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REVIEW

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Bacteriocins from lactic acid bacteria: purification strategies and applications in food and medical industries: a review

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Abstract

Background: Bacteriocins are generally defined as ribosomally synthesized peptides, which are produced by lactic acid bacteria (LAB) that affect the growth of related or unrelated microorganisms. Conventionally, the extracted bacteriocins are purified by precipitation, where ammonium sulphate is added to precipitate out the protein from the solution.

Main text: To achieve the high purity of bacteriocins, a combination with chromatography is used where the hydrophobicity and cationic properties of bacteriocins are employed. The complexity column inside the chromatography can afford to resolve the loss of bacteriocins during the ammonium sulphate precipitation. Recently, an aqueous two-phase system (ATPS) has been widely used in bacteriocins purification due to the several advantages of its operational simplicity, mild process conditions and versatility. It reduces the operation steps and processing time yet provides high recovery products which provide alternative ways to conventional methods in downstream processing. Bacteriocins are widely approached in the food and medical industry. In food application, nisin, which is produced by *Lactococcus lactis* subsp. has been introduced as food preservative due to its natural, toxicology safe and effective against the gram-positive bacteria. Besides, bacteriocins provide a board range in medical industries where they are used as antibiotics and probiotics.

Short conclusion: In summary, this review focuses on the downstream separation of bacteriocins from various sources using both conventional and recent ATPS techniques. Finally, recommendations for future interesting areas of research that need to be pursued are highlighted.

Keywords: Bacteriocins, Probiotics, Chromatography, APTS, Food and medical industry, Food preservative

1 Background

Lactic acid bacteria (LAB) are Gram-positive, rod or cocci-shaped facultative anaerobes which are being increasingly studied for their ability to produce bacteriocin-like inhibitory substances. Bacteriocins are ribosomally assembled peptides that show antimicrobial properties to closely or distantly related bacteria [98, 99]. They are either bacteriocidal or bacteriostatic to obliterate or repress the development of different microorganisms as a way of competition and survival in the microbial community [39].

Bacteriocins produced by each bacteria species are significantly dissimilar from one another in terms of the mode of action, inhibitory spectrum, molecular weight, biochemical properties, and genetic origin [74]. Hence, Klaenhammer, [74] had classified LAB bacteriocins into four distinct classes with further subclasses according to their structural differences: Class I bacteriocins are named lantibiotics (e.g. nisin) due to the presence of the unusual amino acids lanthionines and β-methyllanthionines. Lantibiotics are small (<5 kDa),

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post-translationally modified membrane-targeting peptides,Class II bacteriocins are small (< 10 kDa), membrane-targeting peptides which do not contain lanthionine residues. They are further subdivided into three divisions: (a) *Listeria*-active peptides (e.g. leucocin B-TA1 Ia and pediocin PA-1/AcH), (b) poration complex-forming bacteriocins (e.g. lactococcin G and lactococcin F) which require two peptides for activity and (c) thiol-activated peptides, e.g. lactococcin B whose activity depends on the presence of reduced cysteine residue; Class III bacteriocins are larger (> 30 kDa) and heat-labile usually comes with enzyme activity (e.g. helveticin J, lactacins A and lactacin B); Class IV bacteriocins are convoluted bacteriocins complexed with other chemical moieties like carbohydrates or lipids for their antimicrobial activity (e.g. plantaricin S, lactocin 27 and leuconocin S). This classification scheme had become the backbone for the following categorization of LAB bacteriocins, though different suggestions have been proposed by Kumariya et al. [77].

Bacteriocins work based on a similar mechanism, which is by membrane disruption. Despite the peptide diversity, bacteriocins share a net positive charge which allows the folding into amphiphilic conformation upon interacting with bacterial membranes [146]. In fact, the cationic bacteriocins adsorb to the target cell through the binding to the anionic cell surface receptors like teichoic acid and lipotheichoid acid [104]. From there, bacteriocins dissipate the transmembrane potential and increase the ion permeability through pore formation followed by direct cell lysis, and hence, cell death [154]. Goh and Philip [54] have proved the membrane disruption through the use of SYTOX Green fluorescent dye and a similar conclusion was drawn by Bauer et al. [13] who proposed the cell death was caused by disruption of proton motive force at the cell membrane through the measurement of K^+ efflux.

Nonetheless, these antimicrobial peptides are nature which can be hydrolysed by proteolytic enzymes in the gastrointestinal tract and their effect is strong and rapid even at low concentrations [87]. Recently, different strategies to modify the peptide backbone have been developed to circumvent the low metabolic stability of AMPs and reduce their susceptibility to proteolytic degradation, such as incorporation of D-amino acids, end-tagging by hydrophobic amino acid stretches, intramolecular cyclization, and blocking N- and/or C-terminal ends of the peptide by N-acetylation, C-amidation, or N-pyroglutamate [60, 88]. Several studies had reported D-amino acid substitution can improve the activity of antimicrobial peptides or retain its activity, and more importantly can improve their stability [71],Y. [85, 87]. Thus, bacteriocins produced by LAB were being extensively researched as a safer and promising alternative to chemical additives or antibiotics in various industries. Various strategies were used in the purification of bacteriocin in order to fulfill the demand for large yield and high purity of bacteriocin. Despite major advancements in the development of non-chromatographic separation methods like precipitation and aqueous two-phase separation (ATPS), chromatography continues to be the workhorse of biopharmaceutical industry and serves as the industry gold standard. This review focuses on the pre-purification (precipitation) and purification strategies (chromatography and aqueous two-phase system) of bacteriocins, and its potential application in the food and medical industries.

2 Main text

Precipitation is a pre-purification step for many proteins; hence it is discussed here as a pre-purification process for bacteriocin derived from lactic acid bacteria. Furthermore, two purification strategies have been highlighted, which are chromatography and aqueous two-phase for the purification of bacteriocin derived from lactic acid bacteria. The potential use of bacteriocin in food and medical applications is also reviewed.

2.1 Precipitation

To begin with purification, proteinaceous bacteriocins are first concentrated, corresponding to a reduction in supernatant volume. Proteins are usually precipitated by several methods including the use of ammonium sulphate, polyethylene glycol (PEG) and organic solvents like alcohol or acetone with the most common salting-out agent being the use of ammonium sulphate. Addition of ammonium sulphate increases the ionic strength of solution and decreases the protein solubility hence precipitates out the proteins from the solution [22]. According to Scopes [131], the salt is relatively inexpensive and more readily available compared to PEG hence it is capable of large-scale commercial applications in protein purifications. Besides, ammonium sulphate is very soluble in water and the protein precipitate formed is stable against proteolysis and bacterial degradation.

It is not uncommon to find the use of ammonium sulphate as a protein preliminary purification step in many bacteriocin-related research. For instance, the soluble bacteriocins were isolated from the supernatant of *Lactobacillus salivarius* strain NRRL B-30514 by a combination of ammonium sulphate precipitation and dialysis to produce a crude bacteriocin against *Campylobacter jejuni*. The specific activity increased from 14,000 AU/mg to 29,000 AU/mg with purity of 9.1% [139]. Bauer et al. [13] have purified pediocin PD-1 from *Pediococcus damnosus* NCFB1832 using 70% ammonium sulphate followed by SP Sepharose Fast Flow cation exchange

column. A four-step purification method of ammonium sulphate precipitation (80%), Amberlite XAD-16 column chromatography, Vivaspin size exclusion chromatography and finally reversed-phase HPLC was reported by Goh et al. [54] to purify the bacteriocin A3 from lactic acid bacteria (LAB) *Weissella confusa* A3. The difference in the ammonium sulphate saturation in which protein precipitates is determined empirically, depending on the bacteriocin concentration and molecular weight. Purification of the paracasin SD1 from *Lactobacillus paracasei* SD1 was achieved with 40% ammonium sulfate precipitation followed by chloroform and gel filtration chromatography (Superdex 200 HR 10/30 column) [150]. It was revealed that the specific antimicrobial activity of the paracasin SD1 against *S. mutans* ATCC 25,175 increased from 53.8 AU μg − 1 (in the supernatant) to 46,875 AU μg − 1 (in the active fraction).

Although ammonium sulphate precipitation is popular, there were still limitations in this method. For instance, the lack of clear ammonium sulphate saturation convention in published protocols, incomplete solid form of pellet and the floatation of pellet in high ammonium sulphate concentration [22]. However, some bacteriocins do not precipitate even at a high ammonium sulphate concentration of 75 – 80% since they pose low molecular weight [19]. Other than the floatation of precipitate, the loss of small molecular weight bacteriocin through the dialysis membrane was also a major problem in ammonium sulphate precipitation, causing significant variables in recovery yields as seen in the 10% recovery of plantaricin LR14 [144], 17% recovery of curvaticin L442 [153], 200% recovery for plantaricin S [72] and the 500% recovery of enterocin I [48]. These remarkable increase in bacteriocin activity could be explained by dissociation of protein into its smaller active forms by ammonium sulphate [72]. Some ammonium sulphate precipitates were insoluble in the washing solvents including distilled water, phosphate buffer, Tris–HCl and Triton X20. This problem was resolved by washing with urea or phosphate or acetate buffer containing SDS [133]. Nevertheless, ammonium sulphate precipitation solely is insufficient to achieve high purity of bacteriocin hence the step was usually followed by combinations of cation exchange chromatography, hydrophobic interaction chromatography and reversed-phase high-performance liquid chromatography [93, 161].

Comparing the antibacterial ability of the crude extract from *L. plantarum* zrx03 obtained by ammonium sulphate precipitation and organic solvent extraction, the ethyl acetate extraction method was the optimal solution, which the crude extract obtained had the strongest antibacterial ability. The cell-free supernatant from *L. plantarum* zrx03 was extracted by ethyl acetate, obtaining a crude extraction with a 23.86% recovery and a special activity of 5,775.89 AU/mg against *E. coli* JM109 [82]. Another study also shows that recovery of lacidin from liquid culture of *Lactobacillus acidophilus* OSU133 by the chloroform extraction procedure, compared with ammonium sulphate precipitation method, was > tenfold greater, in which the total recovered AU per L of culture liquid was 4,500,000 [23]. Chung et al. [31] reported that pediocin could be recovered from the culture supernatant of *Pediococcus acidilactici* WRL-1 by mixing with 3 volumes of cold acetone and maintained at − 20 °C for 2 h. The solvent and precipitate were separated by centrifugation, and most (95.2 ± 2.5%) pediocin activity was found in the liquid phase and it was obtained by evaporation. Other solvents (methanol, ethanol, and butanol) were also suitable for this end.

2.2 Chromatography

The purification of bacteriocin-like inhibitory substance (BLIS) using chromatography is shown in Table 1. Generally, protein separation and purification schemes usually include one or more chromatographic steps to achieve the final separation and purification purpose. After peptide concentration, chromatographic methods employing the cationic and hydrophobicity characteristics of bacteriocins were used for further purification [115]. These include ion-exchange chromatography, hydrophobic interaction chromatography, gel filtration chromatography, and reversed-phase high-performance liquid. Plantaricin secreted by *Lactobacillus* spp., enterocin secreted by *Enterococcus* spp. and pediocin secreted by *Pediococcus* spp. have been purified to various degrees of purity and recovery using multi-step chromatographic combinations. However, the understanding of peptide properties and chromatographic conditions is crucial prior to the selection of any chromatographic methods. Some bacteriocins produced in a large initial amount in the culture broth may be ended up with a low purification yield due to the high hydrophobicity of peptide or adsorption of the peptide to the chromatographic column, as seen in the comparison by Guyonnet et al. [58]. For instance, only 10% yield sakacin A was recovered after purifications in affinity chromatography followed by an ultrafiltration prior to reverse-phase high-pressure liquid chromatography (HPLC).

A preliminary step of purification by ammonium sulphate precipitation had shown an almost 90% reduction in yield, leading to a very low final recovery. Ion-exchange chromatography hence has been seen to replace the use of salt precipitation and resulted in the marginal loss of yield from crude bacteriocin [3]. In fact, the use of ion-exchange chromatography alone is able to achieve > 80% final yield. Several studies have shown the purification of

Table 1 Summary of purification methods for purifying bacteriocin produced by lactic acid bacteria

No. of steps	Bacteriocin	Source	Purification method	Yield (%)	Purification fold	References
1	Bacteriocin-like inhibitory substance (BLIS)	*Pediococcus acidilactici* Kp10 isolated from dried curd	ATPS (PEG/Na-citrate)	81.18	8.43	Abbasiliasi et al. [1]
1	BLIS	*Pediococcus acidilactici* Kp10	AMTPS (Triton X-100 (TX-100))	64.3	5.8	Jamaluddin et al. [69]
1	BLIS	*Lactococcus lactis* Gh1	ATPS (PEG2000/Dextran T500)	77.24	2.92	Jawan et al. [70]
1	BLIS	*Lactobacillus bulgaricus* FTDC 1211	PEG 4000 impregnated Amberlite XAD4 resins/ sodium citrate	82.69	3.26	Abdul Aziz et al. [2]
1	Nisin	*Lactococcus lactis* ATCC 11,454	ATPS (PEG/ $MgSO_4$)	–	–	C. Li et al. [84]
1	Nisin	*Lactococcus lactis* ATCC 11,454	ATPS (PEG 4000 and Na_2SO_4)	111.60	–	C. Li et al. [83]
1	Not specified	*Lactobacillus plantarum* ST16Pa isolated from a papaya species	ATPS (PEG/ sodium polyacrylate (NaPA))	93.36	–	da Silva Sabo et al. [37]
2	Bacteriocin LAB-3H	*Latilactobacillus curvatus* LAB-3H	Ammonium sulphate precipitation, cation exchange chromatography	5.21	249.22	Heidari et al. [64]
2	Pediocin PA-1	*Pediococcus acidilactici* PAC 1.0 cultivated in MRS broth	Ion exchange chromatography, hydrophobic interaction chromatography	73	–	Beaulieu et al. [14]
2	Pediocin PD-1	*Pediococcuc damnosus* NCFB 1832 isolated from beer	Ammonium sulphate precipitation, cation exchange chromatography	34	1700	Bauer et al. [13]
2	Pediocin NCDC35	*Pediococcus pentosaceus* NCDC35	Ammonium sulphate precipitation, ion-exchange chromatography	14.4	14.01	Meena et al. [92]
2	Paracasin SD1	*Lactobacillus paracasei* SD1	Ammonium sulfate precipitation, gel filtration chromatography	–	871	P Wannun et al. [150]
3	Enterocin TJUQ1	*Enterococcus faecium* TJUQ1 isolated from pickled Chinese celery	Ammonium sulfate precipitation, reversed-phase chromatography (Sep-Pak C 8) and cation-exchange chromatography	–	35.89	Qiao et al. [118]
3	Enterocin LD3	*Enterococcus hirae* LD3	Ammonium sulphate precipitation, ion-exchange chromatography, gel filtration chromatography	0.37	13.2	Gupta et al. [57]
3	Plantaricin GZ1-27	*Lactobacillus plantarum* GZ1-27	Ammonium sulphate precipitation, gel-filtration chromatography, and RP-HPLC	0.1	10.55	Pei et al. [110]
3	Bacteriocin LF-BZ532	*Lactobacillus fermentum* BZ532	Ammonium sulphate precipitation, cation-exchange chromatography, and RP-HPLC	0.85	4.2	Rasheed et al. [121]
3	Fermencin SD11	*Lactobacillus fermentum* SD11	Ammonium sulfate precipitation, gel filtration chromatography, and reverse-phase high-performance liquid chromatography	–	7,526	Phirawat Wannun et al. [151]
3	Plantaricin KL-1X	*Lactobacillus plantarum* KL-1	Ammonium sulphate precipitation, ion-exchange chromatography, RP-HPLC	0.004	0.04	Rumjuankiat et al. [127]
	KL-1Y			0.8	10.5	
	KL-1Z			0.1	0.9	
3	Sakacin A	*Lactobacillus sakei 2675*	Ion exchange chromatography, hydrophobic interaction chromatography, RP-HPLC	10	–	Guyonnet et al. [58]

Table 1 (continued)

No. of steps	Bacteriocin	Source	Purification method	Yield (%)	Purification fold	References
	Sakacin P	*Lactobacillus sakei 2525*		50	–	
	Enterocin A	*Enterococcus faecalis 336*		66	–	
	Pediocin PA-1	*Pediococcus acidilactici 1521*		25	–	
4	Bacteriocin-zrx01	*Lactobacillus rhamnosus zrx01* isolated from Kefir	Ammonium sulphate precipitation, ion-exchange chromatography, gel filtration chromatography, RP-FPLC	1.76	8.5	Pei et al. [110]
4	Paracin 54	*Lactobacillus paracasei* ZFM54	Ammonium sulphate, strong cation-exchange chromatography, G-25 gel column, and RP-HPLC	1.93	45.64	Zhu et al. [161]
4	Enterocin HDX-2	*Enterococcus faecium* HDX-2 isolated from pickled Chinese cucumber	Ammonium sulphate precipitation, ion-exchange chromatography, hydrophobic interaction chromatography, RP-HPLC	6.38	56.61	Du et al. [43]
4	Plantaricin ZJ5	*Lactobacillus plantarum* ZJ5	Ammonium sulphate precipitation, ion-exchange chromatography, hydrophobic interaction chromatography, RP-HPLC	1.7	139.5	Song et al. [138]

bacteriocin such as Enterocin AS-48 and Pediocin PA-1 could be purified without the need for pre-purification step of ammonium sulphate precipitation as presented in Table 1 [3, 14, 53, 147]. Hence, ammonium sulphate precipitation is unnecessary prior to ion-exchange chromatography because it is reliable for the purification of crude supernatant. Another advantage of this method is the reusability of column matrix. Abriouel et al. [3] had regenerated and reused the matrix for 20 times without any significant loss of its properties on the purification of enterocin AS-48. Ion-exchange method was also combined with other purification methods to resolve the peptide loss which is a problem of ammonium sulphate precipitation and complex chromatographic protocol. For instance, Enterocin LD3 from *Enterococcus hirae* was purified using ion-exchange chromatography and gel filtration chromatography, Enterocin B from *Enterococcus faecium* was purified using cation-exchange chromatography and reverse-phase high-performance liquid chromatography, bacteriocin from *Lactobacillus murinus* AU06 was purified using cation exchange chromatography and hydrophobic interaction chromatography (Table 1). Cheigh et al. [30] had used expanded bed ion-exchange chromatography to purify nisin Z from the unclarified culture broth of *Lactococcus lactis* to 90% recovery and 31-fold purity. This method is simple, cost-effective and reported to give higher recovery of bacteriocins compared to conventional methods by reducing the number of purification steps.

Gel filtration chromatography is a common purification method of bacteriocin, which purifies bacteriocins based on their molecular mass. Because the molecular masses of bacteriocins vary widely, various gel columns, such as Superdex, Sephadex and Sepharose, are available (P [150, 153]. Purification by hydrophobic interaction chromatography was able to greatly increase the specific activity, providing a very high purification fold but contritely almost halving the yield [45]. To counter this issue, the protocol of Gaussier et al. [53] was modified by Beaulieu et al. [14]. The Sep-pack C_{18} column was replaced by a scalable and more efficient octyl-Sepharose column in the purification of pediocin PA-1. HCl was used as the peptide eluent instead of trifluoroacetic acid (TFA) in the hydrophobic and reversed-phase high-performance liquid chromatography columns because the presence of TFA contamination may interfere with the protein structure; HCl is relatively easy removed by a low-coating filtration membrane and did not affect on the protein yield and characterization. The change of column and eluent had increased the amount of pediocin purified, to an overall yield of 73% which is much higher than the 38% recovery by the former, 34% by Bauer et al. [13], 25% by Guyonnet et al. [58] and 15% by Meena et al. [92].

Reversed-phase high-performance liquid chromatography (RP-HPLC) is usually the final step of peptide purification to homogeneity yet is usually associated with loss of activity [97]. Todorov et al. [145] have compared two purification methods in the purification of plantaricin ST31 with method one being ammonium sulphate precipitation, Sep-pack C_{18} cartridge and RP-HPLC and method two utilizing only cation exchange chromatography with 0.8% and 5.9% yield, respectively. The small activity recovery of method one was due to half of the plantaricin ST31 purified by RP-HPLC being converted to its oxygenated form, thus reducing the final yield by 50%. The longer procedure of method one also contributed to the lower yield of plantaricin ST31.

In general, chromatographic methods pose several drawbacks in terms of the lengthy procedures and low yields, usually less than 20% [125]. The pricey equipment required at each step also made the purification difficult to scale up [44]. The more the number of steps, the more peptide were lost during the process as seen in the recovery by 4-steps purification protocol as shown in Table 1, in which the yields of bacteriocins (Enterocin HDX-2, Plantaricin LR14, Paracin 54, Plantaricin ZJ5, and Bacteriocin-zrx01) were in the range from 1.7 to 6.4%. Other than that, the complex composition of the culture medium not only has important effects on the bacteriocin production, but also affects the downstream purification scheme. For instance, [14] had reported difficulties in the chromatographic capturing of bacteriocin from complex supernatant containing high levels of unwanted medium peptides. The use of a semi-defined medium could reduce the excess level of contaminants while providing sufficient nutrients to sustain the growth and production of the bacteriocin-producing strain.

Overall, chromatographic purification employed the cationic and hydrophobicity characteristics of bacteriocins. An ideal protocol for bacteriocin purification should be simple, rapid, low production and recovery cost, and applicable for large-scale production to achieve more than 50% yield and more than 90% purity. The complexity of chromatographic combinations had urged the development of a more efficient and robust procedure to handle a larger amount of bacteriocins from the upstream process.

2.3 Aqueous two-phase system

Throughout the years, the aqueous two-phase system (ATPS) has gained attention in the separation, extraction and purification of biological products [68]. There are two types of major ATPS systems: polymer/polymer system and polymer/salt system. The polymer polyethylene glycol (PEG) is commonly used in both systems due to its low cost and its ability to enhance protein refolding for

higher recovery. The polymer/polymer system (e.g. PEG/dextran) is high cost and high viscosity compared with the polymer/salt system (e.g. PEG/phosphate) which is low cost, low viscosity and provides wider hydrophobicity range of difference [111]. Thus, the polymer/salt system is more preferred in the ATPS bioseparation process. The polymer phase separation occurs when two hydrophilic polymers exhibit incompatibility and become immiscible in aqueous solution above a critical concentration. The critical concentration for phase separation to occur depends on several factors including the type of phase-forming polymer, ionic strength, pH, and temperature of the solution [114]. In a polymer/salt system, the wide density difference between polymer and salt gives rise to the separation of the polymer-rich fraction to the top phase whereas the salt-rich fraction to settle as bottom phase [124]. Although ATPS has been well-established, the studies on the purification of bacteriocin was limited.

The purification of bacteriocin-like inhibitory substance (BLIS) using ATPS is shown in Table 1. Abbasiliasi et al. [1] had attempted to purify a BLIS from the lactic acid bacteria *Pediococcus acidilactici* Kp10 using ATPS as an alternative method to reduce the lengthy, costly and complicated purification steps of conventional chromatographic methods. An ATPS system of PEG/sodium citrate was used by the researchers who had successfully recovered 81.18% of BLIS from the PEG-rich top phase. Citrate was used instead of the common phosphate and sulphate salts because their presence in the effluent poses serious issues to the environment. Citrates are biodegradable, non-toxic and have lower euphoric potential which is more environmentally friendly to be used [1, 134]. Jawan et al. [70] had successfully purified the BLIS from *Lactococcus lactis* Gh1 using PEG2000/Dextran T500 based ATPS. ATPS composed of 10% (w/w) PEG2000 and 8% (w/w) dextran T500, provided the greatest conditions for the extractive BLIS production with purification fold of 2.92 and yield of 77.24%. An ATPS was used to grow *Lactococcus lactis* to relieve lactic acid inhibition, simultaneously producing the nisin. The nisin production (803 IU ml^{-1}) was enhanced by 33% using ATPS, which composition was 11% (w/v) PEG 20,000 and 3.5% (w/v) $MgSO_{4\cdot7}H_2O$ [84]. In 2001, C. Li et al. [83] had conducted ATPS using PEG and sodium sulphate (Na_2SO_4) for nisin recovery, and it was found that the nisin recovery was greatly improved under optimal composition which consisted of 15.99% (w/w) PEG 4000 and 15.85% (w/w) Na_2SO_4 (pH 2), and the optimal ATPS allowed an 11.60% increase of nisin recovery.

The partition of the target protein to either the PEG-rich top phase or salt-rich bottom phase is based on the interaction of hydrophobic interaction and electrostatic forces between the two phases and the protein. Several factors were known to influence the biomolecule partition in ATPS including the molecular weight of polymer used, pH of the system, volume ratio of top to bottom phase and the presence of neutral salts in the system [119].

For the effect of polymer molecular weight, an increase in PEG molecular weight had enhanced the purification of biomolecules as reported by Abbasiliasi et al. [1]. The highest purification factor was recorded using PEG 8000 in both cases as a higher PEG molecular weight indicates more units of PEG involved in biomolecule partitioning due to the increased hydrophobic interaction between polymer and biomolecule. Next, pH of the system not only affects the charge of solute, but also affects the solubility of proteins [8]. Since PEG is positively charged, the negatively charged proteins are more attracted to the PEG phase due to a higher electrostatic difference at higher pH [114, 141].

Besides, the volume ratios of top to bottom phases of the system also influence the ATPS biomolecule partition although located at the same tie-line with the same phase composition. Volume ratio larger than 1 contains diluted PEG hence it is a less effective extractant whereas volume ratio smaller than 1 contains concentrated PEG hence it is a more effective extractant [124]. A lower volume ratio has reduced the partition of undesired protein into the free volume of top phase thus resulted in higher purification factor of target protein [1, 134]. However, if end-product inhibition is a consideration during bacteriocin production, a larger volume ratio could be effective for the in-situ removal of inhibitory end-product [29]. C. Li et al. [84] employed a PEG 20,000/$MgSO_4 \bullet 7H_20$ system with a higher volume ratio of 1.71:1 instead of 0.45:1 to allow the partition of inhibitory lactic acid into the top phase, remaining the nisin-producing *Lactococcus lactis* cells in the bottom phase.

The presence of neutral salts in the system alters the partition of biomolecules due to the differential distribution of salt ions between the top and bottom phases [55]. Improvement of bacteriocin partition in the presence of sodium sulphate and choline-based salts has been reported by da Silva Sabo et al. [37]. The group of researchers employed a PEG 10,000/sodium polyacrylate (NaPA) system for the bacteriocin partition from a clarified fermentation broth of *Lactobacillus plantarum* ST16Pa. The addition of salts into the system had boosted the product yield as well as highest partition coefficient at $K = 32$ although the volume ratio was kept lower than 1. The presence of negatively-charged salts created stronger electrostatic forces in the system which then improved the partition of negatively-charged bacteriocin to the top phase.

ATPS is a new purification method with several advantages in terms of its operational simplicity, mild process conditions and versatility. ATPS has been used as a simple yet powerful single-step purification method to minimalize the number of purification steps. For instance, recovery of BLIS from *Pediococcus acidilactici* Kp10 using ATPS only required one step to recover 81.18% of BLIS [1]. A similar outcome was also drawn by other studies on ATPS for bacteriocin purification as presented in Table 1. Hence, ATPS can simplify the overall lengthy purification protocol by reducing the number of unit operations and processing time yet providing high recovery and purity of products [111]. Next, ATPS provides a mild process condition in which the degradation of proteins at the interface could be minimized. Rapid mass transfer is allowed between the two hydrophilic phase-forming polymers due to low interfacial tension. Residues of neutral salts like choline added to the system to boost product recovery are also very mild and is not a concern to consumers as choline has been used in animal feeds as a dietary supplement [68].

Although ATPS has achieved high recovery and purity yields, there is still a gap between their bench-scale use and potential industrial applications. Hence, ATPS is also a versatile technique in which it can be combined with other techniques to achieve higher yield and purity, as well as scaling up. For instance, the application of ABS in the recovery of biomolecules can be extended to the large-scale bioprocesses by incorporating the two-phase principle in a bioreactor operation for enhanced productivity in the integration of upstream and downstream processing as compared to laboratory scale, which uses test tube or falcon tube (Fig. 1). Besides, membrane-supported liquid–liquid extraction (MEMEX) is an integrated separation method based on ATPS and membrane extraction (Fig. 2). A hydrophobic membrane contactor is added to the ATPS, enabling the extraction of biomolecules without the need of phase separation. Lysozyme and bovine serum albumin (BSA) were successfully extracted by [12] using MEMEX in the presence of a surfactant Tween 20. Since emulsion formation during phase separation was avoided, MEMEX is capable of continuous extraction without centrifugation. For better separation of bacteriocin in ATPS, MEMEX technique can be considered in the future. Next, aqueous two-phase floatation (ATPF) is the combination of ATPS and solvent sublation in which a stream of nitrogen gas bubbles is sparged from the bottom of an ATPS (Fig. 3). The biomolecules are adsorbed on the surface of ascending nitrogen gas bubbles and then released to top phase as the bubbles burst. ATPF was introduced by Sidek et al. [134] for the separation of BLIS from a fermentation broth of *Pediococcus acidilactici* Kp10. The method had reported lesser consumption of organic solvents and better separation efficiency compared to ATPS and solvent sublation. Therefore, integration of ATPS with other techniques had enhanced the current separation methods by having the benefits from both techniques, making these methods more sophisticated and promising alternatives to the conventional separation methods in downstream processing.

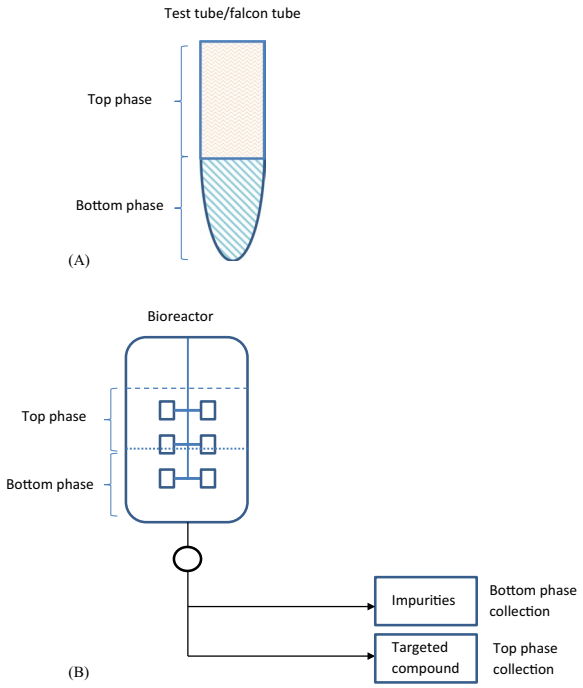

Fig. 1 Schematic diagram of (**a**) laboratory ATPS and (**b**) ATPS bioreactor

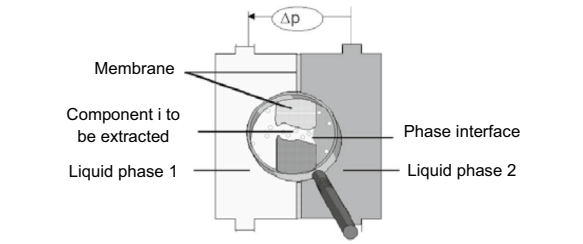

Fig. 2 Principle of membrane-supported liquid–liquid extraction (MEMEX) as explained by [123]. ΔP (Delta P) is a mathematical term symbolizing a change (Δ) in pressure (P).

2.4 Applications in food industry

Food is a major source of energy for living organisms. However, spoilage can easily occur if the food was improperly prepared, sterilized or canned at the

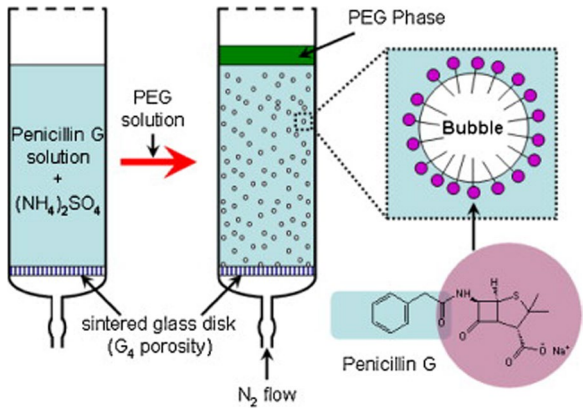

Fig. 3 Separation of penicillin G in the aqueous two-phase system (ATPF) by (Bi et al. 2009)

manufacturing facility and even during transportation to the markets. The increase in international food trade and human travel result in foodborne outbreak, leading to serious health problems and great loss in economics all over the world [65]. Challenges in food safety are higher due to the increasing market demand for higher quality, more natural and minimally processed food [40]. The use of biopreservatives to replace chemical preservatives in food has been adopted by food manufacturers using various organic and naturally occurring antimicrobials [12]. These include organic acids such as lactic acid and citric acid, salts of organic acids such as potassium sorbate and potassium benzoate, chelating agents such as EDTA, proteins such as bacteriocin, lactoferrin and reuterin and plant essential oils such as carvacrol and grape seed extract.

Bacteriocins are gaining interest among scientists as a natural biopreservative in the food industry due to their proteinaceous nature and minimal impact on the nutritional and sensory properties of food [73, 117]. In fact, nisin A is a permitted and commercially available food additive under various companies as Nisaplin®, Chrisin® and Delvoplus® [41]. Nisin is a bactericidal antimicrobial produced by *Lactococcus lactis* subsp. *lactis* known to be effective against Gram-positive bacteria and is widely used for the control of foodborne pathogens including *Listeria monocytogenes* [66] and *Clostridium botulinum* [50]. Nisin acts by pore formation at the cellular membrane, causing the leaching of important cellular compounds including K^+ ions, amino acids and ATP, eventually leading to cell death [63, 117]. There are a few potential applications of nisin in the food industry, including the combinations of nisin with other antimicrobial agents or physical food processing methods and the use of nisin in the antimicrobial packaging of food products.

2.4.1 Combination of nisin and other antimicrobial agents

Due to its limitation to only Gram-positive bacteria, nisin has been combined with other antimicrobial agents to maximise its inhibitory spectrum [27]. This hurdle technology allows the additive or synergistic effect between both preservation factors while minimising the addition of chemically synthesized preservatives. For instance, the activity of nisin against Gram-positive foodborne pathogens is higher in the presence of other natural antimicrobial agents [21]; the dual antimicrobial control of foodborne pathogens also enabled the bactericidal activity of nisin against Gram-negative bacteria at lower concentrations compared to individual antimicrobial agents when used alone [100]. The combination of bacteriocins nisin and pediocin AcH has also been long suggested for overcoming resistant strains [61]. The examples of additive or synergistic effects between nisin and other biopreservatives are summarised in Table 2. Nisin has combined with organic compounds like diacetyl to inhibit the growth of *Enterobacter sakazakii* and *L. monocytogenes* [101]. Combination with an essential oil such as grape seed extract, green tea extract, etc. also showed a good synergistic effect against *L. monocytogenes* [135]. Besides that, nisin could be used together with organic acids or other bacteriocins as dual antimicrobial control of foodborne pathogens [20, 113].

2.4.2 Combination of nisin and high-pressure processing methods

Besides, combined treatment of nisin and high pressure has not only shown its potential in microbial reduction but also in the inactivation of bacterial endospores (Table 3). Bacterial spores are more likely to remain in food even after sterilization because of their high resistance to radiation, heat and chemicals [132], hence causing the spoilage of food and are related to foodborne diseases. The action of nisin alone is more sporostatic than sporicidal, due to the presence of an outer coat and cortex protecting the inner membrane [75, 76]. Therefore, high hydrostatic pressure is applied to enhance the nisin inactivation of spores by destabilizing the membrane structure, allowing the penetration of nisin to its site of action, where pore formation occurs [18]. In fact, the transmission electron micrographs of *Bacillus subtilis* spores by E. Black et al. [17] and Rao et al. [120] have illustrated the higher sensitivity of spores to nisin due to sublethal injuries to the outer coat and cortex by high pressure and moderate heat. This hurdle technology is feasible alternative to heat pasteurization of milk [5] whereby lower pressure, lower temperature, shorter processing time and natural preservative are needed to ensure the commercial sterility and quality of milk and milk products [51].

Table 2 Examples of antimicrobial substances showing dual antimicrobial inhibition with nisin

Antimicrobial substance	Target organism(s)	Substrate	References
Organic compound			
Diacetyl	*Enterobacter sakazakii*	Broth	S. Y. Lee et al. [81]
	L. monocytogenes,	Saline	O'Bryan et al. [101]
Essential oil			
Grape seed extract	*L. monocytogenes*	Cooked shrimp	Zhao et al. [160]
Green tea extract	*L. monocytogenes*	Mixed fruit and vegetable smoothie	Casco et al. [25]
Carvacrol	*L. monocytogenes*	Bologna sausage	Churklam et al. [32]
Oregano	lactic acid bacteria	Grass carp	Zhang et al. [159]
Perilla frutescense	*S. aureus, E. coli, S. enteritidis*, and *P. tolaasii*	Strawberry	Wang et al. [149]
Organic acids			
Citric acid	*L. monocytogenes*	King oyster mushrooms	Yoon et al. [156]
Potassium sorbate	*E. coli* O157:H7	Strawberry puree	Santos et al. [129]
Lactic acid	*E. coli* O157:H7	Enoki mushrooms	Yoon et al. [156]
Chelating agents			
EDTA	*Salmonella*	–	Özdemir et al. [108]
	Salmonella Typhimurium	–	Yüksel et al. [157]
Other bacteriocins			
Buforin I	*B. subtilis, S. epidermidis,* and *A. oryzae*	–	Roshanak et al. [126]
Pediocin	*L. monocytogenes*,	Vegetables	Bari et al. [11]
Lactocin AL705	*Listeria innocua* 7	Broth and a hard cheese	Verdi et al. [148]
Lactoferrin	*L. monocytogenes*, *E. coli*	Broth	Murdock et al. [96]
Endolysin	*L. monocytogenes*	Queso Fresco	Ibarra-Sánchez et al. [67]

Table 3 Examples of the application of nisin under high-pressure conditions showing increased microbial inactivation

Treatment	Target organism(s)	Substrate	Inactivation	References
High pressure and nisin	*E. coli* and *P. fluorescens*	Skim milk	> 8 log CFU/mL reduction	E. P. Black et al. [18]
	L. innocua and *E. coli*	Carrot juice	7-log CFU/mL reduction	Pokhrel et al. [116]
	S. aureus	Cheese	7 log CFU/g reduction	Capellas et al. [24]
	S. aureus	Milk cheese	> 7 log CFU/g reduction on day 3	Arqués et al. [7]
	L. innocua and *E. coli*	Avocado dressing	> 7 log CFU/mL reduction	Manolya E Oner [106, 107]
	S. Enteritidis	Broth	8 log CFU/mL reduction	J. Lee et al. [79]
	S. Enteritidis	Broth	> 9 log CFU/mL reduction	Ogihara et al. [103]
	Escherichia coli	Green juice (celery stalk, apple, cucumber, parsley)	7 log CFU/mL reduction	Manolya E Oner [106, 107]
	Mesophilic spore count	Liquid micellar casein concentrates	2 log CFU/g reduction	García et al. [52]
	Spores of *A. acidoterrestris*	Apple juice	6.15 log CFU/g reduction	Sokołowska et al. [137]
High pressure CO_2 (HPCD) and nisin	Spores of *B. subtilis*	Broth	4.1 log CFU/mL reduction	Rao et al. [120]
	Spores of *B. subtilis*, *G. stearothermophilus*	Metal plate	> 7 log CFU/mL reduction	da Silva et al. [35]
High hydrostatic pressure (HP), moderate heat and nisin	Spores of *B. sporothermodurans*	Broth	5 log CFU/mL reduction	Aouadhi et al. [5]
	Spores of *C. perfringens*	Milk	6 log CFU/mL reduction	Y. Gao et al. [51]
	Paenibacillus sp. and *Terribacillus aidingensis* spores	UHT milk	6 log CFU/mL and 4 log CFU/mL reduction	Kmiha, et al. [75, 76]

2.4.3 Antimicrobial packaging of food products

On the other hand, antimicrobial packaging is a multifunctional hurdle technology employing antimicrobial agents into packaging materials, aiming to improve food preservation by protecting it from external environments and inhibiting the undesired growth of spoilage bacteria at the same time [6, 140. Having a GRAS (generally regarded as safe) status, nisin has been widely immobilized [9] or coated onto packaging matrices in different forms (Table 4). The consistent migration or direct contact of nisin on packaging to the food would result in a rapid, irreversible extension of lag phase and retardation of the proliferation of surface contaminant [46], 152]. However, antimicrobial packaging is still facing several challenges. Firstly, the kinetics governing bacteriocin release from the polymeric matrix is still unclear, leading to the uncontrolled release of nisin from packaging [130]. This results in inconsistent control of bacteria flora and rises the chance of resistance development by the survivors. The unpredictable release of bacteriocins is also ascribed to pH and temperature and substrate water content and hydrophobicity of the environment [91]. Shelf-life and stability of the bioactive films are also a concern in developing an active antimicrobial packaging as the degradation of film is ascribed to uncontrolled release of antimicrobial agents [95]. In short, the potential of antimicrobial packaging can be extrapolated by further understanding the complex kinetics and factors influencing the antimicrobial effectiveness in real food systems [9].

The advances in bioengineering and molecular biology enable the production of recombinant bacteriocin with higher bactericidal activity, which is an adjunct in expanding its inhibitory spectrum [102] and overcoming the development of nisin-resistant mutants [10]. In fact, Smith et al. [136]has proved that a bioengineered nisin derivative, M21A when combined with food-grade additives citric acid or cinnamaldehyde, was able to eliminate the *Listeria monocytogenes* biofilm grown on Tryptic Soy Broth compared to wild-type nisin A. The group of researchers have suggested the substitution of nisin A in commercial Nisaplin® with M21A to augment the bactericidal activity in food products. Nevertheless, additional studies on bioengineered bacteriocins in terms of their production and working conditions and safety are paramount before it can be accepted by the food industry and consumers [112].

2.5 Medical industry

Antibiotics are secondary metabolites of microorganisms that show a broad spectrum of inhibitory activity against other microorganisms. The first antibiotic, penicillin was discovered by Alexander Fleming in 1928, leading to its large-scale production and dissemination for disease control worldwide [141]. Since then, exclusive reliance and overuse or misuse of antibiotics have been ascribed with the emergence of antibiotic-resistant pathogens, jeopardizing the efficacy of currently available antibiotics and consequently posing a huge menace to human health [143]. Therefore, the search for alternative prophylactic

Table 4 Examples of the incorporation of nisin in the antimicrobial packaging of food products

Packaging material	Target organism(s)	Substrate	References
Cellophane surface	Various Gram-positive bacteria	Chopped meat	Guerra et al. [56]
Soy lecithin nanovesicle capsules	*L. monocytogenes*	Milk	da Silva Malheiros et al. [36]
Polyvinyl alcohol-Alyssum homolocarpum seed gum (PVA-AHSG) films	-	–	Marvdashti et al. [90]
Chitosan-cellulose film	*L. monocytogenes*	Cheese	Divsalar et al. [42]
Palmitoylated alginate-based films and beads	*S. aureus*	Beef	Millette et al. [95]
Corn-zein-based coating	*L. monocytogenes*	Hybrid striped bass	Hager et al. [59]
Mater-Bi film	*Listeria monocytogenes, Salmonella enteritidis, Escherichia coli,* and *Staphylococcus aureus*	–	Lopresti et al. [86]
Chitosan-polylactic acid (PLA) composite film	*E. coli* and *S. aureus*	Fish fillet	Chang et al. [28]
Poly(vinyl alcohol)-based nanofibers (NP) with polyethylene (PE)	Total Mesophilic Bacteria	Rainbow trout fillets	Oner et al. [105]
Nisin-chitosan coated on vinyl acetate-ethylene copolymer	*L. monocytogenes*, *M. flavus*, *E. coli*	Milk, Orange juice	C. H. Lee et al. [79]
	Aerobic bacteria, Yeast	Milk, Orange juice	C. H. Lee et al. [80]
ZrO2/poly (vinyl alcohol)-wheat gluten antimicrobial barrier film	*Staphylococcus aureus*	–	Pang et al. [109]
Hydroxypropylmethylcellulose	*Staphylococcus aureus* and *Listeria innocua*	Mozzarella cheese	Freitas et al. [49]

agents is of utmost importance to overcome the problematic issue of microbial resistance [4].

Lactic acid bacteria as probiotics are non-pathogenic, live microorganisms capable of conferring beneficial effects on the host when consumed in adequate amounts. Bacteriocins produced from probiotic bacteria (*Pediococcus*, *lactobacilli*, *bifidobacteria* and *enterococci*) are widely researched for their clinical applications in the prevention and/or treatment of gastroenteritis infections (Table 5). These bacteriocins have gained extensive attention as a potential solution to antibacterial shortcomings as they have a relatively narrow and specific killing spectrum without compromising the surrounding microflora and their proteinaceous nature which is sensitive to digestive enzymes in the GI tract. [38, 78, 82]. More specifically, narrow-spectrum bacteriocins target specific types of bacteria, such as Gram-negative or Gram-positive bacteria. For example, nisin has a narrow spectrum inhibiting only Gram-positive bacteria, including *Clostridium botulinum*, *Listeria monocytogenes*, *Micrococcus*, *Staphylococcus*, etc. Nisin does not generally inhibit gram-negative bacteria, yeasts, or molds [41]. The spectrum of activity of nisin can be expanded to include gram-negative bacteria when it is used in combination with other antimicrobial agents. In fact, bacteriocins when used alone or in combination with other antimicrobial agents are shown effective to inhibit nosocomial or antibiotic-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).

EntDD14 produced from *Enterococcus faecalis* 14, alone or in combination with methicillin successfully reduced by ~30% the biofilm formation capability of MRSA-S1 strain and impacted on the expression of the main genes involved in biofilm formation like nuc and pvl which code, respectively, for nuclease and Panton-Valentine leucocidin. Similar effects were registered for other genes such as cflA, cflB and icaB, coding for bacterial ligands clumping factors A, B and intercellular adhesion factor, respectively [16]. Millette et al. [94] have demonstrated that a reduction of 1.85 log10 CFU/g of VRE was measured at 3 days post-infection in VRE-colonized mice after treatment with the pediocin PA-1/AcH producing *P. acidilactici* MM33. Levels of VRE in the treated mice were undetectable at 6 days postinfection, which indicates pediocin PA-1/AcH can reduce VRE intestinal colonization. Lauková et al. [78] also reported that Enterocin A producing *E. faecium* EK13 protects the digestive tract of rabbits and reduced the counts of *E. coli* and Enterobacteriaceae. Other than that, nisin has been shown to be an effective agent for the treatment of staphylococcal mastitis during lactation. A solution of the bacteriocin nisin (6 μ/mL) was applied to the nipple and mammary areola of the women with clinical signs of *staphylococcal mastitis* and no clinical signs of mastitis were observed among the women of the nisin group on day 14 [47]. Clostridium difficile-associated diarrhoea (CDAD) is a common diarrhoea and is a major type of gastroenteritis infection for the elderly. The results by Rea et al. [122] indicate that lacticin 3147 has potential for the treatment

Table 5 Examples of the bacteriocin in the clinical applications

Bacteriocins	Producing microorganism	Target organism(s)	Clinical application	References
Pediocin PA-1	*P. acidilactici* UL5	*L. monocytogenes*	Prevent intestinal infection in mouse model	Dabour et al. [38]
Bacteriocin CC2	*Enterococcus faecium DSM 20,477*	*Streptococcus mutans*	Prevent oral cavity	Ng et al. [98, 99]
Bacteriocin AS-48	*Enterococcus faecalis* UGRA10	*Trypanosoma cruzi*	Chagas treatment	Martín-Escolano et al. [89]
Bacteriocin AS-48	*Enterococcus faecalis* UGRA10	*Propionibacterium acnes*	Treatment of dermatological diseases	Cebrián et al. [26]
Pediocin PA-1/AcH, Nisin Z	*L. lactic* MM19, *P. acidilactici* MM33	VRE	Reduce intestinal colonization by VRE in mouse model	Millette et al. [94]
Plantaricin EF	*L. plantarum*	*Mucispirillum* and *Parabacteroides*	Treatment of acute inflammatory bowel disease	Yin et al. [155]
enterocin DD14	*Enterococcus faecalis* 14	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Treatment of multidrug-resistant bacteria	Belguesmia et al. [16]
Nisin	*L. lactis* ESI 515	*S. aureus, S. epidermis*	Treatment of staphylococcal mastitis during lactation in human	Fernández et al. [47]
Nisin	*Lactococcus lactis subsp. lactis*	SW1088 tumor cell line	Anti-cancer therapy	Zainodini et al. [158]
Bacteriocin-like inhibitory substance	*Enterococcus faecium* DSM 20,477	*Streptococcus mutans, Candida albicans*	Prevention of dental caries	Ng et al. [98, 99]

of C. difficile infections. The addition of lacticin 3147 to faecal fermentation demonstrated that lacticin is effective in completely eliminating C. difficile from a model faecal environment within 30 min when present at concentrations as low as 18 μg/mL. Bacteriocin also helps in oral health by inhibiting *Streptococcus mutans* and *Candida albicans* [98, 99]. *Streptococcus mutans* is found to be the most virulent species that acts as a dental caries initiator and it was found to have a synbiotic relationship *C. albicans* synergizes in plaque-forming in human oral environment, which makes them more resistant to the human oral environment. BLIS produced by *Enterococcus faecium* CC2 has shown to be effective against these oral pathogens with 76.46% of inhibition.

As potential antimicrobial agents, few bacteriocins have been commercially applied. Although bacteriocins are generally thought to be non-toxic for mammalian cells, enterococcal cytolysin has shown toxicity at high concentrations [34]. Further toxicity tests are vital when considering the clinical feasibility and commercial relevance of bacteriocins. This is because the sensitivity of bacteriocins can differ significantly in vitro and in vivo due to limited understanding of the interaction between bacteriocins, host flora and pathogens in different conditions, and most of the experiments were based on gnotobiotic models which do not represent the actual interactive niche [15]. Next, the concomitant perturbation of indigenous commensal microflora entailed by transient introduction of bacteriocins comes with an unknown long-term effect to the host GI tract [33]. Many studies have been done to investigate toxicity and biosafety of bacteriocins. For example, the enterocin AS-48 produced by Enterococcus strains, when administered to BALB/c mice at concentrations of 50, 100, and 200 mg/kg in the diet for 90 days showed no lethal effect and no toxic effect. Another example is the bacteriocin TSU4 from *Lactobacillus animalis*. This bacteriocin was demonstrated to be safe in a sub-chronic toxicity test in BALB/c mice [128]. On the other hand, the toxicological tests of two bacteriocins (plantaricin E and F) that have been heterologously produced in *L. lactis* NZ3900 showed that these bacteriocins are non-toxic compounds that can be considered as a strong antibiotic candidate [62]. Other than the toxicity testing, the stability and survival of bacteriocins under acidic GI conditions is also a key criterion during the selection of a desired therapeutic agent (Dunne et al. 2001). The future of bacteriocins lies not only in their discovery but also in their testing for toxicity to prove their safe use in a preclinical phase as candidates for therapeutic processes.

3 Conclusions

This study explored the purification methods of bacteriocin, as many researches have proved that bacteriocin has a strong antibacterial activity and is a broad-spectrum bacteriostatic substance, which has high thermal stability and pH stability. From previous studies, there are pros and cons of each purification method. Selection of methods for the bacteriocins purification and quantification is quite wide. Conventional method is still the preferred approach in the current industries, and yet other alternative approaches have been intensively studied. However, a few-step procedures, combining various extraction, precipitation chromatographic, and other methods, is necessary, as the amount of bacteriocin in culture media is quite low. An alternative purification method such as ATPS, which exploited the size, charge, and hydrophobic properties of the target bacteriocin, with a lower cost of purifying materials, was employed. In recent, the bacteriocin research has shown a clear upward trend in response to the potential applications of these antimicrobial peptides in the field of food and medicines. However, there are still less bacteriocins to be marketed and approved for its use in food preservation and treatment for infectious diseases as compared to antibiotic. This may be due to the high cost of purification of bacteriocin, which reduces the demand for bacteriocin. Future work on the application of other purification methods at cheaper cost should be up scaled, in order to convince the industries to apply the method. Therefore, the purification processes have to be tested on a larger scale to develop this natural and high-efficiency preservatives, which can be well used in the food and medical industries.

Abbreviations

LAB: Lactic acid bacteria; ATPS: Aqueous two-phase system; BLIS: Bacteriocin-like inhibitory substance; TFA: Trifluoroacetic acid; RP-HPLC: Reversed-phase high performance liquid chromatography; PEG: Polymer polyethylene glycol; CHO: Chinese hamster ovary; BSA: Bovine serum albumin; ATPF: Aqueous two-phase floatation; GI: Gastrointestinal.

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PGY drafted the work or revised it critically for important intellectual content, ZWL revised the intellectual content, JST made substantial contributions to the conception or design of the work. All authors read and approved the final manuscript.

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