

## Antagonistic Effect of *Lactobacillus* Isolates from Kunnu and Cowmilk on Selected Pathogenic Microorganisms

Olotu Olanrewaju

Department of Microbiology, Federal University of Technology, P.M.B 704 Akure, Nigeria

**Abstract:** Wild strains of *Lactobacillus* species were isolated from Kunun zaki (fermented millet drink) and Fresh Cowmilk. Three of the isolates designated K<sub>1</sub>, K<sub>2</sub> and K<sub>3</sub> were obtained from Kunun zaki while two isolates designated C<sub>1</sub> and C<sub>2</sub> were obtained from the Fresh Cowmilk. Their antagonistic effect was tested against five selected bacteria namely *Escherichia coli* NCIB86, *Staphylococcus aureus* NCIB67, *Klebsiella pneumoniae* NCIB418, *Bacillus cereus* NCIB6349, and *Pseudomonas aeruginosa* NCIB532. The results showed that the isolates were able to inhibit the growth of some of the selected indicator organisms in varying degrees. Isolate K<sub>1</sub> was found to be the most effective with a zone of inhibition of 30mm recorded against *Staphylococcus aureus*. Also observed to be next in effectiveness is isolate K<sub>3</sub> obtained from the same source as isolate K<sub>1</sub> with a zone of inhibition of 20 mm against *Escherichia coli*. However, the least level of inhibition, 3 mm, was recorded against *Klebsiella pneumoniae* by isolate K<sub>3</sub>. Isolate K<sub>2</sub> was also found not to have inhibitory effect on any of the indicator organisms. The inhibition recorded in the case of the isolates that have antagonistic effect may be due to the production of organic acids, bacteriocins and hydrogen peroxide.

**Key words:** Kunun zaki, *Lactobacillus*, inhibition

### Introduction

All free-living animals, including man are hosts for a society comprising an enormous number of individuals – the normal microflora. The normal microflora differs between animal species, between individuals within the same species and also between body sites. The normal microflora changes dramatically during lifetime of the host. Human as well as animals normally are born sterile but shortly after birth, colonization begins and every location is filled up with the fittest microbes from the environment, thus creating a balanced ecological system.

The intestinal flora weighs between 1 and 2 kg, that is roughly the same weight as organs such as liver, brain or lungs, housing about 10<sup>14</sup> bacteria meaning that there are more living bacteria in the flora than there are cells in a normal body. This flora can be divided into two different categories namely the dominant floral population and the subdominant floral population accounting for less than 1% of the total bacterial population, but which according to recent studies may play a non-negligible role in equilibrium of the intestinal ecosystem.

The Lactic Acid Bacteria, if present, constitutes the dominant flora population due to their ability to colonize the human and animal intestinal tract. Exogenous bacteria, probiotic or pathogen, influence all the bacteria within the intestinal flora. The acidity of the stomach maintains a low concentration of bacteria in the upper part of the digestive tract and destroys pathogens. Interactions that occur between various bacterial species are also important in maintaining the equilibrium of the intestinal microflora.

Generally, the Lactic Acid Bacteria (LAB) are the most implicated of the probiotic organisms with respect to intestinal bacterial colonization, particularly those of the genera *Lactobacilli* and Bifidobacteria, which stakes out their territory by secreting acids, thereby creating an environment which is inhospitable to disease-causing bacteria. *Lactobacilli* change the oxidation-reduction potential through its production of metabolites by making the environment less conducive for organisms requiring oxygen. This action contributes to the overall inhibiting effect of these probiotic bacteria.

\* Corresponding author. mailing address: Department of Microbiology, Federal University of Technology, P.M.B 704 Akure, Nigeria, Tel: 234-7030236194, E-mail: olusemi@yahoo.com

## Objectives

Ever since Louis Pasteur formulated the germ theory of disease in the late 1800, humans have been locked in mortal combat with microorganisms. The zealous use of antibiotics, disinfectant chemicals and sanitary packaging attests to our fear and loathing of all things microbial. In this age of antiseptics, it seems ironic that each of our intestinal tracts harbours tens of trillions of bacteria, which by some estimates exceed the total number of cells making up the human body. More ironic is that many of these bacteria are beneficial.

It is however important to carry out *in-vitro* study in order to discover how *Lactobacillus* species exhibit their antagonistic characteristics since they have been reported to possess ranges of beneficial properties which extends to colon cancer prevention, immune system enhancement, food allergy reduction, short chain fatty acid production, to mention but a few.

Hence, the purpose of this project is to:

- i. Embark on an extensive screening programme in order to isolate and characterize *Lactobacillus* species from Kunun zaki and Cowmilk which demonstrated antagonistic activity against food spoilage and pathogenic bacteria
- ii. Study the *in vitro* antagonistic activity of the isolated *Lactobacillus* strains on some selected typed bacteria

## Materials and Methods

**Isolation of *Lactobacilli*.** 1ml of each of the samples of Kunun zaki and Cowmilk were drawn aseptically into test tubes in preparation for serial dilution to provide  $10^{-1}$  which was used for further dilutions to  $10^{-5}$ . About 17g DeMann Rogosa and Sharpe (MRS) agar was dispensed into 240 ml distilled water and autoclaved at 121°C for 15 minutes. 0.5mls of selected two dilution factors from each of the source samples were dispensed aseptically into sterile Petri dishes which has been inoculated with the serially diluted isolates and incubated at 37°C for 24 hours.

**Biochemical characterization.** Biochemical tests were performed on the isolates according to the scheme of Cowan and Steel (1974).

**Detection of antagonistic activity.** The antagonistic activity of the isolated *Lactobacillus* cultures was performed using the agar well diffusion assay described by Schillinger and Lucke (1989) against the selected test organisms

## Results

**Inhibition of the test organisms.** The zones of inhibition measured after incubating the isolates alongside the test organisms for 24 hours to observe their inhibitory effect is shown in Table 3. It was observed that there were larger zones of inhibition in the plates containing *Lactobacillus* isolates from Kunun, especially those of K<sub>1</sub> (30 mm), which inhibited *Staphylococcus aureus* and that of isolate K<sub>3</sub> (20 mm) which has the next larger diameter of zone of inhibition active against *Escherichia coli*. Isolate K<sub>3</sub> obtained from Kunun was able to inhibit the entire indicator organisms provided, though the lowest diameter of zone of inhibition was observed as 3 mm against *Klebsiella pneumoniae*. For the isolate K<sub>2</sub> also obtained from Kunun, it exhibited no antagonistic effect on any of the indicator organisms. Likewise, the zone of inhibition (8 mm) from C<sub>2</sub> isolate from Cowmilk was observed only in *Staphylococcus aureus* out of all the indicator organisms. A clear zone of inhibition of 18mm was observed against *Bacillus cereus* as exhibited by isolate K<sub>1</sub> from Kunun.

This shows that the *Lactobacillus* isolates obtained from Kunun are more effective than isolates from Cowmilk as regards their antagonism or inhibition (Table 3).

A total of 5 isolates were obtained based on their colonial/morphological and biochemical characteristics. These are shown in Tables 1 & 2 below.

Table 1. Morphological characteristics of isolates from Kunun and Cowmilk

Isolates	Color on MRS agar	Shape formed as seen under microscope	Morphology arrangement
K <sub>1</sub>	Dirty-white color on medium surface	Rods arranged in chains	Clustered, straight rods
K <sub>2</sub>	Dirty-white color on medium surface	Rods arranged in chains	Clustered, straight rods
K <sub>3</sub>	Dirty-white color on medium surface	Rods arranged in chains	Clustered, straight rods
C <sub>1</sub>	Cream color on medium surface	Rods arranged in chains	Thick, short rods
C <sub>2</sub>	Cream color on medium surface	Rods arranged in chains	Thick, short rods

Table 2. Biochemical characteristics of *Lactobacillus* isolates

Isolate	Gram strain	Catalase	Coagulase	Indole	Tentative identity of isolate
K <sub>1</sub>	+	-	-	-	<i>Lactobacillus</i> sp.
K <sub>2</sub>	+	-	-	-	<i>Lactobacillus</i> sp.
K <sub>3</sub>	+	-	-	-	<i>Lactobacillus</i> sp.
C <sub>1</sub>	+	-	-	-	<i>Lactobacillus</i> sp.
C <sub>2</sub>	+	-	-	-	<i>Lactobacillus</i> sp.

Table 3. Inhibition of indicator bacteria by *Lactobacilli* isolated from Kunun and Cowmilk samples

Test organisms	K <sub>1</sub>	K <sub>2</sub>	K <sub>3</sub>	C <sub>1</sub>	C <sub>2</sub>
<i>Bacillus cereus</i>	18 mm	NI	4 mm	NI	NI
<i>Escherichia coli</i>	12 mm	NI	20 mm	6 mm	NI
<i>Pseudomonas aeruginosa</i>	NI	NI	4 mm	15 mm	NI
<i>Klebsiella pneumoniae</i>	NI	NI	3 mm	NI	NI
<i>Staphylococcus aureus</i>	30 mm	NI	4 mm	NI	8 mm

N.B: Values represent mean (average) of duplicates. (NI)No Inhibition.

### Discussion

*Lactobacilli* grow best in highly nutritive substrates. They use the nutrient in the substrate for their own metabolism and cell growth and multiply in milk (from one million per millilitre to one billion per millilitre). They are present in the fermented food not only as viable cells and non-colony forming units, but also with the primary and secondary metabolites they have produced during the fermentation process (Robinson, 1991).

The MRS medium used was selective for the isolation of *Lactobacillus* species since they are extremely fastidious. Lindquist (1998) reported that a medium that will support their growth must contain a fermentable carbohydrate and many growth factors. He later recommended the use of Brain Heart Infusion medium for their growth. It was ensured throughout the experiment that only overnight cultures of the *Lactobacillus* isolates were made use of.

*Lactobacillus* isolate (K<sub>2</sub>) obtained from Kunun was observed to be inactive against any of the indicator organisms. In comparison, *Lactobacillus* isolate (K<sub>3</sub>) obtained from the same source inhibited all the indicator organisms (Table 3). Isolate K<sub>1</sub> was able to inhibit *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus* in the order 18 mm, 12 mm and 30 mm respectively. It has the greatest diameter of zone of inhibition directed against *Staphylococcus aureus* (30 mm).

Gilliland and Speck (1977) had earlier reported that *Lactobacilli* showed stronger antibacterial properties

against gram-positive bacteria (*Staphylococcus aureus* and *Clostridium perfringens*) than gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*). When observed strictly, *Lactobacillus* isolates K<sub>1</sub>, K<sub>3</sub> and C<sub>2</sub> inhibited *Staphylococcus aureus* in the order of 30 mm, 4 mm and 8 mm with respect to diameter of zone of inhibition. Isolates K<sub>1</sub>, K<sub>3</sub> and C<sub>1</sub> also inhibited *Escherichia coli* in the order 12mm, 20mm and 6mm. However, this is not conclusive because *Klebsiella pneumoniae* (a gram-negative organism) was only inhibited by isolate K<sub>3</sub> obtained in Kunun.

Jin *et al.* (1996) reported 12 strains of *Lactobacilli* isolated from chicken intestines, used in their experiment to investigate their inhibitory ability against five strains of *Salmonella* and three serotypes of *Escherichia coli*. The *Escherichia coli* serotypes O1:K1 and O78:K80 were found to be the most resistant to the *Lactobacillus* strains. They also stated that of the 12 strains of *Lactobacilli*, *Lactobacillus brevis* C and *Lactobacillus fermentum* C16, isolated from caecum were most effective in inhibiting the growth of the three *Escherichia coli* serotypes with the radius of zones of inhibition ranging from 12.5 mm to 18.0 mm. In this study, zones of inhibition of diameters 12 mm, 20 mm and 6 mm recorded against *Escherichia coli*.

Jin *et al.* (1996) had earlier recorded the inhibition zones of between 2.5 mm and 18.5 mm in the eight pathogens and concluded that the *Lactobacillus brevis* C17 isolated from caecum was the least effective in their inhibitory activity.

Also, *Lactobacilli* isolated from goats' milk inhibited the growth of *Listeria monocytogenes* and *Staphylococcus aureus* through the production of organic acids only (Hechard *et al.*, 1990). The present work also shows the antagonism of *Staphylococcus aureus* by *Lactobacillus* isolates K<sub>1</sub>, K<sub>3</sub> (from Kunun) and C<sub>2</sub> (from Cowmilk), hence it confirms the report of Hechard *et al.* (1990).

Gilliland and Speck (1977) reported that the antimicrobial/antibacterial action produced by *Lactobacillus* was probably due to a combination of factors including acids, hydrogen peroxide and other inhibitory substances such as bacteriocins. This was also supported by Juven *et al.* (1992) who equally reported that a strain of *Lactobacillus acidophilus* 147 from chicken intestine produced lactic acid, hydrogen peroxide and bacteriocins.

The inhibitory activity reported in the present work may be due to the production of these metabolic products reported above.

### Conclusion

This study has demonstrated that *Lactobacillus* with its ability to produce both antimicrobial and antibacterial substances can:

- i. Easily and readily be isolated from various foods depending on their nutrient composition since they require mostly, a fermentable carbohydrate and other complex growth factors for them to thrive
- ii. Exhibit its antagonistic properties towards diverse pathogenic bacteria especially the gram-positive ones.

Since the antimicrobial substances produced by LAB could offer alternatives to chemical food additives, studies should be aimed at opening new possibilities of bacteriocins synthesis to increase their production on an industrial scale. While they are being referred to as safe, these antimicrobial compounds need a safe evaluation before their use as food additives. Also, more attention should be given to food products which could serve as medium for sustaining live and viable cultures of these beneficial organisms.

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