African Journal of
Clinical Medicine and Pharmacy Research

ISSN: 3434-2990

Index: Harvard, Boston, Sydney University, Dimensions, Lens, ResearchGet Scilit, Semantic, Google Scholar, Base etc

https://doi.org/10.58578/AJCMPR.v2i2.5387

Antibacterial Potential of Lactobacillus-Derived Bacteriocin-Like Inhibitory Substances (BLIS) Against Group B Streptococcus (GBS): A Biotherapeutic Approach

Samuel Tamunoiyowuna Cockeye Brown[1], Ikrimah Usman Mohammed[2*], Eze Emmanuel Onyemaechi[3]

[1,2]Federal University Wukari, Taraba State, Nigeria

[3]I. M. Sechenov Medical University Moscow, Russia

ikrimah25@gmail.com

Article Info:

Submitted:	Revised:	Accepted:	Published:
Mar 10, 2025	Mar 25, 2025	Apr 6, 2025	Apr 11, 2025

Abstract

Background: Lactic acid bacteria (LAB) are a diverse group of Gram-positive, non-spore-forming bacteria known for their ability to produce antimicrobial substances such as bacteriocins and bacteriocin-like inhibitory substances (BLIS). **Aim:** This study aimed to isolate, characterize, and evaluate the antimicrobial activity of BLIS produced by LAB against Group B Streptococcus (GBS) from vaginal samples of pregnant women attending Federal University Teaching Hospital Wukari, Nigeria. **Methods:** Vaginal swabs were collected from 50 pregnant women and processed for the isolation of LAB and GBS using selective media and biochemical characterization. Bacteriocin-like inhibitory substances were extracted from LAB isolates and tested against GBS using the disk diffusion method. The effects of temperature and pH on BLIS activity were also evaluated. **Results:** The results revealed that LAB isolates exhibited varying degrees of antimicrobial activity against GBS. The BLIS produced were found to be moderately heat-stable, retaining activity up to 90°C but showing loss at 100°C. BLIS produced by **selected**

AJCMPR Journal is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License

isolates (S8, S4, and S2) maintained antimicrobial activity across a broad temperature range (30°C –100°C) and different pH conditions (5.5–8.0). The inhibitory effects of BLIS remained stable across different pH levels, reinforcing their potential as alternative antimicrobial agents. There was no significant difference in activity between crude and partially purified BLIS, suggesting they may be low-molecular-weight peptides. Antibiotic susceptibility testing showed that GBS isolates were resistant to levofloxacin, amoxicillin, and ampiclox, while ciprofloxacin, erythromycin, chloramphenicol, and streptomycin remained effective. **Conclusion:** This study underscores the potential of LAB-derived BLIS as natural antimicrobial agents for managing GBS infections. The effectiveness and stability of Lactobacillus-derived BLIS in various environmental conditions suggest its suitability for pharmaceutical and food industry applications. Further research on purification and molecular characterization could enhance its use in antimicrobial therapy.

Keywords: Lactic acid bacteria, Bacteriocin-like inhibitory substances, Group B Streptococcus, Antimicrobial activity, Pregnant women, Wukari

INTRODUCTION

Lactic acid bacteria (LAB) are known to be Gram-positive, non-spore-forming rods, cocci and cocco-bacilli non-aerobic but aerotolerant, able to ferment carbohydrates into energy and lactic acid [1]. They are the dominant bacteria of a healthy human vagina, and their presence and number are influenced estrogen production, which under goes age and menstrual cycle dependant changes. A health vaginal ecosystem is dominated by certain species of lactobacillus which exert a significant influence on the microbiology of the vagina [2]. The lactic acid bacteria (LAB) group includes more than 25 *Lactobacillus* genera, including *Lactobacillus*, *Acetilactibacillus*, *Agrilactobacillus*, *Amylolactibacillus*, *Furfurilactobacillus*, *Fructilactobacillus*, *Holzapfelia*, *Latilactobacillus*, *Lactiplantibacillus*, *Loigolactibacillus*, *Paralactobacillus*, *Schleifer ilactobacillus*, etc [3].

According to Yusuf and Hamid [1], LAB belong to the phylum Firmicutes. The different major genera of LAB include: *Lactobacillus*, *Weissella Lactococcus*, *Melissococcus*, *Enterococcus*, *Lactosphaera*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Vagococcus*, *Carnobacterium* and *Tetragenococcus*. Other genera include: *Aerococcus*, *Propionibacterium*, *Microbacterium*, and *Bifidobacterium*. In addition *Lactobacillus*, *Streptococcus*, *Carnobacterium*, *Enterococcus*, *Lactococcus*, *Pediococcus* and *Weissella* are also under in the LAB group [3]. According to Karaoglu *et al.*

[4] among LAB members, the *lactobacilli* are composed of a diverse group of homofermentative and heterofermentative species. The production of bacteriocins by LAB has been known for many years [4].

Bacteriocins are heterogeneous group of peptides or proteins with antimicrobial activity synthesized ribosomally and released extracellularly by bacteria belonging to nearly all taxonomic groups; these are classified according to their genetic, structural and biochemical characteristics [5]. According to the original definition, the term bacteriocin refers to proteins of the colicin type, characterized by lethal biosynthesis, intraspecific activity, and adsorption to specific receptors. Those produced by gram-positive bacteria fit closely to the classical colicin model [4]. Bacteriocins are active metabolic peptides that are ribosomally synthesized by certain lactic acid bacteria (LABs). These are characterized by being non-toxic, and are either electrically neutral or positively charged [3].

Bacteriocins produced by different LABs differ from each other by their unique biochemical, structural, genetic, ecological and metabolic activity [3, 6]. Bacteriocins are found within each of the four major classes of antimicrobial proteins produced by lactic acid bacteria. Class I (lantibiotics) was only recently discovered in the Lactobacillaceae. Lantibiotics are small membrane active peptides (<5 kDa) containing the unusual aminoacid lanthionine. Class II; small heat-stable, non-lanthionine containing membrane-active peptides (<10kDa) characterized. The class III bacteriocins, which have to date only been found in *Lactobacillus*, include heat-labile proteins of large molecular mass. Class IV is a complex bacteriocin group. These proteins are associated with other lipid or carbohydrate moieties, which appear to be required for activity. The bacteriocin was relatively hydrophobic and heat stable [4].

The vaginal microbiome of healthy women is a dynamic ecosystem, and it is colonized by a variety of microorganisms. The composition of the vaginal microbiome is influenced by a number of factors such as age, hormonal levels, sexual activity, hygiene, phase of menstrual cycle, or diet [7, 8]. In healthy premenopausal women, bacteria of the genus Lactobacillus are dominant in the vaginal microbiome at 10^7–10^8 CFU/g of vaginal fluid [8]. The most frequently found are species of *L. crispatus*, *L. gasseri*, *L. iners*, and *L. jensenii*, but this depends on ethnic group or geographic location. There are also differences between pregnant and non-pregnant women; lower strain diversity was found in pregnant women. On the other hand, lactobacilli in pregnant women are more stable than in non-pregnant

women [7-9]. The healthy urogenital tract plays a significant role in protecting against vaginal infections, and vaginal lactobacilli are important because of their protective functions (adhesion to the vaginal tissue and production of antimicrobial substances) [8].

Group B Streptococcus (GBS), also known as *Streptococcus agalactiae*, is a facultative, gram-positive diplococcus [10]. According to Patras and Nizet, [11], *Streptococcus agalactiae* [Group B Streptococcus (GBS)] is an encapsulated Gram-positive bacterium that colonizes the lower gastrointestinal tract, and in females, the urogenital tract, of 20–30% of healthy human adults. *Streptococcus agalactiae* is a leading source of perinatal and neonatal infections globally, accounting for 1.8 new born infections per 1000 live births each year [12]. The organism can be acquired after delivery or in utero via transfer from maternal vaginal or anorectal mucosa [13]. It is estimated that 10 to 30% of all pregnant women are colonized with GBS in their gastrointestinal and genital tracts [10]. Group B Streptococcus utilizes multiple adhesins and stress response mechanisms, defences against other microbes, and immune evasion strategies to achieve persistent or intermittent vaginal colonization. During the peripartum period, GBS gains access to a new host, the immune-deficient neonate, where GBS can again serve as a commensal organism or transition to an invasive pathogen resulting in sepsis or meningitis. GBS displays an arsenal of virulence factors, including a potent hemolytic toxin and multiple surface proteins to invade host tissues, as well as molecular mimicry and proteases to impede host immune recognition and responses [11].

MATERIALS AND METHODS

Description of the Study Area

Wukari Metropolis, the headquarters of Wukari Local Government Area in Taraba State, is crossed by the River Donga and River Benue [14, 15]. It is one of the 16 local government areas in the state, located at latitude 7.53'43'N and longitude 9.47'59'E, with a population ranging from 5,000 to 10,000. Covering 4,308 km² [16, 17]. Wukari is historically significant as the administrative center of the Kwararafa Kingdom and is predominantly inhabited by the Jukun people. The region features Savannah vegetation and an economy driven by farming, fishing, livestock rearing, commerce, and civil service [14, 17, 18]. The main languages spoken include Jukun, Hausa, Fulani, and Tiv [14].

Ethical Considerations

Before collecting the samples, the Head of Department Microbiology provided a letter of introduction that was submitted to the Education Secretary Wukari Local Government through the administration of Federal University Teaching Hospital Wukari for clearance. Samples were gathered, as well as collection schedules at the Federal University Teaching Hospital Wukari.

Collection of Specimen

Vaginal fluids were taken aseptically from the lateral vaginal wall of 50 pregnant women [2] attending Federal University Teaching Hospital Wukari with a sterile swab stick and promptly delivered to the Microbiology Laboratory Federal University Wukari.

Isolation Characterization and Identification of *Lactobacillus Agalactiae*

Samples were delivered for the isolation of *Lactobacillus Agalactiae*. The medium were created in accordance with the manufacturer's instructions [19]. The vaginal swab samples transported in Thioglycollate Broth Medium were inoculated onto Rogosa SL agar and incubated at 37 ºC in a candle jar for 48 hours before the colonies were subculture to obtain pure culture for further analysis [4]. The lactobacilli were identified on the basis of growth on selective Rogosa SL agar (pH 5.2), cell morphology, gram staining, and catalase activity. Further identification of the species of these lactobacilli was performed according to carbohydrate fermentation patterns, esculin hydrolysis and growth at 15 ºC and 45 ºC in the Lactobacilli DeMan Rogosa Sharpe (MRS) broth (Difco), as described in Bergey's Manual of Systematic Bacteriology. Purified cultures were maintained at –80 ºC in MRS broth with 10% glycerol [4].

Isolation, Characterization and Identification of Group B *Streptococcus*

Streptococcus Vaginal Swabs for Group B Streptococcus isolate were immersed in peptone broth for 1 minute before being gently pressed against the edge of the tube. The tube was incubated at 37°C for 24 hours under anaerobic conditions. Growth in the culture broth was streaked onto 5% sheep blood agar plates and incubated at 37°C for 24 hours. Colonies with clear zones were selected and sub-cultured. Gram staining was performed on a freshly produced culture, and the catalase test was seen.

Bacterial Identification

The isolates were identified based on their morphological, cultural, and biochemical characteristics. *Lactobacillus spp*. were identified by comparing observed isolate features to those of lactic acid bacteria as given in Bergey's Manual of Determinative Bacteriology [20]. The catalase test, Christie, Atkins, and Munch-Peterson (CAMP) test and Gram staining were used to identify Group B Streptococcus [21].

Crude Bacteriocin-like Inhibitory Substance (BLIS)

Bacteriocin-like Inhibitory Substance production involves the extracellular release of proteins into the culture broth. Three hundred (300) mL of MRS broth were inoculated with 1% v/v of the overnight cultures of the identified Lactobacillus isolates and anaerobically incubated at 37°C for 48 hours in triplicate. Following incubation, cells were sorted by centrifugation at 5000 g for 30 minutes. The supernatants were adjusted to pH 6.5 with Sodium Hydroxide (NaOH) to eliminate the influence of organic acid, and 5 mg/ml catalase was added to remove the inhibiting effect of hydrogen peroxide. The supernatants were filtered through a 0.45 μm membrane filter to extract crude BLIS from the cell-free supernatant [2, 21]. To produce partially pure BLIS, ammonium sulphate precipitation of bacteriocin (90% saturation) was performed by gradually dissolving 90.45 g of the salt in the cell-free supernatant and stirring until completely dissolved. The precipitate were re-dissolved in 6 ml of 0.05 M Sodium Phosphate buffer (pH 7.0) after centrifugation and then kept under cold condition for further use [21].

Media Preparation

Any media used for microbe isolation must be prepared so that it does not produce erroneous results. The work bench was properly sterilized with 70% alcohol. Material for media preparation was assembled on the workbench. Then, the manufacturer's directions were properly followed. Autoclaving sterilized the media, which was then prepared according to the manufacturer's instructions [18].

Production of Crude Bacteriocin-like Inhibitory Substance (BLIS)

BLIS proteins are secreted into the culture broth. To extract them, 300 ml of MRS broth was inoculated with 1% (v/v) overnight cultures of the identified isolates and incubated anaerobically at 37°C for 48 hours. Following incubation, the cells were removed by centrifugation at 5000 g for 30 minutes. The obtained supernatants were split into two

portions for crude and refined extract preparation. One portion was adjusted to pH 6.5 using NaOH to neutralize organic acids, and catalase (5 mg/ml) was added to eliminate the effects of hydrogen peroxide. Finally, the supernatants were filtered through a 0.45 μm membrane to obtain the crude BLIS extract [2].

Preparation of Group B Streptococcus

The Group B Streptococcus overnight culture was inoculated into the broth and incubated at 37°C for 24 hours. The microbial count was determined using a routine plate count method. A tenfold serial dilution was carried out by transferring 1 mL of the stock solution into 9 mL of sterile normal saline, continuing up to the tenth dilution. From each dilution, 0.1 mL was plated on nutrient agar using the spread plate technique. After overnight incubation, colony counts were recorded. The sensitivity test was conducted using a culture containing approximately 5×10^8 cells/mL [21].

Determination of Inhibitory Spectrum of Bacteriocin

The bacteriocin assay was performed using the disk diffusion method as described by Bhunia [22]. Whatman No. 1 filter paper was punched into 8.0 mm discs, which were then soaked with 100 μl of the extract. These discs were sterilized in a dry heat oven at 140°C for one hour and cooled before use. A sterile glass rod was used to spread 100 μl of an overnight broth culture (containing approximately 5×10^8 cells/ml of the test organism) onto nutrient agar, which was left to stand briefly. The sterilized discs, pre-soaked in 100 μl of the extract, were placed on the inoculated agar using sterile forceps. Sterile phosphate buffer (pH 7.0) served as a control. The plates were incubated aerobically at 37°C for 24 hours, and inhibition zones were observed around the discs, indicating antimicrobial activity. The diameters of these zones were measured in millimeters, with antagonistic activity expressed as the average inhibition zone size.

Physicochemical Characterization of BLIS

Effect of Temperature on BLIS

The impact of crude and partially purified extracts on the target organism was evaluated at different temperatures over a 10-minute period. Each sample was placed in a 3 mL test tube, sealed with cotton, and heated using a water bath to temperatures of 30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C, and 100°C. After heating, the samples were immediately

cooled in a refrigerator before being tested against the target organism using the previously described method [21, 23].

Effect of hydrogen ion concentration (pH) on BLIS

The effects of crude and purified extracts on the target organism were analyzed at different pH levels (5.5, 6.0, 7.5, and 8.0). The pH was adjusted using 1 N NaOH and 1 N HCl with the help of a Hanna pH meter [21, 23]. After adjustment, the samples were filtered through 0.45 μm membrane filters and tested against GBS following the previously described method. The sample's activity at its original pH (7.0) was used as a control [21].

Isolation and Identification of *Lactobacillus* and Group B *Streptococcus*

Samples were cultured on selective media, and isolates were identified through biochemical assays. The presence of *Lactobacillus* and Group B Streptococcus (GBS) was confirmed using Gram staining, catalase testing, and carbohydrate fermentation analysis.

Antimicrobial Activity of *Lactobacillus* Isolates

The antibacterial activity of *Lactobacillus* isolates against Group B Streptococcus (GBS) was evaluated using agar well diffusion and microdilution techniques. The effectiveness of crude and partially purified bacteriocin-like inhibitory substances (BLIS) was determined by measuring inhibition zones and assessing their potency through minimum inhibitory concentration (MIC) analysis.

Statistical Analysis

Descriptive data analysis was used to assess the prevalence and antibacterial activity, with statistical significance set at $p < 0.05$.

RESULTS AND DISCUSSIONS

Among the 50 samples collected from the vaginas of pregnant women in different laboratories and clinics of Wukari Taraba State, some samples showed the microscopic characteristics of *Lactobacillus and streptococcus algalactiae.* Table 1 showed the microscopically characteristics of the 14 isolates used in the study. Some of the isolates did not comply with the biochemical tests while also some do not produce any inhibitory effect. Microscopic examination revealed that the tested isolates were Gram positive, rods appearing in pairs or

in chains. The test organism used (GBS) was Gram positive, spherical in shape and they occur in chains. It had a wide clear zone on nutrient agar.

Table 1: Microscopic characteristics of isolates from sample collected at Federal University Teaching Hospital Wukari

Isolate symbol	Gram reaction	Microscopic appearance	Cellular arrangement
S1	+	Bacilli	Chains
S14	+	Bacilli	Chains
S3	+	Cocci	Chains
S12	+	Cocci	Chains
S4	-	Cocci	Pairs
SB	+	Cocci	Chains
S7	+	Short-Bacilli	Chains
S8	+	Cocci	Pairs
S2	-	Cocci	Pairs
S10	+	Cocci	Pairs
S13	+	Bacilli	Chains
S9	-	Cocci	Pairs
S6	+	Cocci	Chains
S5	+	Bacilli	Chains

The microscopic characteristics (Table 1) confirmed the presence of both Gram-positive and Gram-negative bacterial isolates, with varied cellular arrangements such as chains and pairs. The majority of isolates were Gram-positive bacilli and cocci, which are typical characteristics of ***Lactobacillus* spp.** and **Group B Streptococcus (GBS)**. Similar studies by **Liasi *et al.* [24]** and **Williams *et al.* [25]** have also identified *Lactobacillus* isolates as Gram-positive, rod-shaped, and occurring in chains.

Table 2: Biochemical characteristics of isolates from sample collected at Federal University Teaching Hospital Wukari

Isolate symbol	Colony Morphology	Biochemical test				Sugar fermentation test			
		Catalase Test	Oxidase Test	Coagulase Test	Camp Test	Glucose	Fructose	Galactose	Lactose	Maltose
S1	Smooth, circular non pigmented colonies	-	-	-	+	+	-	+	-	w
S14	Rough, irregular, pigmented colonies	+	-	+	-	+	+	-	+	+
S3	Smooth, flat, non pigmented colonies	-	-	-	+	+	+	w	+	-
S12	Smooth, irregular, pigmented colonies	-	-	+	+	-	+	+	+	+
S4	Raised pigmented, concave colonies	+	+	-	-	+	+	+	+	+
SB	Smooth, non pigmented colonies	-	-	-	+	w	+	+	+	+
S7	Rough ,non pigmented colonies	+	-	-	+	+	+	w	-	+
S8	Smooth, regular, flat colonies	-	-	-	+	+	-	+	-	-
S2	Moderate, non pigmented colonies	-	+	-	-	+	w	+	+	-
S10	Small, smooth, nonpigmented colonies	-	-	-	+	+	+	+	+	+
S13	Rhizoid, pigmented colonies	-	+	-	-	-	+	+	w	+
S9	Pigmented, translucent rhizoid colonies	-	+	-	-	+	+	+	+	-
S6	Non pigmented, smooth colonies	-	+	-	-	+	w	+	+	+
S5	Rough non pigmented colonies	-	+	+	-	-	+	+	`-	-

Keys + = positive; - = negative; w = weak reaction. All the isolates fermented glucose, fructose and galactose.

The biochemical tests (Table 2) further classified the isolates based on their enzymatic activity and carbohydrate fermentation patterns. The catalase-negative isolates, such as S1, S3, S12, and SB, align with the properties of **Lactobacillus spp.**, which are known for their catalase-negative nature due to their fermentative metabolism. Studies by **Hemme & Foucaud-Scheunemann [26]** confirmed that catalase-negative Lactobacillus species contribute to antimicrobial activity through acid and bacteriocin production.

Moreover, the **Camp test** positivity in S1, S3, S12, SB, S7, S8, and S10 supports the identification of **Group B Streptococcus (GBS)** strains, as **GBS is Camp test-positive**, a distinguishing characteristic that differentiates it from other streptococci [27].

Table 3: Positive Samples after Anaerobic Incubation for 48hrs using Peptone Broth

Isolates	Test
S14	Turbid
S1	Turbid
S2	Turbid
S6	Turbid
S4	Turbid
S9	Turbid
S5	Turbid
S12	Turbid
S3	Turbid
S10	Turbid
S7	Turbid

Table 3 presents the turbidity observed after 48-hour incubation, which confirms bacterial viability and growth under anaerobic conditions. The presence of turbidity in isolates such as S14, S1, S6, S4, and S9 suggests that these isolates thrive in low-oxygen environments. This observation aligns with previous findings by Centeno ***et al.*** **[28]**, who reported the facultative anaerobic nature of **Lactobacillus spp.** and **GBS**, allowing them to survive in the gastrointestinal and urogenital tracts.

Figure 1 is the inhibitory spectrum of crude and partially purified extract of *Lactobacillus* species against the test organism. The BLIS from all isolates had their strongest activity at 30°C (Fig 2 - 4). The **Bacteriocin-Like Inhibitory Substances (BLIS)** extracted from ***Lactobacillus*** **spp.** demonstrated significant inhibitory activity against the test organism **GBS** (Figure 1). The **agar well diffusion assay** revealed **clear inhibition zones**, confirming the presence of antimicrobial peptides.

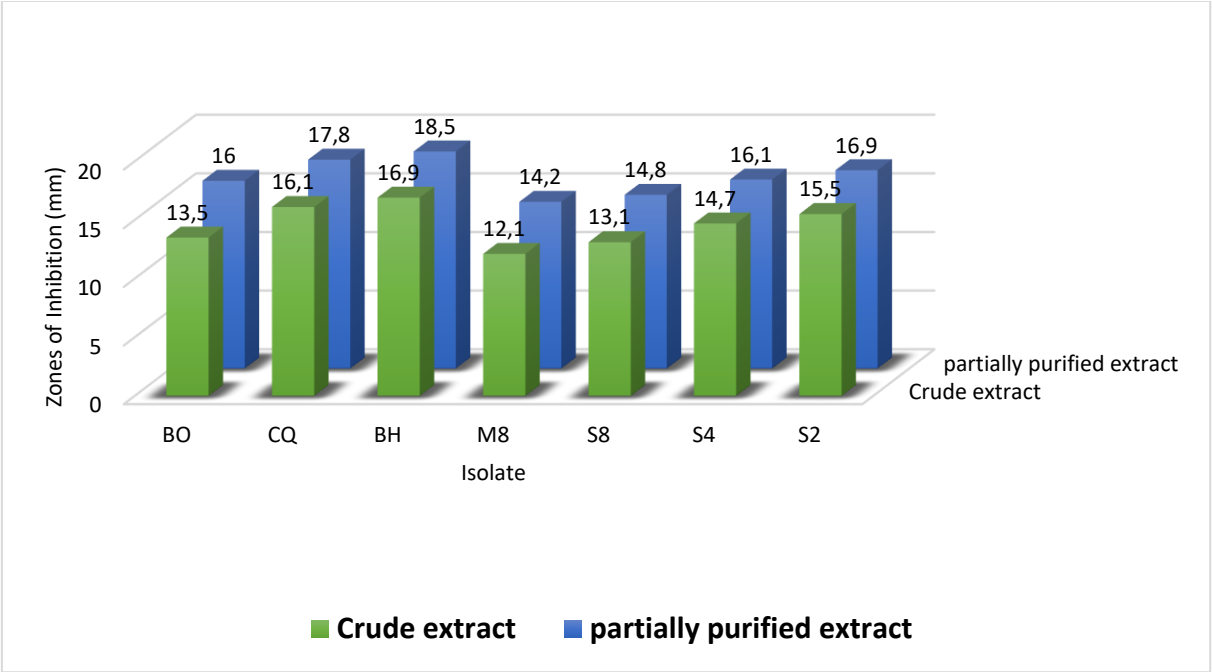


Figure 1: Inhibitory spectrum of crude and partially purified extract of *Lactobacillus* species against the test organism

Figure 2 shows the activity of BLIS from isolate S8, its activity was maintained from 30°C - 50°C in the crude extract, while the activity of partially purified BLIS was stable at 80^0C - 100^0C with an average of 12.5mm zone of inhibition. Figure 3 shows the activity of BLIS in the crude extract to be steady at high temperature while the activity of partially purified BLIS was not steady at all temperature ranges. Partial loss of activity was seen for BLIS from isolate S2 as the temperature increased. The partially purified BLIS lost its activity at 100°C (Figure 4).

The results in Figures 2, 3, and 4 highlight the **temperature stability of BLIS**. The inhibitory activity was retained at temperatures up to **70°C**, but a decline was observed at higher temperatures (90°C and 100°C). Similar findings were reported by **Todorov & Dicks [29]**, where Lactobacillus bacteriocins retained activity at moderate heat but lost potency at extreme temperatures.

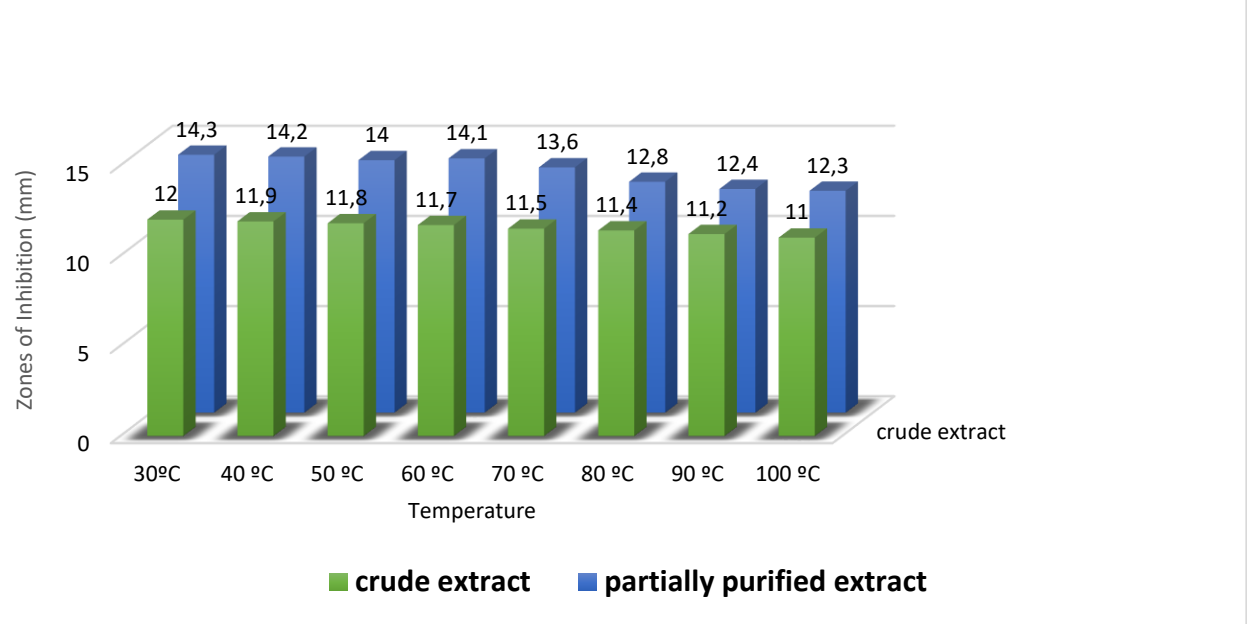


Figure 2: Effect of BLIS Produced by Isolate S8 at Different Temperature Ranges

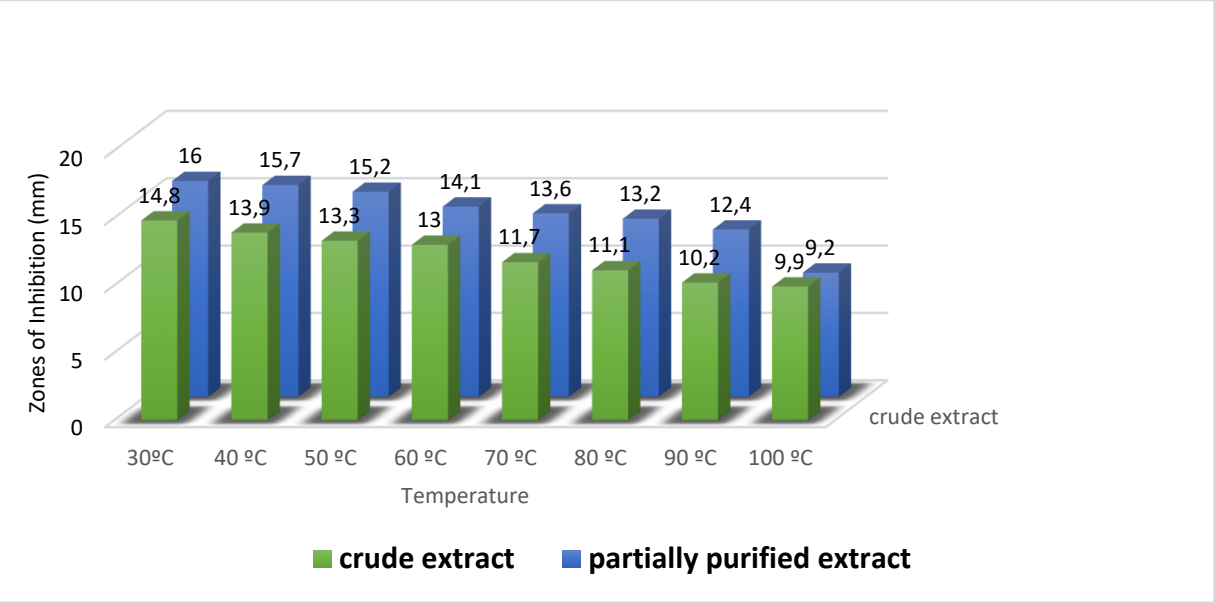


Figure 3: Effect of BLIS Produced by Isolate S4 at Different Temperature Ranges

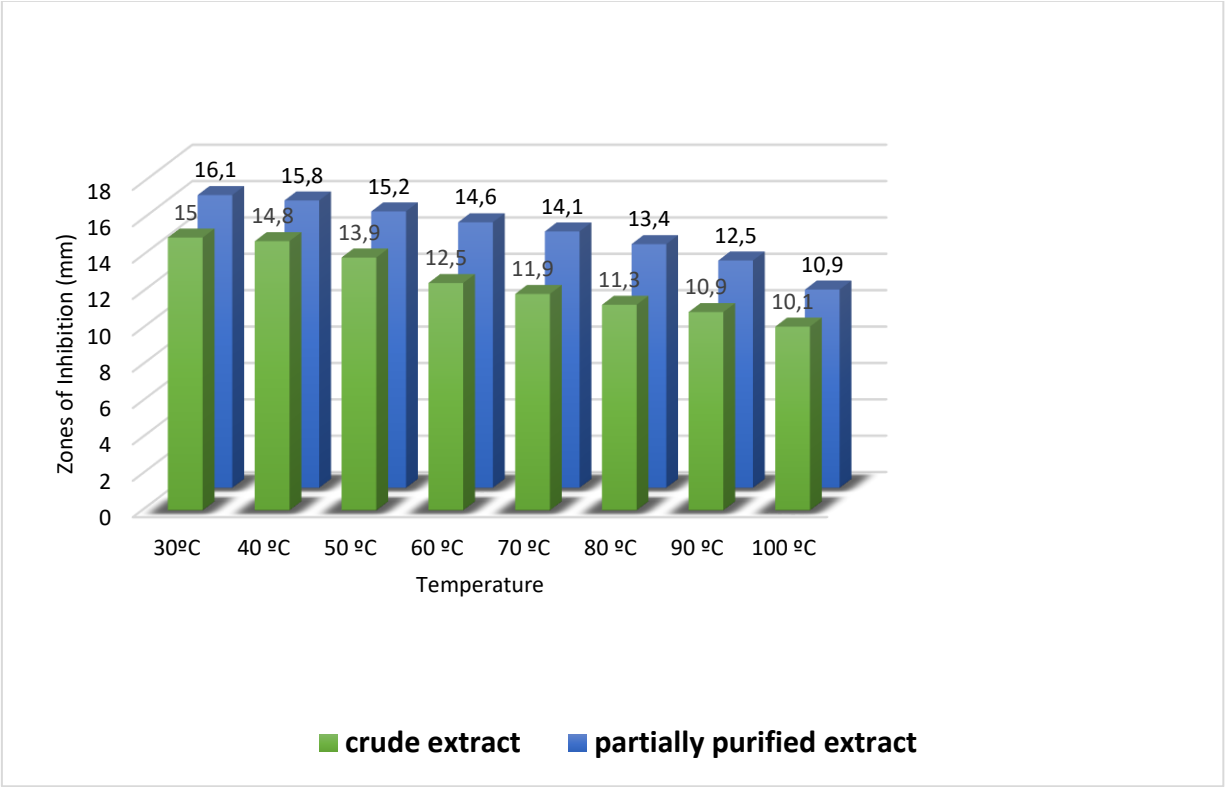


Figure 4. Effect of BLIS Produced by Isolate S2 at Different Temperature Ranges

Each inhibitory compound was assayed for its stability after 1 hour incubation at different pH levels (pH 5.5 – pH 8.0). The partially purified inhibitory compounds were active at a wide range of pH. Isolates S4 showed highest activity at pH 5.5. S8 and S2 were more sensitive against the target organism at pH 6.0. BLIS from isolate S2 had the strongest activity with 17.8 mm zone of inhibition. Generally the activities of the isolates were more active at acidic pH than at alkaline pH. The **pH stability** study (not shown in tables) also indicated that BLIS remained active at a **wide pH range (5.5–8.0)**, consistent with bacteriocins reported by **Mokoena [30]**.

The results of the inhibitory capacity assessment revealed that S2 and S8 exhibited the strongest activity against the target organism. When these two inhibitory compounds were combined, their effectiveness increased, as indicated by larger zones of inhibition. The crude extract of the combined compounds produced a 19.06 mm inhibition zone, while the partially purified extract showed an even greater zone of 21.07 mm. Further analysis of the partially purified extract across different pH levels demonstrated activity over a broad pH

range (5.5 - 8.0). Additionally, the extract maintained its inhibitory effect when exposed to temperatures between 30°C and 100°C for 10 minutes, showing stability particularly within the 30°C to 60°C range.

Table 4: Antibiotics Sensitivity Test for Positive Isolates

Test Isolate	Antimicrobial Agent	Zone of Inhibition (mm)	Disk Content (µg)	Zone Diameter (Interpretative Criteria)		
				S	I	R
S1	Amoxicillin	26	10			✓
	Streptomycin	20	10			✓
	Chloramphenical	22	30	✓		
	Ciprofloxacin	17	5		✓	
	Norflaxacin	18	10	✓		
	Erythromycin	28	10	✓		
	Colistin	26	10	✓		
	Ampiclox	20	20	✓		
	Rifampin	28	5	✓		
	Levofloxacin	26	5	✓		
S14	Ciprofloxacin		5	✓		
S3	Amoxicillin	13	10			✓
	Streptomycin	14	10	✓		
	Chloramphenical	28	30	✓		
	Ciprofloxacin	28	5	✓		
	Norflaxacin	23	10	✓		
	Erythromycin	25	10	✓		
	Colistin	22	10		✓	
	Ampiclox	10	20			✓
	Rifampin	16	5	✓		
	Levofloxacin	22	5	✓		
S12	Amoxicillin		10	✓		
	Streptomycin	24	10	✓		
	Chloramphenical	24	30	✓		
	Ciprofloxacin		5	✓		
	Norflaxacin	18	10			✓
	Erythromycin		10	✓		
	Colistin		10	✓		
	Ampiclox		20	✓		
	Rifampin		5			✓
	Levofloxacin		5	✓		

S4	Amoxicillin		10			✓
	Streptomycin	19	10			✓
	Chloramphenical	20	30			✓
	Ciprofloxacin		5			✓
	Norflaxacin	18	10	✓		
	Erythromycin		10	✓		
	Colistin		10			✓
	Ampiclox		20			✓
	Rifampin		5	✓		
	Levofloxacin		5	✓		
SB	Amoxicillin		10	✓		
	Streptomycin	21	10	✓		
	Chloramphenical		30		✓	
	Ciprofloxacin	16	5	✓		
	Norflaxacin		10			✓
	Erythromycin	18	10	✓		
	Colistin	17	10		✓	
	Ampiclox	15	20	✓		
	Rifampin	14	5			✓
	Levofloxacin		5			✓
S7	Ciprofloxacin	24	5	✓		
S8	Ciprofloxacin	27	5	✓		
S2	Amoxicillin		10			✓
	Streptomycin	21	10	✓		
	Chloramphenical		30			✓
	Ciprofloxacin		5	✓		
	Norflaxacin		10			✓
	Erythromycin		10	✓		
	Colistin		10		✓	
	Ampiclox		20			✓
	Rifampin		5	✓		
	Levofloxacin	18	5	✓		

S10	Ciprofloxacin	24	5	✓	
S13	Ciprofloxacin	20	5	✓	
S9	Amoxicillin		10	✓	
	Streptomycin		10		✓
	Chloramphenical	15	30	✓	
	Ciprofloxacin	17	5		✓
	Norflaxacin		10	✓	
	Erythromycin		10		✓
	Colistin		10		✓
	Ampiclox		20	✓	
	Rifampin		5		✓
	Levofloxacin		5	✓	
S6	Ciprofloxacin	23	5	✓	
S5	Ciprofloxacin	19	5	✓	

Note: Multiple spectrum and single spectrum antibiotic disk was used for this assay, the single spectrum used was Ciprofloxacin.

The **antibiotic sensitivity test** revealed that many isolates were **resistant to Amoxicillin, Ampiclox, and Colistin**, which is concerning due to the increasing **antimicrobial resistance (AMR)**. However, most isolates were sensitive to **Streptomycin, Erythromycin, and Ciprofloxacin**, showing promising treatment options. Resistance to **β-lactam antibiotics (Amoxicillin, Ampiclox)** aligns with reports by **Simeoni *et al.* [31]**, who observed that **GBS strains** possess β-lactamase enzymes that degrade penicillins. Sensitivity to **Ciprofloxacin and Erythromycin** supports previous findings by **Ruh *et al.* [32]**, where similar strains of **GBS** were susceptible to **fluoroquinolones and macrolides**.

***Lactobacillus* strains isolated from dairy and fermented foods** have been reported to produce heat-stable bacteriocins with a broad inhibitory spectrum [33]. **GBS resistance to penicillin and ampicillin** has increased over time, as noted in studies on neonatal

infections [32]. **Bacteriocin-producing *Lactobacillus*** species from probiotic sources have been explored as alternatives to antibiotics [30], which supports the potential application of BLIS in combating drug-resistant pathogens.

During the course of this study, a total number of 50 samples were obtained from the vaginas of pregnant women and identified based on cultural and biochemical characteristics. The biochemical characteristics of this research isolates are comparable with vaginal *Lactobacillus* isolated by some researchers. *Lactobacillus plantrum* is found to be a normal inhabitant of healthy women [2]. Isolate S4 and S2 which were provisionally identified as *L. acidophilus* in this study, has been isolated from other studies [4]. Aslim *et al.* [2] found 16% *L. acidophilus* in healthy women. According to Li *et al.* [34], the rate of vaginal *L. acidophilus* in pregnant women was 76.9%. According to Pascaul *et al.* [35] *L fermentum* is also a normal inhabitant of healthy women. Lactobacillus species are commonly isolated from the vaginas of healthy women. The presumptive Lactobacilli identified in this study closely resemble those reported by previous researchers.

Bacteriocins or bacteriocin-like inhibitory substances (BLIS) produced by *Lactobacillus* serve as a protective mechanism against pathogenic and opportunistic microorganisms in the vagina. Research has highlighted the antimicrobial properties and beneficial effects of bacteriocins/BLIS from vaginal *Lactobacillus* on Gram-positive and closely related organisms [36]. However, their impact on Group B Streptococci (GBS) remains largely unexplored.

The BLIS produced in this study successfully inhibited the growth of the test organism (GBS), aligning with the findings of Strus *et al.* [37], who reported that *Lactobacillus* species could suppress GBS growth. Similarly, Ruiz *et al.* [38] demonstrated that BLIS from *L. fermentum* and *L. rhamnosus* exhibited inhibitory effects on GBS. Furthermore, the combination of BLIS from both *Lactobacillus* species showed stronger inhibitory activity than individual BLIS, a trend also observed in this study.

The antimicrobial activity observed in this study was attributed to bacteriocins or bacteriocin-like inhibitory substances (BLIS), as their inhibitory effects remained unchanged after treatment with catalase or pH adjustment to 6.5. The BLIS produced by the isolates were partially characterized and evaluated for their in vitro antimicrobial activity against the test organism under varying temperature and pH conditions. The BLIS

exhibited moderate heat stability, as their effects were tested for only 10 minutes. However, isolate S4 lost its activity at 100°C within this timeframe, suggesting it is heat-labile.

The results showed no discernible difference between crude and partially purified BLIS activity, indicating that low molecular weight molecules may make up the BLIS. Certain antibiotics, such as ciprofloxacin, erythromycin, chloramphenicol, and streptomycin, demonstrated resistance in the antibiotic assay used to treat infections, while others, such as levofloxacin, amoxicillin, and ampiclox, demonstrated effectiveness. Some antibiotics showed sensitivity that was at the intermediate stage.

CONCLUSION

This study demonstrated the antimicrobial potential of lactic acid bacteria (LAB) isolated from the vaginal microbiota of pregnant women in Wukari, Nigeria, against Group B Streptococcus (GBS). The bacteriocin-like inhibitory substances (BLIS) produced by these LAB isolates exhibited significant inhibitory activity against GBS, suggesting their potential as natural antimicrobial agents. The BLIS were found to be moderately heat-stable and effective across a range of pH levels, further supporting their viability for therapeutic applications. Additionally, the antibiotic susceptibility testing revealed that some GBS isolates were resistant to commonly used antibiotics such as levofloxacin, amoxicillin, and ampiclox, while ciprofloxacin, erythromycin, chloramphenicol, and streptomycin remained effective. These findings highlight the need for alternative antimicrobial strategies, such as LAB-derived BLIS, in managing GBS infections, particularly in pregnant women where GBS colonization poses risks of neonatal infections. Further research is needed to fully characterize the molecular properties of these BLIS, optimize their production, and explore their potential applications in probiotic formulations or alternative antimicrobial therapies. This study contributes to the growing body of evidence supporting LAB as beneficial microorganisms in the human microbiome with promising clinical applications.

Conflict of Interest

There were not any conflicts of interest between the authors from beginning of the study to the end. Everything went well as design and agrees on the proposal.

Disclosure of Funding Source: The research did not received any specific grant from funding agencies in the public, commercial or non-profit sectors.

Acknowledgement

To the authors, without which this work would not have been possible; we did it!

ORCID

Ikrimah Usman Mohammed : https://orcid.org/0009-0001-3575-5783

Samuel Tamunoiyowuna Cockeye Brown : https://orcid.org/0009-0001-4245-206X

REFERENCES

1. Yusuf MA, Hamid TH. Lactic acid bacteria: bacteriocin producer: a mini review. IOSR Journal of Pharmacy. 2013 May;3(4):44-50.
2. Aslim B, Kilic E. Some probiotic properties of vaginal lactobacilli isolated from healthy women. Japanese journal of infectious diseases. 2006 Aug 28;59(4):249-53. doi.org/10.7883/yoken.JJID.2006.249
3. Bhattacharya D, Nanda PK, Pateiro M, Lorenzo JM, Dhar P, Das AK. Lactic acid bacteria and bacteriocins: novel biotechnological approach for biopreservation of meat and meat products. Microorganisms. 2022 Oct 18;10(10):2058. doi.org/10.3390/microorganisms10102058
4. Karaoglu ŞA, Aydin F, Kilic SS, Kilic AO. Antimicrobial activity and characteristics of bacteriocins produced by vaginal lactobacilli. Turkish Journal of Medical Sciences. 2003;33(1):7-13.
5. Rossi F, Pallotta ML. Bacteriocin producing cultures: A sustainable way for food safety improvement and probiotics with additional health promoting effects. International Journal of Medical and Biological Frontiers. 2016;22(1):59. doi:10.1038/nrmicro1273-c1
6. Balay, D.R.; Dangeti, R.V.; Kaur, K.; McMullen, L.M. Purification of Leucocin A for Use on Wieners to Inhibit Listeria monocytogenes in the Presence of Spoilage Organisms. Int. J. Food Microbiol. 2017, 255, 25–31.
7. Borges S, Silva J, Teixeira P. The role of lactobacilli and probiotics in maintaining vaginal health. Archives of gynecology and obstetrics. 2014 Mar;289(3):479-89.
8. Kumherová M, Veselá K, Kosová M, Mašata J, Horáčková Š, Šmidrkal J. Novel potential probiotic lactobacilli for prevention and treatment of vulvovaginal infections. Probiotics and antimicrobial proteins. 2021 Feb;13:163-72.
9. DiGiulio DB, Callahan BJ, McMurdie PJ, Costello EK, Lyell DJ, Robaczewska A, Sun CL, Goltsman DS, Wong RJ, Shaw G, Stevenson DK. Temporal and spatial variation of the human microbiota during pregnancy. Proceedings of the National Academy of Sciences. 2015 Sep 1;112(35):11060-5.
10. Tam T, Bilinski E, Lombard E. Recolonization of group B Streptococcus (GBS) in women with prior GBS genital colonization in pregnancy. The Journal of Maternal-Fetal & Neonatal Medicine. 2012 Oct 1;25(10):1987-9. https://doi:10.3109/14767058.2012.670331

11. Patras KA, Nizet V. Group B streptococcal maternal colonization and neonatal disease: molecular mechanisms and preventative approaches. Frontiers in pediatrics. 2018 Feb 22;6:27. doi: 10.3389/fped.2018.00027

12. de Mesquita Guimarães AM. Population structure, virulence and antibiotic resistance of Streptococcus agalactiae colonizing non-pregnant women. 2021. doi.org/10.1590/S1517-83822013005000030

13. Moussa TA, Elsherif RH, Mohamed YA, Dawoud ME, AboElAref AM. Group B streptococcus colonization of pregnant women: comparative molecular and microbiological diagnosis. Comparative Clinical Pathology. 2013 Nov;22:1229-34. doi 10.1007/s00580-012-1555-x

14. Mohammed IU Isolation and identification of pathogenic bacteria from commercially marketed fish in Wukari, North-Eastern Nigeria. European Journal of Microbiology and Infectious Diseases, 2024; 1(2): 66-72.

15. Brown STC, Ikrimah UM , Isaac JU Antimicrobial Activity of Paw-paw (*Carica papaya*) Leaves and Seed Extracts on Shigella and Salmonella Species. African Journal of Biochemistry and Molecular Biology Research, 2024; 1(1): 105-116.

16. Agwaranze DI, Ikrimah UM, Ugwuala MC. Bacteria Contamination of Rice Husk in Puje Ward, Wukari, Nigeria. Journal of Research in Agriculture and Food Sciences. 2024 May 1;1(1):22-

17. Brown S TC, Ikrimah UM , Isaac JU, Ibrahim JM, Benjamin ND, Ugwuala CM, Nurudeen UA Prevalence of Helicobacter pylori Among Suspected Ulcer Patients Attending General Hospital Wukari, Taraba State. African Journal of Biochemistry and Molecular Biology Research, 2024b; 1(1): 93-102.

18. Brown ST, Ikrimah UM, Agwaranze DI. Microbial population of food waste dump contaminated areas in parts of Taraba State. FUW Trends in Science & Technology Journal. 2023;8(3):040-6.

19. Ikrimah UM, Brown STC, Ibrahim JM, Idris HA, Nurudeen UA. Molecular Characterization and Screening of Antibiogram of Pathogenic Isolated from Sewage Water Source in part of Taraba State, Nigeria. FUW Centre for Research Journal of Science and Technology (FUWCRJST), 2024; 1(1):10-27

20. Ahmad MS, Zargar M, Mir SA, Bhat NA, Baba ZA, Kant RH, Dar ZM, Khan IJ, Bandey S. Morphological and biochemical studies for the identification of Lactobacillus plantarum sp. nov., and Lactobacillus fermentum sp. nov., from municipal waste. J Pharmacogn Phytochem. 2018;7(5):1421-4.

21. Alajekwu OU, Ike AC. Effect of bacteriocin-like inhibitory substances produced by vaginal lactobacilli on group B streptococcus. Nigerian Journal of Biotechnology. 2017 Apr 24;32:20-7.

22. Bhunia AK, Johnson MC, Ray B. Purification, characterization and antimicrobial spectrum of a bacteriocin produced by Pediococcus acidilactici. Journal of Applied Bacteriology. 1988 Oct;65(4):261-8.

23. Hefzy EM, Khalil MA, Amin AA, Ashour HM, Abdelaliem YF. Bacteriocin-like inhibitory substances from probiotics as therapeutic agents for Candida vulvovaginitis. Antibiotics. 2021 Mar 17;10(3):306.

24. Liasi SA, Azmi TI, Hassan MD, Shuhaimi M, Rosfarizan M, Ariff AB. Antimicrobial activity and antibiotic sensitivity of three isolates of lactic acid bacteria from fermented fish product, Budu. Malaysian Journal of Microbiology. 2009;5(1):33-7.

25. Williams ML, Pearl DL, LeJeune JT. Multiple-locus variable-nucleotide tandem repeat subtype analysis implicates European starlings as biological vectors for Escherichia coli O157: H7 in Ohio, USA. Journal of applied microbiology. 2011 Oct;111(4):982-8.

26. Hemme D, Foucaud-Scheunemann C. Leuconostoc, characteristics, use in dairy technology and prospects in functional foods. International Dairy Journal. 2004 Jun 1;14(6):467-94.

27. Zhao X, Oh SH, Yeater KM, Hoyer LL. Analysis of the Candida albicans Als2p and Als4p adhesins suggests the potential for compensatory function within the Als family. Microbiology. 2005 May;151(5):1619-30.

28. Centeno MS, Guerreiro CI, Dias FM, Morland C, Tailford LE, Goyal A, Prates JA, Ferreira LM, Caldeira RM, Mongodin EF, Nelson KE. Galactomannan hydrolysis and mannose metabolism in Cellvibrio mixtus. FEMS microbiology letters. 2006 Aug 1;261(1):123-32.

29. Todorov SD, Dicks LM. Lactobacillus plantarum isolated from molasses produces bacteriocins active against Gram-negative bacteria. Enzyme and Microbial Technology. 2005 Feb 1;36(2-3):318-26.

30. Mokoena MP. Lactic acid bacteria and their bacteriocins: classification, biosynthesis and applications against uropathogens: a mini-review. Molecules. 2017 Jul 26;22(8):1255.

31. Simeoni, D., Rizzotti, L., Cocconcelli, P., Gazzola, S., Dellaglio, F., & Torriani, S. (2008). Antibiotic resistance genes and identification of **Lactobacillus** species from fermented dry sausages. *Journal of Food Protection*, **71**(1), 39–46. https://doi.org/10.4315/0362-028X-71.1.39

32. Ruh E, Zakka J, Hoti K, Fekrat A, Guler E, Gazi U, Erdogmus Z, Suer K. Extended-spectrum β-lactamase, plasmid-mediated AmpC β-lactamase, fluoroquinolone resistance, and decreased susceptibility to carbapenems in Enterobacteriaceae: fecal carriage rates and associated risk factors in the community of Northern Cyprus. Antimicrobial Resistance & Infection Control. 2019 Dec;8:1-0.

33. Gänzle MG. Lactic metabolism revisited: metabolism of lactic acid bacteria in food fermentations and food spoilage. Current Opinion in Food Science. 2015 Apr 1;2:106-17.

34. Li L, Yang HI, Su J. Vaginal Lactobacillus distributions and functions in pregnant women. African Journal of Microbiology Research. 2011 May 18;5(10):1224-30.

35. Pascual LM, Daniele MB, Ruiz F, Giordano W, Pájaro C, Barberis L. Lactobacillus rhamnosus L60, a potential probiotic isolated from the human vagina. The Journal of general and applied microbiology. 2008;54(3):141-8.

36. Razzak MS, Al-Charrakh AH, Al-Greitty BH. Relationship between lactobacilli and opportunistic bacterial pathogens associated with vaginitis. North American Journal of Medical Sciences. 2011 Apr;3(4):185.

37. Strus M, Malinowska M, Heczko PB. In vitro antagonistic effect of Lactobacillus on organisms associated with bacterial vaginosis. The Journal of reproductive medicine. 2002 Jan 1;47(1):41-6.

38. Ruiz FO, Gerbaldo G, García MJ, Giordano W, Pascual L, Barberis IL. Synergistic effect between two bacteriocin-like inhibitory substances produced by lactobacilli strains with inhibitory activity for Streptococcus agalactiae. Current microbiology. 2012 Apr;64:349-56.