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## ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA FROM RAW POULTRY MEAT

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**Abstract:** A total of 15 presumptive isolates of lactic acid bacteria, from poultry raw meat, were isolated and identified. The results of the standard physiological and biochemical tests, 5 identified isolates of *Lactococcus lactis* ssp. *lactis* 1, 3 isolates of *Lactobacillus lactis* ssp. *lactis* 2, 2 isolates of *Lactobacillus fermentum* 1, 2 isolates of *Lactobacillus paracasei* 1, and 3 isolates of *Lactobacillus rhamnosus*. These strains results of this study, indicated that the presence of heterofermentative *Lactobacillus* species in raw poultry meat.

**Key words:** lactic acid bacteria, heterofermentative *Lactobacillus* spp.

### Introduction

The microbiology of meat, meat products and poultry meat has recently been reviewed by *ICMSF* (1980). Isolation and identification of microorganisms from natural resources are an occurring process that have the most powerful means for obtaining cultures and also have commercial purposes. Especially for Lactic Acid bacteria (LAB), which are used all over the world for manufactured a wide varieties fermented foods. This is used especially the lactic acids do not pose any health risks to mankind, and are Generally Recognized as Safe (GRAS) organisms (*Encyclopaedia Britannica Online*, 2007).

Lactic acid bacteria produce various compounds such as organic acids, diacetil, hydrogen peroxide, and bacteriocins or bactericidal proteins during lactic acid fermentations (*Oyetayo et al.*, 2003). There is a tendency to use milder preservation methods, either because of energy-saving, the consumers' preference for mildly cured or cooked products, or their desire for having more 'fresh' meat products, or because of an aversion to the use of preservatives (*Simonsen et al.*, 1988). Bacteriocins are antimicrobial proteinaceous compounds that are inhibitory towards sensitive strains and are produced by both Gram-positive and Gram-negative bacteria (*Tagg et al.*, 1976). The bacteriocins from the GRAS lactic acid

bacteria have arisen a great deal of attention to control pathogens in foods. Lactic acid bacteria exert strong antagonistic activities against many microorganisms, including food spoilage organisms and pathogens. The inhibitory spectrum of some bacteriocins also includes food spoilage and/or food-borne pathogenic microorganisms (*Schillinger et al., 1996*).

The aim of this work was to isolate and identify lactic acid bacteria from raw poultry meat.

## Materials and Methods

**Isolation and identification of lactic acid bacteria.** The lactic acid bacteria were isolated from raw poultry meats, by appropriate dilutions with NaCl fisiological. Decimal dilution of these samples were mixed with MRS medium (AEB, France) and incubated at 37°C for 48-72 h. Pure cultures were maintained in MRS agar (*De Man et al., 1960*) at 4°C for short term use. Eighteen well-isolated colonies were picked up and transferred to MRS broth. They were propagated twice and streaked on MRS broth to check the purity of the isolates and then stored in MRS agar and overlaid with MRS agar for the anaerobic condition. Selection of strains was made in agreement with morphology, Gram stain, viability during storage at 4°C and antimicrobial activity.

The identification of the cultures was based on the characteristics of the lactobacilli as described in Bergey's Manual of Determinative Bacteriology (*Kandler and Weiss, 1986; Holt et al., 1994; Garrity et al., 2004*), fermentation of different carbon sources (API 50 CHL, bioMerieux SA, France), gas production from glucose, growth at different temperatures.

**Sugar fermentation profiles of isolates.** The abilities of these isolated strains to produce acids from different carbohydrates was determined by API 50 CHL test kit (bioMerieux SA, France). The API test strips were prepared as recommended by the kit supplier and scored after incubation for 24 and 48 hours at 37°C. The results were communicated to the APIWEB, which used the phenotypic data to predict a species identity for each isolate. Interpretations of the fermentation profiles were facilitated by systematically comparing all results obtained for the isolates studied with information from the computer-aided database, in which the identification of a microorganism is accompanied by the following information: (i) The percentage of identification (%ID) is an estimate of how closely the profile corresponds to the taxon relative to all the other taxa in the database. (ii) The T-index represents an estimate of how closely the profile corresponds to the most typical set of reactions for each taxon. Its value varies between 0 and 1, and is inversely proportional to the number of atypical tests. (iii) Comments on the quality of identification derived from the %ID and the T-index of the selected taxon (excellent identification %ID > 99.9 and T> 0,75).

Antibiogram of Lactic Acid Bacteria isolates. The isolates were inoculated into MRS broth individually and incubated for 24 h. About 20 ml MRS agar was seeded with the cultures of LAB isolates, mixed well, poured into sterile Petri plates and stored at 4°C for 1 h to solidify the media. OCTA-discs (OXOID) were placed up side down, pressed on the top of the agar plates and kept again at 4° for 1 h. The plates were incubated at 37°C over night. Resistance was defined as the absence of a growth inhibition zone around the discs.

## Results and Discussion

**Lactic acid bacteria microflora.** Eighteen isolates of LAB were isolated from the samples. After series of purification on MRS agar, fifteen isolates were found to be Gram-positive, catalase negative, non-motile bacilli. The results of the isolation and identification of the standard physiological and biochemical tests were identified the isolates as 5 isolates of *Lactobacillus lactis* ssp. *lactis* 1, 3 isolates of *Lactobacillus lactis* ssp. *lactis* 2, 2 isolates of *Lactobacillus fermentum* 1, 2 isolates of *Lactobacillus paracasei* 1, and 3 isolates of *Lactobacillus rhamnosus*.

Table 1 presents the results of the best five final identifications for each type of isolates on API gallery.

**Table 1 Results of the biochemical tests for the identification of the isolated strains by using API gallery**

Isolated strains	Identification	% ID	T-index
<i>Lactobacillus fermentum</i> 1	Acceptable identification	84.4	0.43
<i>Lactobacillus lactis</i> ssp. <i>lactis</i> 1	Acceptable identification	84.7	0.47
<i>Lactobacillus paracasei</i> ssp. <i>paracasei</i> 1	Acceptable identification	84.4	0.46
<i>Lactobacillus rhamnosus</i>	Acceptable identification	80.5	0.46
<i>Lactobacillus lactis</i> ssp. <i>lactis</i> 2	Acceptable identification	84.7	0.35

From Table 1, we assumed that in the raw poultry meat there are some lactic acid bacteria, *Lactobacillus fermentum* 1 (Isolate 1), *Lactobacillus lactis* ssp. *lactis* 1 (Isolate 2), *Lactobacillus paracasei* ssp. *paracasei* 1 (Isolate 3), *Lactobacillus rhamnosus* (Isolate 4), and *Lactobacillus lactis* ssp. *lactis* 2 (Isolate

5). And we can use the bacteria, I mean the lactic acid bacteria for many things, e.g., as preservative, or as starters to produce healthy foods.

In Table 2, presents the results of the antibiotics sensitivity of the five isolates. Isolated strains exhibited antibiotic sensitivity with the inhibition diameters obtained, are between 0 mm and 34 mm.

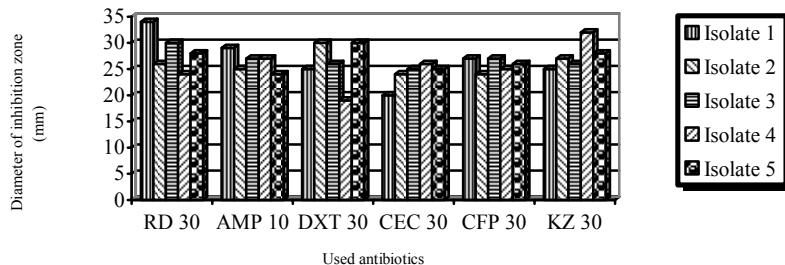
**Table 2 Antibiotic sensitivity of the five isolates**

Antibiotics	Bacterial isolates					
	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	
SPC 100	18	I	17	I	21	S
RD 30	34	S	26	S	30	S
PB 100	8	R	6	R	8	R
TM 5	10	R	6	R	8	R
TE 30	23	S	25	S	27	S
AMP 10	29	S	25	S	27	S
OX 1	15	S	10	R	12	I
K 30	8	R	12	R	10	R
DXT 30	25	S	30	S	26	S
E 15	25	S	28	S	29	S
CN 10	9	R	11	R	10	R
CIP 5	19	S	14	R	15	R
CEC 30	20	S	24	S	25	S
CL 30	16	I	13	R	17	I
CFP 30	27	S	24	S	27	S
KZ 30	25	S	27	S	26	S
					32	S
					28	S

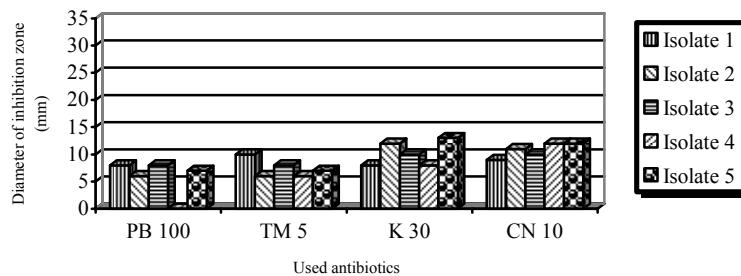
Notes: R = resistance, I = intermediate reaction, S = sensitivity

From Table 2, isolate 1 showed sensitivity reaction to RD 30, TE 30, AMP 10, OX 1, DXT 30, E 15, CIP 5, CEC 30, CFP 30, and KZ 30. And isolate 2 showed sensitivity to RD 30, TE 30, AMP 10, DXT 30, and E 15. Isolate 3 showed sensitivity reaction to SPC 100, RD 30, TE 30, AMP 10, DXT 30 and E 15. Isolate 4 showed sensitivity reaction to RD 30, AMP 10, OX 1 and DXT 30. And isolate 5 showed sensitivity reaction to RD 30, TE 30, AMP 10, DXT 30, E 15, CEC 30, CFP 30, and KZ 30.

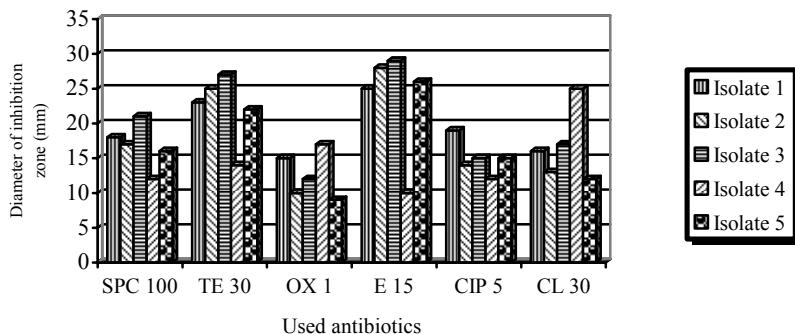
In the Graphs 1 to 3, there are the results about the five isolates to different antibiotics.

**Graph 1.****Diameter of inhibition zone to the RD 30, AMP 10, DXT 30, CEC 30, CFP 30 and KZ 30**

From the Graphic 1, the isolates 1, 2, 3, 4 and 5 has sensitivity to RD 30, and also to the AMP 10, DXT 30, CEC 30, CFP 30 and KZ 30.

**Graph 2.****Diameter of inhibition zone to the PB 100, TM 5, K 30, and CN 10**

From the Graph 2, the isolates 1, 2, 3, 4 and 5, were resistance to the PB 100, TM 5, K 30 and CN 10.

**Graph****3. Diameter of inhibition zone to the SPC 100, TE 30, OX1, E 15, CIP 5, and CL 30**

From the Graph 3, the isolates 1, 2, and 5, were have intermediate reaction, but isolate 3 has sensitivity reaction, and the isolate 4, has resistance to the SPC

100, but to the TE 30, the isolates were sensitivity and only the isolate 4 was resistance. To the OX 1, the isolates 1 and 4 were sensitivity reaction, but isolate 3 was intermediate reaction, and isolate 2 and 5 were resistance. To E 15, only isolate 4 has resistance; but the others (isolate 1, 2, 3, and 5) were sensitivity. The isolate 1, was sensitivity to CIP 5, and isolate 2, 3, 4, and 5 were resistance. And to the antibiotic CL 30, the isolates 1 and 3 were intermediate reaction, but 2 and 5 were resistance, and isolate 4 was sensitivity.

## Conclusion

The results obtained in this study revealed the presence of a wide variety of lactic acid bacteria (LAB) in the raw poultry meat.

Some of the isolated and identified LAB shows outstanding performances that were similar and in some cases was higher performances as biopreservatives, 5 isolates of *Lactobacillus lactis* ssp. *lactis* 1, 3 isolates of *Lactobacillus lactis* ssp. *lactis* 2, 2 isolates of *Lactobacillus fermentum* 1, 2 isolates of *Lactobacillus paracasei* 1, and 3 isolates of *Lactobacillus rhamnosus*.

Antimicrobial compounds produced by LAB have provided these organisms with a competitive advantage over other microorganisms.

In conclusions, 15 LAB isolates from the raw poultry meat, capable of producing good amount of bacteriocins have been anticipated to have enormous potential for applications as fermentor or as biopreservatives to meat and products.

These researches are vital in the sense that functional properties in lactic acid bacteria improved the preservative effect to the meat and meat products. And also the LAB have an essential role in meat fermentation processes, as we known was food preservation of fermented foods, and the isolated strains can positively have impact on their use as starter cultures for fermented food especially for the sausages or other healthy food products, with a view to improving the hygiene and safety of fermented produce.

## Izolacija i identifikacija mlečnokiselinskih bakterija iz svežeg pilećeg mesa

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## Rezime

Ukupno 15 izolata mlečnokiselinskih bakterija iz svežeg pilećeg mesa je izolovano i identifikovano. Rezultati standardnih biohemijskih i fizioloških testova, 5 identifikovanih izolata *Lactococcus lactis* ssp. *lactis* 1, 3 izolata *Lactobacillus*

*lactis* ssp. *lactis* 2, 2 izolata *Lactobacillus fermentum* 1, 2 izolata *Lactobacillus paracasei* 1, i 3 izolata *Lactobacillus rhamnosus*. Ovi izolati mlečnokiselinskih bakterija dobijeni u istraživanju ukazuju na prisustvo heterofermentativnih *Lactobacillus* vrsta u svežem pilećem mesu.

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