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Cloning and expression of bacteriocins of *Pediococcus* spp.: A review

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Abstract

Natural food biopreservatives have always's remained the preference of health conscious consumers. This necessity has led to the studies exploring better alternatives which are more acceptable, economical and safer than chemical preservatives. Bacteriocins are one of such compounds produced by lactic acid bacteria which offer a great potential to contribute in food, health and pharmaceutical industry. Present review focuses the complete biochemical, functional and molecular genetic characterization of bacteriocins produced by *Pediococcus* spp. A great deal of diverse heterologous expression systems have been exploited for cloning, expression and purification of pediocins at laboratory scale but data is lacking for industrial processes. Thus, there is an urgent need to design low cost, industrially viable and continuous system in order to exploit these natural bioactive compounds in food and pharmaceutical industry.

Keywords: Pediocin, *Pediococcus*, bacteriocin, probiotic, lactic acid bacteria, cloning

Introduction

Lactic acid fermentations are deliberately exploited to produce various products such as pickled vegetables, bakery items, wine making, fermented meat, sausages and cultured milk products such as yogurts, cheeses, butter, buttermilk, kefir, koumiss etc. Natural lactic acid fermentations are brought about by lactic acid bacteria (LAB) which includes a large group of relatively fastidious, heterotrophic Gram-positive bacteria that produce lactic acid as an end product of carbohydrate fermentation. Core microbial genera of LAB include *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus* and *Streptococcus* which are grouped together in the family *lactobacillaceae*. Their industrial importance is evidenced by their ubiquitous occurrence in natural food products, Genarally Recognized as Safe (GRAS) status, and ability to exert health benefits beyond basic nutrition. LAB display numerous antimicrobial activities which are mainly exhibited due to production of organic acids, bacteriocins and anti-fungal agents [1-6]. Highly promising results are obtained in the studies underlying the functional importance of bacteriocinogenic LAB as starter culture, consortium members and bioprotective agents in food industry that improve food quality, safety and shelf life [7]. Applications of bacteriocin starter cultures and bacteriocin thereof in various food systems are already addressed in a number of review articles [8-11]. LAB is commonly exploited in the dairy industry as producers of flavoring enzymes and metabolites that contribute to naturally rich flavor and texture of foods. A variety of probiotic LAB species including *Lactobacillus acidophilus*, *L. bulgaricus*, *L. lactis*, *L. plantarum*, *L. rhamnosus*, *L. reuteri*, *L. fermentum*, *Bifidobacterium longum*, *B. breve*, *B. bifidum*, *B. esselnsis*, *B. lactis*, *B. infantis* are currently recommended for development of functional food products with health-promoting capacities [12]. Health claims of various LAB strains include normalization of gastro-intestinal [13-14] and vaginal ecosystem [15-16], improvement of specific and non-specific immune responses [17], detoxification of carcinogens and suppression of tumors and cancers [18-20], reduction of blood pressure in hypertensive patients [21] and cholesterol [22]. Importance of LAB in treatment of milk allergies [23] and improvement of mineral absorption capacity of the intestine is also well documented in the literature [24].

Pediocins: The anti-microbial peptides (AMPs)

Pediococci as saprophytes were first isolated and characterized from plants by Mundt *et al.* [25] as catalase-negative, homofermentative bacteria producing lactic acid as a result of sugar fermentation that can tolerate temperature as high as 50°C

[26]. These highly fastidious, non-motile, non-sporulating facultative anaerobes belong to family *lactobacillaceae* with *P. acidilactici, P. pentosaceus, P. damnosus, P. parvulus, P. inopinatus, P. halophilus, P. dextrinicus, P. cellicola, P. claussenii, P. ethanolidurans* and *P. stilesii* as the representative species. *P. pentosaceus* and *P. acidilactici* are commonly used in the fermentation of vegetables [27] and meats [28].

Anti-microbial peptides or bacteriocins are raised as an integral component of the bacterial defense mechanism and have been identified and characterized in a number of prokaryotic organisms. Bacteriocins have long attracted the interest of food sector as potential natural food preservatives against spoilage and pathogenic bacteria. Pediocins produced by various pediococcal species have gained considerable attention because of their remarkable heat stability, activity over a wide pH range, broader antimicrobial spectrum; higher specificity and effectiveness in very low concentrations [1-3, 9, 10]. A large number of pediocins have been isolated and characterized till date. **Table 1** describes production of pediocins by various Pediococcal strains, class they belong to, association of their genetic determinants with small cryptic plasmids, their biochemical characteristics, mode of action and the antimicrobial spectrum.

TABLE 1. Bacteriocins of *Pediococcus* spp.: their classes, genetic and biochemical features, mode of action and antimicrobial spectrum.

Bacteriocin	Producer organism	Class	MW (kDa)	MW of Bac^+ plasmid	Degraded by	Thermostablity (100-120°C)	pH range	Polypeptide nature	Mode of action	Antimicrobial range	References
Bacteriocins produced by *Pediococcus acidilactici*											
Pediocin AcH	*P. acidilactici* H, E, F, M	IIa	4.6	8.9 kb pSMB74	Trypsin, papain, α-chymotrypsin, protease K, ficin, protease IV, XIV & XXIV	yes	2.5-9.0	net +ve charge; pI 9.6; sequence is KYYGNGVTCGKHSCSVDWGKATTCIINNGAMAWATGGHQGNHKC	Bactericidal & Bacteriolytic	*Aeromonas hydrophila, Bacillus cereus, Brochothrix, Clostridium perfringens, C. botulinum, Enterococcus faecalis, E. faecium, E. hirae, Escherichia, Lactobacillus brevis, L. curvatus, L. leichmanni, L. plantarum, L. viridescens, Listeria monocytogenes, L. innocua, L. seeligeri, Lactococcus lactis, Leuconostoc mesenteroides, Micrococcus sedentarius, Pediococcus acidilactici, P. pentosaceus, Pseudomonas putida, Salmonella, Staphylococcus aureus, S. xylosus, Yersinia*	1, 36-43
Pediocin PA-1	*P. acidilactici* PAC1.0 NRRL-5627	IIa	4.6	9.3 kb pRSQ11	Protease, papain, α-chymotrypsin	yes	2.0-10.0	net +ve charge; pI 8.65; sequence is KYYGNGVTCGKHSCSVDWGKATTCIINNGAMAWATGGHQGNHKC	Bactericidal & Bacteriolytic	*B. cereus, L. bifermentans, L. brevis, L. plantarum, L. lactis, L. dextranicum, L. mesenteroides, L. monocytogenes, P. acidilactici, P. pentosaceus*	1, 26, 40, 44
Pediocin PO_2	*P. acidilactici* PO_2	IIa	4.6	5.5 MD	β-chymotrypsin, protease I & XIV, trypsin, lysozyme	yes	-	-	Bactericidal	*B. coagulans, E. faecalis, L. curvatus, L. monocytogenes, L. mesenteroides, S. aureus, Streptococcus faecalis*	45-49
Pediocin JD	*P. acidilactici* JD-123	IIa	-	-	Trypsin	yes	-	-	Bactericidal	*L. monocytogenes*	50-51

Pediocin PC	*P. acidilactici* PC	IIa	-	8.47 kb	Chymotrypsin, ficin, protease, trypsin	yes	4.0-8.0	-	Bactericidal	*C. perfringens, Listeria, Leuconostoc, Pediococcus*	52, 166
Pediocin SJ-1	*P. acidilactici* SJ-1	IIa	4.0	4.6 MD	α-amylase, α-chymotrypsin, trypsin, protease XIV, papain, proteinase K	yes	3.0-9.0	basic polyeptide; pI in alkaline range	Bactericidal	*C. perfringens, L. brevis, L. plantarum, L. leichmanni, L. monocytogenes*	53
Pediocin L50	*P. acidilactici* L50	IIa	5.25	-	Trypsin, papain, protease II, VI & XIV	yes	2.0-11.0	net +ve charge; partial sequence is	Bactericidal	*B. cereus, C. botulinum, C. perfringens, E. faecalis, L. monocytogenes, S. aureus, L. brevis, L. plantarum, L. sake* 148, *L. innocua, L. lactis, L. mesenteroides, P. acidilactici, P. pentosaceus*	54
Pediocin AcM	*P. acidilactici* M	IIa	4.6	-	Trypsin	yes	1.0-12.0	-	-	*A. hydrophila, B. coagulans, B. cereus, C. perfringens, L. monocytogenes, S. aureus*	55
Pediocin F	*P. acidilactici* F	-	4.46	9.1	Many proteases	yes	Wide	Resistant to organic solvents	-	-	56-57
Pediocin CP2	*P. acidilactici* CP2	IIa	4.63	8.9 kb pCP289	α- chymotrypsin, pepsin, papain, proteinase K, trypsin	yes	2.0-9.0	pI 8.85; resistant to many organic solvents	Bactericidal, Bacteriostatic, Antifungal and Spore inhibitory	*Aspergillus flavus, C. sporogenes, E. faecalis, L. brevis, L. bulgaricus, L. mesenteroides, L. monocytogenes, Micrococcus flavus, Neisseria mucosa, P. acidilactici, P. pentosaceus, Pseudomonas putida, P. aeruginosa, Staphylococcus albus, S. aureus, Streptococcus mutans, S. pyogenes*	58-60
Pediocin SA-1	*P. acidilactici NRRL B5627*	IIa	3.66	-	proteinase K , but resistant to trypsin, α-chymotrypsin, pepsin and papain	yes	-	N-terminal sequence: KYYGXNGVXTXGKHSXVDX	Bactericidal	*B. cereus, C. sporogenes, C. thiaminolyticum, E. faecalis, L. brevis, L. bulgaricus, L. casei, L. curvatus, L. jensenii, L. plantarum, L. sakei, L. lactis, L. monocytogenes, L. innocua, L. mesenteroides, M. flavus, M. luteus, P. acidilactici, P. pentosaceus, S. carnosus*	61
Bacteriocins produced by Pediococci other than *P. acidilactici*											
Pediocin A	*P. pentosaceus* ATCC 43200, ATCC 43201	?	80.0	13.6 MD pFBB61, 10.5 MD pFBB63	Trypsin, pronase, proteinase K	Heat labile	-	-	Bactericidal	*B. cereus, C. botulinum, C. perfringens, C. sporogenes, C. tyrobutyricum, E. faecalis, E. coli, L. monocytogenes, L. innocua, L. sake, L. brevis, L. plantarum, L. lactis, L. mesenteroides, P. acidilactici, P. pentosaceus, Salmonella typhimurium, S. aureus*	1, 62-66

Pediocin N5p	*P. pentosaceus*	-	-	-	Acid protease, α- chymotrypsin, pepsin, ficin, papain	yes	2.0-8.0	-	Bactericidal	*Lactobacillus hilgardii, Leuconostoc oenos, P. pentosaceus E5p*	67-68
Pediocin PD-1	*P. damnosus* NCFB-1832	-	≅ 3.5	-	proteinase K	yes	2.0-10.0	pI 3.5	Bactericidal	*Oenococcus oeni,* several food spoilage and pathogenic bacteria	69
Pediocin ISK-1 (nukacin ISK-1)	*Pediococcus* sp. ISK-1	-	-	-	Acid protease, α- chymotrypsin, pepsin, ficin, papain	yes	3.0-8.0	-	Bactericidal	*Bacillus subtilis, L. casei* ssp. *casei, L. lactis, M. luteus, P. acidilactici*	70-71
Pediocin K1	*Pediococcus* sp. KCA1303-10	IIa	4.2	9.1 kb	Pronase, pepsin, trypsin, lipase	yes	2.0-10.0	-	Bactericidal	*E. faecalis, E. faecium, L. monocytogenes*	72
Pentocin L	*P. pentosaceus* L	-	27	-	-	yes	-	-	-	Broad inhibition spectrum, *B. subtilis, B. cereus*	73
Pentocin S	*P. pentosaceus* S	-	25	-	-	yes	-	-	-	Broad inhibition spectrum, *B. subtilis, B. cereus*	73
Pediocin ACCEL	*P. pentosaceus* ACCEL	IIa	17.5	-	α- chymotrypsin, pepsin, trypsin, papain, proteinase K, pronase, bromelain	yes	2.0-6.0	N-terminal sequence: KYYGNGVTXG-KHSXXVDXG	Bactericidal	*B. subtilis, B. cereus, C. perfringens, L. helveticus, L. plantarum, L. monocytogenes L. lactis, P. pentosaceus, S. faecalis, S. epidermidis*	74
Pediocin ST18	*P. pentosaceus* ST18	IIb	-	-	-	yes	2.0-12.0	Resistant to detergents, EDTA and PMSF. It does not adhere to producer cells	Bacteriostatic	*L. innocua, L. plantarum, Pediococcus* spp.	75

Pediocin SM-1	*P. pentosaceus* SM-1	IIa	5.37	-	α- chymotrypsin, pepsin, trypsin, papain, proteinase K	yes	wide	-	Bactericidal	*C. thiaminolyticum*, *C. sporogenes*, *L. monocytogenes*, *L. innocua*, *Pediococcus* spp., several LAB species	61
Pediocin pK23-2	*P. pentosaceus* K23-2	IIa	5.0	-	Many proteases	yes	-	Resistant to organic solvents	-	Gram-positive bacteria, especially *L. monocytogenes*	76
Pediocin 05-10	*P. pentosaceus* 05-10	IIa	<6.5	-	Many proteases	yes	2.0-10.0	It shows adsorption to both resistant and sensitive cells but not to producer cells	Bactericidal	*Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Listeria*, *Pediococcus*, *Streptococcus*	77
Bacteriocin ST44AM	*P. pentosaceus* ST44AM	IIa	6.5	-	Many proteases	yes	2.0-12.0	Resistant to detergents, urea, NaCl and EDTA	Bactericidal	*E. coli*, *Klebsiella pneumoniae*, *L. monocytogenes*, *L. innocua*, *L. ivanovii* subsp. *ivanovii*, *P. aeruginosa*, other LAB	78

Classification of bacteriocins

Five classes of bacteriocins have been established based on the producing strains, common resistance mechanisms, mechanisms of action, molecular weights and chemistry [29-31]. Class I includes post-translationally modified, small lantibiotic peptides containing a number of modified amino acid residues, and it's further divided in to two subclasses. Class Ia groups peptides with a net positive charge that exert their activity through the formation of pores in bacterial membranes (e.g. Nisin). They constitute pfam domains PF05500 and PF04369, in conjunction with F(ND)L(DEN)(LVI), SLCTPGC and SXXXCPTTX-CXXXC motifs [32]. Class Ib mainly consists of post-translationally modified, small globular peptides with a negative or zero charge (e.g. Mersacidin) which's antimicrobial activity is related to the inhibition of specific enzymes. F(ND)L(DEN)(LVI), FTCCS, GXXXTOBX-C motifs and PF05500 and PF04369 pfam domains have been identified in class Ib bacteriocins. Class IIa specifies small, strongly cationic, heat stable, non-lantibiotic, antilisterial pediocin-like peptides with at least one disulfide bridge (e.g. Pediocin PA-I, Pediocin CP2, Pediocin AcH, Enterocin A). N-terminal YGNGVXC, LSXXELXXIXGG and double glycine motifs and PF04604, PF02052, PF01721 pfam domains characterize class IIa bacteriocins. Class IIb bacteriocins require two different peptides of 25 to 65 kDa, constituting domains PF02052, PF01721 and motifs P(RQ)GXXXTOBX-C, LSXXELXXIXGG and double GG for their activity (e.g. Lactococcin G). Class IIc includes remaining cationic bacteriocins of 30 to 65 kDa which are secretory signal-dependent bacteriocins (e.g. Acidocin B). Large heat labile bacteriocins are clustered together under class III (e.g. Helveticin). Fourth class comprises an undefined mixture proteins, lipids and carbohydrates usually more than 10kDa in size. The existence of the fourth class was supported mainly by the observation that some bacteriocin activities obtained in cell free supernatant, exemplified by the activity of *L. plantarum* LPCO10, were abolished not only by protease treatments, but also by glycolytic and lipolytic enzymes [33]. Pediocin SJ-1, pediocin PO_2 and pediocin K1 lost 50% or more acitivity upon treatment with alpha amylase, lysozyme and lipase respectively (Table 1). Thus, a situation of ambiguity arises whether to keep these heat-stable and anti-listerial bacteriocins in class IIa or class IV (Author's own observation). One additional group of circular bacteriocins of 49-108 kDa, carrying two trans-membrane segments were housed in class V and has been described in BAGEL database [31]. BAGEL is a web-based bacteriocin genome mining tool that helps to identify putative bacteriocin ORFs in microbial genomes by extending various *in silico* computational methods using novel, knowledge-based bacteriocin databases and motif databases. Many bacteriocins are encoded by small genes that are often omitted in the an-

notation process of bacterial genomes. Gassericin A, circulatin A, and carnocyclin A are few examples of circular bacteriocins which may carry two trans-membrane segments that facilitate pore formation in sensitive cells [31, 34-35]. Their unique functional activities as well as circular nature make them potential candidates for developing novel antimicrobial agents. Class I and II bacteriocins are produced as pre-bacteriocins and usually processed during their transport through the cytoplasmic membrane at G(SA) and P(RQ) sites and GG, GG P(RQ) and G(GSA) sites respectively.

Mechanism of pediocin action

AMP's are frequently enriched in cationic amino acid residues and interact very strongly with anionic bacterial membranes. They kill sensitive bacteria by punching holes in their cell membranes, causing a disruption in their trans-membrane potential (PMF) and destroying the delicate balance of which the organisms maintain between themselves and their environment [79]. In a study conducted on membrane vesicles derived from both sensitive and immune cells, liposome delivered pediocin PA-1 elicited efflux of small ions in a concentration dependent manner [79]. Higher concentration of pediocin effectively released, higher molecular weighted substances. They frequently adopt conformations where polar and non-polar residues are segregated properly resulting in a typical amphipathic structure that exhibits more peptide internalization and membrane perturbation. Trans-membrane potential (negative inside) in bacteria, acts as a potential driving force for insertion and internalization of the antimicrobial peptides promoting AMP interaction [80]. Pediocin PA-1 exerts bactericidal or bacteriolytic effect depending on the species of the sensitive cells [81]. Pediocins also act on some sensitive bacterial strains in bacteriostatic manner and thus retard further proliferation of the sensitive cells (e.g. Pediocin ST18, pediocin CP2). Antifungal and spore-inhibitory property of a broad spectrum pediocin CP2 has been explored in a study conducted at Department of Biotechnology, Punjabi University, Patiala, India. Antibacterial activity of bacteriocins produced by *Pediococci* is well documented in literature but, none of the earlier report indicates their antifungal property against *A. niger* isolates [82]. Currently scientists are focusing on these deadly workings of AMPs as a new approach to treat bacterial infections [12-17, 21, 83-85]. A study conducted using nisin indicated its effectiveness and efficiency as alternative therapeutic to antibiotics for the treatment of Staphylococcal mastitis [83, 84]. *In vitro* and *in vivo* studies performed with lysostaphin a class III bacteriocin have shown that this staphylococcin has potential to be used, solely or in combination with other antibacterial agents, to prevent or treat bacterial staphylococcal infectious diseases [83]. Nowadays, purified bacteriocins are available and have shown to posssess anti-neoplastic activity. Pyocin, colicin, pediocin, and microcin are among bacteriocins reported to present such activity. Moreover, modified bacteriocins proved to be effective in a glioblastoma xenograft mouse model [85].

Applications of bacteriocin producing LAB in food industry

Foodborne pathogens can multiply rapidly during extended storage at low temperature and under oxygen stress conditions, which make food unfit for consumption. *Aeromonas hydrophila*, *Bacillus*, *Clostridium botulinum* types B and E, *Escherichia coli*, *Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella enterinais, Shigella, Yersinia enterocolitica* have been isolated from refrigerated foods and implicated in outbreaks of foodborne illness [54,86-88]. Strains of mesophilic organisms such as *Salmonella* and *Escherichia* are capable of proliferation in temperature abused (10-12°C) refrigerated systems. *B. cereus* has been well established as a cause of foodborne illness in humans [89-90]. Pathogenicity of *B. cereus* is associated with tissue destructive/ reactive exoenzyme production. It secretes a proteinaceous enterotoxin and induces a diarrheal syndrome. In addition to food poisoning, it causes a number of systemic and local infections in both immunologically compromised and immunocompetent individuals including aplstic anemia, brain abscesses, endophthalmitis, gas gangrene, meningitis, pneumonia and pseudomembranous tracheobronchitis [91]. Many pediocins are effective in controlling growth and multiplication of such foodborne pathogens and spoilage organisms in various food systems (Table 1). Many studies have highlighted the resistance of Gram-negative species to LAB bacteriocins [89, 92]. Skytta *et al.* [93] reported that some selected strains of *Pediococci:* one of *P. damnosus* and two of *P. pentosaceus* synthesize broad spectrum bacteriocins that effectively kill Gram-negative *Y. enterocolitica, P. fragi* and *P. fluorescens* in minced meat. Increased activity of bacteriocins was observed when they were used in combination with other antagonistics factors. A few reports indicated that sublethal injury due to heating, freezing, low pH exposure, ultrahigh pressure, electroporation, presence of chemical bactericidal agents such as sodium acetate, detergents and chelating agents enhance susceptibility of Gram-negative bacteria such as *A. hydrophila*, *S. typhimurium, Y. enterocolitica*, *E. coli*, *P. putida, P. fluorescens* etc. against LAB bacteriocins [42-43, 94-101]. The presence of bacteriocin-producing LAB could act as a potential barrier to inhibit the growth of spoilage bacteria and foodborne pathogens. Bromberg *et al.* [102] tested 813 strains of LAB which were able to inhibit the growth of *Staphylococcus aureus* CTC33 and/ or *Listeria innocua* Lin11 invitro in meat and meat products against a range of Gram-positive (*B. cereus, C. sporogenes, C. perfringens, E. faecalis, L. plantarum, S. aureus*) and Gram-negative (*E. coli, Pseudomonas sp., S. typhimurium*) test organisms and found that, Of these 128 strains showed various inhibition frequencies.

Today consumers' preference for safe, fresh-tasting, ready-to-eat, minimally-processed foods has created the necessity of exploration of novel and natural alternatives to chemical preservatives, which are useful to control development of food spoilage and pathogenic microorganisms in food systems. Nisin is a

good example of food bio-preservative as well as an additional hurdle factor for increasing the shelf-life of minimal processed foods [103]. Antimicrobial substances produced by LAB offer potential applications in food preservation, food safety as well as to develop "novel" foods, health care, and pharmaceutical products [9, 88]. Bacteriocins could be added to canned/packaged food items in the form of concentrated preparations, or they could be produced in situ by bacteriocin producing starter cultures. Immobilized bacteriocins could be exploited to develop bioactive food packaging materials. Foods are considered as highly complex ecosystems where microbial interactions may influence bacteriocin efficacy and proliferation of harmful bacteria. There is a necessity to understand the global effects of bacteriocins in food ecosystems, to study bacterial genomes which may reveal new sources of bacteriocins and to develop genetically engineered food grade expression systems for development of commercial products.

Therapeutic potential of LAB bacteriocins

In the past 4 to 5 decades, use of antibiotics to fight against infectious diseases caused by microorganisms, has lead to dramatic rise of average life expectancy in humans. Unfortunately, the eventual appearance of strains resistant to multiple antibiotics in disease-causing microbes is an increasing public health problem in recent years. Urogenital problems such as bacterial vaginosis, gastrointestinal infections, pneumonia, septicemia and childhood ear infections are just a few of such diseases that have become hard to treat with antibiotics. Very often, bacteria develop several ways to resist antibiotics and other antimicrobial drugs. Other factors such as poor medical facilities, increasing use and misuse of existing antibiotics in human and veterinary medicine and in agriculture has significantly worsened the problem.

Bacterial vaginosis (BV) is one such problem where an inflammation of vagina occurs when the natural microbial balance of vagina is disturbed. Gardner [104] indicated association of bacteria such as *Gardnerella vaginalis, Prevotella bivia, Peptostreptococcus* spp. *Mycoplasma hominis, Mobiluncus* and a yeast strain *Candida albicans* with bacterial vaginosis. BV can have adverse outcomes of pregnancy [105-112] and enhances susceptibility to infection by HIV [113], HSV type 2 [114] and other sexually transmitted diseases. Goldstein *et al.* [115] had demonstrated that resistance of *G. vaginalis* to metronidazole increased to 68% in year 2000. Recurrence rates of up to 30% within three months after treatment have been reported [116].

Helicobacter pylori infection is another problem that affects almost all patients with duodenal ulcers and 70% of cases with gastric ulcers [117]. Pathogen weakens the protective mucous coating of the stomach and duodenum by secreting urease, protease or phospholipases etc. as virulence traits helping colonization of the pathogen. Both acid and bacteria irritate the lining and cause a sore, or ulcer [118]. Peptic ulcers are usually treated by antibiotics, proton pump inhibitors, antiacids and H2 blockers [12, 119-120]. However, emergence of antibiotic resistance in *H. pylori* due to point mutations and decreased binding of the antibiotics to the ribosomes has raised the concern [121-125].

Lactobacillus paracasei CRL1289 shows strong inhibition of *S. aureus* induced urinogenital infection as tested in a mouse model [126]. Probiotic LAB provides best alternative and attractive proposition to get rid of these opportunistic pathogens of vaginal and gastrointestinal tract. Skarin and Sylwan [127] studied growth inhibitory properties of vaginal lactobacilli against bacterial species associated with BV. Lactacin A164 produced by *L. lactis subsp.* A164, lacticin BH5 produced by *L. lactic subsp.* BH5, bulgaricin BB18 produced by *L. bulgaricus* BB18 and enterocin MH3 produced by *Enterococcus faecium* MH3 have shown strong anti-*Helicobacter pylori* activity in laboratory experiments [128,129]. Thus, bacteriocin producing starter cultures are potential candidates for formulating health promoting functional food products or vaginal creams which might be used to contribute a beneficial effect on the balance of intestinal and vaginal microflora respectively.

Probiotics: Best alternative to antibiotic therapy

Prof. Metchnikoff [130] the Nobel laureate of 1908, introduced the concept of probiotics in his book *"The Prolongation of Life"*. He argued that these friendly living bacteria normalize bowel habits, fight against disease-carrying bacteria and extend normal life span. Term "Probiotic" was first introduced by Kollath [131]. Fuller [132] gave a widely accepted definition of probiotics as *"A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance"*. Since their establishment in various food systems, they are widely recommended in rotavirus diarrhea, to get rid of antibiotic-associated side effects, food allergies, lactose intolerance, atopic eczema, irritable bowel syndrome, inflammatory bowel disease, cystic fibrosis, traveller's diarrhea, dental caries, to enhance proficiency of oral vaccines and to reduce incidence of certain cancers [12, 133]. The protective role of probiotics was established in colon [20, 134] and cervical cancer [135].

The indigenous microbiota plays an important role in protecting the host from colonization by opportunistic pathogens. Earlier studies have highlighted the inhibitory affects of the LAB towards BV associated pathogens [127, 136]. *Lactobacillus* is the predominant genus in the vaginal and endocervical microbial communities [137-139]. A number of studies explored the role of bacteriocin-like substances from vaginal isolates of *Lactobacillus* sps. to inhibit the growth of vaginal pathogens like *E. coli, E. faecalis, E. faecium, G. vaginalis*, *Klebsiella* spp., *N. gonorrhoeae, S. aureus* and *Streptococcus agalactiae* [140-142]. Lactocin 160, a bacteriocin produced by a probiotic vaginal *L.*

rhamnosus has been shown to target cytoplasmic membrane of *G. vaginalis* [143]. The potential use of human lactobacilli as probiotics assigned to restore and maintain a healthy urogenital tract represents a promising alternative to conventional chemotherapy [12, 144-161].

Pediocin production: A plasmid linked trait in pediococci

In last two decades, there have been significant advances in functional genomic analyzes of LAB and biochemical characterization of bacteriocins produced by them. Considerable efforts have been made to functionally characterize bacteriocin operons and to express them in heterologous systems [57, 170, 178-195]. Whole genome sequence and sequences of several cryptic plasmids of *Pediococci* bearing genetic determinants for bacteriocin production can be retrieved from GenBank database of NCBI. As far as genetic characterization is concerned, pediocin PA-1 produced by various *P. acidilactici* strains has been studied extensively. Gonzalez and Kunka [26] showed that pediocin PA-1 operon of *P. acidilactici* PAC1.0 NRRL-5627 is located on 9.3 kb plasmid pRSQ11. Bhunia *et al.* [36] isolated a bacteriocin producing strain *P. acidilactici* H from fermented sausage. Subsequently, in their laboratory, they also identified three more Bac^+ strains; E, F and M, from different sources capable of producing pediocin AcH. Pediocin production trait in all of these strains has been linked to 8.9 kb plasmid pSMB74 [37, 162-164]. *P. acidilactici* strains harbour this high copy number plasmid which is generally lost from the cells under stress [1] and could be transferred to plasmidless *P. acidilactici* strains [163]. Plasmid pSMB74 has been completely sequenced, mapped and fragments have been cloned in a pUC119 vector [165]. In *P. acidilactici* SJ-1, only pediocin SJ-1 structural gene is associated with a 4.6 MDa plasmid, but not its immunity factor [53]. Bacteriocin production in *P. acidilactici* PC too is a plasmid linked feature [52, 166]. Few other reports also indicated the plasmid linkage of bacteriocin activity in *Pediococcus* species. Pediocins such as PO_2, PC, SJ-1, L50, AcM, F, CP2, SA-1, PD-1, K1, ACCEL, SM-1, pK23-2, ST44AM, and 05-10 are other examples where association of bacteriocin production trait has been established with small cryptic plasmids [45, 52-58, 61, 69, 72, 74, 76-78]. In *P. pentosaceus,* production of more than 10 bacteriocins has been reported (Table 1). Pediocin A operon in *P. pentosaceus* FBB61 and *P. pentosaceus* FBB63 has been linked to plasmids of 13.6 and 10.5 MDa sizes, respectively [62, 63, 66]. Pediocin A encoding plasmid pMD136 of *P. pentosaceus* ATCC 43200 was characterized by restriction fragment analysis by Kantor *et al.* [167]. Genetic information regarding production of various bacteriocins in *P. pentosaceus* (N_5P, PD-1, ISK-1, ACCEL, ST18, SM-1, pK23-2, 05-10, bacteriocin ST44AM and pentocins L and S) and their immunity factors is currently not available. Plasmid borne characters have a great potential for genetic manipulations and improvement of strains for conventional starter cultures used in biotechnology industry. Their ability to show antagonism against food spoilage and pathogenic microbes opens up scope for the development of food grade biopreservatives and novel therapeutics. At the same time, such plasmid encoded characters are of interest to food technologists as they could be transferred to selected strains of LAB to develop strongly competitive starter culture bacteria which are capable of predominating over natural flora by direct antagonism along with their superior fermentation characteristics.

Genetic organization of pediocin operon

Pediocin PA-1 of *P. acidilactici* PAC1.0 and pediocin AcH of *P. acidilactici* H have been shown to contain a cluster of four genes with common promoter and terminator sequences [40, 168-169]. *PedA* encodes a 62 amino acids long prepediocin PA-1. Eighteen residue long leader sequence from N-terminal of pre-pediocin is removed during processing and export of pediocin through producer cell membrane. Mature pediocin carries 44 amino acid residues and two intra-molecular disulphide bridges at cys9-cys14 and cys24-cys44 positions [46, 170-171]. *PedB* immunity gene is located downstream to *pedA* and encodes a protein of 112 amino acid residues. *PedC* a 174 amino acid long amphiphilic protein involved along with *pedD* protein in facilitating/accelerating the trans-membrane export of prepediocin in *P. acidilactici* [168]. *PedD* gene specifies a polypeptide of 724 amino acid residues. Deletion analysis and site specific mutagenesis of *pedD* resulted in complete loss of pediocin production, showing its essentiality for secretion in *E. coli* [40]. *PedD* sequence show a very high homology to members of ATP dependent transport proteins and also to a group of eukaryotic proteins involved in multidrug resistance [40]. Very high similarity of *pedD* was already established with *HlyB*, an *E. coli* membrane protein required for the export of hemolysin A [172]. *ComA* (required for competence induction in *Streptococcus pneumonia*) is another member of this family of ATP binding protein with high degree of similarity [40]. These proteins carry an ATP binding motif (GMSGSGKTT) [40]. Pediocin AcH is another well characterized pediocin of *P. acidilactici* H linked to *papABCD* operon involving pediocin AcH structural gene (*papA*), immunity function (*papB*), ABC transport proteins (*papC* and *papD*) that play an important role in translocation and processing of active pediocin AcH [170]. Miller and coworker [172] provided experimental evidence by random mutagenesis that all four cysteine residues in pediocin AcH are necessary for its activity, as they play a vital role in stabilization of the secondary structure of this small peptide. His-kinase and C39-protease are other genes usually found associated with bacterioicn operon and are indirectly involved in production and secretion of active bacteriocins by producer organisms [31].

Cloning and heterologous expression of pediocins

Since the establishment of pediocin production as a plasmid linked trait, studies on cloning these plasmids in heterologous systems have started. **Table 2** summarizes all those efforts

TABLE 2. Cloning and expression of pediocins in heterologous systems.

Pediocin	Producer Organism	Vector	Expression Host	Activity	Comments	References
Pediocin PA-1	*P. acidilactici* PAC 1.0	pSRQ11 and pVA891	*E. coli*	+	Linearized pRSQ11 ligated to linearized pVA891	40
Pediocin AcH	*P. acidilactici* H	Shuttle vector pHPS9	*E. coli χ925* and a *ped⁻ P. acidilactici*	+	Transformed minicells of *E. coli* chi 925 require *papA* and *papD* for pediocin AcH production and secretion	168
Pediocin PA-1	*P. acidilactici* PAC 1.0	-	*L. lactis*	+	Expressed successfully under lactococcal promoter	182
Pediocin	*P. acidilactici*	pMC117	*L. lactis* subsp. *lactis* MM210	+	Electro-transformed *L. lactis* subsp. *lactis* MM217 got ped$^+$ phenotype with no alterations in its cheese making properties	194
Chimeric Pediocin AcH-MBP proteins	*P. acidilactici* H	pPR682 pIH821	Periplasmic leaky *E. coli* E609L	+	> 90% reduction in viable cell counts after 24h IPTG induction in pIH821; whereas 10% viability loss reported in pPR682	170
Pediocin PA-1 with lactococcin A promoter and leader sequence	*P. acidilactici*	pFI2058	*L. lactis* IL1403	+	Recombinant displayed 25% pediocin activity. Additional copies of *lcnC* and *lcnD* introduced to raise activity. Nisin and pediocin coexpressed in *L. lactis* IF5876	183-184
Pediocin	*P. acidilactici*	Shuttle vector PST	*S. thermophilus, E. coli, L. lactis* ssp. *lactis, E. faecalis*	+	Expressed under p2201 and *repA* of *S. thermophilus* Production stable up to 10 sub-culturing only	178
Pediocin PA-1	*P. acidilactici* PAC1.0	yT&A Yeast expression vector	*S. cerevisiae* Y294	+	Expressed using yeast ADH1 promoter & MFa1S signal peptide and bactericidal yeast strain developed for wine, baking and brewing industries	192
Pediocin PA-1	*P. acidilactici* 347	pMG36c, pHB04 with P32 promoters	*L. lactis* IL1403	+	*pedA* and *pedB* genes coexpressed successfully with enterocin A in *L. lactis* IL1403 though at very low levels	185
Pediocin F	*P. acidilactici* F	pQE32	*E. coli*	+	T5 promoter based expression and over expressed upon induction with IPTG and purified by Ni-NTA metal affinity chromatography	57
Pediocin P	*P. pentosaceus* Pep1	pHD1.0	*E. coli* JM109	–	Successfully electro-transformed E. coli JM109 but no activity in recombinant cells	57
Pediocin	*P. acidilactici*	pPC418	*L. lactis* ssp. *lactis, S. thermophilus, E. faecalis*	+	Expressed successfully	186
Rec-pediocin with lactococcin A leader sequence	*P. acidilactici*	pFI2391, pFI2436	*L. lactis*	+	Nisin A inducible promoter and lactococcin A secretory apparatus	187

Pediocin PA-1	*P. acidilactici* PAC 1.0	Yeast expression vector	*Pichia pastoris* KM71H	-	Rec-pediocin aggregated with "collagen-like" material, showed less hydrophobicity, an altered isoelectric point and no biological activity.	193
Chimeras of pediocin PA-1, sakacin P, enterocin A, leucocin A and curvacin A.	*P. acidilactici*	pMG36e	*L. sakei* LB790	+	P32 promoter based expression, C-terminal domain of pediocin like bacteriocins is involved in specific recognition of the C-terminal part of cognate immunity protein and determines the antimicrobial spectrum.	190
Rec-pediocin PA-1 with Bifidobacterial α-amylase signal peptide	*P. acidilactici*	pPSAB (E. coli); pPSAB1 (*B. longum*)	*Bifidobacterium longum* MG1	+	Strong antimicrobial activity in E. coli; approx. 90% pPSAB1 stably maintained in *B. longum* MG1 over 20 successive subculturings without an antibiotic stress	191
Pediocin PA-1 fused with His tagged *DHFR* gene	*P. acidilactici*	pQE40PED	*E. coli* M15	+	Over expressed with IPTG and purified by Ni-NTA metal affinity chromatography and recovered by Factor Xa protease digestion	179
Trx-pediocin PA-1	*P. acidilactici* PAC1.0		*E. coli*	+	Thioredoxin-*pedA* fusion protein lacked biological activity, but upon cleavage by an enterokinase gave biologically active pediocin PA-1	180
6XHis-Xpress-*PedA*	*P. acidilactici* K7	pTZ57R/T subcloned in pRSET-A	*E. coli* BL21 (DE3)	+	PT7 based expression, 8 to 10 times higher purification efficiency achieved with Ni-NTA affinity beads; refolded *invitro* using 5mM β-mercaptoethanol and 1M glycine	181
Chimeric pediocin PA-1, enterocin A and other class IIa bacteriocins	*P. acidilactici*	DNA shuffled library		++	Mutant B1 inhibited a pediocin resistant *L. lactis*. Sequence analysis revealed novel N-terminal sequence TKYYGNGVSCTKSGC in strain B1 as compared to KYYGNGVTCGKHSC of pediocin PA-1	195
Pediocin PA-1	*P. acidilactici* K7	Shuttle vector pAMJ	*L. lactis* MG1363	+	P170 promoter based expression	188
Pediocin PA-1	*P. acidilactici* 347	Lactococcin A secretory apparatus	Lactococci	+	Co-produciton of nisin and rec-pediocin PA-1 (*lcnA* leader, propediocin under the control of *lcnA* secretory machinery)	189

made to clone potentially useful pediocins and till date, a number of research groups have reviewed their sources, production, properties, genetic features, food industry applications, antimicrobial properties etc. [2, 64, 101, 173-177]. Pediocin PA-1 has been cloned and expressed in several bacterial strains including *E. coli* [40, 57, 168, 170, 178-181], *L. lactis* [178, 182-189], *L. sakei* [190], *S.thermophilus* [178, 186], *E. faecalis* [178, 186], *P. acidilactici* [168], *B. longum* [191], in baker's yeast *Saccharomyces cerevisiae* [192] and in methylotrophic yeast *Pichia pastoris* [193].

Cloning and expression of pediocin in *E. coli*

E. coli is the organism of choice for production of rec-proteins, enabling the FDA approval of Eli Lilly's recombinant insulin under the trade name Humulin® in 1982. Afterwards the number of US and European biopharmaceutical companies grew tremendously with their ever increasing number of approved recombinant products which were cultivated in *E. coli* systems.

The expanding choice of *E. coli* expression systems for achieving high level production of rec-proteins is empowered by factors such as voluminous knowledge of their physiological and biochemical properties, availability of genetically engineered *E. coli* strains that facilitate formation of correct disulphide bonds in the reducing environment of cytoplasm and yield high product with least proteolytic degradation. A plethora of protease deficient *E. coli* strains (all B strains including B834, BL21, BLR, Origami™ B, Rosetta™, Tuner™ are deficient in *lon* and *ompT* proteases) have been developed with their well known codon usage, as rare codons in the cloned genes can have adverse outcome on levels of protein synthesis. *E. coli* BL21(DE3) is most widely exploited for heterologous gene expression in *E. coli*. BLR(DE3) is a *recA*⁻ mutant of *E. coli* BL21(DE3) which is commonly used to express genes carrying repetitive sequences [196]. *E. coli* C41(DE3) and *E. coli* C43(DE3) are more promising to deal with membrane proteins than native host *E. coli* BL21(DE3). Origami™ B, Rosetta™ and Tuner™ strains are deficient in *lacY* permease which facilitates uniform entry of the IPTG inducer and allows a homogenous level of induction. *E. coli* strains AD494, AD494(DE3), BLR*trxB*, BLR*trxB*(DE3), Origami, Origami™ B and Rosetta-gami™ have mutations in their glutathione reductase (*gor*) and thioredoxin reductase (*trxB*) genes and have been specially designed to support formation of correct disulfide bonds in rec-proteins [197, 198]. Rosetta™ are engineered to supply rare tRNA for the codons AUA, AGA, AGG, CCC, CUA and GGA on a compatible chloramphenicol resistant plasmid [199]. A very high-level expression is offered by a wide variety of tightly regulated prokaryotic promoters and expression systems (pT7Blue, pBlueStar, pRSFDuet, pSMART, pQE32, pQE40, pET32, pTZ57R/T, pRSET-A etc.). There has been a remarkable increase in the availability of fusion partners (such as T7-tag, S-tag, His-tag, HSV-tag, Trx-tag, CBD-tag, GST-tag, Nus-tag, Dsb-tag etc.) with improved protein folding tools. Recombinant proteins could be secreted by tagging with highly specific sequence tags that facilitate their detection by affinity purification, immuno-fluorescence, immuno-precipitation, western blotting. Extensive workout has been done on the mechanism of controlling gene expression and on obtaining biological activity of the proteins in heterologous *E. coli* systems [200].

While designing the expression systems for pediocins, one should be very particular about the natural sensitivity of the LAB against bacteriocins produced by them. Producer organisms have well developed defense machinery that protects the host from self secreted bacteriocins [40, 79, 201-204]. Thus, a need arises to co-express the pediocin immunity protein when production and secretion of the native pediocin is sought in heterologous strains. However, in some bacterial strains immunity function of *pedB* is not required for expression of biologically active pediocin such examples are many strains of *E. coli* showing resistance to pediocins produced by Gram-positive *Pediococcus* species [40, 168]. Shuttle vector pHPS9 bearing *pedA* gene from *P. acidilactici* H has been introduced in *E. coli* χ925. In transformed minicells of *E. coli* χ925, only *papA* and *papD* are required for pediocin AcH production and secretion, as the recombinant cells are highly resistant to pediocin AcH. T5 promoter based expression system consisting of a Novagen vector pQE32 has been used for expression of pediocin F of *P. acidilactici* F in *E. coli*. It was over expressed upon induction with IPTG and his-tagged protein was extracted from cell lysates using Ni-NTA metal affinity chromatography [57]. Thioredoxin-pediocin PA-1 fusion protein has been expressed in *E. coli*. Fusion protein itself did not show any biological activity, but upon cleavage by an enterokinase, biologically active pediocin PA-1 was obtained [180]. In addition, four to five fold increases in production yield was obtained in comparison to pediocin PA-1 produced naturally by *P. acidilactici* PAC 1.0.

Expression of biologically active form of recombinant pediocin in non-native organisms in a soluble form remains a bottle neck. It depends upon survival tendency, propagation and copy number of recombinant plasmid in transformed host, in addition to half life of the rec-protein in an altered environment and osmotic condition of the cytosol. It has been observed that integral membrane proteins of *E. coli* could interfere with growth and viability of the recombinant cells, when pediocin was over expressed [170]. To overcome this problem, *papA* was fused in-frame to secretary maltose binding protein (MBP) of *E. coli* and coned in *malE* vectors pPR682 and pIH82, whose efficient and powerful secretory signals directed very high level synthesis of MBP chimeric protein [170]. About one third of chimeric proteins were secreted into periplasm and released into the culture medium by periplasmic leaky *E. coli* E609L. However, a very high viability loss of >90% in recombinant *E. coli* E609L transformed with pIH821 and of 10% in *E. coli* E609L transformed with pPR682 was observed after 24h of IPTG induction.

Upon over-expression in heterologous systems, rec-proteins may tend to accumulate in inclusion bodies (IBs) of *E. coli* as a result of reducing conditions of the cytosol. To extract an intracellular protein it is necessary to disrupt the cells and separate IBs [205]. IBs are subsequently washed and resolublized for proper folding of rec-proteins [206]. 6XHis-Xpress-*pedA* carrying pediocin structural gene from *P. acidilactici* K7 was cloned in pTZ57R/T [181]. It was further subcloned in pRSET-A for over expression in *E. coli* BL21(DE3). Recombinant pediocin was purified using Ni-NTA beads and eluted with 0.5M imidazole. *In vitro* refolding of rec-pediocin was carried out in redox system consisting of 5mM β-mercaptoethanol and 1M glycine to achieve its biological activity.

The antimicrobial activity of the heterologous expressed pediocin varied from 0 to 10 fold depending on the expression system used. Osmanagaoglu *et al.* [57] successfully electrotransformed *E. coli* JM109 cells with pHD1.0 bearing pediocin P structural gene from *P. pentosaceus* Pep1, but none of the transformant was able to express and/or release pediocin P. To overcome this, rec-pediocin was fused inframe with alpha-amylase signal peptide of *Bifidobacterium* to construct plasmids pSAB and pSAB1 for transforming *E. coli* and *B. longum* MG1, respectively [191]. Recombinant *E. coli* showed strong antimicrobial activity, while 90% of pSAB1 was stably maintained in *B. longum* MG1 over 20 successive subculturings without an

antibiotic stress. Moon and coworkers [179] fused *pedA* with His-tagged *DHFR* in pQE40PED and transformed *E. coli* M15. Recombinants displayed very high pediocin activity upon overexpression with IPTG and subsequently fusion protein was purified by Ni-NTA affinity chromatography. Recovery of the native pediocin PA-1 from fusion product was achieved by digestion with Factor Xa protease. PT7 based expression system offers 8 to 10 times higher yields with great purification efficiency achieved through Ni-NTA affinity beads [181].

Cloning and expression of pediocin in other microbial systems

Heterologous hosts including *S. thermophilus, L. lactis* subsp. *lactis* and *E. faecalis* have been demonstrated for their ability to express pediocin under p2201 and *repA* of shuttle vector PST [178]. The major limitation of these expression systems is the decreased stability (upto 10 subculturings only) of the cloned genes. A chimeric stretch consisting of lactococcin A promoter, lactococcin A leader sequence and pediocin PA-1 structural gene has been introduced in pFI2058 for constructing a recombinant plasmid which was used to transform *L. lactis* IL1403. Recombinant lactococcal strains displayed only 25% pediocin activity. Thus, in an attempt to raise pediocin yields, additional copies of *lcnC* and *lcnD* were co-introduced in recombinant *L. lactis* IL1403. Using same recombinant pFI2058, a nisin producing *L. lactis* IF5876 was also transformed, where nisin and pediocin PA-1 were coexpressed successfully [183-184]. *PedA* and *pedB* genes of pediocin operon from *P. acidilactici* 347 have been successfully coexpressed with enterocin A in *L. lactis* IL1403 using plasmids pMG36c, pHB04 carrying P32 promoters, but resulting pediocin activity detected in recombinant cells was very low [185]. Rec-pediocin with lactococcin A leader sequence was secreted by recombinant *L. lactis* bearing plasmids pFI2391, pFI2436 under nisin inducible promoters and lactococcin A secretory apparatus [187]. P170 promoter based expression system has also been exploited for over-expression of rec-pediocin in *L. lactis* MG1363 using the shuttle vector pAMJ [188].

DNA shuffling technique has enabled construction of chimeric gene sequences carrying desirable traits. Chimeras of pediocin PA-1, sakacin P, enterocin A, leucocin A and curvacin A were generated by shuffling the genes of five different parental bacteriocins. Subsequent cloning of chimeric constructs in P32 promoter based expression vector pMG36e was accomplished and recombinant *L. sakei* LB790 was generated [190]. Results indicated that some of the variants have dramatically more bacteriocin activity than their native bacteriocins. Results also highlighted the involvement of C-terminal domain of pediocin like bacteriocins in specific recognition of the cognate immunity protein and determination of the antimicrobial spectrum of the secreted bacteriocin.

Attempts have been made to express pediocin in yeast strains *S. cerevisiae* and *P. pastoris*, where active disulphide bond formation can take place; however studies showed low levels of expression [192] and inhibition of its biological activity [193]. Aggregation of the rec-pediocin was observed in *P. pastoris* KM71H, due to its association with collagen-like material. These collagen-pediocin aggregates were less hydrophobic and behaved differently when subjected to isoelectric focusing. Rec-pediocin lost its biological activity due to aggregation [193].

Conclusions

Though pediocin is an equally promising biopreservative as nisin is, its indusrial scale production has not been taken up yet. The main reason is lack of a comparable scale of production. To improve its production heterologous systems have been studied which have used a variety of promoters for enhanced expression, secretory proteins for fusion and peptide tags to facilitate purification. Present review compiled the information available to date, giving variety of production enhancing strategies for improving heterologous pediocin production. Apart from its biopreservative potential in foods, pediocin is an attractive antimicrobial agent against many pathogenic bacteria and hence has pharmaceutic application too. As an additive to cosmetics its property to modulate skin microflora needs to be explored. Its probiotic potential in modulating gut microbiota towards cholesterol lowering, antidiabetic and antihypertensive state promises to make it an important component of neutraceutic and wellness products. For all these applications either GRAS grade whole cells, over secreting copious amounts of pediocin or purified pediocin producted at industrial scale can be used. More research into production aspects is needed in near future.

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