

Marine bacteriocins: a review

Abstract

Marine environment is entirely different from terrestrial environment and exploration of resources of marine origin is of perpetual interest to scientists. In this review we have attempted to concisely present a general idea of bacteriocins. This also includes a review on bacteriocins of marine origin, their use in marine aquaculture and sea food industry.

Keywords: bacteriocins, marine, probiotics, classification

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Introduction

Like all organisms in nature, bacteria too have their own immune system and defense mechanisms. The antagonistic factors like antibiotics, bacteriocins, lysozymes, siderophores, proteases, and/or hydrogen peroxide and the alteration of pH values by organic acids produced either singly or in combination act as defense substances. Bacteriocins are potent antimicrobial peptides and proteins, found in almost every bacterial species examined till date, and within a species tens or even hundreds of different kinds of bacteriocins are produced.¹

The three types of cells in a microbial community are, bacteriocinogenic (produce bacteriocin), sensitive, or resistant to each bacteriocin. Thus in marine environments, all three cell types compete with each other for limited resources, with only a small percentage of bacteriocinogenic cells induced to produce and release bacteriocin. While some sensitive cells are killed immediately by the bacteriocin, others harbor mutations that impart resistance. These resistant cells rapidly displace the producer cells. In contrast to traditional antibiotics that are used in human health applications, bacteriocins mostly target members of the producer species and their closest relatives.² Hence they are classically considered to be narrow spectrum antibiotics. *Halobacteria* and *archaea* too produce their own version of bacteriocins, the halocins.³ Some bacteriocins are capable of inhibiting *archaea*,⁴ but there is no confirmed inhibition of bacteria by a halocin, although there are reports that halophilic *archaea* are capable of inhibiting halophilic bacteria.

Bacteriocin was first discovered by Gratia in 1925,⁵ during his search for ways to kill bacteria. He named it a colicine because it killed *E. coli*. The term bacteriocin was coined by Jacob and coworkers in 1953,⁶ which paved the way for the development of microbial antibiotics and the discovery of bacteriophages, all within the span of a few years. High-throughput sequencing technologies reveal that bacterial diversity is larger than expected in marine microbial ecology and contains an extremely large number of microbial genes of unknown function.⁷ Nevertheless, only a few bacteriocins and bacteriocin-like substances have been described from marine bacteria. In the limited knowledge of marine bacterial biodiversity and the urgent requirement for antibiotic alternatives, the marine bacteriocin research is an open alternative in the near future.

Discussion

Bacteriocin definition

Bacteriocins are ribosomally synthesized proteinaceous

compounds, lethal to closely related species of producing bacteria, the latter being protected by self immunity. These toxins play a critical role in mediating microbial population or community interactions. Bacteriocins may serve as anti-competitors enabling the invasion of a strain into an established microbial community or act as communication molecules in bacterial consortia like biofilms. i.e., they play a defensive role and act to prohibit the invasion of other strains or species into an occupied niche or limit the advance of neighboring cells.⁸ An additional role proposed by Miller & Bassler⁹ for Gram-positive bacteriocins is in quorum sensing. Some bacterial species produce toxins which exhibit numerous bacteriocin-like features, but they are yet not fully characterized; such toxins are referred to as bacteriocin-like inhibitory substances, or BLIS. This review focuses on bacteriocins¹⁰⁻¹⁴ and bacteriocin like substances¹⁵⁻¹⁸ isolated from marine environment and marine food products.^{19,20}

A precise definition of the bacteriocins is obscure and futile. Conventional criteria for definition of bacteriocins were based on the characteristics of colicins. These criteria have been used in varying combinations and applied with different degrees of consistency and proof in defining other bacteriocins: (i) A narrow inhibitory spectrum of activity centered about the homologous species; (ii) a bactericidal mode of action; (iii) the presence of an essential, biologically active protein moiety; (iv) attachment to specific cell receptors; (v) plasmid-borne genetic determinants of bacteriocin production and of host cell bacteriocin immunity; (vi) production by lethal biosynthesis (i.e., commitment of the bacterium to produce a bacteriocin will ultimately lead to cell death).²¹

Marine organisms as a potent source of bioactive compounds

The marine environment differs substantially from terrestrial and fresh water habitats because of its exigent, competitive and aggressive nature. The estimated density of bacteria in seawater and sediment ranges from 10⁵ to 10⁷/mL and 10⁸ -10¹⁰/g respectively.²² Little is known about the diversity of marine microorganisms. The number of species of microorganisms has been estimated from as low as 10⁴ -10⁵ to as high as 10⁶ -10⁷.⁷ Bacteriocins produced by marine bacteria are primarily of interest to researchers due to their potential as probiotics and antibiotics in the seafood industry and marine aquaculture.²³⁻²⁵

The first marine bacteriocin was isolated from *Vibrio harveyi* (formerly *Beneckeia harveyi*) by McCall & Sizemore²⁶ when they screened 795 strains of *Vibrio* spp. isolated from Galveston Island, Texas. The identification of harveyicin led to numerous bacteriocin-

screening studies in marine bacteria, which focused on biochemical characterization of bacteriocins and BLIS.

A study by Wilson et al.,²⁷ on surface-attached bacteria isolated from Sydney Harbor, Australia, revealed that approximately 10% of surface-attached marine bacteria possess antibacterial activity. Proteinase K treatment attributed this inhibitory activity to proteinaceous substances such as bacteriocins or BLIS. Antimicrobial screening of 258 bacterial strains from water and sediment in the Yucatan peninsula revealed 46 strains of genera *Aeromonas*, *Burkholderia*, *Photobacterium*, *Bacillus*, *Pseudomonas*, *Serratia* and *Stenotrophomonas* with antimicrobial activity. Around fifty percent of this antimicrobial activity was attributed to bacteriocins or BLIS.²⁸ A thermostable bacteriocin BL8 from *Bacillus licheniformis* from marine sediment,²⁹ and halocin SH10 produced by an extreme *haloarchaeon* *Natrinema* sp. BTS10 from salt pans of South India³⁰ were reported.

Some bacteria particularly those in the digestive tract, produce

inhibitory compounds that control the colonization of potential pathogens in fish.^{31,32} For instance a heat-labile and proteinaceous substance with a molecular mass of <5kDa was recovered from *Vibrio* sp. obtained from the intestine of a spotnape pony fish.³³ Similarly, bacteria capable of inhibiting growth of pathogenic *Vibrio* sp. were isolated from the digestive tract of halibut (*Hippoglossus hippoglossus*) larvae.³⁴ In another study, of the 1,055 intestinal bacteria derived from 7 coastal fish in Japan, 28 isolates (2.7% of the total) inhibited the human and eel pathogen *V. vulnificus*.³⁵ Marked inhibition was displayed by 15 isolates, consisting of 11 Vibrionaceae representatives, 3 coryneforms, and 1 *Bacillus* strain NM 12; the latter demonstrating the most pronounced antimicrobial activity. A heat labile siderophore of <5kDa molecular weight inhibited the growth of 227 out of 363 (62.5% of the total) intestinal bacterial isolates from 7 fish.³⁶ Bacteriocin producer was also reported from the deep sea shark gut, where a *Bacillus amyloliquefaciens* BTSS3 was shown to produce thermostable, pH tolerant bacteriocin.³⁷ A detailed view is given in Table 1a & 1b.

Table 1a Some characterized marine bacteriocins and their sources

Bacteriocin	Producer strain	Molecular weight	Killing breadth	Source of isolation	Reference
BLIS	<i>Lactobacillus pentosus</i> 39	-	<i>Aeromonas hydrophila</i> , <i>Listeria monocytogenes</i>	Salmonlets	82
Carnocin UI49	<i>Carnobacterium</i> sp.	4.5-5kDa	<i>Lactobacillus</i> , <i>Lactococcus</i> , <i>Pediococcus</i> , <i>Carnobacterium</i>	Fish	10
Divergin M35	<i>Carnobacterium divergens</i> M35	~4.5kDa	<i>Carnobacterium</i> , <i>Listeria</i>	Frozen smoked mussel	12
Divercin V41	<i>Carnobacterium divergens</i> V41	4.5kDa	<i>Carnobacterium</i> , <i>Listeria</i> , <i>Enterococcus</i>	Fish viscera	11
Carnobacteriocin B2	<i>Carnobacterium piscicola</i> A9b	~4.5kDa	<i>Listeria</i>	Cold smoked salmon	19
Piscicocin CS526	<i>Carnobacterium piscicola</i> CS526	~4.4kDa	<i>Tetragenococcus</i> , <i>Leuconostoc</i> , <i>Listeria</i> , <i>Enterococcus</i> , <i>Pediococcus</i>	Frozen surimi	13,14
Piscicocin VIa	<i>Carnobacterium piscicola</i> VI	4.4kDa	<i>Lactobacillus</i> , <i>Listeria</i> , <i>Enterococcus</i> , <i>Pediococcus</i> , <i>Carnobacterium</i>	Fish	15
BLIS	<i>Enterococcus faecium</i> CHG 2-1 and Ch 1-2	-	<i>Enterococcus</i>	Venus clams	16
BLIS	<i>Enterococcus faecium</i> C-K, C-S, M 2-1, and PEF 2-2	-	<i>Listeria</i>	Anchovy, shark fillet, Swordfish fillet	16
Enterocin B like BLIS	<i>Enterococcus faecium</i> ALP7	<6.5kDa	<i>Listeria</i> , <i>Staphylococcus</i> , <i>Bacillus</i> , <i>Enterococcus</i> , <i>Lactobacillus</i> , <i>Lactococcus</i> , <i>Leuconostoc</i>	Non-fermented shellfish	17
BLIS	<i>Lactobacillus lactis</i>	94kDa	<i>Bacillus</i> , <i>Staphylococcus</i> , <i>Enterococcus</i> , <i>E.coli</i> , <i>Pseudomonas</i> , <i>Shigella</i>	Sediment sample	18

Table 1b Some characterized marine bacteriocins and their sources

Bacteriocin	Bacteria	Molecular Wt	Killing Breadth	Source	Reference
Bacteriocin BL8	<i>Bacillus licheniformis</i>	<3kDa	<i>Staphylococcus aureus</i> , <i>Bacillus</i> sp.	Sediment	29
BLIS	<i>Vibrio</i> sp.	<5kDa	<i>Bacillus</i> sp., <i>Vibrio</i> sp., <i>Pseudomonas</i> sp.	Spot nape pony fish	33
BLIS	<i>Vibrio</i> sp.			Halibut larvae (<i>Hippoglossus hippoglossus</i>)	34
Bacteriocin	<i>Bacillus</i> sp. NM12	Siderophore, <5kDa	Fish pathogens	Coastal fish	35
Bacteriocin Bac3	<i>Bacillus amyloliquefaciens</i> BTSS3	~ 3kDa	<i>Bacillus</i> sp., <i>Staphylococcus aureus</i>	Deep sea shark (<i>Centroscyllium fabricii</i>)	37
BLIS	<i>Proteus</i> sp. CT1.1	-	<i>Vibrio</i>	Cobia	90
BLIS	<i>Proteus</i> sp. G1	-	<i>Vibrio</i>	Ornate spiny lobster	90
BLIS	<i>Bacillus cereus</i> D9	-	<i>Vibrio</i>	Subnose pompano	90

Fifteen isolates with confirmed consistent antimicrobial activity recovered from Irish seaweeds, as well as sand and seawater, were spore-forming *Bacillus* sp. While PCR screening was successful in identifying three of the marine bacteria as lichenicidin producers, the rest of the isolates did not harbor structural genes for any of the known *Bacillus* bacteriocins for which PCR primers could be designed. These negative PCR outcomes strongly suggest that these isolates produce novel bacteriocins.³⁸

Bacteriocin classification

The bacteriocin family includes diverse proteins in terms of size, modes of action, microbial targets and immunity mechanisms. In general, bacteriocins are studied based on the Gram designation of their producing species, Gram-negative Vs Gram-positive (Table 2). Additionally, a relatively small number of bacteriocins from *Archaeal species* have also been characterized.

Table 2 Classification of Bacteriocins with examples

	Bacteriocins	Type/Class	Size	Example	Reference
	Colicins	Pore Formers	20-	Colicins A, B	46
		Nucleases	80	Colicins E2, E3	46
Gram negative bacteria	Colicin-like		20-	S-pyocins	45
			80	Klebicins	
	Phage-tail like		>80	Maltocin P28	49
		Post translationally modified		Microcin C7	45
Gram positive bacteria	Microcins	Unmodified	<10	Microcin B17	
		Class IIc - non-ribosomal siderophore-type post-translation modification		Colicin V	50
	Class I	Type A- Linear peptides, positively charged		microcin E492	51
		Type B- Rigid globular peptides, negatively or neutrally charged	<5	Nisin	54
		IIa - contain YGNGVxCxxxxCxV, Narrow spectrum of activity		Subtilisin A	57
	Class II	IIb – require concerted activity of 2 peptides		Pediocin, enterocin	60
IIc – circular peptide bacteriocins		<10	Lactacin F, Lactococcin G	60	
IId – linear, non-pediocin like, single peptide			Carnocyclin A	61	
IIla – bacteriolysin		>10	Epidermicin NIOI	62	
Class III	IIlb – non-lytic bacteriocin		Enterolysin A	63	
	Require lipid or carbohydrate moieties		Helveticin A & J	64	
Archea	Halocins	Microhalocins	<10	Leuconocin S8, lactocin 27	65
		Protein Halocins	>10	Halocin A4, C8, G1	67
	Sulfolobacin	Membrane associated proteins	~20	Halocin H1, H4	67
				Sulfolobacin	71

Bacteriocins of Gram-negative bacteria: Bacteriocins of Gram-negative bacteria are categorized into four main classes: colicins, colicin-like bacteriocins, microcins, and phage-tail like bacteriocins.³⁹ Colicins are so well studied that they have been used as a model system to study bacteriocin structure, function and evolution.⁴⁰⁻⁴³ In general, colicins are thermo-sensitive, protease sensitive proteins that vary in size from 25 to 90kDa.⁴⁴

There are two major colicin types based on their mode of killing; nuclease and pore former colicins. Nuclease colicins (Colicins E2, E3, E4, E5, E6, E7, E8, E9) kill by acting as DNases, RNases, or tRNases and pore former colicins (colicins A, B, E1, Ia, Ib, K) kill sensitive strains by forming pores in the cell membrane. Proteinaceous bacteriocins produced by other Gram-negative species are classified as colicin-like due to the presence of similar structural and functional

characteristics. They can be nucleases (pyocins S1, S2) and pore-formers (pyocin S5) like colicins.^{45,46} S-pyocins of *Pseudomonas aeruginosa*, Klebicins of *Klebsiella species*, 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 which are even argued as defective phage particles.⁴⁷ R and F pyocins of *P. aeruginosa* are some examples of the most thoroughly studied phage-tail like bacteriocins.^{45,48,49}

Pore-forming colicins range in size from 449 to 629 amino acids. Nuclease bacteriocins have an even broader size range, from 178 to 777 amino acids. In colicins, the central domain comprises about 50% of the protein and is involved in the recognition of specific cell surface receptors. The N-terminal domain (>25% of the protein) is responsible for translocation of the protein into the target cell. The remainder of

the protein is a short sequence involved in immunity protein binding. The killing domain and the immunity region are present in this region. Although the pyocins share a similar domain structure, the order of the translocation and receptor recognition domains are exchanged.⁴³

Finally, Gram-negative bacteria produce much smaller (<10kDa) peptide bacteriocins called microcins. They can be divided into three classes: post-translationally modified (microcins B17, C7, J25, and D93)⁵⁰ and unmodified microcins (microcins E492, V, L, H47, and 24). Class IIc bacteriocins are non-ribosomal siderophore-type post-translation modification at the serine-rich carboxy-terminal region, such as microcin E492⁵¹ (Table 2).

Bacteriocins of Gram-positive bacteria: Bacteriocins of gram-positive bacteria are more abundant and even more diverse than those in Gram-negative bacteria,⁵² but differing in two fundamental ways.

1. Bacteriocin production is not necessarily a lethal event as it is for Gram-negative bacteria.
2. This vital difference is due to the transport mechanisms encoded by Gram-positive bacteria to release bacteriocin toxin. Some have evolved a bacteriocin-specific transport system, whereas others employ the *sec*-dependent export pathway.
3. The Gram-positive bacteria have evolved bacteriocin-specific regulation, whereas bacteriocins of Gram-negative bacteria rely solely on host regulatory networks.

Classification of bacteriocins of gram-positive bacteria

Based on size, morphology, physical, and chemical properties, bacteriocins of Gram-positive bacteria are generally divided into four classes.⁵³

Class I bacteriocins: Are post-translationally modified small peptides (<5kDa) incorporating non-traditional amino acids such as dehydrobutyrine, dehydroalanine, lantionine and methyl-lanthione called lantibiotics.⁵⁴ This class is subdivided into Type A and B with the distinction being that members of Type A are linear peptides (nisin)⁵⁵ and positively charged, whereas those in Type B are rigid globular peptides (mersacidin), labyrinthopeptins, such as globular peptide labyrinthopeptin A2,⁵⁶ and sactibiotics, such as globular peptide subtilosin A⁵⁷ either negatively or neutrally charged.

Class II bacteriocins: Are small 30-60 amino acids (<10kDa), heat-stable peptides that are not post-translationally modified and positively charged.⁵⁸ Class II is also subdivided into four subgroups]. The class IIa Listeria-active or pediocin-like peptides containing a conserved N-terminal sequence (YGNGVxCxxxxCxV) or “pediocin box” with two cysteine residues forming disulphide bridge, are the most extensively studied group with a narrow spectrum of activity.⁵⁹ Lactacin F and lactococcin G are part of Class IIb bacteriocins that require the concerted activity of two peptides to be fully active.⁶⁰ Class IIc bacteriocins are circular peptide bacteriocins, such as carnocyclin A.⁶¹ Class IId bacteriocins are linear, non-pediocin-like, single-peptide bacteriocins, including epidermicin NI01.⁶²

Class III bacteriocins: Are generally large (>10kDa), heat-sensitive peptides, subdivided into two subtypes. Type IIIa are bacteriolysins, which are bacteriolytic enzymes such as Enterolisin, which kill sensitive strains by lysis of the cell wall.⁶³ Helveticin J (37kDa) produced by *Lactobacillus helveticus* belongs to Type IIIb, which are non-lytic bacteriocins.⁶⁴

Class IV bacteriocins: Require lipid or carbohydrate moieties for activity. They are also known as complex bacteriocins, with unique

structural characteristics. The first and last amino acids of these bacteriocins are covalently bound, thus having cyclic structures. Examples include leuconocin S 8 and lactocin 27.⁶⁵ Enterocin AS-48 produced by *Enterococcus faecalis* subsp. *Liquefaciens* S-48 was the first characterized bacteriocin of this class.⁶⁶

Bacteriocins of archaea: The *Archaea* also produce unique bacteriocin-like antimicrobial compounds called archaeocins,⁶⁷ but are much less scrutinized than the bacteriocins. So far, two major types of archaeocins have been identified: halocins of halobacteria and sulfolobocins of *Sulfolobus* genus. Halocins can be divided into two classes based on size: the smaller microhalocins (3.6kDa) and larger halocins of 35kDa.⁴ The first halocin discovered was S8, which is a short hydrophobic peptide of 36 amino acids, processed from larger 34kD pro-protein. Halocin production is a universal feature of halobacteria.³ Halocin genes are located on megaplasmids (or minichromosomes). Halocins H4 and S8 are located on ~300 kbp and ~200kbp plasmids, respectively.^{68,69} Their activity is usually detected at the late exponential to early stationary growth phase.

Sulfolobocins are not extensively studied, Prangishvili et al.,⁷⁰ screened sulfolobocin production from *Sulfolobus islandicus* isolated from volcanic vents throughout Iceland. This study predicted that sulfolobocin is a membrane associated protein. Sulfolobocins are also associated with membranous vesicles ranging in size from 90 to 180nm in diameter. Like many bacteriocins, they are thermostable and sensitive to protease treatment. Their mode of action is still unknown.⁷¹

Bacteriocin mode of action

The great variety of their chemical structures allow bacteriocins to affect different essential functions of the living cell (transcription, translation, replication and cell wall biosynthesis), but most act by forming membrane channels or pores destroying the energy potential of sensitive cells. Research on the mode of action of bacteriocins largely focused upon two distinct aspects of bacteriocin action on susceptible bacteria: the kinetics of the physical interaction between bacteriocin and susceptible cells, and the detection of specific biochemical lesions within the affected organisms. In a widely accepted hypothesis of the mode of action of bacteriocins, it was suggested that the interaction of a bacteriocin with a sensitive cell occurs in two stages.⁷² The first stage, probably a reversible phase, corresponds to physical adsorption of bacteriocin to cell-envelope receptors. The removal of the bacteriocin during this stage apparently leaves the cell unscathed as there is no permanent physiological damage. The second stage develops later when irreversible pathological changes are effected via specific biochemical lesions after a measurable time.

Although in many cases the adsorption of bacteriocins are highly specific for susceptible bacteria, some others like the *staphylococcins* 414 and 1580, lactocin LP27 and streptococcin B-74628 lack this adsorption specificity. Each of these bacteriocins adsorbed to bacteria resistant to its killing action. This nonlethal binding may be a reflection of the high surface activity of some bacteriocins. Polypeptide antibiotics such as polymyxin B show this capability of adsorbing nonspecifically to bacteria. Even though adsorption of bacteriocins exists in most cases, non-adsorption to susceptible or to resistant bacteria was also demonstrated by bacteriocin 28 of *C. perfringens*,⁷³ staphylococcin 462 and viridin B.

The antibiotic activity of bacteriocins from Gram-positive bacteria is based on their interaction with the bacterial membrane. The mechanisms of action of peptide antibiotics are diverse, but the bacterial membrane is the target for most bacteriocins.⁷⁴ Most of the class II

bacteriocins disturb the proton motive force (PMF) of the target cell by pore formation. The subclass IIc comprises miscellaneous peptides with various modes of action such as membrane permeabilisation, specific inhibition of septum formation and pheromone activity.

Bacteriocin-induced cell damage

The physiological state of the indicator culture has a strong influence on susceptibility to the lethal action of bacteriocins. Actively multiplying cells were most sensitive to streptococin A-FF22, staphylococin 1580, bacteriocin 28 of *C. perfringens*, and bacteriocin E-1 and bacteriocin X-14 (hemolysin) of *S. faecalis* subsp. *zymogenes*. This indicates a requirement for active cellular metabolism to affect killing of cells. A time dependent morphological change to the sensitive strain was demonstrated by the action of pediocin from *P. acidilactici* on sensitive strain *L. monocytogenes*. Bacteriocin-treated *L. monocytogenes* V7 were almost completely destroyed after 6h. The major morphological changes were apparently due to changes in the cell wall, which started to relax, and ruptured after just 0.5h of treatment with bacteriocin (6,400AU/mL). After 1h and 3h of treatment, ruptures in the cell wall and plasma membrane were more evident with more cell contents escaping. After 6h of treatment, the cell wall was completely irregular and damaged.⁷⁵

Applications in sea food industry

Global production of fish, crustaceans, molluscs and other aquatic animals is ever increasing and reached 158million tonnes in 2012. Aquaculture production continued to show strong growth, with an average annual growth rate of 6.1percent from 36.8million tonnes in 2002 to 66.6million tonnes in 2012 according to Food and Agriculture Organization of United Nations (FAO) 2014.⁷⁶ Consumer demand for fish continues to climb, especially in affluent nations, which imported around 33million tonnes of fish in 2012. Moreover fish is an ingredient in pet food, health supplements, fishmeal and many non-food products manufactured on a global scale. Since pressure on seafood resources is set to increase further, fisheries that are poorly managed may quickly collapse. Spoilage and disease are the main challenges in seafood industry, both due to microorganisms.

Food preservation

The application of *Lactococcus lactis* subsp. *lactis* KT2W2L, a nisin Z producer for biopreservation of cooked, peeled and ionized tropical shrimps during storage at 8°C was studied.⁷⁷ Karthik et al.,⁷⁸ studied the efficacy of bacteriocin producing *Lactobacillus* sp. AMET1506, as a biopreservative for shrimp under different storage conditions. Nisin-coated plastic films suppressed the growth of other aerobic and anaerobic spoilage microorganisms in a concentration-dependent manner.⁷⁹ Diop et al.,^{80,81} reported that *L. lactis* subsp. *lactis* strain CWBIB1410, a nisin producer was used as a starter culture to improve the traditional Senegalese fish fermented guedj. They also suggest that this new fish fermentation strategy can enhance the safety of guedj. Anacarso et al.,⁸² studied the ability of *Lactobacillus pentosus* 39, a BLIS producing strain to control the growth of *Aeromonas hydrophila* ATCC14715 and *Listeria monocytogenes*, under simulated cold chain break conditions. One area of active research in seafood aquaculture is the utilization of bacteriocins as antimicrobials.

Probiotics in aquaculture

Probiotics are defined as “live microorganisms, which when consumed in adequate amounts, confer a health benefit on the host”.⁸³ The majority of probiotics in use today include species

of lactic acid bacteria (LAB), including lactobacilli, as well as bifidobacteria, nonpathogenic *Escherichia coli*, bacilli and yeasts such as *Saccharomyces boulardii*.⁸⁴ Antibiotic over use in aquaculture disease control results in the emergence of bacterial resistance and transfers the bacterial resistance genes to unexposed strains by alterations in the existing genome or horizontal gene transfer through plasmids or bacteriophages. This highlights the need for alternatives for antibiotics in aquatic disease management. Probiotics use in aquaculture for elimination of antimicrobial drug is increasing. Bacteria such as *Vibrio* sp., *Pseudomonas* sp., *Bacillus* sp. and several *Lactobacilli* sp. have been used successfully as probiotics in mollusk, crustacean, and finfish aquaculture, with most identified from aquatic animals, culture environment or from the intestine of different aquatic species.⁸⁵ Since probiotic research in aquaculture is still in its infancy and gaining acceptance in the industry, much research is needed to understand and resolve the controversies, such as real environmental demonstrations on successful usage of probiotics, their mode of action, and mechanism *in vivo*. The application of terrestrial bacteria in aquaculture has limited success because characteristics of bacteria depend upon their niche environment. Thus, identification of potential probiotics from marine environments where they grow optimally is a better approach.

Aeromonas media A199 controlled *Vibrio tubiashii* infection in Pacific oyster, *Crassostrea gigas* larvae by bacteriocin-like inhibitory substances, which antagonized several pathogenic bacteria in culture.^{86,87} *Alteromonas macleodii* 0444 was studied as a probiotic for controlling *V. coralliilyticus* and *Vibrio pectenicida* in flat oyster, *Ostrea edulis*, larvae⁸⁸ and also against *Vibrio splendidus* infection in Green shell mussel *Perna canaliculus* larvae leading to increased survival.⁸⁹

Bacteriocinogenic bacteria were isolated from ornate spiny lobsters (*Panulirus ornatus*), black tiger shrimp (*Penaeus monodon*), cobia (*Rachycentron canadum*) and snubnose pompano (*Trachinotus blochii*). Two candidate probiotic formulations with bacteriocinogenic bacteria showed beneficial effects on aquaculture-raised juveniles of ornate spiny lobsters (*Panulirus ornatus*) challenged with *V. owensii* DY05.⁹⁰ Thus in all aspects either the bacteriocin or the producer organism served as a food preservatives or immune enhancer in marine food industry.

Conclusion

Seafood industry is a growing part of the economy, but its economic value is diminished by infections which reduce the growth and survival of commercial species or decrease quality. These impacts are most evident in the stressful and crowded conditions of aquaculture, which dominates seafood production. For instance, marine diseases of farmed oysters, shrimp, abalone, and various fishes, particularly Atlantic salmon, cost billions of dollars each year. Farmed species often receive infectious diseases from wild species and can return infectious agents to wild species. Disease control in marine aquaculture farms is the main concern in all the countries where seafood is a major source of income. The movement of exotic infectious agents to new areas continues to be the greatest concern.

Bacteriocins and bacteriocin producing bacteria isolated from marine environment can play a pivot role in those places where we want to reduce the use of chemical antibiotics. Though the spectrum of action is small for bacteriocins, the probiotic bacteria can serve as an alternative. Thus a better understanding of bacteriocins, their classification and mechanism of action is worthwhile. The use of nisin and pediocin as food preservative is well studied and applied in

many countries. The requirement is still open and hence exploration of marine resources for bacteriocin is still in the lime light.

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Conflict of interest

The author declares no conflict of interest.

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