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ANTIMICROBIAL EFFICACY OF *Lactobacillus plantarum* STRAIN AGAINST THE B*. cereus, B. subtilis, S. aureus* AND *E.coli* STRAINS

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Abstract

The current study aimed to detect the antimicrobial effect of cell free supernatant (CFS) of *Lactobacillus plantarum* strain against Gram +ve and Gram –ve bacterial strains. The strain of *Lactobacillus plantarum* was isolated using selective media MRS agar. The strain was characterized on the basis of the gram staining, colony morphology, the biochemical tests and the DNA sequencing based method of 16S ribotyping. A total of four test strains (The three already isolated and reported strains (*E.coli, S. aureus and B. subtilis*) and the one recently identified novel strain (*B. cereus*) were used for the analysis of antagonistic activity of bacteriocin produced by *L. plantarum* strain. The CFS of *L. plantarum* showed zone of inhibition against all the test strains (Gram +ve and Gram –ve bacteria). The conditions favoring the growth of bacteria were associated with the antimicrobial efficacy of CFS. Bacteriocin activity of CFS remained effective after exposure to temperature stress. Wide range of antagonistic potential of CFS of *L. plantarum* provides an alternative for antibiotics in pharmaceutical industry. Heat resistant feature of bacteriocin suggests its application in food industry.

Keywords: Antimicrobial activity. Bacteriocin. *Bacillus cereus*. *Lactobacillus plantarum*. Molecular identification.

1. Introduction

Lactobacilli enhance the stability of gut permeability, renovate micro flora in intestine and upgrade the inflammatory reaction due to production of different metabolites including lactic acid, propionic acid, acetic acid, H2O2, diacetyl, reuterin, antimicrobial peptides (AMPs) and the bacteriocins (Iorizzo et al. 2020) Their feature of antagonistic action against Gram -ve and Gram +ve bacteria, causing the food spoilage, indicates their importance as natural bio preservative (Nawaz et al. 2009; Riaz et al. 2010). In poultry industry, these are used for their diverse benefits associated with the growth performance and meat quality of broiler chicks (Abbas et al. 2021). The different strains of *L. plantarum* produce a variety of bacteriocins (Goel et al. 2020). Formerly, a large number of bacteriocins has been identified and purified for the microbial control (Banik et al. 2019).

L. plantarum is a greatly adaptable species of Lactobacilli with largest genome. These are often found in fermented products as well as in gut biota. These enhance the vitamins concentrations in the fermentation products, moderate the host immune response and form new vitamins in the human gut (Liu et al. 2018). Their antimicrobial effects could be utilized to enhance the shelf life of stored food. It could

also be a replacement for the antibiotics for the treatment of pathogenic infections (Zubair and Nawaz 2017).

The purpose of current work is to screen and identify the bacteriocin producing Lactobacillus. Further a strain of *Bacillus cereus* was also screened out and recognized. Present study pursues the control of pathogenic strain through the cell free supernatant of isolated Lactobacilli. Optimization conditions like temperature and pH for bacteriocin production were also studied. Moreover characterization of bacteriocin was performed to check its possible use as antibacterial agent.

2. Material and Methods

Screening and Identification of Lactobacillus strain and Test Strain

Yogurt sample was collected from the cafeteria of University of Sargodha. It was used for the isolation of *Lactobacillus* strains following the method as mentioned earlier (Akhtar et al. 2020). Phenotypic parameters of colonies included shape, size, margin, color, elevation, opacity and texture. Biochemical characterization was based on Gram staining, catalase test, cytochrome oxidase test, growth at different temperature and pH, sugar fermentation tests and growth on selective media. All tests were performed for identification of strains in accordance to Bergey's Manual of Determinative Bacteriology (Ewing 1986). Sugar fermentation ability was checked by using different sugars including the mannose, arabinose, mellibiose, sucrose, glucose, rhamnose, amygdalin and sorbitol (Merck, Germany). Additional biochemical features were defined through arginine dihydrolase (ADH), gelatinase, tryptophan deaminase (TDA), Voges Proskauer (VP), citrate, hydrogen sulphide (H2S), urease, o-Nitrophenyl-D-galactoside (ONPG), oxidase, ornithine decarboxylase (ODC) and Indole test (Merck, Germany). Molecular characterization was performed on the basis of DNA sequencing for 16S ribotyping. For this purpose services of First BASE Laboratories Sdn Bhd (604944-X) Malaysia were availed with the help of Advance biosciences Lahore. Tools including PCR and 3730xl genetic analyzer were utilized for 16S rRNA ribotyping. The resulting sequence analysis was completed through tools of bioinformatics (BLAST at NCBI). The phylogenetic position was detected through the same tool of bioinformatics.

Food supplements having rice in their composition were used for the isolation of test strain, *B. cereus*. That was further cultured and was identified through phenotypic, biochemical and molecular characterization. The molecular characterization was based on 16S ribotyping. Nutrient agar (MP BIOMEDICALS, LLC, France) was used for the growth of test strain. Three already identified and reported strains including *E. coli*, *B. subtilis* and *S. aureus* isolated in previous studies, were also used as test strains (Akhtar et al. 2020).

Characterization of Bacteriocin and Optimization of Conditions for Its Yield

Lactobacillus strain was used for isolation of cell free supernatant (CFS). The presence of bacteriocin in supernatant was detected as described previously (Akhtar et al. 2020). Biuret and Ninhydrin test were performed for the presence of proteinaceous components of supernatant. For verification, the proteinase enzyme test was performed to evaluate the bacteriocin activity (Vijayendra et al. 2010). Zones of inhibition were measured to check the antimicrobial activity of *Lactobacillus* (Jamuna and Jeevaratnam 2004). The culturing parameters (incubation period, temperature, pH) were adjusted and examined for getting the maximum growth associated with the yield of bacteriocin as per previously described methods (Akhtar et al. 2020). Optimization of bacteriocin production was assessed by using the parameters of pH and temperature. For this purpose, the *Lactobacillus* strain inoculated in MRS broth (three test tubes for each temperature) were incubated at different temperatures (30°C, 35°C, 40°C, 45°C, 50°C) for detection of antimicrobial activity of CFS using well diffusion assay. The effect of pH (3, 5, 7, 9 and 11) was also detected using same method involving test strains. SDS PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) was performed following the standard protocol (EZTM Pre stained protein ladder marker; cat # PM001; size~12kDa to~160kDa; bright reference bands:~27kda to~100kDa) to determine the molecular weight of the bacteriocin. After electrophoresis gel was stained with coomassie brilliant blue R-250. For the

detection of heat stability, CFS of the culture MRS broth (Lab M Ltd, UK) was heated at 50°C, 60°C, 70°C, 80°C, 90°C, 100°C and at autoclaving conditions separately. After heat treatment these samples were used for the detection of antibacterial efficacy using well diffusion assay.

Statistical Analysis

Mean values of ZOI observed at various pH and temperature were compared through one way ANOVA to find significance difference. Multiple comparisons were done through post hoc Duncan's test.

3. Results

The current study aimed to detect the presence of bacteriocin in cell free supernatant of a novel strain of *Lactobacillus.* The antimicrobial efficacy of its CFS was analyzed using a novel strain as a test strain, isolated for the same study. Three other previously isolated strains (*E. coli, B. subtilis* and *S. aureus*) were also used for the same purpose (Akhtar et al. 2020).

Table 1. Results of 16S ribotyping for the molecular characterization of strains.

Subject	Identification	Score			Identities			Strand
Accession numbers	Gene	Bit	Raw	E-value	Match	Total	Pct(%)	
KP296674.1	Lactobacillus plantarum strain R1 16S ribosomal RNA gene, partial sequence	1254	679	0.0	686	689	99	Plus/Plus
KY041686.1	Lactobacillus plantarum strain Cys5-4 16S ribosomal RNA gene, partial sequence	1646	891	0.0	909	917	99	Plus/Minus
HM573365.1	Bacterium EB466 16S ribosomal RNA gene, partial sequence	1242	672	0.0	685	691	99	Plus/Plus
KF601958.1	Bacillus cereus strain SBTBc-001 16S ribosomal RNA gene, partial sequence	1628	881	0.0	897	904	99	Plus/Minus

Screening and Identification of Novel Strain of Lactobacillus and Test Strain *Bacillus cereus*

Gram staining results indicate that both of these strains were Gram positive rods. The results for colony morphology and the biochemical reactions including the fermentation tests, show that *Lactobacillus* strain belong to *Lactobacillus plantarum* while the test strain belongs to group of *Bacillus cereus.* Molecular characterization was based on 16S ribotyping. The sequence was analyzed for identification of strains on the basis of genetic homology. Strain of *Lactobacillus* identified on the basis of biochemical test was having similarity with subjects with accession numbers KP296674.1 and KY041686.1 showing the highest score of 1254 and 1646 bits for forward and reverse primers respectively and got 99% similarity index with *Lactobacillus plantarum*. The result of other sequencing sample showed the homology to *B. cereus* with subjects having accession numbers HM573365.1 and KF601958.1 showing a highest score of 1242 and 1628 bits for forward and reverse primers respectively with 99% similarity index. The sequencing results are shown in Figure 1 and Figure 2. The data of sequencing for 16S ribotyping is shown in Table 1 and Table 2.

Table 2. References for the results of 16S ribotyping (BLAST).

Reference for results of 16S ribotyping results using nucleotide BLAST	Accession no
https://www.ncbi.nlm.nih.gov/nucleotide/827027115?report=genbank&log$=nuclalign&blast_rank=1&RID=0	KP296674.1
https://www.ncbi.nlm.nih.gov/nucleotide/1092886438?report=genbank&log$=nuclalign&blast_rank=1&RID=0	KY041686.1
https://www.ncbi.nlm.nih.gov/nucleotide/302180026?report=genbank&log$=nuclalign&blast_rank=1&RID=0	HM573365.1
https://www.ncbi.nlm.nih.gov/nucleotide/545599244?report=genbank&log$=nuclalign&blast_rank=1&RID=0	KF601958.1

Figure 1. Results for *L. plantarum* 16S ribotying.

Detection and Optimization of Conditions for Higher Yield of Bacteriocin

The growth of *L. plantarum* rises with increase in incubation period. Maximum growth was recorded at 120 hours. With the change in the temperature, growth rate is also affected. Maximum growth was observed at 30 to 40°C. The effect of pH was also noticed on the strain. Maximum growth was achieved at pH ranging from 5 to 7.

The positive results for Biuret and Ninhydrin tests confirm the existence of proteinaceous components in the CFS. According to observation of well diffusion assay based analysis, CFS treated with proteinase enzyme lost its inhibitory activity against test strain.

The CFS showed ZOI against test strains. Figure 3 depicts the ZOI of CFS against novel strain *Bacillus cereus*. Table 3 shows antibacterial activity of CFS yielded by *Lactobacillus plantarum* against test stains at different temperatures. Clear ZOI formed at 30°C, 35°C and 40°C indicates the antibacterial activity of the bacteriocin at these temperatures. However, at temperature 45°C and 50°C, no ZOI was noticed. Result of SDS PAGE showed that molecular weight of bacteriocin is almost 10 KDa. In other experiment involving the incubation of *Lactobacilli* strains at 37°C, CFS heated at 50°C, 60°C, 70°C, 80°C, 90°C and autoclaved produces clear ZOI. It shows that no loss of antimicrobial activity of bacteriocin occurred. Table 4 shows that from 3 to 9 pH range antimicrobial activity of CFS was observed against test strains. At pH 11 no ZOI formed, showing loss of antimicrobial activity at pH 11.

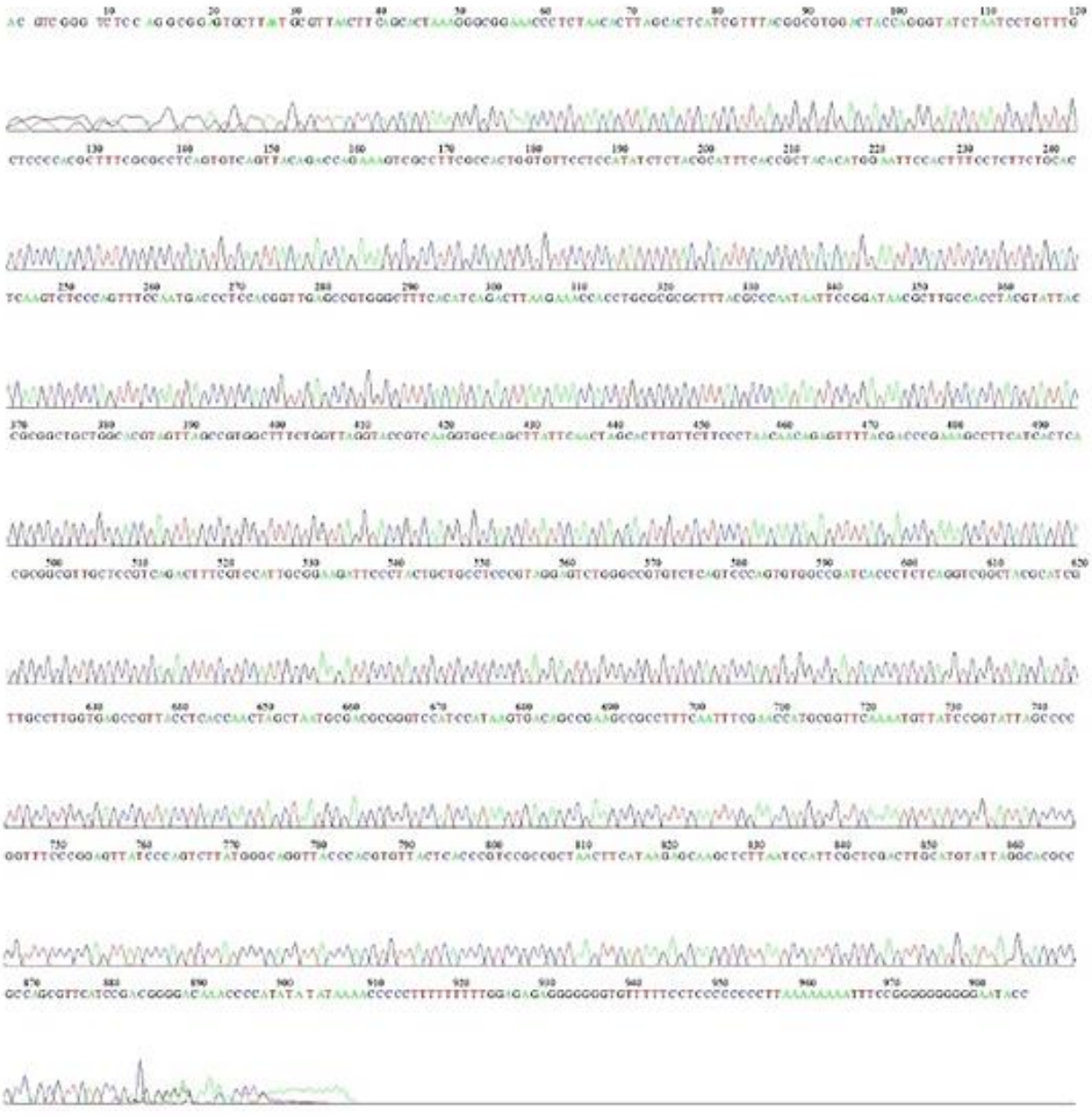

Figure 2. 16S ribotyping peaks for unknown test strain.

Table 3. Antagonistic activity of bacteriocin yielded by *Lactobacillus plantarum* against test strains at different temperatures (Strain wise analysis).

Sr. No.	Temperature (°C)	Zone of inhibition (mm)			
		B. subtilis Mean± S.E (N= 3)	*S. aureus* Mean± S.E (N= 3)	*E. coli* Mean± S.E (N= 3)	*B. cereus* Mean± S.E (N= 3)
1	30	2.40±0.87[a]	2.95±0.69[a]	3.50±1.08[a]	4.16±1.08[a]
2	35	3.41±1.03[a]	2.33±1.28[a]	2.80±0.23[a]	3.98±0.68[a]
3	40	2.81±0.81[a]	2.78±0.35[a]	2.68±0.71[a]	4.27±1.23[a]
4	45	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]
5	50	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]

One way ANOVA followed by post hoc Duncan' s test was performed to compare strain wise means. p≤0.0001. Means with same alphabets are not statistically significantly different. Distilled water was used as negative control.

Figure 3. Antibacterial activity of bacteriocin against *B. cereus.*

Table 4. Influence of variation in pH on antagonistic activity of bacteriocin produced by *Lactobacillus plantarum* against test strains (Strain wise analysis).

Sr. No.	pH	Zone of inhibition (mm)			
		B. subtilis Mean± S.E (N= 3)	*S. aureus* Mean± S.E (N= 3)	*E. coli* Mean± S.E (N= 3)	*B. cereus* Mean± S.E (N= 3)
1	3	5.33±0.09[b]	5.52±0.20[a]	2.00±0.76[b]	3.62±0.13[c]
2	5	7.96±0.35[a]	5.51±0.15[a]	2.98±0.34[b]	5.32±0.10[a]
3	7	4.81±0.47[b]	6.13±0.17[a]	4.37±0.15[a]	4.48±0.10[b]
4	9	2.48±0.14[c]	3.03±0.52[b]	2.67±0.10[b]	1.53±0.12[d]
5	11	0.00±0.0[d]	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[e]

One way ANOVA followed by post hoc Duncan's test was performed to compare strain wise means. p≤0.0001. Means with same alphabets are not statistically significantly different. Distilled water was used as negative control.

4. Discussion

Antibacterial activity of bacterial strains provides the basics for their selectivity as probiotic, starter culture and bio preservative agents against pathogenic bacteria. The current study deals with the isolation of bacteriocinogenic *Lactobacillus* strain. Other aspect of investigation was to analyze the range of antagonistic activity against Gram +ve and Gram -ve bacteria. Furthermore, effects of temperature and pH were analyzed for maximum yield of bacteriocin.

In current study *Lactobacillus* strain was screened out from yogurt. Molecular identification based on 16S ribotyping recognized the isolated strain as *Lactobacillus plantarum*. The method of bacterial identification on the basis of 16S ribotyping is a reliable method. Peng et al. (2017) identified *P. pentosaceus* on the basis of 16S ribotyping isolated from fermented fish and shrimp.

Jose et al. (2015) stated that CFS of *Lactobacilli* did not show antagonistic effects against *E.coli.* Sharma et al. (2017) revealed that *Lactobacilli* isolated from curd did not display inhibition against *S. aureus*, *L. monocytogenes*, *K. pneumoniae* and *E. coli*. Nevertheless the strains of *Bacillus cereus*, *S. flexneri* and *S. enterica serovar Typhi* were inhibited by CFS to some extent. Little activity was observed against *Proteus mirabilis* and *Pseudomonas aeruginosa*. Few strains isolated from curd were found active against *Streptococcus mutans*. Isolates of *Lactobacilli* from human milk exhibited no susceptibility against any test strain (Sharma et al. 2017). Present study reported that *Lactobacillus* isolated from yogurt has antimicrobial effects against the test strains *B. cereus*, *S. aureus*, *B. subtilis* and *E. coli*.

Sure et al. (2016) studied the antimicrobial action of bacteriocin produced by *L. viridescence*. They reported antimicrobial properties against the Gram +ve and -ve bacteria. Susceptibility was observed against *S. aureus*, *E. coli*, *Pseudomonas aeruginosa*, *B. megaterium* and *B. cereus* (Sure et al. 2016). Present study reported antimicrobial effects against Gram +ve and Gram -ve bacteria. Bacteriocin yielded by *P. pentosaceus* showed antimicrobial activity against Gram +ve bacteria such as *B. subtilis* ESBCC 01, *E. faecalis* ATCC 29212, *L. monocytogenes* and no significant activity was observed counter to Gram -ve bacteria (Peng et al. 2017). In contrast CFS of *L. plantarum* was found active against the entire Gram +ve (*S. aureus*, *B. cereus* and *B. subtilis*) and Gram -ve test strains (*E. coli*) involved in the study.

Temperature plays a vital role in growth of bacteria and synthesis of bacteriocin. Jana et al. (2019) reported that *Lactococcus lactis* JC10 produced maximum bacteriocin at optimized temperature of 30°C to 35°C. Very less bacteriocin production was observed at 45°C and no production was observed at 65°C (Jana et al. 2019). Our study reported the similar results. Optimized temperature was recorded between 30°C to 40°C. At 45°C and 50°C no bacteriocin production was recorded. Ihum et al. (2019) found that *L. plantarum* NRIC 0383 produce maximum bacteriocin at optimized temperature of 35°C. It was also reported that increase in temperature caused the decreased bacteriocin activity. The optimized temperature was recorded 37°C for bacteriocin production in *Lactobacillus viridescence* which is contradictory to present study (Sure et al. 2016).

Present study shows that *L. plantarum* produced the maximum level of bacteriocin at acidic pH ranging from 5 to neutral pH 7. In contrast, Hwanhlem et al. (2017) reported optimum pH below 5 for *Lactococcus lactis subsp. lactis* KT2W2L. Ihum et al. (2019) revealed that pH 6 is the optimum pH for *Lactobacillus plantarum* NRIC 0383. Jana et al. (2019) observed optimized bacteriocin production at pH 6.8. *Lactobacillus viridescence* produced bacteriocin at optimized pH of 7 (Sure et al. 2016). Current study revealed the bacteriocin production at alkaline pH 9. At this pH, the decreased bacteriocin production occurred comparatively to acidic and neutral pH. Jana et al. (2019) reported bacteriocin production at pH 12.5. In contrary to Jana et al. (2019) at pH 11 *L. plantarum* did not produce any bacteriocin. Present study is in agreement to Amortegui et al. (2014) which reported bacteriocin production from acidic pH 5 to neutral pH 7. The current investigation described that stability of bacteriocin produced by *L. plantarum* is up to neutral 7 pH. At alkaline pH it lost its stability and no antagonistic activity was observed against test strains. This result is in contradiction to Peng et al. (2017) and Wang et al. (2014) who reported broad range of pH stability from 2 to 10 and 2 to 12 respectively.

In a study Fossi et al. (2017) isolated three strains of *Lactobacilli* including *L. plantarum*, *L. rhamnosus* and *L. brevis*. Cell free supernatant of these strains did not lose its antibacterial activity after treatment with NaOH and catalase. It was concluded that inhibitory effects are not because of organic acids and hydrogen peroxide. Similarly inhibitory activity disappeared when treated with proteolytic enzymes which showed protein nature of substances. It was learnt that substances causing the inhibitory effects are the bacteriocins which are of proteinaceous nature (Fossi et al. 2017). Present study is in consonance to the outcomes of Fossi et al. (2017). Bacteriocin lost its activity when treated with proteinase K enzyme which was in accordance to previous studies (Wang et al. 2014; Peng et al. 2017).

Current study revealed 10 KDa molecular weight of heat resistant bacteriocin in CFS. Hassan et al. (2020) also reported heat stable bacteriocin (BLp) with molecular weight of 10 KDa produced from the *L.*

plantarum. Similarly Afrin et al. (2021) reported molecular weight of different *Lactobacilli* strains and found 10 KDa for *L. plantarum.* Zangeneh et al. (2020) reported the molecular weight of bacteriocin produced from the *L. plantarum* less than 10 KDa. In contrast Imade et al. (2021) revealed the molecular weight of bacteriocins of 23 KDa isolated from Lactic acid bacteria. Zubair and Nawaz (2017) reported 13 kDa bacteriocin produced from the *L. acidophilus*. Zhang et al. (2018) reported molecular weight of bacteriocins of almost 6.73 KDa.

In current study bacteriocin was found stable when heated at 50°C, 60°C, 70°C, 80°C, 90°C, 100°C and 121°C for 30 minutes. Present study is in consonance to the Jang et al. (2014) which described that heating at 80°C, 100°C and 121°C did not disturb bacteriocin susceptibility. Different observations were reported by Peng et al. (2017) regarding the heat stability of bacteriocin. According to them, the bacteriocin was stable from 4°C to 60°C. It lost its activity when heated at 80°C and 100°C for 2 hours. While heating at 121°C for only 20 minutes lost the activity (Peng et al. 2017).

5. Conclusions

On the basis of the current pilot project, it can be determined that the bacteriocin synthesized by the strain of *L. plantarum* has antagonistic potential against the strains of Gram +ve and -ve bacteria (*B. cereus*, *S. aureus*, *B. subtilis*, and *E. coli*). The novel strain producing the bacteriocin was identified on the basis of 16S ribotyping. Likewise, a test strain of *B. cereus* was also identified on the basis of 16S ribotyping. The yield of bacteriocin can be enhanced by providing the appropriate temperature and pH for supporting the growth of *L. plantarum* strain. The bacteriocin synthesized by the observed strain is heat stable.

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References

ABBAS, S., et al. Probiotic potential of locally isolated strain *Lactobacillus brevis* MF179529 and its comparison with commercial probiotics in chicken model. *Pakistan Journal of Agricultural Sciences.* 2021, **58**, 135-141. https://doi.org/10.21162/PAKJAS/21.869

AFRIN, S., et al. Characterization and profiling of bacteriocin-like substances produced by lactic acid bacteria from cheese samples. *Access Microbiology*. 2021, **3**(6). https://doi.org/10.1099/acmi.0.000234

AKHTAR, S., NAWAZ, S.K. and ZUBAIR, F. Optimizing the bacteriocin production in strain of *Lactobacillus pentosus* isolated from cheese. *International Journal of Biosciences.* 2020, **17**, 506-517. https://doi.org/10.12692/ijb/17.6.506-517

AMORTEGUI, J., et al. Characterization of a new bacteriocin from *Lactobacillus plantarum* LE5 and LE27 isolated from ensiled corn. *Applied Biochemistry and Biotechnology.*2014, **172**(7), 3374-3389. https://doi.org/10.1007/s12010-014-0757-x

BANIK, A., et al. Microbiological quality of ready-to-eat food from Dhaka, Bangladesh. *Current Research in Nutrition and Food Science Journal.* 2019, **7**(1), 161-168. https://doi.org/10.12944/CRNFSJ.7.1.16

EWING, W.H. The genus *Escherichia*. Identification of *Enterobacteriaceae*. 1986, **4**, 93-134.

FOSSI, B.T., et al. Screening for Bacteriocins Producing Probiotic Bacteria from Fermented Sap of Palm Trees (*Elaeis Guineesis* and *Raffia Sudanica*): Production and Partial Characterization of Bacteriocins. *Journal of Applied Biotechnology and Bioengineering.* 2017, **2**(1), 00017. https://doi.org/10.15406/jabb.2017.02.00017

GOEL, A., HALAMI, P.M. and TAMANG, J.P. Genome analysis of *Lactobacillus plantarum* isolated from some Indian fermented foods for bacteriocin production and probiotic marker genes. *Frontiers in microbiology.* 2020, **11**, 40. https://doi.org/10.3389/fmicb.2020.00040

HASSAN, M. U., et al. Characterisation of bacteriocins produced by *Lactobacillus spp.* isolated from the traditional Pakistani yoghurt and their antimicrobial activity against common foodborne pathogens. *BioMed research international*, *2020*. https://doi.org/10.1155/2020/8281623

HWANHLEM, N., et al. Inhibition of food-spoilage and foodborne pathogenic bacteria by a nisin Z-producing *Lactococcus lactis subsp. lactis* KT2W2L. *LWT-Food Science and Technology.* 2017, **82**, 170-175. https://doi.org/10.1016/j.lwt.2017.04.052

IHUM, T.A., et al. Bacteriocin Production by *Lactobacillus plantarum* NRIC 0383, Measured in Terms of Its Antagonistic Activity against Bacteria Pathogens of Selected Vegetables. *Microbiology Research Journal International.* 2019, **27**, 1-7. https://doi.org/10.9734/MRJI/2019/v27i630117

IMADE, E.E., et al. Lactic acid bacterial bacteriocins and their bioactive properties against food-associated antibiotic-resistant bacteria. *Annals of Microbiology*. 2021, **71**(1), 1-14. https://doi.org/10.1186/s13213-021-01652-6

IORIZZO M, et al. Antimicrobial activity against *Paenibacillus* larvae and functional properties of *Lactiplantibacillus plantarum* strains: Potential benefits for honeybee health. *Antibiotics*. 2020, **9**(8), 442. https://doi.org/10.3390/antibiotics9080442

JAMUNA, M. and JEEVARATNAM, K. Isolation and characterization of *Lactobacilli* from some traditional fermented foods and evaluation of the bacteriocins. *The Journal of General and Applied Microbiology.* 2004, **50**, 70-90. https://doi.org/10.2323/jgam.50.79

JANA, S.C., CHATTERJEE, M. and RAYCHAUDHURI, U. Optimization of Media and Culture Conditions for Improved Production of Bacteriocin by Using Conventional One-Factor-At-A-Time (OFAT) Method. *EC Microbiology.* 2019, **15.4**, 251-258.

JANG, S., et al. Identification of an anti-listerial domain from *Pediococcus pentosaceus* T1 derived from Kimchi, a traditional fermented vegetable. *Food Control.* 2014, **43**, 42-48. https://doi.org/10.1016/j.foodcont.2014.02.040

JOSE, N.M., BUNT, C.R. and HUSSAIN, M.A. Comparison of microbiological and probiotic characteristics of *Lactobacilli* isolates from dairy food products and animal rumen contents. *Microorganisms.* 2015, **3**(2), 198–212. https://doi.org/10.3390/microorganisms3020198

LIU, Y.W., LIONG, M.T. and TSAI, Y.C. New perspectives of *Lactobacillus plantarum* as a probiotic: The gut-heart-brain axis. *Journal of Microbiology.* 2018, **56**(9), 601-613. https://doi.org/10.1007/s12275-018-8079-2

NAWAZ, S.K., RIAZ, S. and HASNAIN S. Screening for anti-methicillin resistant Staphylococcus aureus (MRSA) bacteriocin producing bacteria. *African Journal of Biotechnology.* 2009, **8**,365-368.

PENG, C., et al. Characterization of anti-listerial bacteriocin produced by lactic acid bacteria isolated from traditional fermented foods from Cambodia. *International Food Research Journal*. 2017, **24**(1), 386-393.

RIAZ S., NAWAZ S.K. and HASNAIN S. Bacteriocins produced by *L. Fermentum* and *L. acidophilus* can inhibit cephalosporin resistant *E. coli. Brazilian Journal of Microbiology.* 2010, **41**, 643-648. https://doi.org/10.1590/S1517-83822010000300015

SHARMA, C., et al. Antibacterial effects of *Lactobacillus* isolates of curd and human milk origin against food-borne and human pathogens. *3 Biotech*. 2017, **7**(1), 1-9. https://doi.org/10.1007/s13205-016-0591-7

SURE, K.P., et al. Production and characterization of bacteriocin produced by *Lactobacillus viridescence* (NICM 2167). *Brazilian Archives of Biology and Technology.* 2016, 59. https://doi.org/10.1590/1678-4324-2016150518

VIJAYENDRA, S.V.N., RAJASHREE, K. and HALAMI, P.M. Characterization of a heat stable anti-listerial bacteriocin produced by vancomycin sensitive *Enterococcus faecium* isolated from idli batter. *Indian Journal of Microbiology.* 2010, **50**, 243-246. https://doi.org/10.1007/s12088-010-0030-0

WANG, G., et al. Partial characterisation of an anti-listeria substance produced by *Pediococcus acidilactici* P9. *International Dairy Journal.* 2014, **34**(2), 275-279. https://doi.org/10.1016/j.idairyj.2013.08.005

ZANGENEH, M., KHORRAMI, S. and KHALEGHI, M. Bacteriostatic activity and partial characterization of the bacteriocin produced by *L. plantarum sp.* isolated from traditional sourdough. *Food Science & Nutrition*. 2020, **8**(11), 6023-6030. https://doi.org/10.1002/fsn3.1890

ZHANG, J., et al. Purification and partial characterization of bacteriocin Lac-B23, a novel bacteriocin production by *Lactobacillus plantarum* J23, isolated from Chinese traditional fermented milk. *Frontiers in Microbiology*. 2018, **9**, 2165. https://doi.org/10.3389/fmicb.2018.02165

ZUBAIR, F. and NAWAZ, S.K. Antibacterial activity of bacteriocin isolated from *Lactobacillus acidophilus* against throat infections causing bacteria. *International Journal of Biosciences.* 2017, **10**, 372-379. https://doi.org/10.12692/ijb/10.4.372-379

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