KnE Life Sciences

ICSAFS Conference Proceedings
2nd International Conference on Sustainable Agriculture and Food Security: A Comprehensive Approach
Volume 2017

Knowledge E
Engaging minds

Conference Paper

Isolation and Identification of Lactobacillus Bacteria from Culled Hens Meat for Meat Biopreservator

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Abstract

Using chemical bio-preservatives is being frowned at because of their probable adverse effects on the health of consumers. Isolation and identification of microorganisms from natural resources are an occurring process that have most powerful means for obtaining cultures and also have commercial purposes. The aim of this study was to get bio-preservatives from poultry meat, by isolation and then identification of lactic acid bacteria. Lactic acid bacteria do not pose any health risks to human, and are generally recognized as safe (GRAS) organisms. The lactobacillus were isolated from raw poultry meat by appropriate dilutions with NaCl fisiological, and the decimal dilution were mixed with MRS medium and then incubated at 37°C for 48-72 h. Pure cultures were maintained in MRS broth agar at 4°C for short term use. Thirty well-isolated colonies were picked up and transferred to MRS broth. Selection of strains was made in agreement with morphology, Gram-stain, viability during storage at 4°C and antimicrobial activity, was found twenty isolate. The identification of the cultures was based on the characteristics of the lactobacilli as described in Bergey's manual of determinative bacteriology, fermentation of different carbon sources, gas production from glucose, growth at different temperatures. For anti-biogram, the isolates were inoculated into MRS broth individually and incubated for 24h. The plates were incubated at 37°C overnight. Resistance was defined as the absence of a growth inhibition zone around the discs. Results indicated that 20 isolate of Lactic acid bacteria were identified: 3 isolates of *Lactobacillus fermentum*, 2 isolates of *Lactobacillus paracasei* ssp. *paracasei*, 5 isolates of *Lactobacillus plantarum*, 3 isolates of *Lactobacillus rhamnosus*, 2 isolates of *Lactobacillus lactis* ssp. *lactis* 1, and 5 isolates of *Lactobacillus lactis* ssp. *lactis* 2. Characterization of the microbial metabolic product for antimicrobial agents reveals that lactic acid bacteria has responsibility for the inhibition of the indicator organisms, and can be used as meat biopreservator.

Keywords: isolation, identification, lactobacillus, poultry meat, biopreservator.

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Received: 28 July 2017
Accepted: 14 September 2017
Published: 23 November 2017

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the ICSAFS Conference Committee.

1. Introduction

Meat as food, as well as processed meat products is one of the human needs for life. However, the high protein content makes meat and processed meat products can not last long, because it is easily destroyed by microorganisms. For that, we need a method or materials to preserve meat and processed meat products. During this time, a chemical used as a preservative, but the use of chemical preservatives have a risk to consumer health. It is estimated that the use of chemical preservatives deleterious to the health of consumers. Therefore, we need to find a way to preserve meat and processed meat products using natural preservative.

Lactic acid bacteria have been known to have a potency to preserve foodstuffs. It is like organic acids from the fermentation of sugars that lead to increased durability of fermented foods. The lactic acid bacteria have some preserving effects, by the reduction of pH and production of lactate and acetate as organic acids which are the primary inhibitory actions. Decreasing the pH value and produces organic acids, such as lactic acid and acetic acid, an inhibitor of bacterial decay; because few of these bacteria are in-resistant at low pH. Lactic acid bacteria also produce inhibitory substances, hydrogen peroxide, diacetyl, bacteriocins, and some secondary reaction products as hypothiocyanate [1].

Lactobacillus bacteria is one of the lactic acid bacteria that have an important role in preserving foods, preventing food poisoning [2].The lactic acid bacteria are a group of Gram-positive bacteria united by a constellation of morphological, metabolic, and physiological characteristics. Lactic acid bacteria produce various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocins during lactic acid fermentation [3]. The general description of the bacteria included in the group is Gram-positive, non-sporing, non-respiring cocci or rods, which produce lactic acid as the major end-product during the fermentation of carbohydrates. The boundaries of the group have been subject to some controversy, but historically the genera Lactobacillus, Leuconostoc, Pediococcus, and Streptococcus form the core of the group [4]. The classification of lactic acid bacteria into different genera is largely based on morphology, mode of glucose fermentation, growth at different temperatures, configuration of the lactic acid produced, ability to grow at high salt concentrations, and acid or alkaline tolerance [5]. One of the genera that fit the general description of the typical LAB in most respects is Lactobacillus. Classification of lactic acid bacteria is largely based on phenotypical and biochemical characters. Lactobacillus is the largest genus that well characterized with either homo- or hetero-lactic fermentation and comprising about 50 species; whereas each of the other six genera is monospecific

or contains only a few species. Most of the genera in the group exhibit unique characteristics which facilitate their differentiation and identification [6].

The lactobacilli, has great importance in manufacturing some products, cheese manufacturing or sausages manufacturing, besides have some preserving effects; because lactic acid bacteria has faster development and decrease the pH so produce microbiologically fermented products. Starter production in the industrial scale indicate future trends of lactic acid bacteria use in fermented products. This isolation and identification of lactic acid bacteria will be solved the problems to identify the genetic determinant of certain physiological characteristics in order to obtain a GRAS microorganism.

2. Materials and Methods

2.1. Isolation and identification of lactobacillus

The lactobacillus were isolated from fresh culled hens meat, 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 at 4°C for short term use. Twenty 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 [7]. 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 [8–11], fermentation of different carbon sources (API 50 CHL, bioMerieux SA, France), gas production from glucose, growth at different temperatures.

2.2. 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 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

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

Isolated strains	Identification	% ID	T-index
Lactobacillus fermentum	Acceptable identification	84.4	0.43
Lactobacillus paracasei ssp. *paracasei*	Acceptable identification	80.5	0.46
Lactobacillus plantarum	Acceptable identification	80.5	0.46
Lactobacillus rhamnosus	Acceptable identification	73.5	0.81
Lactobacillus lactis ssp. *lactis* 1	Acceptable identification	84.7	0.77
Lactobacillus lactis ssp. *lactis* 2	Acceptable identification	80.5	0.43

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).

2.3. 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 upside down, pressed on the top of the agar plates and kept again at 4°C for 1 h. The plates were incubated at 37°C overnight. Resistance was defined as the absence of a growth inhibition zone around the discs.

3. Results and Discussion

3.1. Lactic acid bacteria microflora

Thirty isolates of Lactic Acid Bacteria were isolated from the samples, and after series of purification on MRS agar, twenty 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 six isolates strains as 3 isolates of *Lactobacillus fermentum*, 2 isolates of *Lactobacillus paracasei* ssp. *paracasei*, 5 isolates of *Lactobacillus plantarum*, 3 isolates of *Lactobacillus rhamnosus*, 2 isolates of *Lactobacillus lactis* ssp. *lactis* 1, and 5 isolates of *Lactobacillus lactis* ssp. *lactis* 2.

Table 2: Antibiotic sensitivity of the bacterial isolates.

Nr	Antibiotics	Bacterial isolates strains											
		I		II		III		IV		V		VI	
1	AMP 10	23	S	27	S	24	S	25	S	19	S	20	S
2	CEC 30	22	S	18	S	19	S	20	S	21	S	22	S
3	CFP 30	24	S	22	S	24	S	22	S	25	S	26	S
4	CIP 5	11	R	13	R	15	R	15	R	10	R	12	R
5	CL 30	15	I	14	R	15	I	20	S	19	S	20	S
6	CN 10	6	R	8	R	6	R	8	R	10	R	9	R
7	DXT 30	20	S	28	S	20	S	28	S	18	S	19	S
8	E 15	6	R	26	S	26	S	6	R	11	R	12	R
9	K 30	6	R	6	R	8	R	6	R	11	R	13	R
10	KZ 30	23	S	30	S	23	S	25	S	20	S	22	S
11	OX 1	13	S	10	R	12	I	13	S	15	S	14	S
12	PB 100	6	R	1	R	6	R	1	R	5	R	8	R
13	RD30	24	S	26	S	30	S	28	S	22	S	24	S
14	SPC 100	20	S	16	I	10	R	16	I	11	R	13	R
15	TE 30	13	R	22	S	25	S	23	S	21	S	23	S
16	TM 5	1	R	4	R	6	R	6	R	9	R	10	R

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

From Table 1, there were the best six final identifications for each type of isolates strains on API gallery. The identified isolated strains were *Lactobacillus fermentum*, *Lactobacillus paracasei* ssp. *paracasei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus lactis* ssp. *lactis* 1 and *Lactobacillus lactis* ssp. *lactis* 2. *Lactobacillus fermentum* is thermophilic and heterofermentative bacteria, *Lactobacillus plantarum*, *Lactobacillus rhamnosus, Lactobacillus lactis* ssp. *lactis* 1 and *Lactobacillus lactis* ssp. *lactis* 2 are mesophilic and heterofermentative bacteria. If the starters has some factors determining the shelf life of products then they could improve the product developments.

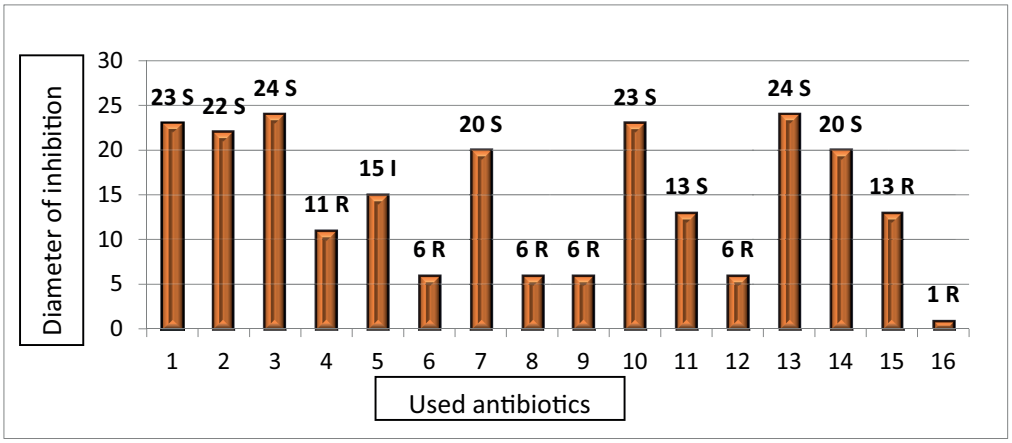
Table 2 presents the results of the antibiotics sensitivity of the six isolates strains. Isolated strains exhibited antibiotic sensitivity with the inhibition diameters obtained, are between 0 mm and 34 mm.

For Amphiciline (AMP 10), the resistance is <16 mm, no intermediate, and the sensitive is >17 mm. Cefachlor (CEC 30), the resistance is <14 mm, intermediate between 15– 17 mm and the sensitive is >18 mm. For Cefoperazone (CFP 30) the resistance is <15

mm, intermediate between 16–20 mm and the sensitive is >21 mm. Ciprofloxacine (CIP 5), the resistance is <15 mm, intermediate between 16–20 mm and the sensitive is >21 mm. Cefalexin (CL 30), the resistance is <14 mm, intermediate between 15–17 mm and the sensitive is >18 mm. Gentamicine (CN 10) the resistance is <12 mm, intermediate between 13–14 mm and the sensitive is >15 mm. Doxicicline (DXT 30) the resistance is <12 mm, intermediate between 13–15 mm and the sensitive is >16 mm. Erytromicine (E 15), the resistance is <13 mm, intermediate between 14–22 mm and the sensitive is >23 mm. Kanamicine (K 30) the resistance is <13 mm, intermediate between 14–17 mm and the sensitive is >18 mm. Cefazoline (KZ 30) the resistance is <14 mm, intermediate between 15–17 mm and the sensitive is >18 mm. Oxaciline (OX 1), the resistance is <10 mm, intermediate between 11–12 mm and the sensitive is >13 mm. Polimixine (PB 100), the resistance is <8 mm, intermediate between 9–11 mm and the sensitive is >12 mm. Rifampicine (RD 30) the resistance is <16 mm, intermediate between 17–19 mm and the sensitive is >20 mm. Spectinomicine (SPC 100), the resistance is <13 mm, intermediate between 14–17 mm and the sensitive is >18 mm. Tetracicline (TE 30), the resistance is <14 mm, intermediate between 15–18 mm and the sensitive is >19 mm. Trimethoprim (TM 5), the resistance is <10 mm, intermediate between 11–15 mm and the sensitive is >16 mm [6].

From Table 2, Isolate I showed sensitivity reaction to Amp 10, CEC 30, CFP 30, DXT 30, KZ 30, OX 1, RD 30, SPC 100, intermediate to CL 30, and resistance to CIP 5, CN 10, E 15, K 30, PB 100, TE 30, and TM 5. And the Isolate II, showed sensitivity to AMP 10, CEC 30, CFP 30, DXT 30, E 15, KZ 30, RD 30, and TE 30, but showed intermediate to SPC 100, and resistance to CIP 5, CL 30, CN 10, K 30, OX 1, PB 100, and TM 5. Isolate III showed sensitivity to AMP 10, CEC 30, CFP 30, CN 10, DXT 30, E 15, KZ 30, RD 30, and TE 30, intermediate to CL 30 and OX 1, and resistance to K 30. Isolate IV showed sensitivity reaction to AMP 10, CEC 30, CFP 30, CL 30, DXT 30, KZ 30, OX 1, RD 30, and TE 30; showed intermediate to SPC 100, and resistance to CIP 5, CN 10, E 15, K 30, PB 100, and TM 5. The isolate V showed sensitivity reaction to AMP 10, CEC 30, CFP 30, CL 30, DXT 30, KZ 30, OX 1, RD 30, and TE 30, resistance to CIP 5, CN 10, E 15, K 30, PB 100, SPC 100, and TM 5. Isolate VI showed sensitivity reaction to AMP 10, CEC 30, CFP 30, CL 30, DXT 30, KZ 30, OX 1, RD 30, and TE 30; and showed resistance to CIP 5, CN 10, E 15, K 30, PB 100, SPC 100, and TM 5. In the Figures 1 to 6, there are the results about the six isolates strains to sixteen different antibiotics.

From the Figure 1, the isolates I, has sensitivity to eight antibiotics, one intermediate and seven resistance. Isolate I showed sensitivity reaction to Amp 10, CEC 30, CFP 30, DXT 30, KZ 30, OX 1, RD 30, SPC 100; intermediate to CL 30, and resistance to CIP 5, CN 10, E 15, K 30, PB 100, TE 30, and TM 5. Figure 2 shows the results of the diameter inhibitions of Isolates II.



Notes : R = resistance, I = intermediate, S = sensitive

1. AMP 10	5. CL 30	9. K 30	13. RD30
2. CEC 30	6. CN 10	10. KZ 30	14. SPC 100
3. CFP 30	7. DXT 30	11. OX 1	15. TE 30
4. CIP 5	8. E 15	12. PB 100	16. TM 5

Figure 1: Diameter of inhibition zone of Isolate I to all antibiotics.

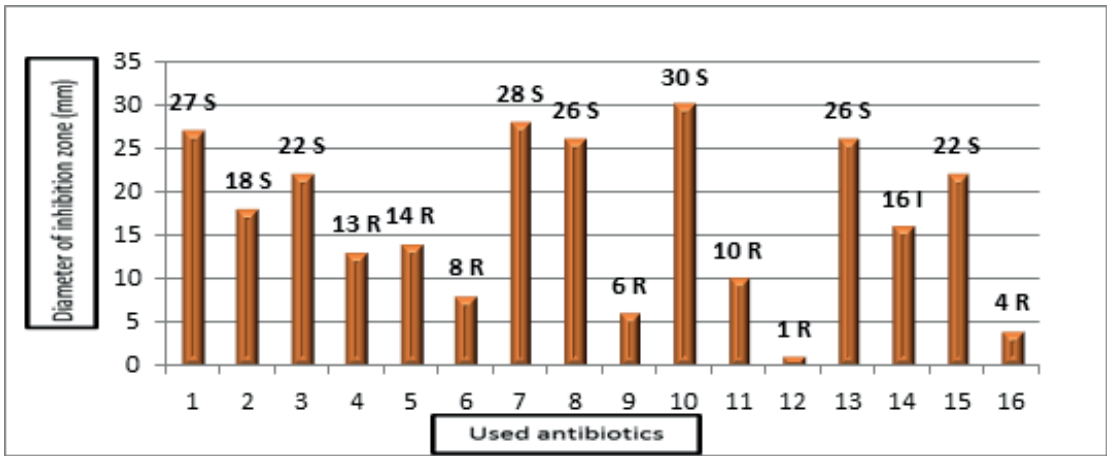


Figure 2: Diameter of inhibition zone of Isolate II to all antibiotics.

From the Figure 2, the Isolates II were resistance to six antibiotics, three intermediate and seven sensitivities. The Isolate II, showed sensitivity to AMP 10, CEC 30, CFP 30, DXT 30, E 15, KZ 30, RD 30, and TE 30, but showed intermediate only to SPC 100, and resistance to CIP 5, CL 30, CN 10, K 30, OX 1, PB 100, and TM 5. Figure 3 showed diameter of inhibition of the Isolate III.

From the Figure 3, the Isolates III had intermediate reaction to 2 antibiotics, but had sensitivity reaction to eight antibiotics, and had resistance to six antibiotics. Isolate III showed sensitivity to AMP 10, CEC 30, CFP 30, CN 10, DXT 30, E 15, KZ 30, RD 30, and TE 30, intermediate to CL 30 and OX 1, and resistance to K 30. Figure 4 showed the results of Isolates IV to sixteen different antibiotics.

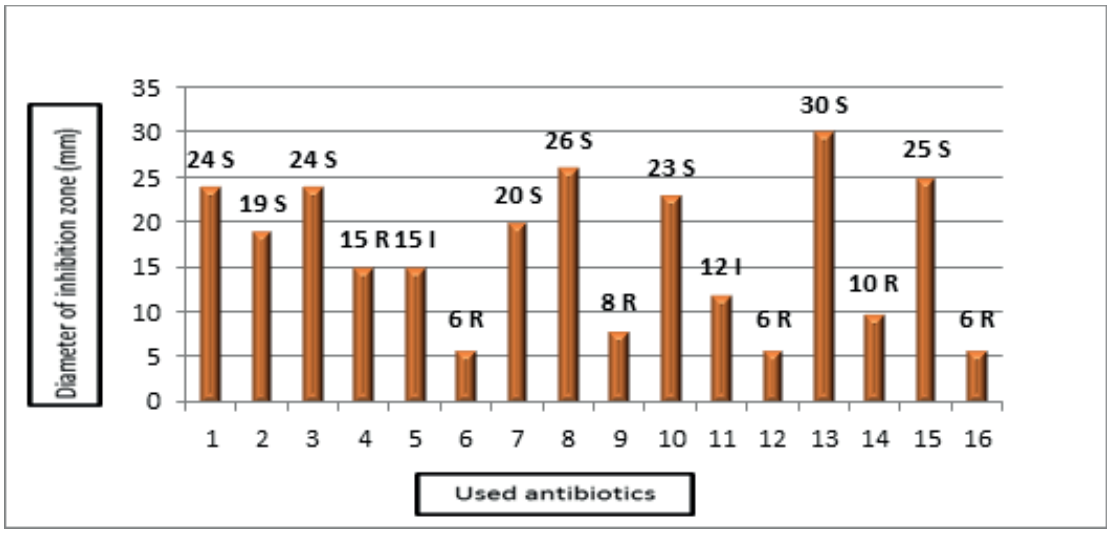


Figure 3: Diameter of inhibition zone of Isolate III to all antibiotics.

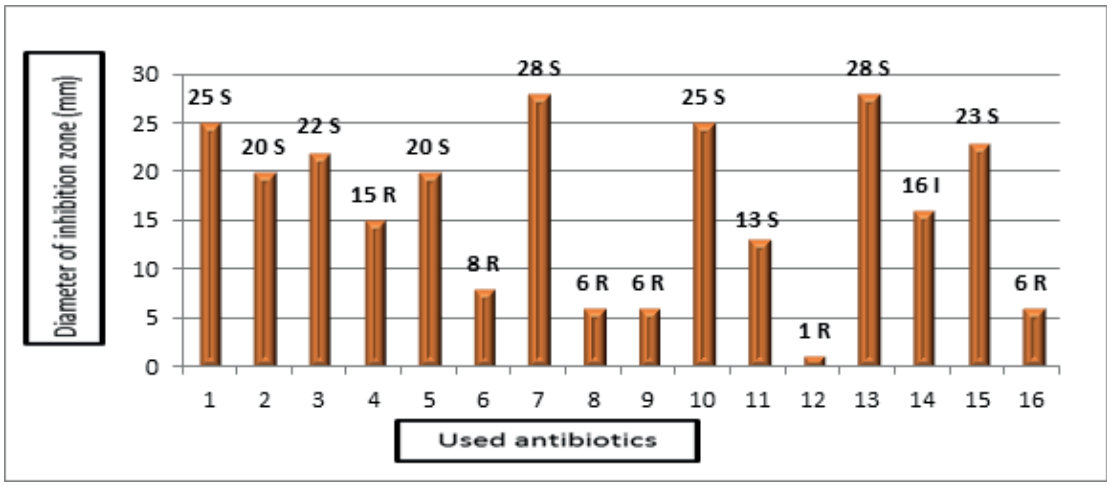


Figure 4: Diameter of inhibition zone of Isolate IV to all antibiotics.

From the Figure 4, the Isolates IV had sensitivity to nine antibiotics, one intermediate and six resistance. Isolate IV showed sensitivity reaction to AMP 10, CEC 30, CFP 30, CL 30, DXT 30, KZ 30, OX 1, RD 30, and TE 30, showed intermediate to SPC 100, and resistance to CIP 5, CN 10, E 15, K 30, PB 100, and TM 5. Figure 5 showed the presence of inhibition zone of Isolate V to all antibiotics.

From the Figure 5, the Isolates V were found to be resistance to seven antibiotics. It had no intermediate and sensitivities to nine antibiotics. The Isolate V showed sensitivity reaction to AMP 10, CEC 30, CFP 30, CL 30, DXT 30, KZ 30, OX 1, RD 30, and TE 30, then showed resistance to CIP 5, CN 10, E 15, K 30, PB 100, SPC 100, and TM 5. Figure 6 showed the diameter of inhibition zone of Isolate VI to all antibiotics.

From the Figure 6, the Isolates VI, had no intermediate reaction to antibiotics, but had sensitivity reaction to nine antibiotics, and had resistance to seven antibiotics. Isolate VI showed sensitivity reaction to AMP 10, CEC 30, CFP 30, CL 30, DXT 30, KZ 30, OX 1,

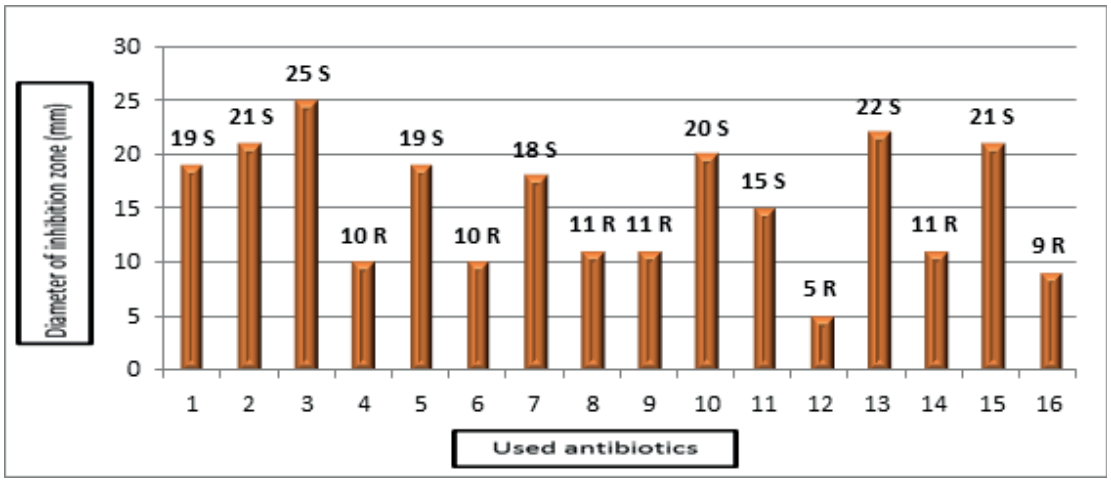


Figure 5: Diameter of inhibition zone of Isolate V to all antibiotics.

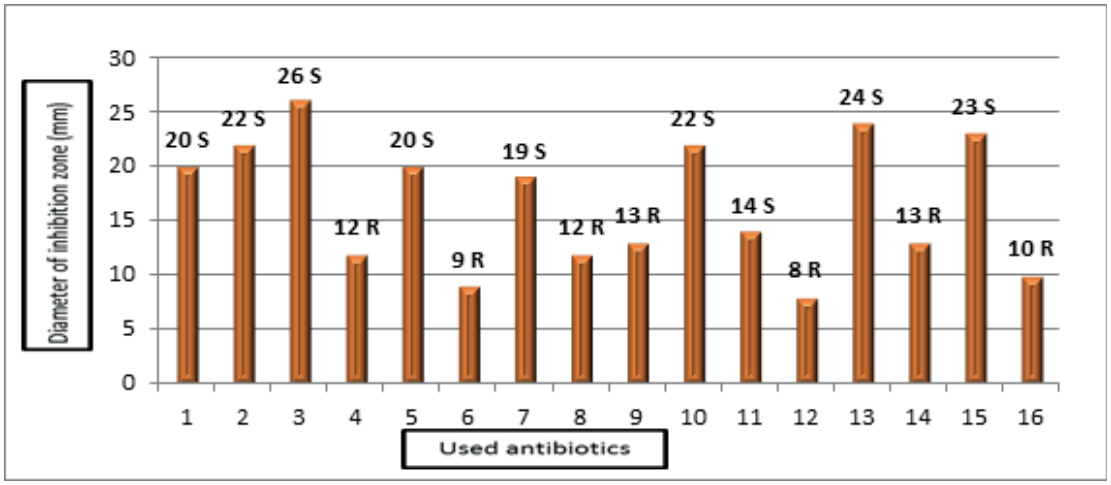


Figure 6: Diameter of inhibition zone of Isolate VI to all antibiotics.

RD 30, and TE 30, then showed resistance to CIP 5, CN 10, E 15, K 30, PB 100, SPC 100, and TM 5.

4. Conclusions

The research is vital in the sense that functional properties in lactic acid bacteria improve the preservative effect to the meat and meat products. The results obtained in this study revealed the presence of a wide variety of Lactic acid bacteria (LAB) in the poultry meat. Some of the isolated and identified LAB (*Lactobacillus fermentum*, *Lactobacillus paracasei* ssp. *paracasei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus lactis* ssp. *lactis* 1 and *Lactobacillus lactis* ssp. *lactis* 2) showed outstanding performances that were similar and in some cases was higher performances as biopreservatives. *Lactobacillus* bacteria is one of the lactic acid bacteria, have had an important role in preserving foods and preventing food poisoning, by the reduction

of pH and production of lactate and acetate as organic acids which are the primary inhibitory actions. Lactic acid bacteria also produce inhibitory substances, hydrogen peroxide, diacetyl, bacteriocins, and some secondary reaction products as hypothiocyanate. Antimicrobial compounds produced by LAB have provided these organisms with a competitive advantage over other microorganisms. In conclusions, twenty LAB isolates from the poultry meat were capable of producing enough amount of bacteriocins that have been anticipated to have enormous potential for applications as biopreservatives. The lactic acid bacteria have an essential role in meat fermentation processes, known as food preservation of fermented foods, and the isolated strains can positively have impact on their use as starter cultures for fermented food especially for meat products, with a view to improve the hygiene and safety of fermented produce. The results of this study open the possibility for the application of lactic acid bacteria in some industrial use. In sausage products, isolated and purified antimicrobial substances could be added.

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