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## Evaluation of the antagonistic potential of lactic acid bacteria isolated from compost against *Escherichia coli*, *Staphylococcus aureus* and *Clostridium difficile*

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Bacteriocins from lactic acid bacteria (LAB) have recently attracted the attention of scientific community for their safe application in food preservation and most importantly for their potential application as therapeutic agents. In this study, LAB isolated from compost were evaluated for their antimicrobial activity against *E. coli*, *S. aureus* and *C. difficile*. Furthermore, the different synthesized antimicrobial agents were characterized. Agar overlay technique and agar well diffusion assay have been applied in order to investigate the antimicrobial activity of LAB against the indicator organisms. Heat and proteolytic treatments were applied for the characterization and for studying the stability of the different antimicrobial compounds secreted by LAB. The strains that efficiently inhibited the indicator organisms have been identified by API 50 CHL system. Molecular characterization of the antimicrobial substances was performed using SDS-PAGE electrophoresis. The findings of this study revealed an antagonistic effect against *S. aureus* and *C. difficile*. The produced antimicrobial substances were heat stable. The antagonistic activity displayed against *S. aureus* was completely destroyed following treatment with proteinase K whereas; the antimicrobial activity exhibited against *C. difficile* was not inhibited by proteinase K. The strains that displayed inhibitory activity against *S. aureus* were identified by API 50 CHL test as *Lactobacillus plantarum* 2 and *Lactobacillus curvatus* with 96.6% and 99.1% similarity rates, respectively. SDS-PAGE analysis of the antimicrobial compounds produced by *Lactobacillus curvatus* and *Lactobacillus plantarum* showed distinct bands with different molecular masses (>30 kDa). Based on the obtained results, these bacteriocins could be considered as a promising tool for future applications in both food industry and medicine.

**Keywords:** Lactic acid bacteria, Compost, Antimicrobial activity, *S. aureus*, *C. difficile*, Bacteriocins.

### INTRODUCTION

The growing prevalence of food-borne diseases represents a major public health and economic issue worldwide (Jahan 2012). Each year, 600 million people get sick from contaminated food and 420 000 deaths are registered annually, involving 125 000 children under the age of five, according to a recent report

by the world health organization (WHO 2015).

Nowadays, consumers become more aware about the quality as well as the nutritional value of their food. Moreover, they are more concerned about processing as well as preservation technologies applied to their food. Thus, in order to satisfy consumer's desire for natural food free of chemicals and additives which have been

extensively questioned regarding their toxic and carcinogenic potential, food industry is persistently looking for alternatives to provide safe food characterized by long shelf life while keeping both nutritional and organoleptic properties (Sanlibaba and Gucer 2015).

On the other hand, huge attention has recently been given towards finding new antimicrobials as alternatives to current antibiotics; due the growing prevalence of drug-resistant pathogens which represent a serious threat to human health (Allen et al. 2014). Each year, 25,000 deaths are recorded in Europe from multidrug-resistant bacterial infections and the annual related costs are estimated at around 1.5 billion euros (Blair et al. 2015).

Lactic acid bacteria (LAB) which are generally known as safe, have recently received much attention due to their antagonist effects against spoilage and foodborne pathogens as well as their inhibitory potential against human and animal pathogens such as: MRSA as well as VRE (Perez et al. 2014). LAB are defined as Gram-positive, non-spore forming and non-motile bacteria. They are identified based on their coccus or rods shape and their negative catalase activity. Lactic acid bacteria which synthesize lactic acid as their final fermentation product, can be classified into homofermentative or heterofermentative, based on their metabolic pathways regarding the fermentation of carbohydrates (Rattanachaiakunsopon and Phumkhachorn 2010). *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus* and *Bifidobacterium* genera represent the most frequently utilized LAB strains in the food industry (Liu et al. 2014). The preservative potential exerted by LAB is primarily due to the synthesis of several antibacterial compounds such as diacetyl, acetaldehyde, hydrogen peroxide, bacteriocins as well as organic acids namely lactic acid (Yang et al. 2012). However, compared to the different antimicrobial agents synthesized by LAB, bacteriocins have recently received immense attention and have been broadly investigated due to their safe application in food preservation as well as their potential application as therapeutic agents (Güllüce et al. 2013).

In addition to the aforementioned, a particular interest has been recently shown with respect to the utilization of probiotics from unconventional sources for human as well as animal health which therefore, may enable identifying new probiotic

strains (Sornplang and Piyadeatsoontorn 2016). The isolation of LAB from non-dairy products mainly plants and soil has received considerable attention due to their diverse metabolic profile and potential application as starter adjuncts or starters in dairy industry as well as their distinctive flavor-forming activities (Alemayehu et al. 2014; Teneva-Angelova and Beshkova 2015). Although, the diversity and abundance of lactic acid bacteria within various unconventional sources was previously demonstrated by several works, only few investigations have been performed on bacteriocinogenic LAB from compost (Chen et al. 2005; Yanagida et al. 2006; Ekundayo 2014).

The main goal of the current study was to evaluate the antimicrobial properties of LAB derived from compost which is well known for its diverse metabolic and microbial profile. Thus, in order to identify novel bacteriocins and bacteriocinogenic strains possessing desired functional characteristics, the antagonistic potential of LAB against *E. coli*, *S. aureus* and *C. difficile*, was assessed in the present study.

## MATERIALS AND METHODS

### Isolation of lactic acid bacteria from compost

This step was performed following the method of Cai et al. (2012); Ekundayo (2014) with slight modifications. 5 g of compost was homogenized with 45 ml PBS using stomacher blender (Stomcher80 Laboratory System, Seward Ltd, UK). Serial 10-fold dilutions were performed. The initial dilution was prepared by adding 1ml of the homogenized compost into 9 ml PBS. After that, 1ml of the initial dilution was transferred and added to the next sterile universal for the second dilution. Similarly, 1ml of each dilution was transferred to the next universals for  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$  dilutions. 100  $\mu$ l of each dilution was plated onto both MRS and BSM agar for further testing against *E. coli* and *S. aureus*. These plates were incubated anaerobically for 48 hours at 37°C. MRS Agar was used in order to encourage the growth of LAB and is selective for lactobacilli however; leuconostocs as well as pediococci may grow in this media. Compared to tomato juice, MRS Agar gives profuse growth of lactobacilli in particular *Lactobacillus fermenti* and *Lactobacillus brevis*, which are considered as slow growing and fastidious strains (Bujalance et al. 2006; Oxoid 2016). BSM agar containing a supplement was utilized for selective isolation of bifidobacteria

(Ferraris et al. 2010).

### Screening for antimicrobial activity

#### Agar overlay technique

The method described by Suwanjinda et al. (2007) was used with slight modifications. Several tubes containing 6 ml of melted TSA agar with 0.6 ml Tris HCL and 100 µl of indicator bacteria were prepared. Melted TSA agar tubes were held in hot water bath at 47°C.

The indicator bacteria *E.coli* MG 1655 and *S.aureus* 6571 NTUCC 91 used in this assay were prepared by inoculating a single colony in 9 ml TSB and incubating aerobically at 37°C for 24 hours. The content of each tube was poured into plates which have been later incubated aerobically at 37°C for 24 hours. Colonies exhibiting zone of inhibition were subcultured onto fresh MRS or BSM agar plates for further investigation.

#### Agar well diffusion assay

In order to confirm the previous results obtained with the agar overlay technique, the agar well diffusion assay was conducted. This assay was applied according to the method previously described by Yang et al. (2012) with minor modifications. 100 µl of overnight culture of each indicator organism including *E.coli*, *S. aureus* and *C.difficile* (NTUCC2) was inoculated by spreading the inoculum over the surface of Iso-sensitest agar or Mueller Hinton Agar. Several wells of 5mm diameter were performed using a sterile tip and 40 µl of the cell free supernatant (CFS) was added to each well. As for the preparation of cell free supernatant, each isolate which previously showed clear zone of inhibition using the agar overlay method was cultured overnight in 30 ml MRS or BSM broth. Centrifugation at 4700 rpm for 10 min was conducted in order to remove cells. The supernatant was then checked for pH and adjusted at 7.0 with 1mol/L NAOH. The prepared supernatants were filtered using 0.2 µm sterile syringe filters (Minisart; Sartorius Ltd., Epsom, UK). Plates tested against *E.coli* and *S. aureus* were incubated aerobically at 37°C for 24 hours whereas, plates evaluated against *C.difficile* were incubated anaerobically at 37°C for 48h.

#### Characterization and study of the stability of the antimicrobial agents

#### Effect of heat treatment

In order to test the stability of the different antimicrobial agents, the supernatants were heated at 100°C for 10 min in a heat block, following the approach used by Sifour et al. (2012); Nordin and Abu Bakar (2013) with only minor changes. These supernatants were then tested against the indicator organism by the agar well diffusion assay.

#### Treatment with proteinase K

The antimicrobial activity of the supernatants treated with proteinase K was tested using well diffusion assay following the method applied by Rawal et al. (2013) with some modifications. 100 µl of the supernatants and 1 µl of proteinase K (20 mg/ml) were added to Eppendorf tubes and then incubated for 30 min at 37°C in water bath. 100 µl MRS broth added to 1 µl proteinase K were used as a control after being incubated for 30 min at 37°C in water bath.

#### Bacterial identification using API 50 CHL

The colonies that efficiently inhibited the indicator organism were further determined using API 50 CHL system (bioMérieux®). This system is applied for the identification of the genus *Lactobacillus* as well as the related genera.

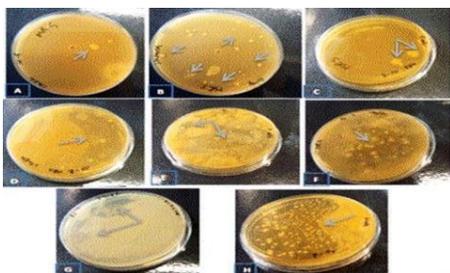
#### Molecular characterization of the antimicrobial compounds using sds-page electrophoresis

The antimicrobial agents secreted by both *Lactobacillus plantarum* 2 and *Lactobacillus curvatus* were characterized using SDS-PAGE Electrophoresis. Both supernatants that previously showed antagonistic effect against *S.aureus* have been concentrated using centrifugal filtration. 3 kDa, 10 kDa and 30 kDa centrifugal filters (Millipore, Carrigtwohill, Co. Cork, Ireland) were used. The different concentrated samples obtained as well as the filtrates were tested for their antimicrobial activity against *S.aureus* using agar well diffusion assay. The concentrated samples that exhibited antimicrobial effect against the indicator organism were analyzed by SDS-PAGE electrophoresis following the method previously described by Laemmli (1970). The polyacrylamide gels used consisted of separating gel 12% and stacking gel 5% . 1X SDS-PAGE Running buffer was utilized. 10X SDS-PAGE Running Buffer which consisted of 250 mM Tris, 1.92 M Glycine, and 1% SDS; pH 8.3 was diluted to a 1X solution using distilled water.

To have detectable amounts of proteins for Sodium dodecyl sulfate polyacrylamide gel electrophoresis, the samples were precipitated. 1/10 volume of 100% Trichloroacetic acid was added to the samples which were later kept at 4°C for 2 h and centrifuged at 13000 rpm for 30 min at 4°C. The supernatants were discarded and 30 µL of 1X SDS page sample buffer (50 mM Tris-HCl (pH 6.8), 10% glycerol, 2% SDS, 0.1% bromophenol blue and 100 mM dithiothreitol) as well as 1 µL of saturated Tris were added to the pellets, respectively. After that, the samples were boiled at 100°C for 5 min. Finally, these proteins were separated by SDS-PAGE electrophoresis. A volume of 20 µL of each sample was loaded on gel and 5 µL of Precision Plus Protein Dual Xtra Standards (Bio-Rad) was utilized as a molecular weight standard. SDS-PAGE gels were run at 15 mA for 10 min and then, at 30 mA for 2 h. After that, the gel was stained with Coomassie Brilliant Blue R-250 staining solution (Bio-Rad) for overnight and then destained with a solution containing 10% acetic acid and 30% methanol for 1 h in order to remove excess of stain for better visualization of the bands. The destained gels were then visualized using ChemiDoc™ MP imaging system (Bio-Rad). Molecular weight estimation of the unknown proteins was conducted by plotting the Log molecular weight versus R<sub>f</sub>, based on the bands formed in the gel.

## RESULTS

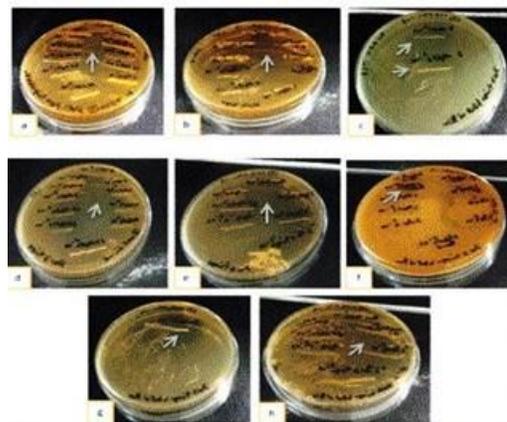
All colonies isolated from compost were tested for their antagonistic effect against *E.coli* and *S.aureus* using the agar overlay assay. Overall, several colonies showed clear zone of inhibition against the indicator organism. Different colonies exhibiting large and small zones of inhibition against the indicator organisms were noticed on both MRS and BSM agar plates using the agar overlay method (Figure 1).



**Figure 1: Agar overlay assay showing colonies exhibiting large and small zones of inhibition against *E.coli* and *S.aureus*.**

A, B, D, F : Colonies isolated onto MRS agar plates from  $10^{-5}$ ,  $10^{-4}$ ,  $10^{-2}$ ,  $10^{-3}$  dilutions, respectively, tested against *S.aureus*. G: Colonies isolated onto BSM agar plate from  $10^{-2}$  dilution tested against *S.aureus*. C, E, H: Colonies isolated onto MRS agar plates from  $10^{-5}$ ,  $10^{-3}$ ,  $10^{-2}$  dilutions, respectively, tested against *E.coli*.

Positive results were noticed in eight plates including seven MRS agar plates and one BSM agar plate. Five plates containing colonies from  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  dilutions showed antimicrobial zones of inhibition against *S.aureus* whereas; three plates having colonies respectively, from  $10^{-2}$ ,  $10^{-3}$  and  $10^{-5}$  dilutions showed antimicrobial effects against *E.coli*. The colonies which exhibited clear zones against both *S. aureus* and *E.coli* have been tested again by the agar overlay assay by transferring these colonies into fresh MRS agar plates using single streak technique to confirm their antagonistic effect against the indicator organisms (Figure 2).

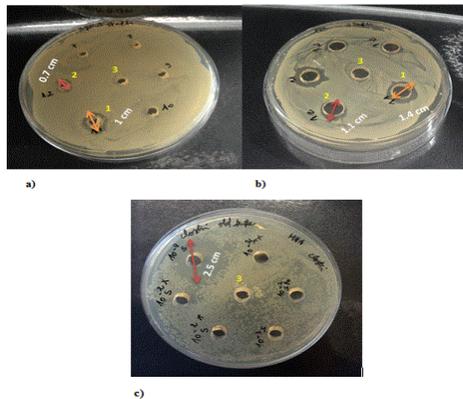


**Figure 2: Agar overlay assay using single streak technique showing colonies exhibiting clear zones of inhibition against *S.aureus* and *E.coli*.**

Plates a, b, h: isolates tested against *S.aureus*. Plates c, d, e, f, g: isolates tested against *E.coli*.

Overall, eight plates involving colonies from  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  dilutions displayed clear antimicrobial zones against the indicator organisms including three plates showing positive results against *S.aureus* and five had antimicrobial activity against *E.coli*. In order to confirm these results, the well diffusion assay was then performed against *E.coli* and *S.aureus* as well as *C.difficile* as a third indicator organism.

The well diffusion assay revealed that only two colonies derived from  $10^{-4}$  and  $10^{-5}$  dilutions had antimicrobial effects against *S.aureus* with zones of inhibition of 1cm and 0.7 cm, respectively (Figure 3).

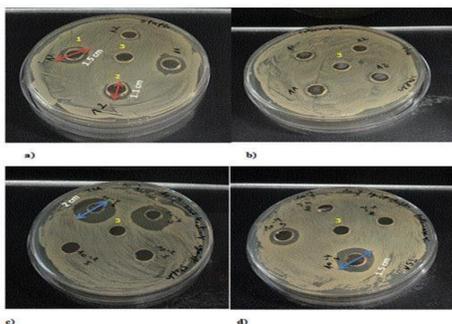


**Figure 3: well diffusion assay.**

This assay performed against *S.aureus* (a,b) and *C.difficile* (c) showed clear zones of inhibition. Two strains of LAB exhibited higher (1) and lower (2) antimicrobial activity against *S.aureus*. The results were compared to a negative control (3).

However, no antimicrobial activity was noticed against *E.coli*. These positive results were confirmed by repeating the agar well diffusion test using new prepared supernatants. Zones of inhibition of 1.4 cm and 1.1 cm diameter were noticed with the first and the second supernatant, respectively (Figure 3). Interestingly, LAB strains from compost exhibited a considerable antimicrobial activity against *C.difficile*. However, these strains have been contaminated and therefore, could not be further identified (Figure 3).

The stability of the antimicrobial activity displayed against *S.aureus* was evaluated by heat treatment at 100 °C. The results showed that the produced antimicrobial substances were heat stable (Figure 4).

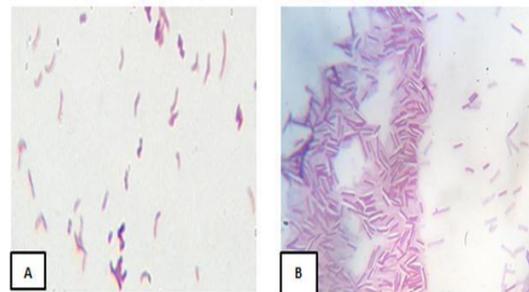


**Figure 4: Characterization of the different antimicrobial agents.**

Characterization of the substances that exhibited higher (1) and lower (2) antagonistic effect against *S.aureus* by heat treatment (a) and proteinase K treatment (b) and against *C. difficile* by heat (c) and proteinase K (d) treatments. The results were compared to a negative control (3).

Zones of inhibition of 1.5 and 1.1 cm diameter were noticed. As for treatment with proteolytic enzymes, no inhibitory activity was observed against the indicator organism when the cell free supernatants were exposed to the action of proteinase K. These results confirm the proteinaceous nature of the synthesized antimicrobial agents (Figure 4). Additionally, the characterization of the antimicrobial agents that efficiently inhibited *C.difficile* revealed that these compounds were heat stable and were not inhibited by proteinase K (Figure 4). Unfortunately, these substances could not be further characterized due to contamination affecting the producing strains.

The morphological characterization of the isolates that previously exhibited antimicrobial activity against *S.aureus* revealed gram positive, non-spore forming, rod-shaped bacteria (Figure 5).



**Figure 5: Microscopic observation of the Gram stained isolates showing gram positive, non-spore-forming, rod-shaped bacteria (magnification x 1000).**

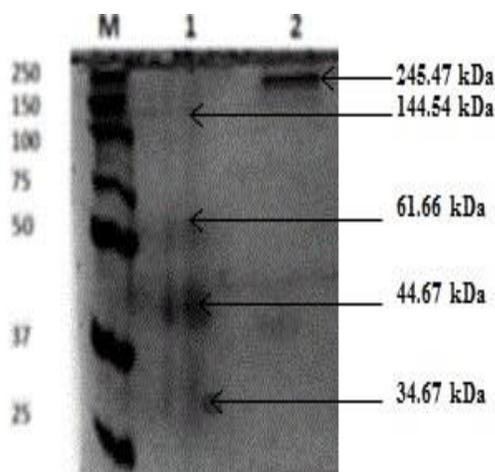
The examined isolates are those that exhibited higher (A) and lower (B) antimicrobial activity against *S.aureus*.

After a 48-hour incubation period, the strains that previously showed antagonistic activity against *S.aureus* were determined as *Lactobacillus plantarum* 2 and *Lactobacillus curvatus* by API 50 CHL test with 96.6% and 99.1% similarity rates, respectively. A positive test is indicated by colour change from purple to yellow and to black regarding the esculin test (Table 1).

**Table 1: Identification of the isolates using API 50 CHL Kit and API web**

API kit (50 CHL)	Strain 1 <i>Lactobacillus curvatus</i> (99.1%)	Strain 2 <i>Lactobacillus plantarum</i> 2 (96.6%)
Control	-	-
Glycerol	-	+
Erythritol	-	-
D-arabinose	-	-
L-arabinose	-	-
D-ribose	+	+
D-xylose	-	-
L-xylose	-	-
D-adonitol	-	-
Methyl-βD-xylopyranoside	-	-
D-galactose	+	-
D-glucose	+	+
D-fructose	+	+
D-mannose	+	+
L-sorbose	-	-
L-rhamnose	-	-
Dulcitol	-	-
Inositol	-	-
D-mannitol	+	+
D-sorbitol	-	+
Methyl-αD-mannopyranoside	-	-
Methyl-αD-glucopyranoside	-	-
N-acetylglucosamine	+/-	+
Amygdalin	-	-
Arbutin	-	-
Esculin	+	+
Salicin	-	-
D-cellobiose	-	-
D-maltose	+	+
D-lactose	+	-
D-melibiose	-	-
D-saccharose	+	+
D-trehalose	+	-
Inulin	-	-
D-melezitose	-	-
D-raffinose	-	-
Starch	-	-
Glycogen	-	-
xylitol	-	-
gentiobiose	-	-

D-turanose	+	-
D-lyxose	-	-
D-tagatose	-	-
D-fucose	-	-
L-fucose	-	-
D-arabitol	-	-
L-arabitol	-	-
gluconate	-	-
2- ketogluconate	-	-
5-ketogluconate	-	-



**Figure 6: SDS- PAGE gel visualized by ChemiDoc™ MP imaging system (Bio-Rad).**  
 Lane M: molecular standard. Lane 1: Indicates the antimicrobial substances produced by *L.curvatus*.  
 Lane 2: shows the antimicrobial substances produced by *L.plantarum*.

SDS-PAGE analysis of the antimicrobial substances produced by *Lactobacillus curvatus* and *Lactobacillus plantarum* revealed distinct bands of different molecular weights (Figure 6). SDS-PAGE analysis of sample 1 from *L.curvatus* showed four proteins with molecular weights ranging from 34.67 to 144.54 kDa whereas sample 2 derived from *Lactobacillus plantarum* 2 showed a single protein band with molecular weight of 245.47 kDa.

## DISCUSSION

In the present study, lactic acid bacteria were isolated from compost, an interesting source recognized for its diverse microbial and metabolic profile. Bacteriocinogenic LAB from compost were evaluated for their antimicrobial effects against *E. coli*, *S.aureus* and *C. difficile*. These pathogens cause a spectrum of disease that range from mild diarrhea to life-threatening complications (Khan et al. 2015). Although many factors contribute to the severity of these infections, antimicrobial therapy is one of the most important causes and gained a huge amount of interest over the past decade. The emergence of strains that are resistant to different antimicrobial agents poses a serious public health problem and therefore, alternative antimicrobial approaches should be adopted (Spellberg and Gilbert 2014). Bacteriocins derived from LAB have recently attracted the attention of scientific community for their safe application in food preservation and most importantly for their potential application as therapeutic agents (Zacharof and Lovitt 2012; Dicks et al. 2011). Although several types of bacteriocins have been previously identified and characterized, Nisin is the only bacteriocin that is commercially available as a bio-preservative (Gautam and Sharma 2009). On the other hand, similar to many other compounds, bacteriocins should go through rigorous investigation and validation process before being approved for future

application as therapeutic agents (Hammami et al. 2013).

Recently, there has been growing interest regarding the use of probiotics from alternative sources defined as unconventional sources for both human and animal health which could enable the identification of new probiotic strains (Sornplang and Piyadeatsoontorn 2016).

The findings of this study showed that LAB strains exhibited antagonistic effects against both *S.aureus* and *E.coli*, using the agar overlay method. However, an antimicrobial activity has been noticed only against *S.aureus* by using agar well diffusion assay. LAB strains also displayed higher antimicrobial effect against *C.difficile* by agar well diffusion assay however; further identification could not be achieved due to a contamination that affected the producing strains.

The antimicrobial spectrum of LAB against both Gram-negative and Gram-positive bacteria has been widely investigated using different methods. Interestingly, Djadouni and Kihal (2012) investigated the antagonistic effect of LAB isolated from meat, dairy products as well as agro-industrial wastes, against ten indicator strains including *Escherichia coli*, *Clostridium sp.*, *Salmonella typhimurium* and *para-typhimurium* B, *Pseudomonas sp.*, *Bacillus megaterium*, *Listeria ivanovii*, *Staphylococcus aureus* and *Streptococcus sp.* Using the agar spot method, this study showed that strain LBbb0141 possessed broad spectrum activity against all indicator strains. Although a large number of studies has been previously performed regarding the characterization of bacteriocins produced by LAB derived from dairy and meat products, only few works have been conducted on bacteriocinogenic LAB from unconventional sources. A study carried out by Yanagida et al. (2006) showed for the first time a bacteriocin being synthesized by *Lactobacillus animalis* and therefore, suggesting that soil represents an interesting source for potential identification of novel bacteriocinogenic LAB strains.

The abundance and diversity of LAB in soil were also confirmed by Chen et al. (2005) which indicated a high amount of lactic acid bacteria in soil. In this study, thirty-two strains were determined as lactic acid bacteria including four *L.plantarum*, ten *Lactococcus lactis*, two *Lactobacillus paracasei*,

one *Weissella paramesenteroides*, one *Leuconostoc fallax*, three *Leuconostoc mesenteroides*, ten *Enterococcus spp* as well as one *Lactobacillus brevis*. Furthermore, Ekundayo (2014) isolated lactic acid bacteria from rhizosphere soils of different fruit trees. Overall, a total number of seventeen LAB isolates has been identified. In the present study, the highest antimicrobial effect against *S.aureus* was noticed with *Lactobacillus curvatus*. This was also found by Papamanoli et al. (2003) which demonstrated that a percentage of 50 and 29% of *L. curvatus* and *L. plantarum* strains isolated from fermented dry sausages efficiently inhibited two *S. aureus* strains. *Lactobacillus curvatus* is considered as one of the most frequent lactic acid bacteria associated with meat products.

It is utilized in starter and biopreservative cultures for sausage fermentation, processed and fresh meat and fish. Phylogenetically, *Lactobacillus curvatus* is closely related to different species which are also associated with meat environments namely *Lactobacillus sakei*, *Lactobacillus fuchuensis* as well as *Lactobacillus graminis*, species (Hebert et al. 2012). According to Henning et al. (2015), *Lactobacillus curvatus* FS47, Beef3 from ground beef and FS44-B from ground pork could not inhibit *Staphylococcus aureus* ATCC 12600, ISP 178 and *Enterococcus faecalis* ATCC 19433; however, *Lactobacillus curvatus* strains showed higher antimicrobial activity against *Listeria monocytogenes*.

Although numerous *L. curvatus* bacteriocins from sausages, retail meats, Kimchi as well as goat cheese have been previously described, bacteriocins from alternative sources have been poorly studied (Ahmadova et al. 2013). Thus, the present study greatly contributed to the evaluation of the antagonistic potential of LAB from compost against various indicator organisms in order to identify new bacteriocins with broad inhibitory spectrum.

With respect to the stability of bacteriocins, the findings of this study revealed that the antimicrobial agents produced by *L.curvatus* and *L.plantarum* 2 were found to be heat stable after heat treatment at 100°C for 10 min. Additionally, these antimicrobial substances have lost their activity by proteinase K treatment which confirms the proteinaceous nature of these substances.

*L.curvatus* strain Y108 derived from a traditional Japanese pickle was previously characterized by Kawahara et al. (2010). The results of this study

showed that the antimicrobial activity remained stable after incubation for 30 min at 60°C however; the antimicrobial activity of the supernatant decreased by 52% after incubation at 100°C for 1h. The antimicrobial activity was completely destroyed after treatment with several proteolytic enzymes as well as lipase however;  $\alpha$ - amylase partially inhibited the antibacterial activity.

No effect was noticed regarding treatment with catalase. Additionally, bacteriocin from *Lactobacillus plantarum* Lp6SH derived from Sha'a" which is a traditional fermented beverage was characterized by Marie et al.(2012).

In this study, the thermostability of this bacteriocin was evaluated by heating the bacteriocin at 100°C for 120 min. The results indicated that the bacteriocin produced by *L.plantarum* Lp6SH was heat stable. However, this bacteriocin lost its antimicrobial activity after treatment with several proteolytic enzymes.

Referring to the literature, bacteriocins have been divided into different classes based on the producer organism, their chemical structure, physical properties, mode of action as well as their molecular sizes (Güllüce et al. 2013). However, bacteriocin classification is constantly changing due to the increased number of new bacteriocins with unique properties being discovered (Perez et al. 2014).

SDS-PAGE analysis of the antimicrobial compounds secreted by *Lactobacillus curvatus* and *Lactobacillus plantarum* showed distinct bands with different molecular masses (>30 kDa). However; Tomé et al. (2009) showed that bacteriocins ET06, ET31 as well as ET30 produced by *Lb. curvatus* strains from Cold-Smoked Salmon had molecular weights of 2.8 kDa, 4.5 kDa and 3.1 kDa respectively. Moreover, bacteriocin BM-1 synthesized by *Lactobacillus plantarum* BM-1 derived from traditionally fermented chinese meat was characterized by Zhang et al. (2013).

The molecular weight of this bacteriocin was estimated to be 4.0 kDa by SDS- PAGE electrophoresis. Encouragingly and in comparison with other works, the findings of the current research may suggest a possible new class of bacteriocins produced by *Lactobacillus plantarum* and *Lactobacillus curvatus*. However, more investigations are required to confirm the obtained results.

## CONCLUSION

In summary, the results of the present work may suggest a new group of bacteriocins since those produced by both *L.curvatus* and *L.plantarum* had large molecular weights and were heat stable. However, more investigations are needed to confirm these findings. Furthermore, characterization at the molecular and genetic levels of these bacteriocins should be addressed.

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

## ACKNOWLEDGEMENT

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## AUTHOR CONTRIBUTIONS

NEH CHALAL designed the study, performed the lab work, analysed data, and wrote the manuscript. The author read and approved the final version of the manuscript.

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