

Optimization of bacteriocin production by *Lactobacillus plantarum* using Response Surface Methodology

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ABSTRACT

Bacteriocin production is influenced by various factors such as carbon and nitrogen sources as well as fermentation conditions including pH, temperature, and agitation—these factors aid in optimizing bacteriocin production and improving its inhibitory activity against pathogens for great economic significance. The study investigates the effect of growth conditions on bacteriocin production by *Lactobacillus plantarum*. The response surface methodology was applied to optimize and determine the interaction among the process variables in bacteriocin production by *Lactobacillus plantarum* and determine their optimum levels. Chloroform–Methanol (2:1 v/v) was used for crude bacteriocin extraction through the liquid-liquid extraction method, and its antimicrobial activity was assessed. The sample has shown inhibitory activity against all the organisms, i.e., *E. coli*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa* good diffusion. Compared to control and ciprofloxacin, the sample at all concentrations (Lab 9, 1%, 2%, 3%) has shown better inhibitory activity in pH-7 and-8 at 37⁰ C against *S.aureus* in good diffusion. The selected factors were carbon and nitrogen source, temperature, and pH. The bacteriocin was produced at maximum activity in MRS broth supplemented with 1% dextrose and 1% ammonium nitrate. RSM analysis found that the optimal temperature for bacteriocin production was 36°C at a pH of 6.5 using 1% inoculum. At the same time, the increase in the percentage of inoculum (2% and 3%) did not affect bacteriocin production. The quadratic model found that temperature and pH profoundly affected bacteriocin production.

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Introduction

Lactic acid bacteria (LAB) are microorganisms responsible for the natural fermentation of dairy products and are being explored for their potential use as adjuvants and bio preservatives in food products (1). LAB also has considerable inhibitory activity against pathogenic bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and microorganisms (*Listeria monocytogenes* and *Salmonella* spp) that causes food spoilage (2) The preservative effect of LAB is primarily due to the production of organic acids (such as lactic acid) which results in lowered pHs (3). LAB also produces antimicrobial compounds such as hydrogen peroxide, carbon dioxide, diacetyl, acetaldehyde, D-isomers of amino acids, reuterin, and bacteriocins (3).

Lactobacilli produce specific antimicrobial compounds such as bacteriocins, forming an important prevents bioactive class that food spoilage (4). Bacteriocins are protein or protein complexes produced by bacteria and have antimicrobial activity against closely related species and various gram-positive and gram-negative bacteria, including food spoilage bacteria and pathogens (5). The bacteriocins from LAB are generally recognized as safe and have evolved a great deal of attention as a novel method to control pathogens in foodstuffs (6). For these reasons, bacteriocins have recently attained much attention for use as natural or so-called 'biopreservatives' in foods.

Previous research studied the effect of nitrogen and carbon sources on the production of plantaricin ST31, plantaricin 423, plantaricin UG1, plantaricin KW30, and plantaricin ST13BR (7-10). However, studies on the effects the growth conditions for optimal production of bacteriocins are lacking. Previous studies reported that bacteriocins produced by LAB such as pediocin PD-1, enterocin P, enterocin AS-48, and sakP have demonstrated that growth pH and temperature are often regulated (11-14). With this hiatus, we evaluated the effects of nutrients and some environmental factors such as medium pH and temperature on bacteriocin production by *Lactobacillus plantarum* by applying the RSM model.

Materials and Methods

Bacterial strains, media, and growth conditions

The bacteriocin-producing strain used in this study was goat milk isolate (*L. plantarum*) which was isolated. Bacterial cultures of *L. plantarum* were revived by inoculating 1% (v/v) frozen stock cultures into 10 mL MRS broth (Lactobacillus MRS Broth-Cat No: 49190) and incubated at 37°C under static conditions. The cultures were then streaked onto the MRS agar plate and incubated for another 48h. A single pure colony was then inoculated and incubated in 10 mL MRS broth for 48 h, then sub-cultured into 10-mL MRS broth for another 24 h of incubation. (15)

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Extraction of Bacteriocin

Chloroform–Methanol (2:1 v/v) was used for crude bacteriocin extraction through liquid-liquid extraction. However, precipitate produced at solvent-aqueous inter-phase was collected aseptically, the solvent was evaporated, and precipitate was kept in the buffer for antimicrobial study. Lab 9 cultures were dissolved in the ratio of 2:1 (Chloroform: Methanol) & (5ml was used for liquid-liquid partition chromatography). Soluble fractions were separated using a separating funnel. L-L fractions were kept for evaporation in a water bath at 70°C. L-L fractions were analyzed by agar well diffusion method to check the bacteriocin production (16).

Determination of antimicrobial activity

E.coli, *S.aureus*, *K.pneumoniae*, and *P.aeruginosa* cell suspension were prepared and grown on Peptone broth and cultures were incubated for 24hrs at 37°C. Cultures were incubated for 24-48 hrs at 27°C. The cell suspensions of all the cultures were adjusted to 1-2x 10⁶cells/ml. Later, *E.coli*, *S.aureus*, *K.pneumoniae* and *P.aeruginosa* were inoculated on Soya bean Casein Digested agar plates (90 mm). Then, the test compounds i.e., sample (25µl), standard ciprofloxacin (25µl), Itracanzole (25µl) for *E.coli*, *S.aureus*, *K.pneumoniae* and *P.aeruginosa* were added to the 5mm well on agar plates. The treated plates with *E.coli*, *S.aureus*, *K.pneumoniae* and *P.aeruginosa* were incubated in the aerobic chamber at 37°C for 24h and were observed for the zone of inhibition around the wells. Bacteriocin activity was expressed as an arbitrary unit per ml (AU/ml) and calculated from the formula Diameter of the zone of clearance*1000/ Volume taken in the well. (17)

Optimization of carbon and nitrogen sources for bacteriocin production

To achieve the maximum bacteriocin production by *L. plantarum*, the various media components like carbon sources (fructose, sucrose, and lactose individually at 1.0%) and nitrogen sources (ammonium acetate, ammonium nitrate and sodium citrate individually at 1.0%) were substituted in the production medium. Appropriate control (MRS medium) was also maintained. Then, all the media were inoculated aseptically with *L. plantarum* and kept in an orbital shaker (120 rpm) at 37°C for 48 h. After 48 h incubation absorbance was measured at 600 nm using spectrophotometer. Cells were centrifuged at 10,000 × g for 15 min at 4°C. After that the cells free supernatant was collected from each tube and examined for bioactivity to determine the bacteriocin production over shrimp pathogens by agar well diffusion assay

Effect of pH and cultivation temperature on bacteriocin production and determination of its antimicrobial activity

The influence of pH on bacteriocin production by the *L. plantarum* was examined. pH, such as 6.0, 7.0 and 8.0 were fixed using 1N NaOH and 1N HCl in the culture medium (MRS Broth). Culture (1%, 2% and 3%) was then aseptically inoculated with MRS Broth and kept in an orbital shaker (120 rpm) for 48h. Similarly, bacteriocin production with the bacterium was optimized by varying the incubation temperature individually viz., 30, 37 and 42°C. Later, bacteriocin production (AU/ml) and bacterial growth were determined.

S. aureus cell suspension was prepared and grown on peptone broth and cultures were incubated for 24hrs at 37°C. The cell suspension of the culture was adjusted to 1-2x 10⁶cells/mL and inoculated on Soya bean Casein Digested agar plates (90 mm). Then, the test compounds i.e., sample (25µl), standard ciprofloxacin (25µl), and Itracanzole (25µl) for *S.aureus*, *K.pneumoniae* were added to the 5mm well on agar plates. The treated plates with *S.aureus* were incubated in the aerobic chamber at 37°C for 24hrs and were observed the for the zone of inhibition around the wells. Bacteriocin activity was expressed as arbitrary unit per ml (AU/ml) and calculated from the formula Diameter of the zone of clearance*1000/ Volume taken in the well.

In this RSM, the 2D contour plot and 3D response surface curves are the graphical representation of the regression equation. Each figure showed the effect of two variables (pH and temperature) on the bacteriocin production at various concentrations. The response surface curves were plotted to understand how the variables interacted and to determine the optimum level of each variable for maximum response. In 3D-response surface graphs, the elliptical form of the contour reflects the mutual interactions of all variables in producing a significant effect on the responses. (18,19)

Statistical methods

Data is analyzed using statistical software **SPSS version 21** and **Microsoft Excel**. Difference among the groups was analysed by ANOVA . P-value less than or equal to 0.05 indicates statistical significance.

Results

The zone of inhibition observed with test sample (Lab 9), standard (ciprofloxacin), and control (Meoh+Chcl3) against different test organisms are summarized in Table 1. The sample has shown inhibitory activity against all the organisms i.e., *E. coli*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa* in well diffusion.

The influence of different carbon sources on bacteriocin production was evaluated using various media components like carbon sources (fructose, sucrose, and lactose individually at 1.0%) (Fig. 1a). The three carbon sources tested were suitable for bacterial growth; however, dextrose was examined for bioactivity to determine the bacteriocin production over pathogens by agar well diffusion assay than lactose, sucrose, and control (MRH broth media) groups, respectively. The effect of different nitrogen sources on bacteriocin production is shown in Fig. 1a. The highest bacteriocin activity was seen in bacterial cells grown in MRS broth supplemented with ammonium nitrate than that observed in other nitrogen sources (Figure 1b).

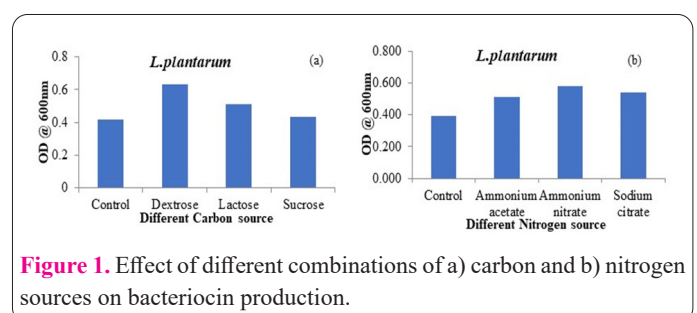


Figure 1. Effect of different combinations of a) carbon and b) nitrogen sources on bacteriocin production.

The influence of different pH and temperatures on bacteriocin production in MRS broth at pH from 6 to 8 at each temperature i.e., 30°C, 37°C, and 42°C is shown in Table 2. The sample (Lab 9) at all concentrations (1%, 2%, 3%) has shown better inhibitory activity in pH-7, and pH-8 at 37°C against *S.aureus* in well diffusion.

The results of ANOVA for the current model indicated its high statistical significance (Table 4). The model's goodness of fit was checked by the correlation coefficient multiple $R=0.825183$, determination coefficient $R^2=0.680927$, and adjusted $R^2=0.512006$, the values of which confirmed the moderate agreement between the experimental the predicted values explained by this model.

Lesser p-values suggest more impact of assessed factors on the final output. The response surface quadratic model found that temperature and pH profoundly affected the bacteriocin production. While other interaction coefficients such as % of inoculum were insignificant (Table 5).

Discussion

Bacteriocin production is influenced by various factors such as carbon and nitrogen sources as well as fermentation conditions including pH, temperature, agitation. These factors aid in optimizing bacteriocin production and improving its activity for great economic significance. It was reported that bacteriocin production usually occurs during the mid-exponential phase and increases to reach a maximal level at the end of the exponential phase or the beginning of the early-stationary phase (20). Hence, in our study, we studied the effect of growth media, carbon and nitrogen sources, pH, and temperature on bacteriocin production by *L. plantarum* by RSM methodology.

According to literature, MRS medium supports abundant growth of all lactobacilli from oral, faecal, dairy, and other sources lactobacilli. The presence of peptones and dextrose in MRS medium supplies nitrogen and carbon. While tween 80, acetate, magnesium, and manganese in MRS medium provide growth factors aids in cultivation of a variety of lactobacilli. These ingredients may also inhibit the growth of some organisms other than lactobacilli (21). As a results, we used MRS medium for successful isolation of LABs for bacteriocin production.

Research reported that most of the bacteria preferentially utilize glucose for bacteriocin production against complex sugars due to less catabolic steps (22). In our study, *L. plantarum* grown in MRS medium in the presence of dextrose produced more bacteriocin levels at 0.632 OD when compared to lactose or sucrose. While Todorov reported bacteriocin ST23LD production was stimulated by maltose at a concentration of 20 g/l and higher. While the presence of glucose (20 g/l), sucrose (20 g/l) and maltose (10 g/l) yielded a bacteriocin level of 2910 AU/OD (23). Amer et al., reported lactose produced ~7-fold and ~6-fold more bacteriocin than sucrose and glucose, respectively when supplemented with 0.5% carbon source in an M17 broth media (20). Further, galactose and raffinose although resulted in good growth produced low levels of bacteriocin (20). Other studies reported that bacteriocins such as *plantaricin* KW30 9 and *plantaricin* ST 317 were stimulated at higher glucose concentrations. Cheigh et al., reported *Lactobacillus lactis* subsp. *lactis* A164 isolated from kimchi produced 4-fold greater nisin-like bacteriocin in M17 broth when supplemented with lactose than other

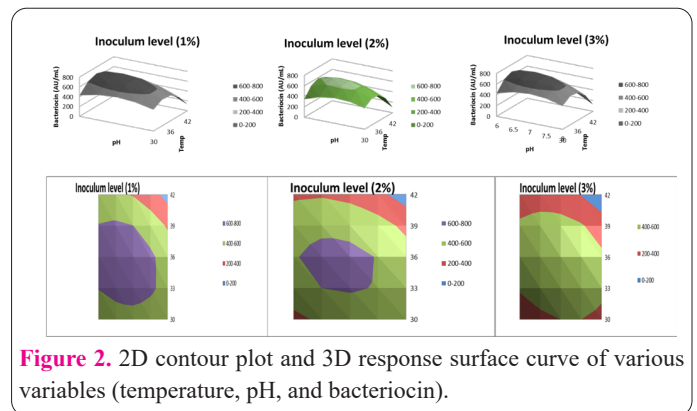


Figure 2. 2D contour plot and 3D response surface curve of various variables (temperature, pH, and bacteriocin).

carbon sources (24).

In our study, maximal bacteriocin production and inhibitory activity were observed with ammonium nitrate in MRS media compared to ammonium acetate and sodium citrate. Todorov et al reported that the combination of yeast extract and tryptone in 1:1 ratio produced optimal bacteriocin ST23LD production. While strain ST341LD grown in the presence of tryptone as a sole nitrogen source, a combination of tryptone and meat extract or tryptone plus yeast extract produced bacteriocin levels of 5672 AU/OD indicating tryptone as the key nitrogen source for optimal bacteriocin ST341LD production (24). Similarly plantaricin 423 in MRS broth supplemented with bacteriological peptone, followed by casino acids, tryptone, and meat extract showed optimal bacteriocin production (8). As far as we could determine, this is the first report of ammonium nitrate being the choice of nitrogen source in producing *L. plantarum* bacteriocins. Amer et al., reported that the highest bacteriocin activity (12,246 AU/ml) was observed in bacterial cells cultured in M17 broth supplemented with 1.0% yeast extract and the activity was ~2-fold more significant than that seen in other nitrogen sources for bacteriocin production by strain *L. acidophilus* AA11 (20).

In our study, 1% *L. plantarum* culture stimulated maximum bacteriocin production in MRS broth at an initial pH of 6, 6.5 and 7 at a temperature of 36°C. However, an increase in temperature and pH eventually decreased the bacteriocin production. Concerning 2% and 3% *L. plantarum* did not increase the bacteriocin production, indicating that the production mainly depends on the pH and temperature. Overall, our results conclude that bacteriocin production by *L. plantarum* is stimulated maximum at a pH of 6.5 and at a temperature of 36°C. On the other hand slightly repressed at a pH of 7 and above and at temperatures above or below 36°C. Todorov et al demonstrated that optimal bacteriocin ST23LD was produced at 2930 AU/OD in MRS broth at a pH of 6.5. whereas the bacteriocin ST23LD activity (1460 AU/OD) was reduced to 50% in the same medium adjusted to an initial pH of 6.0.18 While with respect to bacteriocin ST341LD, the production was inhibited when the strain was grown at a pH of 6.5, however stimulated at pH of 6.0 or 5.5 (23). The results were consistent with another study by Todorov et al., for other bacteriocins produced by *L. plantarum*.7 Cheigh et al demonstrated that the maximum biomass produced by *Lactococcus lactis* subsp. *lactis* A164 was obtained at 37 °C and the optimal temperature for the bacteriocin production was 30°C (25). Furthermore, the optimal pH for growth and bacteriocin production was maximum at 6.0 and the show was nearly the same level at pH 5.5 and 6.5 (25,26).

Table 1. Inhibitory activity of test compounds against test organisms.

Test Organisms	Test Compounds	Conc. per well (µg/ml)	Zone of inhibition (mm)	Arbitrary Unit (U/ml)
<i>Escherichia coli</i>	Control (Meoh+Chcl3)	-	-	-
	Ciprofloxacin (Standard)	2.5	28	1120
	<i>L.plantarum</i> (Lab 9)	100%	13	520
<i>Staphylococcus aureus</i>	Control (Meoh+Chcl3)	-	-	-
	Ciprofloxacin (Standard)	2.5	27	1080
	<i>L.plantarum</i> (Lab 9)	100%	14	560
<i>Klebsiella pneumoniae</i>	Control (Meoh+Chcl3)	-	-	-
	Ciprofloxacin (Standard)	2.5	25	1000
	<i>L.plantarum</i> (Lab 9)	100%	18	720
<i>Pseudomonas aeruginosa</i>	Control (Meoh+Chcl3)	-	-	-
	Ciprofloxacin (Standard)	2.5	25	1000
	<i>L.plantarum</i> (Lab 9)	100%	14	560

Meoh+Chcl3, Methanol – Chloroform

Table 2. Inhibitory activity of test sample against *Staphylococcus aureus*.

pH and Temperature	Test Compounds	Conc. per well(µg/ml)	Arbitrary units(U/ml)
pH 6 30°C	Control	-	-
	Ciprofloxacin (Standard)	2.5	1000
	Lab 9 (1%)	100%	400
	Lab 9 (2%)	100%	440
	Lab 9 (3%)	100%	520
pH 6 37°C	Control	-	-
	Ciprofloxacin (Standard)	2.5	960
	Lab 9 (1%)	100%	320
	Lab 9 (2%)	100%	380
	Lab 9 (3%)	100%	560
pH 6 42°C	Control	-	-
	Ciprofloxacin (Standard)	2.5	960
	Lab 9 (1%)	100%	320
	Lab 9 (2%)	100%	400
	Lab 9 (3%)	100%	480
pH 7 30°C	Control	-	-
	Ciprofloxacin (Standard)	2.5	1040
	Lab 9 (1%)	100%	280
	Lab 9 (2%)	100%	320
	Lab 9 (3%)	100%	440
pH 7 37°C	Control	-	-
	Ciprofloxacin (Standard)	2.5	1040
	Lab 9 (1%)	100%	600
	Lab 9 (2%)	100%	680
	Lab 9 (3%)	100%	800
pH 7 42°C	Control	-	-
	Ciprofloxacin (Standard)	2.5	960
	Lab 9 (1%)	100%	360
	Lab 9 (2%)	100%	400
	Lab 9 (3%)	100%	400
pH 8 30°C	Control	-	-
	Ciprofloxacin (Standard)	2.5	960
	Lab 9 (1%)	100%	320
	Lab 9 (2%)	100%	560
	Lab 9 (3%)	100%	640
pH 8 37°C	Control	-	-
	Ciprofloxacin (Standard)	2.5	920
	Lab 9 (1%)	100%	440
	Lab 9 (2%)	100%	520
	Lab 9 (3%)	100%	640
pH 8 42°C	Control	-	-
	Ciprofloxacin (Standard)	2.5	920
	Lab 9 (1%)	100%	-
	Lab 9 (2%)	100%	-
	Lab 9 (3%)	100%	-

Table 4. Analysis of variance for the current regression model.

ANOVA	df	SS	MS	F	P-value
Regression	9	648424.2	72047.13	4.03104	0.006511365
Residual	17	303842.5	17873.09		
Total	26	952266.7			

Table 5. ANOVA table for the response surface quadratic model.

	Coefficients	Standard Error	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-16118.3	3760.024	0.000499	-24051.29003	-8185.38	-24051.3	-8185.376641
A Temperature	550.9069	121.6942	0.000298	294.1547065	807.6592	294.1547	807.6591861
B pH (units)	1961.667	803.0695	0.025793	267.3381322	3655.995	267.3381	3655.995201
C Inoculum (%)	306.6667	419.2279	0.474435	-577.826904	1191.16	-577.827	1191.160237
AA	-6.52381	1.566598	0.00065	-9.829041925	-3.21858	-9.82904	-3.218577123
BB	-106.667	54.57882	0.06731	-221.8179214	8.484588	-221.818	8.48458807
CC	-10	54.57882	0.856792	-125.1512547	105.1513	-125.151	105.1512547
A x B	-13.0428	6.402603	0.057518	-26.55112459	0.465498	-26.5511	0.46549768
A x C	-0.77982	6.402603	0.904488	-14.28812765	12.72849	-14.2881	12.72849462
B x C	-25	38.59306	0.525777	-106.4242331	56.42423	-106.424	56.42423309

RSM is an effective approach for optimizing the fermentation medium for bacteriocin production (27,28). In the present study, the experimental results clearly showed that the bacteriocin synthesis was primarily influenced by dextrose and ammonium nitrate concentration, initial pH, and temperature. The combination of carbon and nitrogen source concentration for the highest accumulated bacteriocin may need also be estimated further. Also in future meta-analysis regarding different techniques available and yield of the bacteriocin can be studied.

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Nil.

Interest conflict

Authors don't have any conflict of interest.

References

- Lactic Acid Bacterium - an overview | ScienceDirect Topics Available from: <https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/lactic-acid-bacterium>. Accessed May 10, 2022.
- Muñoz-Atienza E, Gómez-Sala B, Araújo C, Campanero C, del Campo R, Hernández PE, Herranz C, Cintas LM. Antimicrobial activity, antibiotic susceptibility and virulence factors of Lactic Acid Bacteria of aquatic origin intended for use as probiotics in aquaculture. *BMC Microbiol* 2013; 13:15.
- Sanlibaba P, Güçer Y. Antimicrobial activity of lactic acid bacteria. 2015; 3:451–457.
- Sowani H, Thorat P. Antimicrobial Activity Studies of Bacteriocin Produced by Lactobacilli Isolates from Carrot Kanji. *Online J Biol Sci* 2012; 12:6–10.
- Meade E, Slattery MA, Garvey M. Bacteriocins, Potent Antimicrobial Peptides and the Fight against Multi Drug Resistant Species: Resistance Is Futile? *Antibiotics* 2020; 9:32.
- Garcia Lira A. Antimicrobial Effects of Bacteriocins Produced by Lactic Acid Bacteria Against *Listeria* on Cantaloupe (Cucumis Melo) Surface. LSU Masters Theses Available from: https://digitalcommons.lsu.edu/gradschool_theses/5025. 2019.
- Todorov S, Gotcheva B, Dousset X, Onno B, Ivanova I. Influence of Growth Medium on Bacteriocin Production in *Lactobacillus plantarum* ST31. *Biotechnol Biotechnol Equip* 2000; 14:50–55.
- Verellen TLJ, Bruggeman G, Van Reenen CA, Dicks LMT, Vandamme EJ. Fermentation optimization of plantaricin 423, a bacteriocin produced by *Lactobacillus plantarum* 423. *J Ferment Bioeng* 1998; 86:174–179.
- Kelly W j., Asmundson R v., Huang C m. Characterization of plantaricin KW30, a bacteriocin produced by *Lactobacillus plantarum*. *J Appl Bacteriol* 1996; 81:657–662.
- Todorov SD, van Reenen CA, Dicks LMT. Optimization of bacteriocin production by *Lactobacillus plantarum* ST13BR, a strain isolated from barley beer. *J Gen Appl Microbiol* 2004; 50:149–157.
- Nel HA, Bauer R, Vandamme EJ, Dicks LM. Growth optimization of *Pediococcus damnosus* NCFB 1832 and the influence of pH and nutrients on the production of pediocin PD-1. *J Appl Microbiol* 2001; 91:1131–1138.
- Herranz C, Martínez JM, Rodríguez JM, Hernández PE, Cintas LM. Optimization of enterocin P production by batch fermentation of *Enterococcus faecium* P13 at constant pH. *Appl Microbiol Biotechnol* 2001; 56:378–383.
- Abriouel H, Valdivia E, Gálvez A, Maqueda M. Influence of physico-chemical factors on the oligomerization and biological activity of bacteriocin AS-48. *Curr Microbiol* 2001; 42:89–95.
- Aasen IM, Møretro T, Katla T, Axelsson L, Storror I. Influence of complex nutrients, temperature and pH on bacteriocin production by *Lactobacillus sakei* CCUG 42687. *Appl Microbiol Biotechnol* 2000; 53:159–166.
- Iyapparaj P, Maruthiah T, Ramasubburayan R, Prakash S, Kumar C, Immanuel G, Palavesam A. Optimization of bacteriocin production by *Lactobacillus* sp. MSU3IR against shrimp bacterial pathogens. *Aquat. Biosyst.* 2013 Dec;9(1):1-0.
- Cheng M, Gong SG, Lévesque CM. Rapid Isolation and Purification of Secreted Bacteriocins from *Streptococcus mutans* and Other Lactic Acid Bacteria. *Bio-protocol*. 2020 Nov 20;10(22):e3824-.
- Tzu-Hsing Lin, Tzu-Ming Pan, characterization of an antimicrobial substance produced by *Lactobacillus plantarum* NTU 102, *J Microbiol Immunol Infect*; 2019. pp. 409-417
- Desai A. Strain identification, viability and probiotics properties of *Lactobacillus casei*, Thesis. Australia: school of Biomedical and health sciences, Victoria University, Werribee campus Victoria; 2008. pp. 48–53.

19. Soleimanian-Zad S, Mirlohi M, Dokhani S, et al. Investigation of acid and bile tolerance of native lactobacilli isolated from fecal samples and commercial probiotics by growth and survival studies. *Iranian J of Biotech.* 2009;7:4 230–240.
20. Abo-Amer AE. Optimization of bacteriocin production by *Lactobacillus acidophilus* AA11, a strain isolated from Egyptian cheese. *Ann Microbiol* 2011; 61:445–452
21. Dehydrated, culture, media, *Lactobacillus*, peptose Available from: <https://www.fishersci.ca/shop/products/bd-difco-dehydrated-culture-media-lactobacilli-mrs-broth-3/p-4869073>. Accessed May 14, 2022.
22. Lee J-H, O’Sullivan D. Genomic Insights into Bifidobacteria. *Microbiol Mol Biol Rev* MMBR 2010; 74:378–416.
23. Todorov SD, Dicks LMT. Effect of medium components on bacteriocin production by *Lactobacillus plantarum* strains ST23LD and ST341LD, isolated from spoiled olive brine. *Microbiol Res* 2006; 161:102–108.
24. Todorov SD, van Reenen CA, Dicks LMT. Optimization of bacteriocin production by *Lactobacillus plantarum* ST13BR, a strain isolated from barley beer. *J Gen Appl Microbiol* 2004; 50:149–157.
25. Cheigh C-I, Choi H-J, Park H, Kim S-B, Kook M-C, Kim T-S, Hwang J-K, Pyun Y-R. Influence of growth conditions on the production of a nisin-like bacteriocin by *Lactococcus lactis* subsp. *lactis* A164 isolated from kimchi. *J Biotechnol* 2002; 95:225–235.
26. Daba GM, El-Dien AN, Saleh SA, Elkhateeb WA, Awad G, Nomiya T, Yamashiro K, Zendo T. Evaluation of *Enterococcus* strains newly isolated from Egyptian sources for bacteriocin production and probiotic potential. *Biocatal. Agric. Biotechnol.* 2021 Aug 1;35:102058.
27. Tiwari S, Srivastava S. Statistical Optimization of Culture Components for Enhanced Bacteriocin Production by *Lactobacillus plantarum* LR/14. *Food Biotechnol* 2008; 22:64–77.
28. Cladera-Olivera F, Caron GR, Brandelli A. Bacteriocin production by *Bacillus licheniformis* strain P40 in cheese whey using response surface methodology. *Biochem Eng J* 2004; 21:53–58.