Microbiology and Nature
Volume 2, Issue 2 pages 213-224
ISSN 2664-388X
https://doi.org/10.26167/7fs9-bp95

Microbiol. Nat.

Société Ivoirienne de Microbiologie

MICROBIOLOGY AND NATURE
Journal homepage: www.microbiologyandnature.com

Antimicrobial potential of Lactobacillus strains against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, three human opportunistic pathogenic bacteria

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Received, June 10th 2024, Revised July 17th 2024, Accepted August 20th 2024, published online August 27th

Abstract

Some species Lactobacillus (Lb) can inhibit the growth of undesirable bacteria by producing metabolites such as bacteriocins. The aim of this study was to evaluate the capacity of Lb species to inhibit the growth of three reference pathogenic strains (Staphylococcus aureus ATCC 29213, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853). Two hundred and thirteen (213) strains of Lb were isolated and identified by MALDI-TOF from raw cow's milk, curdled milk, cassava ferments and Döderlein flora. These strains belonged to nine (9) species (Lb plantarum, Lb casei, Lb delbrueckii spp bulgaricus, Lb helveticus, Lb fermentum, Lb rhamnosus, Lb reuteri, Lb acidophilus, Lb paracasei). Antibacterial activity was assessed using the solid-state diffusion method on Mueller Hinton agar medium. The Lb isolated from the cassava ferment showed the best antibacterial activity against *S. aureus* (60.29 %), while the curd strains showed the best antibacterial activity against E. coli (49.31 %) and *P. aeruginosa* (49.31 %). Strains isolated from raw cow's milk showed the lowest antibacterial activity with 13.6 % against *P. seudomonas*. The Lb species that gave broad activity spectra between 10-19 mm were Lb plantarum (raw cow's milk, manioc ferment); Lb bulgaricus (curdled milk) and Lb acidophilus (Döderlein flora). The most sensitive pathogenic bacterium was S. aureus, with rates ranging from 38.23 % to 60.29 % and the least sensitive was *P. aeruginosa*, with rates ranging from 13.16 % to 32.88 %. Their antibacterial activity could be used to fight the emergence and spread of multi-resistant bacteria.

Keywords : Lactobacillus, Bacteriocin, Inhibition, Reference strains

Résumé

Certaines espèces de *Lactobacillus* (*Lb*) peuvent inhiber la croissance des germes indésirables par la production de métabolites tels que les bactériocines. L'objectif de cette étude était de tester le pouvoir inhibiteur des espèces de *Lb* sur la croissance de trois souches de référence (*Staphylococcus aureus* ATCC 29213*, Escherichia coli* ATCC 25922 *et Pseudomonas aeruginosa* ATCC 27853). Deux cent treize (213) souches de *Lb* ont été isolées et identifiées par MALDI-TOF à partir du lait cru de vache, lait caillé, ferments de manioc et flore de Döderlein. Ces souches appartenaient à neuf (9) espèces (*Lb plantarum, Lb casei, Lb delbrueckii spp bulgaricus, Lb helveticus, Lb fermentum, Lb rhamnosus, Lb reuteri, Lb acidophilus, Lb paracasei*). L'activité antibactérienne a été réalisée par la méthode de diffusion en milieu solide sur la gélose Mueller Hinton. Les *Lb* isolées du ferment de manioc ont montré les meilleures activités antibactériennes contre *S. aureus* (60.29 %) tandis que les souches du lait caillé ont montré les meilleures activités *antibactériennes sur Escherichia coli (49.31 %) et P. aeruginosa (49.31 %). Celles isolées du lait cru de vache présentaient l'activité antibactérienne la plus faible avec 13.6 % contre P. seudomonas*. Les espèces de Lb qui ont donné de larges spectres d'activités compris entre 10-19 mm étaient Lb plantarum (lait cru de vache, ferment de manioc) ; Lb bulgaricus (lait caillé) et Lb acidophilus (flore de Döderlein). La bactérie pathogène la plus sensible était S. aureus dont les taux était de 38.23 % à 60.29 % et la moins sensible était P. aeruginosa avec des taux de 13.16 % à 32.88 %. Leur pouvoir antibactérien pourrait être utilisé dans la lutte contre l'émergence et la dissémination des bactéries multirésistantes.

***Mots-clés**: Lactobacillus, Bactériocine, Inhibition, Souches de référence.*

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Introduction

The occurrence of antibiotic resistance in microbial pathogens worldwide is becoming a huge public health problem termed the antibiotics resistance crisis (Ventola, 2015). This crisis depitecd by the world health organization (WHO) as an existential threat to humanity is potentially compromising the available therapeutic treatments against bacterial infections (IACG, 2019). Multi-resistant bacteria characterized by their ability to resist to more than two family of antibiotics, not only increase the cost of treatment and extend hospital stays (Fosseprez, 2013), but also are responsible for 1.27 millions deaths each year (Murray et al., 2022).

The emergence and spread of resistant bacteria throughout the world is closely linked to the massive and often irrational use of antibiotics. Indeed, one of the main factors contributing to the spread of antibiotic resistance is the excessive and uncontrolled use of antibiotics in hospitals, in the human community, on farms and in agricultural activities that considerably modify the microbial ecology and tends to increase the prevalence of multi-resistant bacteria (Leverstein-Van Hall et al., 2011).

As a solution, the search of novel active antibiotic molecules to conterbalance the spread of antibiotic resistance is of high medical importance. In this context, bacteriocins stand among the main candidates as alternative to antibiotics in the fight against pathogenic and multi-resistant bacteria (Makhloufi, 2011; Hernández-González et al., 2021).

Interestingly, bacteria of the genus Lactobacillus are known as one of source of bacteriocins production (Mokoena, 2017). These bacteria are Gram-positive, immobile, facultative anaerobic and homofermentative bacilli. In addition, they are ubiquitous in many biotopes, particularly in fermented foods (Hammi, 2016) and constitute a heterogeneous group of microorganisms that produce lactic acid as the main product of metabolism. These colonise numerous food products such as dairy products, meat, plants and cereals. They are also part of the intestinal and vaginal flora of humans and animals (Lachi & Kellas, 2019). Bacteria of the genus Lb sp are widely used in the agri-food industry for their fermentation capacity. They contribute to the organoleptic quality of foods during the fermentation process.

The metabolites produced by Lb also increase the shelf life of foods (Dortu & Thonart, 2009 ; Requena & Buist, 2000 ; Mechai, 2009). Moreover, Lb have the ability to inhibit the growth of undesirable microorganisms by producing several metabolites with antimicrobial properties such as organic acids, carbon dioxide, diacetyl and particularly bacteriocins (Dortu & Thonart, 2009).

The aim of this paper was to analyze the inhibition capacity of Lb species isaloted from fews samples, againsts the growth of three reference bacterial strains, namely Staphylococcus aureus ATCC 29213, Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 known as opportunistic pathogenic bacteria.

Materials and Methods

Bacterial strains

The bacterial strains used in this study were composed of Lb strains from which we analyzed the antimicrobial activity against reference strains notably S. aureus ATCC 29213, E. coli ATCC 25922 and P. aeruginosa ATCC 27853. Lb strains tested for antimicrobial activity were isolated from raw cow's milk, curdled milk, casava ferment and dôderlein flora whereas the pathogenic opportunistic ATCC strains (S. aureus ATCC 29213, E. coli ATCC 25922 and P. aeruginosa ATCC 27853), used as target of antimicrobial activity from Lb strains, were provided by the laboratory of the National Reference Centre of the Pasteur Institute of Côte d'Ivoire.

Culture conditions and isolation of Lactobacillus (Lb)

Samples of three (3) food matrices (raw cow's milk, curdled milk and manioc ferment) and Döderlein flora were collected from various localities in the city of Abidjan in Côte d'Ivoire.

For isolation of bacteria, 10 g or 10 ml of solid or liquid

sample were added to 90 ml of buffered peptone water. The mixture was homogenised in a stomacher bag. This solution constituted the stock solution. From this stock solution, 1 mL was taken and mixed with 9 mL of buffered peptone water in a test tube. This gave the 10-1 dilution. Then 1 ml of the previous dilution was added to 9 ml of buffered peptone water to obtain the next dilution. The other decimal dilutions were made up to 10-8. Then 0.1 mL of each dilution was plated onto MRS agar medium, incubated in a jar under anaerobic conditions for 24-48 h, both at 37°C and 44°C. The resulting colonies on plate were identified as lactic acid bacteria via their key basic biochemical feature, notably Gram positive, rod shaped and catalase negative.

Bacterial Identification by mass spectrometry (MALDI-TOF)

To further identify the isolates, the colony of Lb sp in subculture for 24 hours was removed using a 10 µl calibrated loop and then placed on the spot on the plate. Each plate can be used to identify 24 strains. A matrix (α -Cyano-4-hydroxycinnamic acid) was used to cover each spot. The operation was carried out using the reference strain of Escherichia coli DH5 alpha, which was used as a quality control for the MALDI-TOF identification manipulation. The plate was then inserted into the mass spectrometer. Results were analyzed on the computer workstation (laboratory computer system) associated with the spectrometer (Liu et al., 2023).

Preparation of the Lactobacillus (Lb) culture extract

Lb strains were grown in MRS broth for 24 h at 37°C. Then 20 mL of the culture were used for centrifugation at 4000 rpm for 15 min, the supernatant containing the crude extract was neutralised with 0.1 N NaOH to obtain a pH of 6.5. This eliminated the effect of organic acids accumulated in the culture medium. The obtained culture extract, notably cells free supernatant was tested against the pathogenic strains to ckeck the potential presence of bioactives antimicrobial, presumed bacteriocin.

Growing the references pathogenic strains

Strains stored in deep agar using haemolysis tubes at -20°C were transferred to test tubes containing brain heart broth (BCC) medium, then incubated at 37°C for 24 hours. The resulting microbial culture were used to inoculate different solid media depending on the type of pathogenic strain notably Chapman for S. aureus, cetrimide for P. aeroginosa, and Eosine Methylene Blue (EMB) for E. coli. Pathogenic strains were then grown at 37°C for 24 h and used as target for analysis of Lb antimicrobial activity.

Analysis of antimicrobial activity in the crude extract supernatant from Lactobacillus (Lb) strains

The antimicrobial activity of the strains was analyzed by the solid-state diffusion method using Müeller Hinton (MH) agar (Barefoot & Klaenhammer, 1983) on which wells of 5 mm diameter and 4 mm dept were aseptically made. These 5 mm diameter wells were made on Müeller Hinton agar (MH) using the upper end of a sterile Pasteur. This test was carried out on MH agar plates. A sterile cotton swab was immersed into the bacterial suspension of the pathogenic strains. The content of the swab was used to seed MH agar in tightly packed streaks, using the cadrant method and carefully avoiding the wells. Inoculated plates were dried in an oven for 15 min. After removing the plates, the wells were loaded with 100 mL of supernatant of the strain to be tested. The agar plates were left on the bench for 1 hour then incubated at 37°C for 24 hours. The antimicrobial activity of Lb strains was detected and measured via the diameters of inhibition zones around the well (Schillinger & Lucke, 1989 ; Pulsani et al., 1979).

Results

Isolation of Lactobacillus (Lb) sp.

From a total of 180 samples analyzed, 213 strains of Lb sp were isolated on MRS from different matrices, 80 (37.50 %) strains from raw cow milk, 33 (15.49 %) strains from curdled milk, 70 (32.86 %) strains from manioc ferments, 30 (14.08 %) strains from human Doderlein flora. All were selected for identification by MALDI-TOF (Table I)

Table I: Number of samples analysed and *Lactobacillus* (*Lb*) isolation rate for each matrice

	raw cow milk	curdled milk	Cassava ferment	Doderlein flora
Number of samples analysed	50	40	60	30
Number of strains isolated n (%)	80 (37.50 %)	33 (15.49 %)	70 (32.86 %)	30 (14.08 %)

Identification of Lactobacillus (Lb) by MALDITOF (Mass Spectrometry)

Microscopic observation after Gram staining identified two forms: shells and rods. The rod-shaped forms represent 90 % of the total number of bacteria and are represented by Lb sp strains (all Gram-positive, immobile, catalase-negative bacilli).

The identification by MALDI-TOF allowed us to identify a total of nine (09) species of Lb : Lb plantarum, Lb casei, Lb delbrueckii spp bulgaricus, Lb helveticus, Lb fermentum, Lb rhamnosus, Lb reuteri, Lb paracasei, Lb Acidophilus isolated from four matrices (raw cow's milk, curdled milk, cassava ferment and human Döderlein flora). (Table II).

Seven species of Lb have been identified from raw cow's milk. Among these, Lb plantarum was the most dominant species with a rate of 37.25 %, followed by Lb casei whose rate was 31.5 %. On the other hand, Lb acidophilus, Lb rhamnosus and Lb reuteri were the minor species with weak proportions ranging between 6.25 % and 2.5 % (Table II).

Unlike the curdled milk sample, the identification revealed the presence of five (5) Lb species and the most dominant species was Lb fermentum which alone represented 45.45 %. In addition, two other species, namely Lb bulgaricus and Lb casei, were also represented with respective rates of 24.2 % and 15.5 %. Then Lb plantarum was weakly represented with a rate of 6.1 % (Table II).

As for the sample of cassava ferments, four (4) Lb species have been identified. Indeed, Lb plantarum was the most dominant with a rate of 50 % followed by Lb rhamnosus and Lb reuteri which had the same identification rates of 21.43 % and finally the minor species was Lb casei with a rate of 7.15 % (Table II).

While five (5) Lb species were identified in the samples of the flora of Döderlein, among which Lb acidophillus was the most dominant with a percentage of 46.7 % while Lb bulgaricus was weakly represented with a rate of 6.7 %. It should also be noted that the Lb plantarum species had a dominance of 23.3 % (Table II).

Table II : Different species of *Lactobacillus (Lb)* isolated from each matrice and identified by MALDI-TOF**.** The number should be written in english

Species	Raw cow's milk n (%)	Curdled milk n (%)	Cassava ferment n (%)	Flora of döderlein n (%)
Lb Plantarum	**30 (37.25)**	2 (6.1)	**35 (50)**	**7 (23.3)**
Lb Acidophillus	5 (6.25)	NI	NI	**14 (46.7)**
Lb Helveticus	NI	3 (9.1)	NI	NI
Lb Bulgaricus	NI	**8 (24.43)**	NI	2 (6.7)
Lb Casei	**25 (31.25)**	**5 (15.15)**	5 (7.15)	NI
Lb Paracasei	8 (10)	NI	NI	3 (10)
Lb Rhamnosus	5 (6.25)	NI	**15 (21.43)**	NI
Lb Reuteri	5 (6.25)	NI	**15 (21.43)**	NI
Lb Fermentum	NI	**15(45.45)**	NI	4 (13)
Total	80	33	70	30

Lb : *Lactobacillus ;*

La : *Lactococcus* ;

NI : Not Identified ;

n : number of isolates

Antimicrobial activity of *Lactobacillus* (*Lb)* species

The inhibitory activity of *Lb* species isolated from raw cow's milk, curdled milk, Cassava ferment and Döderlein flora against the three pathogenic strains tested are shown in **Fig 1** The results show a good inhibitory effect of the lactic strains against the pathogenic species. The inhibition diameter varied between 10 and 18 mm depending on the pathogen strain tested. Inhibition is positive when the inhibition diameter is greater than 1 mm around the well (**Schillinger & Lucke, 1989**) **(Fig 1).**

The results obtained show that the *Lb* species isolated from the different matrices have, in general good inhibitory activity against the three reference tested bacteria, namely *S. aureus* ATCC 29213, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 (**Table III, IV, V** and **VI**), with the exception of the *Lb plantarum* strains isolated from the Döderlein flora against all the reference strains tested (**Table VI**). In raw cow's milk, 13 strains of *Lb plantarum* had antibacterial activity (sensitive and very sensitive) against *S. aureus* ; 11 strains against *E. coli* and 5 strains against *P. aeruginosa*, while 4 strains of *Lb casei* had antibacterial activity (sensitive and very sensitive) against *S. aureus* and 4 strains against *E. coli* (**Table III**). Among the *Lb* species isolated from curdled milk and tested for their antibacterial power against reference strains. 25 strains of *Lb bulgaricus* were (sensitive and very sensitive) to *S. aureus* ; 27 strains against *E. coli* and 20 strains against *P. aeruginosa*. The strains of *Lb fermentum* (9) had antibacterial activity (sensitive and very sensitive) against *S. aureus* ; 8 strains against *E. coli* ; 4 strains against *P. aeruginosa*. 6 (six) strains of Lb helveticus showed antibacterial activity (sensitive and very sensitive) against *S. aureus* (4 strains) and *E. coli* (2 strains) (**Table IV**). *Lb plantarum* strains isolated from cassava ferments showed the strongest antibacterial activity with 28 strains against *S. aureus*, 20 strains against *E. coli* and 12 strains against *P. aeruginosa*. *Lb rhamnosus* species (17 strains) and *Lb reuteri* (9 strains) showed antibacterial activity against the three (3) reference strains tested (**Table V**). The *Lb acidophilus* species isolated from the Döderlein flora showed good antibacterial activity against all the strains tested. 13 strains against *S. aureus* ; 14 strains against *E. coli* and 7 strains against *P. aeruginosa* (**Table VI**).

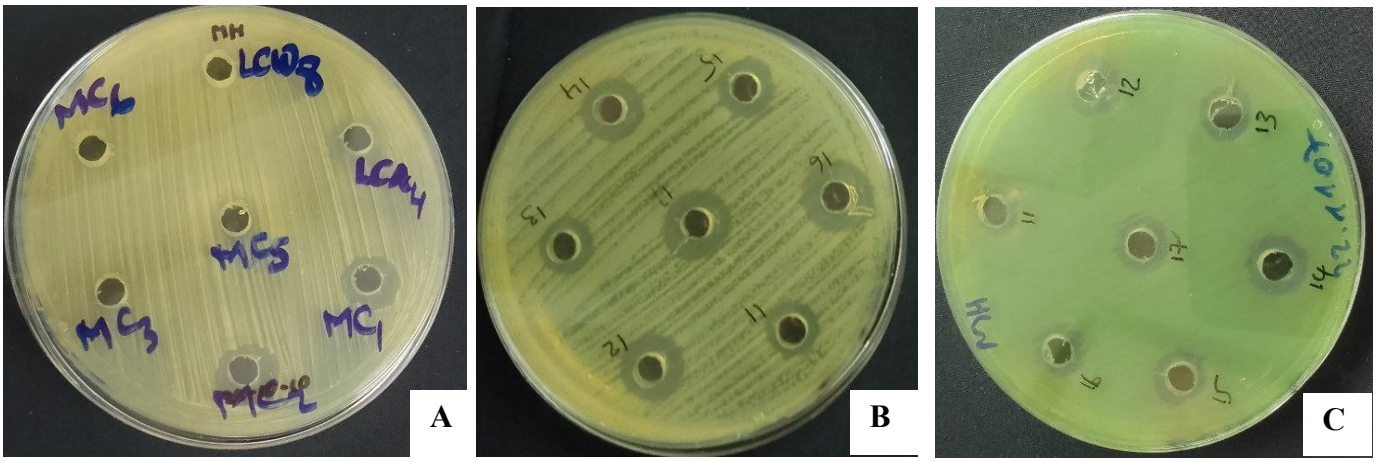

Fig1 : Zones of inhibition of *Lactobacillus* species in relation to the three reference strains.

Lactobacillus plantarum (MC2 ;17) ; *Lactobacillus fermentum* (MC1 ;16) ; *Lactobacillus acidophilus* (MC5 ; 14) ; *Lactobacillus casei* (LCW8 ; 15) ; *Lactobacillus bulgaricus* (MC6 ; 13) ; *Lactobacillus ruteri* (LCA4 ; 11) ; *Lactobacillus rhamnosus* (MC3 ; 12)

(A) *Staphylococcus aureus* ATCC 29213; (B) *Escherichia coli* ATCC 25922, (C) *Pseudomonas aeruginosa* ATCC 27853

Table III : Diameter of halo inhibition of *Lactobacillus* (*Lb*) species isolated from raw cow's milk against pathogenic strains milk against pathogenic strains

	Lb plantarum* (n=23 strains*)			***Lb casei* (n=15 strains)**		
Diameter Inhibition(mm)	Resistant (< 9)	sensitive (10-14)	Very sensitive (15-19)	Resistant (< 9)	sensitive (10-14)	Very sensitive (15-19)
against Staphylococcus aureus	10	10	3	11	3	1
against Escherichia coli	12	9	2	11	2	2
against Pseudomonas aeruginosa	18	5	0	15	0	0

n : number of strains

Table IV : Diameter of halo inhibition of *Lactobacillus* (*Lb*) species isolated from curdled milk against the three pathogenic strains

	***Lb bulgaricus* (n=45 strains)**			***Lb fermentum* (n=18 strains)**			***Lb helveticus* (n=10 strains)**		
Diameter Inhibition (mm)	Resistant (< 9)	sensitive (10-14)	Very sensitive (15-19)	Resistant (< 9)	sensitive (10-14)	Very sensitive (15-19)	Resistant (< 9)	sensitive (10-14)	Very sensitive (15-19)
against Staphylococcus aureus	20	15	10	9	6	3	6	2	2
against Escherichia coli	18	11	16	10	2	6	8	0	2
against Pseudomonas aeruginosa	25	13	7	14	4	0	10	0	0

n : number of strains

Table V : Diameter halo inhibition of *Lactobacillus (Lb)* species isolated from cassava ferment against the three pathogenic strains

	***Lb plantarum* (n=36 strains)**			***Lb rhamnosus* (n=19 strains)**			***Lb ruteri* (n=13 strains)**		
Diameter Inhibition (mm)	Resistant (< 9)	sensitive (10-14)	Very sensitive (15-19)	Resistant (< 9)	sensitive (10-14)	Very sensitive (15-19)	Resistant (< 9)	sensitive (10-14)	Very sensitive (15-19)
against Staphylococcus aureus	8	26	2	10	9	0	9	4	0
against Escherichia coli	16	20	0	14	5	0	11	2	0
against Pseudomonas aeruginosa	24	12	0	16	3	0	10	3	0

n : number of strains

Table VI : Diameter of halo inhibition of *Lactobacillus (Lb)* species isolated from the Döderlein flora against the three pathogenic strains.

	***Lb acidophilus* (n=25 strains)**			***Lb plantarum* (n=9 strains)**		
Diameter Inhibition (mm)	Resistant (< 9)	sensitive (10-14)	Very sensitive (15-19)	Resistant (< 9)	sensitive (10 -14)	Very sensitive (15-19)
against Staphylococcus aureus	12	8	5	9	0	0
against Escherichia coli	11	5	9	9	0	0
against Pseudomonas aeruginosa	18	4	3	9	0	0

n : number of strains

The *Lb* isolated from the cassava ferments samples had the highest antibacterial activity against the *S. aureus* ATCC 29213 with a level of 60.29 %. In contrast, the *Lb* strains isolated from the Döderlein flora had the lowest antibacterial activity with a rate of 38.23 % (Fig. 2).

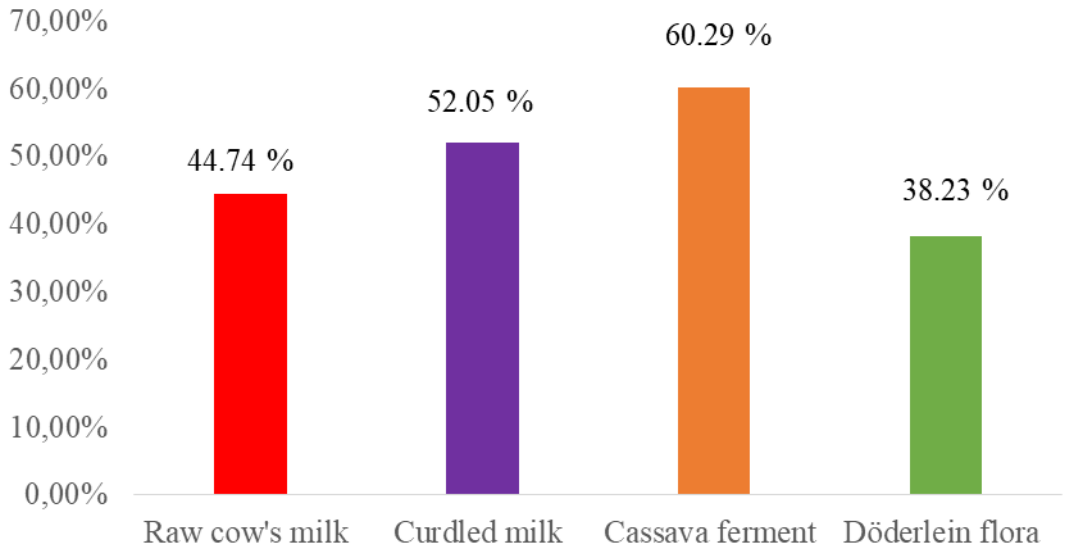

Fig. 2 : Proportion of isolated *Lactobacillus* (*Lb*) strains from different matrices having antibacterial activity against *Staphylococcus aureus* ATCC 29213.

Lb isolated from curdled milk showed the most dominant antibacterial activity with a proportion of 49.31 % against *E. coli* ATCC 29213. The antibacterial activity of *Lb* strains isolated from samples of Döderlein flora, cassava ferment and raw cow's milk tested agains *E. coli* was 41.18 % ; 39.70 % and 39.47 % respectively (Fig3).

Lb strains isolated from curdled milk had the highest antibacterial activity against *P. aeruginosa* ATCC 27853 with a percentage of 32.88 %. *Lb* isolated from raw cow's milk had the lowest antibacterial activity with a rate of 13.16 %. (Fig 4).

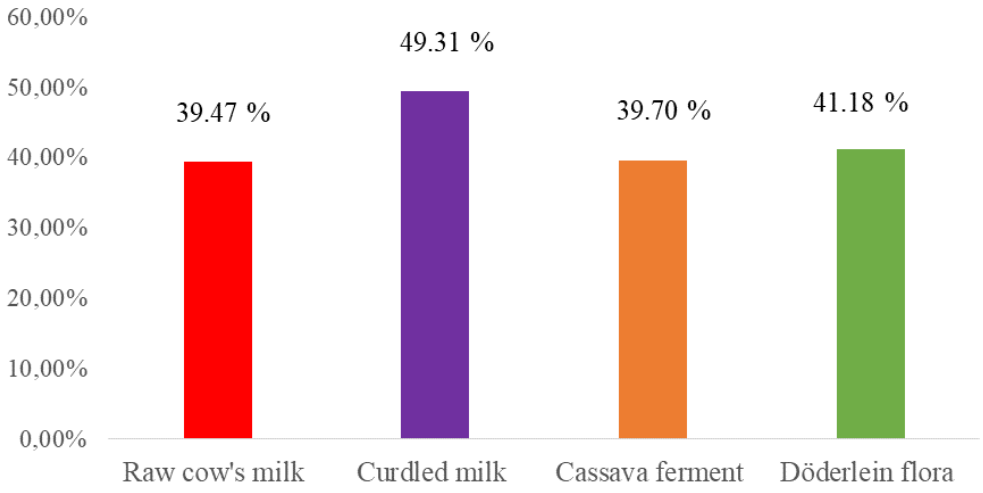

Fig. 3 : Proportion of isolated *Lactobacillus* (*Lb*) strains from different matrices having antibacterial activity against *Escherichia coli* ATCC 25922.

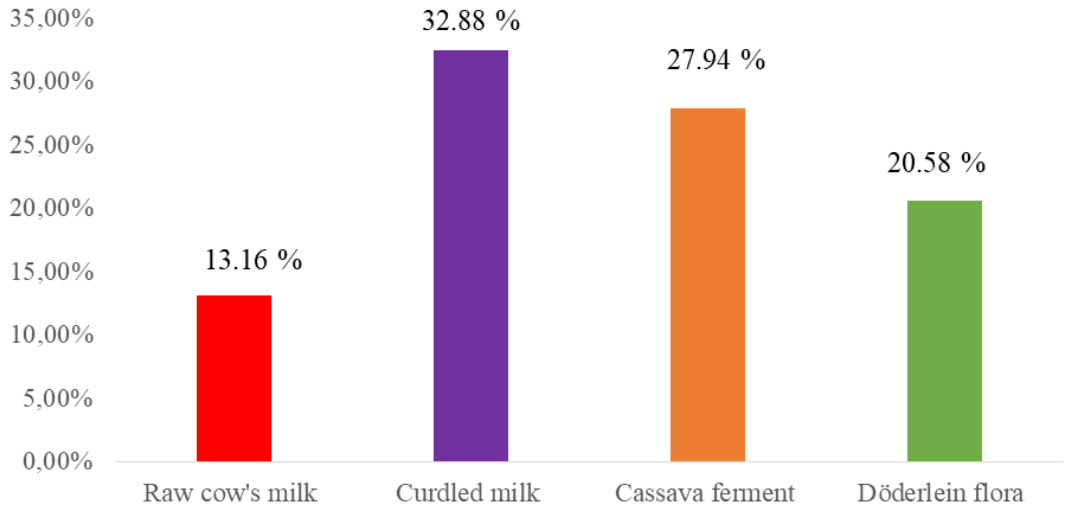

Fig. 4 : Proportion of isolated *Lactobacillus* (*Lb*) strains from different matrices having antibacterial activity against *Pseudomonas aeruginosa* ATCC 27853.

Discussion

In the present study, we have isolated and identified Lb species from raw cow's milk, curdled milk, manioc ferment and Döderlein flora. The results obtained enabled us to identify nine (9) Lb species, namely Lb plantarum, Lb casei, Lb acidophillus, Lb helveticus, Lb bulgaricus, Lb ruteri, Lb rhamnosus, Lb paracasei and Lb fermentum. Our results are in line with those reported by Mami et al. (2008), who identified virtually the same Lb species isolated from raw goat's milk.

Working under experimental conditions that eliminated the effect of lactic acid and hydrogen peroxide, the inhibitory activity of the Lb species studied revealed a broad spectrum against the three pathogenic strains S. aureus, E. coli and P. aeruginosa. The various Lb species isolated from several biotopes (raw cow's milk, curdled milk, cassava ferment and Döderlein flora), tested for their inhibitory powers, showed good activity against the targeted microbial strains. Similarly, Allouche et al. (2010), reported good inhibitory activity of the Lb species tested against S. aureus, E. coli, P. aeruginosa and Bacillus subtilis. This notable antimicrobial activity could be due to the quality of the milks studied and the natural lactic ferment of the milks that we used. The inhibitory potency of the Lb plantarum strains isolated from raw cow's milk and the cassava ferment tested against the targeted strains possess a broad spectrum of significant inhibition, with diameters ranging from 10 to 19 mm. Likewise, previous study from Belhamra, (2017) reported a Lactobacillus strains with remarkable antimicrobial activity against both E. coli and S. aureus. This activity could be explained by the presence in cell-free supernatant of a diversity of antibacterial agents produced during microbial culture.

Previous studies have shown that out of seven (7) Lb strains isolated from raw cow's milk and dromedary minced meat, one Lb strain isolated from raw cow's milk has inhibition zones against the pathogenic S. aureus strain (Bouzaid et al., 2016). The high presence of Lb plantarum strains with antimicrobial activity in the samples could be due to the production of organic acids, hydrogen peroxide, phages and/or bacteriocins (Todorov & Dicks, 2005 ; Guessas et al., 2005).

Surprisimgly, some strains of Lb acidophilus isolated from the Dôderlein flora were among the less effective against the pathogenic strains tested notably S. aureus and E. coli. Cherifi and Chetioui, (2019) also showed the absence of antimicrobial activity of this species against both strains. Furthermore, the lack of sensitivity observed in E. coli towards the Lb plantarum species from Dôderlein flora, corroborates those obtained by (Boulouf, 2016 ; Mami et al., 2010 ; Tagg et al., 1976). In contrast, Lb plantarum strains isolated from raw cow's milk and casava ferment have very interesting activities. In fact, they have broad activity spectra on all the pathogenic germs tested, with zones of greater inhibition (Bahri, 2014). This could be explained by the presence of bacteriocins in the bacterial strains tested. Several authors (Itoh et al., 1995; Tahara & Kanatani, 1996) have shown that it is also possible for the Lb acidophilus strain to exert only an inhibitory activity against bacteria taxonomically close to the producing strain.

The results of the antibacterial activity of Lb strains isolated from curdled milk and tested against E. coli ATCC 25922 were the most dominant with a rate of 49.31 %, similarly to those reported by (Goa et al., 2022). These authors showed a strong inhibitory activity against E. coli ATCC 25922 of Lb strains isolated from fermented milk.

The antibacterial activities of Lb isolated from our curd samples, against S. aureus ATCC 29213 and P. aeruginosa ATCC 27853 were respectively 52.05 and 32.88 %. However, Lb from fermented camel milk was reported to be active against a larger range of bacteria notably S. aureus ATCC 25923, Salmonella sp., E. coli ATCC 25922, Bacillus subtilis and Enterobacter cloacae (Bouguerra, 2021). Additionnally, a high level of antimicrobial activity in Lb strains from curdled milk was also reported by (Lachi & Kellas, 2019) ;

this could be explained by the fact that the Lb in curdled milk help to ensure product quality, to better control the fermentation process and to reduce the risk of infection.

Conclusion

Identification of the Lb strains isolated from the different matrices showed a diversity of Lactobacillus species. Most of the Lb species had antibacterial activity against the three reference strains (*Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853). However, *Lb* strains isolated from cassava ferments gave the best antibacterial activity against *S. aureus*, whereas *Lb* isolated from raw cow's milk showed low antibacterial activity against *Pseudomonas aeruginosa*. In addition, *Lb fermentum* species isolated from cassava ferments showed the broadest spectrum of activity. Furthermore, the reference strain *S. aureus* was the most sensitive of the reference strains tested and the least sensitive bacterium was *P. aeruginosa*. The metabolites (potentially bacteriocins) responsible for the antibacterial activity of these *Lb* strains could be an alternative to the use of antibiotics and thus help to fight against the emergence and spread of multi-resistant bacteria.

Funding of the study

This study was carried out with the financial support of the Institut Pasteur de Côte d'Ivoire.

Conflict of Interest

The authors declare that there are no conflicts of interest in this article.

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