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Study Bacteriocin Production and Optimization Using New Isolates of *Lactobacillus* spp. Isolated from Some Dairy Products under Different Culture Conditions

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ABSTRACT

Lactobacilli belong to the group of lactic acid bacteria (LAB), that have several distinguished abilities such as production of lactic acid, enzymes such as *β*-Galactosidase and natural antimicrobial substances called bacteriocins. Bacteriocin is a biopreservative agent potential of suppressing growth of some contaminant bacteria in food industry but its commercial availability is limited and costly. The study aimed to select isolates of *Lactobacillus* spp. potential for producing bacteriocins to suppress the growth of *Escherichia coli* ATCC 25922 and *Bacillus subtilis* NCIB3610, and to optimize the process of bacteriocin production. Results obtained in this study showed that *L. acidophilus* isolate CH1 was selected as the best candidate for bacteriocin among the four isolates that tested. The largest amounts of the bacteriocins were synthesized only in MRS medium was supplemented with K_2HPO_4 (1.0%), Tween 80 (1%), Beef extract (1%), glucose, cyctein and peptone extract (1%). The optimization of culture conditions for bacteriocin production areas showed that corn steep liquor medium was the best medium for all isolates against *Bacillus subtilis* while no effect was observed on *Escherichia coli* ATCC 25922 except when used MRS medium. The optimum conditions for bacteriocin production were pH 6.0, temperature 34˚C with 4% Phenyl acetamide showing the greatest growth inhibition areas.

Keywords: *Lactobacillus acidophilus*; Lactic Acid Bacteria; Bacteriocins Production

1. Introduction

Lactic acid bacteria (LAB) produce a number of antimicrobial substances such as organic acids, free fatty acids, ammonia, reuterin, diacetyl, hydrogen peroxide and bacteriocin, which have the capacity to inhibit the growth of food spoilage and pathogenic organisms [1]. Bacteriocins are proteinaceous and ribosomally synthesized antibacterial compounds produced by certain LAB during lactic acid fermentations that exhibit bactericidal activity against closely related species [2,3]. In recent years, a renewed interest in bacteriocin like activities has led to the discovery, isolation, and purification of bacteriocins from both gram-negative and gram-positive bacteria. They are now being considered for a variety of antimicrobial uses in foods and medicine [4]. Some bacteriocins produced by lactic acid bacteria, such as nisin, inhibit not only closely related species but are also effective against foodborne pathogens and many other gram-positive spoilage bacteria [5]. For this reason, bacteriocins have attracted considerable interest for use as natural food preservatives in recent years, which have led to the discovery of ever increasing potential sources of these protein inhibitors.

LAB bacteriocins are divided into three main groups, based on their amino acid sequence, mode of action, heat tolerance, biological activity, presence of modified amino acids, and secretion mechanism. The classes I and II are further divided into subgroups, and the members of these classes are the most studied because they are so widespread among the LAB and due to their heat stability. The class III bacteriocins are heat-labile and therefore less interesting in the terms of food processing and protection. Quite recently a new classification has been proposed by Cotter *et al*. [6]. In this scheme the most dramatic change is the removal of class III bacteriocins to their own group of "bacteriolycins", hence making the group of bacteriocins smaller and more strictly defined.

Lactobacillus bacteriocins are found within each of the four major classes. Class I bacteriocins (antibiotics) were discovered in the lactobacillaceae by Mortvedt *et al*. [7]. These bacteriocins are small membrane-active peptids

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(<5 kDa) containing an unusual amino acids, lanthionine. The class II bacteriocins are small heatstable, non-lanthionine containing and membrane-active peptides (<10 kDa). The class III bacteriocins, have been found in Lactobacillus, include heat labile proteins of large molecular mass. The class IV bacteriocins are a group of complex proteins, associated with other lipid or carbohydrate moieties, which appear to be required for activity. They are relatively hydrophobic and heat stable [8].

Different bacteriocin exhibits different inhibition profile on food spoilage and pathogenic microorganisms. Therefore, they could be natural replacements for synthetic food preservatives [9]. In order to increase the productivity of bacteriocins, a better understanding of factors affecting their production is essential. Bacteriocin production has been reported to be affected by several factors including carbon and nitrogen sources; and fermentation conditions, such as pH, temperature and agitation [9].

The optimization of bacteriocin production and enhancement of its activity are economically important to reduce the production cost. Thus, the aims of this study were to formulate industrial media for bacteriocin production by four lactic acid bacteria isolates and the optimization of culture conditions for maximizing bacteriocin production.

2. Materials and Methods

2.1. Isolation and Identification of Lactic Acid Bacteria

The lactic acid bacteria were isolated from raw milk and Ras cheese, by appropriate dilutions with NaCl physiological. Dilutions (10^{-1} - 10^{-6}) were prepared and plated on de Man Rogosa agar (MRS agar) medium (Hi Media Laboratory Pvt. Ltd. Mumbai, India) to isolate the *Lactobacillus* spp and incubated at 37˚C for 48 - 72 h [10]. The strains were sub-cultured onto MRS agar slant incubated at 30˚C for 24 h and preserved in 20% glycerol at −80˚C. One of the isolates was selected for further studies. It exhibited strong inhibitory activity against indicator strains. It was identified on the basis of growth, cell morphology, gram staining and catalase activity. Further, identification was performed according to carbohydrate fermentation patterns and growth at 15˚C and 45˚C in the de Man Rogosa Sharpe (MRS) broth based on the characteristics of the lactobacilli as described in Bergey's Manual of Determinative Bacteriology [11-14] and fermentation of different carbon sources (API 50 CHL, bioMerieux SA, France). The ability of these isolated strains to produce acids from different carbohydrates was determined by API 50 CHL test kit (bioMerieux SA, France).

2.2. Selection of Isolates Producing Bacteriocin

2.2.1. Treatment of Bacteria Prior to Production of Bacteriocin

The isolates were tested for their production of Bacteriocin. *E. coli* ATCC 25922 and *Bacillus subtilis* NCIB3610 were used as indicator microorganism in all assays. Indicator microorganisms used are propagated for 48 h in the Nutrient agar media, and at the temperatures indicated 30˚C.

2.2.2. Detection of Antibacterial Activity

The antimicrobial activity of the isolates during the growth phase against Gram negative bacterium *E. coli* ATCC 25922 and Gram positive bacterium *Bacillus subtilis* NCIB3610 was evaluated by deferred methods: 1) well-diffusion assay [15] and 2) Tetrazolium/formazan-test method [16].

2.3. The Maximization of Bacteriocins Production

2.3.1. Determination of Bacteriocin Production at Different Culture Conditions

The effects of different temperatures and initial pH on the bacteriocin production were tested. MRS broth (10 mL) was inoculated with each isolate and incubated at different temperatures such as 10˚C, 20˚C, 30˚C, 40˚C and 50˚C to study the effect of different temperatures on the bacteriocin production. The effect of initial medium pH on bacteriocin production was determined by adjusting the MRS broth to different pH levels of 2, 4, 6, 8 and 10, respectively. Each tube was inoculated with 2.0% v/v of an 18 h-old culture of the four isolates and incubated at 30˚C for 96 h, without agitation.

2.3.2. Influence of Medium Component on the Production of Bacteriocins

The effect of medium ingredients on bacteriocin production was evaluated using composed media. The supplements studied were tryptone, yeast extract, beef extract, triammonium citrate sodium acetate, $MgSO_4$-$7H_2O$, K_2HPO_4, NaCI, glucose and tween 80 (1%, 2% and 3%) for each. Then, cells were removed by centrifugation at 6 000 rpm for 20 min. the culture media was adjusted to pH 7.0 using 1 M NaOH to exclude the antimicrobial effect of organic acids, followed by filtration of the supernatant through a 0.2 ml pore-size cellulose acetate filter.

2.3.3. Influence of Different Media on the Production of Bacteriocins

The effect of different medium on bacteriocin production was evaluated using media at 30˚C for 48 hours.

- Selective medium (MRS media) as control.
- Medium (A) Corn steep liquor-Lactose medium [17].
- Medium (B) Corn steep liquor-Lactose medium [18].
- Medium (D) Corn steep liquor medium [19].
- Medium (E) Glycerol-molasses-liquid medium [17].

Broth media were used as seed culture (10% of the total volume of the fermentation medium). The culture was adjusted to pH 7.0.

2.4. Optimization of Bacteriocins Activity

2.4.1. Production of Crude Bacteriocin Samples

Lactobacillus species were cultured in 1000 ml MRS broth (pH 7.0) for 48 h at 30˚C. For extraction of bacteriocins, a cell-free solution was obtained by centrifuging (6000 rpm for 20 min. at 4˚C) the culture and was adjusted to pH 7.0 [20,21].

2.4.2. Effect of Temperature on Crude Bacteriocins Activity

In order to test the heat resistance, 10 ml of bacteriocin preparation was heated for 30 minutes at 30˚C, 60˚C, 90˚C and 121˚C respectively. Residual bacteriocin activity was detected against *E. coli* and *Bacillus subtilus* at each of these temperatures [22] by teterazoluim chloride method.

2.4.3. Effect of pH on Crude Bacteriocins Activity

According to the method described by Karaoglu *et al.* [8], sensitivity of the cell-free supernatant to different pH values was tested by adjusting the pH of the bacteriocins in the range of pH 2 to 10 with sterile IN Noah and IN HC1. Residual activity of each of the samples was determined against the indicator organism by agar-well diffusion assay.

2.4.4. Effect of Surfactants on Crude Bacteriocins Activity

The effect of surfactants on the bacteriocins was tested by adding SDS, CTAB, EDTA and Tween 80 (0.5% v/v final concentration), to crude bacteriocins. Untreated bacteriocin preparation (positive control). All samples were incubated at room temperature for 2 hours then tested for residual antimicrobial activity by teterazolium formazan test.

2.4.5. Effect of Organic Solvents on Crude Bacteriocins Activity

Crude bacteriocin preparations were mixed with organic solvents including acetone, butanol, chloroform, ethanol, methanol and propanol at a final concentration of 0.5%. Untreated bacteriocins preparation were used as (positive control). All samples were incubated at room temperature for 2 hours and tested for residual antimicrobial activity by teterazolium formazan test.

2.4.6. Effect of Metal Ions on Crude Bacteriocins Activity

In a separate experiment the effect of metal salts on bacteriocin was examined by adding $AgNO_3$, $CuSO_4$, FeSO, $MgSO_4$, $MnCl_2$, and $ZnSO_4$ (Merck) to 10 ml of crude bacteriocin preparation (0.5% final concentration). Untreated bacteriocin preparation (positive control). All samples were incubated at room temperature for 2 hours and tested for residual antimicrobial activity [23,24] by teterazolium formazan test.

2.4.7. Effect of Different Concentration of NaCl on Crude Bacteriocins Activity

In a separate experiment the effect of different concentration of NaCl (2%, 4%, 6%, 8%, 10%) on bacteriocins were examined by adding to 10 ml of crude bacteriocins preparation. Untreated bacteriocin preparation (positive control). All samples were incubated at room temperature for 2 hours and tested for residual antimicrobial activity [24] by agar-well diffusion assay.

2.4.8. Effect of Different Concentration of Amino Acids on Crude Bacteriocins Activity

In a separate experiment the effect of different concentration of 21 amino acids compound (essential amino acids) (2%, 4%, 6%, 8% and 10%) on bacteriocins were examined by adding to 10 ml of crude bacteriocins preparation. Untreated bacteriocin preparation (positive control). All samples were incubated at room temperature for 2 hours and tested for residual antimicrobial activity by agar-well diffusion assay.

2.4.9. Effect of Different Concentration of Vitamins on Crude Bacteriocins Activity

In a separate experiment the effect of different concentration of vitamins (50%) such as (B12 and B complex) on bacteriocins were examined by adding (1) to 10 ml of crude bacteriocins preparation. Untreated bacteriocin preparation (positive control). All samples were incubated at room temperature for 2 hours and tested for residual antimicrobial activity by agar-well diffusion assay.

2.5. Statistical Analysis

Data are presented as the mean ± standard deviation, and n represents the number of the isolates and the control.

3. Results and Discussion

3.1. Isolation and Identification of Bacteriocinogenic Strains

Sixteen isolates of LAB were isolated from the samples.

After series of purification on MRS agar, four isolates were found to be Gram-positive, catalase negative, non-motile bacilli. In addition, all strains were tested for growth at 10˚C for 10 days, 45˚C for 48 h, and CO_2 production from glucose [25]. **Table 1** presents the results of the final identifications for each type of isolates with API gallery as stated below: *L. acidophilus* M2, *L. acidophilus* CH1, *L. fermentum* M1 *and L. pentosus* CH2.

3.2. Bacteriocin Production

The antibacterial activity of bacteriocins against food borne pathogenic, as well as spoilage bacteria has raised considerable interest for their application in food preservation [26]. Application of bacteriocins may help reduce the use of chemical preservatives and/or the intensity of heat and other physical treatments, satisfying the demands of consumers for foods that are fresh tasting, ready to eat, and lightly preserved. In the present study the average diameter of the inhibition zones measured ranged from 2 - 20 mm in size (**Table 2**). Among the isolates, *L. fermentum* M1 and *L. acidophilus* CH1 were bacteriocin effectively inhibited the *Bacillus subtilis* NCIB3610 with maximum inhibitory activity, compared to the other tested bacteria while, the impact of these strains were less against *E. coli* ATCC 25922. In addition, *L. acidophilus* M2 exhibited stronger inhibition activity on *Bacillus subtilis* NCIB3610 than *E. coli* ATCC 25922 but its effect was less than the effect of *L. acidophilus* CH1 that exemplifies a difference within the same species. The present *L. pentosus* CH2 isolate showed inhibitory activity against *Bacillus subtilis* NCIB3610 on the other hand it was less inhibitory activity against *E. coli* ATCC 25922. Such observations [27,28] made earlier are in tune with the results of the present study which confirmed that the bacteriocins of Gram-positive bacteria generally exhibit antagonistic activity against Gram-positive bacteria and the activity against Gram negative bacteria is an unusual phenomenon and has been reported for the bacteriocins produced by *Lactobacillus plantarum* [29], the isolates were screened for antimicrobial spectrum against Gram-positive and Gram-negative bacteria using the AWD method.

3.3. Effect of Culture Conditions and Medium Composition on Bacteriocin Production

The culture conditions and composition of the growth medium are very important for the production of individual bacteriocins [5]. Several media have been evaluated by numerous authors to improve bacteriocin synthesis [30] because these peptides are not always produced in standard or enriched culture media. Lactic acid bacteria are fastidious microorganisms that require rich media containing milk, whey ultrafiltrate, or complex synthetic media such as MRS [10], M17 [31] or LAPTg [32] for growth. Therefore, the isolation of a peptide(s) in rich-medium supernatant is an additional problem, making the purification of the bacteriocin a relatively complicated protocol. The present study was primarily aimed to determine cultural conditions for obtaining better and stable bacteriocins production. *L. acidophilus* CH1 was able to produce bacteriocins, which had a wide inhibitory spectrum towards both Gram-negative and Gram-positive food spoilage and pathogenic bacteria. Results show that bacteriocin was produced when nutrients were available for metabolic activity. **Tables 3** and **4** showed that maximum activity was noted at pH 6.0, temperature 30˚C. Bacteriocin production is frequently regulated by pH and growth temperature, as has been shown in several studies involving the pediocin AcH [33]. From the results proved that it could be used in acidic foods like pickle or yoghurt. It might be secondary metabolites.

The composition of medium influencing the production of bacteriocin by *Lactobacillus* isolates. **Table 5** showed that MRS seemed to be more suitable medium

Table 1. Pre-identification of some isolates.

Strain	Preliminary tests					API Identification
	Gram staining	Catalase test	Growth at 10˚C	Growth at 45˚C	CO_2 Production	
M2	+	−	−	+	−	***L. acidophilus***
CH1	+	−	−	+	−	***L. acidophilus***
CH2	+	−	−	+	+	***L. pentousus***
M1	+	−	+	+	−	***L. fermentum***

Table 2. Bacteriocin production by four isolates Detected by well-diffusion assay.

Strains	Diameter of the inhibition-zone (mm) for *B. subtilis*	Diameter of the inhibition-zone (mm) for *E. coli*
L. fermentum M1	20 ± 0.01	3 ± 0.02
L. acidophilus M2	18 ± 0.02	16 ± 0.03
L. acidophilus CH1	20 ± 0.01	18 ± 0.01
L. pentousus CH2	15 ± 0.01	2 ± 0.02

Data are presented as mean ± SD.

Table 3. Effect of different temperature on production.

Temp. / Strains	Diameter of the inhibition-zone (mm) for *E. coli* ATCC 25922				Diameter of the inhibition-zone (mm) for *Bacillus subtilis* NCIB3610			
	***L. fermentum* M1**	***L. acidophilus* M2**	***L. acidophilus* CH1**	***L. pentosus* CH2**	***L. fermentum* M1**	***L. acidophilus* M2**	***L. acidophilus* CH1**	***L. pentosus* CH2**
Control	20 ± 0.01	3 ± 0.01	9 ± 0.01	5 ± 0.01	3 ± 0.01	4 ± 0.01	16 ± 0.01	**2** ± 0.01
10	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
20	**3** ± 0.01	**4** ± 0.01	**6** ± 0.01	**4** ± 0.01	**5** ± 0.01	**4** ± 0.01	**14** ± 0.01	**6** ± 0.01
30	**4** ± 0.01	**3** ± 0.01	**16** ± 0.01	**7** ± 0.01	**4** ± 0.01	**5** ± 0.01	**21** ± 0.01	**7** ± 0.01
40	**3** ± 0.01	**5** ± 0.01	**9** ± 0.01	**3** ± 0.01	**3** ± 0.01	**2** ± 0.01	**15** ± 0.01	**6** ± 0.01
50	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Data are presented as mean ± SD.

Table 4. Effect of different pH on production.

pH / Strains	Diameter of the inhibition-zone (mm) for *E. coli* ATCC 25922				Diameter of the inhibition-zone (mm) for *mBacillus subtilis* NCIB3610			
	***L. fermentum* M1**	***L. acidophilus* M2**	***L. acidophilus* CH1**	***L. pentosus* CH2**	***L. fermentum* M1**	***L. acidophilus* M2**	***L. acidophilus* CH1**	***L. pentosus* CH2**
Control	2 ± 0.01	3 ± 0.01	9 ± 0.01	5 ± 0.01	3 ± 0.01	4 ± 0.01	16 ± 0.01	**2** ± 0.01
2	**1 ± 0.01**	**0.0**	**2** ± 0.01	**0.0**	**0.0**	**1** ± 0.01	**3** ± 0.01	**0.0**
4	**2** ± 0.01	**1** ± 0.01	**3** ± 0.01	**1** ± 0.01	**0.0**	**1** ± 0.01	**4** ± 0.01	**0.0**
6	**2** ± 0.01	**3** ± 0.01	**10** ± 0.01	**7** ± 0.01	**4** ± 0.01	**4** ± 0.01	**20** ± 0.01	**7** ± 0.01
8	**1** ± 0.01	**2** ± 0.01	**5** ± 0.01	**2** ± 0.01	**2** ± 0.01	**2** ± 0.01	**10** ± 0.01	**10** ± 0.01
10	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Data are presented as mean ± SD.

for the bacteriocin production. Similar results were observed by [34,35]. Results in **Table 5** also indicate that larger amounts of the bacteriocins were synthesized only in MRS medium supplemented with K_2HPO_4 (1.0%), Tween 80 (1%), Beef extract (1%), glucose, cyctein and peptone extract (1%), while addition of tri-ammonium citrate, sodium acetate and magnesium sulphate, had no effect on bacteriocin production. Thus variation in the concentration of constituents/ supplementation of cultivation media might have an influence on the amount of bacteriocin produced by microorganisms. Similar observations have been made previously. Daba *et al*. [21] obtained similar results in the production of mensenterocin 5. Biswas *et al*. [33] compared the production of pediocin ACH by *Pediococcus acidilactici* H cultivated in TGE broth, MRS broth and several modifications of it. Modification of nutrients of cultivation media should be considered for maximal production of bacteriocin that has potential use as a food biopreservative [33]. Similar results were recorded for nisin [36] and pediocin AcH [33]. The reason for increased bacteriocin production is not clear and yet to be ascertained. Most of the bacteriocin producing organisms requires stabilizers or a unique medium composition for bacteriocin synthesis. It is probable that the yeast extract may in part serve to inactivate an inhibitor of bacteriocin synthesis [37]. Being a surfactant Tween 80, might enable the discharging of the bacteriocin from the cell surface of the producer strain. This finding was supported by the increased bacteriocin production in the medium supplemented with different concentrations of yeast extract plus Tween-80.

An earlier study by the senior author [28] revealed that in *L. plantaram* MTCC1746, maximum bacteriocin production could be achieved by providing 1.5% yeast extract and 1.5% Tween-80. The addition of $MgSO_4$ could make a slight impact on the production of bacteriocin. Activity of 1000 AU/mL was observed by the addition of this substrate at a lower concentration of 0.02% to 0.04%. The higher concentrations 1%, 2% and 3%, however, bring about reduction in bacteriocin production.

3.4. Influence of Different Media on the Production of Bacteriocins

Several complex culture media of high cost have been used for bactenocins production. In the current study, we have used an effluent from the food industry (Corn sleep

Table 5. Effect of adding some nutrient components in MRS media on bacteriocins production by *Lactobacillus* spp. isolates.

Medium Constituents	%	Diameter of the inhibition-zone (mm)							
		E. coli ATCC 25922				*Bacillus subtilis* NCIB3610			
		M1	M2	CH1	CH2	M1	M2	CH1	CH2
MRS (control)		2	3	9	5	3	4	16	**2**
	1	0.00	3	9	0.00	0.00	0.00	10	0.00
MRS + Yeast extract	2	0.00	0.00	0.00	0.00	0.00	0.00	9	0.00
	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1	10	0.00	13	0.00	0.00	0.00	25	0.00
MRS + Beef extract	2	15	0.00	17	10	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1	0.00	0.00	0.00	0.00	5	7	24	0.00
MRS + Peptone extract	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1	0.00	0.00	0.00	0.00	0.00	0.00	13	0.00
MRS + Glucose	2	0.00	0.00	15	0.00	0.00	0.00	11	0.00
	3	9	19	11	9	0.00	0.00	0.0	0.00
	1	0.00	0.00	12	0.00	0.00	0.00	23	0.00
MRS + Tween80	2	0.00	0.00	0.00	0.00	0.00	0.00	15	0.00
	3	0.00	0.00	5	9	8	12	20	0.00
	1	1	2	3	5	9	0.00	19	0.00
MRS + Sodium acetate	2	2	2	4	2	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1	4	0.00	0.00	0.00	0.00	5	10	0.00
MRS + Tri-ammonium citrate	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1	0.00	11	12	5	6	6	16	0.00
MRS + $MgSo_4 \cdot 7H_2O$	2	0.00	5	0.0	0.00	7	0.0	0.0	0.00
	3	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.00
	1	12	10	20	0.00	0.00	0.00	15	0.00
MRS + Cyctein	2	0.00	0.00	15	0.00	0.00	0.00	11	0.00
	3	0.00	0.00	**25**	15	0.00	0.00	10	0.00
	1	0.00	0.00	0.00	10	9	20	27	0.00
MRS + K_2HPO_4	2	0.00	0.00	0.00	9	0.00	9	13	0.00
	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

liquar (CSL), CSL with glucose and glysrol culture media for bacteriocins production of at low costs. These media were used for bactenocin production by four lactic acid bacteria isolates (*Lactobacillus fermentum* M1, Lactobacillus acidophilus M2, *Lactobacillus acidophilus* CH1 and *Lactobacillus pentosus* CH2). Production of bacteriocins at 30˚C and at a pH 6.5 were carried out in different media MRS, Medium (A) Corn steep liquor—Lactose medium, Medium (B) Corn steep liquor—Lactose medium, Medium (C) Corn steep liquor medium, Medium (D) Glycerol-molasses-liquid medium for bacteriocins (**Table 6**). A maximum growth rate were shown in MRS and CSL for all isolates and a maximum bacteriocrn activity (inhibition zone mm of *Bacillus subtilis* NCIB3610) was appeared by-isolate *Lactobacillus acidophilus* CH1 in medium C, but the maximum bacteriocm activity by isolate CH1. On the other hand isolates CHl and M1 were given the maximum bacteriocm(s) activity in meadium C. The lowest amounts of bacteriocins activity were produced in date by M1 isolate. Although this fact suggests the possible effect of substrate inhibition, it could also be related to the control that the supplied sugar substrate exerts on the bacteriocm biosynthesis. Biswas *et al*. [33] reported that MRS medium is a better medium for cell growth and bacteriocins production than other media. Generally, maximum production corresponds to against pathogenic microbe such as *Bacillus subtilis* NCIB3610 and *E. coli* ATCC 25922.

Table 6. Effect of different media on production of bacteriocins.

Strains	Diameter of the inhibition-zone (mm) *for Bacillus subtilis* NCIB3610				Diameter of the inhibition-zone (mm) for *E. coli* ATCC 25922			
Media	*L. fermentum* M1	*L. acidophilus* M2	*L. acidophilus* CH1	*L. pentosus* CH2	*L. fermentum* M1	*L. acidophilum* M2	*L. acidophilus* CH1	*L. pentosus* CH2
MRS control	2	3	9	5	3	4	16	2
A	8	0.0	10	0.0	0.0	0.0	0.0	0.0
B	8	5	20	12	0.0	0.0	0.0	0.0
C	5	25	32	20	0.0	0.0	0.0	0.0
D	10	0.0	16	0.0	0.0	0.0	0.0	0.0

Medium (A) Corn steep liquor-Lactose medium. Medium (B) Corn steep liquor-Lactose medium with some modifications. Medium (C) Corn steep liquor medium. Medium (D) Glycerol-molasses-liquid medium.

Therefore increased cell concentrations in a high cell-density reactor is expected to increase bactenocm production. In general bactencin production by lactic acid bacteria occurs during the active growth phase [29,38]. Conditions favouring bacterial growth and high cell densities are frequently beneficial to bactenocin production as well [38]. However, a high cell yield does not necessarily result in a high bactenocin activity since the latter may be limited by a low specific bacteriocin production, *i.e*. a low bacteriocin production per gram of cells [28]. Hence, there exists a rather complex relationship between environmental conditions and bactenocin activity levels and no generalisation about the optimum conditions for bactenocin production can readily be made. The kinetics of both cell growth and bactenocin production in function of the environmental situation have to be studied to obtain a better understanding of the production mechanism.

3.5. Optimization of Bacteriocin Activity

3.5.1. Effect of Different Temperatures on the Crud Bacteriocin

The effect of different temperatures on crud bacteriocin have been clarified in the **Tables 7-10**. These tables clearly highlights of effect of different temperature 30˚C, 60˚C and 90˚C /30min on crud bacteriocin from four LAB: *L. fermentum* M1, *L. acidophilus* M2, *L. acidophilus* CH1and *L. pentosus* CH2 according to the teterazolium chloride methods. As can be seen, LAB were isolated from local raw milk and Ras cheese using MRS agar. According to **Table 7**, the isolated *Lactobacillus fermentum* M1 was showed antimicrobial activity against *E.coli* ATCC 25922 which showed the largest of growth inhibitor% around 76.08% in temp. 60˚C/30min bacteriocin but the smallest of the antimicrobial activity was 39.34% in temp. 90˚C/30min wherever *Lactobacillus fermentum* was showed antimicrobial activity against *Bacillus subtilis* NCIB3610 was 35.79% in temp. 60˚C/30min but the smallest of the antimicrobial activity was 30% in temp. 30˚C/30min. **Table 8**, in addition, the strains *L. acidophilus* M2 which showed the largest growth inhibition% was 80.32% in temp. 60˚C/30min against *Bacillus subtilis* NCIB3610 but the smallest was 65.81% in temp. 90˚C/30min wherever, the same strain was the largest growth inhibitor% 87.34% in temp. 60˚C/30min but the smallest was 11.12% in temp. 30˚C/30min against to *E. coli*.

According to **Table 9** The isolated *Lactobacillus acidophilus* CH1 was showed antimicrobial activity against *E.coli* ATCC 25922 which showed the largest of growth inhibitor% around 59.56% in temp. 60˚C/30min but that showed the smallest 18.08% in temp. 90˚C/30min. Bacteriocin wherever *Lactobacillus acidophilus* CH1 was showed antimicrobial activity against Bacillus subtilis NCIB3610 was 87.08% in temp. 30˚C/30min but the smallest of the antimicrobial activity was 18.08% in temp. 90˚C/30min. **Table 10**, in addition, the strains *L. pentosus* CH2 which showed the largest growth inhibition% was 85.98% in temp. 60˚C/30min against *Bacillus subtilis* NCIB3610 but the smallest was 0.00% in temp. 60˚C/30min wherever, the same strain was the largest growth inhibitor% 98.40% in temp. 90˚C/30min against to *E. coli*.

An optimal temperature of 25˚C for the production of bacteriocin by *Leuconostoc carnosum* LA54A was found by Geisen *et al*. [39]. Vignolo *et al*. [40] showed that production of lactocin 705 by *Lactobacillus casei* CRL 705 increased as the culture temperature was reduced: for every temperature tested (15˚C ± 30˚C), bacteriocin production levels were identical but biomass increased with the temperature. Moreover, the lower the temperature, the higher the volumic production, particularly for mesenterocin 52 A. This indicated that bacteriocin production was stimulated by temperatures unfavorable for growth, particularly the low temperatures.

3.5.2. Effect of Different Levels of pH on the Crud Bacteriocin

The effects of different pH such as 2, 4, 6, 8 and 10 on crude of bacteriocins were studied. In MRS broth, pH2

Table 7. Effect of temperature on crude bacteriocins.

	***L. fermentum* M1**					
	***Bacillus subtilis* NCIB3610**			***E. coli* ATCC 25922**		
	O.D	**Growth %**	**Growth inhibitor %**	**O.D**	**Growth %**	**Growth inhibitor %**
Control	0.813	100	0.00	1.350	100	0.00
30˚C/30min	0.569	69.99	**30.01**	0.531	39.33	60.67
60˚C/30min	0.522	64.21	**35.79**	0.323	23.93	**76.08**
90˚C/30min	0. 55	67.65	32.34	0.090	6.66	**39.34**

Table 8. Effect of temperature on crude bacteriocins.

	***L. acidophilus* M2**					
	***Bacillus subtilis* NCIB3610**			***E. coli* ATCC 25922**		
	O.D	**Growth %**	**Growth inhibitor %**	**O.D**	**Growth %**	**Growth inhibitor %**
Control	0.813	100	0.00	1.350	100	0.00
30˚C/30min	0.232	28.54	71.46	0.943	69.85	30.15
60˚C/30min	0.160	19.68	**80.32**	0.171	12.66	**87.34**
90˚C/30min	0.278	34.19	**65.81**	1.20	88.88	**11.12**

Table 9. Effect of temperature on crude bacteriocins.

	***L. acidophilus* CH1**					
	***Bacillus subtilis* NCIB3610**			***E. coli* ATCC 25922**		
	O.D	**Growth %**	**Growth inhibitor %**	**O.D**	**Growth %**	**Growth inhibitor %**
Control	0.813	100	0.00	1.350	100	0.00
30˚C/30min	0.105	12.92	**87.08**	1.007	74.59	25.41
60˚C/30min	0.411	50.55	49.45	0.546	40.44	**59.56**
90˚C/30min	0.666	81.92	**18.08**	1.096	81.19	**18.82**

Table 10. Effect of temperature on crude bacteriocin.

	***L. pentosus* CH2**					
	***Bacillus subtilis* NCIB3610**			***E. coli* ATCC 25922**		
	O.D	**Growth %**	**Growth inhibitor %**	**O.D**	**Growth %**	**Growth inhibitor %**
Control	0.813	100	0.00	1.350	100	0.00
30˚C/30min	0.122	15	84.99	0.221	16.37	83.63
60˚C/30min	0.114	14.02	**85.98**	0.221	16.37	83.63
90˚C/30min	1.229	151.17	−51.17	2.154	1.59	**98.40**

increased the activity of bacteriocins isolated from *Lactobacillus acidophillus* CH1 against to *E. coli* ATCC 25922 was 12 mm but the *L. acidophillus* M2, *L. fermentum* M1 and *L. pentosus* CH2 isolates were around (5, 9, 10) mm, wherever four isolates were decreased pH against to *Bacillus subtilis* NCIB3610 (**Table 11**). Among them, in pH 6, the largest activity of bacteriocin from *Lactobacillus acidophillus* CH1, *L. acidophillus* M2, *L. fermentum* M1 and *L. pentosus* CH2 against *E. coli* ATCC 25922 was shown 11, 11, 28 and 15 mm, but isolates were decreased pH against to *Bacillus subtilis* NCIB3610. Among them, in pH 8, the largest activity of bacteriocin from *Lactobacillus acidophilus* CH1, *L. acidophillus* M2, *L. fermentum* M1 and *L. pentosus* CH2 against *E. coli* ATCC 25922 was shown 13, 10, 26 and 20 mm, but isolates were decreased pH against to *Bacillus subtilis* NCIB3610. In pH 10, no activity for bacteriocin from *Lactobacillus* spp. against *E. coli* ATCC 25922 and *Bacillus subtilis* NCIB3610. So that, the activity of different bacteriocins were shown in pH6 against *E. coli* ATCC 25922 and the isolates were decreased against to *Bacillus subtilis* NCIB3610.

Thus, under uncontrolled pH conditions, a lower temperature coincided with a higher maximum bacteriocin production, a result also obtained by De Vugst *et al*. [41] with the bacteriocin from *Lactobacillus amylovorus*.

Table 11. Effect of pH on crude bacteriocins.

pH / Strains	*E. coli* ATCC 25922				*Bacillus subtilis* NCIB3610			
	L. fermentum M1	*L. acidophilus* M2	*L. acidophilus* CH1	*L. pentosus* CH2	*L. fermentum* M1	*L. acidophilus* M2	*L. acidophilus* CH1	*L. pentosus* CH2
2	10	9	12	5	6	8	9	5
4	9	11	9	9	10	8	10	10
6	11	11	28	20	5	5	33	5
8	13	10	26	15	5	5	9	5
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

3.5.3. Effect of Surfactant on Crude Bacteriocins

Tables 12-15 clearly highlights of effect of different minerals such as SDS, ETDA, Tween80 and CTAB on crud bacteriocin from four LAB such as *L. fermentum* M1, *L. acidophilus* M2, *L. acidophilus* CH1and *L. pentosus* CH2 according to the teterazolium chloride methods. As can be seen, LAB were isolated from local raw milk and Ras cheese using MRS agar. According to **Table 12** The isolated *Lactobacillus fermentum* M1 was showed antimicrobial activity against *E. coli* ATCC 25922 which showed the largest of growth inhibitor% around 73.56% in Tween80 on bacteriocin but the smallest of the antimicrobial activity was 34.23% in CTAB wherever *Lactobacillus fermentum* was showed antimicrobial activity against *Bacillus subtilis* NCIB3610 was 47.48% in Tween80 but the smallest of the antimicrobial activity was 12.42% in SDS. **Table 13**, in addition, the strains *L. acidophilus* M2 which showed the largest growth inhibition% was 78.59% in SDS against *Bacillus subtilis* NCIB3610 but the smallest was 11.44% in Tween80 wherever, the same strain was the largest growth inhibitor% 74.52% in CTAB but the smallest was 39.4% in Tween80 against to *E. coli*.

According to **Table 14**, the isolated *Lactobacillus acidophilus* CH1 was showed antimicrobial activity against *E. coli* ATCC 25922 which showed the largest of growth inhibitor% around 78.29% in Tween80 but that showed the smallest 20% in CTAB. Bacteriocin wherever *Lactobacillus acidophilus* CH1 was showed antimicrobial activity against *Bacillus subtilis* NCIB3610 was 75.77% in Tween80 but the smallest of the antimicrobial activity was 34.44% in SDS. **Table 15**, in addition, the strains *L. pentosus* CH2 which showed the largest growth inhibition% was 49.57% in CTAB against *Bacillus subtilis* NCIB3610 but the smallest was 7.13% in Tween80 wherever, the same strain was the largest growth inhibitor% 80.59% in CTAB against to *E. coli* but the smallest of the antimicrobial activity was 40.89% in ETDA. Similar observation was made earlier in *L. acidophilus* [42].

3.5.4. Effect of Organic Solvents on Crude Bacteriocins Activity

Tables 16-19 clearly highlights of effect of different solvent such as ethanol, Isopropanol, Isoamylchlorde and chrolform on crud bacteriocin from four LAB such as *L. fermentum* M1, *L. acidophilus* M2, *L. acidophilus* CH1and *L. pentosus* CH2 according to the teterazolium chloride methods.

As can be seen, LAB were isolated from local raw milk and Ras cheese using MRS agar. According to **Table 16**, the isolated *Lactobacillus fermentum* M2 was showed antimicrobial activity against *E. coli* ATCC 25922 which showed the largest of growth inhibitor% around 60.67% in ethanol bacteriocin wherever *Lactobacillus fermentum* was showed antimicrobial activity against *Bacillus subtilis* NCIB3610 was 93.85% in Iso amyl chloride but the smallest of the antimicrobial activity was 30% in ethanol. **Table 17**, in addition, the strains *L. acidophilus* M2 which showed the largest growth inhibition% was 88.98% in chroloform against *Bacillus subtilis* NCIB3610 but the smallest was 46.87% in ethanol wherever, the same strain was the largest growth inhibitor% 87% in isopropanol but the smallest was 11.12% in Iso amyl chloride against to *E. coli*.

According to **Table 18**, the isolated *Lactobacillus acidophilus* CH1 was showed antimicrobial activity against *E. coli* ATCC 25922 which showed the largest of growth inhibitor% around 85.19% in ethanol but that showed the smallest 38.45% in isoamylchlorde. Bacteriocin wherever *Lactobacillus acidophilus* CH1 was showed antimicrobial activity against *Bacillus subtilis* NCIB3610 was 87.69% in ethanol but the smallest of the antimicrobial activity was 14.69% in chloroform. **Table 19**, in addition, the strains *L. pentosus* CH2 which showed the largest growth inhibition% was 33.21% in ethanol against *Bacillus subtilis* NCIB3610 but the smallest was 0.00% in chloroform wherever, the same strain was the largest growth inhibitor% 70.52% in ethanol but the smallest was 40.74% in isopropanol against to *E. coli*.

3.5.5. Effect of Some Minerals Salt on Crude Bacteriocins

Tables 20-23 clearly highlights of effect of different minerals such as $AgNo_3$, $CuSo_4$, $FeSo_4$, $MgSo_4$, $MnCl_2$ and $ZnSo_4$ on crud bacteriocin from four LAB isolates according to the teterazolium chloride methods. Tables

Table 12. Effect of surfactant on crude bacteriocins from *Lactobacillus fermentum* M1.

Isolates / Surfactant	*Lactobacillus fermentum* M1					
	Baccillus subtilis NCIB3610			*E. coli* ATCC 25922		
	O.D	Growth %	Growth inhibitor %	O.D	Growth %	Growth inhibitor %
Control	0.813	100	0.00	1.350	100	0.00
SDS	0.712	87.58	**12.42**	0.739	54.74	45.26
ETDA	0.576	71.09	28.91	1.766	130.81	−30.81
Tween80	0.427	52.52	**47.48**	0.357	26.44	**73.56**
CTAB	0.465	57.19	42.80	0.888	65.78	**34.23**

Table 13. Effect of surfactant on crude bacteriocins from *Lactobacillus acidophillus* M2.

Isolates / Surfactant	*L. acidophillus* M2					
	Baccillus subtilis NCIB3610			*E. coli* ATCC 25922		
	O.D	Growth %	Growth inhibitor %	O.D	Growth %	Growth inhibitor %
Control	0.813	100	0.00	1.350	100	0.00
SDS	0.174	21.40	**78.59**	0.549	40.67	59.34
ETDA	0.272	33.46	66.54	1.463	108.37	−8.37
Tween80	0.720	88.56	**11.44**	0.818	60.59	**39.4**
CTAB	0.583	71.71	28.29	0.344	25.48	**74.52**

Table 14. Effect of surfactant on crude bacteriocins from *Lactobacillus acidophillus* CH1.

Isolates / Surfactant	*L. acidophillus* CH1					
	Baccillus subtilis NCIB3610			*E. coli* ATCC 25922		
	O.D	Growth %	Growth inhibitor %	O.D	Growth %	Growth inhibitor %
Control	0.813	100	0.00	1.350	100	0.00
SDS	0.533	65.56	**34.44**	0.359	26.59	73.41
ETDA	0.494	60.76	39.24	1.670	123.70	−23.70
Tween80	0.197	24.23	**75.77**	0.293	21.70	**78.29**
CTAB	0.292	35.92	64.08	1.080	80	**20**

Table 15. Effect of surfactant on crude bacteriocins from *Lactobacillus pentosus* CH2.

Isolates / Surfactant	*L. pentosus* CH2					
	Baccillus subtilis NCIB3610			*E. coli* ATCC 25922		
	O.D	Growth %	Growth inhibitor %	O.D	Growth %	Growth inhibitor %
Control	0.813	100	0.00	1.350	100	0.00
SDS	0.512	62.98	37.02	1.907	141.25	−14.25
ETDA	0.503	61.87	38.13	0.798	59.11	**40.89**
Tween80	0.755	92.87	**7.13**	2.499	185.11	−85.11
CTAB	0.410	50.43	**49.57**	0.262	19.41	**80.59**

Table 16. Effect of different solvent on crude bacteriocin.

Different Solvents	*Lactobacillus fermentum* M1					
	Bacillus subtilis NCIB3610			*E. coli* ATCC 25922		
	O.D	Growth %	Growth inhibitor %	O.D	Growth %	Growth inhibitor %
Control	0.813	100	0.00	1.350	100	0.00
Ethanol	0.569	69.99	30.01	0.531	39.33	60.67
Isopropanol	0.522	64.21	35.79	1.323	98	2
Iso amyl chlode	0.05	6.15	93.85	0.90	66.66	33.34
Chroloform	0.554	68.14	31.86	0.00	0.00	0.00

Table 17. Effect of different solvent on crude bacteriocin.

Different Solvents	*Lactobacillus acidophilus* M2					
	Bacillus subtilis NCIB3610			*E. coli* ATCC 25922		
	O.D	Growth %	Growth inhibitor %	O.D	Growth %	Growth inhibitor %
Control	0.813	100	0.00	1.350	100	0.00
Ethanol	0.10	12.30	87.69	0.20	14.81	85.19
Isopropanol	0.683	84.00	15.99	0.767	56.81	43.19
Iso amyl chloride	1. 90	233.7	−33.7	0.831	61.56	38.45
Chroloform	0.692	85.12	14.89	0.739	54.74	45.26

Table 18. Effect of different solvent on crude bacteriocin.

Different Solvents	*Lactobacillus acidophilus* CH1					
	Bacillus subtilis NCIB3610			*E. coli* ATCC 25922		
	O.D	Growth %	Growth inhibitor %	O.D	Growth %	Growth inhibitor %
Control	0.813	100	0.00	1.350	100	0.00
Ethanol	0.432	53.14	**46.87**	0.943	69.85	30.15
Isopropanol	0.10	12.30	87.69	0.171	12.66	**87.34**
Iso amyl chlode	0.278	34.19	65.81	1.20	88.88	**11.12**
Chroloform	0.090	11.07	**88.93**	2.00	148.15	−48.15

Table 19. Effect of different solvent on crude bacteriocin.

Different Solvents	*Lactobacills pentosus* CH2					
	Bacillus subtilis NCIB3610			*E. coli* ATCC 25922		
	O.D	Growth %	Growth inhibitor %	O.D	Growth %	Growth inhibitor %
Control	0.813	100	0.00	1.350	100	0.00
Ethanol	0.543	66.79	33.21	0.398	29.48	70.52
Isopropanol	0.765	94.09	5.91	0.800	59.26	40.74
Iso amyl chlode	0.625	76.88	23.12	0.699	51.78	48.23
Chroloform	0.00	0.00	0.00	0.421	30.52	69.48

showed that LAB were isolated from local raw milk and Ras cheese using MRS agar. According to **Table 20** The isolated *Lactobacillus fermentum* M1 was showed antimicrobial activity against *E. coli* ATCC 25922 which showed the largest of growth inhibitor% around 86.89% in $MgSo_4$ on bacteriocin but the smallest of the antimicrobial activity was 12.23% in $FeSo_4$ wherever *Lactobacillus fermentum* was showed antimicrobial activity against *Bacillus subtilis* NCIB3610 was 87.20% in $FeSo_4$ but the smallest of the antimicrobial activity was 39.36% in $CuSo_4$. **Table 21** showed that the *L. acidophilus* M2 isolate which showed the largest growth inhibition% was 95.57% in $FeSo_4$ against *Bacillus subtilis* NCIB3610 but the smallest was 46.74% in $CuSo_4$ wherever, the same strain was the largest growth inhibitor% 77.70% in $MgSo_4$ but the smallest was 40.37% in $FeSo_4$ against to *E. coli*.

According to **Table 22**, the *Lactobacillus acidophilus* CH1 isolate was showed antimicrobial activity against *E.coli* ATCC 25922 which showed the largest of growth inhibitor% around 97.92% in $FeSo_4$ but that showed the smallest 66.89% in $MgSo_4$. Bacteriocin wherever *Lactobacillus acidophilus* CH1 was showed antimicrobial activity against *Bacillus subtilis* NCIB3610 was 75.03% in $ZnSo_4$ but the smallest of the antimicrobial activity was 5.41% in $CuSo_4$. **Table 23**, in addition, the strains *L. pentosus* CH2 which showed the largest growth inhibition% was 97.17% in $FeSo_4$ against *Bacillus subtilis* NCIB3610 but the smallest was 15.5% in $ZnSo_4$ wherever, the same strain was the largest growth inhibitor% 93.11% in $MgSo_4$ against to *E. coli* but the smallest of the antimicrobial activity was 13.34% in $AgSo_4$.

3.5.6. Effect of Different Concentration of NaCl on Crude of Bacteriocins

The effects of different concentration of NaCl on crude of bacteriocins were studied. In MRS broth, 2% NaCl increased the activity of bacteriocins isolated from *Lactobacillus pentosus* CH2 against to *E.coli* ATCC 25922 was 14 mm, *L. fermentum* M1 was shown15 mm against to *Bacillus subtilis* NCIB3610 (**Table 24**). Among them, bacteriocin from *Lactobacillus pentosus* CH2 against

Table 20. Effect of some mineral salt on crude bacteriocins from *Lactobacillus fermentum* M1.

	***Lactobacillus fermentus* M1**					
	***Bacillus subtilis* NCIB3610**			***E. coli* ATCC 25922**		
	O.D	**Growth %**	**Growth inhibitor %**	**O.D**	**Growth %**	**Growth inhibitor %**
control	0.813	100	0.00	1.350	100	0.00
$AgNO_3$	0.844	103.8	−3.8	2.221	164.5	−64.5
$CuSO_4$	0.493	60.63	39.36	2.221	164.5	−64.5
$FeSO_4$	0.104	12.79	87.20	1.185	87.76	**12.23**
$MgSO_4$	0.301	37.03	62.98	0.177	13.11	**86.89**
$MnCl_2$	0.216	26.57	73.43	2.301	170.44	−70.44
$ZnSO_4$	0.424	52.15	47.85	0.975	72.22	27.77

Table 21. Effect of some mineral salt on crude bacteriocins from *Lactobacillus acidophillus* M2.

	***Lactobacillus acidophillus* M2**					
	***Bacillus subtilis* NCIB3610**			***E. coli* ATCC 25922**		
	O.D	**Growth %**	**Growth inhibitor %**	**O.D**	**Growth %**	**Growth inhibitor %**
control	0.813	100	0.00	1.350	100	0.00
$AgNO_3$	0.448	55.10	44.89	1.305	96.67	3.34
$CuSO_4$	0.433	53.26	**46.74**	1.793	132.8	−32.8
$FeSO_4$	0.036	4.43	**95.57**	0.805	59.63	**40.37**
$MgSO_4$	0.09	11.07	88.92	0.301	22.29	**77.70**
$MnCl_2$	0.833	102.46	−2.46	2.040	151.11	−51.11
$ZnSO_4$	0.297	36.53	63.47	2.20	162.96	−62.96

Table 22. Effect of some mineral salt on crude bacteriocins from *Lactobacillus acidophillus* CH1.

	***Lactobacillus acidophillus* CH1**					
	***Bacillus subtilis* NCIB3610**			***E. coli* ATCC 25922**		
	O.D	**Growth %**	**Growth inhibitor %**	**O.D**	**Growth %**	**Growth inhibitor %**
control	0.813	100	0.00	1.350	100	0.00
$AgNO_3$	0.665	81.79	18.20	2.096	155.25	−55.25
$CuSO_4$	0.769	94.59	**5.41**	2.301	170.44	−70.44
$FeSO_4$	0.265	32.59	67.40	0.028	2.07	**97.92**
$MgSO_4$	0.301	37.02	62.97	0.447	33.11	**66.89**
$MnCl_2$	0.700	86.10	13.89	2.484	184	−84
$ZnSO_4$	0.203	24.97	**75.03**	2.444	181.03	−81.03

Table 23. Effect of some mineral salt on crude bacteriocins from *Lactobacillus pentosus* CH2.

	***Lactobacillus pentosus* CH2**					
	***Bacillus subtilis* NCIB3610**			***E. coli* ATCC 25922**		
	O.D	**Growth %**	**Growth inhibitor %**	**O.D**	**Growth %**	**Growth inhibitor %**
control	0.813	100	0.00	1.350	100	0.00
$AgNO_3$	0.543	66.79	33.21	1.170	86.67	**13.34**
$CuSO_4$	0.685	84.26	15.74	2.461	182.3	−82.3
$FeSO_4$	0.023	2.83	**97.17**	0.334	24.74	75.26
$MgSO_4$	0.201	24.73	75.27	0.093	6.89	**93.11**
$MnCl_2$	0.687	84.50	**15.5**	2.470	182.9	−82.96
$ZnSO_4$	0.230	28.3	71.7	0.155	11.48	88.51

E. coli ATCC 25922 was shown 12 mm, *L. fermentum* M1 was shown13 mm against to *Bacillus subtilis* NCIB3610 was showed activity in the presence of 4% NaCl concentration, but this activity at 6% and 8% NaCl. Two bacteriocins from *L. pentosus* CH2 and *L. acidophilus* M2 sawed 11 or 10 mm. According to 10% NaCl concentration were shown no increase in their activity, but were inhibited by more than 1% NaCl in MRS media. The supplementation with NaCl, bacteriological peptone and beef extract have resulted in reduced activity. In contrast to the present observation, growth as well as bacteriocin production in the presence of bacteriological peptone or casamino acids and NaCl was reported to be higher by previous researchers [43,44].

3.5.7. Effect of Different Concentration of Amino Acids Component on Crude Bacteriocin Activity

The effects of different concentrations of 21 esintial amino acid such as 2%, 4%, 6%, 8% and 10% on crude of bacteriocins were studied **Table 25**. In MRS broth, 2% increased the activity of bacteriocins isolated from *Lactobacillus pentosus* CH2 against to *E. coli* ATCC 25922 was 9 mm but the *L. fermentum* M1 was around (8 mm) activity, the *L. acidophillus* M2 and *L. pentosus* CH2 were no activity. The activity of *L. fermentum* M1 was showed 13 mm against to *Bacillus subtilis* NCIB3610. Among them, in 4%, the largest activity of bacteriocin from *Lactobacillus pentosus* CH2 against *E. coli* ATCC 25922 was shown 10 mm, but isolates were no activity against to *Bacillus subtilis* NCIB3610. Among them, 6%, the largest activity of bacteriocin from *L. acidophillus* M2 against *E. coli* ATCC 25922 was shown 11 mm, but isolates were no activity against to *Bacillus subtilis* NCIB3610. 8%, the largest activity of bacteriocin from *L. fermentum* M1 against *E. coli* ATCC 25922 was shown 11 mm, but isolates were no activity against to *Bacillus subtilis* NCIB3610. In 10%, no activity for bacteriocin from *Lactobacillus sp*. against *E. coli* ATCC 25922 and *Bacillus subtilis* NCIB3610. So that, the activity of different bacteriocins were shown in 2% against *E. coli* ATCC 25922 and *Bacillus subtilis* NCIB3610.

3.5.8. Effect of Different Vitamins Component on Crude Bacteriocin Activity

The effects of different vitamins such as B_{12} and B complex on crude of bacteriocins were studied **Table 26**. In MRS broth, B_{12} increased the activity of bacteriocins isolated from *Lactobacillus acidophillus* CH1 against to *E. coli* ATCC 25922 was 10 mm but the three other isolates no activity were observed, wherever four isolates were shown 10 to 11 mm against to *Bacillus subtilis* NCIB3610. Among them, in B complex, bacteriocin from *Lactobacillus acidophilus* CH1 against *E. coli* ATCC 25922 was shown 13 to 14 mm, the largest activity of bacteriocins for *L. fermentum* M1 and *L. acidophilus* CH1were shown15 mm against to *Bacillus subtilis* NCIB3610 and the smallest was showed 5 mm in *L. acidophilus* M2 *and L. pentosus* CH2. This result was agree with Adenike *et al*. [45].

Table 24. Effect of different concentration of Nacl on crude bacteriocin.

Na Cl / Strains	*E. coli* ATCC 25922				*Bacillus subtilis* NCIB3610			
	L. fermentum M1	*L. acidophils* M2	*L. Acidophils* CH1	*L. pentosus* CH2	*L. Fermentum* M1	*L. Acidophils* M2	*L. Acidophils* CH1	*L. pentosus* CH2
Control	2	3	9	5	3	4	16	**2**
2%	**10**	**11**	**11**	**14**	**15**	**14**	**10**	**10**
4%	**9**	**6**	**10**	**12**	**13**	**11**	**9**	**11**
6%	**0.0**	**0.0**	**9**	**11**	**5**	**10**	**9**	**10**
8%	**0.0**	**0.0**	**0.0**	**0.0**	**10**	**9**	**7**	**5**
10%	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Table 25. Effect of adding 21 amino acid on crude bacteriocin.

21 amino acid / Strains	*E. coli*				*B. subtilis*			
	L. fermentum 10	*L. acidophilus* 110	*L. acidophilus* 111	*L. pentosus* 160	*L. fermentum* 10	*L. acidophilus* 110	*L. acidophilus* 111	*L. pentosus* 160
2%	**8**	**0.0**	**0.0**	**9**	**13**	**12**	**11**	**0.9**
4%	**7**	**5**	**7**	**10**	**0.0**	**0.0**	**0.0**	**0.0**
6%	**0.0**	**11**	**9**	**0.0**	**7**	**5**	**6**	**0.0**
8%	**11**	**10**	**7**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
10%	**9**	**0.0**	**0.0**	**10**	**9**	**7**	**5**	**0.0**

Table 26. Effect of adding some vitamins on crude bacteriocin.

Vitamins / Strains	B_{12}		B comple	
	E. coli	*B. subtilis*	*E. coli*	*B. subtilis*
L. fermentum 10	0.0	11	14	11
L. acidophilus 110	0.0	10	13	5
L. acidophilus 111	10	11	14	11
L. pentosus 160	0.0	11	10	5

4. Conclusions

Bacteriocin production was strongly dependent on pH, nutrients source and temperature various physicochemical factors seemed to affect bacteriocin production as well as its activity.

The bacteriocin suspension of *Lactobacillus* spp. grown in MRS broth had the best inhibitory effect against wide spectrum of bacteria. The present study demonstrated the production of the bacteriocin by four lactobacilli isolates under different culture conditions. Its antimicrobial potency, pH stability, activity retention in low and high temperatures suggested its wide applicability in acidic pH conditions and in pre-processed food products. Further research though, should be performed to develop extraction techniques for lactic acid and bacteriocins and test further their production on the nutrient media.

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