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## Inhibitory potentialities of lactic acid bacteria isolates from quail caeca (*Cortunix cortunix japonica*) on the growth of antibiotic resistant pathogens in Côte d'Ivoire

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### Abstract

The emergence of multi-antibiotic resistant microorganisms is a major threat to public health. The search for alternative molecules to the use of classical antibiotics has become a necessity. The present work explores the possibility of using bioactive substances produced by lactic acid bacteria isolated from the gastrointestinal tract of quail, giving them the ability to combat resistant pathogens. To this end, microbiological analysis of quail caeca on MRS agar for lactic acid bacteria isolates was carried out. Subsequently, direct and indirect confrontation tests carried out on solid and liquid MRS media (Difco, USA) against pathogenic strains of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enterica* serogroup O:8, by means of lactic acid bacteria isolates, revealed their potential antibacterial activity. Of the 29 bacterial isolates obtained after microbiological analysis, ten (10) lactic acid bacterial isolates showed excellent antibacterial potential against the target pathogens, with zones of inhibition ranging from 3.5±0 to 25.5 ±0.5 mm. The lactic acid bacterial isolates C6S3, C4S2, C6S4 and C13S4 proved to be holders of substances with antagonistic activity against the growth of the 3 target pathogens, with diameters of the inhibition zones varying from 5.5 ± 1 to 13 ± 0.5 mm for the *Escherichia coli* strain, from 4 ± 0 to 13 ± mm for the *Salmonella* serogroup O: 8 and from 3.5 ± 0.5 to 7 ± 0 mm for the *Staphylococcus aureus* strain. Therefore, the suspicion of a plausible presence of bioactive substances with antibacterial characteristics opens up avenues of exploration of the extracellular content of these isolates, with the simple aim of listing potential constituents that could be excellent alternatives in the fight against multidrug resistance to antibiotics.

**Keywords:** quail caeca (*Cortunix cortunix japonica*), lactic acid bacteria, bioactive substances, resistant pathogens.

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### Introduction

Food is an environment in which several bacteria can coexist. Some of these microorganisms, such as *Escherichia coli*, *Salmonella* and *Campylobacter*, cause food poisoning. Antibiotics have been an excellent means of blocking all these harmful microorganisms for some decades. However, there is a great danger of resistance developing in these microorganisms (Benmouna, 2012) <sup>[5]</sup>. Indeed, this resistance in pathogenic bacteria can be transferred to other bacteria, causing disease in humans. Thus, the possible transfer of this resistance to humans remains a growing problem worldwide. Infections caused by these resistant bacteria have been associated with increased morbidity and mortality in humans (Helms *et al*, 2002) <sup>[17]</sup>.

There are many disease problems associated with bacterial infections in poultry. Also, the overuse and misuse of antibiotics for the treatment of these diseases is the cause of the emergence of resistance in the pathogens involved. (Coffie, 2008) <sup>[11]</sup>.

From this point of view, the control of bacterial infections through the consumption of animals, in particular poultry, becomes complex, which poses a real public health problem, hence the need to look for alternative ways to this use.

As an alternative to the overuse of antibiotic molecules, lactic acid bacteria would seem to be the most appropriate.

Indeed, lactic acid bacteria, found in many microbial ecosystems, have the particularity of predominating in rather rich environments such as milk and its derivatives, plants, human and animal cavities. Also, the digestive tract of mammals, like that of birds, contains a microbial population extremely rich in lactic acid bacteria, with the capacity to produce inhibiting substances against these pathogens, thus limiting the proliferation of pathogenic germs in the intestinal tract of these animals (Andrieu, 1995; Pascual *et al.*, 1999) <sup>[22]</sup>. As a result, the search for new alternatives to the use of these antibiotic molecules, focusing on lactic bacteria with antibacterial potential, is becoming an avenue to explore.

## Materials and Methods

### Sampling of quails *Coturnix coturnix japonica*

Samples of digestive tracts of quails were taken in a poultry farm in SANTAI, in the peri-urban area of Bingerville (Côte d'Ivoire). Non-random sampling was used to take three samples over a period of three weeks on the farm, at a rate of five samples per week. Once the quails had been slaughtered and eviscerated, the various digestive tracts were collected using sterile gloves and scalpels, then placed in sterile plastic bags taken individually and deposited in a cooler containing cold accumulators at 4°C.

### Isolation of lactic acid bacteria strains from quail caeca

Once in the laboratory, 2g of caeca are taken and weighed with a METTLER electronic balance of 0.001 precision and put in 18 mL of MRS broth (Difco, USA), which constitutes the stock solution at 10<sup>-1</sup>. Subsequently, successive dilutions of the stock solution are plated on MRS agar plates at a rate of 1 mL per dilution.

### Phenotypic identification of lactic acid bacteria isolates

Preliminary identification tests, i.e. macroscopic examination for shape and colour by the Gram staining technique, the appearance of presumptive colonies observable with the naked eye on MRS agar (Difco, USA) and physiological tests, such as the catalase test and the fermentative type of the presumptive colonies, made it possible to confirm their membership of the lactic acid bacteria group.

### Screening of lactic acid bacteria isolates with inhibitory activities

The inhibitory power of the lactic acid bacteria isolates was demonstrated by direct and indirect contact with indicator strains that are multi-resistant to antibiotic molecules, including *Salmonella enterica* serogroup O:8 and *Escherichia coli* strains from the Central Veterinary Laboratory of Bingerville; and the *Staphylococcus aureus* strain from the Bio banque of the Pasteur Institute of Côte d'Ivoire (Table 1).

**Table 1:** Antibiotic resistance profile of indicator strains in this study

Indicator strains	Antibiotic resistance profile
<i>Salmonella enterica</i> sérogruppe O: 8	A-AMC-TIC-CF-CTX-G-C-SXT-TE-NAL-CIP
<i>Escherichia coli</i>	FOX-TET-SXT-AMP
<i>Staphylococcus aureus</i>	G-FOX-TE-VA-FA

A: Amoxicillin; AMC: Amoxicillin/Clavulanic acid; TIC: Ticarcillin; C: Chloramphenicol; CF: Cephalotin; FOX: Cefoxitin; CTX: Cefotaxime; G: Gentamycin; SXT: Sulfonamides; TE: Tetracycline; NAL: Nalidic acid; CIP: Ciprofloxacin; AMP: Ampicillin; VA: Vancomycin; FA: Fusidic acid

### Direct method

The Fleming *et al.* (1975) <sup>[15]</sup> method was used to screen for antagonistic bacteria by evaluating the antagonistic effect of strains inoculated in keys (test strains) against strains inoculated en masse (indicator strains). This method was modified in this work when applied by Bonny *et al.* (2021) <sup>[6]</sup>. From 24-hour-old colonies of each target strain, a bacterial suspension with a turbidity of 0.5 McFarland is formed, then diluted 1:10 in MRS agar (Difco, USA), maintained in supercooling. After solidification of the seeded agar and prior pouring onto the plate, the 24-hour starter culture isolates of lactic acid bacteria were added by touch, using a sterile Pasteur pipette. The Petri dishes are read after 24 hours of incubation at 37°C, in aerobic conditions.

### Indirect method

This method is used to determine the nature of the inhibitory substance produced by the lactic acid bacteria isolates. The growth of the target bacteria is inhibited by the action of the inhibiting substances released by the lactic acid bacteria isolates.

### Pre-culture preparation of the test bacteria

Prior to the use of the pathogen strain, 3 to 5 colonies were suspended in 10mL of nutrient broth for 18-24 hours at 37±1°C. The resulting overnight culture was used as inoculum.

### Preparation of the active supernatant

Isolates of lactic acid bacteria inoculated in 1mL of MRS broth (Difco, USA) were incubated at 37°C for 24 hours. Subsequently, the bacterial suspensions are resuspended in 10 mL of MRS broth (Difco, USA) and incubated at 37°C for 18 hours. The suspensions are centrifuged at 6000 rpm for 30 min. The recovered supernatant was filtered through a 0.45µm pore size membrane. The pellet was washed twice with distilled water.

The supernatant obtained after filtration is divided into two volumes, the first is neutralised with a 1N NaOH solution to obtain a pH of 6.50 and the second is left at the initial pH of between 4 and 4.6.

### Conduct of the test

On plates coated with a suspension of the pathogen strain, initially prepared at OD=0.08 at 625nm (Ausubel *et al.*, 1991), 6 mm diameter wells are dug into Petri dishes containing MRS agar (Difco, USA). These wells are then filled with filtered and untreated supernatant, filtered and neutralised supernatant and sterile distilled water (negative control) respectively. After diffusion of each fraction, the Petri dishes are incubated for 24 hours at 37°C.

The diameters of the inhibition zones ( $Z_i$ ) appearing around the wells are measured (average of two perpendicular diameters). The inhibition diameter is determined by the formula described by Schillinger and Lücke (1989) [22].

$$Z_i = \text{diameter of the resulting inhibition zone} - \text{well diameter}$$

### Identification of lactic acid bacteria isolates with high antibacterial power by MALDI-TOF-MS

Subcultures on MRS agar (Difco, USA) of lactic acid bacteria colonies that showed antibacterial activity on the target pathogens were used for MALDI-TOF MS analysis. Most colonies were grown the day before. Approximately 50 µg of fresh cells were taken from a single colony, without agar residue through the use of inoculation loops and transferred to stainless steel wells. Immediately, the bacterial material was extracted with 0.3 mL of matrix solution (10 mg of 2,5-dihydroxybenzoic acid in 1 mL of water: acetonitrile [1:1], acidified with 1% trifluoroacetic acid). Positive ion mass spectra were recorded for each strain using a MALDI-TOF mass spectrometer (AXIMA CFRplus, Shimadzu, Germany). For desorption of the components, a nitrogen laser beam ( $\lambda = 337$  nm) was focused on the model. The acceleration voltage was set at 20 kV, the delay time was 950 ns. All measurements were performed in the delayed extraction mode, allowing the determination of high resolved mass values ( $m/z$ ; mass-to-charge ratio). The analyses were performed in the positive ion mode site, yielding mainly molecular ions ( $[M + H]^+$ ).

### Statistical analysis

All data spectra obtained by MALDI-TOF mass spectrometry were processed by the instrument software with baseline correction, peak filtering and smoothing. The resulting site peak lists were exported to SARAMIS software (AnagnosTec). The peak lists of individual samples were compared to the superspectra database, generating a ranked list of matching spectra.

### Results

#### Isolation and identification of lactic acid bacteria isolates from quail caeca

After aseptic isolation of the quail caeca, the bacterial colonies obtained from the enrichment broth were subjected to a series of tests (macroscopic, microscopic and physiological) resulting in the isolation of a total of 29 presumptive lactic acid bacteria isolates. All 29 lactic acid bacterial isolates tested negative in the catalase test. Gram staining and microscopic observation allowed the differentiation between bacilli and cocci. Thus, of the 29 lactic acid bacteria isolates obtained, twenty-five (25) isolates were detected as Gram-positive bacilli, i.e. a rate of 86.2%, while the other four (4) (13.8%) were detected as Gram-positive cocci (Table 2).

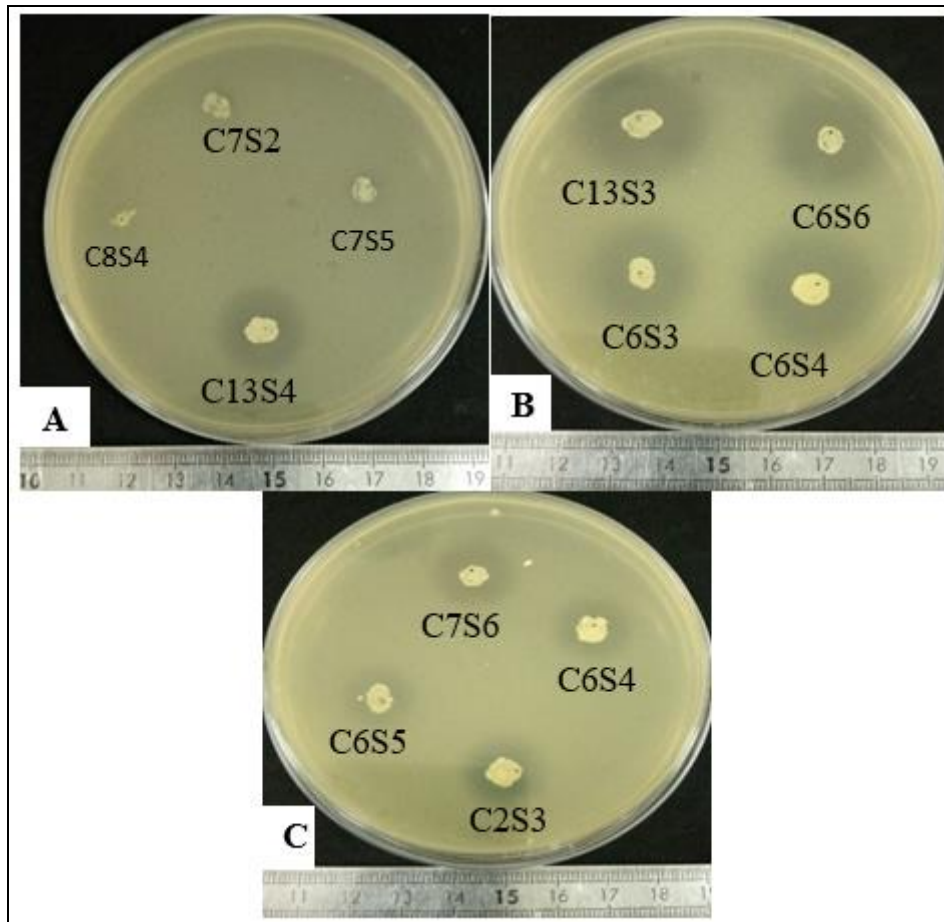
The study of the fermentation type, which is the key to the phenotypic identification of the lactic bacteria isolates, carried out on glucose culture medium, allowed them to be differentiated into homofermentative and heterofermentative.

To this end, the 29 lactic acid bacteria isolates showed two types of fermentation: twenty-six (26) lactic acid bacteria isolates were found to be hetero-fermentative, i.e. a rate of 89.66 %, and three (3) as homofermentative, i.e. a rate of 10.34 %.

#### Screening of lactic acid bacteria isolates with inhibitory activities

The direct antagonism test carried out on all the lactic acid bacteria isolates revealed their ability to inhibit the growth of target pathogens. The inhibition of pathogen growth is reflected in the appearance of a clear zone (halo) around the lactic acid bacteria spot (Figure 1). Thus, out of the twenty-nine (29) lactic acid bacteria isolates, ten (10) isolates (C2S3, C4S2, C6S4, C6S6, C10S7, C13S1, C13S3, C13S4, C6S3 and C13S6) showed antagonistic activity against the *Staphylococcus aureus* strain, with diameters of the zones of inhibition varying from  $8 \pm 0$  to  $13 \pm 0$  mm; i.e. a rate of 34.5 %. Twenty-one (21) lactic acid bacteria isolates, apart from C2S4, C4S4, C7S2, C7S5, C8S4, C12S1, C7S3 and C10S6, showed antagonistic activity against the pathogens *Salmonella enterica* serogroup O: 8 and *Escherichia coli*, with inhibition zone diameters ranging from  $8 \pm 0$  to  $20.5 \pm 0.5$  mm for *Salmonella* and from  $8.5 \pm 0.5$  to  $25.5 \pm 0.5$  mm for *E. coli*, i.e. a rate of 72.4%. However, 02 isolates (6.9%) of lactic acid bacteria (C7S3 and C10S6) showed no antagonistic activity on the 03 target pathogens (Table 3).

From the above, it can be observed that the *Staphylococcus aureus* strain is the one that showed more resistance to the action of these lactic acid bacteria isolates, during its growth in agar medium. Therefore, the 10 lactic acid bacteria isolates tested (34%) that showed antagonistic activity on the 03 target pathogen strains were selected for the search for probable inhibiting substances.



**Fig 1:** Antagonistic activity of LAB isolates towards target pathogens by the direct method LAB: Lactic acid bacteria; A: *Staphylococcus aureus*; B: *E.coli*; C: *Salmonella enterica* serogroup O:8

**Table 2:** Diameters (mm) of inhibition of lactic acid bacteria isolates by the direct method

No. of isolates	Forms/ Fermentative type	<i>E. coli</i>	<i>S. aureus</i>	<i>S. enterica</i> O:8
C2S3	Bacillus/Heterofermentative	25.5±0,5	13	13.5 ±0.5
C4S2	Bacillus/Heterofermentative	16±1	10±1	16±1
C6S3	Bacillus/Heterofermentative	20±1	11.5±0,5	16.5±0.5
C6S4	Bacillus/Heterofermentative	22.5±0.5	11±1	11.5±0.5
C6S6	Bacillus/Heterofermentative	19.5±0.5	9	15.5±0.5
C10S7	Bacillus/Heterofermentative	14	8.5±0.5	15.5±0.5
C13S1	Bacillus/Heterofermentative	16±1	9.5±0.5	11±1
C13S3	Bacillus/Heterofermentative	21.5±0.5	8	13.5±0.5
C13S4	Bacillus/Heterofermentative	20.5±0.5	16	16.5±0.5
C13S6	Bacillus/Heterofermentative	23±1	11.5±0.5	18.5±0.5
C6S1	Bacillus/Heterofermentative	19±1	0	15.5±0.5
C1S2	Bacillus/Heterofermentative	8.5±0.5	0	9
C1S6	Bacillus/Heterofermentative	9	0	10.5±0.5
C4S1	Cocci/ Heterofermentative	11	0	20.5±0.5
C4S3	Bacillus/Heterofermentative	14	0	18.5±0.5
C6S5	Cocci/ Heterofermentative	14.5±0.5	0	12.5±0.5
C7S1	Bacillus/Homofermentative	17	0	16
C7S6	Cocci/ Heterofermentative	8.5±0.5	0	13.5±0.5
C7S8	Bacillus/Heterofermentative	12	0	15
C8S3	Bacillus/Heterofermentative	12.5±0.5	0	19±1
C13S8	Bacillus/Heterofermentative	16	0	16.5±0.5
C2S4	Bacillus/Heterofermentative	0	0	13.5±0.5
C4S4	Bacillus/Heterofermentative	0	0	13±1
C7S2	Bacillus/Heterofermentative	11.5±0.5	0	0
C7S5	Bacillus/Homofermentative	13.5±0.5	0	0
C8S4	Bacillus/Heterofermentative	12	0	0
C12S1	Bacillus/Heterofermentative	0	0	8

C7S3	Bacillus/Heterofermentative	0	0	0
C10S6	Cocci/ Homofermentative	0	0	0

### Research of inhibiting substances in isolates of lactic acid bacteria with antibacterial activities

The antagonism test carried out by the indirect method on the ten (10) selected LAB isolates (C2S3, C4S2, C6S4, C6S6, C10S7, C13S1, C13S3, C13S4, C6S3 and C13S6) made it possible to target the location of the potential inhibiting substances responsible for the inhibiting power of the LAB isolates. Inhibition of pathogen growth is manifested by the presence of clear zones around the wells (Figure 2). Thus, from the supernatants obtained after addition of 1N NaOH of the 10 isolates, four (4) LAB isolates, including C6S3, C4S2, C6S4 and C13S4, were found to possess a substance with antagonistic activity against the 3 targeted pathogens. The diameters of the inhibition zones obtained with the supernatants of the 04 treated isolates varied from  $5.5 \pm 1$  to  $13 \pm 0.5$  mm for the *E.coli* strain, from 4 to 13 mm for the *Salmonella* strain and from  $3.5 \pm 0.5$  to 7 mm for the *Staphylococcus aureus* strain. The largest diameters of the inhibition zones ( $13 \pm 0.5$  mm, 13 mm and 7 mm) were observed with the 1N NaOH-treated supernatant of the C4S2 lactic acid bacterial isolate, on all three target pathogens (Table 3). These results could reflect the ability of the 04 Lactic Acid Isolates to inhibit the growth of the 03 target pathogens, through the action of organic acids and/or bacteriocins. However, the other six lactic acid bacteria isolates, namely C13S1, C10S7, C13S3, C13S6, C6S6 and C2S3, did not show any antagonistic activity on the targeted pathogens, which could reflect the fact that the inhibitory effect of hydrogen peroxide was eliminated by the action of 1N NaOH in the various supernatants of these isolates.

The non-neutralised supernatants of the ten (10) lactic acid bacteria isolates were 100 % effective on the target pathogens, with inhibition zone diameters ranging from 1 to 7 mm (Table 4). More specifically, the zone diameters obtained with the treated supernatants of these isolates ranged from 2 to 7 mm for the *E. coli* strain, from 1.5 to  $5.5 \pm 0.5$  mm for the *Staphylococcus aureus* strain and from 1 to  $3.5 \pm 0.5$  mm for the *Salmonella enterica* O:8 strain. The inhibitory effect of these types of supernatants could be due to potential substances such as hydrogen peroxide, organic acids or bacteriocins. In comparison with the diameters obtained, the supernatants not treated with NaOH show a low activity compared to the supernatants treated with NaOH.

In sum, the lactic acid bacteria isolates that showed very good antibacterial activity on the target pathogens were C6S3, C4S2, C6S4 and C13S4.

**Table 3:** Inhibition diameters (mm) of neutralised supernatants of lactic acid bacteria isolates by the well method

No. of isolates	<i>E.coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella enterica</i> O:8
C6S3	$10.5 \pm 0.5$	$10.5 \pm 0.5$	$5.5 \pm 0.5$
C4S2	$13.5 \pm 0.5$	13	7
C6S4	$5.5 \pm 0.5$	4	4
C13S4	$7 \pm 1$	$7 \pm 0.5$	$3.5 \pm 0.5$
C13S1	0	0	0
C10S7	0	0	0
C13S3	0	0	0
C13S6	0	0	0
C6S6	0	0	0
C2S3	0	0	0

**Table 4:** Inhibition diameters (mm) of non-neutralised supernatants of lactic acid bacteria isolates by the well method

No. of isolates	<i>E.coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella enterica</i> O:8
C6S3	$5.5 \pm 0.5$	3	2
C4S2	7	$5.5 \pm 0.5$	$1.5 \pm 0.5$
C6S4	4	$2.5 \pm 0.5$	$1.5 \pm 0.5$
C13S4	$5.5 \pm 0.5$	3	$2 \pm 1$
C13S1	$3.5 \pm 0.5$	2	2
C10S7	$4 \pm 1$	$3.5 \pm 0.5$	$2.5 \pm 0.5$
C13S3	4	$5.5 \pm 0.5$	$3.5 \pm 0.5$
C13S6	3	1.5	1
C6S6	2	$2 \pm 0.5$	$1.5 \pm 0.5$
C2S3	$2.5 \pm 0.5$	$2.5 \pm 0.5$	$2.5 \pm 0.5$

### Identification of lactic acid bacteria isolates with high antibacterial power by MALDITOF-MS

MALDITOF-MS mass spectrometry, performed on isolates of lactic acid bacteria with high antibacterial power, revealed their identity.

Two genera of lactic acid bacteria were determined, namely *Weissella confusa* (C6S3 and C6S4) and *Lactobacillus fermentum* (C4S2 and C13S4).

## Discussion

The identification of bacterial colonies isolated from quail (*Cortunix cortunix Japonica*) caeca, based on the study of their morphological, microscopic and biochemical characteristics, allowed the selection of small colonies, whitish in colour with a regular outline. The catalase test allowed the selection of 29 bacterial colonies, mainly composed of bacilli at a rate of 86 % and 14 % of cocci, all with positive Gram staining. These results are contrary to those reported by Franciosi *et al.* (2009) <sup>[15]</sup> on the one hand and Cheriguene (2008) <sup>[9]</sup> on the other hand, which show a high rate of coccles compared to bacilli in isolates of lactic acid bacteria from raw cow's and goat's milk. The study of the fermentation type, an important test for the identification of bacteria and based on the production or not of CO<sub>2</sub>, made it possible to distinguish isolates of lactic bacteria presenting either a homofermentative metabolism (no production of CO<sub>2</sub>) or a heterofermentative one (production of CO<sub>2</sub>), from the metabolism of glucose. Thus, of the 29 lactic bacteria isolates, a strong dominance of heterofermenters (89.66 %) is observed. This high rate of heterofermentative strains, according to De Roissart and Luquet (1994) <sup>[11]</sup>, reflects the fact that they use the pentose phosphate pathway and are able to metabolise sugars into lactic acid, ethanol or acetic acid and CO<sub>2</sub>. Isolates of homofermentative lactic acid bacteria would reflect their ability to produce lactic acid only, from the fermentation of carbohydrates by glycolysis (De Roissart and Luquet, 1994) <sup>[11]</sup>. All these factors would increase the acidifying power of lactic bacteria, thus making the environment unfavourable for the growth of pathogens (Lachi and Kellas, 2019) <sup>[18]</sup>. The antagonistic activity of the 29 lactic acid bacteria isolates against *Staphylococcus aureus*, *Salmonella enterica* serogroup O:8 and *E. coli* revealed 83 % of the lactic acid bacteria isolates with activity against *E. coli* and *Salmonella enterica* serogroup O:8. Only 34.5 % of the isolates tested showed antagonistic activity against *Staphylococcus aureus*. This low rate of inhibition observed in the case of *Staphylococcus aureus*, under the action of lactic acid bacteria isolates is reported by Bougoddima and Sebaha (2019) <sup>[10]</sup>. Indeed, staphylococci are pathogenic strains that are highly resistant to the metabolites produced by lactic acid bacteria isolates. Furthermore, it should be noted that the inhibitory effect of lactic acid bacteria isolates was significantly different according to the type of wall, more significant in Gram positive than in Gram negative bacteria. Also, the lactic acid bacterial isolates that showed inhibitory action on all target pathogens were better for *E. coli* (25.5±0.5 mm), *Staphylococcus aureus* (11.5±0.5 mm) and *Salmonella enterica* serogroup O:8 (18.5±0.5 mm), compared to the different diameters of the inhibition zones observed by Yateem *et al.* (2008) <sup>[23]</sup>, in isolates of lactic acid bacteria, isolated from camel milk in Kuwait, whose antagonistic effect was exerted only on gram-negative bacteria such as *Salmonella* sp (12 mm) and *E. coli* (16 mm). However, Ammor *et al* (2006) <sup>[1]</sup> describe only a limited antagonistic effect of lactic acid bacteria isolates on Gram-positive bacteria such as *Staphylococcus aureus*.

Of the 29 lactic acid bacteria isolates, only 10 isolates (34 %) showed excellent activity against the 3 pathogenic strains tested. This excellent antibacterial activity of these lactic acid bacterial isolates could be explained by their ability to synthesise certain inhibitory substances, such as organic acids (lactic acid, acetic acid, etc.), CO<sub>2</sub>, reuterin, hydrogen peroxide, diacetyl and bacteriocins (Bellil *et al*, 2014; De Vuyst and Vandamme, 1994; Dortu and Thonart, 2009) <sup>[4, 14, 15]</sup>. Therefore, in order to identify the substances responsible for the inhibitory activity, the well diffusion method was performed on the raw supernatants and the supernatants neutralised with NaOH 1N. The results obtained from the raw culture supernatants of the lactic acid bacteria isolates, showing the presence of inhibition zones around the wells, indicate an inhibitory activity of the supernatants of the 10 isolates selected by the spot method. The direct contact by spots could be attributed to an effect of nutritional competition between cells, without synthesis of inhibitory metabolites or to a too low concentration of inhibitory agent in the filtrates. Similar results were obtained by Ammor *et al* (2006) <sup>[1]</sup> and Mami *et al* (2008) <sup>[19]</sup>.

The inhibitory effect of the supernatants not treated with NaOH, for all the lactic bacteria isolates tested, would reflect the probable existence of inhibitory substances, such as organic acids, CO<sub>2</sub>, reuterin, hydrogen peroxide, diacetyl and bacteriocins, naturally produced in these lactic bacteria strains (Bouferroum *et al*, 2020) <sup>[9]</sup>. Therefore, the substances responsible for the inhibition of these lactic bacteria isolates would be of extracellular origin. As for the inhibitory effect of the neutralised supernatants of isolates C13S4, C13S1, C10S7, C13S6, C6S6, and C2S3 by NaOH on the target pathogens, this would indicate that the variation in the pH of the supernatants after the addition of NaOH would be at the origin of the loss of the inhibitory activity of hydrogen peroxide. From this point of view, organic acids and/or bacteriocins would be the basis of their inhibiting effect. This hypothesis, supported by Lachi and Kellas (2019) <sup>[18]</sup>, would attribute the inhibiting power of these isolates to their acidifying property, a phenomenon involved more particularly in the inhibition of the growth of pathogenic flora and spoilage germs (*Pseudomonas*, *Leuconostoc*, etc.). Also, the studies conducted by Lachi and Kellas (2019) <sup>[20]</sup> on the one hand, and by Yateem *et al.* (2008) <sup>[23]</sup> on the other hand, have highlighted the production of bacteriocins by isolates of lactic bacteria from dairy products.

The existence of antagonistic activity in strains of lactic acid bacteria, such as *Weissella confusa* and *Lactobacillus fermentum*, identified via the MALDITOF-MS technique in quail caeca (*Cortunix cortunix Japonica*), against the 3 pathogen strains tested in this work, would open up avenues of research for the exploration of new exploitable avenues to alleviate the problems of the emergence of antibiotic resistance in the Ivorian poultry sector. Indeed, these two types of lactic acid bacteria isolated from quail caeca are known according to the work of Heravi *et al.*(2011) <sup>[17]</sup> on the one hand and (Miraka and Milhkel, 2009) on the other hand, as potential probiotics isolated from the gastrointestinal tract of chickens and human origin, capable of being used as a feed supplement in place of antibiotic molecules.

### Conclusion

The exploration of naturally occurring antimicrobials is receiving increasing attention due to the alarming incidence of bacterial resistance that threatens human life.

The research for alternative ways of combating the spread of multidrug resistance in pathogenic strains, both for humans and animals and in the environment, is becoming a prerequisite for the research community. To this end, the search for new generations of antibiotics, less expensive and available, in lactic acid bacteria, opens up ways and means of combating the emergence of food pathogens.

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### Conflicts of interest

The authors declare no conflict of interest in the completion of this work.

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