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Probiotics' Antibacterial Activity on Beef and Camel Minced Meat at Altered Ranges of Temperature

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***Abstract*—**Because of their inhibitory effects, selected probiotic Lactobacilli may be used as antimicrobial against some hazardous microorganisms responsible for spoilage of fresh minced beef (cattle) minced meat and camel minced meat. Lactic acid bacteria were isolated from camel meat. These included 10 isolates; 1 *Lactobacillus fermenti*, 4 *Lactobacillus plantarum*, 4 *Lactobacillus pulgaricus*, 3 *Lactobacillus acidophilus* and 1 *Lactobacillus brevis*. The most efficient inhibitory organism was *Lactobacillus plantarum* which can be used as a propiotic with antibacterial activity. All microbiological analyses were made at the time 0, first day and the second day at altered ranges of temperature [4±2 ^{0}C (chilling temperature), 25±2 ^{0}C, and 38±2 ^{0}C]. Results showed a significant decrease of pH 6.2 to 5.1 within variant types of meat, in addition to reduction of Total Bacterial Count, Enterococci, *Bacillus cereus* and *Escherichia coli* together with the stability of Coliforms and absence of *Staphylococcus aureus*.

***Keywords*—**Antibacterial, camel meat, inhibition, probiotics.

I. Introduction

THROUGHOUT 1900 AD, the faith of probiotics appeared when Ellie Metchnikoff contemplated the long life of Bulgarian peasants as a result of taking in fermented milk and milk products. Probiotics are considered a set of microorganisms which passively influence the host's health. The term "probiotics" is a composite word from Latin and Greek that literally means 'for life'. Probiotics have many beneficial effects related to preservation of food, especially milk and meat products. Other beneficial effects include saving the stability of flavour, and enhancing the nutritive value of food [1].

Lactic Acid Bacteria (LAB) have been used extensively for manufacturing a wide variety of fermented foods. As the lactic acids produced from these bacteria do not pose any health risks, they are Generally Recognized as Safe (GRAS) organisms. Apart from lactic acids, these bacteria also produce various types of compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocins or bactericidal proteins during lactic acid fermentations [2].

Bacteriocins are antimicrobial protein compounds which exert inhibitory activities towards a broad spectrum of pathogenic microorganisms, including food spoilage and foodborne bacteria. They are produced by both Gram-positive and Gram-negative bacteria [3]. In recent times, the bacteriocins from the GRAS LAB have a great scientific role in controlling pathogens in foods. Therefore, LAB are reported as biopreservative agents with great economic importance [4]-[6].

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Meat is one of the most desirable types of food; as it contains the highest level of protein, as well as it has high nutritive value. It is one of the perishable types of food because it has high nutrients which are used to support the growth of many micro-organisms.

Meat is subjected to contamination by different microbes due to raw material, grinding of meat where the contamination could spread throughout the entire muscle, post processing handling or different equipment, lack of refrigeration facilities, temperatures above 20 ºC, and lack of enough suitable methods of transportation which are used from the point of production till marketing plus improper storage [7]. The process of meat deterioration starts at the time of slaughtering and progresses till consuming. The combination of low temperature with LAB strains could increase the length of meat shelf life [8].

The aim of this study is to investigate the ability of Lactobacillus species probiotic to induce bacterial inhibition on both beef minced meat (cattle) and camel minced meat at altered ranges of temperatures.

II. Materials and Methods

A collection of camel meat samples (ten in number) for isolation of Lactobacilli was performed from different slaughter halls in Assiut, Egypt. Transportation formed by using the refrigerated box (4 ºC). Camel and beef (cattle) meat were purchased from Assiut slaughter halls and used as meat system test.

A. Enumeration and Identification of Lactobacilli [9]

15-20 ml sterile De Man Rogosa Sharpe (MRS) agar was poured into sterile petri dishes containing one ml of the diluted test sample. The medium was allowed to solidify on a flat surface for 5-10 minutes and was incubated at 37 ºC for 48-72 hrs in CO_2 incubator. Colonies with Lactobacilli-like morphology were counted and the number of cfu/g was determined, isolated and purified on MRS agar. Isolates were examined for general characters of *Lactobacilli*, which are Gram-positive, catalase-negative, non-motile, non-spore forming rods, being able to clot milk, and not able to produce indole.

B. Biochemical Identification

Catalase, oxidase, indole production test, growth at different temperatures like 15, 37 and 45 ºC, carbohydrate fermentation for *Lactobacilli* spp., nitrate reduction test, arginine hydrolysis and growth at 4% NaCl were performed to the isolates.

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C. Antibacterial Activity of Probiotic Bacteria on Some Pathogens

1. Indicator Organisms Used

Gram-negative bacteria (*E. coli* (NCTC No. 12023) and *B. cereus* and *St. aureus* (NCTC No. 7447) as Gram-positive bacteria were used as indicator bacteria for detection of the antibacterial activity. All strains mentioned above were obtained from High Quality Media unit (HQM) in Animal Health Research Institute in Dokki, Egypt; the pathogens were maintained in Brain Heart Infusion Agar (BHIA) butt-slants in screw-capped tubes kept at 4 ºC.

2. Preparation of Test Pathogens [9]

An actively growing test microorganism (indicator) was cultured for 24 hrs at 35 ºC in a Tryptone Soya broth (OXOID). The microorganism was dipped with a sterile cotton swab, then spanned various times and pressured thoroughly on the top of the fluid level on the inside of the tube's wall to discard inocula leftover from the swab.

3. Preparation of Probiotic Strains [9]

The well isolated colony was chosen from MRS agar plate culture. A loop was used to touch the growth, which was then transmitted into sterile 5 ml MRS broth in a tube. The broth culture was incubated for 24 hrs at 35 ºC.

4. Bacteriocin Activity Assay [10]

Cells were separated by centrifugation for 10 minutes at 5000 rpm. The cell free supernatant pH was amended with sterile 0.2 N NaOH to reach 5.5. The activity of bacteriocin in the supernatant was tested by agar well diffusion assay.

5. Agar Well Diffusions Method

20 ml of molten nutrient agar medium was cooled at 47 ºC and seeded with 1% overnight culture of the indicator organism. Seeded agar was poured into sterile petri dish and allowed to solidify at room temperature. Wells of 7 mm diameter were cut in the solidified agar using a sterile metal cork borer and filled with 100 µL of supernatant bacteriocins. The plates were left at 4-5 ºC for two hours to allow diffusion of the substances and then incubated in CO_2 incubator for 24 hrs at the temperature optimum for the indicator organisms, 37 ºC. Absence or presence of inhibition zones as well their diameters were recorded.

D. Meat System Test

Camel meat and cattle were purchased from Assiut slaughter halls. The amount of each was 1 kg. Deboning and slicing were done, followed by mincing using sterilized mincer with checking pH before inoculation.

1. Inoculated Meat Samples [6]

L. plantrum was selected from the other strains as it was the most efficient inhibitory activity against different types of bacteria. It was grown on MRS for 24 hrs at 30 ºC. Dilutes up to 10^6 from this culture were plated on MRS to determine the cell concentration. A dilute of 10^6cfu/g was used and inoculated.

The minced meat was mixed with glucose (5%w/w) and introduced in sterile plastic bags, then stored at altered ranges of temperature [chilling temperature (4±2 ºC), 25 ºC, and at (38±2ºC)] at different storage days (day zero, first day and second day). Minced meat as a control sample was stored without the addition of glucose.

2. Determination of Physiochemical Characteristics [6]

The pH of the samples was measured by a pH meter apparatus (Crison Micro pH 2000).

3. Microbiological Examination

a. Preparation of Serial Dilution

Tenfold serial dilution of minced meat was prepared according to [11].

b. Total Bacterial Count [11]

One ml of each dilution was carefully transferred into 15 ml of previously melted and cooled (45±1 °C) standard plate agar. The inoculated plates were upturned and incubated at 32±1 ºC after hardening. Colony forming units were calculated for detection of aerobic plate count.

c. Total Coliforms Count (MPN) [12]

d. Presumptive Test for Coliforms Group (MPN/g) [12]

In three replicate tubes of lauryl sulphate tryptose (LST) broth provided with upturned Durham's tubes, 1 ml of several dilutions (1:10, 1:100 and 1:1000) was inoculated. The inoculated LST tubes were incubated at 35 ± 0.5 °C for 24±2 hrs. All the tubes showing the gas were submitted to confirmatory tests for counting total coliform.

e. Confirmatory Tests for Coliforms Group

All positive LST tubes were sub-cultured in Brilliant Green Bile lactose (BGB) broth with inverted Durham's tubes. The inoculated tubes were incubated at 35±0.5 °C for 48±2 hrs. BGB tubes showing gas production were recorded and considered positive for coliforms. Numbers of coliforms/g were calculated from MPN tables for three tubes dilutions [13].

f. Confirmed Test for Fecal Coliforms [12]

The positive LST tubes showing gas production were sub-cultured into *Escherichia coli* broth (EC broth) tubes with inverted Durham's tubes The inoculated EC both tubes were incubated at 45 °C for 48±2 hrs. All positive tubes showing gas production collected in Durham's tubes were recorded.

g. Escherichia Coli Count [12]

Positive EC broth tubes were sub-cultured by streaking on Leviene's-Eosin Methylene Blue (L-EMB) agar plates. The inoculated L-EMB plates were incubated for 24±2 hrs at 35 °C. *E. coli* appeared in the form of metallic sheen with dark center typically nucleated. Positive EMB plates for *E. coli* were recorded and the numbers of *E. coli*/g were calculated from MPN tables for 3 tubes dilutions.

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h. Identification of the Suspected E. coli Colonies

i. Microscopic Examination [14]

Films were prepared from the pure culture of suspected colonies then, stained with Gram's stain and examined microscopically.

j. Biochemical Reactions

k. Sugar Fermentation Reaction [15]

Pure cultures of the isolated organisms were inoculated into peptone water containing 0.5-1% of the following sterile filtered sugars (xylose, lactose, glucose, mannitol, arabinose, raffinose, dulcitol and sorbitol), as well as, inverted Durham's tubes. Tubes were incubated at 37 ºC and reactions were noticed daily for up to 7 days. The appearance of yellow color indicated sugar fermentation.

l. Triple Sugar Iron (TSI) Agar Reaction [16]

A pure culture of suspected colonies was picked up and inoculated into TSI agar by stabbing the butt of the tube and streaking the surface of the slant. The tubes were incubated at 37 °C for 18-24 hrs. The results were documented as acid production (yellow butt and/or slant), alkaline production (red slant), gas production (bubbles and/or cracks in the medium or the medium pushed up in the tube) and H_2S production (black discoloration).

m. Urease Test [15]

The ability of the microorganism to hydrolyze urea was detected by heavy streaking the surface of Christensen's urea agar slant with a pure culture of the tested organism. The inoculated slants were incubated at 35 °C for 18-24 hrs up to 3 days or more. A positive reaction was indicated by red discoloration of the medium, while negative tubes retained the original yellow colour.

n. Indole Production Test [15]

Tryptone water tubes were inoculated with a pure culture of the tested organism. Inoculated tubes were incubated at 35 °C for 18-24 hrs, and then drops of Kovac's reagent were added down the inner wall of the tubes. Development of a bright fuchsine red colour appeared at the interface of the reagent and the broth within seconds after adding the reagent indicated a positive test.

o. Methyl Red Test [15]

MR-VP broth was inoculated with a pure culture of the tested organism, and then incubated at 35 °C for 48-96 hrs. After incubation, (0.5 ml) 5 drops of the methyl red reagent were added directly to the broth. Development of stable red color of the medium indicated a positive test. Negative tubes were yellow in color.

p. Voges-Proskauer Test [15]

Tubes of MR-VP broth were inoculated with a pure culture of the tested organism. Inoculated tubes were incubated for 24 hrs at 35 °C. At the end of the incubation period, one ml of the broth was transferred to a clean test tube and 0.6 ml of 5% α-naphthol, followed by 0.2 ml of 40% KOH addition. The tube was shaken gently and allowed to be undisturbed for 10-15 min. Positive results were indicated by the development of a red color within 15 min or more after addition of the reagents. The test should not be read after standing for over 1 hr.

q. Citrate Utilization Test [15]

The slant surface of Simmons citrate agar tubes was tightly streaked with a pure culture of the test organism. The bottom was also inoculated by stabbing. The inoculated tubes were incubated at 37 °C for up to 48 hrs. Blue discoloration of the medium indicated positive results, while negative tubes had a green colour with no growth.

r. Enumeration of Enterococci Count [17]

One-tenth (0.1 ml) of the prepared dilutions of each sample was dispensed into the dry surface of KF streptococcal agar plates (duplicate plates were used), and evenly distributed until complete absorption using plating technique. The inoculated plates were incubated at 35±1 °C for 48±2 hrs. All red and pink colonies were counted and recorded as Enterococci.

s. Enumeration of Staphylococcus aureus Count [18]

Over a dry surface of Baired-Parker agar plates (duplicate plates were used), 0.1 ml from each of the prepared dilutions of samples under investigation was transferred and evenly spread using surface plating technique. Inoculated Baired-Parker agar plates were incubated at 37 °C for 24 hrs. Suspected colonies showing black, shiny with narrow white margins and surrounded by clear zones extending into the opaque medium were counted. The plates were then re-incubated for additional 24 hrs before being counted for further growth. A significant number of the suspected colonies were submitted to confirmatory tests.

t. Enumeration of Bacillus spp.

From the already prepared serial dilution, 0.1 ml was transferred to Mannitol egg yolk polymyxin agar (MYP) according to [19].

III. Results

Ten isolates obtained from camel minced meat according to their morphological characteristics and biochemical properties which collectively showed that these are common features of Lactic acid bacteria [9].

Isolates R1 were identified *as L. fermenti*, R2 -R4 -R7 -R10 as *L. plantarum*, R 3 as *L. pulgaricus*, R5, R8, R9 *L. acidophilus* and R6 as *L. brevis*.

The varied ranges of inhibition zones are observed against the pathogenic strains of bacteria. The diameter of each zone was measured in millimeters (inhibition diameter, mm) (Table I).

All the diameters of inhibition zones against *St. aureus* ranged from 16 mm to 23 mm as well as *B. cereus* while *E. coli* from 18 mm to 22 mm (Table I), (Fig. 1).

Results about the *in vivo* assay revealed a pH drop from 6.2 to 5.1 in inoculated cattle minced meat while inoculated camel

minced meat showed the decrease of pH from 6.3 to 5.1 at altered ranges of temperature, within two days of the incubation (Tables II, III). In the non-treated samples, the pH reached 7.3 units in the same periods. This means the spoilage of meat with the appearance of off odour, changing in colour and texture. The pH decrease in the inoculated assay to 5.1 was due to lactic acid formation by LAB; as there is no off odour, changing in colour and texture.

The microbial profiles plotted to show TBC decreased 3log units at chilling temperature in cattle minced meat (Fig. 2), where camel minced meat showed 2.5 log units (Fig. 5). The initial TBC of the mixture was around $4x10^9$ cfu/g which decreased to $3x10^7$ cfu/g at the second day of inoculation. Low decrease pattern at the same temperature observed for Enterococci. Enterococci reduced 0.6log units and 1log units in cattle minced meat and camel minced meat respectively on the first day (Figs. 2, 5).

TABLE I
ANTIBACTERIAL ACTIVITY OF PROBIOTIC BACTERIA ON PATHOGENIC BACTERIA

Sample	Species	Zone diameter(mm) of inhibition		
		St. aureus	*B. cereus*	*E. coli*
R1	*L.fermenti*	21	20	21
R2	*L. plantarum*	20	23	20
R3	*L. pulgaricus*	21	18	19
R4	*L. plantarum*	20	16	21
R5	*L. acidophilus*	18	20	20
R6	*L. brevis*	16	22	23
R7	*L. plantarum*	21	22	22
R8	*L. acidophilus*	20	21	18
R9	*L. acidophilus*	19	22	19
R10	*L. plantarum*	23	21	20

The second day showed better results as reduction reached 1 log unit for cattle minced meat, but 1.3 log unit for camel minced meat. *Bacillus* species had high initial count $1x10^7$ cfu/g. In spite they are psychrotrophic microorganisms; it diminished at the chilling temperature to $3x10^5$ cfu/g from $9x10^8$cfu/g.

Some microorganisms can cause deterioration in fresh meat not only *Enterococci* but also *St. aureus* and coliforms. In this article, there was absence of *St. aureus* at the inoculated and control samples, otherwise the coliforms showed stability for more than 1100 cfu/g on two types of meat all degrees of all degrees of temperature at the same duration of two days.

The same screen appeared for the presence of *E. coli* in cattle minced meat as it decreased from 11 cfu/g to less than 3 cfu/g in cattle minced meat. The highest number in camel minced meat was about 36 cfu/g. The inoculated samples showed the least reduction in the number of *E. coli* at all ranges of temperature.

Minimum inhibitory activity of *L. plantarum* cleared at high temperatures whether 25±2 ºC or 38 ±2 ºC against TBC as it failed to prevent its level from increasing (9.5log cfu/g to 11.5 log cfu/g), (9.5log cfu/g to 11.3log cfu/g), (9.5log cfu/g to 11.2log cfu/g) and (9.5log cfu/g to11.7logcfu/g) in both cattle and camel minced meat, respectively on the first day. Nearly the same levels appeared on the second day (Figs. 3, 4, 6, 7) in spite of acidic pH (5.2) at inoculated samples (Tables II, III).

Fig. 1 Inhibition zones of LAB against some pathogenic bacteria under study: R1 as *L. fermenti*, R2 -R4 -R7 -R10 as *L. plantarum*, R 3 as *L. pulgaricus*, R5 *L. acidophillus*

TABLE II
PH VALUES OF INOCULATED AND NON-INOCULATED WITH *L. PLANTARUM* (10^6) IN CATTLE MINCED MEAT SAMPLES AT DIFFERENT RANGES OF TEMPERATURE

pH	Chilling temp (control)	Chilling temp	25 ºC temp (control)	25 ºC temp	38 ºC temp (control)	38 ºC temp
Zero day	6.2	6.2	6.2	6.2	6.2	6.2
First day	5.3	5.2	5.3	5.3	5.2	5.2
Second day	6.4	6.2	7.1	6.3	7.3	6.1

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TABLE III
pH VALUES OF INOCULATED AND NON-INOCULATED WITH *L.PLANTARUM* (10^6) IN CAMEL MINCED MEAT SAMPLES AT DIFFERENT RANGES OF TEMPERATURE

pH	Chilling temp (control)	Chilling temp	25 °C temp (control)	25 °C temp	38 °C temp (control)	38 °C temp
Zero day	6.3	6.3	6.3	6.3	6.3	6.3
First day	5.6	5.5	6.7	5.3	6.7	5.2
Second day	6.6	6.5	7	6.6	7	5.1

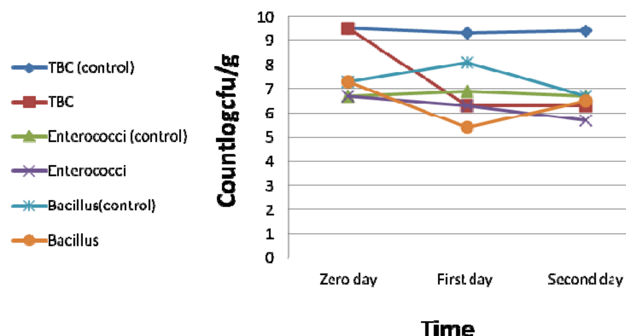

Fig. 2 Inhibitory activity *of L. plantarum* on different microorganisms in cattle minced meat at chilling temperature (4±2 °C)

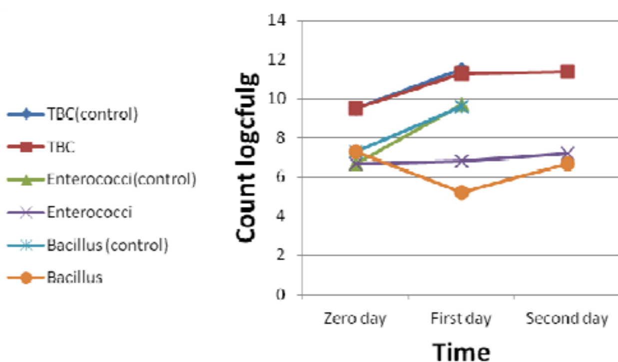

Fig. 3 Inhibitory activity of *L. plantarum* on different microorganisms in cattle minced meat at 25 ±2 °C

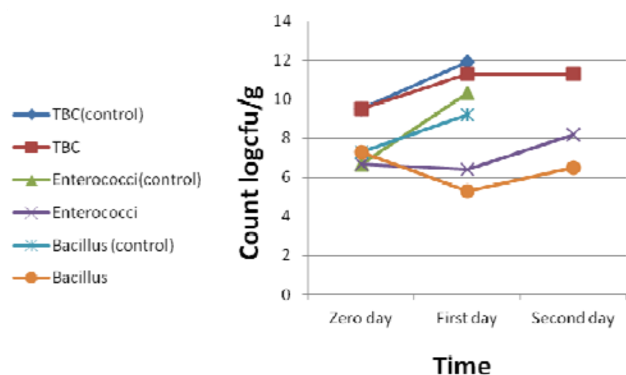

Fig. 4 Inhibitory activity of *L. plantarum* on different microorganisms in cattle minced meat at 38±2 °C

For all that, at these ambient temperatures Enterococci on the first day and second day (6.5log cfu/g, 6.1log cfu/g) showed an immediate bactericidal effect of *L. plantarum* in comparison with 9.4log cfu/g and 9.3log cfu/g at 25±2 °C and later 38±2 °C in camel minced meat (Figs. 6, 7). Besides, there was corresponding decrease in cattle minced meat (2.9log units cfu/g) on the first day only, within the same temperatures (Figs. 3, 4). This effect is totally eliminated at non inoculated samples during different ranges of temperature at the different time.

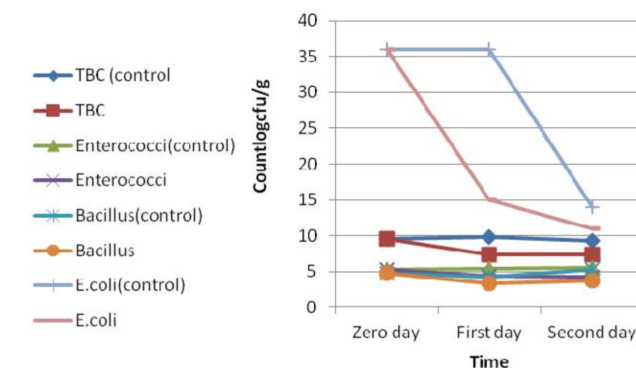

Fig. 5 Inhibitory activity of *L. plantarum* on different microorganisms in camel minced meat at chilling temperature (4±2 °C)

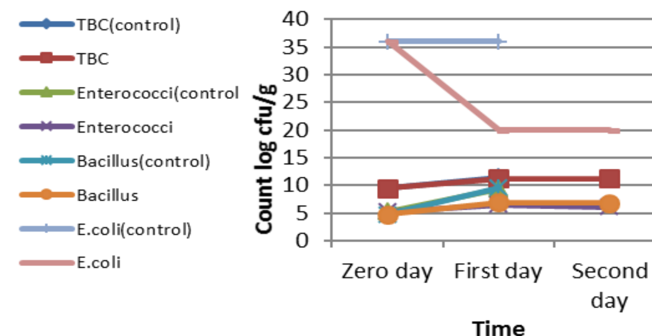

Fig. 6 Inhibitory activity of *L. pantaraum* on different microorganisms in camel minced meat at 25 ±2 °C

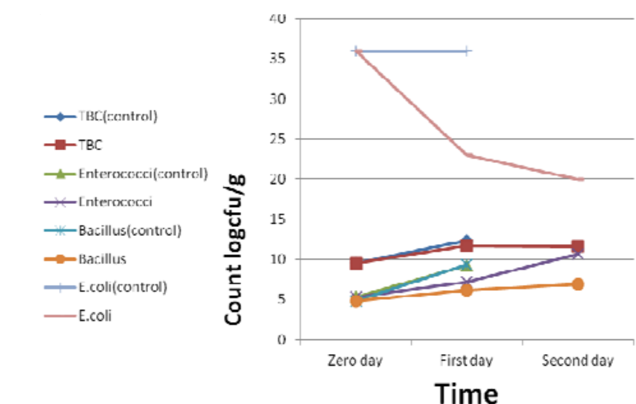

Fig. 7 Inhibitory activity *of L. plantarum* on different microorganisms in camel minced meat at 38±2 °C

The influence of *L. plantarum* as antibacterial

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microorganism also appeared towards *B. cereus* in cattle minced meat and camel minced meat. The high decline level revealed at the former (4.4log units cfu/g) at 25±2 ºC (Fig. 3), in addition to 6.7log cfu/g was the number of *B. cereus* at 38±2 ºC on the second day. The latter vanished (2.7log units) cfu/g at 25±2 ºC (Fig. 6), while it decreased to 3.2 log units cfu/g (38 ±2ºC) (Fig. 7); all of these results appeared on the first day. On the second day in the inoculated cattle minced meat samples the *B. cereus* counts were $6x10^6$ cfu/g which are less than the counts at zero time ($1x10^7$ cfu/g). *B. cereus* was nearly $4x10^6$cfu/g 38±2 ºC.

IV. Discussion

LAB originally isolated from meat and meat products are probably the best candidates for improving the microbiological safety of these foods because they are well adapted to the conditions in meat and should be more competitive than LAB from other sources [20]. LAB isolated from meat and meat products are able to compete with target culture when they are incubated together as they are most originally adapted to the condition of the meat. This characteristic media used to improve the microbiological safety of these types of foods, therefore this article looked for inhibitory activities of the ten isolates from fresh meat against Gram positive and Gram negative bacteria.

The isolation of different species of LAB formed from camel meat. *L. plantarum* showed a high frequency of inhibition in the media to *St. aureus*, *B. cereus* and *E. coli* where the inhibition zone diameter reached to 23 mm. This corresponds to [21] and agreed with [22]; where the LAB strains, which were isolated from Burkina Faso fermented milk, had showed nearly similar inhibition zone diameters against the same used indicator bacteria (*E. coli* and *St. aureus*).

The inhibition variety of bacteria by *Lactobacillus* bacteria was due to a combination of many factors produced LAB e.g. production of lactic acid reduced pH of meat and also inhibitory substances which are responsible for the most antimicrobial activity [23]. The pH decreased drastically during the first day in cattle minced meat; less than 6 units in the treated and untreated sample, while the range of ultimate pH values of camel meat ranged between 5.7units and 6units [24]. Similarly, pork meat samples inoculated with either *St. carnosus* or *L. alimentarus* showed a pH reduction from 6.4 to 5.5 within 24 hrs when stored at 20 ºC [25].

A drop in pH may be due to lactic acid production by *L. plantarum*. [7]. The shelf life of meat products could be extended; by the inoculation of *L. plantarum* under refrigeration after dipping in sugar cane molasses can be extended to 5 ºC by inhibiting psychotropic and mesophilic microorganisms [21].

Lactobacillus bacteria were used as biopreservative at the refrigeration temperature and subtropical temperature [26]. The fermentation performed in these studies at 20 ºC stopped the growth of the pathogens and spoilage of microorganisms [26]. Recently, using a combination of Lactobacillus bacteria and changing temperature can be considered as an integral part of hurdle technology.

Our results are confirmed by [27], where the bacteriocin activity of *L. plantarum* was very stable at 4 ºC.

A significant reduction by *L. innocua* was observed with a combination of low pH 5.5 and nisin at 20 ºC [28].

Highest antibacterial activities against *E. coli*, *St. aureus* were suggested by species of *L. corustoru, L. plantarum, L. casei, and L. fermentum* [29].

Nearly similar results revealed in this study to those reported by [7]; *L. dlbreuki* was isolated from camel meat for preservation at pH (4.0-4.2) and it led to the drastic reduction in SPC (Standard plate count), Coliforms, Enterococci and *Staphylococci,* within 3 days at 22 ºC [7].

Lactic acid is the supernatant of *L. fermentum*. The effect of 30% of lactic acid was clear on orange juice since it decreased the viable count of microorganisms by 3log units in comparison with control at 4 ºC for 21 days. This effect was increased, with increasing the concentration of the preservatives, while there was no effect at 25 and 37 ºC at all storage periods [30].

The stability to the number of coliforms was coinciding, with the using of the different strains of LAB, as biopreservative bacteria; which preserve the whole fish, meat, or minced meat products. The total plate count estimated for three days and the count of coliforms reduced, but it was not inhibited [31].

Our evidence is supported by [32]; where pH control samples in their study increased to 7.54 on the third day; due to the growth of meat- borne spoilage and pathogenic bacteria. The antibacterial effect of plantarcin is produced by *L. plantarum* against *B. cereus*, *E. coli* and *St. aureus*.

In [31], *L. plantarum* count reached to 8.0log/g, but the complete inhibition of the bacteria was not achieved. It was referred that this case might be due to the presence of dominant Gram-negative lipolytic bacteria; such as *Flavobacterium* and *Pseudomonas* species. *L. plantarum* 15H isolated from traditional dairies microbiota, showed the most efficient antagonistic pathogens [33].

The influence of LAB strains of variation of inhibition related to many different aspects as generating lactic acid which declines pH of meat, in addition of the inhibitors like bacteriocins and hydrogen peroxide, which are liable for most antimicrobial activity [34].

V. Conclusion

The vision of the new generations is the fulfillment of the healthiest liveliness; from here probiotics are widely used; due to the increasing of the consumer demands; for the natural products and the application of a natural inhibitory substances, as a food preservative. Probiotics adds to the extension of shelf life and the improvement of the food quality; by using microbes or their metabolites. The results presented in this article provide a clearer idea on the potential of antimicrobial *Lactobacillus strains* selected; which represents a way

forward, for the production of antimicrobial substances, that are used in the fermentation and biopreservation of the food.

This study showed that probiotics strains could be used for the inhibition of the microorganisms; which is responsible for the deterioration and the spoilage of meat. In the course of time, probiotics can be taken into an account for biopreservation.

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References

[1] Neethu Kamarudheen, Christy George, Charles Lekhya Priya and K. V. Bhaskara Rao. "Biopreservation of meat by probiotic bacteria isolated from dairy products", Der Pharmacia Lettervlo, 6 (6), p. 266-271, 2014. www.scholarsresearchlibrary.com. 06/01/ 2014.

[2] V.O., Oyetayo, F.C., Adetuyi., F.A. Akinyosoye, "Safety and Protective effect of *Lactobacillus acidophilus* and *Lactobacillus casei* used as probiotic agent *in vivo*", *African Journal of Biotechnology,* vol. 2, p. 448-452, 2003.

[3] Tagg, A.S Dajani, L.W. Wannamaker."Bacteriocins of Gram-positive bacteria", *Bacteriology Review,* vol. 40, 722-756, 1976.

[4] M.A. Daeschel, "Antimicrobial substance from lactic acid bacteria for use as food preservatives", *Food Technology,* vol.43, p.164-166, 1989.

[5] H. Bolm, and C. Morvedt", Antimicrobial substances produced by food associated, microorganisms", *Biochemical Society Transactions,* vol.19, p. 694-698, 1991.

[6] J.C. Piard and M.J. Desmazeaud, "Inhibiting factors produced by lactic acid bacteria. Part 2. Bacteriocins and other antimicrobial substances", vol.72, p.113-142, 1992.

[7] Kalalou, M. Faid and A.T. Ahami, "Extending the shelf life of fresh minced camel meat at ambient temperature by *Lactobacillus delbruekii* subsp. *delbruekii*", *Electronic Journal of Biotechnology*, vol.7, p. 246-251, 2004.

[8] Y. Babji and T.R.K d Murthy, "Effect of inoculation of mesophilic lactic acid bacteria on microbial and sensory changes of minced goat meat during storage under vacuum and subsequent aerobic storage", *Meat Science*, vol. 54, p. 197-202, 2000.

[9] J. D De man, Rogosa, M. A. a M.E. Sharpe, "A medium for the Cultivation of Lactobacilli", *Journal Appl Bact*., vol.23, p.1135, 1960.

[10] Geis. J. Singh and M. Teuber, "Potential of lactic streptococci to produce bacteriocin", *Applied and Environmental Microbiology*, vol.45, p. 205-211, 1983.

[11] A.P.H.A. "American Public Health Association", Compendium of Methods for the Microbiological Examination of Foods. 2nd Ed., *American Public Health Association,* Washington, DC, USA, 1992.

[12] A.O.A.C. "Association of Official Analytical Chemist", Official methods of analysis. 15th Ed. P.O. Box 540, Benjamin Franklin Station, Washington, 1990.

[13] FDA “Food and Drug Administration”, Bacteriological Analytical Manual, 8thEd. Washington, DC: US Food and Drug Administration, 2002.

[14] A.P.H.A. "American Public Health Association", Standard methods for the examination of dairy products. 15th ed., APHA (American Public Health Association), Washington, DC, USA, 1985.

[15] E.W Koneman; P.C.; Schrechenberger; S.D.; Aleen; W.C Winn, and W.M Janda. Color Atlas and Textbook of Diagnostic Microbiology. 4th Ed., Winters, R. (ed.) J. P. Lippincott Company, Philadilphia, 1992.

[16] E.J. Baron; L.R Peterson, and S.M. Finegold, Bailey and Scott's, Diagnostic Microbiology, 9th ed.,st. Louis, Baltimore, USA, Chapter 34, p. 457-473,1994.

[17] R. H. Deible and P. A. Hartman, "The Enterococci. In Compendium of methods for the microbiological examination of foods." M.L., Speck (ed.), 2nd Ed., American Public Health Association, 1982.

[18] A.C. Baird-Parker, "An improved diagnostic and selective medium for isolation of coagulase positive staphylococci", *Journal Applied Bacteriology*, vol. 25, p.12-19, 1962.

[19] G.A. Lancette and M. Harmon, "Enumeration and confirmation of Bacillus cereus in foods", *Journal Association Official Analytic Chem*istry, vol.63, p. 581-586, 1980.

[20] U. Schillinger and F Lucke, "Antimicrobial activity of *Lactobacillus sake* isolated from meat", *Journal Applied Environmental Microbiology,* vol .55, p. 1901 – 1906, 1989.

[21] Ângela M. Fiorentini; Ernani S. Sant’Anna; Anna C.S. Porto; Jaciara Z. Mazo; Bernadette D.G.M. Franco, Influence of bacteriocins produced by *Lactobacillus plantarum* BN in the shelf-life of refrigerated bovine meat", *Brazilian Journal of Microbiology,* vol.32, p. 42-46, 2001.

[22] A .Savadogo, C .Ouattara, I .Bassole, AS Traore, "Antimicrobial activities of lactic acid bacteria strains isolated from Burkina Faso fermented milk", *Pakistan Journal of nutrition*, vol. 3, p.174–9, 2004.

[23] M. A. H. Al-Allaf, A. M. M. Al-Rawi and A. T. Al-Mo ,"Antimicrobial activity of lactic acid bacteria isolated from minced beef meat against some pathogenic bacteria", *Iraqi Journal of Veterinary Sciences*, vol. 23, p.115-117, 2009.

[24] I. T. Kadim and O. Mahgoub, "Meat quality and composition of *Longissimus thoracis* from Arabian camel (Camelus dromedaries) and Omani beef": A comparative study. In First conference of the international society of camelids research and development (ISOCARD) (p. 118). Al-Ain, United Arab Emirates, 2006.

[25] E.C. Okolocha, L. Ellerbroek, "The influence of acid and alkaline treatments on pathogens and the shelf life of poultry meat", *Food Control*, vol. 16, p. 217–225, 2005.

[26] H.; Minor-Pérez; Ponce-Alquicira, E.; Macíasbravo, S. and Guerrero-LEgarreta, I, "Changes in fatty acids and microbial populations of pork inoculated with two biopreservative strains", *Meat Science*, vol. 66, p. 793-800, 2004.

[27] J. Nowroozi, M. Mirzaii, M .Norouzi, "Study of Lactobacillus as Probiotic Bacteria", Iranian *Journal of Public Health,* vol. 33, p. 1-7, 2004.

[28] Ananou, S., M. Maqueda, M. Martínez-Bueno and E. Validivia, "Biopreservation and ecological approach to improve the safety and shelf-life of foods". Communicating Current Research and Educational Topics and Trends in *Applied Microbiology*, p. 475 - 486, 2007.

[29] Zohreh Mashak, "Antimicrobial Activity of Lactobacillus Isolated from Kashk-eZardand Tarkhineh, Tw oIranian Traditional Fermented Food", *International Journal Enteric Pathog*ens, vol. 4, p. 34692, 2016.

[30] Wala’a Sh. Ali Nibras N.Mahmood Ali M.Hasan, Rayim S. Abbood, "Effect of some natural preservatives on some home made fresh fruit juices", *Advances in Environmental Biology*, vol.10, p. 23-2, 2016.

[31] Kannappan Sudalayandi, and Kannar S. Manja, "Repressive efficacy of lactic acid bacteria against the human pathogenic and fish-borne spoilage microbiota of fresh Indian mackerel fish chunks", *African Journal of Biotechnology*, vol. 11, p. 15695-15701, 2012.

[32] HJ Yusuf, MC Varadaraj, "Antibacterial effect of plantaricin LP84 on food-borne pathogenic bacteria occurring as contaminants during idli batter fermentation", World *Journal of Microbiology and Biotechnol*ogy, vol. 15, p.33-38, 1999.

[33] Babak Haghshenas, Minoo Haghshenas, Yousef Nami, Ahmad Yari Khosroushahi, Norhafizah Abdullah, Abolfazl Barzegari, Rozita Rosli, Mohammad Saeed Hejazi,"Probiotic Assessment of *Lactobacillus plantarum* 15HN and *Enterococcus mundtii* 50H Isolated from Traditional Dairies Microbiota". *Advanced Pharmaceutical Bulletin*, vol.6, p. 37-47, 2016.

[34] Lekha Ravindran, Niveda Manjunath, Rachel Prianka Darshan and Suba G A Manuel," *In vitro* Study Analysis Of Antimicrobial Properties Of Lactic Acid Bacteria Against Pathogens", *Journal Bio Innovation*, vol. 5, p. 262-269, 2016.

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