescens* possess both plasmid and chromosomally encoded bacteriocins (Riley & Wertz, 2002b). Just as we see differences in the function of Gram-negative, Gram-positive, and Archaeal bacteriocins, we can also trace these distinctions to the genetic level.

In general, the full function of bacteriocins produced by Gram-negative bacteria is encoded via three tightly linked genes, the toxin, immunity, and lysis genes (Fig. 2A). However, there are significant differences in the genetics of colicins, colicin-like bacteriocins, phage-tail-like bacteriocins, and microcins (Fig. 2). For example, the colicin gene cluster consists of the three bacteriocin-related genes in close proximity, whereas colicin-like pyocin S3 does not possess a lysis gene (Fig. 2A-B). Two representative phage-tail like bacteriocins, R and F-type pyocins, R2 and F2, are encoded in a large gene cluster that spans more than 40 kb (Nakayama et al., 2000) and includes 44 open reading frames (Nakayama et al., 2000). The open reading frames include regulatory, lysis, R, and F pyocin genes (Fig. 2C).

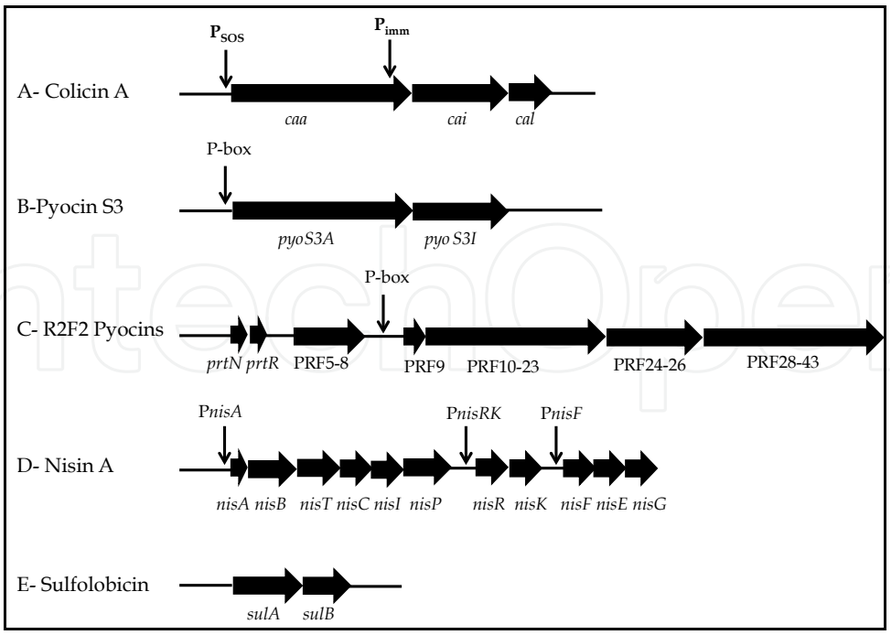

Fig. 2. Genetic organization of bacteriocins
(Horizontal arrows represent genes; not to scale)
A- Genetic organization of colicin gene cluster. P_{SOS} and P_{imm} represent promoter regions of the SOS and immunity genes. Gene *caa* encodes colicin A; *cai* encodes the immunity gene; and *cal* encodes the lysis gene (adapted from Cascales et al., 2007).
B-Genetic organization of colicin-like gene cluster. P-box refers to the binding site for transcriptional regulator (PrtN); *pyoS3A* gene encodes pyocin S3; *pyoS3I* encodes the immunity gene (adapted from Duport et al., 1995).
C- Genetic organization of phage-tail like bacteriocin gene cluster. *prtN* and *prtR* encode transcriptional activator (PrtN) and repressor (PrtR), respectively. PRF 9 and PRF24-26 encodes the lysis genes; PRF10-23 and PRF28-43 encode the R2 and F2 pyocin structural genes, respectively (adapted from Nakayama et al., 2000).
D-Genetic organization of lantibiotic gene cluster. P_{nisA}, P_{nisRK}, and P_{nisF} encode promoter genes for *nis A*, *nisRK,* and *nisFEG*, respectively; *nisA* encodes nisin A precursor; *nisR* and *nisK* encode proteins for nisin biosynthesis; *nisB, nisC*, *nisT*, and *nisP* encode proteins for nisin processing and translocation; *nisI*, *nisF*, *nisE*, and *nisG* encode immunity proteins (adapted from Kuipers et al., 1993; Mierau & Kleerebezem, 2005).
E-Genetic organization of archaeocin gene cluster. Sulfolobicin is composed of SulA and SulB proteins, encoded by *sulA* and *sulB* genes (adapted from Ellen et al., 2011).

The Gram-positive bacteriocins are more complicated genetically, with genes that encode post-translational modification of the toxin. The genetic organization varies between the Gram-positive bacteriocin classes as well – such as the requirement of two peptides for the full activation of Class II bacteriocins. An example is provided by the nisin gene cluster (Fig. 2D), which includes 11 genes (nis*ABTCIPRKFEG*) encoding functions such as synthesis of the nisin precursor (*nis*A), regulation of nisin biosynthesis *(nis*RK), the processing and translocation of nisin (*nis*BCTP), and immunity (*nis*IFEG) (Kuipers et al., 1993; Mierau & Kleerebezem, 2005).

The genetic organization of archaeocins is relatively unknown in comparison to other bacteriocins. A recent study showed that sulfolobicin of *Sulfolobus acidocaldarius* is comprised

of two proteins, SulA and SulB, which are encoded by *sulA* and *sulB* genes, respectively (Fig. 2E). These two proteins are associated with membrane vesicles in the extracellular medium (Ellen et al., 2011). The gene organization of archaeocins is also unique. Sun et al. (2005) showed that the gene *halC8* encoded both halocin C8 and its immunity protein HalI (Sun et al., 2005). It differs from other bacteriocins in that typically separate genes encode bacteriocin toxin and immunity proteins.

2.4 Bacteriocin biosynthesis

Bacteriocins are often produced under stress conditions, such as nutrient limitation and overpopulation (Riley & Gordon, 1999). The biosynthesis of Gram-negative bacteriocins is regulated by the host; often involving the SOS system. The SOS system is comprised of RecA and LexA proteins. LexA is a transcriptional repressor, which binds to the bacteriocin promoter and prevents its transcription. In stress conditions such as DNA damage and UV exposure expression of the *recA* gene is induced. RecA binds to LexA and therefore prevents the repression of bacteriocin expression (Cascales et al., 2007). The expression of colicin-like S-pyocins and phage-tail like RF pyocins also depends on RecA, except these genes possess a P-box in their promoter region instead of an SOS box (Nakayama et al., 2000; Sano et al., 1993). Further, there is no LexA-dependent repression of bacteriocin expression. Activated RecA cleaves the PtrR transcriptional repressor protein, which leads to expression of the transcriptional activator *ptrN* gene. PtrN binds to the P-box and induces the expression of the pyocin genes (Matsui et al., 1993; Michel-Briand & Baysse, 2002). Alternatively, Gram-positive bacteria possess bacteriocin-specific biosynthesis pathways. For example, nisin regulates its own biosynthesis in cell-density dependent conditions (Eijsink et al., 2002). Finally, there is much less known about archaeocins. Regulation of their biosynthesis is still under investigation.

3. Bacteriocins of marine bacteria

Bacteriocins produced by marine bacteria have generated a great deal of excitement due primarily to their potential to serve as probiotics and antibiotics in the seafood industry (Galvez et al., 2008; García et al., 2010; Pilet & Leroi, 2011). A recent antimicrobial screen of 258 bacterial strains isolated from water and sediment in the Yucatan peninsula revealed that 46 strains belonging to the genera *Aeromonas*, *Bacillus, Burkholderia*, *Photobacterium*, *Pseudomonas*, *Serratia*, and *Stenotrophomonas* possessed antimicrobial activity. Approximately fifty percent of this antimicrobial activity was due to bacteriocins or BLIS (De la Rosa-Garcia et al., 2007). Further, Wilson et al. (2000) investigated surface-attached bacteria isolated from Sydney Harbor, Australia. He showed that approximately 10% of surface-attached marine bacteria possess antibacterial activity. Proteinase K treatment showed that this inhibitory activity was associated with proteinaceous substances such as bacteriocins or BLIS (Wilson et al., 2010). Given the fact that bacteriocins and BLIS have been characterized in most culturable bacterial species, it is tempting to speculate about the diversity of new substances the marine environment will reveal.

The first bacteriocin isolated from marine microorganisms was detected in *Vibrio harveyi* (formerly *Beneckea harveyi*). McCall and Sizemore (1979) screened a total of 795 *Vibrio* spp. strains isolated from Galveston Island, Texas for bacteriocin production (McCall & Sizemore, 1979). This study revealed that approximately 5% of the *Vibrio* spp. possessed a

high molecular weight bacteriocin-like killing agent. Further investigation revealed that the source of the bacteriocin-like killing was a plasmid. It was also determined that the killing range was limited to strains of *B. harveyi*. This bacteriocin was named harveyicin (McCall & Sizemore, 1979).

In 1982, Hoyt and Sizemore investigated the chemical nature of harveyicin. They showed that it is a proteinaceous substance, sensitive to protease, trypsin, and papain treatment, and resistant to pepsin and lipase treatment. Furthermore, it is stable at room temperature and -20 °C for several weeks and several months, respectively. However, the protein did lose killing activity after heat treatment at 55 °C for 4 hours (Hoyt & Sizemore, 1982). Hoyt and Sizemore (1982) also investigated the role of harveyicin in bacterial competition in enteric and planktonic environments at various temperatures (4-39°C), pH's (5-9.5), and salt concentrations (1.75% and 3.5%). They also performed a competition assay on nonluminous bacteriocinogenic (SYLum-) and luminous bacteriocin sensitive strains (SYcured) in equal concentrations (10^6 cells/ml). The ratio of harveyicin producer strain (SYLum-) to the harveyicin sensitive strain (SYcured) peaked in an enteric environment (25 °C, high salinity (3%), and alkaline pH (pH 9.5)). The harveyicin producer strain also outcompeted the harveyicin sensitive strain at an acidic pH (pH 5.0) (Hoyt and Sizemore, 1982). Given the fact that the natural habitat of these species is in the guts of fish (an acidic environment), bacteriocin production may serve as a competitive advantage to the bacteriocin producer strain in this environment.

The identification of harveyicin led to numerous bacteriocin-screening studies in marine bacteria, which focused primarily on biochemical characterization of bacteriocins and bacteriocin-like inhibitory substances (Bagenda et al., 2008; Bhugaloo-Vial et al., 1996; Carraturo et al., 2006; Hosseini et al., 2009; Hoyt & Sizemore, 1982; Longeon et al., 2004; McCall & Sizemore, 1979; Messi et al., 2003; Metivier et al., 1998; Moro et al., 1997; Nilsson et al., 2002; Pinto et al., 2009; Pirzada et al., 2004; Prasad et al., 2005; Rajaram et al., 2010; Selvin et al., 2004; Shehane & Sizemore, 2002; Stoffels et al., 1992; Sugita et al., 1997; Suzuki et al., 2005; Tahiri et al., 2004; Valenzuela et al., 2010; Yamazaki et al., 2005; Zai et al., 2009). The majority of these studies focused on the killing breadth of the producing strains due to their potential for use as antimicrobials and probiotics. However, the authors generally did not further characterize the identified bacteriocins/BLIS, although see Table 3 for rough classifications (Table 3).

The relatively few characterized marine bacteriocins are primarily isolated from *Carnobacterium* species, which are ubiquitous, Gram-positive lactic acid bacteria isolated from marine organisms (such as fish and marine sponges), from cold and temperate environments, as well as from terrestrial environments including Canadian winter soil, permafrost ice, composite piles, and horse manure (Leisner et al., 2007). *C. divergens* and *C. maltaromaticum* (formerly known as *C. piscicola*) are the most-studied species from this genus.

Carnobacterium species can produce bacteriocins at low temperatures and high salt concentrations (Buchanan & Bagi, 1997). Further, the bacteria survive in fish products and have low acidifying capacity (Tahiri et al., 2009). Thus, these species have been the focus of intense research due to their potential as probiotics (Leisner et al., 2007; Rihakova et al., 2009). Piscicocin V1a, V1b, divercin V41, piscicocin CS526, divergicin M35, carnocin U149, and carnobacteriocin B2 are some of the bacteriocins isolated from marine *Carnobacterium* species (Table 3) (Bhugaloo-Vial et al., 1996; Duffes et al., 1999; Metivier et al., 1998; Stoffels et al., 1992; Suzuki et al., 2005; Tahiri et al., 2004; Yamazaki et al., 2005). These bacteriocins share similar characteristics with the Class II bacteriocins of Gram-positive bacteria.

Bacteriocin	Producer Strain	Enzyme Sensitivity	Enzyme Resistance	Molecular Weight	Killing Breadth	Source of Isolation	Reference
BLIS	*Aeromonas hydrophila*	NA	NA	NA	*Staphylococcus aureus,* MRSA	Water sample from a water tank containing alligators	Moro et al., 1997
BLIS	*Aeromonas hydrophila*	NA	NA	NA	*Staphylococcus, Listeria, Streptococcus, Lactobacillus*	Water samples	Messi et al., 2003
Carnocin U149	*Carnobacterium* sp.	NA	NA	4.5-5 kDa	*Lactobacillus, Lactococcus, Pediococcus, Carnobacterium*	Fish	Stoffels et al., 1992
Divergicin M35	*Carnobacterium divergens* M35	α-chymotrypsin, Proteinase K, Pronase E	Trypsin	~4.5 kDa	*Listeria, Carnobacterium*	Frozen smoked mussel	Tahiri et al., 2004
Divercin V41	*Carnobacterium divergens* V41	Pronase E	Catalase	4.5 kDa	*Listeria, Carnobacterium, Enterococcus*	Fish viscera	Métivier et al., 1998; Duffes et al., 1999
Carnobacteriocin B2	*Carnobacterium piscicola* A9b	Proteases, Proteinase K, Trypsin	Pepsin, Lipase, Phospholipase C, α-amylase	~4.5 kDa	*Listeria*	Cold smoked salmon	Nilsson et al., 2002
Piscicocin CS526	*Carnobacterium piscicola* CS526	α-chymotrypsin, Papain, Proteinase K, Actinase, Trypsin	Catalase, RNase, Lipase	~4.4 kDa	*Listeria, Enterococcus, Pediococcus, Tetragenococcus, Leuconostoc*	Frozen surimi	Yamazaki et al., 2005; Suzuki et al., 2005
Piscicocin V1a	*Carnobacterium piscicola* V1	NA	NA	4.4 kDa	*Listeria, Enterococcus, Pediococcus, Leuconostoc, Lactobacillus, Carnobacterium*	Fish	Bhugaloo-Vial et al., 1996
Piscicocin V1b (or carnobacteriocin BM1)	*Carnobacterium piscicola* V1	NA	NA	4.5 kDa	*Listeria, Enterococcus, Pediococcus, Lactobacillus, Carnobacterium*	Fish	Bhugaloo-Vial et al., 1996
BLIS	*Enterococcus feacium* CHG 2-1 and Ch 1-2	NA	NA	NA	*Enterococcus*	Venus clams, Horse mackerel	Valenzuela et al., 2010
BLIS	*Enterococcus feacium* CHG 2-2, CHG 2-3, C 2-3, PE 1-2, PE 2-1, PE 3-2, M 2-1, and M 2-2	NA	NA	NA	*Enterococcus, Listeria*	Venus clams, Dogfish fillet, Swordfish fillet, Shark fillet	Valenzuela et al., 2010
BLIS	*Enterococcus feacium* C-K, C-S, M 2-1, and PEF 2-2	NA	NA	NA	*Listeria*	Anchovy, Shark fillet, Swordfish fillet	Valenzuela et al., 2010
Enterocin B-like BLIS	*Enterococcus feacium* ALP7	Trypsin, Proteinase K, Pronase E, Papain	Lipase, α-amylase	<6.5 kDa	*Listeria, Staphylococcus, Bacillus, Enterococcus, Lactobacillus, Lactococcus, Leukonostoc*	Non-fermented shellfish	Pinto et al., 2009
BLIS	*Enterococcus feacium* LHICA46	Proteinase K	Pepsin, Pancreatin	NA	*Bacillus, Carnobacterium, Listeria, Staphylococcus*	Refrigerated turbot	Hosseini et al., 2009
Enterocin A	*Enterococcus feacium* PE 2-2	NA	NA	NA	*Enterococcus, Listeria, Staphylococcus*	Swordfish fillet	Valenzuela et al., 2010

Table 3. Bacteriocin and BLIS activity characterized from marine bacteria (NA: Not available)

Bacteriocin	Producer Strain	Enzyme Sensitivity	Enzyme Resistance	Molecular Weight	Killing Breadth	Source of isolation	Reference
BLIS	Gram-positive marine bacterium ZM-81	Pronase, Trypsin	Pepsin, Lyzozyme, Lipase	>10 kDa	Gram-positive marine bacterium ZM-19	Water sample, Karachi coast, Pakistan	Pirzada & Ali, 2004
BLIS	*Lactobacillus lactis*	Proteinase K, Pepsin	α-amylase, DNase, RNAse, Lipase	94 kDa	*Bacillus, Staphylococcus, Enterococcus, Escherichia coli* , *Pseudomonas, Shigella*	Sediment sample from the Bay of Bengal, Indian Ocean	Rajaram et al., 2010
Pediocin PA1-like BLIS	*Pediococcus pentosaceus* ALP57	Trypsin, Proteinase K, Pronase E, Papain	Lipase, α−amylase	<6.5 kDa	*Listeria, Bacillus, Enterococcus, Lactobacillus, Lactabacillus, Lactococcus, Leukonostoc*	Non-fermented shellfish	Pinto et al., 2009
BLIS	*Pediococcus pentosaceus* Iz3.13	α-chymotrypsin, Proteinase K	Catalse, Lipase, , α-a	4.6 kDa	*Clostridium botulinum*	Fermented izushi	Bagenda et al., 2008
BLIS P-153	*Pseudoalteromonas* spp. X153	NA	NA	87 kDa	*Pseudomonas, Escherichia coli, Staphylococcus, Propionibacterium, Candida, Pityrosporum, Vibrio* spp., *Deleya, Halomanas, Cytofaga* spp., *Bacillus* spp., *Pseudomonas* spp.	A pebble collected at St. Anne du Portzic, France	Longeon et al., 2004
Phocaecin PI80	*Streptococcus phocae*	Trypsin, Protease, Pepsin, Chymotrypsin	Catalase, Peroxidase, Diastase	9.2 kDa	*Listeria, Vibrio*	Indian white shrimp	Kumar & Arul, 2009
BLIS	*Streptomyces* sp. BTL-7	NA	NA	NA	*Micrococcus, Pseudomonas, Escherichia coli, Bacillus, Salmonella, Staphylococcus, Vibrio, Klebsiella, Clostridium*	Marine sponge	Selvin et al., 2004
BLIS	*Vibrio* sp. Strain NM10	Trypsin, Proteinase K	α−chymotrypsin, Protease, Lysozyme, Achromopeptidise, α-amylase, Ribonuclease A,	< 5 kDa	*Bacillus* spp., *Corynefoms, Enterobacteriaceae, Flavobacterium* spp., *Pseudomonas* spp., *Vibrio* spp., *Pasteurella*	Intestine of a spotnape ponyfish	Sugita et al., 1997
BLIS	*Vibrio anguillarum* AVP10	Proteinase, Proteinase K, Trypsin	Lipase	NA	*Vibrio* spp.	Catfish	Zai et al., 2009
BLIS-BC2	*Vibrio cholerae*	Protease, α-amylase	Lipase	1.35 kDa	*Vibrio, E. coli*	Water samples, Wilmington, NC	Shehane & Sizemore, 2002
Harveyicin	*Vibrio harveyi* SY	Trypsin, Papain	Pepsin, Lipase	~24 kDa	*Vibrio*	Galveston Island, Texas	McCall & Sizemore, 1979; Hoyt & Sizemore, 1982
BLIS	*Vibrio harveyi* VIB 571	Proteinase K, Pepsin, Trypsin, Pronase E, Lipase	Lysozyme, α-amylase	~32 kDa	*Vibrio* spp.	Sea bass	Prasad et al., 2005
BLIS-400	*Vibrio mediterranei* 1	Proteinase K	Trypsin, α-chymotrypsin	63-65 kDa	*Aeromonas, Vibrio*	Seafood	Carraturo et al., 2006
BLIS-BC1	*Vibrio vulnificus*	Protease, α-amylase	Lipase	7.5 kDa	*Vibrio*	Water samples, Wilmington, NC	Shehane & Sizemore, 2002
BLIS-IW1	*Vibrio vulnificus*	Protease, α-amylase	Lipase	9.0 kDa	*Vibrio*	Water samples, Wilmington, NC	Shehane & Sizemore, 2002

Table 3. Continued

BLIS from the marine environment are a relatively uncharacterized group of inhibitory substances. While they may not be well characterized, they are abundant. Indeed, numerous BLIS have been identified from marine species belonging to *Vibrio*, *Aeromonas*, *Carnobacterium*, *Lactococcus*, *Streptomyces*, *Pseudoalteromonas*, *Enterococcus*, and *Pediococcus* genera (Table 3) (Bagenda et al., 2008; Carraturo et al., 2006; Longeon et al., 2004; Messi et al., 2003; Moro et al., 1997; Pinto et al., 2009; Pirzada et al., 2004; Prasad et al., 2005; Rajaram et al., 2010; Satish Kumar & Arul, 2009; Selvin et al., 2004; Shehane & Sizemore, 2002; Sugita et al., 1997; Valenzuela et al., 2010; Zai et al., 2009). These species are important in the seafood industry and in human health.

The bacteriocins and BLIS isolated from marine microorganisms are diverse. However, they do share common characteristics with bacteriocins from Bacteria and Archaea (Fig. 3). They can be small peptides (5-10 kDa) like microcins of Gram-negative bacteria, microhalocins of halobacteria, and class I and II bacteriocins of Gram-positive bacteria. They can also be larger in size (10-90 kDa) like colicins and colicin-like bacteriocins of Gram-negative bacteria. The majority of marine BLIS have been tested against a number of proteases including trypsin, proteinase K, and pronase A, which are commonly used to identify bacteriocin activity. Some have unique characteristics. For example, BLIS-IW1, BLIS-BC1, and BC2 from *Vibrio* species possess a carbohydrate moiety while BLIS VIB 571 from *V. harveyi* has a lipid moiety (Shehane & Sizemore, 2002). It is not clear if these moieties are

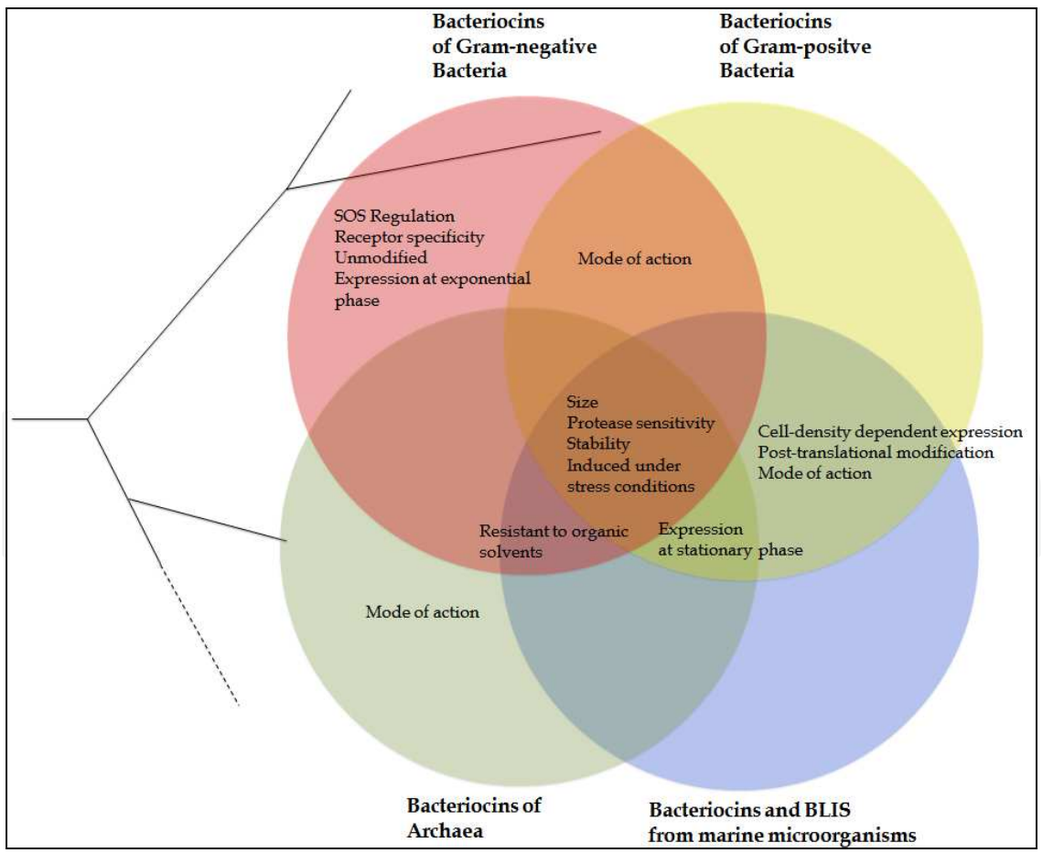

Fig. 3. Shared and unique characteristics of bacteriocins and BLIS from marine Bacteria with bacteriocins of terrestrial Bacteria and Archaea

involved in BLIS activity. The mode of actions of these BLIS is unknown and their killing ranges are variable; either narrow - like bacteriocins of Gram-negative bacteria - or quite broad - like that observed in bacteriocins of Gram-positive bacteria (Bhugaloo-Vial et al., 1996; Moro et al., 1997; Sugita et al., 1997; Suzuki et al., 2005; Yamazaki et al., 2005).

Further, the stability of BLIS activity varies. BLIS IW1 is inactive after cold storage at -70C; like phage-tail-like bacteriocins. Others, such as BLIS-BC1, BLIS-BC2, and BLIS-400, are still active after cold treatment, as is observed in colicin and colicin-like bacteriocins. Some BLIS are also resistant to treatment with organic solvents. For example, BLIS-400 is resistant to organic chemicals including ethanol, methanol, acetone, and chloroform (Carraturo et al., 2006). This feature is similar to some bacteriocins isolated from Gram-positive bacteria and Archaea (Carraturo et al., 2006). Finally, most BLIS from marine bacteria are produced during the stationary phase of growth, similar to bacteriocins from Gram-positive bacteria (Pinto et al., 2009; Tahiri et al., 2004).

4. Applications and implications of marine bacteriocins

The international seafood industry is one of the world's most profitable commodities, worth more than $75 billion per year (Food and Agriculture Organization of the United Nations [FAO], 2006). Fish and seafood are major proteins in some areas of the world. In 2006, of the 143 million tons of total fishery production (including fish, crustacean, and mollusks), 110 million tons was for direct human consumption (Pilet & Leroi, 2011). To meet this demand, we have seen a marked rise in aquaculture (the farming of aquatic plants and animals) in the last decade (FAO, 2006). Recently, there have also been dramatic changes in the seafood industry due to technological advances, consumer habits, and globalization of the food market (Galvez et al., 2008). In particular, there has been an increase in consumer preference for foods that are minimally processed or preserved (especially those that claim health-promoting benefits). Consumers are also demanding that these foods be fresh tasting and ready-to-eat (Galvez et al., 2008). The demands on the industry to provide fresh, minimally preserved products in the ever-growing globalized food market is requiring a longer and more complex food-chain and increasing the risk of microbial contamination and spoilage (García et al., 2010).

4.1 Challenges in the seafood Industry: Spoilage and disease

With the expansion of the seafood industry and the rise in seafood consumption, spoilage and disease in fish are the main challenges the industry faces (Gram & Dalgaard, 2002; Toranzo et al., 2005). Microorganisms are the major cause of spoilage and diseases in the seafood industry. It is estimated that nearly 25% of all the seafood produced is lost to microbial spoilage (Baird-Parker, 2000). Microbes cause changes in the sensory properties of the seafood (smell, taste, color), which make it less appealing and often dangerous to eat (Gram & Dalgaard, 2002). Disease severely affects the production and trade of farmed seafood, creating high economic impacts for many countries (Bondad-Reantaso et al., 2005).

4.2 Common microbial diseases in aquaculture

Fear of disease, as well as climate change, are acting as deterrents to aquaculture farming (Bondad-Reantaso et al., 2005). It has been shown that bacteria exhibit greater pathogenesis at higher temperatures, leading to greater and more virulent disease in aquaculture (Desriac

et al., 2010). The bacterial microflora of fish can become pathogenic under stress conditions such as sudden temperature changes, overcrowding, and poor water quality conditions and thus can cause diseases in fish. Furunculosis, vibriosis, columnaris disease, streptococcosis, pasteurellosis, fish tuberculosis, and enteric septicemia are common microbial diseases observed in economically important aquaculture fish species. These diseases are predominantly caused by *Aeromonas*, *Vibrio*, *Cytophaga*, *Streptococcus*, *Pasteurella*, *Mycobacterium*, and *Edwardsiella* genera (Table 4).

Microorganism	Hosts	Diseases	Antibiotic Treatment	References
Aeromonas	Salmonids, catfish, carp, tilapia, trout, perch, goby	Frunculosis MAS Erythrodermatitis, Ulcer	Florfenicol Sulfadimethoxine and Ormetoprim Oxytetracycline dihydrate	Noga, 2010
Vibrio	Salmonids, cod, ayu, Japanese eels	Vibriosis Cold water vibriosis, Hemorrhagic septicmia Skin ulcers	Oxytetracycline Potentiated sulfonomides Oxolinic acid	Noga, 2010 Toranzo et al., 2005
Cytophaga	Salmonids, catfish, tilapia, stripped bass	Columnaris disease	Oxytetracycline dihydrate Florfenicol	Noga, 2010
Streptococcus	Stripped bass, tilapia, turbot, barramundi, Atlantic Salmon	Streptococcosis	Oxytetracycline Amoxicillin Erythromycin	Noga, 2010 Toranzo et al., 2005
Pasteurella	Yellow tail, seabass	Pasteurellosis	Oxytetracycline	Noga, 2010 Toranzo et al., 2005
Mycobacterium	Striped bass, seabass, Atlantic salmon	Fish tuberculosis	Ampicillin Erythromycin thiocyanate	Noga, 2010 Jacobs et al., 2009
Edwardsiella	Channel catfish	Enteric septicemia Fish gangrene	Florfenicol Sulfadimethoxine and Ormetoprim	Noga, 2010 Mohanty & Shaoo, 2007

Table 4. Fish pathogens, corresponding diseases, and antibiotic treatment regimens

Furunculosis (skin infection) and motile Aeromonas septicemia (MAS) are two major fish diseases caused by *Aeromonas* species (Noga, 2010). Nonmotile, psychrophilic *Aeromonas salmonicida* is the causative agent of furunculosis in salmonids (O'Brien et al., 1994). Besides furunculosis in salmonids, it causes erythrodermatitis in carp and ulcers in trout (Noga, 2010). Furthermore, motile mesophilic *Aeromonas hydrophila* and *Aeromonas veronii* are the causative agents of MAS in carp, tilapia, perch, catfish, and salmon. They also cause ulcer disease in catfish, cod, carp, and goby (Noga, 2010).

Vibrio species cause systemic infections in fish (Vibriosis). The common symptoms are loss of appetite and skin ulcers, which are associated with septicemia. *Vibrio anguillarum* and *Vibrio salmonicida* are the causative agents of vibriosis and cold water vibriosis in salmon and cod, respectively (Colwell & Grimes, 1984). Further, *Vibrio vulnificus* causes hemorrhagic septicemia in Japanese eels and *V. damsela* causes skin ulcers (Toranzo et al., 2005).

Columnaris disease affects the skin and gills of freshwater fish including salmonids, catfish, striped bass, and tilapia (Noga, 2010). Infection causes degradation of the cartilage tissue, the major cause of death. *Cytophaga columnaris* as well as *Bacillus columnaris, Flexibacter columnaris,* and *Flavobacterium columnare* are the causative agents of columnaris disease (Noga, 2010).

Streptococcosis is a fish disease caused by *Streptococcus* species. Some of the clinical symptoms of streptococcosis are anorexia, loss of orientation, erratic swimming, lethargy, and hemorrhaging (Noga, 2010). *Streptococcus iniae* is the causative agent of streptococcosis in at least 27 species of farmed finfish. The worldwide impact of streptococcosis was estimated globally to be around $100 million (USD) (Agnew &Barnes, 2007).

Pasteurellosis (pseudotuberculosis) is one of the most important diseases in Japanese aquaculture affecting commercial yellowtail, ayu, black sea bream, red sea bream, and hybrid striped seabass (Noga, 2010). *Pasteurella piscidium* is the causative agent of this disease (Romalde, 2002). Hemorrhaging around the gills and lesions in the skin, liver, and kidneys are common symptoms in acute and chronic forms of this disease (Noga, 2010).

Mycobacteriosis (or fish tuberculosis) can be observed in nearly 200 species including salmonids, seabass, turbo, cod, and halibut (Noga, 2010). *Mycobacterium marinum* is the most common *Mycobacterium* species causing tuberculosis. Greyish-white nodules in the spleen, liver, and kidney, and hemorrhagic lesions are among the symptoms of this disease (Jacobs et al., 2009).

Edwardsiellosis (emphysematous putrefactive disease) is commonly observed in carp, tilapia, eel, catfish, salmon, and trout (Noga, 2010). *Edwardsiella tarda* and *Edwardsiella ictaluri* are the main species causing Edwardsiellosis. Gas-filled lesions in the skeletal musculature are the major clinical sign of this disease (Mohanty & Sahoo, 2007). Further, *Edwardsiella ictaluri* is associated with enteric septicemia in channel catfish (Mohanty & Sahoo, 2007)

4.3 Current practices in the seafood industry to combat spoilage and disease

The seafood industry employs numerous techniques to eliminate microorganisms from their products. The oldest and still widely used form of seafood preservation is drying/salting. Besides keeping the seafood chilled in cold-water or on ice, this is the most low-tech preservation technique. There are many variations to this method such as wet-salting or additional acidification, but most achieve the same results. In this method, the fish is dried (with or without salt), which creates an environment devoid of vast nutrients (and possibly with high salinity). This prevents most bacterial growth, but spoilage can still occur due to filamentous fungi growth or insect infestation. Yeast is also able to grow in heavily wet-salted fish (Gram & Dalgaard, 2002).

Other preservation methods include washing with disinfectants, including chlorinated water, iodophores, salts, organic compounds, aldehydes, hydrogen peroxide, quaternary ammonium compounds, and antiseptic dyes (Calo-Mata et al., 2007; Shao, 2001). Disinfectants are mostly used to kill fungi and parasites. However, they may also select for antibiotic resistance in bacteria (Murray et al., 1984). Seafood can also be marinated in an acidic solution to prevent bacterial growth. Vacuum-packing and preservatives such as sorbate and benzoate have also been employed to prevent microbial growth (Einarsson & Lauzon, 1995; Gram & Dalgaard, 2002). Recently, complex solutions such as carbon-dioxide packing, spray-drying of antimicrobials, and radiofrequency heating have been applied to fight these ever-present problems of spoilage and contamination (Calo-Mata et al., 2007; Galvez et al., 2007; Gram & Dalgaard, 2002).

The use of vaccines and antibiotics in aquaculture is aimed at preventing the initial colonization by microorganisms. The means of administration of these prophylactics is simple, they are either added to the water or feed, or given by injection (Shao, 2001). A large body of research in the 1970s resulted in vaccines against numerous seafood pathogens, primarily species of *Vibrio* (Shao, 2001). Although vaccines are effective (and cost-effective), there are still no vaccines against shrimp and mollusc pathogens (Subasinghe, 2009).

An alternative to vaccination is the use of antibiotics to prevent bacterial infections. Tetracycline has become one of the most popular antibiotics in aquaculture due to its low-cost, low-toxicity, and high efficacy. Further, florfenicol, sulfadimethoxine/ormetoprim, oxytetracycline, and sulfonamides, are used to treat common bacterial infections (Table 4). However, the rampant use of antimicrobials in this industry has created massive selective pressure for bacteria to develop resistance (World Health Organization [WHO], Fact sheet 194). While these drugs are effective in killing bacteria, they also play a much more nefarious role in aquaculture. Antibiotics have varying half-lives, meaning they degrade at different rates. Some antibiotics degrade slowly and thus proliferate in the aquatic environment (Cabello, 2006). Worse still, the drugs flow into open waterways, sewage systems, sediments, and can even remain in the flesh of the farmed seafood (Benbrook, 2002; Cabello, 2006). These antibiotics continue to impose selective pressures leading to resistance until they are eventually degraded. This resistance is not only seen in the bacteria that inhabit the seafood produced in antibiotic-using aquaculture facilities, but in the animals neighboring the facilities as well (Benbrook, 2002). It has also been shown that these resistant bacteria are able to horizontally transfer their resistance-conferring genes to other human pathogens (Benbrook, 2002). For these reasons, governing agencies, such as the US Food and Drug Administration (FDA) and Environmental Protection Agency (EPA), strictly regulate the use of antibiotics in aquaculture.

Due to the problems associated with antibiotic use, the seafood industry is exploring the use of probiotics to promote the growth of healthy microflora in the seafood that can combat infecting pathogens. Probiotics are live microbial feed supplements that beneficially affect the host animal by improving its intestinal health (Fuller, 1989). While this definition needs a bit of tweaking to fit the seafood industry, it does still apply.

One area of active research in seafood aquaculture is the utilization of bacteriocins as antimicrobials. Bacteriocins have a long history of use in dairy or meat applications and there is an increasing number of studies on the effect of bacteriocins as antimicrobials in the seafood industry (Table 5) (Aasen et al., 2003; Al-Holy et al., 2004; Budu-Amoako et al., 1999; Einarsson & Lauzon, 1995; Elotmani & Assobhei, 2004; Luders et al., 2003; Neetoo et al., 2008; Nilsson et al., 1997; Nykanen et al., 2000; Szabo &Cahill, 1999; Tahiri et al., 2009; Tsironi & Taoukis, 2010; Zuckerman & Ben Avraham, 2002). These studies have focused largely on the effects of nisin, a Gram-positive bacteriocin that has been generally recognized as safe (GRAS) by the FDA. Early studies of nisin indicated that it delayed growth of *L. monocytogenes* in cold-smoked salmon. Later research revealed that the addition of CO_2 atmospheric packing significantly increased the effectiveness of nisin against *Listeria* (Nilsson et al., 1997).

There has also been encouraging research into nisin-coated packaging. Neetoo et al. (2008) investigated the effect of nisin-coated plastic films on the survival of *L. monocytogenes* on vacuum-packed cold smoked salmon. This study showed that nisin-coated plastic films

Bacteriocin	Target	Seafood product	Reference
Bavaricin A	Extended shelf-life	Shrimp	Einarsson et al., 1995
Carnocin U149	Extended shelf-life	Shrimp	Einarsson et al., 1995
Divergicin M35	*L. moncytogenes*	Salmon	Tahiri et al., 2009
Nisin	*L. moncytogenes*	Salmon	Nilsson et al., 1997
Nisin	*L. moncytogenes*	Salmon	Nilsson et al., 1997
Nisin	*L. moncytogenes*	Salmon	Szabo and Cahill, 1999
Nisin	*L. moncytogenes*	Salmon	Neetoo et al., 2008
Nisin	*L. moncytogenes*	Salmon	Zuckerman and Ben Avraham, 2002
Nisin	*L. moncytogenes*	Trout	Nykanen et al., 2000
Nisin	*L. moncytogenes*	Lobster	Budu-Amoako et al., 1999
Nisin	*L. innocua*	Caviar and ikura	Al-Holy et al., 2004
Nisin	Aerobic bacteria	Salmon	Zuckerman and Ben Avraham, 2002
Nisin	Bacterial flora	Sardines	Elotmani et al., 2004
Nisin	Extended shelf-life	Fish	Tsironi and Taoukis, 2010
Nisin Z	Extended shelf-life	Shrimp	Einarsson et al., 1995
Pediocin	*L. moncytogenes*	Salmon	Szabo and Cahill, 1999
Sakacin P	*L. moncytogenes*	Salmon	Aasen et al., 2003
Sakacin P	*E. coli*	Salmon	Luders et al., 2003

Table 5. Examples of bacteriocin trials in seafood products (Adapted from Galvez et al. 2008).

reduced the number of *L. monocytogenes* by 3.9 log CFU/cm^2 at 4 ^{o}C and 10 ^{o}C after 56 and 49 days of incubation, respectively. Further, this study also showed that nisin-coated plastic films suppressed the growth of other aerobic and anaerobic spoilage microorganisms in a concentration-dependent manner (Neetoo et al., 2008).

The combination of nisin with heat has also been shown as an effective method to prevent *L. monocytogenes* contamination. Budu-Amoako et al. (1999) applied nisin along with moderate heating on cold-packed lobster and showed a reduction of L. *monocytogenes* by 3-5 logs in comparison to nisin and/or heat treatment alone (Budu-Amoako et al., 1999). Further, Al-Holy et al. (2004) used a radio-frequency heating method to apply heat treatment in conjunction with nisin. The combination of nisin and radio-frequency heating caused reduction of *L. innocua* by 100% (Al-Holy et al., 2004). With an industry moving away from traditional preservation techniques, bacteriocins (such as nisin) offer a promising alternative as antimicrobials in the seafood industry.

4.4 Bacteriocin potential in the seafood industry

There are a number of factors that play a significant role in the potential to use bacteriocins as probiotics and/or bio-preservatives in the seafood industry. The natural microbiota of the seafood needs to continue to be surveyed for its sensitivity to bacteriocins. This information should be incorporated into the guidelines for bacteriocin use in order to use these proteins prudently against relevant pathogens. The environmental conditions, such as pH and temperature, during seafood growth and processing could also affect the activity of applied bacteriocins and requires further investigation (Galvez et al., 2007).

Despite these factors, research on aquatic microorganisms has shown that bacteriocin production and diversity in aquatic environment is abundant (Bagenda et al., 2008; Bhugaloo-Vial et al., 1996; Carraturo et al., 2006; Hosseini et al., 2009; Hoyt & Sizemore, 1982; Longeon et al., 2004; McCall & Sizemore, 1979; Messi et al., 2003; Metivier et al., 1998; Moro et al., 1997; Nilsson et al., 2002; Pinto et al., 2009; Pirzada et al., 2004; Prasad et al., 2005; Rajaram et al., 2010; Selvin et al., 2004; Shehane & Sizemore, 2002; Stoffels et al., 1992; Sugita et al., 1997; Suzuki et al., 2005; Tahiri et al., 2004; Valenzuela et al., 2010; Yamazaki et al., 2005; Zai et al., 2009). Bacteriocins have numerous qualities that make them attractive as alternatives to antibiotics. They have been shown to be non-toxic to eukaryotic cells and are GRAS, making them a safe alternative to traditional antimicrobials (Galvez et al., 2008). It has also been shown that purified bacteriocins do not effect the sensory qualities of seafood and that they are stable up to a salinity concentration of 10%. Additionally, the relatively narrow killing spectrum of bacteriocins compared to traditional antibiotics limits the selective pressure for bacteria to evolve resistance to these antimicrobials and thus reduces the incidence of drug-resistant pathogens.

Because of the above stated reasons, some have suggested that bacteriocins should be applied to foods by spray-drying as either dried bacteriocins or probiotic bacteriocinogenic strains (Calo-Mata et al., 2007; Galvez et al., 2007). For example, Brillet et al. (2005) has shown that bacteriocin producer *Carnobacterium divergens* V41 can be used as a biopreservative to inhibit the growth of *Listeria monocytogenes* in cold smoked salmon (Brillet et al., 2005). This study showed that spray application of *C. divergens* V41 on commercial smoked salmon did not affect the sensory qualities of the salmon (Brillet et al., 2005). Additionally, Schobitz et al. (1999) directly applied a BLIS from *Carnobacterium piscicola* into vacuum-packed meat, which inhibited the growth of *L. monocytogenes* in the vacuum-packed meat after 14 days of storage at 4 ^{o}C (Schobitz et al., 1999). These studies aid in the argument that bacteriocins should be used as a biopreservation technique in the seafood industry. This technology has already emerged in the terrestrial food industry as we see with nisin (an FDA approved food additive) and Microgard™ (a milk-based BLIS).

It has also been suggested that bacteriocins could be combined with current methods of antimicrobial treatment and preservation to produce synergistic effects, such as incorporating bacteriocins into bio-active packaging (Calo-Mata et al., 2007; Galvez et al., 2007; Pilet & Leroi, 2011). For example, bacteriocins can be impregnated into gel coatings and/or polyethylene films and can be applied to seafood during packaging (Neetoo et al., 2008). The application of bacteriocins on packaged seafood is steadily being seen as a very promising biopreservation method (Aasen et al., 2003; Al-Holy et al., 2004; Budu-Amoako et al., 1999; Einarsson & Lauzon, 1995; Elotmani & Assobhei, 2004; Luders et al., 2003; Neetoo et al., 2008; Nilsson et al., 1997; Nykanen et al., 2000; Szabo &Cahill, 1999; Tahiri et al., 2009; Tsironi & Taoukis, 2010; Zuckerman & Ben Avraham, 2002). In fact, immobilization of bacteriocins on coating materials for biopreservation may actually reduce the cost of packaging due to the reduced amount and cost of the antibacterial needed to attach to the film (Galvez et al., 2008). Creating combinations of bacteriocins and current methods used in the seafood industry has the potential to increase the guarantee of freshness by assuring the inhibition of spoilage causing microorganisms.

One trouble that the industry was having was the scale-up of these bacteriocins to levels that were high enough for use in pilot studies and/or on the industrial scale. However, there is

technology in the pipeline that will make this an issue of the past (Galvez et al., 2008). As the exploration of the aquatic environments of our planet increases, we are sure to find new and exciting bacteriocins, which could play a vital role in antimicrobial effects and biopreservation in the seafood industry.

5. Conclusions and future research

Bacteriocins have been the focus of an extensive number of studies for the past sixty years due to their important role in nature and more recently, their potential for use as therapeutics and probiotics. Most studies of bacteriocins initially focused on phenotypic and molecular characterization of these toxins. However, due to their high potency and relatively narrow killing spectrum, they were quickly recognized as a natural alternative to antibiotics.

Knowledge regarding bacteriocins and their potential applications from terrestrial bacteria is vast. Gram-negative bacteriocins such as colicins and microcins of enteric bacteria and pyocins of *P. aeruginosa* have great promise in human and veterinary medicine. In addition, Gram-positive bacteriocins such as nisin, pediocin, and lacticin have been developed for use as food preservatives.

The focus of this chapter was to explore the bacteriocin and BLIS activity characterized from marine microorganisms and assess their potential applications in aquaculture. The bacteriocins and BLIS from marine microorganisms are under-studied relative to their terrestrial counterparts. Thus far, most studies are limited to the identification of BLIS activity and characterization of its killing breadth. Some studies have further characterized these proteinecous killing agents and classified them based on similarities to known bacteriocins. However, the abundance and diversity of bacteriocins in marine microorganisms remains to be fully explored. We predict that a wealth of interesting bacteriocin proteins can be easily identified as the screening efforts proceed.

Further, there is increased interest in the use of bacteriocins as alternatives to classical antibiotics in aquaculture. Bacteriocins are highly potent against marine pathogens and environmentally safe, due to the fact that they do not create intensive selection pressures for antibiotic resistance. Clearly, bacteriocins could prove extremely beneficial to the seafood industry and more research should be dedicated to exploring their potential applications as probiotics and therapeutics. Given the fact that all species of bacteria have the potential to produce bacteriocins, and only a handful have thus far been identified from marine microorganisms, we are confident that existing studies have exposed only the tip of the iceberg, in terms of bacteriocin diversity and potential use in the seafood industry.

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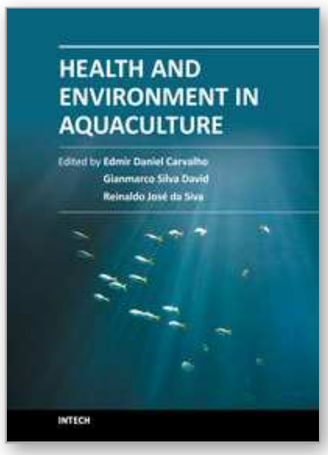

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Aquaculture has been expanding in a fast rate, and further development should rely on the assimilation of scientific knowledge of diverse areas such as molecular and cellular biology, and ecology. Understanding the relation between farmed species and their pathogens and parasites, and this relation to environment is a great challenge. Scientific community is involved in building a model for aquaculture that does not harm ecosystems and provides a reliable source of healthy seafood. This book features contributions from renowned international authors, presenting high quality scientific chapters addressing key issues for effective health management of cultured aquatic animals. Available for open internet access, this book is an effort to reach the broadest diffusion of knowledge useful for both academic and productive sector.

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