



## Research Article

# Antibacterial Activity of Lactobacillus Species Isolated from Poultry Waste (Droppings) Against Poultry Pathogens

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## Abstract

This study aimed to provide information on antibacterial activity of lactobacilli from poultry wastes against poultry pathogens. A bacteriocin-producing lactobacilli strain were isolated from poultry droppings. Activity against poultry pathogens was carried out using both agar well diffusion and disk paper method. Eight species of lactobacilli were isolated, characterized and identified from poultry waste and were screened for their antibacterial potency. Out of the eight species only *L. buchneri*, *L. helveticus*, *L. delbreuchi* and *L. acidophilus* were active against all the tests organisms. The zone of inhibition ranging from  $3.00 \pm 0.00$  to  $35.17 \pm 0.17$  mm with the highest potency observed on *L. delbruckii* ( $35.17 \pm 0.17$  mm) against *E. coli* while the least zone of inhibition against *Salmonella typhi* with  $3.00 \pm 0.00$  mm. All isolated lactobacilli demonstrated inhibition against one or more selected pathogens, they can, therefore, be used as a means to control of poultry pathogens. The results suggest the bacteriocins from isolated species could be useful for the production of antibacterial agents in poultry production.

**Key words:** Poultry, antibacterial, lactobacillus, metabolites, pathogens, isolates

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The place of poultry in the livestock sector of any nation cannot be under rated. This is important not only in term of economic activities, but also in the quest for attaining food security in terms of protein consumption, both meat and eggs (Banjoko *et al.*, 2014). Eggs, a major products of poultry production, are one of the most nutritious and complete foods known to man. Hence, the poultry sector could be a panacea to protein deficiency, which is a major challenge to food security and is particularly critical in Nigeria. This is critical in Nigeria where the current per capita consumption of animal protein is only 10 g day<sup>-1</sup> compared to the 34 g day<sup>-1</sup> recommended by the FAO as the minimum for healthy living (FAO., 2014, Owen and Dike, 2013). The high turnover rate and the quest for white meat have given more credence to poultry among livestock farming. The need to meet up with the demand for poultry meat has stimulated the large-scale production of poultry and subsequent use of veterinary drugs, especially antimicrobials (Ezenduka *et al.*, 2014).

Currently, one of the problems confronting operation and progress in the poultry production is the antimicrobial resistance pose by the indiscriminating use of antibiotics. Although the economic and health advantages of using antibiotics have revolutionized intensive poultry production (Oluwasile *et al.*, 2014), the increase used of antibiotics as a part of the poultry and other livestock production industries to treat and prevent both bacterial and fungi infections has led to the problem of the development of bacterial antibiotic resistance over time. This then create the necessity of the search for alternative agents that will not pose any harmful effects such agent may include bacteriocins from *Lactobacillus* spp. Lactobacilli are the major type of lactic acid bacteria, which have been shown to act as a preservative as well as a probiotic agent (Kumar *et al.*, 2014). Probiotics are products used as dietary supplements to enhance the growth and health of humans and animals. They have been shown to be important in disease control, as digestion aids, immune booster and in supplementing or replacing the use of antimicrobial compounds in the field of health (Chantharasophon *et al.*, 2011). The present study was carried out with the objective to examine the antibacterial activity of *Lactobacillus* species isolated from poultry droppings in the treatment of poultry pathogens.

## MATERIALS AND METHODS

**Collection of samples:** Poultry waste (dropping) was collected at Federal University of Technology Akure research and

teaching farm from six week old broilers. The sample was collected in polythene nylon and transported to laboratory. The sample was serially diluted by dissolving 1 g in 9 mL of distilled water and 1 mL was inoculated into deMan Rogosa Sharpe (MRS) agar.

**Test organisms:** The test organisms (poultry pathogens) used in this work were collected from the Department of Microbiology Federal Institute of Industrial Research Oshodi Lagos and were preserved under refrigeration condition until use. These include *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 25923, *Salmonella typhi* ATCC 6539, *Shigella flexneri* ATCC 12022.

**Standardization of inoculum:** The inocula were prepared from the stock cultures, which were maintained on nutrient agar slant at 4°C and subcultured onto nutrient broth using a sterilized wire loop. The density of suspension inoculated onto the media for susceptibility test was determined by comparison with 0.5 McFarland standard of Barium chloride solution (Cheesbrough, 2006).

**Isolation and identification of lactobacilli:** The isolation of lactobacilli from poultry dung was done using MRS (peptone, meat extract, yeast extract, glucose, tween 80) medium. Briefly, 1 g of dung was serially diluted and 1 mL was inoculated anaerobically at 37°C for 48 h. The observed colony was tested for catalase. Growth from MRS agar cultures was subculture for pure isolate and then grown on MRS broth (Nowroozi *et al.*, 2004). *Lactobacillus* species of these isolates were identified by comparing their sugar fermentation patterns (Oyetayo *et al.*, 2003) with the scheme described in Bergey's Manual of Systematic Bacteriology.

**Preparation of cell free supernatant:** The method of Elamathy and Kanchana (2012) was used. The bacteriocin producing strain was grown in MRS broth anaerobically at 37°C for 48 h. A cell free solution was obtained by centrifuge the culture at 12000 g for 12 min under 40°C followed by filtration of the supernatant through whatmann filter paper. The supernatant was then store in refrigerator.

**Antimicrobial activity of LAB:** The method of Duraiswamy *et al.* (2010) was employed. The agar well diffusion assay, a 48 h culture of the indicator strain was used to inoculate nutrient agar growth media at 37°C. Wells of 6 mm diameter were cut into agar plates and 50 µL of culture supernatant fluid containing antibacterial activity were added to each well. Supernatant fluid was obtained by growing the

inhibitory producer strain 48 h in MRS broth at 37°C. Cells were then removed by centrifugation and the supernatant fluid placed in the wells and allowed to diffuse into the agar for 24 h at 4°C. The plates were then incubated at optimum growth temperature of the indicator strains and examined after 24 h for inhibition zone. The plates were incubated for 24 h at 37°C. Antimicrobial activity was evaluated by measuring the inhibition zone in millimeter in diameter and recorded. Plates were incubated and observed for zones inhibition.

## RESULTS

Table 1 and 2 shows the morphology and biochemical characteristics of the isolated lactobacilli. All are rod shaped with positive reaction to Gram reaction. In reaction to sugar utilization, 87% of the isolates can utilize arabinose, 50% can utilize fructose and mannitol, 62% utilized sucrose and maltose, 100% can utilize lactose and glucose while 75% can utilize galactose respectively. Among the isolates only 37.5% are motile while 50% can utilize citrate. None of the isolates form spore nor produce indole. While 50% can grow under the temperature of 20°C, all can grow at 37°C. As some of the isolates grow in certain salt concentration some cannot grow, however 50% of the isolate completely grow in all the salt concentration.

**Antagonistic activity of lactobacillus metabolites:** The result of antibacterial activity of lactobacillus metabolite was shown

in Table 3 after 24 h of incubation. The agar well diffusion and paper disk method was used to carry out the antibacterial activities against the test organisms namely *Escherichia coli*, *Staphylococcus aureus*, *Shigella flexneri* and *Salmonella typhi*. All the metabolites showed good inhibitory potency against selected pathogens. The zone of inhibition ranging from 12.00±0.00 to 35.17±0.29 mm with the highest potency observed on *L. delbrückii* (35.17±0.29 mm) against *E. coli* while the the least zone of inhibition (12.00±0.00 mm) was observed against *Staphylococcus aureus*, *Shigella flexneri* and *Salmonella typhi* by *L. caucasicus* and *L. buchneri*.

Among the metabolites, *L. pastori* and *L. acidophilus* exhibited antibacterial potency against *Salmonella typhi* only. In term of resistance *E. coli*, *Staphylococcus aureus* and *Shigella flexneri* showed sign of resistance to *L. pastori* and *L. acidophilus*. *E. coli* also exhibited sign of resistance to *L. caucasicus* and *L. pastori*.

Disk method was presented in Table 4 the zone of inhibition ranging from 3.00±0.00 to 26.67±0.64 mm with *platanrum* showed potency against *Salmonella typhi*. All the pathogens showed resistance to *L. pastori* and *L. acidophilus*. *Escherichia coli*, *Staphylococcus aureus* and *Shigella flexneri* also showed resistance to *L. pastori* and *L. platanrum*.

## DISCUSSION

Lactic Acid Bacteria (LAB) are one of the most important groups of microorganisms to mankind, being part of normal

Table 1: Morphological and colonies characteristics of Isolated *Lactobacillus* spp.

Isolates	Colony shape	Elevation	Edge	Optical characteristics	Colony surface	Pigmentation
A	Rod	Flat	Circular	Translucent	Smooth	Milky
B	Long rod	Flat	Irregular	Translucent	Smooth	Grey
C	Short rod	Raised	Rough	Translucent	Smooth	Milky
D	Short rod	Flat	Rough	Translucent	Smooth	Milky
E	Rod	Flat	Rough	Opaque	Smooth	Whitish
F	Rod	Flat	Circular	Opaque	Smooth	Milky
G	Rod	Flat	Circular	Opaque	Smooth	Grey
H	Slender rod	Flat	Rough	Translucent	Smooth	Grey

Table 2: Biochemical tests

Isolates	Sugars				Other tests				Temperature		Salt concentration					GR						
	AR	FR	MA	SU	LA	ML	GA	GL	CIT	MOT	SPO	IND	20°C	37°C	1%	2%	3%	4%	5.6%	6.5%	+ve rod	
A	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	+ve rod
B	+	-	-	-	+	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	+ve rod
C	+	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+ve rod
D	+	-	+	-	+	-	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+ve rod
E	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+ve rod
F	-	-	-	+	+	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	+ve rod
G	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+ve rod
H	+	+	-	+	+	+	-	+	+	-	-	-	+	+	-	-	-	+	+	+	+	+ve rod

A: *Lactobacillus caucasicus*, B: *Lactobacillus pastori*, C: *Lactobacillus buchneri*, D: *Lactobacillus helveticus*, E: *Lactobacillus plantarum*, F: *Lactobacillus delbrevechi*, G: *Lactobacillus acidophilus*, H: *Lactobacillus brevis*, AR: arabinose, FR: Fructose, MA: Mannitol, SU: Sucrose, LA: Lactose, ML: Maltose, GA: Galactose, GL: Glucose, CIT: Citrate, MOT: Motility, SPO: Spore, IND: Indole, GR: Gram reaction, +: Growth occurred, -: No growth occur

Table 3: Antibacterial effect of lactobacillus metabolites against selected pathogens (agar well diffusion method)

Metabolite of Isolates <i>Typhi</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Shigella flexneri</i>	<i>Salmonella typhi</i>
A	0.00±0.00 <sup>Aa</sup>	12.00±0.00 <sup>Bb</sup>	12.00±0.00 <sup>Bb</sup>	13.00±0.00 <sup>Bc</sup>
B	0.00±0.00 <sup>Aa</sup>	0.00±0.00 <sup>Aa</sup>	0.00±0.00 <sup>Aa</sup>	14.00±0.00 <sup>Cb</sup>
C	18.00±0.00 <sup>Cb</sup>	12.00±0.00 <sup>Ba</sup>	12.00±0.00 <sup>Ba</sup>	12.00±0.00 <sup>Aa</sup>
D	18.33±0.17 <sup>Dd</sup>	14.12±0.44 <sup>Ca</sup>	17.33±0.17 <sup>Ec</sup>	16.83±0.17 <sup>Fb</sup>
E	0.00±0.00 <sup>Aa</sup>	14.33±0.17 <sup>Cc</sup>	13.00±0.00 <sup>Cb</sup>	15.00±0.00 <sup>Dd</sup>
F	35.17±0.17 <sup>Ed</sup>	28.17±0.17 <sup>Eb</sup>	27.53±0.33 <sup>Fa</sup>	32.67±0.17 <sup>Gc</sup>
G	0.00±0.00 <sup>Aa</sup>	0.00±0.00 <sup>Aa</sup>	0.00±0.00 <sup>Aa</sup>	13.07±0.07 <sup>Bb</sup>
H	14.00±0.00 <sup>Ba</sup>	18.00±0.00 <sup>Dd</sup>	15.00±0.00 <sup>Dd</sup>	16.00±0.00 <sup>Ec</sup>
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Data are presented as Means ± SE, n = 3, mean with the same superscript letter (s) along the same row (lower case) or column (upper case) are not significantly different ( $p \leq 0.05$ ), A: *L. caucasicus*, B: *L. pastoriurus*, C: *L. buchneri*, D: *L. helveticus*, E: *L. platanrum*, F: *L. delbreuchi*, G: *L. acidophilus*, H: *L. brevis*

Table 4: Antibacterial effect of lactobacillus metabolites against selected pathogens (Disk method)

Metabolites of Isolate	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Shigella flexneri</i>	<i>Salmonella typhi</i>
A	0.00±0.00 <sup>Aa</sup>	0.00±0.00 <sup>Aa</sup>	0.00±0.00 <sup>Aa</sup>	0.00±0.00 <sup>Aa</sup>
B	0.00±0.00 <sup>Aa</sup>	0.00±0.00 <sup>Aa</sup>	0.00±0.00 <sup>Aa</sup>	6.67±3.33 <sup>Cb</sup>
C	14.97±0.00 <sup>Cc</sup>	0.00±0.00 <sup>Aa</sup>	0.00±0.00 <sup>Aa</sup>	11.79±0.02 <sup>Db</sup>
D	12.00±0.00 <sup>Bd</sup>	0.00±0.00 <sup>Aa</sup>	9.90±0.00 <sup>Cb</sup>	10.90±0.01 <sup>Db</sup>
E	0.00±0.00 <sup>Aa</sup>	0.00±0.00 <sup>Aa</sup>	0.00±0.00 <sup>Aa</sup>	10.90±0.01 <sup>Db</sup>
F	19.77±0.14 <sup>Db</sup>	16.83±0.43 <sup>Ca</sup>	26.67±0.37 <sup>Dd</sup>	20.00±0.00 <sup>Ec</sup>
G	0.00±0.00 <sup>Aa</sup>	0.00±0.00 <sup>Aa</sup>	0.00±0.00 <sup>Aa</sup>	0.00±0.00 <sup>Aa</sup>
H	0.00±0.00 <sup>Aa</sup>	9.34±0.24 <sup>Bc</sup>	9.00±0.00 <sup>Bc</sup>	3.00±0.00 <sup>Bb</sup>
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Data are presented as Means ± SE, n = 3, mean with the same superscript letter (s) along the same row (lower case) or column (upper case) are not significantly different ( $p \leq 0.05$ ), A: *L. caucasicus*, B: *L. pastoriurus*, C: *L. buchneri*, D: *L. helveticus*, E: *L. platanrum*, F: *L. delbreuchi*, G: *L. acidophilus*, H: *L. brevis*

flora, contained antimicrobial substance that has inhibitory effect on growth of pathogens (Darsanaki *et al.*, 2012). The isolated, tested Lactobacilli in this study displayed a varied antibacterial potency against selected poultry pathogens. Lactobacilli are known for their production of various antimicrobial compounds (Pangallo *et al.*, 2008). The production of these compounds by intestinal microflora is probably one of the most important mechanisms responsible for the antagonistic phenomenon (Gomes *et al.*, 2006) and therefore it is essential to examine this property in probiotic candidates.

Among the isolated lactobacillus spp. *Lactobacillus helveticus* showed good inhibitory spectrum against all the test organisms. This result was similar to what obtained by Nikolova *et al.* (2009) while working on the antibacterial activity of the *Lactobacillus helveticus* against *E. coli* and reported that this inhibitory effect has generally been attributed to the production of lactic acid during the Lactobacillus growth. *Lactobacillus plantarum* showed strong inhibition against selected pathogen. This broad spectrum of *L. plantarum* has been reported by different researchers; (Alvarado *et al.*, 2006; Lade *et al.*, 2006; Bharathi *et al.*, 2011). However, in this study *L. plantarum* did not show any inhibitory effect against *E. coli* this may be connected to the fact that the antimicrobial effect of some strains of Lactobacillus may be completed with the production of relevant concentration of lactic acid in the micro environment,

which in combination also inhibit the growth of Gram-negative pathogenic bacteria such as *E. coli* this is in agreement with the work of Nouri who reported that the *Lactobacillus* spp. isolated from gizzard and crop did not show any inhibitory effect against *E. coli* (Nouri *et al.*, 2010). Although Gilliland and Speck (1977) had earlier reported that Lactobacilli showed stronger antibacterial properties against Gram positive bacteria (*S. aureus*) than Gram negative bacteria (*E. coli* and *Shigella flexneri*). Anas *et al.* (2008) reported strong inhibitory effect of *Lactobacillus plantarum* in the work title antibacterial activities of *Lactobacillus* spp. Isolated from Algeria raw goat milk against *S. aureus*. Ravaei *et al.* (2013) reported *L. plantarum* as a strong inhibitor to *Salmonella thyphimurium*. Among the mechanisms causing antibacterial effect by lactobacillus species, the *in vitro* investigable mechanisms include production of bacteriocin, bacteriocin like substances, hydrogen peroxide and excretion of lactic acid that have examined by this study were not effective against *E. coli* this could be due to fact that the antimicrobial effect of some strains may be completed with the production of relevant concentration of lactic acid or different metabolites in the microenvironment, which in combination inhibit the growth of Gram-negative pathogenic bacteria (Alakomi *et al.*, 2000).

*Lactobacillus acidophilus* against indicator organisms showed resistant except for *S. typhi*. This result was not in agreement with Lonkar *et al.* (2005). He reported that

*Lactobacillus acidophilus* was active against *E. coli* in a related study carried out by Mobarez *et al.* (2008). *Lactobacillus acidophilus* isolated from yoghurt exhibited antibacterial activities against *S. aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Bacillus cereus*. An earlier study with bacteriocin from *L. acidophilus* has been shown to have intermediate activity against *S. aureus* (Aslim *et al.*, 2005). The ability of *L. acidophilus* to prevent proliferation of pathogenic bacteria in the gut environment has been documented (Vila *et al.*, 2009). In addition to lactic acid, *L. acidophilus* has the capacity to produce numerous metabolites that kill pathogenic bacteria. For example bacteriocidal proteins termed bacteriocin are produced by some strain of *L. acidophilus* (Gukasian *et al.*, 2002). Among *Lactobacillus*, strain belong to species of *L. acidophilus* are frequently used as a probiotic agent (Klaenhammer and Kullen, 1999). Bacteriocin production and antibacterial activity has shown for *L. acidophilus* isolated from intestinal tract (Barefoot and Klaenhammer, 1983).

*Lactobacillus delbrueckii* showed broad inhibitory effect against all the selected pathogens. This finding is in agreement with Bharathi *et al.* (2011). Likewise *L. brevis* was part of those inhibiting both Gram positive and Gram negative bacteria in this study. This is in agreement with other related researchers including. Lade *et al.* (2006), who reported that *Lactobacilli* species are effective against *E. coli* while, study some properties of bacteriocins produced by *Lactobacillus* species isolated from agro-based waste. Likewise, Alvarado *et al.* (2006) reported that *Lactobacillus* strains isolated from traditional Mexican foods are able to show inhibition against at least one pathogenic indicator microorganism while working on food-associated lactic acid bacteria with antimicrobial potential from traditional foods. The primary antimicrobial effect exerted by *Lactobacillus* is due to a combination of many factors e.g., production of lactic acid with reduce pH, production of various antimicrobial compounds, which can be classified as (1) Low-molecular-mass compounds such as hydrogen peroxide and carbon dioxide and (2) High-molecular-mass compounds such as bacteriocins which are responsible for the most antimicrobial activities (Ahmed *et al.*, 2012). The levels of production of organic acids by *Lactobacillus* depend on species or strain, culture composition and growth conditions (Ogunbanwo, 2005; Ammor *et al.*, 2006). Antimicrobial action of bacteriocins occurs in steps-adsorption of the bacteriocin on cell wall, its transport across the cell membrane and finally its action within the cytoplasm (Garcha and Sharma, 2013).

## CONCLUSION

*Lactobacillus* isolates exhibiting antibacterial activity in this study constitute an interesting trait that deserves more investigation to demonstrate the probiotic function that can improve the health as well as increase performance parameters in poultry production

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