Isolation of Amylolytic Lactic Acid Bacteria from Cow, Goat and Sheep Milk in Yola Town, Adamawa State, Nigeria

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Abstract:- In this study, lactic acid bacteria (LAB) were isolated from fresh milk samples from cow, sheep, and goat using MRS agar as a growth medium. *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Enterococcus gallinarum*, and *Enterococcus faecium* were the species of LAB that were isolated and identified using the Vitek 2 compact system. While *Lactobacillus fermentum*, *Enterococcus faecium*, and *Lactobacillus plantarum* were isolated from goat milk, *Lactobacillus acidophilus*, *Lactobacillus gallinarum*, and *Lactobacillus plantarum* were isolated from cow milk. However, from sheep milk, only *Lactobacillus plantarum* and *Lactobacillus acidophilus* were isolated. All the isolates were subjected to amylase production test using modified MRS media and the results showed that *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Lactobacillus fermentum* produced amylase while *Enterococcus gallinarum* and *Enterococcus faecium* did not produce amylase.

Keywords:- Milk, LAB, Vitek 2, Amylase and Modified MRS.

I. INTRODUCTION

One of the most important types of bacteria in the food industry are the lactic acid bacteria (LAB). People all over the world have long consumed them in dairy products, and the majority of them are categorized as “generally recognized as safe” (GRAS) microorganisms because they are non-pathogenic, appropriate for industrial and technological processes, tolerant of bile and acid, and capable of producing antimicrobial substances (Shehata et al., 2016). *Pediococcus*, *Enterococcus*, *Lactobacillus*, *Streptococcus*, *Leuconostoc*, and other bacteria are among the cocci and bacilli in the LAB group (Mazzoli et al., 2014). Application areas for lactic acid bacteria (LAB) include food, agriculture, and medicine (Bintsis, 2018). Furthermore, lactic acid bacteria produce a variety of enzymes, including lipases, ureases, peptidases, amylases, and proteases.

Amylase producing lactic acid bacteria known as amylolytic lactic acid bacteria (ALAB) secrete amylases, which make it easier to hydrolyze and ferment starch into lactic acid in a single step (Reddy et al., 2008). The manufacture of food additives like organic acids (like lactic acid) and enzymes (like alpha amylase) is done in the food processing industries using ALAB enzymes (Panda and Ray, 2016). The ALAB produce amylases, which are starch-modifying enzymes with a wider range of uses than chemical starch hydrolysis in the food processing sectors (Smerilli et al., 2015). Among these enzymes in the realm of biotechnology is amylase. From a fungal base, amylase appears to have been the first enzyme manufactured industrially in 1894. In the treatment of digestive diseases, it was utilized as a medicinal acid (Shamugasundaram et al., 2015). According to Sachdev et al. (2016), amylase is one of the leading enzymes used in industry from decade and that microorganisms such as *Bacillus* species are the major source of production of amylase because of the ease of availability, manipulation and operation. In contrast to chemical starch hydrolysis in the starch processing sector, a wide range of commercially available microbial amylases now hold potential applications. Among other bacteria that produce amylase, the most recently found ALAB strain *Lactobacillus paracasei* B41 was the first amylolytic representative of the *L. casei* group (Bhanwar and Gangul, 2014).

Milk is the primary source of nutrition for young mammals (including breastfed human infants) before they are able to digest solid food (Van Winckel et al., 2011). The primary players in milk fermentation are lactic acid bacteria, which work with amylase and other produced enzymes to convert lactose to lactic acid. This increased acidity makes the growth conditions of microorganisms other than LAB increasingly unfavourable (Fernandez et al., 2015).

Considering cattle, sheep and goats rearing as major occupation in Adamawa state of Nigeria, fresh milk is always in abundant supply. Therefore, isolating amylase producing lactic acid bacteria from cow, sheep and goat milk in the state will create an avenue for exploring and harnessing the available resources within the state. Consequently, the aim
of this research work was to isolate and identify the amylase producing lactic acid bacteria from cow, goat and sheep milk from Yola, Adamawa state of Nigeria.

II. METHODOLOGY

A. Sample Collection, Isolation and Identification of Bacteria Isolates

Five samples of fresh cow, goat, and sheep milk each were collected in sterile containers, transported to the laboratory, and left for a 24-hour fermentation period. In total, fifteen samples were obtained. Serial dilutions were made for all the samples and appropriate dilutions (10⁻⁵ to 10⁻⁹) inoculated on MRS agar (Dextrose-20, Agar 12., Proteose peptone-10, Beef extract-10, Yeast extract-5, Sodium acetate-5, Ammonium citrate-2, Dipotassium phosphate-2, Tween 80-1, Magnesium sulphate-0.10 and Manganese sulphate-0.05 all g/L, pH-6.5 at 25°C) (TITAN BIOTECH LTD India) for 24 to 48 hours at room temperature (30-37°C). Pure isolates were obtained by sub-culturing a representative sample of distinct colonies spotted on MRS agar. The bacteria isolates were identified using the Vitek 2 compact system (BIOMERIEUX, France) in addition to the catalase test and microscopic identification.

B. Detection of Amylase Activity

In order to detect amylase activity, the isolated lactic acid bacteria were grown on modified MRS media containing 0.5% peptone, 0.7% yeast extract, 0.2% NaCl, 2% starch, and 1.5% agar. Following a 24-48-hour incubation period at 37°C, Gram's iodine solution (iodine: 0.2%, KI: 0.4%, distilled water: 100 mL) was added and the results were monitored (Madhav et al., 2011).

III. RESULTS

➢ Results of Bacteria Isolates from Cow, Goat and Sheep Milk

Table 1: Catalase Test and Microscopic Identification of the Isolates

<table>
<thead>
<tr>
<th>S/N</th>
<th>ISOLATE CODE</th>
<th>CATALASE TEST</th>
<th>GRAM REACTION</th>
<th>SHAPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C1</td>
<td>–</td>
<td>+</td>
<td>Rods</td>
</tr>
<tr>
<td>2</td>
<td>C2</td>
<td>–</td>
<td>+</td>
<td>Rods</td>
</tr>
<tr>
<td>3</td>
<td>C3</td>
<td>–</td>
<td>+</td>
<td>Rods</td>
</tr>
<tr>
<td>4</td>
<td>G1</td>
<td>–</td>
<td>+</td>
<td>Rods</td>
</tr>
<tr>
<td>5</td>
<td>G2</td>
<td>–</td>
<td>+</td>
<td>Rods</td>
</tr>
<tr>
<td>6</td>
<td>G3</td>
<td>–</td>
<td>+</td>
<td>Rods</td>
</tr>
<tr>
<td>7</td>
<td>S1</td>
<td>–</td>
<td>+</td>
<td>Rods</td>
</tr>
<tr>
<td>8</td>
<td>S2</td>
<td>–</td>
<td>+</td>
<td>Rods</td>
</tr>
</tbody>
</table>

KEY: C=Cow Milk Isolate, S= Sheep Milk Isolate, G= Goat Milk Isolate + = Positive, - = Negative

Table 2: Lactic Acid Bacteria Identified using the Vitek 2 Compact System from the Milk Samples

<table>
<thead>
<tr>
<th>S/No</th>
<th>Source Lactic Acid Bacteria</th>
<th>Lactic Acid Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C1</td>
<td>Lactobacillus fermentum</td>
</tr>
<tr>
<td>2</td>
<td>C2</td>
<td>Lactobacillus plantarum</td>
</tr>
<tr>
<td>3</td>
<td>C3</td>
<td>Enterococcus gallinarum</td>
</tr>
<tr>
<td>4</td>
<td>G1</td>
<td>Lactobacillus plantarum</td>
</tr>
<tr>
<td>5</td>
<td>G2</td>
<td>Lactobacillus fermentum</td>
</tr>
<tr>
<td>6</td>
<td>G3</td>
<td>Enterococcus faecium</td>
</tr>
<tr>
<td>7</td>
<td>S1</td>
<td>Lactobacillus acidophilus</td>
</tr>
<tr>
<td>8</td>
<td>S2</td>
<td>Lactobacillus plantarum</td>
</tr>
</tbody>
</table>

KEY: C = Cow Milk, G = Goat Milk and S = Sheep Milk
Results of Lactic Acid Bacteria That Produce Amylase

Plate 1: Detection of Lactic Acid Bacteria’s Amylase Activity on a Modified MRS Agar Plate

Table 3: Amylase Producing Lactic Acid Bacteria

<table>
<thead>
<tr>
<th>S/N</th>
<th>ISOLATE CODE</th>
<th>ISOLATE NAME</th>
<th>AMYLASE PRODUCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C1</td>
<td>Lactobacillus fermentum</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>C2</td>
<td>Lactobacillus plantarum</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>C3</td>
<td>Enterococcus gallinarum</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>G1</td>
<td>Lactobacillus plantarum+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>G2</td>
<td>Lactobacillus fermentum+</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>G3</td>
<td>Enterococcus faecium-</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>S1</td>
<td>Lactobacillus acidophilus+</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>S2</td>
<td>Lactobacillus plantarum+</td>
<td></td>
</tr>
</tbody>
</table>

KEY: C = isolate from cow milk, G = isolate from goat milk, S = isolate from sheep milk, (+) = produce amylase and (-) = do not produce amylase

IV. DISCUSSION

Based on this finding, the bacteria isolated were Gram positive and catalase negative. Altogether, a total of six rods and two cocci bacteria were isolated (Table 1). The isolates identified were Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus acidophilus, Enterococcus gallinarum and Enterococcus faecium. L. plantarum was found in all the three milk samples while L. fermentum were found in goat and sheep milk. L. acidophilus was however, found only in cow milk sample. Similarly, Enterococcus gallinarum was present in cow milk only while Enterococcus faecium was isolated only from goat milk. All the isolates are members of the lactic acid bacteria (LAB). This agrees with Agagunduz et al. (2021), who reported that a variety of live lactic acid bacteria species can be found in fermented dairy products. Tyokusa and Owuama (2023), also isolated lactic acid bacteria (Enterococcus gallinarum and Enterococcus faecium) and additional bacteria from cow and goat milk that are Gram positive. Raw cow milk was used to isolate and identify LAB genera, including Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, and Bifidobacterium species (Fesseha et al., 2021). Similarly, the raw cow milk was also used to isolate the LAB namely: Enterococcus, Lactobacillus, Leuconostoc, Streptococcus, Lactococcus, and Pediococcus species (Wassie and Wassie, 2016). Guessa and Kihal (2004), also isolated LAB namely Lactococcus sp, Streptococcus thermophilus, Leuconostoc sp, Lactobacillus curvatus, L. helviticus, L. plantarum, L. reuteri, L. casei, L. brevis, L. bulgaricus, L. paracasei and L. caidophilus from goat milk.

The Lactobacilli genera: Lactobacillus plantarum, L. pentosus, L. delbrueckii, L. helveticus, and L. paracasei associated with a fermented goat milk product from Tajikistan were isolated by Cho et al. (2018). In contrast, Lactobacillus plantarum YN.1.3, a lactic acid bacterium, was isolated by Yelnetty et al. (2020), from goat milk. According to Chen et al. (2020), Hu sheep milk was used to isolate...
Lactococcus lactis, Leuconostoc lactis, and Sphingomonas lactis, and Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus acidophilus, and Bacillus amyloliquefaciens are the four species of lactic acid bacteria (LAB) that were isolated from sheep milk and identified using physiological, biochemical, and 16S rRNA sequencing study (Patil et al., 2019).

All the Lactobacillus species isolated produced amylase while Enterococcus species did not produce amylase (Table 3). Amylolitic Lactobacillus species have been isolated from milk and other sources. Lee et al. (2006), isolated L. acidophilus and L. fermentum with high amylase activity from intestinal contents of pigs. Also, South African barley was used to isolate strains of Lactobacillus plantarum that produced amylase (Hattingh et al., 2015). Likewise, from traditional fermented Dadih, Elida et al. (2022), isolated the lactic acid bacteria that produce amylase: Lactobacillusparacasei subsp. paracasei ML3, Lactobacillusplantarum mlH4, Lactococcuslactis subsp. lactis Mh6, Lactobacillusplantarum Mh8, and Lactobacillusparacasei subsp. paracasei MI14.

The amylase activities of the lactic acid bacteria isolates, Lactobacillus fermentum, Lactobacillus plantarum, and Lactobacillus acidophilus is shown on plate 1. Amylases are a class of enzymes that break down starch into simple sugars, and a wide range of species produced these proteins (Gomez-Villegas et al., 2021). Amylases' main function is to hydrolyse the glycosidic bonds that hold starch molecules together, converting complicated carbohydrates into simpler sugars.

Alpha, beta, and gamma amylases are the three primary classes of amylases, and they all target different parts of the carbohydrate molecule (Akinfemiwa et al., 2023). Thus, the degradation of the starch present in the medium resulting in the clear zones (the loss of blue colour of iodine-starch complex) around the colony of isolates as seen on plate 1 is as a result of the amylases produced by the three LAB isolates. By analyzing the halo zone surrounding the colonies, which had a diameter of 0.9 mm and 1.23 mm, Padmavathi et al. (2018), found out that Lactobacillus fermentum and Lactobacillus species G3_4_1TO2, isolated from various materials such as milk, curd, and bovine colostrum, produced amylase enzyme.

Garcia-Cano et al. (2020), identified endo and exoamylases as among the enzymes that break down starch. The inner section of amylase and amylopectin contains α-1,4 glycosidic linkages that endoamylase cleave to release oligosaccharides and dextrin as α-anomeric derivatives. Like glucoamylase or α-glucosidase, exoamylase cleave the α-1,4 and α-1,6 bonds of the exterior glucose residues, releasing glucose as the end product.

One important feature of LAB that contributes to the fermentation of starch into lactic acid is its amylolytic activity. The primary cause of the amylolytic activity in ALAB is the alpha-amylase (amyA) genes. The amyA gene has 1746 base pairs, a 459-amino acid catalytic domain, and a 97-amino acid starch-binding domain (SBD) (Wang et al., 2006). A 954 amino acid protein with an open reading frame of 2862 bp was found to be encoded by the whole nucleotide sequence of the Lactobacillus amylovoransamyA gene (Giraud and Cuny, 1997).

V. CONCLUSION

Fresh milk from cows, sheep, and goats obtained from Adamawa State, Nigeria, like any other place, is a good source of amylase producing lactic acid bacteria such as Lactobacillus fermentum, Lactobacillus plantarum, and Lactobacillus acidophilus. The importance of this enzyme in food and other industries cannot be over emphasized. It is therefore important to harness the readily available milk in Adamawa state for the commercial production of the amylase enzyme.

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