Pak. j. sci. ind. res. Ser. B: biol. sci. 2025 **68B**(3) 262-268

Assessment of Amylase Production Potentials of Lactic Acid Bacteria Isolated from Wheat Sourdough

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(received April 10, 2023; revised April 25, 2023; accepted April 30, 2023)

Abstract. Consequent from increasing demand for amylase enzyme in the food industry, this study assessed the amylase production potentials of lactic acid bacteria (LAB) isolated from wheat sourdough. LAB were isolated from wheat sourdough samples and screened for amylolytic ability using the plate assay method. Isolates that were amylolytic were characterized and identified using standard methods. Submerged fermentation was carried out using the amylolytic lactic acid bacteria (ALAB) isolates at 30°C for 120 h. Reducing sugar yields and amylase activity were determined using the 3, 5-dinitrosalicylic acid method. The lactic acid bacteria isolates and their occurrences were as follows: *L. plantarum* 3(27.3%), *L. brevis* 3(27.3%), *L. fermentum* 2(18.2%), *L. pentosus* 1(9%) *L. mesenteroides* 1(9%) and *P. pentosaceus* 1(9%). A total of 11 isolates exhibited amylolytic activity with zones of starch hydrolysis in the range of 18-46 mm. *L. plantarum* TM3 and *L. brevis* KT1strains produced the highest reducing sugar concentrations (0.63mg/mL and 0.61 mg/mL respectively). Peak amylase activities of the ALAB isolates were observed at 24 h incubation time and ranged from 8.76 to 30.67 µmol/min/mL with *L. plantarum* TM3 and *L. brevis* KT1strains recording the highest amylase activities of 30.67 µmol/min/mL and 30.20 µmol/min/mL respectively. Findings from this study show that wheat sourdough is a good source of amylase-producing LAB strains such as *L. plantarum* TM3 and *L. brevis* KT1that could be exploited in starch degradation processes especially in the food industry.

Keywords: amylase, amylolytic, lactobacillus, lactic acid bacteria, sourdough, wheat

Introduction

Proteins that enhance biochemical and chemical reactions, known as enzymes, serve as natural catalysts and are widespread in plants, animals and micro-organisms. They play crucial roles in various processes such as cheese-making, beer brewing and wine production (Ahmad *et al*., 2013). In Nigeria, the reliance on imported enzymes has led to their high cost and limited availability for industrial applications (Onyimba *et al*., 2022). The demand for industrial enzymes is steadily increasing, with microbial sources, particularly lactic acid bacteria (LAB), being significant contributors (Adrio and Demain, 2014).

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Lactic acid bacteria, characterized as non-sporing, gram-positive, anaerobic or facultative aerobic rods or cocci are essential in the natural fermentation of foods, enhancing their nutritional value and functional properties (Ngene *et al*., 2019; Wassie and Wassie, 2016). LAB's safe metabolic activity and their generally regarded as safe (GRAS) status make them valuable for microbial food processing in developing countries like Nigeria (Bourdichon *et al*., 2012).

Certain strains of lactic acid bacteria, referred to as amylolytic lactic acid-producing bacteria (ALAB), have been identified as efficient producers of extracellular alpha-amylase enzymes (Reddy *et al*., 2008; Sanni *et al*., 2002). This has sparked interest in utilizing ALAB for amylase production, expanding their applications beyond lactic acid production in the starch processing

industry (Amapu *et al*., 2016). The amylases from ALAB find diverse applications in industries such as beverages, food, starch and textiles (Ajita *et al*., 2014; Ribotta and Le Bail, 2007). ALAB, commonly found within genera like *Lactobacillus, Lactococcus, Streptococcus, Pediococcus, Carnobacterium* and *Weissella* are part of the microbiota in various foods, including dairy and non-dairy products (Bhanwar and Ganguli, 2014).

In Nigeria, ALAB strains have been isolated from different amylaceous fermented foods like kunun zaki, burukutu, ogi and fufu, with strains such as *L. plantarum, L. fermentum, L. brevis, L. acidophilus, L. amylovorus* and *L. delbrueckii* being commonly identified (Panda and Ray, 2015; Reddy *et al*., 2008). The awareness of ALAB's potential applications in starch-based food processing has grown, suggesting their use as functional starter cultures, especially as alternatives to industrial malt in fermentation processes. This approach allows a single strain of ALAB to combine amylase production and acidification, ensuring better control of fermentation and improving product quality standards (Panda and Ray, 2015).

ALAB has also been employed for partial starch hydrolysis to enhance the energy density of cereal gruels and mixtures of cereal and legumes. With health concerns surrounding the use of chemical oxidants like potassium bromate in bread improvement, the supplementation of flour and dough with microbial amylases, including those from ALAB, has become more common in the baking industry (Aleid *et al*., 2015). The extracellular amylases from ALAB are utilized as sourdough starters, contributing to the taste, aroma and shelf life of bakery products (Panda and Ray, 2015; Reddy *et al*., 2008). Additionally, the addition of alpha-amylase to dough has proven beneficial in increasing fermentation rates and improving bread volume and texture (Amapu, 2016; Van der Maarel *et al*., 2002).

Furthermore, ALAB's amylase has potential applications in the production of composite bread, where expensive wheat flour is substituted with flours from starchy cereals and tubers, especially cassava. The multifaceted potential of ALAB's amylase underscores the need for ongoing efforts to discover and select efficient strains for various applications in the food industry (Sumalu, 2018).

In the context of sourdough, a tangy and acidic dough obtained through spontaneous fermentation of flour and salted water, lactic acid bacteria (LAB) play a dominant role. LAB coexist synergistically with yeasts in the sourdough process, adapting to the acidic environment and significantly influencing the fermentation process (Novotni *et al*., 2021; Rocha *et al*., 2016). This study focuses on investigating the amylase-producing capabilities of lactic acid bacteria isolated from wheat flour sourdough.

Materials and Methods

Sample collection. Wheat flour was purchased from three different markets (Terminus, Katako and Kwararafa) in Jos metropolis. The samples were transported in separate clean polythene bags to the department of science laboratory technology central laboratory for use in the study.

Preparation of dough. Dough samples were prepared by manually mixing 1000 g of flour with 1000 mL of distilled water under aseptic conditions (Saeed, 2009; Teiking, 2005). Two dough samples were prepared from flour obtained from each of the three locations to give a total of six dough samples.

Fermentation of dough to produce sourdough. The dough samples were allowed to ferment in an incubator at 40 °C for 120 h.

Lactic acid bacteria isolation. Lactic acid bacteria were isolated from the sourdough samples using the serial dilution/plating method. Stock samples of sourdough were prepared by suspending 10 g each of each sample in 90 mL of de Man-Rogosa-Sharpe (MRS) broth. The stock samples were incubated anaerobically at 37 °C for 24 h. Ten-fold serial dilutions of each of the stock samples were carried out in sterile bottles in line with standard procedures up to the 10^{-4} dilution. One mm each of the 10^{-2} and 10^{-4} dilutions of each sample were pour plated on MRS agar. This was done by placing 1 mL each of the two dilutions in separate sterile Petri plates, pouring about 15 mL of sterile MRS agar cooled to about 45 °C into the plates, mixing the contents of the plates by gentle rotation and allowing the contents to solidify. The plates were then incubated anaerobically at 37 °C for 48 h Isolates obtained were purified by sub-culturing repeatedly on sterile MRS agar under the same conditions. The isolates were then transferred unto MRS agar slants and preserved at 4 °C in a refrigerator until use.

Identification of lactic acid bacteria. The LAB Isolates were characterised and identified based on their

macroscopic, microscopic and biochemical/physiological characteristics. Characterisation was based on gram reaction, catalase test, growth ability at 15 °C, 25 °C, 37 °C, 45 °C, growth ability at 2%, 4%, 6% NaCl and carbohydrate fermentation profile using API 50 CH system (Biomerieux, France).

Primary screening for amylolytic abilities of LAB isolates. Starch hydrolysis test following the method employed by Sun *et al*. (2010) was used to determine the amylolytic abilities of the bacteria isolates. Lactic acid bacteria isolates were spot inoculated onto sterile MRS agar fortified with 2% soluble starch and the agar plates were incubated for 24 h at 30 °C. The plates were then flooded with Gram's iodine and observed for clear zones around the colonies surrounded by deep blue-colored zones of starch-iodine complex which becomes visible within seconds. Bacterial isolates with zones of clearing around them were sub-cultured on MRS agar to get pure cultures which were used for further analysis.

Fermentation and crude enzyme extraction. A basal medium containing 1% soluble starch (w/v) and 0.5% (w/v) yeast extract was prepared. Each LAB isolate that exhibited amylolytic activity was grown separately in 50 mL of the basal medium in a shaker incubator at 30 °C for 120 h. At 24 h intervals, sterile pipettes were used to aseptically transfer 5 mL aliquots of the fermenting medium into test tubes and the media were centrifuged at 4000 rpm for 30 min. The supernatant was filtered through sterile cheese cloth and the culture filtrates of the LAB isolates were regarded as crude amylase enzyme extracts.

Determination of reducing sugar concentration and enzyme activity. Reducing sugar concentration and amylase activity were determined using the 3,5-Dinitrosalicyclic acid (DNS) method as employed by Abu *et al.* (2005).

For determination of reducing sugar, a maltose standard curve was prepared as follows: A maltose stock solution was first prepared by weighing 0.1g of maltose into a 250 mL conical flask and diluting with 100 mL distilled water. Aliquots of 0.2, 0.4, 0.6, 0.8 and 1 mL of the stock solution were transferred into separate test tubes and the contents of each tube made up to 1.0 mL volume with distilled water. One mm of DNS was added to each tube and the mixtures were boiled for 5 min after which they were allowed to cool. Absorbance of the cooled mixtures were determined at 540 nm and a maltose standard curve of absorbance against concentration was then plotted using microsoft excel 2010 software.

To determine enzyme activity of the crude enzyme extracts, 1 mL of crude enzyme extract was taken and added to a mixture of 0.5 mL of 1% (w/v) soluble starch solution (1g of soluble starch in 100 mL of distilled water and 0.1 mL of phosphate buffer with pH of 7.4) in a test tube. The reaction mixture was vortexed and kept in a water bath at 60 °C for 1 h. This was followed by addition of 0.4 mL distilled water to the reaction mixture. A blank which contained 0.1 mL of 1M phosphate buffer, 0.5 mL of 1% starch solution, 0.4 mL of distilled water and 1 mL of DNS was provided. After the 1 h reaction time, the reaction mixtures were allowed to cool to room temperature and absorbance taken at 540 nm. A unit of amylase activity (U) was defined as the amount of enzyme able to hydrolyze a gram of soluble starch within 60 min under the experimental conditions.

Statistical analysis. The statistical analysis of amylase activities involved one-way analysis of variance (ANOVA) using microsoft excel version 2010. Significance was determined by p-values, considering values less than 0.05 as statistically significant and for cases with significant differences, means were separated using the least significant difference (LSD) method.

Results and Discussion

From the sourdough samples, a total of 11 lactic acid bacteria (LAB) were isolated. The isolates exhibited typical characteristics of LAB. Colonies of the isolates were either white or cream coloured. Gram reaction of the isolates showed them to be gram-positive rods or cocci. The isolates were catalase-negative, with most of them being able to grow at 15, 25, 37 and 45 °C and at 2, 4 and 6% NaCl. Whereas 8 (73%) of the isolates were heterofermentative as shown by their ability to produce gas, 3 (27%) were homofermentative. This finding is in concurrence with that of Robert *et al*. (2009) who reported the predominance of heterofermentative LAB (76%) among sourdough LAB isolates. Similarly, Sevgili *et al*. (2021) reported that 73.33% of LAB isolated from sourdoughs from Anatoli in Turkey were heterofermentative. The LAB isolates from the present study belonged to the genera *Lactobacillus, Pediococcus* and *Leuconostoc*, with majority (82%) of the isolates belonging to the

Lactobacillus genus. The LAB isolates and their occurrences were as follows: *Lactobacillus plantarum* (27.3%), *Lactobacillus brevis* (27.3%), *Lactobacillus fermentum* (18.2%), *Lactobacillus pentosus* (9%), *Leuconostoc mesenteroides* (9%) and *Pediococcus pentosaceus* (9%) The morphological and physiological characteristics of the LAB isolates are presented in Table 1. The LAB isolated in this study have previously been isolated from sourdoughs by different authors (Sevgili *et al*., 2021; Dashen *et al*., 2020; Amapu *et al*., 2016). LAB microbiota varies from one sourdough ecosystem to another and this is largely dependent on geography and cultural practices (De Vuyst and Vancanneyt, 2007). In spite of this variability in the LAB microbiota of sourdoughs, some LAB species occur more frequently. In this study, *L. plantarum* and *L. brevis* had similar higher frequencies of occurrence among the isolates. Both LAB occurred in sourdough produced from wheat flours obtained from three different markets (Terminus, Katako and Kwararafa) in Jos. Amapu *et al*. (2016) had similarly reported that *L. plantarum* was one of the dominant species in wet milled cereals that it was one of the dominant species in fermented carbohydrate foods including cassava, *ogi and fufu*. The high occurrence of *L. brevis* reported in the present study corroborates the findings of Sevgili *et al*. (2021) who reported that *L. brevis* had the highest occurrence frequency (43.33%) in sourdoughs from different geographical regions in Turkey.

Over centuries, lactic acid bacteria have been widely used in producing fermented foods. With the discovery of the amylase potential of many LAB strains, there has been heightened interest in the use of locally sourced amylolytic LAB (ALAB) from starchy fermented foods for various industrial purposes. In line with this development, LAB were isolated from sourdough samples and tested for their amylolytic potentials. The amylolytic abilities of ALAB isolated from the sourdough samples in this study are presented in Table 2. The isolated LAB showed various levels of amylolytic activity measured as zones of hydrolysis. The zones of hydrolysis caused by the ALAB isolates were in the range of 18-46 mm with *Lactobacillus plantarum* having the highest amylolytic ability (46 mm). This amylolytic

Table 2. Amylolytic abilities of lactic acid bacteria isolated from wheat flour sourdough

Isolate code	Zone of hydrolysis (mm)
TM1	35
TM2	42
TM3	46
TM4	37
KT1	45
KT2	43
KT3	18
KW1	24
KW2	40
KW3	44
KW4	38

TM = Terminus; KT = Katako; KW = Kwararafa.

Table 1. Morphological and physiological characteristics of amylolytic lactic acid bacteria isolates

Isolate code	Colonial morphology	Gram reaction	Temperature (°C)				Growth in NaCl(%)			Catalase test	Gas from glucose	Inference
			15	25	37	45	2	4	6			
TM1	Creamy	+ rods	+	+	+	+	+	+	+	-	+	Lactobacillus fermentum
TM2	White	+ rods	+	+	+	+	+	+	+	-	+	Lactobacillus brevis
TM3	White	+ rods	+	+	+	+	+	+	+	-	-	Lactobacillus plantarum
TM4	Creamy	+ rods	+	+	+	-	+	+	+	-	+	Lactobacillus pentosus
KT1	White	+ rods	+	+	+	+	+	+	-	-	+	Lactobacillus brevis
KT2	White	+ rods	+	+	+	+	+	+	+	-	-	Lactobacillus plantarum
KT3	Creamy	+ cocci	+	+	+	-	+	+	+	-	+	Leuconostoc mesenteroides
KW1	White	+ cocci	+	+	+	+	+	+	+	-	-	Pediococcus pentosaceus
KW2	White	+ rods	+	+	+	+	+	+	+	-	+	Lactobacillus brevis
KW3	White	+ rods	+	+	+	+	+	+	+	-	-	Lactobacillus plantarum
KW4	White	+ rods	+	+	+	+	+	+	+	-	+	Lactobacillus fermentum

+ = Positive; - = Negative; TM = Terminus; KT = Katako; KW = Kwararafa.

capacity is comparable to the amylolytic value of 48 mm reported by Amapu *et al.* (2016) for *L. plantarum* isolated from naturally fermented maize and the 45 mm value reported by Fossi and Tavea (2013) for LAB isolates from soil samples from corn and *Garri* mills.

There were variations in the reducing sugar yields of the amylolytic lactic acid bacteria (ALAB) isolates. *Lactobacillus plantarum* TM3 produced the highest reducing sugar concentration of 0.63 mg/mL, closely followed by *L. brevis* KT1 with a yield of 0.61 mg/mL. *Leuconostoc mesenteroides* KT3 had the lowest sugar concentration of 0.18 mg/mL. The observed differences in sugar yields indicate different rates of starch hydrolysis by the different LAB. In most cases, LAB isolates that produced larger zones of hydrolysis on starch agar brought about higher reducing sugar yields and had higher amylase activity. Figure 1 shows the amylase activities of the ALAB isolates. Differences in the amylase activities of the isolates were statistically significant ($P < .05$). Maximal amylase activity was recorded at 24 h for all the ALAB isolates after which amylase activity decreased continually with time beginning from 48 h. This trend was probably because of accumulation of sugar resulting from breakdown of starch during the early stage of fermentation; subsequently, the sugar is gradually used up as carbon and energy source. Peak amylase activities of the isolates ranged from 8.76 to 30.67 µmol/min/mL with *L. plantarum* TM3 having the highest amylase activity (30.67 µmol/min/mL). Whereas *Lactobacillus brevis* KT1 had the next highest amylase activity (30.20 µmol/min/mL), *Leuconostoc mesenteroides* KT3 had the lowest activity of 8.76 µmol/min/mL. The amylase activities of the LAB isolates were comparatively higher than the 0.38 to 1.10 U/mL reported by Amapu *et al.* (2016) for LAB isolates from wet milled cereals, cassava flour, and fruits. They were also higher than the 0.440 to 2.810 U/mL reported by Tchekessi *et al*. (2014) for LAB isolated from traditional fermented products in Benin. Lactic acid bacteria have been reported to produce high levels of á-amylase with starchy flours as inducers, the reason being that starchy flours contain proteins and vitamins which are required for growth, enzymes and acids production (Tatsinkou and Tavea, 2013). The relatively high amylase activity of some of the LAB isolates in this study, especially *L. plantarum* TM3 and *L. brevis* KT1 makes them to be potential candidates for use as starter cultures in aspects of food processing that require starch degradation.

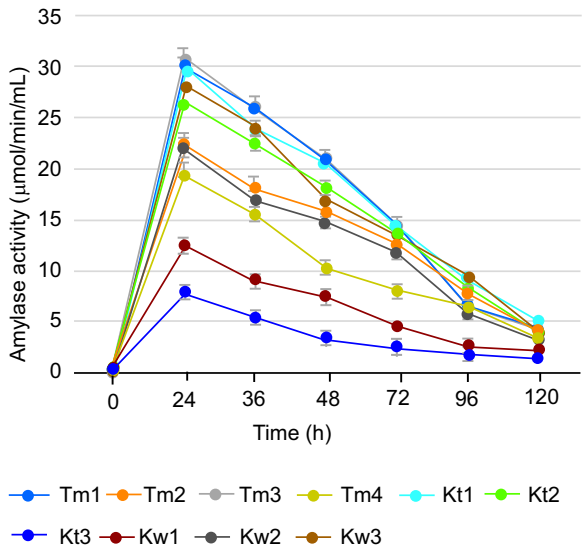

Fig. 1. Amylase yields at 60 °C of lactic acid bacteria isolated from sourdoughs made from flours obtained from different locations in Jos. Tm = Terminus; Kt = Katako; Kw = Kwararafa.

Conclusion

The study has revealed that sourdough produced from flours obtained from different areas in Jos are sources of amylolytic lactic acid bacteria such as *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus pentosus*, *Leuconostoc mesenteroides* and *Pediococcus pentosaceus*. *L. plantarum* and *L. brevis* were the predominant amylase-producing lactic acid bacteria in the sourdough samples and *L. plantarum* TM3 and *L. brevis* KT1 strains possessed higher amylase-producing potentials over the other isolates. These isolates could be optimized for use as starter cultures in bread making and in other food fermentation processes. Future work would include further confirmation of the identities of the lactic acid bacteria isolates through molecular characterization.

Conflict of Interest. The authors declare that they have no conflict of interest

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