



Cite this: *RSC Adv.*, 2017, 7, 29395

## Fermentation factors influencing the production of bacteriocins by lactic acid bacteria: a review

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Lactic acid bacteria (LAB) are of major interest in the food industry primarily by virtue of their biopreservative properties. LAB have ability to produce various types of antimicrobial compounds, the most important being bacteriocins. Bacteriocins and bacteriocin-producing cultures have the potential to increase the shelf-life of foods and contribute towards decreasing the incidence of food-borne diseases. In this respect, food preservation through *in situ* production of bacteriocins by LAB introduced into the food system would be the most logical approach. However, there is a need to understand the relationship between bacterial growth and bacteriocin production in various types of food system. Bacteriocin production by LAB is dependent on a number of factors such as the types of carbon and nitrogen sources and their concentrations in the media formulation. Other factors which need to be considered are the culture conditions which include pH, temperature and aeration which greatly influence the cultivation performance of bacteriocins producing LAB. Economic aspects pertaining to the optimization of fermentation process for the enhancement of bacteriocin production should also be given due considerations. Failure to acknowledge or recognize this hidden economic element would be a substantial financial loss to the industry especially from the point of view that the product is costly and highly sought after. Thus, the fermentation factors which influence the production of bacteriocins by LAB and the approaches to improve the production not only in term of yield and productivity but also in term of economic and regulation are reviewed in this paper.

Received 2nd October 2016

Accepted 27th March 2017

DOI: 10.1039/c6ra24579j

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## Introduction

The demand for foods with minimum processing or foods without chemical preservatives is fast gaining momentum in view of increasing concerns on health.<sup>1</sup> It is well known that various pathogenic and spoilage microorganisms, already present in foods could survive and multiply when there is minimal processing and/or in the absence of preservatives. In

this respect natural biopreservatives such as bacteriocins which are non-detrimental to human health, have surfaced as an alternative to processing and the use of chemical preservatives in foods.

Bacteriocins are extracellularly released and ribosomally synthesized low molecule mass peptides or proteins with bactericidal or bacteriostatic mode of action, in particular against a wide range of mostly closely related Gram-positive bacteria and even against food-borne pathogens, but the producer cells are immune to their own bacteriocins.<sup>2</sup> The use of bacteriocins or bacteriocins producing lactic acid bacteria (LAB) with a wide range of antimicrobial activity could improve safety, control the fermentation microflora, accelerated maturation and increase the shelf life of the products, inhibit the growth of certain pathogenic bacteria during the fermentation and ripening periods, which overall improve the safety aspects of these products.<sup>3,4</sup>

Bacteriocins are known to be produced by many Gram-positive and Gram-negative microorganisms. However bacteriocins produced by Gram-positive microorganisms such as LAB are preferred principally attributed to their preservative properties especially in the food industry. This preference is also partly due to their broader inhibitory spectrum compared to

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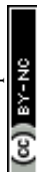
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that of Gram negative microorganisms. Applications of bacteriocins in the food industry had markedly increased with increasing concern in the use of chemical preservatives (*e.g.* nitrites) which are detrimental to human health. Bacteriocins are also generally regarded as safe (GRAS) substances which could be used as food additives or natural preservatives.

The search for bacteriocins with the ability to inhibit the growth of bacterial pathogens such as *L. monocytogenes* is of particular interest in the food industry. LAB could inhibit the growth of the pathogens through the activity of bacteriocins synthesized by these microorganisms. Many strains of LAB have been referred to the European Food Safety Authority (EFSA) for safety assessment without raising any safety concerns. Consequently, they have been included in the QPS (Qualified Presumption of Safety) list authorized for use in the food and feed chain within the European Union. The same applies to the US, where they display the GRAS status assigned by the U.S. Food and Drug Administration (FDA).<sup>5</sup> Thus the identification and characterization of bacteriocin-producing LAB has grown rapidly in the last decade.<sup>6</sup> Bacteriocins had demonstrated remarkable diversity in their effects on numerous bacterial species. Bacteriocins produced by LAB are considered as safe natural biopreservatives in view of the fact that proteases in the gastrointestinal tract could easily degrade it,<sup>7</sup> hence harmless to the human body and surrounding environment.<sup>8,9</sup> From the point of view of the industry there is a need for an inexpensive, large scale bacteriocin production suitable for various food applications.<sup>10,11</sup> Bacteriocin utilization as a preservative could be in two ways; (i) incorporation of bacteriocin-producing-starter culture, and the incorporation of bacteriocin extract or in the pure form. The latter would require an optimal and efficient fermentation which is heavily dependent on factors which are both specific and multiple for maximum production of bacteriocin<sup>12</sup> with low cost and efficient extraction and purification methods. From the commercial standpoint, the priority will always be the production of the highest quality, at the lowest cost and in the shortest possible time.

Bacteriocin production by LAB usually follows primary metabolite growth-associated kinetics which occurs during the exponential growth phase and ceases once stationary phase is reached.<sup>13</sup> This implies that bacteriocin production is dependent on the bacterial total biomass. However, a high cell yield does not necessarily result in a high bacteriocin activity since the latter may be limited by a low specific bacteriocin production, *i.e.* a low bacteriocin production per gram of cells.<sup>14</sup> Thus there exist a rather complex relationship between environmental conditions and bacteriocin production.<sup>15</sup> Relationship between bacteriocin production and growth also depend upon the strain used. In some cases a correlation exist between peptide and biomass production,<sup>16</sup> while in other cases bacteriocin production only starts when stationary phase is reached.<sup>17</sup> The yield per unit biomass is influenced by several factors which include the producing strain, medium composition (carbohydrate and nitrogen sources, cations, and *etc.*), fermentation conditions (pH, temperature, agitation and aeration) as well as mode of fermentation (batch, fed-batch and continuous fermentations).<sup>18</sup> Unfavorable conditions referred

to as stress factors are also known to markedly affect bacteriocin production.<sup>19</sup>

Optimization of fermentation conditions is a complex approach but critically essential for high performance bacteriocin production at commercial scale. In order to develop high performance fermentation process, a better understanding of the influencing factors that affect growth of bacteriocin-producing LAB and their ability to produce bacteriocin is essential. The influencing factors may be strain dependent and could vary with different types of bacteriocin. The effects of two important factors – medium compositions and cultivation conditions, which influence bacteriocin production during fermentation of various LAB strains<sup>20</sup> are discussed in this review.

## Effect of medium composition

### Complex media

Abundant selection of complex media (CM) for the cultivation of LAB are available in today's market. These include the de man rogosa and sharpe (MRS), brain heart infusion (BHI), NaLa (sodium lactate), M17 and trypticase soy broth yeast extract (TSBYE).<sup>21</sup> The complex basal media which are commonly used in the cultivation of LAB for bacteriocin production are summarized in Table 1. Most media are designed for specific strains, *e.g.* M17 for lactococci and MRS for lactobacilli. These media promote exuberant growth and enhance bacteriocin synthesis. However, the use of these media could be uneconomical for industrial application due to their high cost, specific preparation steps and long incubation time. Cost is primarily due to the expensive nitrogen sources such as beef extract, yeast extract and peptone.<sup>22</sup> There are also unutilized proteins at the end of fermentation which could affect the efficiency of the subsequent downstream processing for bacteriocin extraction and purification.

Being the growth media for a broad range of bacteria, these complex media are also not optimal in composition and concentration for use in specific strains for bacteriocin production processes. These limitations include the availability of certain essential molecules which are required for cell metabolism, the production of organic acids that cause a reduction in culture pH resulting in antimicrobial effects, lack of nutrients during exponential growth, lack of essential minerals such as Fe<sup>2+</sup> and Ca<sup>2+</sup>, as well as lack in different carbon sources which are required or preferred by some LAB strains. These limitations are due to the fact that LAB strains have a wide range of variations in their growth requirements and that cause much of complexity forming general growth media for LAB. On the other hand, low nutrient concentrations may cause fast depletion in the essential nutrient which may negatively affect growth whereas high nutrient concentration such as salts could also negatively affect growth or could be insoluble in water.<sup>23</sup> Based on the above information, optimization of medium formulation or compound is required for improvement of bacteriocin production.

In view of the fact that bacteriocin production by LAB is growth-associated, it can be suggested that its production could be improved with the improvement of cell growth through the





Table 1 Complex media commonly used for cultivation of LAB and bacteriocin production

Culture media	Producing strain	Bacteriocin	Remarks	Reference
Diluted whey, concentrated whey, MRS	<i>P. acidilactici</i> NRRL B-5627 and <i>L. lactis</i> subsp. <i>lactis</i> CECT539	Pediocin and nisin	Nutrient sources were not adequate to increase production in diluted whey. Growth and productions in whey were significantly lower compared to MRS broth	Guerra <i>et al.</i> <sup>64</sup>
MRS, TGE, BHI, Elliker, TSB, APT, tomato juice broth, M17 + lactose, M17 + glucose	<i>L. actococcus lactis</i> subsp. <i>lactis</i> A164	Nisin-like bacteriocin	Production was 4-fold higher in M17 supplemented with lactose compared to other carbon sources	Cheigh <i>et al.</i> <sup>71</sup>
APT broth, Elliker broth, M 17 broth, MRS broth, M-MRS broth, M-MRS + 2% Glu broth	<i>E. faecium</i> NKR-5-3	Enterocin	M17 broth gave the highest cell density and production	Wilaipun <i>et al.</i> <sup>139</sup>
MRS broth and LAPTg (peptone + tryptone + glucose + yeast extract + Tween 80)	<i>L. salivarius</i> CRL 1328	Bacteriocin of <i>L. salivarius</i> subsp. <i>salivarius</i> CRL 1328	Highest growth was obtained in MRS while highest production was obtained in LAPTg	Tomás <i>et al.</i> <sup>99</sup>
Feather meal, industrial fibrous soybean, dried cheese whey powder, grape bagasse BHI	<i>B. licheniformis</i> P40	BLIS P40	Highest production was obtained with cheese whey followed by industrial fibrous soybean residue. Production was not detected in feather meal and grape bagasse	Cladera-Olivera <i>et al.</i> <sup>31</sup>
MRS, M17 and BHI	<i>L. lactis</i> subsp. <i>lactis</i> ST34BR	Bacteriocin ST34BR	Production in MRS was higher compared to BHI and M17	Todorov & Dicks <sup>140</sup>
BHI, M17, soy milk, molasses, MRS supplemented with (tryptone, saccharose or vitamin C)	<i>E. faecium</i> ST311LD	Bacteriocin ST311LD	Low level of activity was recorded in BHI, M17 and in soy milk. Production was not recorded in molasses. The highest production was obtained in MRS	Todorov & Dicks <sup>141</sup>
MRS broth, BHI broth, M17 broth, soy milk, molasses	<i>L. plantarum</i> (ST23LD and ST341LD)	Bacteriocin (ST23LD and ST341LD)	Highest production was obtained in MRS. Low activity was recorded in M17, BHI, soy milk and molasses, despite relatively good growth	Todorov & Dicks <sup>92</sup>
M17 and MRS	<i>P. acidilactici</i> KKKU 197	Bacteriocin KKKU197	<i>P. acidilactici</i> KKKU 197 displayed higher activity when grown in MRS compared to M17, although cell growth was similar	Arbuwan <i>et al.</i> <sup>142</sup>
MRS broth, BHI broth, M17 broth, soy milk, skim milk and molasses	<i>L. plantarum</i> (JW3BZ and JW6BZ) <i>L. fermentum</i> (JW11BZ and JW15BZ)	Bacteriocins (bacJW3BZ and bacJW6BZ) bacteriocins bacJW11BZ and JW15BZ	Low production was recorded in BHI and M17. Highest production was obtained in MRS	Mollendorff & Wilhelm <sup>143</sup>
TGE and MRS	<i>P. acidilactici</i> H	Pediocin Ach	TGE resulted in higher production levels compared to MRS broth	Papagianni & Anastasiadou <sup>144</sup>
MRS and TGE	<i>P. acidilactici</i> H	Pediocin Ach	Production in MRS was about 15% lower compared to TGE, although the final cell concentration was greater in MRS	Khay <i>et al.</i> <sup>145</sup>
Improving MRS (trypton, yeast extract, glucose, Tween, pH = 6), normal MRS	<i>E. durans</i> E204	BLIS E204	Production was two-fold higher in improved MRS compared to normal MRS. Cell growth in improved MRS was 1.15 times higher compared to MRS	Khay <i>et al.</i> <sup>145</sup>

optimization of growth medium formulation such as carbon/nitrogen ratio. This is supported by a report that the amount of carbon (glucose) and nitrogen source greatly influenced the bacteriocin synthesis during the growth cycle of *L. mesenteroides* L124 and *L. curvatus* L442.<sup>24</sup> Another study showed that the optimization of medium formulation based on CM medium was successfully applied to increase the volumetric bacteriocin activity by *L. lactis* by two-fold.<sup>21</sup> Medium optimization is not only important for enhancing bacteriocin production but it is also an important factor in terms of cost reduction. Culture medium could account for up to 30% of the total production cost in commercial fermentations.<sup>25</sup>

Optimization of formulation of growth medium is one of the key factors that need to be considered in the enhancement of any fermentation processes. Medium formulation for industrial scale fermentations should fulfill a number of criteria: it should be cost-effective, high product yield, short fermentation time and ease of downstream purification processes.<sup>26</sup> However, the medium that gives the highest product yield may not in most cases be the most cost-effective. Choice of the preferred medium is therefore dependent on the situation and in most cases a trade-off between the different factors. Formulation of medium is not only aimed at improvement of bacteriocin production but also to stabilize its production. Medium components such as sodium chloride (NaCl), ethanol and high carbon source concentrations have been used to stabilize bacteriocin production.<sup>20</sup> Medium pH has also been shown to significantly affect bacteriocin stability.<sup>27</sup>

Some medium components are used to induce stressful environment that could enhance bacteriocin production. This is brought about by either stabilizing the bacteriocin or by preventing the aggregation of bacteriocin molecules. NaCl and ethanol are two components that have the above-mentioned effects but with variable results. The presence of NaCl and ethanol was inhibitory for the production of some bacteriocins while stimulatory in others.<sup>28</sup> Verluyten *et al.*,<sup>29</sup> reported that stress conditions due to nutrient limitation also could stimulate curvacin A production by *L. curvatus* LTH 1174.

Sugars, vitamins and nitrogen sources could be added to the culture medium as supplements to maximize bacteriocin production. Under normal circumstances there is usually an improvement of bacterial growth by the addition of supplements in the medium. However oversupply could lead to the inhibition of both bacterial growth and bacteriocin production. Alternatively the best-adapted culture medium could be formulated for maximum bacteriocin production. LAB is fastidious nutritional requirement microorganism. The fastidious characteristics of LAB could influence on nutritional requirements and metabolic capacity. In addition, fastidious nutritional requirements may also limit the ability to optimize and control the metabolic activities of LAB.<sup>23</sup>

The medium rich in yeast extract and protein hydrolysates is required for growth of LAB and good bacteriocin production. Good cell growth and bacteriocin production are complementary to one another.<sup>30</sup> However, high bacteriocin production need not necessarily depend on optimal cell growth.<sup>31,32</sup> Although it has been generally accepted that bacteriocin

production requires a complex medium,<sup>33</sup> relatively simple medium could also be used for high production of bacteriocin. Many studies have determined the nutritional requirements of various bacterial strains in a completely defined medium<sup>34–37</sup> but only very limited number of bacteriocins have been produced using a defined medium.<sup>38</sup> In general, a semi-synthetic media containing complex peptidic sources such as MRS, Tryptone glucose yeast extract (TGYE) or all purpose tween (APT) are required for bacteriocin production.

### Agriculture, food and industrial wastes

The high cost of protein sources invariably poses a major problem with respect to application of specific technology for commercial applications. At the same time waste products from the food industry could be an environmental problem due to their high biochemical oxygen demand (BOD). Hence the incorporation of protein-rich food wastes in the culture media for the production of bacteriocin using LAB could play a major role towards solving the waste disposal problem while at the same time a techno-economically viable approach could be adopted for the production of bacteriocin on a commercial scale.<sup>39</sup>

The commercial media such as ATP, MRS and TGYE which are the common media for the production of bacteriocins and their metabolites by LAB are characterized by their components. These media, normally rich in salts and proteins varies in concentration and diversity of origin. These media are expensive and not balanced in term of C/N ratio and other components. Thus, they are not suitable for industrial applications and may not support the production of the target bacteriocin. Since the C/N ratio is not balanced, removal of the unconsumed protein materials which still remain in the culture at the end of fermentation is still a problem in the discharge of the liquid waste and at the same time hinder the purification of the bacteriocins from the culture broth.<sup>40</sup>

The use of low-cost protein fractions will bring about a cost reduction in large-scale production processes. The use of food waste generated by the processing of resources from marine origin to obtain the protein fractions will enable a close productive cycle: recycling of a pollutant waste and obtaining products (bacteriocins) with high added value useful for preservation of foodstuffs.<sup>41</sup>

Inexpensive medium for bacteriocin production by LAB could be formulated using industrial waste or by-products such as molasses, soy, hydrolysed wheat flour, cheese whey and corn steep liquor (CSL).<sup>26,42,43</sup> A number of criteria should be considered when opting to use industrial waste in the formulation of fermentation medium. These include the carbon or nitrogen content, price, availability and level of impurities. Research conducted on the use various wastes in medium formulation for bacteriocins production are summarized in Table 2. Food-based industrial by-product media rich in carbon and/or nitrogen with reduced cost have been used for bacteriocin production by LAB with reasonable success.<sup>31</sup> In spite of the high level of impurities but being of food origin these low-cost media have made bacteriocins production most appropriate for application in the food industry.



Table 2 The use of various wastes in medium formulation for production of bacteriocins by LAB<sup>a</sup>

Wastes	Bacteriocin	Producing strain	Reference
WP + 0.2 M KCl WP + 1% (w/v) YE and 0.1% (w/v) Tween 80 MPW	<i>L. lactis</i> UL719	Nisin Z	Desjardins <i>et al.</i> <sup>146</sup>
DW	<i>L. lactis</i> subsp. <i>lactis</i> CECT 539 <i>P. acidilactici</i> NRRL B-5627	Nisin Pediocin	Guerra & Castro <sup>147</sup>
OP	<i>L. lactis</i> subsp. <i>lactis</i> CECT 539 <i>P. acidilactici</i> NRRL B-L. <i>lactis</i> 5627	Nisin Pediocin	Guerra <i>et al.</i> <sup>64</sup> Guerra and Pastrana <sup>148</sup>
CW	<i>L. lactis</i> <i>P. acidilactici</i>	Nisin Pediocin	Vázquez <i>et al.</i> <sup>149</sup>
Demineralized whey protein fermentation liquor	<i>L. lactis</i> subsp. <i>lactis</i> ATCC 11454 <i>L. lactis</i> DPC3147	Nisin Lacticin 3147	Liu <i>et al.</i> <sup>46</sup> Crispie <i>et al.</i> <sup>150</sup>
CP	<i>L. lactis</i> subsp. <i>lactis</i> ATCC 11454	Nisin	Liu <i>et al.</i> <sup>151</sup>
WP	<i>L. plantarum</i> UG1	Plantaricin UG1	Enan & Amri <sup>152</sup>
V and FMR	<i>L. lactis</i> CECT 539 <i>P. acidilactici</i> NRRL B-5627	Nisin Pediocin	Vazquez <i>et al.</i> <sup>49</sup>
FBE	<i>L. lactis</i> subsp. <i>lactis</i> ATCC 11454		Furuta <i>et al.</i> <sup>153</sup>
SW	<i>L. lactis</i> UQ2	Nisin	Gonzalez-Toledo <i>et al.</i> <sup>154</sup>

<sup>a</sup> WP: whey permeate; DW: deproteinized whey; YE: yeast extract; MPW: mussel-processing waste; TGE: tryptone/glucose extract; OP: octopus peptone; CP: cull potatoes V V & FMR: visceral and fish muscle residues; CDS: condensed distillers soluble; FBE: fermented barley extract; SE: sweet whey.

Whey is a by-product of the dairy industry and contains rich nutrients such as lactose, soluble proteins and minerals salts. Unfortunately whey and its nutritional qualities have traditionally been treated as waste and represent an important disposal and pollution issue because of its high biological and BOD. Consequently, it is of interest to use this by-product as a fermentation substrate for the production of value-added products.<sup>44</sup> Substantially high nisin activity was obtained after 9 h batch fermentation supplemented with whey.<sup>45</sup> The use of a mixed culture of *L. lactis* and *Saccharomyces cerevisiae* to whey-based medium to stimulate the production of nisin have been reported.<sup>46</sup> Cheese whey has also been successfully used for bacteriocin production by several LAB strains.<sup>47</sup> Potatoes juice could be used as alternative substrate for *P. acidilactici*, *P. pentosaceus*, and *L. sakei* cultivation aimed at the reduction of microbiological contaminations in sausages.<sup>3</sup> Subsequently, rye wholemeal has also been used as a substrate for the production of bacteriocin by *L. sakei* KTU05-6.<sup>48</sup>

The utilization of waste from the slaughter house for bacteriocin production appears to be another attractive option to solve the environmental problem. The residues of animals generated from meat industries, trout, swordfish, tuna or cephalopod wastes allow the simple, rapid preparation of protein hydrolysates or autohydrolysates which are suitable for the formulation of medium for LAB fermentation. Different peptones obtained from the fish viscera and muscle residues promote growth of LAB for the production of nisin and pediocin.<sup>49</sup>

### Role of carbon and nitrogen in LAB cultivation

In fermentation, carbon and nitrogen sources are required for both growth and product formation. The characteristic features and nature of carbon and nitrogen play a major role in microorganism metabolism.<sup>50</sup> Nitrogen limitation phenomenon would

switch to a carbon limitation phenomenon when there is an increment of nitrogen source concentration while the carbon source concentration is maintained. This could be explained by the fact that the carbon source is readily used up within a shorter period at higher nitrogen concentration and becomes limited in subsequent fermentation stages, suggesting that the yield of biomass would decrease with increasing nitrogen concentration. Nitrogen in one form or another is required for all processes involving biological growth especially with reference to synthesis of cellular protein and nucleic acid production. During fermentation, appropriate nitrogen supplementation is required by the microflora for its metabolism.

### Types and concentrations of carbon source

The effect of carbon sources on the production of antimicrobial substances by various LAB strains are summarized in Table 3. By virtue of its size and rapid uptake, glucose is the main carbon source for all microorganisms which are rapidly utilized for energy conversion.<sup>15</sup> Glucose is the preferred carbon source to stimulate bacteriocin production, in which, most researchers had demonstrated high bacteriocins yield in association with the presence of glucose in growth media and not other monosaccharide's.<sup>51</sup> High yield of bacteriocins production in some LAB strains is associated with the inclusion of other sugars to growth medium rather than the use of glucose alone as they may have a complex enzymatic system that allow them to use other carbohydrates. This has been demonstrated in *E. faecium* which showed variable sugar utilization compared to glucose for bacteriocin production. Similar effect of sucrose and lactose are also confirmed for nisin production by *L. lactis* subsp. *lactis*.<sup>52</sup>

Several LAB strains such as *L. lactis*<sup>53</sup> and *S. pyogenes*<sup>54</sup> appear to use glucose preferentially for nisin Z and streptococin SA-





Table 3 Effect of carbon sources on the production of bacteriocin by LAB<sup>a</sup>

Producer LAB strain; bacteriocin/BLIS	Class	Bacteriocin production			References
		Carbon sources	Before addition	After addition	
<i>L. mesenteroides</i> L124, bacteriocin L124	NM	MRS (MRS + 0.4% yeast extract + 1% peptone) glucose	2560 (AU mL <sup>-1</sup> )	2560 (AU mL <sup>-1</sup> )	Production of bacteriocin with increasing glucose concentration from 2% to 6% (w/v) was not significantly different ( $P > 0.05$ )
		MRS (MRS + 0.4% yeast extract + 1% peptone) glucose		2560 (AU mL <sup>-1</sup> )	
		MRS (MRS + 0.4% yeast extract + 1% peptone) glucose		2560 (AU mL <sup>-1</sup> )	
		MRS (MRS + 0.4% yeast extract + 1% peptone) glucose		2560 (AU mL <sup>-1</sup> )	
<i>L. curvatus</i> L442, bacteriocin L442	NM	MRS (MRS + 0.4% yeast extract + 1% peptone) glucose	1280 (AU mL <sup>-1</sup> )	1280 (AU mL <sup>-1</sup> )	Production of bacteriocin with increasing concentration of glucose from 2% to 6% (w/v) was not significantly different ( $P > 0.05$ )
		MRS (MRS + 0.4% yeast extract + 1% peptone) glucose		1280 (AU mL <sup>-1</sup> )	
		MRS (MRS + 0.4% yeast extract + 1% peptone) glucose		1280 (AU mL <sup>-1</sup> )	
		MRS (MRS + 0.4% yeast extract + 1% peptone) glucose		1280 (AU mL <sup>-1</sup> )	
<i>L. plantarum</i> ST194BZ; bacteriocin ST194BZ	NM	MRS without glucose, supplemented with below carbon source	6400 (AU per OD)		Highest bacteriocin production was obtained in medium with 20 g L <sup>-1</sup> maltose. Production of bacteriocin in medium using 20 g L <sup>-1</sup> of glucose, sucrose or lactose was not significantly different ( $P > 0.05$ )
		Glucose (MRS)		1600 (AU per OD)	
		Saccharose		6400 (AU per OD)	
		Lactose		6400 (AU per OD)	
		Mannose		800 (AU per OD)	
		Maltose		12 800 (AU per OD)	
		Gluconate		800 (AU per OD)	
		MRS without glucose, supplemented with various carbon sources		2910 (AU per OD)	
		Fructose		1468 (AU per OD)	
		Sucrose		2910 (AU per OD)	
		Lactose		726 (AU per OD)	
		Mannose		726 (AU per OD)	
		Maltose		5861 (AU per OD)	
<i>L. plantarum</i> ST341LD; bacteriocin ST341LD	NM	Gluconate	726 (AU per OD)		Highest bacteriocin production was obtained in medium with 20 g L <sup>-1</sup> maltose. Production of bacteriocin in medium using 20 g L <sup>-1</sup> of lactose, mannose or gluconate was not significantly different ( $P > 0.05$ )
		MRS without glucose, supplemented with various carbon sources		2835 (AU per OD)	
		Glucose		350 (AU per OD)	
		Fructose		700 (AU per OD)	
		Sucrose		1425 (AU per OD)	
		Lactose		1425 (AU per OD)	
		Mannose		2835 (AU per OD)	
Maltose		700 (AU per OD)			
<i>Micrococcus</i> sp. GO5; micrococcin GO5	NM	Not added (MRS)	63 (AU mL <sup>-1</sup> )		Highest bacteriocin production was obtained in medium consisting of 2% of lactose or sucrose. Production of bacteriocin in medium using 2% glucose, mannose, dextrin, fructose or maltose was not significantly different ( $P > 0.05$ )
		Glucose		126 (AU mL <sup>-1</sup> )	
		Lactose		256 (AU mL <sup>-1</sup> )	
		Sucrose		256 (AU mL <sup>-1</sup> )	
		Mannose		126 (AU mL <sup>-1</sup> )	



Table 3 (Contd.)

Producer LAB strain; bacteriocin/BLIS	Class	Carbon sources	Bacteriocin production		Addition amounts	Remarks	References		
			Before addition	After addition					
<i>L. salivarius</i> CRL 1328; <i>L. salivarius</i> CRL 1328	NM	Dextrin		126 (AU mL <sup>-1</sup> )	2%		Tomás <i>et al.</i> <sup>156</sup> Bacteriocin production was stimulated by glucose. Lactose did not significantly ( $P > 0.05$ ) influence bacteriocin production		
		Fructose		126 (AU mL <sup>-1</sup> )	2%				
		Maltose		126 (AU mL <sup>-1</sup> )	2%				
		Sorbitol		63 (AU mL <sup>-1</sup> )	2%				
<i>E. mundtii</i> CWBI-B1431; BLIS	NM	Glucose	NM	0.25 log AU mL <sup>-1</sup> units, $p$ value: 0.02	0, 1 and 2%		Highest production was obtained with lactose. BLIS could be produced with all the carbon sources tested		
		Lactose	NM	0.10 log AU mL <sup>-1</sup> units, $p$ value: 0.36	0, 1 and 2%				
		G + L	NM	-0.26 log AU mL <sup>-1</sup> units, $p$ value: 0.05	—				
<i>E. faecium</i> CWBI-B1430; BLIS	NM	M17LG (M17 + 0.5% lactose and 0.5 glucose)	$17 \times 10^3$ (AU mL <sup>-1</sup> )				Highest production was obtained with lactose. BLIS could be produced with all the carbon sources tested		
		Various types of carbon source was added to M17 base broth							
		Arabinose		$0.11 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)			
		Fructose		$0.11 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)			
		Galactose		$0.88 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)			
		Glucose		$0.55 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)			
		Lactose		$3.66 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)			
		Maltodextrin		$0.33 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)			
		Maltose		$0.11 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)			
		Raffinose		$0.11 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)			
		Saccharose		$0.55 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)			
		Xylose		$0.44 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)			
		M17LG (M17 + 0.5% lactose and 0.5 glucose)	$8.66 \times 10^3$ (AU mL <sup>-1</sup> )						
		Different types of carbon source was added to M17 base broth							
Arabinose		$0.11 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)					
Fructose		$0.55 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)					
Galactose		$0.11 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)					
Glucose		$0.55 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)					
Lactose		$7.22 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)					
Maltodextrin		$0.22 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)					
Maltose		$0.55 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)					
Raffinose		$0 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)					
Saccharose		$0.55 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)					
Xylose		$0.22 \times 10^3$ (AU mL <sup>-1</sup> )		1.0% (w/v)					
<i>L. acidophilus</i> AA11; acidocin D20079	II	Without carbon source	$\sim 100$ (AU mL <sup>-1</sup> )				M17 broth supplemented with 0.5% lactose (M17L) was the preferred medium for bacteriocin production. Production in medium with lactose was 7-fold and 6-fold higher than medium with sucrose and glucose, respectively		
		M17	$\sim 2000$ (AU mL <sup>-1</sup> )						
		Lactose		$\sim 12\ 100$ (AU mL <sup>-1</sup> )		0.5%			
		Glucose		$\sim 2000$ (AU mL <sup>-1</sup> )		0.5%			
		Sucrose		$\sim 1800$ (AU mL <sup>-1</sup> )		0.5%			
		Fructose		$\sim 700$ (AU mL <sup>-1</sup> )		0.5%			
		Maltose		$\sim 1500$ (AU mL <sup>-1</sup> )		0.5%			
Galactose		$\sim 1500$ (AU mL <sup>-1</sup> )		0.5%					



Table 3 (Contd.)

Producer LAB strain; bacteriocin/BLIS	Class	Carbon sources	Bacteriocin production		Addition amounts	Remarks	References
			Before addition	After addition			
<i>L. lactis</i> ; bacteriocin	NM	Xylose		~1700 (AU mL <sup>-1</sup> )	0.5%		Ramachandran <i>et al.</i> <sup>1,58</sup> Production of bacteriocin was significantly increased with xylose as carbon source
		Arabinose		~1500 (AU mL <sup>-1</sup> )	0.5%		
		Mannose		~1600 (AU mL <sup>-1</sup> )	0.5%		
		Raffinose		~1600 (AU mL <sup>-1</sup> )	0.5%		
		Media contain glucose	~1.8 (U mL <sup>-1</sup> )		20 (g L <sup>-1</sup> )		
		Glucose		~1.875 (U mL <sup>-1</sup> )	NM		
		Maltose		~0.812 (U mL <sup>-1</sup> )	NM		
		Lactose		~1.562 (U mL <sup>-1</sup> )	NM		
		Starch		~1.31 (U mL <sup>-1</sup> )	NM		
		Xylose		~2.37 (U mL <sup>-1</sup> )	NM		
<i>L. brevis</i> bacteriocin DF01; bacteriocin DF01	NM	Sucrose		~0.68 (U mL <sup>-1</sup> )	NM		Lee <i>et al.</i> <sup>122</sup> Bacteriocin production with glucose and other carbon sources was not significantly different ( $P < 0.05$ ). The highest production was achieved in medium containing glucose
		MRS contain glucose	320 (AU mL <sup>-1</sup> )		2%		
		Lactose		0	2%		
		Fructose		80 (AU mL <sup>-1</sup> )	2%		
		Sucrose		0	2%		
		Mannose		0	2%		
		Dextrin		0	2%		
		Mannitol		0	2%		
		MRS medium was supplemented with various carbon sources in place of glucose			2.0% (w/v)		
		Fructose		418.2 (AU mL <sup>-1</sup> )	2.0% (w/v)		
<i>P. acidilactici</i> ; pediocin	II	Maltose		227.26 (AU mL <sup>-1</sup> )	2.0% (w/v)	Highest bacteriocin production was obtained in medium containing dextrose	
		Galactose		320.45 (AU mL <sup>-1</sup> )	2.0% (w/v)		
		Sorbitol		188.62 (AU mL <sup>-1</sup> )	2.0% (w/v)		
		Xylose		368.18 (AU mL <sup>-1</sup> )	2.0% (w/v)		
		Sucrose		229.53 (AU mL <sup>-1</sup> )	2.0% (w/v)		
		Dextrose		447.73 (AU mL <sup>-1</sup> )	2.0% (w/v)		
		Rhamnose		188.62 (AU mL <sup>-1</sup> )	2.0% (w/v)		
		Lactose		229.53 (AU mL <sup>-1</sup> )	2.0% (w/v)		
		MRS		~(320–385) (AU mL <sup>-1</sup> )			
		Fructose		~(375–490) AU mL <sup>-1</sup>	1.0% (w/v)		
<i>L. sp.</i> MSU3IR; bacteriocin MSU3IR	NM	Maltose		~(355–495) AU mL <sup>-1</sup>	1.0% (w/v)	Highest bacteriocin activity was obtained with lactose and the minimum activity was recorded in mannitol supplied medium	
		Sucrose		~(485–525) AU mL <sup>-1</sup>	1.0% (w/v)		
		Lactose		~(560–695) AU mL <sup>-1</sup>	1.0% (w/v)		
		Mannitol		~(330–440) AU mL <sup>-1</sup>	1.0% (w/v)		
		Xylose		~(355–445) AU mL <sup>-1</sup>	1.0% (w/v)		
		MRS		320 (AU mL <sup>-1</sup> )			
		Glucose		320 (AU mL <sup>-1</sup> )	2%		
				640 (AU mL <sup>-1</sup> )	3%		
				160 (AU mL <sup>-1</sup> )	4%		
<i>E. durans</i> E204; BLIS E204	NM					Highest bacteriocin activity was obtained with 2% glucose	

<sup>a</sup> NM: not mentioned.



FF22 production, respectively. In *L. lactis*, glucose supports higher specific growth rates, faster substrate consumption and greater product formation compared to other carbon sources.<sup>55</sup> Papagianni *et al.*<sup>56</sup> reported a direct relationship between nisin production and the rate of glucose consumption by *L. lactis*.<sup>57</sup> demonstrated that ruminal *S. bovis* uses glucose and sucrose preferentially to maltose and cellobiose and the utilization of these sugars was later shown to be regulated by specific phosphotransferase transport systems (PTS).<sup>58</sup> However, Russell<sup>59</sup> verified that glucose PTS could not account for the glucose consumption rates of rapidly growing cultures and a low-affinity facilitated diffusion mechanism was responsible for glucose transport at high substrate concentrations.

Starch has a negative effect on bacteriocin production which could be due to the attachment of bacterial cells to the surface of starch molecules which could cease utilization of the latter.<sup>60</sup> Most bacteria are able to ferment mono- and di-saccharides and only a limited number possess the ability to ferment complex carbohydrates such as starch. Amylolytic LAB have the ability to secrete amylase which hydrolyse starch to fermentable sugars and these type of LAB have been used in simultaneous saccharification and fermentation processes.<sup>61</sup> High molecular weight carbon and energy sources are broken down into smaller molecules by microorganism to be converted into amino acids, nucleotides, vitamins, carbohydrates and fatty acid to build these basic materials into proteins, coenzymes, nucleic acids, micropeptides, polysaccharides and lipids used for growth.<sup>62</sup> Some bacteria are not able to ferment complex carbon sources, hence it is necessary to have a pre-treatment step where the sugars are hydrolysed into fermentable sugars. Hydrolysis can be achieved by either adding commercial enzymes such as glucoamylase to the broth or by using heat treatment at low pH.<sup>61</sup> The problem with such treatments is the extra cost and complexity involved in the production process which render the procedure less favorable from both economical and production point of view. The ability of LAB to metabolize different carbon sources is based on the specific activities of the enzymes involved in carbohydrate degradation. In many cases, the addition of sugars lead to decrease in bacteriocin production which can be explained with the unfavorable conditions for growth and to some extent with the osmotic stress leading to decreased in growth rate.<sup>63</sup> Osmotic stress, which increases the energy demand, apparently reduces the maximum secretion of bacteriocin, indicating that the energy is required in excess for the synthesis.

Production of bacteriocin is also influenced by the concentrations of carbon source.<sup>64,65</sup> Bacteriocin production is increased with increasing glucose concentration up to certain level. However, bacteriocin production is not stimulated at high glucose concentration due to the saturation of glucose transport inside the cells. In batch fermentation of *L. lactis* nisin production is inhibited at high glucose concentration ( $25 \text{ g L}^{-1}$ ) due to a decrease in the rate of glucose uptake as reported.<sup>56</sup> Pattnaik *et al.*<sup>66</sup> also reported a decrease in bacteriocin production by *B. licheniformis* 26L-10/3RA at high glucose concentrations and hypothesized that this inhibition was caused by catabolite repression.

## Types and concentrations of nitrogen source

The effect of nitrogen sources on the production of antimicrobial substances by various LAB strains are summarized in Table 4. In most cases the presence of a nitrogen source is crucial for bacteriocin production. Nitrogen source used as supplements include yeast extract, beef extract, peptone, malt sprouts and soybean.<sup>27</sup> The response of microorganism to different concentrations of nitrogen depends largely on the type of nitrogen sources. Some microorganisms are not capable of synthesizing organic nitrogen compounds from the readily available nutrient in the media. Hence, this microorganisms especially those with complex nutritional requirement would need essential growth factors. These are in the form of essential nutrients which are necessary for *viz.* synthesis of nucleic acid which require purines and pyrimidines, synthesis of proteins which require amino acids and vitamins which are required as coenzymes and functional groups of certain enzymes. Instead of metabolizing the growth factors as a source of carbon or energy they are taken up by the cells and utilized to perform specific roles in metabolism. Catabolism of amino acid is known to have the capability of providing energy in an environment with limited nutrients. In terms of the catabolic pathways, the role of amino acids in LAB is not fully understood. Some LAB, through the arginine deiminase (ADI) pathway is known to produce extra energy by degrading arginine to citrulline, ornithine and ammonium. Although organic nitrogen is a complex nitrogen source made up of peptides and free amino acids, it is taken up directly from the medium by the cells. Hence this complex nitrogen source is incorporated into protein. Alternatively it is transformed into other cellular nitrogenous constituents.<sup>67</sup> By contrast the cell spends more energy and time in synthesizing amino acids for protein synthesis from inorganic nitrogen sources.<sup>68,69</sup>

Organic nitrogen sources such as peptone, beef extract, and yeast extract are commonly used to support good growth of LAB.<sup>70</sup> An organic nitrogen source, especially yeast extract, is considered to be of vital importance by virtue of its stimulatory effect on microbial cell growth. Yeast extract is the water soluble components of yeast cells made up primarily of amino acids, peptides, carbohydrates and salts. The excellent stimulation for growth is attributed to the presence of a high number of growth factors in the yeast extract compared to any other protein hydrolysates prepared by enzymatic hydrolysis.<sup>71</sup> The amino acids or peptides in yeast extract could act as inducer or precursor for bacteriocin synthesis by LAB. The use of yeast extract which is rich in vitamins, minerals, amino acids and other easily consumable nitrogen sources, is not economical in a large scale fermentation process due to relatively high cost of this nitrogen source. The principal nutrients in the yeast extracts are purine and pyrimidine bases and group B vitamins.<sup>72</sup>

Peptone is another major organic nitrogen source widely used in microbiological media. Peptone is defined as water-soluble, non-heat coagulable protein hydrolysates which contain a mixture of peptides, proteoses and free amino acids.<sup>73</sup> The media recommended for the cultivation of LAB are those



Table 4 Effect of nitrogen sources on the production of bacteriocin by LAB<sup>a</sup>

Producer LAB strain; bacteriocin/BLIS	Class	Bacteriocin production			References
		Nitrogen sources tested	Before addition	After addition	
<i>L. mesenteroides</i> L124, bacteriocin L124	NM	MRS (MRS + 3% glucose + 1% yeast extract) peptone (MRS + 3% glucose + 1% yeast extract) peptone	2560 (AU mL <sup>-1</sup> )	5120 (AU mL <sup>-1</sup> )	Production of bacteriocin increased with the addition of peptone but the production with 2% and 4% peptone was not significantly different ( $P > 0.05$ )
				2% (w/v)	
				4% (w/v)	
<i>L. curvatus</i> L442, bacteriocin L442	NM	MRS (MRS + 3% glucose + 1% yeast extract) peptone (MRS + 3% glucose + 1% yeast extract) peptone	1280 (AU mL <sup>-1</sup> )	2560 (AU mL <sup>-1</sup> )	Production of bacteriocin was increased with the addition of 2% peptone while the production in MRS and media containing 4% peptone was not significantly different ( $P > 0.05$ )
				2% (w/v)	
				4% (w/v)	
<i>L. plantarum</i> ST194BZ; bacteriocin ST194BZ	NM	MRS broth without organic nutrient was supplemented with various types of nitrogen source Glucose (MRS) Tryptone Meat extract Yeast extract Tryptone + meat extract Tryptone + yeast extract Meat extract + yeast extract Tryptone + meat extract + yeast extract	6400 (AU mL <sup>-1</sup> )	12 800 (AU mL <sup>-1</sup> )	Bacteriocin production was stimulated by the presence of tryptone
				20 (g L <sup>-1</sup> )	
				20 (g L <sup>-1</sup> )	
				20 (g L <sup>-1</sup> )	
				20 (g L <sup>-1</sup> )	
				12.5 + 7.5 (g L <sup>-1</sup> )	
				12.5 + 7.5 (g L <sup>-1</sup> )	
				12 800 (AU mL <sup>-1</sup> )	
				800 (AU mL <sup>-1</sup> )	
				12 800 (AU mL <sup>-1</sup> )	
				10 + 5 + 5 (g L <sup>-1</sup> )	
				20 (g L <sup>-1</sup> )	
				20 (g L <sup>-1</sup> )	
<i>L. plantarum</i> ST231LD; bacteriocin ST231LD	NM	MRS broth without organic nutrient was supplemented with various types nitrogen source Tryptone Meat extract Yeast extract Tryptone + ME Tryptone + YE ME + YE YE TP	2922 (AU per OD)	2922 (AU per OD)	Highest production was achieved with combination of yeast extract and tryptone
				1454 (AU per OD)	
				2922 (AU per OD)	
				2922 (AU per OD)	
				12.5 + 7.5 (g L <sup>-1</sup> )	
				5860 (AU per OD)	
				1454 (AU per OD)	
				6.5 (mg L <sup>-1</sup> )	
				11.6 (mg L <sup>-1</sup> )	
				17.45 (mg L <sup>-1</sup> )	
<i>L. sakei</i> CCUG 42687; Sakacin P	II		0 (g L <sup>-1</sup> )	2.5 (g L <sup>-1</sup> )	Production was increased with increasing tryptone concentration although the effect was markedly lower compared to yeast extract
				8.3 (mg L <sup>-1</sup> )	
				10.2 (mg L <sup>-1</sup> )	
				12 (mg L <sup>-1</sup> )	
				10 (g L <sup>-1</sup> )	
				15 (g L <sup>-1</sup> )	
				15 (g L <sup>-1</sup> )	



Table 4 (Contd.)

Producer LAB strain; bacteriocin/BLIS	Class	Bacteriocin production		Addition amounts	Remarks	References
		Nitrogen sources tested	Before addition			
<i>L. plantarum</i> ST341LD; bacteriocin ST341LD	NM	MRS broth without organic nutrient was supplemented with various types of nitrogen source			Tryptone was the key nitrogen source needed for optimal bacteriocin production	Todorov & Dicks <sup>92</sup>
		Tryptone	5672 (AU per OD)	20 (g L <sup>-1</sup> )		
		Meat extract	2842 (AU per OD)	20 (g L <sup>-1</sup> )		
		Yeast extract	1427 (AU per OD)	20 (g L <sup>-1</sup> )		
		Trypnone + ME	5672 (AU per OD)	12.5 + 7.5 (g L <sup>-1</sup> )		
<i>Micrococcus</i> sp. GO5; micrococccin GO5	NM	Trypnone + YE	5672 (AU per OD)	12.5 + 7.5 (g L <sup>-1</sup> )	Highest production was achieved in tryptone-containing medium	Kim <i>et al.</i> <sup>32</sup>
		ME + YE	2842 (AU per OD)	10 + 10 (g L <sup>-1</sup> )		
		Basal medium contained MRS all nitrogen sources were omitted and 2% glucose was replaced with 2% lactose. The basal medium was supplemented with various types of nitrogen sources				
		Not added	18.32 (AU mL <sup>-1</sup> )			
		Beef extract	129.95 (AU mL <sup>-1</sup> )	1% (w/v)		
<i>L. salivarius</i> ; salivaricin CRL 1328	NM	Peptone	65 (AU mL <sup>-1</sup> )	1% (w/v)	Bacteriocin production with yeast extract and tryptone was not significantly different Higher interacting effect for bacteriocin production was detected between yeast extract and tryptone Production was markedly influenced by a combination of yeast extract, tryptone and meat peptone	Tomás <i>et al.</i> <sup>156</sup>
		Skim milk	6.66 (AU mL <sup>-1</sup> )	1% (w/v)		
		Urea	10 (AU mL <sup>-1</sup> )	1% (w/v)		
		NH4Cl	65 (AU mL <sup>-1</sup> )	1% (w/v)		
		Tryptone	261.56 (AU mL <sup>-1</sup> )	1% (w/v)		
		Ammonium sulfate	35 (AU mL <sup>-1</sup> )	1% (w/v)		
		soytone	130 (AU mL <sup>-1</sup> )	1% (w/v)		
		Yeast extract	130 (AU mL <sup>-1</sup> )	1% (w/v)		
		Ammonium citrate	36.65 (AU mL <sup>-1</sup> )	1% (w/v)		
		Yeast extract	0.67 log AU mL <sup>-1</sup> units, <i>p</i> value: 0.00	0, 1 and 2% (w/v)		
<i>E. mundtii</i> CWBI-B1431; BLIS	NM	Tryptone	NM	0.66 log AU mL <sup>-1</sup> units, <i>p</i> value: 0.00	Highest BLIS production was obtained with beef extract	Aguilar-Galvez <i>et al.</i> <sup>157</sup>
		Meat peptone	NM	0.47 log AU mL <sup>-1</sup> units, <i>p</i> value: 0.00		
		YE + T	NM	-0.68 log AU mL <sup>-1</sup> units, <i>p</i> value: 0.00		
		YE + MP	NM	-0.18 log AU mL <sup>-1</sup> units, <i>p</i> value: 0.17		
		T + MP	NM	-0.34 log AU mL <sup>-1</sup> units, <i>p</i> value: 0.013		
M17LG (M17 + 0.5% lactose and 0.5 glucose)	NM	Various type of nitrogen source was added to M17LG, medium without nitrogen source	17.1 × 10 <sup>3</sup> (AU mL <sup>-1</sup> )	0, 1 and 2% (w/v)	Production was markedly influenced by a combination of yeast extract, tryptone and meat peptone	
			Beef extract	3.675 × 10 <sup>3</sup> (AU mL <sup>-1</sup> )		



Table 4 (Contd.)

Producer LAB strain; bacteriocin/BLIS	Class	Bacteriocin production		Addition amounts	Remarks	References		
		Nitrogen sources tested	Before addition				After addition	
<i>E. faecium</i> CWBI-B1430; BLIS	NM	Beef peptone		1.2 × 10 <sup>3</sup> (AU mL <sup>-1</sup> )				
		Casein		0.15 × 10 <sup>3</sup> (AU mL <sup>-1</sup> )				
		Casein peptone		0.975 × 10 <sup>3</sup> (AU mL <sup>-1</sup> )				
		Protease		0.9 × 10 <sup>3</sup> (AU mL <sup>-1</sup> )				
		Soy peptone		1.2 × 10 <sup>3</sup> (AU mL <sup>-1</sup> )				
		Yeast extract		0.9 × 10 <sup>3</sup> (AU mL <sup>-1</sup> )				
		M17LG (M17 + 0.5% lactose and 0.5 glucose)	8.625 × 10 <sup>3</sup> (AU mL <sup>-1</sup> )				Highest BLIS production was obtained with beef extract	Aguilar-Galvez <i>et al.</i> <sup>157</sup>
		Various types of nitrogen source was added to M17LG, medium without nitrogen source						
		Beef extract		2.25 × 10 <sup>3</sup> (AU mL <sup>-1</sup> )				
		Beef peptone		1.125 × 10 <sup>3</sup> (AU mL <sup>-1</sup> )				
<i>L. acidophilus</i> AA11, acidocin D20079	II	Casein		0.3 × 10 <sup>3</sup> (AU mL <sup>-1</sup> )				
		Casein peptone		0.45 × 10 <sup>3</sup> (AU mL <sup>-1</sup> )				
		Protease		0.45 × 10 <sup>3</sup> (AU mL <sup>-1</sup> )				
		Soy peptone		0.675 × 10 <sup>3</sup> (AU mL <sup>-1</sup> )				
		Yeast extract		1.05 × 10 <sup>3</sup> (AU mL <sup>-1</sup> )				
		Control (M17L medium)	12 320 (AU mL <sup>-1</sup> )					
		Basal medium	0 (AU mL <sup>-1</sup> )					
		(Basal medium was based on M17L medium, in which, all nitrogen sources were eliminated and then supplemented with various types of nitrogen source						
		Beef extract		400 (AU mL <sup>-1</sup> )				
		Yeast extract		12 320 (AU mL <sup>-1</sup> )				
<i>L. Lactis</i> , bacteriocin	NM	Tryptone		7360 (AU mL <sup>-1</sup> )				
		Peptone		1680 (AU mL <sup>-1</sup> )				
		Protease peptone		6320 (AU mL <sup>-1</sup> )				
		Casitone		3040 (AU mL <sup>-1</sup> )				
		Casein		480 (AU mL <sup>-1</sup> )				
		Soytone		6320 (AU mL <sup>-1</sup> )				
		Nitrogen source in basal media: peptone, meat extract and yeast extract	~1.8 (U mL <sup>-1</sup> )					
		Peptone		2.875 (U mL <sup>-1</sup> )				
		Urea		0.875 (U mL <sup>-1</sup> )				
		Yeast extract		1.87 (U mL <sup>-1</sup> )				
<i>L. brevis</i> DF01; bacteriocin DF01	NM	Potassium nitrate		1.625 (U mL <sup>-1</sup> )				
		Ammonium chloride		0.81 (U mL <sup>-1</sup> )				
		Sodium nitrate		1.18 (U mL <sup>-1</sup> )				
		MRS	320 (AU mL <sup>-1</sup> )					
		MRS + YE	640					
Highest bacteriocin production was obtained in medium containing yeast extract	Abo-Amer <sup>96</sup>							
Peptone was the preferred nitrogen source for bacteriocin production. Organic nitrogen gave higher bacteriocin yield as compared to inorganic nitrogen sources	Ramachandran <i>et al.</i> <sup>158</sup>							
Highest bacteriocin production was achieved with 1% YE. Reduced bacteriocin production was observed with the addition of	Lee <i>et al.</i> <sup>122</sup>							



Table 4 (Contd.)

Producer LAB strain; bacteriocin/BLIS	Bacteriocin production				References
	Class	Nitrogen sources tested	Before addition	After addition	
<i>P. acidilactici</i> ; pediocin	II	MRS + (BE + YE)	320	(1.5 + 1)%	yeast extract. Reduced bacteriocin production was also observed with the addition of other nitrogen sources such as BE, TP and PP mixed with YE Highest bacteriocin production was observed in medium containing soya peptone Neera <i>et al.</i> <sup>159</sup>
		MRS + (TP + YE)	320	(0.5 + 1)%	
		MRS + (TP + YE)	320	(1.5 + 1)%	
		MRS + (PP + YE)	320	(1.5 + 1)%	
<i>E. durans</i> E204; BLIS E204	NM	MRS was used as basal medium, in which, yeast extract, beef extract, peptone and ammonium citrate were excluded. This basal medium was supplemented with various types of nitrogen source			Highest BLIS production was obtained in medium containing 2.0% tryptone, 2.0 to 3.0% yeast extract Khay <i>et al.</i> <sup>145</sup>
		Skim milk	228.5 (AU mL <sup>-1</sup> )	2.5% (w/v)	
		Casein peptone	250 (AU mL <sup>-1</sup> )	2.5% (w/v)	
		Soya peptone	500 (AU mL <sup>-1</sup> )	2.5% (w/v)	
		Whey protein	370 (AU mL <sup>-1</sup> )	2.5% (w/v)	
		Soya bean meal	250 (AU mL <sup>-1</sup> )	2.5% (w/v)	
		MRS	320 (AU mL <sup>-1</sup> )		
		Tryptone	320 (AU mL <sup>-1</sup> )	1% (w/v)	
			640 (AU mL <sup>-1</sup> )	2% (w/v)	
			160 (AU mL <sup>-1</sup> )	3% (w/v)	
Yeast extract		320 (AU mL <sup>-1</sup> )	0.5% (w/v)		
		320 (AU mL <sup>-1</sup> )	1% (w/v)		
Meat extract		640 (AU mL <sup>-1</sup> )	2% (w/v)		
		640 (AU mL <sup>-1</sup> )	3% (w/v)		
L. sp. MSU3IR; bacteriocin MSU3IR	NM	MRS	~(320–365) (AU mL <sup>-1</sup> )		Highest bacteriocin production was obtained in medium containing ammonium acetate while the lowest bacteriocin production was obtained in medium containing sodium nitrate. Increase in bacteriocin production was due to inorganic nitrogen source Iyapparaj <i>et al.</i> <sup>105</sup>
		Ammonium acetate	~(360–600) (AU mL <sup>-1</sup> )		
	Ammonium chloride	~(315–500) (AU mL <sup>-1</sup> )			
	Ammonium nitrate	~(305–410) (AU mL <sup>-1</sup> )			
	Sodium sulphate	~(240–400) (AU mL <sup>-1</sup> )			
	Sodium citrate	~(0–470) (AU mL <sup>-1</sup> )			
	Sodium nitrate	~(0–410) (AU mL <sup>-1</sup> )			

<sup>a</sup> NM: not mentioned; YE: yeast extract; TP: tryptone; PP: peptone.

that contain several peptones at high concentration, in which, only a small proportion of peptones supplied are consumed during the fermentation.<sup>74</sup> Peptones in commercial media are also necessary for bacteriocin production but the efficiencies (substrate consumed/initial substrate) of these media are usually low suggesting the proportions of nutrients in it is unbalanced.<sup>70</sup>

Attempts have been made to balance the medium by the usual procedure of reducing the initial protein level to a slight excess with regard to consumption. However, often the results are marked drops in the production of biomass and typical metabolites, in particular bacteriocins. Furthermore, protein materials which remain after the incubation constitute superfluous expenditure and hinder subsequent purification of the bacteriocins. Replacement of these proteins by inorganic sources of nitrogen does not produce acceptable results and are not suitable for adjusting the initial protein level to the predicted amount for consumption. As peptones do not represent a source of organic nitrogen but rather a source of amino acids or peptides with specific roles, only a fraction added is really important. Therefore, the use of low-cost protein fractions will bring about a reduction in large-scale production costs.<sup>74</sup>

The replacement of half amount of yeast extract with either beef extract or malt extract resulted in the reduction in biomass from *L. sakei* CCUG 42687 and bacteriocin production.<sup>75</sup> Moreover, growth and bacteriocin production were markedly improved with the replacement of tryptone with bacteriological peptone or soytone, while the use of a fish hydrolysate reduced growth of *L. sakei*.<sup>76</sup> When peptone, beef extract, and yeast extract were replaced in food grade medium by Baker's yeast peptone, *L. plantarum* was able to grow whereas other lactobacilli strains such as *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. delbrueckii* subsp. *lactis* failed to grow.<sup>77</sup>

LAB are incapable of growing in medium using mineral nitrogen devoid of exogenous amino acids. It is however responsive to changes in the availability of nitrogen in its environment brought about by regulating the proteolytic system to ensure that there is a proper nitrogen balance within the cell. The level of individual nutrients in the extracellular environment has some influence in the synthesis of many exo-proteins.<sup>78</sup> Nitrogen limitation appears to be attributed to the fastidious nutritional requirement of the LAB which is especially true when there could be a significant level of nitrogen which remained at the end of fermentation. In this respect energy (carbon) metabolism and biosynthesis (nitrogen) metabolism in LAB do not overlap paving the way for metabolic engineering. In this respect it is possible to change either metabolism which do not influence the other provided energy generation or biosynthesis of cell material remain intact.<sup>64,75</sup> Enhanced bacteriocin production due to increasing nitrogen content could be attributed to the increased in peptide and/or growth factors in the nitrogen sources which is an essential element or an inducer in the synthesis of bacteriocin.

## Surfactants

The effects of surfactant on the production of antimicrobial substances by various LAB strains are summarized in Table 5. In some cases the addition of surfactants increased the concentration of bacteriocins produced as a consequence of cell growth acceleration. Surfactants may enhance the sensitivity of the indicator strain and form micelles with proteinaceous compounds thus stabilizing the bacteriocins.<sup>79,80</sup>

Tween 80 as a surfactant is the most important medium component for the enhancement of bacteriocin production by some LAB strains.<sup>27</sup> This is brought about by Tween 80 stimulating the secretion of peptides through its influence on membrane fluidity. This product is a non-ionic detergent and a water-soluble ester of oleic acid in which growth of microorganisms is enhanced with their presence. Oleic acid has been known to be an essential growth factor for several microorganisms while non-ionic detergents containing oleic acid, free oleic acid and *cis*-vaccenic acid can be used to replace the requirement for biotin by lactobacilli.<sup>81</sup> The presence of Tween 80 in the culture helped to incorporate oleic acid into the cell membrane and oleic acid is then converted into cyclopropane fatty acids.<sup>82</sup> It is believed that the role of cyclopropane fatty acids is to increase fluidity of LAB membranes as in the case of polyunsaturated fatty acids and to protect LAB from different environmental conditions *viz.* low pH, deleterious effects of oxygen, and extreme temperatures.<sup>83</sup> Several reports claimed that Tween 80 improved the production of bacteriocin by preventing the aggregation of their molecules.<sup>84</sup> Tween 80 could change the surface tension of the producer cell and facilitates the discharge/release of bacteriocin from the cell surface.<sup>85</sup> This could be brought about by the formation of micelles in the presence of proteins in the medium which stabilized the production of bacteriocin.

Addition of SDS in culture medium resulted in enhanced bioactivity which could be due to the increased permeability of the cell membrane of indicator organism or due to the break up of bacteriocin complex into active subunits with enhanced lethal effect.<sup>86</sup> Bacteriocin production is enhanced in the presence of a polysorbate non-ionic surfactant Tween 20 by suppressing the adhesion of bacteriocin-producing cells and due to its effect on membrane fluidity and stimulation of the secretion of proteins.<sup>87</sup> The presence of Tween 20 in the culture enhanced the activity of the bacteriocin produced by *L. sakei/curvatus* ACU-1.<sup>88</sup>

## Effect of cultivation condition

### Temperature

Temperature play an important role on bacteriocin production. The optimum temperature for growth and bacteriocin production are correlated as observed in lactocin A,<sup>89</sup> enterocin 1146 and lactocin S,<sup>90,91</sup> nisin Z,<sup>53,92</sup> plantaricin<sup>93</sup> and enterocin 1146.<sup>94</sup> The optimal temperature for bacteriocin production may not be similar to that of optimal temperature for bacterial growth.<sup>21,30,95,96</sup> There are also reports on maximum bacteriocin production at suboptimal growth temperatures. In the case of amylovorin L471, slow growth at low temperature was suggested





Table 5 Effect of Tween 80 on the production of antimicrobial substances by LAB<sup>a</sup>

Producer LAB strain	Antimicrobial substance	Class	Effect	Bacteriocin production (AU mL <sup>-1</sup> )			References
				Before addition	After addition	Amount of tween added	
<i>P. damnosus</i> NCFB 1832	Pediocin PD-1	NM	Positive	1300	4000	3% (v/v)	Nel <i>et al.</i> <sup>160</sup>
<i>E. faecium</i> NKR-5-3	Bacteriocin NKR-5-3	NM	Positive	800	3200	0.1% (v/v)	Wilapun <i>et al.</i> <sup>139</sup>
<i>L. lactis</i> subsp. <i>lactis</i> ST34BR	Bacteriocin ST34BR	NM	Negative	NM	>50% reduced	NM	Todorov & Dicks <sup>140</sup>
<i>L. mesenteroides</i> E131	Bacteriocin E131	NM	No	2560	2560	0.1% (v/v)	Xiraphi <i>et al.</i> <sup>161</sup>
<i>L. mesenteroides</i> subsp. <i>mesenteroides</i> ST33LD	Bacteriocin ST33LD	NM	Positive	NM	>50% increased	NM	Todorov & Dicks <sup>141</sup>
<i>L. lactis</i>	Nisin	NM	Positive	~25 000	~51 000	5 (g L <sup>-1</sup> )	Liu <i>et al.</i> <sup>46</sup>
<i>L. plantarum</i>	Bacteriocins ST28MS & ST26MS	NM	Negative	NM	>50% reduced	NM	Todorov & Dicks <sup>162</sup>
<i>L. plantarum</i> ST194BZ	Bacteriocin ST194BZ	NM	Positive	NM	>50% increased	NM	Todorov & Dicks <sup>155</sup>
<i>L. rhamnosus</i> ST461BZ & ST462BZ	Bacteriocins ST461BZ & ST462BZ	NM	Positive	NM	>50% increased	NM	Todorov & Dicks <sup>163</sup>
<i>L. casei</i> CRL 705	Lactocin 705	II	Positive	0	2133	0% (v/v)	Vignolo <i>et al.</i> <sup>164</sup>
				533	533	0.05% (v/v)	
				1066	1066	0.1% (v/v)	
				2133	2133	0.5% (v/v)	
				2133	2133	1.0% (v/v)	
<i>L. plantarum</i> JW3BZ	Bacteriocin JW3BZ	NM	Positive	3200	25 600	0 (mL L <sup>-1</sup> )	von Mollendorff <i>et al.</i> <sup>165</sup>
					25 600	0.05 (mL L <sup>-1</sup> )	
					25 600	0.10 (mL L <sup>-1</sup> )	
					51 200	0.15 (mL L <sup>-1</sup> )	
					51 200	0.20 (mL L <sup>-1</sup> )	
<i>L. plantarum</i> JW6BZ	Bacteriocin JW3BZ	NM	Positive	3200	25 600	0 (mL L <sup>-1</sup> )	von Mollendorff <i>et al.</i> <sup>165</sup>
					25 600	0.05 (mL L <sup>-1</sup> )	
					25 600	0.10 (mL L <sup>-1</sup> )	
					51 200	0.15 (mL L <sup>-1</sup> )	
					51 200	0.20 (mL L <sup>-1</sup> )	
<i>L. fermentum</i> JW11BZ	Bacteriocin JW11BZ	NM	No effect	6400	6400	0 (mL L <sup>-1</sup> )	von Mollendorff <i>et al.</i> <sup>165</sup>
					6400	0.05 (mL L <sup>-1</sup> )	
					6400	0.10 (mL L <sup>-1</sup> )	
					6400	0.15 (mL L <sup>-1</sup> )	
					6400	0.20 (mL L <sup>-1</sup> )	
<i>L. fermentum</i> JW15BZ	Bacteriocin JW15BZ	NM	Positive	3200	12 800	0 (mL L <sup>-1</sup> )	von Mollendorff <i>et al.</i> <sup>165</sup>
					12 800	0.05 (mL L <sup>-1</sup> )	
					12 800	0.10 (mL L <sup>-1</sup> )	
					25 600	0.15 (mL L <sup>-1</sup> )	
					25 600	0.20 (mL L <sup>-1</sup> )	
<i>L. sakei</i>	Sakacin A	II	No	480	480	0, 1, 2 (g L <sup>-1</sup> or % v/v)	Trinetta <i>et al.</i> <sup>166</sup>
<i>L. sakei</i> ST22CH	Bacteriocin ST22CH	NM	Positive	1600	6400	0 (g L <sup>-1</sup> )	Todorov <i>et al.</i> <sup>167</sup>
					1600	1 (g L <sup>-1</sup> )	
					1600	2 (g L <sup>-1</sup> )	
					3200	3 (g L <sup>-1</sup> )	
					6400	5 (g L <sup>-1</sup> )	



Table 5 (Contd.)

Producer LAB strain	Antimicrobial substance	Class	Effect	Bacteriocin production (AU mL <sup>-1</sup> )		Amount of tween added	References
				Before addition	After addition		
<i>L. sakei</i> ST153CH	Bacteriocin ST153CH	NM	Positive	0	1600	0 (g L <sup>-1</sup> )	Todorov <i>et al.</i> <sup>167</sup>
					0	1 (g L <sup>-1</sup> )	
					800	2 (g L <sup>-1</sup> )	
					800	5 (g L <sup>-1</sup> )	
<i>L. sakei</i> ST154CH	Bacteriocin ST154CH	NM	Positive	0	1600	0 (g L <sup>-1</sup> )	Todorov <i>et al.</i> <sup>167</sup>
					1600	1 (g L <sup>-1</sup> )	
					800	2 (g L <sup>-1</sup> )	
					1600	5 (g L <sup>-1</sup> )	
<i>E. durans</i> E204	BLIS E204	NM	Positive		320	0.1% (MRS)	Khay <i>et al.</i> <sup>145</sup>
					640	0.5%	
					160	1%	

<sup>a</sup> NM: not mentioned.

to be attributed to the release of more energy for bacteriocin production by *L. amylovorus* DCE 471.<sup>91</sup> As for sakacin P, higher bacteriocin production at low temperatures was reported to be due to different rate-limiting reactions which are temperature-dependent resulting in better utilization of carbon and/or energy. Growth rates was low at low temperature, which in turn increased the availability of essential metabolites (ATP included) for bacteriocin production. Increased in degradation or inactivation of the bacteriocin at high temperatures is another explanation.<sup>84</sup> An interesting observation is the temperature sensitivity of sakacin A, which is regulated by a three-component regulatory system,<sup>97</sup> although the optimal temperature was not as low as that required for sakacin P.<sup>84</sup>

Optimum incubation temperature and pH on growth of various LAB strains and production of bacteriocin is summarized in Table 6. The optimum incubation temperature for high yield bacteriocin production must be evaluated on individual basis and on the basis of strain-dependence. The optimum temperature for bacteriocin production in most strains isolated to date ranged from 30 °C to 37 °C.<sup>98</sup> However, at high temperature (44 °C) synthesis or production of bacteriocin by microorganism could be curtailed despite possible cell growth under those conditions.<sup>14,99</sup> Research by Lim<sup>100</sup> confirmed the work of Messens *et al.*<sup>101</sup> who suggested the rate of bacteriocin inactivation increased with high temperature which is probably the result of higher protease activity or a more pronounced cell-bacteriocin or bacteriocin-bacteriocin interaction.

## pH

The metabolic activity of LAB is greatly affected by the medium and culture pH. LAB are generally fastidious on artificial media but they grow readily in most food substrates, produce acids and reduce the culture pH rapidly to a point where other competing microorganisms are no longer able to grow. However the formation of organic acids depended on the type of fermentation, duration, temperature, substrate and LAB strain.<sup>48,102</sup> LAB is more acid tolerant compared to other types of bacteria and are more tolerant to a lower and wider pH range (Table 6). It is well known that culture pH greatly influenced the growth of LAB and bacteriocin production apart from influencing cell aggregation, cell absorption of bacteriocin and/or proteolytic degradation. Bacteriocin is produced within a specific pH range which varies with the producer strains and can be quite different from the pH range at which the bacteriocin is stable and active.<sup>103</sup> pH controls enzymatic reaction – hence due to low pH and accumulation of lactic acid or exhausted energy source, cell growth ceases or stops and so does bacteriocin production. Since immunity of the bacteriocin producer cells is based on the production of immunity peptides whose genes are co-transcribed with the bacteriocin structural genes its level will also decrease when bacteriocin production ceases.<sup>104</sup>

Optimal pH for the growth or bacteriocin production is markedly dependent on the characteristics of microbial strains used. Optimal pH for some bacteriocins production ranged from pH 5.5 to 6.0 while for others being less than pH 5.<sup>105</sup>



Table 6 Effect of temperature and pH on growth of various LAB strains and production of bacteriocin<sup>a</sup>

Producer LAB strain/bacteriocin	Class	Optimum temp. for: (°C)		Optimum pH for		References
		Growth	Production	Growth	Production	
<b>Lactococci</b>						
<i>L. lactis</i> subsp. <i>lactis</i> A164/Nisin-like bacteriocin	NM	37	30	6.0	6.0	Cheigh <i>et al.</i> <sup>71</sup>
<i>L. lactis</i> ssp. <i>lactis</i> 1(NN-MD1-7)/bacteriocin	NM	35	35	6.5	6.5	Chanprasert & Gasaluck <sup>168</sup>
<i>L. lactis</i> /bacteriocin	NM	30	30	6.0	6.0	Ramachandran <i>et al.</i> <sup>158</sup>
<b>Lactobacilli</b>						
<i>L. salivarius</i> CRL 1328/bacteriocin CRL 1328	NM	37	37	6.5	6.5–8.0	Tomás <i>et al.</i> <sup>99</sup>
<i>L. curvatus</i> LTH 1174/curvacin A	II	34.5	20–27	6	5.1	Messens <i>et al.</i> <sup>101</sup>
<i>L. acidophilus</i> AA11/acidocin AA11	II	37	30	6.5	6.5	Abo-AMEL <sup>96</sup>
<i>L.</i> spp. ( <i>L. acidophilus</i> M2, <i>L. acidophilus</i> CH1, <i>L. fermentum</i> M1 and <i>L. pentosus</i> CH2)/bacteriocin	NM	34	34	6.0	6.0	Mahrous <i>et al.</i> <sup>169</sup>
<b>Pediococci</b>						
<i>P. acidilactici</i> ITV 26/pediocin	II	40	35	5	5	Calderón-Santoyo <i>et al.</i> <sup>170</sup>
<i>P. acidilactici</i> PA003/pediocin	II	35	35	6.5	6.1	Zhang <i>et al.</i> <sup>83</sup>
<b>Enterococci</b>						
<i>E. faecium</i> CWBI-B1430 and <i>E. mundtii</i> CWBI-B1431/enterocin-like bacteriocin	NM	37	37	7.0	7.0	Aguilar-Galvez <i>et al.</i> <sup>157</sup>
<i>E. faecium</i> B3L3/bacteriocin B3L3	NM	37	37	8	8	Yusuf & Hamid <sup>171</sup>
<b>Leuconostocs</b>						
<i>L. mesenteroides</i> L124/bacteriocins L124	NM	30	25	6.0–6.5	5.5	Mataragas <i>et al.</i> <sup>30</sup>

<sup>a</sup> NM: not mentioned.

These pH values are however different from the optimal pH for bacterial growth. Bacteriocin production being dependent on pH is an indication that pH could regulate the expression of biosynthetic gene similarly observed for several classes of genes.<sup>33</sup> With low growth rate there is better utilization of energy resulting in enhanced bacteriocin production. The enzymatic reactions in the utilization of energy are regulated by pH. Reduction in pH may decrease the enzymatic reaction, which in turn, reduce the growth rate of the bacterium. The positive effect is therefore on bacteriocin synthesis as a result of net increase of essential metabolites including ATP.<sup>84</sup> The acidification of medium during fermentation could also affect to protein solubility and hydrolysis of polypeptides.<sup>106</sup>

It can be deduced from the above reports that the most critical physiochemical factors which play a crucial role and exert a significant effect on bacteriocin production by bacteriocinogenic strains are pH and temperature. Bacteriocin production was highest at pH ranging from 5.5 to 6.0 with temperature slightly lower than that for optimal growth.<sup>30,107,108</sup> Production of some bacteriocins is enhanced at low pH (5.0). Since the synthesis of bacteriocin is enhanced at a relatively low growth rates, bacteriocin production by some LAB is enhanced at non-optimal growth conditions. Increased in growth rate did not necessarily enhanced bacteriocin production. Lower growth rates at sub-optimal pH or temperature values indicate potentially better utilization of energy and essential metabolites.<sup>75,84</sup> At high temperatures or pH, bacteriocin production is lower because energy needs for maintenance purposes are higher when temperature or pH increases. Maintenance operations such as turnover of macromolecules (DNA, RNA) and maintenance of the potential along the membrane of cells are growth dependent and faster growth rates mean more energy is required for maintenance. However, there are cases of better bacteriocin production at temperatures close to that of optimum for growth.<sup>107,108</sup> The optimum pH and temperature for cell growth did not correspond well with those requirements for the enhancement of bacteriocin synthesis.<sup>99</sup>

### Aeration and agitation

The availability of oxygen has a great influence on microbial growth. Microorganisms vary with respect to their requirements and tolerance toward molecular oxygen. LAB are facultative anaerobic microorganisms where they are capable of modifying a central part of their metabolic pathways according to the availability of oxygen in the medium. This ability allows their classification into homo- and heterolactics.<sup>109</sup> With no oxygen required for growth this element in fact has a negative effect on the growth of these microbes. Being aerotolerant microorganisms growth of LAB is generally slightly affected by oxidative stress.<sup>20</sup>

Some bacteria especially those that are anaerobic are not capable of synthesizing cytochromes and other heme containing enzymes. *L. acidophilus* and *bifidobacterium* spp. lack this capability which is crucial to the electron transportation chain. They are thus unable to synthesize ATP by respiratory means and have to depend strictly on a fermentative mode of metabolism. Due to lack of participation of an external electron

acceptor (oxygen in aerobic bacteria) in anaerobes, the organic substrate undergoes a balanced series of oxidative and reductive reactions mediated by pyridine nucleotides such as NADH.

Substrate level phosphorylation is the main source from which anaerobes derived its energy and in this sense regeneration of NAD<sup>+</sup> from NAD is of critical importance. Growth rate of LAB on glucose is enhanced in the presence of O<sub>2</sub> more so when catalase is present to eliminate the H<sub>2</sub>O<sub>2</sub> formed. This observation indicates the rate at of ATP production from sugars is a growth-rate limiting factor in LAB cultures.<sup>110</sup>

Generally, production of bacteriocins in LAB cultures is a reflection of either a moderately or highly restrictive condition with regards to the availability of oxygen in the medium.<sup>109</sup> The situation could be more complex if the facultative anaerobic nature of the LAB is taken into consideration. Effects of aeration and agitation on bacteriocin production are strain dependent (Table 7). Amiali *et al.*,<sup>111</sup> claimed that nisin A production by *L. lactis* UL719 in fed batch fermentation was enhanced with aeration largely due to increase in cell-bound activity. On the other hand, aeration has been reported to be antagonistic to the production of nisin A,<sup>112</sup> lactosin S,<sup>89</sup> and LIQ-4 bacteriocin.<sup>113</sup> Furthermore, the production of nisin by *L. lactis* requires anaerobiosis, or that “typical conditions” imply moderate agitation or absence of agitation.<sup>114–116</sup> Cabo *et al.*,<sup>117</sup> found, with the same strain, maximum production under clearly aerobic conditions. Production at the maximum biomass point quadrupled when the oxygen saturation percentage was increased from 50 to 100% saturation. Vázquez *et al.*,<sup>109</sup> reported that under the extreme conditions the production of bacteriocin continued after the biomass reached the asymptotic value; this did not occur under intermediate conditions. The observation suggests that bacteriocin can change its metabolic character (primary or secondary) in response to the conditions of aeration. Reduced bacteriocin activity with increasing degree of agitation has been reported which could be due to chemical degradation and effects on gene expression.<sup>118</sup> The growth of *P. acidilactici* Kp10 increased with increasing agitation speed from 100 to 800 rpm but production of bacteriocin was only increased up to 400 rpm and significantly reduced at agitation of above 500 rpm.<sup>103</sup> It was also reported that a culture without agitation was preferred for Pediocin Iz3.13 production by *P. pentosaceus* Iz.3.13.<sup>119</sup> Several pediocin-like bacteriocins contain methionine residues whose sulfur atom could be oxidized resulting in bacteriocin destabilisation in solution with the presence of oxygen.<sup>120</sup>

Agitation is required to improve oxygen supply to the culture during the cultivation in stirred tank bioreactor. However, agitation is also related to shear rate effect. The degree of agitation has several effects on microbial growth which include cell wall disruption, changes in growth morphology, variations in the rates of growth and rates of formation of the desired product.<sup>121</sup>

### Optimization process for improvement of bacteriocin production

Optimization of the fermentation process parameters is possible for the improvement of the production of bioactive





Table 7 Effect of aeration and agitation on bacteriocin production by various LAB strains<sup>a</sup>

Bacteriocin	Class	Producer LAB strain	Remarks/effect		References
			Without agitation/aeration	With agitation/aeration	
Nisin BSA	NM	<i>L. lactis</i> subsp <i>lactis</i> BSA	<b>Without agitation</b>		Dussault <i>et al.</i> <sup>172</sup>
			Skim milk (100%, v/v)	512 (AU mL <sup>-1</sup> )	
Bacteriocin	NM	<i>L. mesenteroides</i> subsp. <i>mesenteroides</i> ATCC 8293	<b>Without agitation</b>		Danial <i>et al.</i> <sup>173</sup>
			MRS broth (100%, v/v)	6650 (AU mL <sup>-1</sup> )	
Nisin	I	<i>L. lactis</i> (MTCC 440)	<b>Without agitation</b>		Mall <i>et al.</i> <sup>174</sup>
			0 rpm	24.5 (mm)	
			100 rpm	23.75 (mm)	
			150 rpm	23.1 (mm)	
			200 rpm	21.25 (mm)	
			250 rpm	20.2 (mm)	
			<b>Moderate agitation</b>		
			0 rpm	NM	
			100 rpm	121.88 (µg mL <sup>-1</sup> )	
			140 rpm	96.88 (µg mL <sup>-1</sup> )	
180 rpm	<50 (µg mL <sup>-1</sup> )				
Plantaricin UG1	II	<i>L. plantarum</i> UG1	<b>Micro-agitation</b>		Enan & Amri <sup>152</sup>
			0 rpm	5880 (AU mL <sup>-1</sup> )	
			50 rpm	5900 (AU mL <sup>-1</sup> )	
			100 rpm	6200 (AU mL <sup>-1</sup> )	
			150 rpm	5600 (AU mL <sup>-1</sup> )	
			200 rpm	5440 (AU mL <sup>-1</sup> )	
			250 rpm	4880 (AU mL <sup>-1</sup> )	
			300 rpm	5000 (AU mL <sup>-1</sup> )	
			<b>No effect</b>		
			pH-temp. (°C)-agit. (rpm)	0 (AU)	
Pediocin	II	<i>P. acidilactici</i> ITV 26	<b>Without agitation</b>		Caldeón-Santoyo <i>et al.</i> <sup>170</sup>
			5-30-0	0 (AU)	
			5-30-200	~3600 (AU)	
			5-40-0	~20 (AU)	
			5-40-200	~2200 (AU)	
			7-30-0	~2400 (AU)	
			7-30-200	~2700 (AU)	
			7-40-0	~2200 (AU)	
			7-40-200	~1600 (AU mL <sup>-1</sup> )	
			6-35-0	52.5 (AU mL <sup>-1</sup> )	
6-35-100	118.2 (AU mL <sup>-1</sup> )				
6-35-200	118.2 (AU mL <sup>-1</sup> )				
<b>No effect</b>					
Bacteriocin KKI 197	NM	<i>P. acidilactici</i> KKU 197	<b>Without agitation</b>		Arbuwan <i>et al.</i> <sup>142</sup>
			0 rpm	~1600 (AU mL <sup>-1</sup> )	
Pediocin Iz3.13	NM	<i>P. pentosaceus</i> Iz.3.13	<b>Without agitation</b>		Kobayashi <i>et al.</i> <sup>119</sup>
			pH, 7	30 (AU mL <sup>-1</sup> )	
BLIS LA07	NM	<i>L. paracasei</i> LA07	<b>Without agitation</b>		Abbasliasi <i>et al.</i> <sup>175</sup>
			pH, 6	40 (AU mL <sup>-1</sup> )	
			pH, 5	52.5 (AU mL <sup>-1</sup> )	
			pH, 4.5	67.5 (AU mL <sup>-1</sup> )	
			No effect		



Table 7 (Contd.)

Bacteriocin	Class	Producer LAB strain	Remarks/effect		References			
			Without agitation/aeration	With agitation/aeration	Without agitation/aeration	With agitation/aeration		
BLIS Kp10	NM	<i>P. acidilactici</i> Kp10	0 rpm – anaerobic	1.38 ± 0.04 (mm)	1.56 ± 0.06 (mm)	Abbassiliasj <sup>176</sup>		
			100 rpm – anaerobic		1.48 ± 0.03 (mm)			
			200 rpm – anaerobic					
			0 rpm – aerobic	1.3 ± 0.02 (mm)				
			100 rpm – aerobic		1.48 ± 0.03 (mm)			
			200 rpm – aerobic		1.48 ± 0.03 (mm)			
			BLIS secretion was highly sensitive to agitation conditions and enhanced under moderate agitation speeds					
			0 rpm	NM				
			100 rpm		6821.93 (AU mL <sup>-1</sup> )			
			250 rpm		6885.94 (AU mL <sup>-1</sup> )			
400 rpm		12 222.10 (AU mL <sup>-1</sup> )						
600 rpm		2953.20 (AU mL <sup>-1</sup> )						
800 rpm		5057.25 (AU mL <sup>-1</sup> )						
Lactocin S	I	<i>L. sake</i> L45	Markedly decreased			Mortvedt-Abildgaard <i>et al.</i> <sup>89</sup>		
Amilovorin DCE 471	NM	<i>L. amylovorus</i> DCE 471	Decreased	2000–3000 (BU mL <sup>-1</sup> )	<200 (BU mL <sup>-1</sup> )	De Vuyst <i>et al.</i> <sup>91</sup>		
			0%	3200 (AU mL <sup>-1</sup> )	3200 (AU mL <sup>-1</sup> )			
			40%		1600 (AU mL <sup>-1</sup> )			
			80%					
Nisin Z	I	<i>L. lactis</i> IO-1	Enhanced			Chinachoti <i>et al.</i> <sup>177</sup>		
			Fermentation in flask					
			0	NM				
			120 rpm		1680 (AU mL <sup>-1</sup> )			
			210 rpm		3720 (AU mL <sup>-1</sup> )			
			320 rpm		4040 (AU mL <sup>-1</sup> )			
			340 rpm		4270 (AU mL <sup>-1</sup> )			
			410 rpm		4180 (AU mL <sup>-1</sup> )			
			Fermentation in jar fermenter					
			0	NM				
			100 rpm		3380 (AU mL <sup>-1</sup> )			
			200 rpm		3720 (AU mL <sup>-1</sup> )			
			320 rpm		3940 (AU mL <sup>-1</sup> )			
540 rpm		3590 (AU mL <sup>-1</sup> )						
800 rpm		3390 (AU mL <sup>-1</sup> )						
1000 rpm		3410 (AU mL <sup>-1</sup> )						
Nisin Z	I	<i>L. lactis</i> UL719	Dissolved oxygen concentration of 60% was optimal for production			Amiali <i>et al.</i> <sup>111</sup>		
Bacteriocin LIQ-4	NM	<i>S. faecalis var. liquefaciens</i>	~5750 (AU mL <sup>-1</sup> )	40 960 (AU mL <sup>-1</sup> )		Kuhnen <i>et al.</i> <sup>113</sup>		
			Maximum yields were produced under microaerophilic conditions	NM				
Nisin Z	I	<i>L. lactis</i> UL719	Aeration demonstrated a stimulatory effect on bacteriocin production			Desjardins <i>et al.</i> <sup>146</sup>		
			Continuous free culture	1490 (IU mL <sup>-1</sup> )	2560 (IU mL <sup>-1</sup> )			
			Immobilized cell culture	1090 (IU mL <sup>-1</sup> )	2430 (IU mL <sup>-1</sup> )			

<sup>a</sup> NM: not mentioned.

Table 8 Statistical analysis for optimization of bacteriocin production by various LAB strains<sup>a</sup>

Experimental and statistical method	Parameters/variables optimized	Producer LAB strain	Bacteriocin/BLIS	Class	Bacteriocin production		References
					Before optimization	After optimization	
FFD and RSM-CCD	Medium composition (sucrose, soybean peptone, yeast extract, KH <sub>2</sub> PO <sub>4</sub> , NaCl, and MgSO <sub>4</sub> ·7H <sub>2</sub> O)	<i>L. lactis</i> ATCC11454	Nisin A	I	1074 (IU mL <sup>-1</sup> )	2150 (IU mL <sup>-1</sup> )	Li <i>et al.</i> <sup>178</sup>
Plackett-Burman design and CCD-RSM	Culture condition and medium composition (glucose, yeast extract, tryptone, MnSO <sub>4</sub> , Tween 80, incubation period, K <sub>2</sub> HPO <sub>4</sub> , and pH)	<i>L. plantarum</i> LR/14	Plantaricin LR14	I	80 (AU mL <sup>-1</sup> )	612 (AU mL <sup>-1</sup> )	Tiwari & Srivastava <sup>179</sup>
RSM and ANN-GA	Medium composition (glucose, peptone, yeast extract, KH <sub>2</sub> PO <sub>4</sub> , NaCl, MgSO <sub>4</sub> ·7H <sub>2</sub> O)	<i>L. lactis</i>	Nisin	I	2568.70 (IU mL <sup>-1</sup> )	21 423 (IU mL <sup>-1</sup> )	Guo <i>et al.</i> <sup>134</sup>
Plackett-Burman design and RSM	Culture condition and medium composition (glucose, yeast extract, tryptone, triammonium citrate, sodium acetate, MgSO <sub>4</sub> , MnSO <sub>4</sub> , K <sub>2</sub> HPO <sub>4</sub> , pH, incubation period and Tween 80)	<i>E. faecium</i> LR/6	Enterocin LR/6	NM	300 (AU mL <sup>-1</sup> )	606 (AU mL <sup>-1</sup> )	Kumar & Srivastava <sup>180</sup>
OVAT and RSM-CCD	Medium composition (carbon sources, nitrogen sources and NaCl concentration)	<i>E. faecium</i> MC13	Bacteriocin MC13	NM	6400 (AU mL <sup>-1</sup> )	25 600 (AU mL <sup>-1</sup> )	Kanmani <i>et al.</i> <sup>181</sup>
Taguchi design	Culture condition and medium composition (temperature, oxygen, yeast extract and peptone)	<i>E. faecium</i>	Enterocin A	II	0 (AU mL <sup>-1</sup> )	3200 (AU mL <sup>-1</sup> )	Mirhosseini & Emtiazi <sup>17</sup>
RSM-CCRD	Culture condition temperature, pH and incubation time	<i>P. acidilactici</i> MPK1	Bacteriocin MPK1	NM	8 (mm)	23 (mm)	Patil <i>et al.</i> <sup>159</sup>
RSM-CCD	Culture condition (pH, temperature, incubation period)	<i>Lactobacillus</i> spp. (LBC216)	Bacteriocin LBC216	NM	5200 (AU mL <sup>-1</sup> )	11 200 (AU mL <sup>-1</sup> )	Arokiyama & Sivakumara <sup>182</sup>
RSM-CCD	Culture condition and media composition (temperature, initial pH, and sucrose concentration)	<i>E. faecium</i> DB1	Bacteriocin DB1	NM	1280 (AU mL <sup>-1</sup> )	2560 (AU mL <sup>-1</sup> )	Choi <i>et al.</i> <sup>183</sup>
OFAT and CCD-RSM	Culture condition (temperature, pH, incubation time, inoculum size)	<i>L. casei</i> LA-1	Bacteriocin LA-1	NM	2844 (AU mL <sup>-1</sup> )	4652.15 (AU mL <sup>-1</sup> )	Kumar <i>et al.</i> <sup>184</sup>
FFD RSM-CCD	Culture condition and medium composition (glucose, yeast extract, MgSO <sub>4</sub> , temperature and initial pH)	<i>L. brevis</i> DF01	Bacteriocin DF01	NM	320 (AU mL <sup>-1</sup> )	1280 (AU mL <sup>-1</sup> )	Lee <i>et al.</i> <sup>122</sup>
ANN	Culture condition (temperature, inoculum size and agitation speed)	<i>P. acidilactici</i> Kp10	BLIS	NM	888.6 (AU mL <sup>-1</sup> )	5118.5 (AU mL <sup>-1</sup> )	Abbasiliasi <i>et al.</i> <sup>103</sup>

<sup>a</sup> One-variable-at-a-time (OVAT); one-factor-at-a-time method (OFAT); fractional factorial designs (FFD); response surface methodology (RSM); fractional factorial designs (FFD); central composite rotatable design (CCRD); artificial neural network-genetic algorithm (ANN-GA); NM: not mentioned.

peptides, which are of great interest for the design of functional foods and nutraceuticals. Although finding an optimal production process is a complex problem, but it is essential for the development of economically viable commercial bacteriocin production which leads to high yields and cost-effective process. High production yields are achieved by optimization of culture conditions or medium composition. The growth of bacteria and the accumulation of their metabolites are strongly influenced by the environment and medium compositions such as culture pH, carbon and nitrogen sources, growth factors, and minerals. Detection of these major factors and their optimization for biotechnological processes including multivariables are difficult.<sup>122</sup> Optimization of fermentation process is time consuming, labor intensive, involves high cost and requires many experimental trials. With the introduction of new mutants and strains, these trials are necessary for industrial applications. The trials entail combinations of different factors and sequence of processes. The influence of medium components also need to be investigated to ensure that the growth conditions which produce the biomass and the physical state are best suited for product formation.

Two different systems, close-end and open-ended, are normally used in the optimization process. In the closed-end system optimization analysis is directed towards a fixed number and types of component parameters. Being a simple strategy many different possible components and/or parameters not given due consideration could be beneficial to the process. In the open-ended system analysis is on any number and types of components and/or parameters. This system makes no assumption which components/parameters are best suited for the process. The common practice adopted has always been to start off with the open-ended system; the best components/parameters are selected and subsequently followed by the close-ended system.

Specific requirements with reference to the production of bacteriocins have been reported.<sup>123–125</sup> Bacteriocin titers can be modified by altering the cultivation conditions of the producing bacterium and certain combinations of influencing factors could be optimized to enhance bacteriocin production.<sup>126</sup> With regards to the complexity of the factors within the food environments itself an in-depth knowledge of the interacting factors influencing the production of bacteriocin need to be understood for subsequent application in the optimization process. Most studies carried out to date claimed validation by statistical analysis and a combination of variables. However their values and limits were arbitrarily chosen based primarily on personal experience.<sup>103</sup>

Conventional methods in fermentation optimization require treating each factor separately which is laborious, incomplete and time consuming. If several factors are to be considered simultaneously their interactions are not discernible even for the dominant ones. These conventional approaches did not yield reliable results either. In this respect, experimental factorial design has been successfully applied for the optimization of various biomanufacturing processes which could also be used to investigate the interacting factors.<sup>103,127,128</sup>

Response surface methodology (RSM), a non-conventional approach is a collection of statistical and mathematical

methods that could be used to quantify the interaction between different factors. This approach provides statistically reliable results with fewer number of experiments and are very useful for the development, improvement and optimization of the biomanufacturing processes.<sup>129–131</sup> RSM is based on analysis of responses induced by specific factors. This methodology is also useful in determining the direction of subsequent experiment towards an optimum point where the equation can be determined from the optimization point of the response surface.<sup>103,132</sup> This method was successfully applied in many areas of biotechnology, including some studies on bacteriocin production (Table 8).

Artificial neural network (ANN) on the other hand has recently emerged as one of the most efficient methods for empirical modeling and prediction in solving complex systems such as bacteriocin production. Several studies have demonstrated that the accuracy for the prediction of ANN models was far more superior compared to RSM using the same experimental design. ANN does not require prior specification of a suitable fitting function. ANN has the universal approximation capability which means that it can approximate almost all types of non-linear functions including quadratic functions. The ability of ANN to predict process characteristics with little prior knowledge is desirable which simplifies their implementation and increases their modeling potential. This property makes ANN a powerful and flexible tool that is well-suited for modeling biochemical processes.<sup>103,133–135</sup> Several reports have demonstrated that the predictive accuracy of ANN model was superior to RSM model using the same experimental design.<sup>134,136–138</sup> However, ANN is known as a black box modeling approach. The effect of factors on response values and the interaction effect among the factors cannot be studied by ANN model.

Several statistical methods such as response surface methodology (RSM) and artificial neural network (ANN) have been applied in the optimization of LAB fermentation for improvement of bacteriocin production (Table 8). Substantial improvement in bacteriocin production was achieved with optimal medium compositions and/or culture conditions as optimized using RSM and ANN.

## Conclusions

Production of bacteriocin by LAB is greatly influenced by medium formulation and culture conditions. Growth of LAB and bacteriocins production is not only affected by the type of carbon (C) and nitrogen (N) sources but also by their concentrations and ratios. The presence of surfactant in the culture medium also improved the production of bacteriocins by some LAB strains. Besides the culture pH, aeration and oxygen supply also exert significant effect on growth of some LAB strains and bacteriocin production. Statistical methods such as RSM and ANN have been used successfully to optimize the culture condition and medium formulations for improvement of growth of LAB and bacteriocin production.



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