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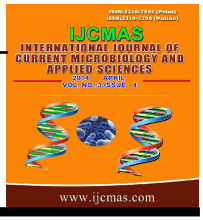

Original Research Article

Purification and characterization of a novel class IIA bacteriocin produced by *Lactobacillus curvatus* DN86 isolated from Saudi chicken CECA

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Keywords

Lactic acid bacteria, *Lactobacillus curvatus*, Saudi chicken cecum microbiota, bacterial antagonism, Class II a bacteriocins

A B S T R A C T

The composition and dynamics of the intestinal microbiota contribute positively to host health, growth, and maturation by acting as a barrier to colonization by pathogens. Understanding the microbial ecology of chicken gut is an important issue in the development of exclusive cultures or probiotics. The microbiota of thirtyceca of Saudi chickens was identified. Following their preliminary confirmation as lactic acid bacteria, 240 strains were identified by combining morphological criteria, biochemical tests, and molecular methods including intergenic 16S-23SrDNA-PCR and 16S rDNA. Most of the lactic acid bacteria isolated belonged to the genus *Lactobacillus* among them *Lactobacillus curvatus*(38.1%), *Lactobacillu ssakei*(20.3%), *Lactobacillus salivarius*(9.4%), *Lactobacillus reuteri*(3.1%), and other lactic acid bacteria strains including *Enterococcus faecalis*(21.9%), *Leuconostoc mesenteroides*(4.5%) and *Streptococcus* sp. (2.7%).One isolate DN86 was active against many food-borne pathogenic and food spoilage bacteria such as *Listeria*, *Staphylococcus*, *Bacillus*, S*erratia* sp.and *Enterococcus.* Along side the determination of Saudi chicken ceca microbiota, this study focused on the characterisation of the bacteriocin DN86 produced by the isolate DN86.It was purified to homogeneity by ammonium sulphate precipitation, sep-pack column and reverse-phase liquid chromatography. Mass spectrometry analyseby MALDI-To Frevealed a molecular mass of 5162.17 Da. Taken together, these results describe a possible new class IIabacteriocin produced by *Lactobacillus curvatis*.

Introduction

Lactic acid bacteria constitute important members of the microbial population in chicken intestine, crop, and feces and play an important role in maintaining the ecological equilibrium between the different species of microorganisms inhabiting these environments. The lactic acid bacteria found in poultry feces were

Streptococcus and *Lactobacillus* species (Nazaf et al. 2008; Souza et al. 2007). Several lactic acid bacteria strains were isolated from the digestive tracts of healthy broilers (Karimi-Torshizi et al. 2010), among which *Lactobacillus fermentum* TMU121, *Lactobacillus rhamnosus* TMU094, and *Pediococcu spentosaceous*TMU457 were considered as potential probiotics due to their antagonistic effects against *Escherichia coli* and *Salmonella* species.Lactic acid bacteria play an important role in the food industry, because they significantly contribute to the flavour and texture (McKay and Baldwin, 1990). They considerably contributed to the nutritional value of the food products (Topisirovic et al. 2006). There exists an increasing interest in meat products containing specific bacterial species with potential health-improving properties (Ammor et al. 2004; Tahiri et al. 2004; Yamazaki et al. 2005; Svetoch et al. 2008). Lactic acid bacteria also produces a number of antimicrobial substances that might be of importance for food and feed fermentation and preservation for which they are of economic significance. Lactobacilli strains are commercially available as probiotics for use in poultry and some have recently been used to reduce *Salmonella enterica* serovar in chicks and turkey poults (Menconi et al. 2011).

Lactic acid bacteria can reduce *Salmonella* species in assays conducted in broiler chicks (Hugas et al. 1993, Surachon et al. 2011). Probiotics are administered orally to poultry to help the birds fight illness and disease (Patterson and Burkholder 2003). One of the desired features of probiotic strains is the production of broad-spectrum bacteriocins (Connerton et al. 2011), competitive exclusion (Rantala and Nurmi 1973), vaccines (Ruiz-Moyano et al. 2011), and bacteriocin treatment (Stern et al. 2005; Stern et al. 2006, Svetoch et al. 2011).

Besides metabolic end products, some lactic acid bacteria strains also secrete antimicrobial proteinaceous compounds, termed bacteriocins, which kill closely related bacteria. Bacteriocins, which are ribosomally synthetized, are produced by various lactic acid bacteria (Tagg et al. 1976; Atrih et al. 2001). Bacteriocins have the ability to inhibit closely related and sometimes more distantly related strains of bacteria and thusplay a major role in the natural defence systems of several bacterial species (Jack et al. 1995). A general classification of bacteriocins has been suggested based on the presence or not of posttranslational modifications (Amortegui et al. 2014; Kemperman et al. 2003).Class I bacteriocins or lantibiotics contain lanthionine or ß-methyl-lanthionine, while unmodified antimicrobial peptides are grouped in class II bacteriocins (Cotter et al. 2013). This class II bacteriocins could be further subdivided in five groups: antilisterial one-peptide bacteriocins with a YGNGV motif (class IIa); two-peptide bacteriocins (class IIb); circular bacteriocins (class IIc); unmodified, linear, non-pediocin-like, single-peptide bacteriocins (class IId); andthe microcin E492-like bacteriocins (class IIe) (Cotter et al. 2013). A third class corresponding to nonbacteriocin lytic proteins or bacteriolysins has been proposed by Cotter et al. (2005). Since lactic acid bacteria are generally regarded as safe, they or their bacteriocins could be safely used in food production and food preservation. Bacteriocins produced by lactic acid bacteria were intensively investigated. Some lactic acid bacteria bacteriocins can inhibit the growth of Gram-positive pathogenic and spoilage bacteria as well as yeasts (Amortegui et al.

2014,Hu et al. 2013; Lakshminarayanan et al. 2013; Martinez et al. 2013;Atanassova et al., 2003; Ennaharet al., 2000;Klaenhammer, 1988). Besides, it has been reported that bacteriocins also inhibit the growth of some Gram-negative species (Messaoudi et al. 2011; Stern et al. 2005;Stevens et al. 1991).Recently, Strain GO5, a bacteriocin-producing bacterium, was isolated from green onion kimchi and identified as *Micrococcus* sp. (Kim et al. 2005). However, the main attention has been paid so far to the meat product of the lactic acid bacteria strains that are routinely used in industrial processes. Therefore, the study of the lactic acid bacteria isolated from the Saudipoultry ceca would be very interesting.

The aim of this work was to characterize the bacteriocin produced by *Lactobacillus curvatus*DN86, a lactic acid bacteria isolated from Saudi poultry ceca, in terms of antimicrobial spectrum of activity and molecular weight.The influence of heat and denaturing agents (pH, enzymes) on its activity will also be described.

Materials and Methods

Animal sampling and isolation of Cecalactic acid bacteria

The Saudi chickens (*Almaraay, Alyoum, Attazej, Alwatania*) used in our study were fed a commercial corn-soy diet (70% corn, 20% soy) containing vitamin-mineral and anti-oxidant supplementation devoid of animal protein and growth-promoting antibiotics. Chickens were reared under controlled management conditions (diet, room temperature, cleaning). Thirty healthy 8-week-old Saudi chickens were killed by cervical dislocation. The isolation of strains were done as described by Messaoudi et al. (2011).

Strains, culture medium and chemicals

The medium and temperature growth of all indicator strains used in this studyare listed in the Table 1. Acetonitrile, HPLC *grade*, was provided by *Chromanorm*. Others chemicals were provided by *Sigma Aldrish*(USA).

DNA extraction and molecular identification using 16S–23SPCR and 16SrDNA sequencing

Total DNA was extracted using the Qiagen DNeasy Tissue Kit. Molecular identification was initially carried out by16S-23S PCR amplification using the primers 16S-p2 (5'-CTTGTACACACCG CCCGTC-3') and 23S-p7 (5'-GGTACTTA GATGTTTCAGTTC-3') as previously described (Kabadjova et al. 2002). Isolates exhibiting two 16S-23S intergenic spacer region (ISR) fragments, expected to be *Lactobacillus* strains, were subjected to a second round of PCR using the previously designed lactobacilli- specific primers LbLMA1 (5'-CTCAAAACTAAACAAA GTTTC-3') and R16-1 5'-CTTGTACA CACCGCCCGTCA-3') (Dubernet et al. 2002). Since 16S-23S-PCR amplification allows bacterial identification only atthe genus level. The complete 16S rRNA gene was amplified using the set of primers fD1 (5’ AGAGTTTGATCCTGGCTC 3’) and rD1 (5’ TAAGGAGGTGATCCAGGC 3’) (Weisburg et al., 1991). A clone library of the 16S rDNA amplified with primers fD1 and rD1 of all strains was constructed in *Escherichia coli* JM 109 by using *p*Drive Cloning Kit (Qiagen) and the insert of positive clones was sequenced. The primers T7-pro and SP6 flanking the multiple cloning site of pDrive DNA were used to sequence both DNA strands. The resulting sequences were assembled into a unique configuration with BioEdit sequence alignment software and then

submitted to the NCBI database. The computer program CLUSTAL (Thompson et al. 1994)was used for sequence alignment and the Basic Local Alignment Search Tool 2 program (BLAST) for sequence representation and similarity searches in the GenBank database.

Preliminary characterization of the bacteriocin

For thermosensitivity assays, culture supernatants were treated at different temperatures for several durations. The samples were assayed foractivity after heating at 60, 110 and 121°C for 15, 30 or 60min. Effect of pH on bacteriocin activity was determined by adjusting the pH of the supernatant with diluted HCl or NaOH. Samples were incubated for 2 h at 30°C and the pH was adjusted to 6.5. For protease susceptibility assays, culture supernatants were incubated for 2h with hydrolases (proteinase K, pronase, proteases, pepsin, chymotrypsin, trypsin, papain, catalase, lysozyme and lipase) at a final concentration of 1mg ml^{-1}(Mitéva et al. 1998). Antimicrobial activity of the cell-free extract and the fractions was evaluated by the agar well diffusion method (Compos et al. 2006) using the indicator strains listed in Table 1.

Bacteriocin purification procedure

*Lactobacillus curvatus*DN86strain was grown in 1litter of tryptone glucose agar (TGA) at 37°C to stationary phase. The cells were removed by centrifugation at 10 000 g for 15 min, and the cell-free supernatant fluid was used as the starting material for bacteriocin purification. Ammonium sulphate concentration was adjusted to 80 % saturation, stirred overnight and centrifuged at 14000 g for 45 min. The precipitate was collected and dissolved in 100 ml of phosphate buffer 10 mM, pH 6.8 and was desalted by dialysis (dialysis membrane *Spectra/Por, USA*. MW cut-off: 1000) against 5 L of phosphate buffer 10 mM, pH 6.8 for 18 h. The final volume of dialysate was applied on *sep-pack* column (*Waters*), eluted at 40 % acetonitrile, lyophilized and suspended in 400 µl phosphate buffer 10 mM pH 6.8. The active fraction was then purified by HPLC (*applied Biosystem* with a 5 µm column C18). The column was maintained at 30°*C* with a column heater. After equilibration of the column with water/ trifluoroacetic acid 0.1% (vol/vol), with a flow rate of 0.8 ml min^{-1}. Peptides were eluted by increasing the concentration gradient of this solvent (0.1 % trifluoroacctic acid in acetonitrile) as follows: 0-5 min: 0 % (vol/vol) acetonitrile; 5-40 min: 0 to 60 % (vol/vol) acetonitrile. Peptides were monitor red spectro-photometrically at 220 and 280 nm. Fractions corresponding to all peaks were collected independently, lyophilized, and assayed for bacteriocin activity.

Mass spectrometry analysis

The lyophilized fractions were analysed with a mass spectrometer model *Voyager-DERP (Perkin-Elmer, USA).* We used the MALDI-ToF system (Matrix-Assisted Laser Desorption Ionisation time-of-flight). The matrix used was the cyano-hydroxycinnamic acid (CHCA).

Results and Discussion

Identification of lactic acid bacteriaisolates

The preliminary identification by using morphological criteria and biochemical tests showed that the 240 colonies obtained on MRS medium are gram-positive, facultative-anaerobe and catalase

negative. They were considered lactic acid bacteria isolates and thus subjected to a preliminary molecular identification using the 16S-23S PCR approach. More information was obtained by taking advantage of the robust sequencing of 16S rDNA genes. Sequencing of the16S rDNA genes of the lactic acid bacteria isolates indicated the presence of *Lactobacillus curvatus* (38.1%), *Lactobacillus sakei* (20.3%), *Lactobacillus salivarius* (9.4%), *Lactobacillus reuteri*(3.1%), and other lactic acid bacteria strains included of *Enterococcus faecalis*(21.9%), *Leuconostoc mesenteroides* (4.5%) and *Streptococcus* sp. (2.7%). These sequences showed high homology scores (98 and 100%) to sequences available in GenBank.

Screening for bacteriocinogenic lactic acid bacteria

About 240lactic acid bacteria strains, isolated from Saudi poultry cecawere examined for their bacteriocin-like activity by the agar well diffusion test. Subsequently, the isolateDN86 which showed the strongest bacteriocin activity against *Listeria*, *Staphylococcus*, *Bacillus*, S*erratia* sp. and *Enterococcus* was retained for further experiments(Table 1).

Sensitivity to hydrolases, pH and heat treatments of *Lactobacillus curvatus* DN86 culture supernatant

Inhibitory activity levels of culture supernatants were not modified after a treatment for 15, 30 or 60 min at 60°C (Table 2). However, the activity was reduced at higher temperatures, since supernatants retained only 54 %, 23 % and 17.2 % of the activity after a treatment respectively for 15, 30 or 60 min at 110°C. Only 8 % of activity was conserved after a treatment of 15 min at 121°C. Inhibitory activity was fully observed for supernatants adjusted to pH values from 5.0 to 8.0. On both sides, activity was dramatically reduced. Inhibitory activity was totally lost by proteinase K, pronase, pepsin, protease, trypsin, alpha-chymotrypsine and papaine treatments, whereas lipase, catalase and lysozyme had no effect on this activity (Table 2). This result strongly suggests that a heat stable proteinaceous compound was responsible for the inhibitory activity of the culture supernatant of *Lactobacillus curvatus* DN86. Thus, we designed purification steps to isolate the biomolecule that we named bacteriocin DN86.

Bacteriocin purification

The proteinaceous compounds secreted by *Lactobacilluscurvatus*DN86 was purified to homogeneity by ammonium sulphate precipitation, C18 *sep-pack* column chromatography followed by two steps of reverse-phase chromatography. The final step of purification by silica C18 reverse phase chromatography gives a single symmetrical peak with antimicrobial activity (Fig.1). The 23.17 min peak was collected and the proteinaceous compound was tested.

Mass determination

The inhibitory fraction after reverse-phasehigh-performance liquid chromatography (RP-HPLC) was analysedfurther by MALDI-ToF.It was to be a peptide with a mass of 5162.17 Da(Fig. 2). The bacteriocin DN86, has a Molecular weight <10kb, themostable,

Fig.1 The second step of HPLC purification of bacteriocin DN86. Reverse-phase HPLC chromatography of the active fraction obtained after *sep-pack* C18 column purification. Individual column fractions were analyzed for bacteriocin activity. Activity was detected only in the greatest peak (23.17 min). The dashed line indicates acetonitrile gradient used to elute the bacteriocin activity (at 30%)

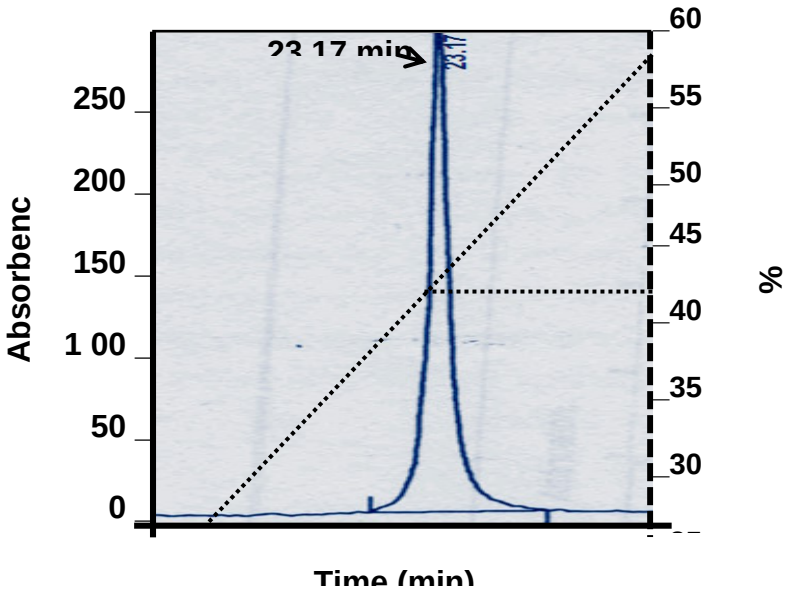

Fig.2 MALDI-ToF mass spectrometry analysis of purified bacteriocin DN86. The molecular mass indicated above the major pick corresponded to the mono-protoned form of this bacteriocin.

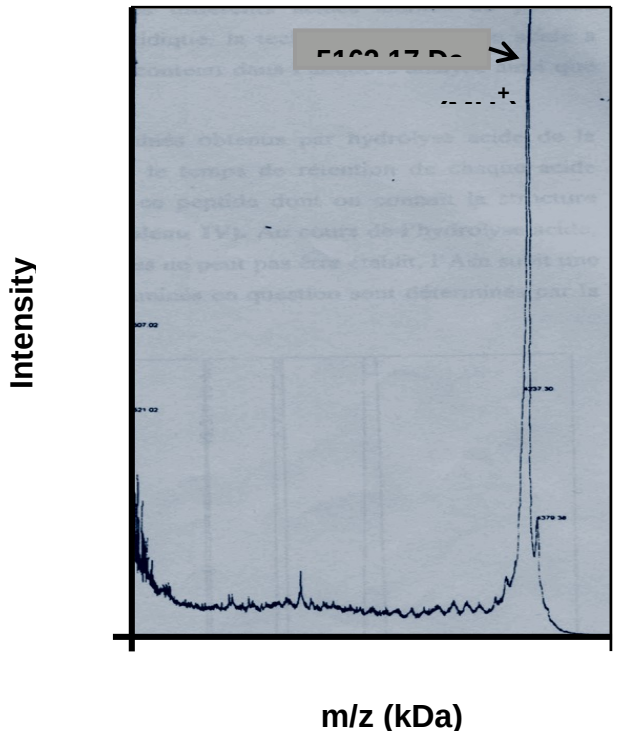

Table.1 Strains used in this study

Microorganism	Growth conditions	Growth medium	Bacterial antagonism of the isolate DN86
Bacillus subtilis ATCC 8633	37°C, aerobic	BHI	+
Enterococcus faecalis JH 22	37°C, aerobic	BHI	+
*Hafnia*sp.	30°C, aerobic	BHI	-
Lactobacillus acidophilus DSM 20079	37°C, anaerobic	MRS	-
*Lactobacillus casei*DSM 20011	37°C, anaerobic	MRS	-
*Lactobacillus curvatus*DSM 20181	30°C, anaerobic	MRS	-
Lactobacillus delb. subsp. *delbrueckii*DSM 20074	30°C, anaerobic	MRS	-
*Lactobacillus fermentum*DSM 20052	37°C, anaerobic	MRS	-
*Lactobacillus helveticus*CIP 103146^{T}	42°C, anaerobic	MRS	-
*Lactobacillus plantarum*ATCC 14917	37°C, anaerobic	MRS	-
*Lactobacillus sakei*ATCC 15531	30°C, anaerobic	MRS	-
Lactococcuscremoris ATCC 11603	37°C, anaerobic	M17	-
Lactococcuslactis ATCC 11454	37°C, anaerobic	M17	-
Leuconostoc mes. subsp.*Mesenteroides*DSM 20240	30°C, anaerobic	MRS	-
*Listeria ivanovii*BUG 496	30°C, aerobic	BHI	+
Micrococcus flavus ATCC 10240	30°C, aerobic	BHI	-
Serratia sp.	30°C, aerobic	BHI	+
Staphylococcus aureus ATCC 25923	30°C, aerobic	TGA	+

DSM, Deutsche SammlungvonMicroorganismenundZellkulturenGmbH, Braunschweig, Germany; CIP, Collection de l'Institut Pasteur, Paris, France; INRA, Institut National de Recherche Agronomique; Jouy, France; ATCC, American Type Culture Collection.

Table.2 Effects of enzymes, pH, and temperature on the activity of bacteriocin DN86

Treatment	Bacterial antagonism of the isolate DN86
Enzymes (0.5 mg/ml)	
Lipase, catalase or lysozyme	+
Proteinase K, pronase, pepsin, protease, trypsin, □- chymotrypsine or	−
Control (not treated with enzymes, 30°C)	+
pH	
2, 3, 4	−
5, 6, 7, 8	+
9, 10, 11, 12	−
Control (pH not adjusted, 30°C)	+
Heat	
15, 30 or 60 min at 60°C	+
15, 30 or 60 min at 110°C	+/−
15 min at 121°C	+/−
Control (bacteriocin at 4°C)	+

+: activity; −: no activity; +/−: loss of activity

anti-*Listeria* activity, it must belong to the class IIabacteriocin.

Understanding the microbial ecology of chicken gut is an important issue in the development of exclusive cultures or probiotics. The bacterial diversity characteristic of the cecum is of major importance in mediating interactions among the members of the intestinal microbiota and with colonizing pathogens. In this study, we established the lactic acid bacteriamicrobiota present in the ceca of Saudi chickens and then to investigate the antagonism of these bacteria against *Listeria*, *Staphylococcus*, *Bacillus*, S*erratia* sp. and*Enterococcus.* The composition and dynamics of the intestinal microbiota contribute positively to host health, growth, and maturation by acting as a barrier to colonization by pathogens. The use of antibiotics is an issue of current concern because of the emergence of antibiotic resistance in human and zoonotic pathogens. Zhang et al. (Zhanget al. 2007) isolated 41 strains of *Lactobacillus salivarius* with strong antagonism against *Salmonella* and

Campylobacter, they did not demonstrate the nature of this antagonism. In our study, among the 240 colonies analyzed, our isolates belonged to the genus *Lactobacillus*, among them *Lactobacillus curvatus* (38.1%), *Lactobacillus sakei*(20.3%), *Lactobacillus salivarius*(9.4%), *Lactobacillu sreuteri*(3.1%), and other lactic acid bacteria strains included of the genus *Enterococcus faecalis*(21.9%), *Leuconostoc mesenteroides*(4.5%), *Streptococcus*sp. (2.7%). The bacterial population in the digestive tract of chickens (Gonget al. 2002) reported a predominance of *Lactobacillus* strains (68.7%) in the ileum and jejunum with a little proportion of *Streptococcus* (6.6%) and *Enterococcus* (6.4%). Research on the ceca of Tunisian chickens revealed the presenceof the genus *Lactobacillus*, among them *Lactobacillus sakei*(33.3%), *Lactobacillus salivarius*(19.4%), *Lactobacillus reuteri*(8.6%), and *Lactobacillus curvatus*(8.6%). The other lactic acid bacteria strains included those of the genus *Weissella*(16.7%), *Enterococcus faecalis*(5.3%), *Leuconostoc mesenteroides*(2.7%), *Lactococcus graviae*(2.7%), and *Streptococcus* sp. (2.7%) (Messaoudi et al. 2011). These proportions are very different from the study of Gong in the cecum, with *Lactobacillus* strains contributing only 8.2%, *Streptococcus* strains 0.7%, and *Enterococcus* strains 1% of the bacterial population (Gonget al. 2002). However, different authors have reported different findings regarding the composition of the microbiota in the chicken digestive tract. The lactobacilli comprise only a small proportion (5 to 6%) (Bjerrum et al. 2002) while Dumonceaux et al. (2006) have found a large number of lactobacilli strains (25%) with a high degree of diversity. In addition, the two studies also identified different species, with Bjerrum et al. (Bjerrum et al. 2002) reporting the presence of *Lactobacillus salivarius*, *Lactobacillusagili* sand *Lactobacilluskitasatoni s*and Dumonceaux et al. (Dumonceaux et al. 2006)*Lactobacillus crispatus*, *Lactobacillus buchneri, Lactobacillus johnsonii,Lactobacillus vaginalis*and *Lactobacillus salivarius*subsp. *salivarius*. In our work, the predominant cecal isolates of lactic acid bacteria were *Lactobacilluscurvatus,* and *Lactobacillussakei* and we didnot detect the presence of *Weissella*sp. nor *Lactobacillusdelbrueckii, Lactobacillus acidophilus*, *Lactobacillus crispatus*, *Lactobacillusreuteri,* and *Lactobacillusaviarius*as reported by Lu et al. (2011).

A bacteriocin like inhibitor substance was detected in the culture supernatant of *Lactobacilluscurvatus*DN86 isolated from Saudi poultry ceca. To identify this compound, a method designed for the isolation of hydrophobic peptides, led us to purify the inhibitor substance permitting its identification (Guyonnet et al. 2000; Ferchichi et al. 2001). The purified product is sensitive to proteases, but insentitive to other hydrolases, confirming its proteinaceous nature. The compound secreted by *Lactobacilluscurvatus*DN86 was namedbacteriocin DN86. The mass determined by mass spectrometry analysis of this molecule was 5162.17 Da. Bacteriocin DN86 is anti-*Listeria*, it belongs to the class IIabacteriocins (Klaenhammer 1993). Bacteriocin DN86 is very active, render this bacteriocin attractive as an anti-*Listeria* compound to protect food. Bacteriocin DN86 inhibit *Listeria*, *Staphylococcus*, *Bacillus*, S*erratia* sp., *Enterococcus.* Bacteriocin DN86 is an anti-*Listeria*bacteriocin

produced by a lactic acid bacteria strain described so far. These characteristics strongly suggest that this strain is a good candidate to be incorporated in starter to protect preserved meat against *Listeria* contamination. lactic acid bacteria produce a wide range of bacteriocins with antagonism against gram-negativebacteria, including *Campylobacter*(Stern et al. 2006; Messaoudi et al. 2011).

Studies on poultry gastro intestinal tract (GIT) microbiota indicate that the GIT is host to various beneficial and harmful bacteria, including lactic acid bacteria and putrefactive bacteria. Putrefactive bacteria are viewed as harmful bacteria for they decompose proteins, produce foul-smelling substances, and cause diarrhea (Ben Salah et al. 2012). lactic acid bacteria are considered as beneficial bacteria particularly because of their production of organic acids, and bacteriocin or bacteriocin-like substances. Probiotics are live microbial supplements that beneficially affect the health of the host by improving the balance of the microflora in the intestinal tract(Ben Salah et al. 2012). The genus *Lactobacillus* is the most named species of lactic acid bacteria. It is commonly found in abundance in the upper gastro intestinal tract of humans and animals and is widely used as a food additive for the fermentation and preservation of food particularly because it displays a number of properties and mechanisms of action that are different from those of conventional antibiotics.In the poultry GIT, lactic acid bacteria constitutes the most common type of bacteria in the intestine(Messaoudi et al 2011). The adhesive ability of lactic acid bacteria has often been investigated through in vitro animal or human models using intestinal and epithelial cell lines, such as Caco-2(Lee et al. 212), T84 (Dahan et al. 2003), LoVo(Shen et al. 2005), and HT-29 (Gopel et al. 2001).Several reports indicated that the administration of probiotics to animals modulate the cells of theimmune system(Ghosh et al. 2004). These immune-regulating agents have also been reported for their ability to change cytokine secretion from a pro-inflammatory to an anti-inflammatory profile (Chambers et al. 2011; Mohamadzadeh et al. 2005; Schultz et al. 2002).

Considering the promising properties and potential of probiotics from *Lactobacillus* strains, future study will be undertaken to investigate and evaluate the probiotic potential of this collection of lactic acid bacteria strains, isolated from different cecum of indigenous poultry in Saudi Arabia. Knowledge on the survival, colonization of potential probiotic strains in the GIT, and the fate of probiotic-derived active components, is important during the evaluation of the positive and negative effects of candidate strains. The survival of different probiotic strains in different parts of the GIT has, for instance, been reported to vary, with a number of strains being rapidly killed in the stomach while others having the ability to persist and pass through the whole gut in high numbers(Marteau et al. 1993).

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