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RESEARCH ARTICLE

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ACTIVITY RANGE DETERMINATION AND OPTIMIZATION OF VARIOUS ENVIRONMENTAL CONDITIONS FOR BACTERIOCIN PRODUCTION BY LACTOBACILLUS ACIDOPHILUS AND LACTOBACILLUS PLANTARUM

ABSTRACT:

Lactic acid bacteria (LAB) are Gram-positive, catalase-negative, oxidase negative, non-sporulating, microaerophilic bacteria, referred as probiotics as there are many species from this bacterial group produce several antibacterial compounds including bacteriocins. Various bacteriocins differ in their activity range where they are either effect only on related species and genera (narrow activity range) or effect on other unrelated genera (wide activity rang). Bacteriocin production was found to be dependent on different factors environmental and cultural conditions as well as the producer strain itself. This study determined the activity range of *Lactobacillus acidophilus* ATCC 4356 and *Lactobacillus plantarum* atcc 8014, optimized the different conditions for the maximum bacteriocin production and finally, characterized the produced bacteriocins partially.

KEY WORDS:

Bacteriocin, probiotic, Lactobacillus, environmental conditions and Activity range

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INTRODUCTION:

The meaning of probiotics, “pro” is “favour” and “bio” means “life”, which is the antonym of antibiotics (Sahu *et al.,* 2008). The Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) defined probiotics as “live microorganisms which confer a health benefit on the host when consumed in adequate amounts as part of food” (FAO/WHO, 2006).

Lactic acid bacteria (LAB) have been thought to be helpful for human heath in the gut as probiotics (Halami, 2004; Nishida *et al.,* 2016). As they provide beneficial effects to host animals via improving its intestinal microbial balance. Further, it exhibits an antagonist effect on pathogens in the gastrointestinal tract (Ilavenil *et al.,* 2015). Lactic acid bacteria (LAB) are Gram-positive, catalase-negative, oxidase negative, non-sporulating microaerophilic bacteria whose main fermentation product from carbohydrates is lactate. They possess several interesting properties of great economic importance, such as lactose utilization, proteinase activity, bacteriophage defence mechanisms, and production of many inhibitory compounds such as organic acids, hydrogen peroxide and bacteriocin (de Vuyst and Vandamme, 1994a; Dunyu, 2011).

It recently was noted that activity of LAB stimulating immune systems is important. Probiotics fight pathogens via different strategies as competition on adherence sites or production of inhibitory compounds including organic acids, hydrogen peroxide, bacteriocins and many other inhibitory compounds (Bian *et al.,* 2010; Brul and Coote, 1999; Helander *et al.,* 1997; Niku-Paa801480148014vola *et al.,* 1999; Oelschlaeger, 2010; Schnürer and Magnusson 2005).

The term of bacteriocin historically was applied to antibiotic like compounds with inhibitory property specificity primarily restricted to bacterial strains of the same genus or closely related genera (Reeves, 1979). The first bacteriocin was detected in *E. coli* by Gratia (1925), and the bacteriocins world was mainly

made up of bacteriocins from gram negative bacteria for a long period of time then Gram positive bacteria were studied for bacteriocin production. Bacteriocins known to have antimicrobial activities against food-spoiling bacteria and food-borne pathogens (Jingping *et al.,* 2016)

The names of bacteriocins generally derived from the producing genus or species as colicin from *E. coli,* plantarcins from *Lactobacillus plantarum* strains, acidocins from *Lactobacillus acidophilus* strains and lactococcin from *Lactococcus lactis* (Main, 2014). Most bacteriocins characterized by heat stability, activity within a wide pH rang from acidic to alkaline level (de Vuyst and Vandamme, 1994a&b). Bacterial strains which produce bacteriocins protect themselves from the toxic effect of their own bacteriocin through the production of specific immunity proteins (Deegan *et al.,* 2006). Bacteriocins of gram negative bacteria known to be active against very closely related species, while those of gram positive bacteria found to be of a wide activity range which may be include both gram positive and negative strains (Main, 2014).

Bacteriocin production affected greatly by the different environmental factors including the growth medium ingredients and their concentrations, the pH of the cultural medium, the inoculum size of the producing strain itself, as well as the growth temperature.

This study aimed to determine the activity range (either wide or narrow) of the two probiotics under study and optimize different growth conditions to achieve the maximum bacteriocin activity level from the two probiotics *Lactobacillus acidophilus* ATCC 4356 and *Lactobacillus plantrum ATCC 8014*.

MATERIAL AND METHODS:

Bacterial strains and growth medium:

The two probiotics under investigation, *Lactobacillus acidophilus* ATCC 4356 and *Lactobacillus plantarum ATCC 8014*, and three bacterial strains included in this study for the activity range determination (*E. coli* ATCC 51659, *Streptococcus mutans* ATCC 25175, *Klebsiella pneumonia* ATCC10031) were bought from the American Type Culture Collection, while the rest seven tested bacterial strains (*Staphylococcus aureus* (MRSA), *Klebsiella sp.*, *E. coli*, *Streptococcus sp.*, *salmonella sp.*, *Proteus sp.*, and *Pseudomonas sp*.) were clinically isolated.

The lactic acid bacterial strains were grown on MRS cultural medium (gm/L): 10 gm peptone, 8 gm beef extract, 4 gm yeast extract, 20 gm D- glucose, 2 gm dipotassium hydrogen phosphate, 5 gm sodium acetate, 2 gm di-ammonium hydrogen citrate, 0.2 gm Magnesium sulphate, 0.02 manganese sulphate, 1 ml tween and the pH was adjusted to 6 ± 0.2, while all pathogenic bacterial strains were grown on nutrient broth medium.

Activity range determination and tester strain selection:

After activating the two lyophilized probiotics under study**,** their antibacterial activities were checked using different gram positive and negative pathogens as indicators for bacteriocin production using the agar well diffusion bioassay after adjusting the two overnight fermentation broths -free from cells- pH at 5.5 (using 1M NaOH to prevent the inhibitory effect of lactic acid) **(**Todorov and Dicks, 2006). Different volumes from each fermentation broth were used (100, 150, 200, and 250 µl) to determine the activity range of each tested probiotic and to select the best testers that will be used in the rest of this study.

Production of bacteriocins at different pH values and different growth temperatures:

Lactobacillus acidophilus ATCC 4356 and *Lactobacillus plantarum ATCC 8014* each was grown in sterile 10 ml MRS broth overnight and used to inoculate autoclaved volumes of 10 ml MRS broth and incubated overnight at different growth temperatures (25, 30, 37, and 45ºc). And also autoclaved volumes of 10 ml MRS which were adjusted to pH 4.5, 5, 5.5, 6, 6.5, 7, 7.5, and 8 with 1 M HCl or 1 M NaOH were inoculated and incubated overnight at the appropriate temperature for each strain (Ogunbanwo *et al.,* 2003; Todorov and Dicks, 2006).

Then the bacteriocin activities were checked from all the inoculated experiments' cell free fermentation broth via the agar well diffusion bioassay using MRSA and *Psedomonase sp*. as the tester pathogenic strains after adjusting the pH of all the fermentation broths to 5.5.

Effect of the inoculum size on bacteriocin production:

An overnight broth from the two probiotics were used to inoculate volumes of 10 ml sterile MRS broth with various amounts (100, 200, 400, 600, 800, and 1000 µl) then all the inoculated tubes were incubated overnight at the appropriate growth temperature for each strain.

Effect of the medium ingredients on the bacteriocin production:

An overnight broth from each lactic acid bacterial strain was used for inoculating 10 ml of the following media: (A) MRS broth, lacking the organic nutrients, supplemented with peptone (22 gm/l), tryptone (20.0 g/l), beef extract (22.0 g/l), yeast extract (22.0 g/l), peptone (13.8 gm/l) plus beef extract (8.8 gm/l), tryptone (12.5 g/l) plus beef extract (7.5 g/l), peptone (13.2 gm/l) plus yeast extract (8.8 gm/l), tryptone (12.5 gm/l), yeast extract (7.5 gm/l) beef extract (14.6 g/l) plus yeast extract (7.4 g/l), tryptone (10.0 g/l), meat extract (5.0 g/l) and yeast extract (5.0 g/l), or a combination

of peptone (10 gm/l) plus beef extract (8 gm/l) plus yeast extract (4 gm/l) as in the normal medium, respectively; (B) MRS broth (oxoid), i.e. with 20.0 g/l glucose; (C) MRS broth lacking glucose, supplemented with 20.0 g/l maltose, mannose, sucrose, fructose, molasses, and galactose, respectively; (C*) MRS lacking glucose, supplemented with 1: 4% from the best sugar which gave the highest bacteriocin activity in comparison with the same concentrations of glucose as a control sugar; (D) MRS broth with (0, 0.5, 1, or 2 ml/l) tween; (E) MRS with three concentrations from the different tested minerals (0, 2, 4 gm/l K_2HPO_4, 0, 0.2, 0.4 gm/l $MgSO_4$; 0, 0.02, 0.04 gm/l $MnSO_4$; 0, 2, 4 gm/l tri-ammonium citrate; and 0, 5, and 10 gm/l sodium acetate (Todorov and Dicks, 2005; Zacharof and Lovitt, 2010). Finally, the best conditions for each *lactobacillus sp.* was tested together for the bacteriocin production activity.

Sensitivity of the produced bacteriocin to heat, pH and enzymes:

Aliquots from the crude bacteriocins (cell free supernatants), (1 ml) from each strain were subjected to different temperatures for different periods of time (65°C and 95°C for 20, 40, and 60 minutes, 100°C for 10, 20, and 30 minutes and 121°C for 20 minutes (autoclaving) (Kyoung *et al.,* 2002; Lash et al., 2005; Goudarzia *et al.,* 2014; Sharma *et al.,* 2006).

Another set of fermentation broths aliquots from the two lactobacilli were adjusted to various pH values ranging from 1 to 13 (at increments of one pH unit) using either 1 M HCl or 1 M NaOH, and incubated for 2 h at 37°C. The pH-treated samples were neutralized to pH 5.5 before measuring the residual bacteriocin activity (Goudarzia *et al.,* 2014; Kyoung *et al.,* 2002; Lash *et al*., 2005; Marie *et al*., 2011; Oh *et al.,* 2000;).

Finally, aliquots of 1 ml also from the different fermentation broths were treated with trypsin (1 mg/ml), proteinase K and (1 mg/ ml) and catalase (1 mg/ml; all Sigma) and incubated at 37°C for 1 hour. Then all samples were boiled 3 minutes to inactivate the enzymes and the residual bacteriocin activity was measured (Fang *et al.,* 2014; Goudarzia *et al.,* 2014; Kyoung *et al.,* 2002; Lash *et al*., 2005; Marie *et al.,* 2012;). All experiments were done in four replicas.

RESULTS AND DISCUSSION:

Upon the activity range determination, it was found that the fermentation broths from both *Lactobacillus acidophilus* atcc 4856 and *Lactopacillus plantarum* atcc 8014 exhibited a broad spectrum activity. As they showed activity towards both gram positive and negative pathogens as shown in table 1.

Table 1. Antibacterial activity range determination of (A) *Lactobacillus acidophilus* ATCC 4356 and (B) *Lactobacillus plantarum* ATCC 8014.

(A)

The tested pathogen	inhibition zone diameter with different amounts of tested cell free supernatant (mm)															
	100 µl				150 µl				200 µl				250 µl			
	R1	R2	M	S.E (±)	R1	R2	M	S.E (±)	R1	R2	M	S.E (±)	R1	R2	M	S.E (±)
MRSA	3	3	2.8	0.2	7	6	6.5	0.2	8	9	8.5	0.4	12	13	13	0.4
Streptococcus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E.coli atcc 1659	0	0	0	0	2	2.5	2.5	0.2	4	4	4	0	5	5	5	0
Klebseilla pnemonia atcc 10031	0	0	0	0	0	0	0	0	1	1	1	0	1.5	2	1.8	0.2
Streptococcus mutans atcc 25175	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Klebseilla sp.	0	0	0	0	1	1	1	0	2	2	2	0	4	4	4	0
Salmonella sp.	0	0	0	0	1	2	1.5	0.4	5	4.5	4.8	0.2	6	7	6.5	0.4
E. coli	1	2	1.5	0.4	4	3	3.5	0.4	4	4	4	0	5	5	5	0
Pesomonase sp.	2	2	1.8	0.2	2	4	3	0.8	4	5	4.5	0.4	5	6	5.5	0.4
Proteus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(B)

The tested pathogen	Inhibition zone diameter of with different amounts of tested cell free supernatant (mm)															
	100 µl				150 µl				200 µl				250 µl			
	R1	R2	M	S.E(±)	R1	R2	M	S.E(±)	R1	R2	M	S.E(±)	R1	R2	M	S.E(±)
MRSA	0	0	0	0	4	5	4.5	0.4	6	7	7	0.4	8	8	8	0
Streptococcus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E.coli atcc 1659	1	1	1	0	1	2	1.5	0.4	1	2	1.5	0.4	2	2	2	0
Klebseilla pnemonia atcc 10031	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus mutans atcc 25175	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Klebseilla sp.	0	0	0	0	0	0	0	0	1	1	1	0	1	2	1.5	0.4
Salmonella sp.	0	0	0	0	1	1	1	0	2	2	2	0	4	4	4	0
E. coli	0	0	0	0	0	0	0	0	1	1	1	0	2	2	2	0
Pesomonase sp.	2	3	3	0.4	3	4	3.5	0.4	5	6	6	0.4	8	8	8	0
Proteus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

The results showed that MRSA was the most sensitive tester organism when different volumes from the fermentation broths of *Lactobacillus acidophilus ATCC 4356* and *Lactopacillus plantarum* ATCC 8014 were used. Although *Psedomonas sp.* was less sensitive than MRSA but both of them were chosen to be tester organisms for the bacteriocin production bioassay to represents the two bacterial groups (gram positive and negative).

It was observed that the pH of the growth medium had a great effect on the level of the produced bacteriocin, where the maximum bacteriocin activity for *Lactobacillus acidophilus atcc 4856* was expressed in

medium of pH values 6 and 6.5 while higher and lower pH values resulted in less antibacterial activities or complete depletion of the activity (Table 2A); however the highest bacteriocin activities of *Lactobacillus plantarum* ATCC 8014 recorded when it was cultured in medium with pH value 5.5 expressing 133.3 and 104.5% activity against MRSA and *Psedomonas sp*., respectively (Table 2B) these results agreed with those recorded in several researches as the pH range required for bacteriocin production for different lactic acid bacteria is found to be between (5.5 to 7.5) (Arokiyamary and Sivakumar, 2011; Biswas *et al.,* 1991; Hoda *et al.,* 2013; Rawal *et al.,* 2013; Vinderola and Reinheimer, 2000).

Table 2. Effect of growth medium initial pH on bacteriocin production from (A) *Lactobacillus acidophilus* ATCC 4356 and (B) *Lactobacillus plantarum* ATCC 8014 against MRSA and *Psedomonas sp.*

(A)

pH	Inhibition zone diameter (mm) *vs.* MRSA					Inhibition zone diameter (mm) *vs. Psedo. sp*.				
	R1	R2	M	S.E (±)	Activity %	R1	R2	M	S.E (±)	Activity %
4.5	0	0	0	0	0	0	0	0	0	0
5	3	2	2.5	0.4	33.3	2	1	1.5	0.4	25
5.5	4	3	3.5	0.4	46.7	3	2	2.5	0.4	41.7
6 *	7	8	7.5	0.4	**100**	6	6	6	0	**100**
6.5	7	8	7.5	0.4	**100**	6	6	6	0	**100**
7	7	7.5	7.3	0.2	96.7	5.5	4.5	5	0.4	83.3
7.5	7	7	7	0	93.3	6	4	5	0.8	83.3
8	7.5	6.5	7	0.4	93.3	5.5	4.5	5	0.4	83.3

(B)

pH	Inhibition zone diameter (mm) *vs.* MRSA					Inhibition zone diameter (mm) *vs. Psedomonas sp.*				
	R1	R2	M	S.E (±)	Activity %	R1	R2	M	S.E (±)	Activity %
4.5	7	7	7	0	77.8	0	0	0	0	0
5	8	9	8.5	0.4	94.4	4	2	3	0.8	45.5
5.5	12	12	12	0	**133.3**	6	5.5	5.75	0.4	**104.5**
6 *	9	9	9	0	100	6	5	5.5	0.4	100
6.5	9	9	9	0	100	5.5	5	5.25	0.2	95.5
7	8.5	9	8.75	0.2	97.2	5.5	5	5.25	0.2	95.5
7.5	4	5	4.5	0.4	50	4	3	3.5	0.4	63.6
8	3	4	3.5	0.4	38.9	1	2	1.5	0.4	27.3

(*)= The normal pH value of MRS growth medium.

On determination the optimum growth temperature for bacteriocin production it was found that different lactobacilli have different optimum growth temperatures for maximum bacteriocin production, in this work the optimum temperature for *Lactobacillus acidophilus* ATCC 4356 and its 37ºC (Table 3A) just like recorded for some lactobacilli by Makhloufi *et al*. (2004) and Rawal *et al.* (2013), while for *Lactobacillus plantrum* atcc 8014 the most suitable incubation temperature was 30ºC (Table 3B) and this temperature was recorded also for many strain of lactic acid bacteria as that reported by Hoda *et al.* (2013), Holt *et al.* (1994), Iyapparaj *et al.* (2013) and Moonchai *et al.* (2005).

Table 3. Effect of growth temperature on bacteriocin production from (A) *Lacto. acidophilus* ATCC 4356 (B) *Lactobacillus plantarum* ATCC 8014 against MRSA and *Psedomonas sp.*

(A)

Growth temp.ºC	Inhibition zone diameter (mm) *vs* MRSA					Inhibition zone diameter (mm) *vs. Psedomonas sp*.				
	R1	R2	M	S.E (±)	Activity %	R1	R2	M	S.E (±)	Activity %
25	0	0	0	0	0	0	0	0	0	0
30	5	4	4.5	0.4	52.9	1	2	1.5	0.4	30
37 *	9	8	8.5	0.4	100	5	5	5	0	100
45	0	0	0	0	0	0	0	0	0	0

(B)

Growth temp.ºC	Inhibition zone diameter (mm) *vs.* MRSA					Inhibition zone diameter (mm) *vs Psedomonas sp.*				
	R1	R2	M	S.E (±)	Activity %	R1	R2	M	S.E (±)	Activity %
25	5	4	4.5	0.4	52.9	4	2.5	3.3	0.6	59.1
30 (*)	9	8	8.5	0.4	100	6	5	5.5	0.4	100
37	5	4.5	4.75	0.2	55.9	4.5	4	4.3	0.2	77.2
45	0	0.5	0.25	0.4	2.9	0	0	0	0	0

(*)= The control growth temperature for each strain.

For the effect of inoculum size on antibacterial activity it was found that *Lactobacillus acidophilus* ATCC 4356 bacteriocin activity didn't affect by the inoculum size as there were no difference in the diameter of all the observed inhibition zones with different inoculum sizes While for *Lactobacillus plantarum* ATCC 8014 it was found that the inoculum size has a great effect on the amount of the produced bacteriocin, where with increasing the inoculum size the diameter of the observed inhibition zones were increased till a certain limit (200 µl) than any increase in the inoculum size caused a decrease in the recorded bacteriocin activity, so we can say that the inoculum size effect depend on the tested strain as reported in several studies (Arokiyamary and Sivakumar, 2011).

Upon testing the effect of the different sugars on bacteriocin activity level for the tested lactobacilli, It could be said that the glucose is the key sugar for bacteriocin production in the both tested lactobacilli but it is more effective in bacteriocin production when present in a dimer form or in combination of other sugars as the highest activities for *Lactobacillus acidophilus* atcc 4856 were achieved in the presence of galactose (which converted into glucose during its metabolism) followed by sucrose (contain glucose subunit), while for *Lactobacillus plantarum* ATCC 8014 maltose - which is two glucose subunits- proved to be the best carbon source for bacteriocin production followed by sucrose (contain a glucose subunit) then glucose and molasses (contains sucrose, with glucose subunit, and glucose), while in presence of mannose (c_2 epimer of glucose) and fructose the bacteriocin activities of the two lactobacilli were decreased (Table 4). these results agreed with those obtained by Aasen *et al.* (2000), Arokiyamary and Sivakumar (2011), Rawal *et al.* (2013), Todorov (2008), and Todorov and Dicks (2006).

Table 4. Effect of different carbon- sources on bacteriocin production from (A) *Lacto. acidophilus* ATCC 4356 and (B) *Lactobacillus plantarum* ATCC 8014 against MRSA and *Psedomonas sp.*

(A)

C-source	Inhibition zone diameter (mm) *vs.* MRSA					Inhibition zone diameter (mm) *vs. Psedo. sp.*				
	R1	R2	M	S.E (±)	Activity %	R1	R2	M	S.E (±)	Activity %
Glucose *	8.3	6	7.15	0.9	100	6	4.5	5.25	0.4	100
Maltose	6	4.5	5.25	0.6	73.4	5	4	4.5	0.4	85.7
Mannose	7	5.5	6.25	0.6	89.3	4	5	4.5	0.4	85.7
Lactose	6	4.5	5.25	0.6	73.4	4.5	4	4.25	0.2	81
Sucrose	9.7	7.5	8.6	0.9	122.9	6	5	5.5	0.4	104.8
Fructose	3	1.8	2.4	0.4	34.3	4.5	3	3.75	0.6	71.4
Molasses	4.5	3	3.75	0.6	53.6	5	4.5	4.75	0	90.5
Galactose	10	8.5	9.25	0.6	132.1	6.5	6	6.25	0.2	119

(B)

C-source	Inhibition zone diameter (mm) *vs.* MRSA					Inhibition zone diameter (mm) *vs. Psedomonas sp.*				
	R1	R2	M	S.E (±)	Activity %	R1	R2	M	S.E (±)	Activity %
Glucose *	10	6	8	0.4	100	4	5	4.5	0.4	100
Maltose	13	8.5	10.8	0.2	134.4	6	6.5	6.3	0.2	138.9
Mannose	7	3	5	0.4	62.5	3	3	3	0.4	66.7
Lactose	12	7	9.5	2	118.8	6	6	6	0	133.3
Sucrose	6.3	7	6.7	0.4	83.1	5	6	5.5	0.4	91.7
Fructose	5	4	4.5	1.2	56.3	3	3.5	3.3	0.2	72.2
Molasses	6	8	7	0.2	87.5	4	3.5	3.8	0.2	83.3
Galactose	7.5	6	6.8	0.6	84.4	4	3	3.5	0.4	77.8

(*) = The control sugar that found in the MRSA growth medium

It was observed that there was a maximum cell growth and production of bacteriocin up to certain sugar concentration then any increase in sugar concentration result in a decrease in the bacteriocin activity when the bacteriocin production test was carried out using different concentration of sugar (Matsusaki *et al.*, 1996; Rawal *et al.*, 2013). The highest bacteriocin activities were recorded for *Lactobacillus acidophilus* atcc 4856 on supplementation of the MRS broth medium with 1% galactose although all the recorded activities for different galactose concentrations were higher than those

recorded for the best glucose concentration (2%), except 4% galactose showed a little activity. (Table 5A). While for *Lactobacillus plantarum* ATCC 8014 all the tested maltose concentrations showed activities higher than the best glucose concentration (2%) and the highest bacteriocin activities were recorded in the presence of 3% maltose (Table 5B).

Table 5. Effect of different concentrations of best carbon- source; for (A) *Lacto. acidophilus* ATCC 4356 (galactose) and (B) *Lactobacillus plantarum* ATCC 8014 (maltose) compared with glucose against MRSA and *Psedomonas sp.*

(A)

% of C-source	Inhibition zone diameter (mm) *vs* MRSA				Activity %	Inhibition zone diameter (mm) *vs Psedo. sp*				Activity %
	R1	R2	M	S.E (±)		R1	R2	M	S.E (±)	
1% Glucose	12	12	12	0	88.9	5	4	4.5	0.4	69.2
2% Glucose*	12	15	13.5	1.2	100	7	6	6.5	0.4	100
3% Glucose	12	12	12	0	88.9	6	6	6	0	92.3
4% Glucose	9	7	8	0.8	59.3	4	3	3.5	0.4	53.8
1% Galactose	17	17	17	0	125.9	10	8	9	0.8	138.5
2% Galactose	16	16	16	0	118.5	8	8	8	0	123
3% Galactose	14	14	14	0.4	103.7	8	7	7.5	0.4	115.4
4% Galactose	12	12	12	0	88.9	6	5	5.5	0.4	84.6

(B)

% of C-source	Inhibition zone diameter (mm) *vs.* MRSA				Activity %	Inhibition zone diameter (mm) *vs. Psedomonas sp.*				Activity %
	R1	R2	M	S.E (±)		R1	R2	M	S.E (±)	
1% Glucose	9	10	9.5	0.4	67.9	4	5	4.5	0.4	90
2% Glucose*	11.5	12.5	12	0.8	100	6	4	5	0.8	100
3% Glucose	11	12	11.5	0.4	82.1	4	5	4.5	0.4	90
4% Glucose	8	9	8.5	0.4	60.7	2	3	2.5	0.4	50
1% Maltose	16	15	15.5	0.4	129.2	8	5	6.5	1.2	130
2% Maltose	16	16	16	0	133.3	7	7	7	0	140
3% Maltose	17	18	17.5	0.4	145.8	9	7	8	0.8	160
4% Maltose	12	13	12.5	0.4	104.2	6	7	6.5	0.8	130

(*) = The control percent of the control sugar that found in the MRSA growth medium.

Most of the lactic acid bacteria which produce bacteriocins require stabilizers or a unique medium composition for bacteriocin synthesis. It is probable that the yeast extract and/or tryptone may in part serve to inactivate an inhibitor of bacteriocin synthesis in some strains (Hoda *et al.*, 2013). It was found that the best nitrogen source should be used for bacteriocin production is yeast extract in the presence of tryptone for the *Lactobacillus acidophilus* atcc 5648. While for *Lactobacillus plantarum* ATCC 8014, the highest bacteriocin activities were achieved upon supplementation the cultural medium with tryptone plus yeast and beef extracts. These results agreed with data obtained in other studies for several probiotics as those of Arokiyamary and Sivakumar (2011), Todorov and Dicks (2005 & 2006), and Verellen *et al.* (1998).

Table 6. Effect of different N-sources on bacteriocin production from (A) *Lactobacillus acidophilus ATCC 4356* and (B) *Lactobacillus plantarum* ATCC 8014 against MRSA and *Psedomonas sp.*

(A)

N-source	Inhibition zone diameter (mm) *vs.* MRSA				Activity %	Inhibition zone diameter (mm) *vs. Psedomonas sp.*				Activity %
	R1	R2	M	S.E (±)		R1	R2	M	S.E (±)	
Peptone+ Yeast + Beef (*)	11	8	9.5	1.2	100	5	5	5	0	100
Tryptone + Beef	0	0	0	0	0	0	0	0	0	0
Yeast + Beef	10.5	8.5	9.5	0.8	100	4	3	3.5	0.4	70
Beef	0	0	0	0	0	0	0	0	0	0
Tryptone + Yeast + Beef	6.3	10	8.15	1.5	85.8	4	2	3	0.8	60
Yeast	10	8.5	9.3	0.6	97.4	6	3.5	4.8	1	95
Peptone	10.5	8	9.3	1.2	97.4	6	4	5	0.8	100
Tryptone	0	0	0	0	0	0	0	0	0	0
Peptone + Beef	10	7.5	8.8	1	92.1	5	4	4.5	0.4	90
Peptone+ Yeast	11.5	7.5	9.5	1.6	100	8.5	7	7.8	0.6	155
Tryptone +Yeast	11	9	10	0.8	105.3	11	8.5	9.8	1	195

(B)

N-source	Inhibition zone diameter (mm) vs MRSA				Activity %		Inhibition zone diameter (mm) *vs Psedomonas sp.*				Activity %
	R1	R 2	M	S.E (±)			R1	R2	M	S.E (±)	
Peptone + Yeast + Beef *	9.4	10	9.7	0.4	100		5	6	5.5	0.4	100
Tryptone + Beef	11	10	10.5	0.8	108.2		6.5	6	6.25	0.2	113.6
Yeast + Beef	8	11	9.5	1.2	98		6	5.5	5.75	0.2	104.5
Beef	9	7	8	1.2	82.5		5	4	4.5	0.4	81.8
Tryptone + Yeast + Beef	15	14	14.5	0.4	**149.5**		7	7	7	0	**127.3**
Yeast	10	11	10.5	0.4	108.2		6	7	6.5	0.4	118.2
Peptone	9	8	8.5	0.4	87.6		4	6	5	0.8	90.9
Tryptone	10	8	9	1.2	92.8		6	6	6	0	109.1
Peptone + Beef	9	8.5	8.8	0.4	90.2		5	5.5	5.25	0.2	95.5
Peptone+ Yeast	10.5	8.5	9.5	0.8	98		6	6	6	0	109.1
Tryptone +Yeast	11	8	9.5	0.8	98		6	6	6	0	109.1

(*) = The control nitrogen source that found in the MRSA growth medium

Upon testing the effect of the tween, being a surfactant Tween 80 could change the surface tension of the producer cell so increasing the rate of bacteriocin release from the cell surface to a certain level then any increase in the tween concentration was found to cause either no change or decrease in the obtained activity (Hoda *et al.,* 2013; Todorov, 2008; Verellen *et al.,* 1998).

Lactobacillus acidophilus atcc 5648 was found to express the maximum bacteriocin activity in the presence of 0.05% tween -less than the normal level found in the MRS medium- (Table 7A), while *Lactobacillus plantarum* ATCC 8014 needed to the normal tween level in the MRS medium to give the maximum antibacterial activity (Table 7B). These results resemble those reported that the Inclusion of Tween 80 in the growth medium increased the production of bacteriocins from lactobacilli, as in case of plantaricin 423 (Verellen *et al.,* 1998), pediocin AcH (Biswas *et al.,* 1991) and lactocin 705 (Vignolo *et al.,* 1995; Todorov and Dicks, 2006).

Table 7. Effect of different tween concentrations on bacteriocin production from *Lacto. acidophilus* ATCC 4356 and (B) *Lactobacillus plantarum* ATCC 8014 against MRSA and *Psedomonas sp.*

(A)

Tween %	Inhibition zone diameter (mm) *vs.* MRSA				Activity %	Inhibition zone diameter (mm) *vs. Psedomonase sp.*				Activity %
	R1	R2	M	S.E (±)		R1	R2	M	S.E (±)	
0	7	2.5	4.8	1.8	63.3	4	1.5	2.8	1	57.9
0.05	11	7.5	9.3	1.4	**123.3**	8	5.5	6.8	1	**142.1**
0.1(*)	9	6	7.5	1.2	100	6	3.5	4.8	1	100
0.2	9	5.5	7.3	1.4	96.7	5.5	3.8	4.6	1	97.4

(B)

Tween %	Inhibition zone diameter (mm) *vs* MRSA				Activity %	Inhibition zone diameter (mm) *vs. Psedomonas sp.*				Activity %
	R1	R2	M	S.E (±)		R1	R2	M	S.E (±)	
0	5.3	4.5	4.9	0.3	59.4	2.3	3.5	2.9	0.5	49.2
0.05	7	4	5.5	1.2	66.7	4.75	4	4.4	0.3	74.2
0.1(*)	8.5	8	8.3	0.2	**100**	5.3	6.5	5.9	0.5	**100**
0.2	8	5.5	6.8	1	81.8	5	5.5	5.3	0.2	89

(*) = The control tween concentration in MRS growth medium.

On studying the effect of different minerals concentrations it was found that the maximum bacteriocin activities for both the tested lactobacilli recorded when the MRS broth supplemented with the normal levels recommended in the medium, except in case of $MgSO_4$ as its depletion was resulted in the highest activities of *Lactobacillus acidophilus* atcc 5648 against the two testers and the activity was decreased with increasing the $MgSO_4$ concentration (TableS 8A& 8B). Generally, it could be said that all mineral ions have an impact on bacteriocin production these results agreed with other studies concerning different lactic acid bacteria as

those of Arokiyamary and Sivakumar (2011), Enan *et al.* (1996), Hoda *et al.* (2013), Powel *et al.* (2007), Todorov (2008), Todorov *et al.* (2000), and Todorov *et al.* (2012), while the opposite was reported by Ogunbanwo *et al.* (2003) as they recorded that the presence of different minerals including Mn, as well as Mg, tri-ammonium citrate, sodium acetate, and potassium phosphate has no effect on the bacteriocin production.

Table 8.A. Effect of different minerals concentrations on bacteriocin production from *Lacto. acidophilus* ATCC 4356 against MRSA and *Psedomonas sp*.

K2HPO4

K_2HPO_4 %	Inhibition zone diameter (mm) *vs.* MRSA				Activity %	Inhibition zone diameter (mm) *vs Psedo. sp.*				Activity %
	R1	R2	M	S.E (±)		R1	R 2	M	S.E (±)	
0	6.5	8.5	7.5	0.8	88.2	4	5	4.5	0.8	72
0.2 *	8	9	8.5	0.4	**100**	6.5	6	6.3	0.2	**100**
0.4	4.5	5	4.8	0.4	55.9	2	2	2	0	32

MgSO4

Mg %	Inhibition zone diameter (mm) *vs* MRSA				Activity %	Inhibition zone diameter (mm) *vs Psedomonas sp.*				Activity %
	R1	R2	M	S.E (±)		R1	R2	M	S.E (±)	
0	9.5	11	10.3	0.6	**124.2**	6	5	5.5	0.4	**137.5**
0.2 *	9	7.5	8.3	0.6	100	4	4	4	0	100
0.4	7	7.5	7.3	0.2	87.9	1	0	0.5	0.4	12.5

MnSO4

Mn %	Inhibition zone diameter (mm) *vs.* MRSA				Activity %	Inhibition zone diameter (mm) *vs. Psedomonas sp.*				Activity %
	R1	R2	M	S.E (±)		R1	R2	M	S.E (±)	
0	4	4	4	0.4	50	1.5	2	1.75	0.4	31.8
0.005 *	7.5	8.5	8	0.4	**100**	4	7	5.5	1.2	**100**
0.01	5	5	4.5	0.8	56.3	2	2.5	2.3	0.4	40.9

ammonium citrate

Tri-amm. citrate %	Inhibition zone diameter (mm) *vs.* MRSA				Activity %	Inhibition zone diameter (mm) *vs. Psedomonase sp.*				Activity %
	R1	R2	M	S.E (±)		R1	R2	M	S.E (±)	
0	0	0	0	0	0	0	0	0	0	0
0.2 *	7	9	8	0.8	**100**	6.5	5	5.8	0.6	**100**
0.4	5	4	4.5	0.4	56.25	3	1	2	0.8	34.8

Sodium acetate

Sodium acetate %	Inhibition zone diameter (mm) *vs.* MRSA				Activity %	Inhibition zone diameter (mm) *vs. Psedomonas sp.*				Activity %
	R1	R2	M	S.E (±)		R1	R2	M	S.E (±)	
0	1	2	1.5	0.4	15	3	3	3	0	60
0.5 *	11	9	10	0.8	**100**	6	4	5	0.8	**100**
1	8	7	7.5	0.4	75	3.5	4	3.8	0.2	75

(*) = The control mineral concentration in MRS growth medium.

Table 8.B. Effect of different minerals concentrations on bacteriocin production by *Lactobacillus plantarum* ATCC 8014 against MRSA and *Psedomonas sp.* bacteria.

K2HPO4

K_2HPO_4 %	Inhibition zone diameter (mm) *vs.* MRSA				Activity %	Inhibition zone diameter (mm) *vs Psedomonase sp.*				Activity %
	R1	R2	M	S.E (±)		R1	R2	M	S.E (±)	
0	4	4	4	0	43.2	0	1	0.5	0.4	9.1
0.2 *	8.5	10	9.3	0.6	**100**	6	5	5.5	0.4	**100**
0.4	0	0	0	0	0	0	1	0.5	0.4	9.1

MgSO4

Mg %	Inhibition zone diameter (mm) *vs.* MRSA				Activity %	Inhibition zone diameter (mm) *vs. Psedo. sp.*				Activity %
	R1	R2	M	S.E (±)		R1	R2	M	S.E (±)	
0	5.3	4	4.7	0.5	53.1	3.5	2.5	3	0.4	50
0.2 *	9	8.5	8.8	0.2	**100**	6	6	6	0.4	**100**
0.4	6.5	5	5.8	0.6	65.7	5.5	5	5.3	0.2	87.5

MnSO4

Mn %	Inhibition zone diameter (mm) *vs.* MRSA				Activity %	Inhibition zone diameter (mm) *vs. Psedomonase sp.*				Activity %
	R1	R2	M	S.E (±)		R1	R2	M	S.E (±)	
0	0	2	1	0.8	11.8	1	4	2.5	1.2	50
0.005 *	8	9	8.5	0.4	**100**	4	6	5	0.8	**100**
0.01	0	1	0.5	0.4	5.9	1	2	1.5	0.4	30

ammonium citrate

Tri- amm. citrate %	Inhibition zone diameter (mm) *vs* MRSA				Activity %	Inhibition zone diameter (mm) *vs. Psedomonas sp.*				Activity %
	R1	R2	M	S.E (±)		R1	R2	M	S.E (±)	
0	0	0	0	0	0	0	0	0	0	0
0.2 *	9	11	10	0.8	**100**	6	4	5	0.8	**100**
0.4	0	2	1	0.8	10	4	3	3.5	0.4	70

sodium acetate

Sodium acetate %	Inhibition zone diameter (mm) *vs* MRSA				Activity %	Inhibition zone diameter (mm) *vs Psedomonas sp.*				Activity %
	R1	R2	M	S.E (±)		R1	R2	M	S.E (±)	
0	0	0	0	0	0	1	3	2	0.8	40
0.5 *	11	10	10.5	0.4	**100**	6	4	5	0.8	**100**
1	9	8	8.5	0.4	81	2	3	2.5	0.4	50

(*) = The control mineral concentration in MRS growth medium.

In conclusion, it could be said as shown in the previous studies of bacteriocin production in various LAB that bacteriocin production by the tested strains can be influenced by many factors either cultural of environmental as pH, temperature, composition of the growth media and inoculum size (Arokiyamary and Sivakumar, Biswas *et al.,* 1991; Eijsink *et al.,* 1996; 2011; Nilsen *et al.,* 1998; Laukova, 1992).

After determining the optimum state for each factor separately affecting the bacteriocin production activity, there were new four growth media fabricated and tested for bacteriocin production under the optimization of the different growth factors (pH, incubation temperature and inoculum size), two new media were fabricated for each strain. The experiments proved the efficiency of the normal MRS in bacteriocin production under the normal cultivation conditions (pH, temperature and inoculum size) as recorded in other studies (Table 9A&B).

Table 9. Effect of best growth conditions together on bacteriocin production by (A) *Lacto. acidophilus* ATCC 4356 and (B) *Lactobacillus plantarum* ATCC 8014 against MRSA and *Psedomonas sp.*

(A)

Growth media	Inhibition zone diameter (mm) *vs.* MRSA				Activity %	Inhibition zone diameter (mm) *vs. Psedomonas sp.*				Activity %
	R1	R2	M	S.E (±)		R1	R2	M	S.E (±)	
MRS	20	22	21	0.8	100	10	12	11	0.8	100
mMRS1	19	21	20	0.8	95.2	9	9	9	0	81.8
mMRS2	20	18	19	0.8	90.5	4.5	0	2.25	0.2	43.2

(B)

Growth media	Inhibition zone diameter (mm) *vs.* MRSA				Activity %	Inhibition zone diameter (mm) *vs. Psedomonas sp.*				Activity %
	R1	R2	M	S.E (±)		R1	R2	M	S.E (±)	
MRS	21	24	22.5	1.2	100	16	18	17	0.8	100
mMRS4	21	22	21.5	0.4	95.5	17	15	16	0.8	94.1
mMRS5	16	15	15.5	0.4	68.9	9	8	8.5	0.4	50

Upon partial characterization of the produced bacteriocins it was found that the obtained bacteriocins could be said to be highly thermo stable as they exhibit activity towards the tester bacteria after applying different heat treatments and remained active even after autoclaving (Table 10A&B), these results like those were reported for many bacteriocins of different strains of *Lactobacillus acidophilus*, *Lactobacillus plantarum* and many other lactobacilli (Deraz *et al.,* 2011; Fang *et al.,* 2014; Han *et al.,* 2007; Hoda *et al.,* 2013; Marie *et al.,* 2012; Rawal *et al.,* 2013; Sankar *et al.,* 2012).

Table 10. Effect of different temperatures on activity of crude bacterocin produced by (A) *Lactobacillus acidophilus* ATCC 4856 and (B) *Lactobacillus plantarum* ATCC 8014 against MRSA and *Psedomonas sp*.

(A)

Temp.	Exposure time (minutes)	Inhibition zone diameter (mm) For *Lacto. acido. vs.* MRSA				Activity %	Inhibition zone diameter (mm) For *Lacto. acido. vs. Psed. sp.*				Activity %
		R1	R2	M	S.E (±)		R1	R2	M	S.E (±)	
Control		9	9	9	0	100	5	5	5	0	100
65 ºC	20	7.5	8	7.75	0.2	86.1	5	4	4.5	0.4	90
	40	5	6	5.5	0.4	61.1	3	3	3	0	60
	60	4	5	4.5	0.4	50	3	2	2.5	0.4	50
	20	8	7	7.5	0.4	83.3	3	3	3	0	60
	40	6	5	5.5	0.4	61.1	3	3	3	0	60
95 ºC	60	3	2	2.5	0.4	27.8	1	2	1.5	0.4	30
	10	5	5	5	0	55.6	4	3	3.5	0.4	70
	20	5	5	5	0	55.6	3	2	2.5	0.4	50
100 ºC	30	1	2	1.5	0.4	16.7	3	1	2	0.8	40
121 ºC	20	7	6	6.5	0.4	72.2	5	4	4.5	0.4	90

(B)

Temp.	Exposure time (minutes)	Inhibition zone diameter (mm) For *Lacto. plant. vs.* MRSA				Activity %	Inhibition zone diameter (mm) For *Lacto. plant. vs. Psed. sp*				Activity %
		R1	R2	M	S.E (±)		R1	R2	M	S.E (±)	
Control		10	10	10	0	100	5	5	5	0	100
	20	10	9	9.5	0.4	95	5	4	4.5	0.4	90
	40	10	9	9.5	0.4	95	5	4	4.5	0.4	90
65 ºC	60	10	9	9.5	0.4	95	5	4	4.5	0.4	90
	20	10	9	9.5	0.4	95	5	4	4.5	0.4	90
	40	10	9	9.5	0.4	95	4	4	4	0	80
95 ºC	60	10	9	9.5	0.4	95	4	4	4	0	80
	10	10	9	9.5	0.4	95	5	4	4.5	0.4	90
	20	10	9	9.5	0.4	95	5	4	4.5	0.4	90
100 ºC	30	10	9	9.5	0.4	95	4	3	3.5	0.4	70
121 ºC	20	10	5	7.5	0.4	50	5	4	4.5	0.4	90

On studying the effect of different pH values on the activity of the produced bacteriocin, it was found that all the tested samples produce bacteriocins that are active upon a wide range of pH values (from acidic to alkaline), as *Lactobacillus acidophilus* ATCC 4356 was active within pH range 2: 8 giving 72: 111% activity against MRSA and pH 4: 13 giving 60: 110% activity against *Psedomonas sp.* as pH values lower or higher these values resulted in a sharp decrease in the activity and against the two testers, while with *Lactobacillus plantrum* ATCC 8014 the best activity for the bacteriocin was observed within pH range 5: 8 against the gram positive indicator recording 85: 100 % activity , and within pH range 2: 8 against the gram

negative indicator expressing 60: 100% activity as pH values above and below these ranges resulted in a detectable decrease in the activity to be less than 50% (Table 11A& B). Arokiyamary and Sivakumar (2011), Deraz *et al.* (2011), Han *et al.* (2007), and Hoda *et al.* (2013) in addition to many other studies all of them reported that there are some bacteriocin don't lose their activity upon a wide range of pH values although the activity may decrease and/or increase with pH change as reported for the tested samples in this study.

Table 11. Effect of pH values on activity of crude bacterocins produced by (A) *Lacto. acidophilus* ATCC 4356 and (B) *Lactobacillus plantarum* ATCC 8014 against against MRSA and *Psedomonas sp.*as tester bacteria.

(A)

	Inhibition zone diameter (mm) For *Lacto. acido. vs.* MRSA						Inhibition zone diameter (mm) For *Lacto. acido. vs Psed. sp.*				
pH	**R1**	**R2**	**M**	**S.E (±)**	**Activity %**	**pH**	**R1**	**R2**	**M**	**S.E (±)**	**Activity %**
Control	**9**	**9**	**9**	**0**	**100**	**Control**	**5**	**5**	**5**	**0**	**100**
1	1	1	1	0	11.1	1	0	0	0	0	0
2	6	7	6.5	0.4	72.2	2	2	2	2	0	40
3	6	7	6.5	0.4	72.2	3	2	2	2	0	40
4	6	7	6.5	0.4	72.2	4	3	3	3	0	60
5	9	9	9	0	100	5	4	5	4.5	0.4	90
6	10	10	10	0	**111.1**	6	5	6	5.5	0.4	**110**
7	10	10	10	0	**111.1**	7	5	6	5.5	0.4	**110**
8	10	9	9.5	0.4	105.6	8	5	5	5	0.4	100
9	4	4	4	0	44.4	9	4.5	5.5	5	0.4	100
10	4	4	4	0	44.4	10	5	5	5	0.4	100
11	4	4	4	0	44.4	11	4	6	5	0.8	100
12	1	1	1	0	11.1	12	4	3	3.5	0.4	70
13	1	1	1	0	11.1	13	4	3	3.5	0.4	70

(B)

	Inhibition zone diameter (mm) For *Lacto. plant. vs.* MRSA					Inhibition zone diameter (mm) For *Lacto. plant. Vs. Psed. sp.*				
pH	**R1**	**R2**	**M**	**S.E (±)**	**Activity %**	**R1**	**R2**	**M**	**S.E (±)**	**Activity %**
Control	**11**	**9**	**10**	**0.8**	**100**	**5**	**5**	**5**	**0**	**100**
1	1	2	1.5	0.4	15	1	1	1	0	20
2	3	2	2.5	0.4	25	3	2	2.5	0.4	50
3	4	3	3.5	0.4	35	3	3	3	0	60
4	5	4	4.5	0.4	45	3	3.5	3.25	0.2	325
5	10	10	10	0	**100**	5	5	5	0	**100**
6	10	10	10	0	**100**	5	5	5	0	**100**
7	10	10	10	0	**100**	5	5	5	0	**100**
8	9	8	8.5	0.4	85	5	5	5	0	**100**
9	5	4	4.5	0.4	300	3	1	2	0.8	40
10	5	4	4.5	0.4	45	2	1	1.5	0.4	30
11	4	3	3.5	0.4	35	1	1	1	0	20
12	0	0	0	0	0	1	1	1	0	20
13	0	0	0	0	0	1	1	1	0.4	20

Finally, the activity of three different enzymes against the fermentation broths from the two studied strains (*Lactobacillus acidophilus* ATCC 4356 and *Lactobacillus plantarum* ATCC 8014) were determined, catalase was tested to ensure that the resulted activity wasn't due to the hydrogen peroxide; proteinase K and trypsin was tested to report the proteinaceous nature of the produced bacteriocins, results are shown in table (12 A&B).

Table 12. Effect of catalase, protease K and trypsin enzymes on activity of crude bacteriocins produced by (A) *Lactobacillus acidophilus* ATCC 4356 and (B) *Lactobacillus plantarum* ATCC 8014 against MRSA and *Psedomonas sp*.

(A)

	Inhibition zone diameter (mm) For *Lacto. acido. vs.* MRSA					Inhibition zone diameter (mm) For *Lacto. acido. vs. Psedo. sp.*				
Enzyme	R1	R2	M	S.E (±)	Activity %	R1	R2	M	S.E (±)	Activity %
Control	10	10	10	0	100	5	5	5	0	100
Catalase	9	10	9.5	0.4	**95**	5	5	5	0	**100**
Protease K	0	0	0	0	0	0	0	0	0	0
Trypsin	0	0	0	0	0	0	0	0	0	0

(B)

	Inhibition zone diameter (mm) For *Lacto. plant. vs.* MRSA					Inhibition zone diameter (mm) For *Lacto. plant. vs. Psedo. Sp*				
Enzyme	R1	R2	M	S.E (±)	Activity %	R1	R2	M	S.E (±)	Activity %
	10	10	10	0	100	5	5	5	0	100
Catalase	10	10	10	0	**100**	5	5	5	0	**100**
Protease K	0	0	0	0	0	0	0	0	0	0
Trypsin	0	0	0	0	0	0	0	0	0	0

It was recorded that the activity of the two strains didn't change or reduce with a very small ratio when the different fermentation broths were treated with catalase enzyme, which indicate that the observed antibacterial activities were due to bacteriocin production. While a complete loss of the activity was recorded after the treatment of different fermentation broths from the two tested strains with different proteolytic enzymes (proteinas K and Trypsin) as there were no detected inhibition zones in the agar plates seeded with the tester pathogens, and this proves the proteinaceous nature of the tested substance, which is a general characteristic of bateriocins.

REFERENCES:

Aasen IM, Møretrø T, Katla T, Axelsson L, Storrø I. 2000. Influence of complex nutrients, temperature and pH on bacteriocin production by *Lactobacillus sakei* CCUG 42687. Appl. Microbiol. Biotechnol., 53(2): 159–166.

Arokiyamary A, Sivakumar PK. 2011. Characterization of bacteriocin produced by dominant *Lactobacillus spp.* associated with certain traditional dairy products. Int. J. Recent Sci. Res., 2(6): 167-172.

Bian L, Molan A, Maddox I, Shu Q. 2010. Antimicrobial activity of *Lactobacillus reuteri* DPC16 supernatants against selected food borne pathogens. World J. Microbiol. Biotechnol., 27(4): 991-998.

Biswas SR, Ray P, Johnson MC, Ray B. 1991. Influence of growth conditions on the production of a bacteriocin, pediocin AcH, by *Pediococcus acidilactici* H. Appl. Environ. Microbiol., 57(4): 1265–1267.

Brul S, Coote P. 1999. Preservative agents in foods: Mode of action and microbial resistance mechanisms. Int. J. Food Microbiol., 50(1-2): 1-17.

de Vuyst L Vandamme EJ. 1994b. Antimicrobial potential of lactic acid bacteria. In: "Bacteriocins of lactic acid bacteria: microbiology, genetics and applications. (de Vuyst L, Vandamme EJ. ed.)". Blackie Academic & Professional, London, United Kingdom, pp. 91–142.

de Vuyst L, Vandamme EJ. 1994a. Lactic acid bacteria and bacteriocins: their practical importance. In: "Bacteriocins of lactic acid bacteria: microbiology, genetics and applications. (de Vuyst L, Vandamme EJ. ed.)". Blackie Academic & Professional, London, United Kingdom, pp. 1–11

Deegan LH, Cotter PD, Colin H, Ross P. 2006. Bacteriocins: biological tools for bio-preservation and shelf-life extension. Int. Dairy J., 2006: 1058-1071.

Deraz SF, Karlsson EN, Hedström M, Andersson MM, Mattiasson B. 2005. Purification and characterisation of acidocin D20079, a bacteriocin produced by Lactobacillus acidophilus DSM 20079. J. Biotechnol., 117(4): 343-354.

Dunyu X. 2011. Application of Probiotics and green tea extract in Post-harvest processes of *Pacific oysters* (*Crassostrea gigas*) for reducing *Vibrio parahaemolyticus* and extending shelf life. M Sc. Thesis, Food Science and Technology, Oregon State University, US.

Eijsink VGH, Brurberg MB, Middelhoven PH, Nes IF. 1996. Induction of bacteriocin production in Lactobacillus sake by a secreted peptide. J. Bacteriol., 178(8): 2232–2237.

Enan G, el-Essawy AA, Uyttendaele M, Debevere J. 1996. Antibacterial activity of Lactobacillus plantarum UG1 isolated from dry sausage: characterization, production and bactericidal action of plantaricin UG1. Int. J. Food. Microbiol., 30(3): 189–215.

Fang Z, Hongfei Z, Fengling B, Piotr D, Yuen L, Bolin Z. 2014. Purification and characterisation of the bacteriocin produced by *Lactobacillus plantarum*, isolated from Chinese Pickle. Czech J. Food Sci., 32(5): 430–436.

FAO/WHO. 2006. Probiotics in food-health and nutritional properties and guidelines for evaluation. FAO Food and Nutrition Paper. Rome, Italy: Food and Agriculture Organization of the United Nation and World Health Organization.

Goudarzia L, Kermanshahi RK, Z. Mousavinezhad Z. 2014. Antimicrobial activity of bacteriocin produced by *lactobacillus* bacteria against *Proteus* spices. Sci. J. Microbiol., 3(9): 96-103.

Gratia A. 1925. Sur un remarquable exemple *d'antagonisme entre deux souches de colibacille.* Comptes Rendus des Seances de la Societe de Biologie et des ses Filiales, 93: 1040-1042.

Halami PM. 2004. Biotechnological approaches on the fermentative production of bacteriocin as bio-preservative. A thesis submitted to the University of Mysore for the degree of doctor of philosophy in biotechnology.

Han KS, Kim Y, Kim SH, Oh S. 2007. Characterization and Purification of Acidocin 1B, a Bacteriocin Produced by Lactobacillus acidophilus GP1B. J. Microbiol. Biotechnol., 17(5): 774-783.

Helander I, von Wright A, Mattila-Sandholm T. 1997. Potential of lactic acid bacteria and novel antimicrobials against Gram-negative bacteria. Trends Food Sci. Technol., 8(5):146-150.

Hoda M, Abeer M, Abd El-Mongy M, El-Batal AI, Hamza HA. 2013. Study Bacteriocin Production and Optimization Using New Isolates of *Lactobacillus spp.* Isolated from Some Dairy Products under Different Culture Conditions. Food Nutr. Sci., 4(3): 342-356.

Holt JG, Krig NR, Staley JT, Williams ST. 1994. Gram positive Cocci. Bergey'z Manual of Determinative Bacteriolog, 9th Edn., Baltimore, Maryland, USA, pp. 528-540.

Ilavenil S, Park HS, Vijayakumar M, Arasu MV, Kim da H, Ravikumar S, Choi KC. 2015. Probiotic potential of *Lactobacillus* Strains with antifungal activity isolated from animal manure. Sci. World J., 2015: 802570.

Iyapparaj P, Maruthiah T, Ramasubburayan R, Halami S, Kumar C, Immanuel G, Palavesam A. 2013. Optimization of bacteriocin production by Lactobacillus sp. MSU3IR against shrimp bacterial Pathogens. Aquat. Biosyst., 9(1): 12.

Jingping G, Yanyang S, Xing X, Ying W, Wenxiang P. 2016. Purification and Partial Characterization of a Novel Bacteriocin Synthesized by Lactobacillus paracasei HD1-7 Isolated from Chinese Sauerkraut Juice. Sci. Rep., 14(6):19366.

Kyoung SH, Jee YI, Se JO, Woo MJ, Sae HK. 2002. Bacteriocin Produced by Lactobacillus acidophilus ATCC 4356 - Characterization and Purification. Food Sci. Biotechnol., 11(5): 531-536.

Lash BW, Mysliwiec TH, Gourama H. 2005. Detection and partial characterization of a broad-range bacteriocin produced by *lactobacillus plantarum* ATCC 8014. Food microbial., 22(2-3): 199-204.

Laukova A. 1992. The effect of cultue media on bacteriocin production in some bacterial strains. Vet. Med. Praha, 37(12): 661-666.

Main PJ. 2014. Investigating the bacteriocin library of *Lactobacillus plantarum* A-1. Thesis for master degree presented at Massey University, Manuwatu Campus, Palmerston North New Zealand.

Makhloufi KM, Carré-Mlouka A, Peduzzi J, Lombard C, van Reenen CA, Dicks LMT, Rebuffat S. 2013. Characterization of Leucocin B-KM432Bz from Leuconostoc pseudomesenteroides Isolated from Boza, and Comparison of its Efficiency to Pediocin PA-1. PLoS ONE 8(8): e70484.

Marie KP, François ZN, Abbasi A, Anwar F, Ali SA, Victor SD, Félicité TM. 2012. Characterization of a Bacteriocin Produced by *Lactobacillus plantarum* Lp6SH Isolated from" Sha'a", a Maize-Based Traditionally Fermented Beverage from Cameroon. Int. J. Biol., 4(2): 149-158.

Marie KP, François ZN, Florence FA, Victor SD, Félicité TM. 2011. Characterization of bacteriocin produced by *Lactobacillus rhamnosus*1K isolated from traditionally fermented milk in the western highlands region of Cameroon. New York Sci. J., 4(8): 121-128.

Matsusaki H, Endo N, Sonomoto K, Ishizaki A.1996. Lantibiotic nisin Z fermentative production by *Lactococcus lactis* IO-1: relationship between production of the lantibiotic and lactate and cell growth. Appl. Microbiol. Biotechnol., 45(1-2): 36–40.

Moonchai S, Madlhoo W, Jariyachavalit K, Shimizu H, Shioya S, Chauvatcharin S. 2005. Application of a mathematical model and dierential evolution algorithm approach to optimization of bacteriocin production by *Lactococcus lactis* C7. Bioprocess Biosyst. Eng., 28(1): 15–26.

Niku-Paavola ML, Laitila A, Mattila-Sandholm T, Haikara A. 1999. New types of antimicrobial compounds produced by *Lactobacillus plantarum.* J. Appl. Microbiol., 86(1): 29-35.

Nilsen T, Nes IF, Holo H. 1998. An exported inducer peptide regulates bacteriocin production in *Enterococcus faecium* CTC492. J. Bacteriol., 180(7): 1848–1854.

Nishida S, Ono Y, Sekimizu K. 2016. Lactic acid bacteria activating innate immunity improve survival in bacterial infection model of silkworm. Drug Discov. Ther., 10(1): 49-56.

Oelschlaeger T. 2010. Mechanisms of probiotic actions. A review. Int. J. Med. Microbiol., 300(1): 57-62.

Ogunbanwo ST, Sanni AI, Onilude AA. 2003. Influence of cultural conditions on the production of bacteriocin by *Lactobacillus brevis* OG1. Afr. J. Biotech., 2(7): 179–184.

Oh S, Kim SH, Worobo RW. 2000. Characterization and purification of a bacteriocin produced by a potential probiotic culture, *Lactobacillus acidophilus* 30SC. J. Dairy Sci., 83(12): 2747–2752.

Powell JE, Witthuhn RC, Todorov SD, Dicks LMT. 2007. Characterization of bacteriocin ST8KF produced by a kefir isolate *Lactobacillus plantarum* ST8KF. Int. Dairy J., 17(3): 190-198.

Rawal K, Bhavsar N, Raol G, Raol BV, Patel JD. 2013. Bacteriocin: Production and optimization by *Lactobacillus* species. J. Microbiol. Biotech. Res., 3(6): 64-76.

Reeves P. 1979. The concept of bacteriocins. Zentralbl. Bakteriol. Orig. A., 244(1): 78-89.

Sahu MK, Swarnakumar NS, Sivakumar K, Thangaradjou T, Kannan L. 2008. Probiotics in aquaculture: importance and future perspectives. Indian J. Microbiol., 48(3): 299-308.

Sankar NR, Priyanka VD, Reddy PS, Rajanikanth P, Kumar VK, Indira M. 2012. Purification and Characterization of Bacteriocin Produced by *Lactobacillus plantarum* Isolated from Cow Milk. Int. J. Microbiol. Res., 3(2): 133-137.

Schnürer J, Magnusson J. 2005. Antifungal lactic acid bacteria as biopreservatives. Trends Food Sci. Technol., 16(1-3): 70-78.

Sharma N, Kapoor G, Neopaney B. 2006. Characterization of a new bacteriocin produced from a novel isolated strain of *Bacillus lentus* NG121. Antonie Van Leeuwenhoek, 89(3-4): 337-343.

Todorov SD, Dicks LMT. 2005. Effect on Growth medium on bacteriocin production by *Lactobacillus plantarum* ST194BZ, a strain isolated from boza. Food Technol. Biotechnol., 43(2): 165-173.

Todorov SD, Dicks LMT. 2006. Effect of medium components on bacteriocin production by *Lactobacillus plantarum* strains ST23LD and ST341LD, isolated from spoiled olive brine. Microbiol. Res., 161(2): 102—108.

Todorov SD, Gotcheva B, Dousset X, Onno B, Ivanova I. 2000. Influence of growth medium on bacteriocin production in *Lactobacillus plantarum* ST31. Biotechnol. Biotech. Eq., 14(1): 50–55.

Todorov SD, Oliveira RPS, Vaz-Velho M. 2012. Media Optimization of Bacteriocin ST22Ch Production by *Lactobacillus Sakei* ST22Ch Isolated from Salpicao, a Traditional Meat-Product from Portugal. Chem. Eng. T., 27: 283-288.

Todorov SD. 2008. Bacteriocin production by *Lactobcillus plantrum* AMA-K isolated feom ANASI, a zimbabwean fermented milk product and study of the adsorption of bacteriocin AMA-K to *Listeria sp.* Braz. J. Microbiol., 39: 178-187.

Verellen TLJ, Bruggeman G, Van Reenen CA, Vandamme EJ. 1998. Fermentation optimization of plantaricin 423, a bacteriocin produced by *Lactobacillus plantarum* 423. J. Ferment. Bioeng., 86(2): 174–179.

Vignolo GM, de Kairuz MN, de Ruiz Holgado AAP, Oliver G. 1995. Influence of growth conditions on the production of lactocin 705, a bacteriocin produced by Lactobacillus casei CRL 705. J. Appl. Microbiol., 78(1): 5–10.

Vinderola CG, Reinheimer JA. 2000. Enumeration of *Lactobacillus casei* in the presence of *Lactobacillus acidophilus*, bifidobactria and lactic starter bacteria in fermented dairy products. Int. Dairy J., 10(4): 271- 275.

Zacharof MP, Lovitt RW. 2010. Development of an optimised growth strategy for intensive propagation, lactic acid and bacteriocin production of selected strains of *Lactobacilli* genus. IJCEA, 1(1): 55-62.

تقدير النشاط البكتيري والظروف المناسبة لإنتاج البكتريوسين بواسطة كلا من بكتريا البروبيوتيك *لاكتوباسيلاس أسيدوفيلس والبلانتارم*

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بكتيريا حمض اللاكتيك بكتيريا موجبة الجرام، سالبة اختبار الأكسيداز والكاتالاز، لا تكون جراثيم ولديها القدرة بان تنمو في وجود كمية قليلة من الأكسجين (بكتيريا أليفة قلة الأكسيجين). يطلق عليها أسم البروبيوتك حيث أنه يوجد العديد من الأنواع المنتمية لهّذه المجموعة البكتيرية تنتج العديد من المواد ذات التأثير المضاد للبكتيريا كمادة البكتيريوسين. البكتيريوسينات المختلفة تتميز بأن كل منها له نطاق نشاط محدد أما ان يكون نطاق نشاط ضيق المجال بحيث أن المواد المضادة للبكتيريا تؤثر على الأنواع أو الأجناس التي لها صلة بالبكتيريا المنتجة فقط، أو أن يكون نشاط واسع المجال بحيث أن المواد المضادة للبكتيريا لها تأثير يمتد ليشمل الأجناس المختلفة عن جنس النوع المنتج. لقد وجد أن أنتاج البكتيريوسين يعتمد على العوامل البيئية المختلفة وكما يعتمد على ظروف النمو المختلف بالإضافة الى السلالة البكتيرية المنتجة نفسها. الدراسة الحالية تهدف الى تحديد نطاق النشاط المضاد للبكتيريا لسلالتين من البكتيريا: اللاكتوباسيلاس أسيدوفيلاس المعرفة برقم 4356 في المركز الأمريكي لتجميع المزارع وبكتيريا اللاكتوباسيلاس بلانتارم المعرفة برقم 8014 في المركز الأمريكي لتجميع المزارع، وضبط ظروف النمو البيئية المختلفة للحصول على أعلى إنتاجية من البكتيريوسين بالإضافة الى عمل توصيف جزئي للبكتيريوسينات المنتجة بواسطة هاتين السلالتين.