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Nutritional and Molecular Studies of Alkaliphilic Tolerant Potential Amylase Producing Lactic Acid Bacteria Isolated from Indigenous Foods and Dumpsites.

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Abstract
Alkaliphilic organisms are important in industries but most of these organisms are obtained from Bacillus species and the like that require special scrutiny before use in fermentation of food related products. Lactic acid bacteria are generally regarded as safe (GRAS) and are therefore readily applied in industrial fermentation with confidence. Due to high demand for the products of alkaliphilic organisms and industrial acceptability of lactic acid bacteria, there is a need to source for GRAS microorganisms that can withstand pH ranges. The aim of this study was to isolate alkaliphilic tolerant lactic acid bacteria from fermented cassava mash, `ogi` and dump site samples with a view to determining their nutritional requirement as well as molecular identity. Lactic acid bacteria were isolated from the samples using standard methods. The isolates were subsequently characterized morphologically, biochemically and molecularly. Effects of different carbon, nitrogen sources, initial temperature and pH as well as metal ions (Na^+, Ca^{2+}, Mg^{2+}, Zn^{2+} and Cu^{2+}) on the growth of the isolates were examined. Data obtained were analyzed using one-way analysis of variance (ANOVA). Results obtained indicate that extracted DNA sequence of A3, A7, A8 and A10 were related to those of Lactobacillus brevis FJ476121.1, Pediococcus acidilactici CP096031.1, Pediococcus pentasaceus AB362605.1 and Pediococcus acidilactici CP053421.1 respectively with the sequences within the GenBank. 1% fructose elicited growth for all the bacteria detected except Lactobacillus brevis and ammonium nitrate was most preferred by all the bacteria detected apart from Pediococcus pentasaceus strain. Lactobacillus brevis FJ476121.1 and Pediococcus acidilactici CP053421.1 were the most alkaliphilic tolerant bacteria detected with OD of 0.285±0.04 and 0.198±0.04 respectively. Different metal ions significantly influenced the growth of isolates in this study. The results in this study indicate potential applicability of the detected bacteria for industrial processes.

Keywrods: Alkaliphilic bacteria, *Lactobacillus brevis, Pediococcus acidilactici,* Dumpsites and Lactic acid bacteria

INTRODUCTION
The most concentrated and widespread occurrences of organisms are generally observed in "moderate" environments (Ankit *et al*., 2017). Groups of organisms specifically adapted to these unique conditions inhabit extreme environments; these organisms are commonly referred to as alkaliphiles, halophiles, thermophiles and acidophiles, depending on the type of extreme environment they live in (Nagendra *et al*., 2022). The major goal of microbial ecology is to understand microbial diversity in natural habitats; therefore, knowledge of both

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micro-organisms and habitats is essential (Ankit *et al*., 2017). Alkaliphiles are interesting groups of extremophilic organisms that thrive at pH of 9.0 and above. Extremophilic microorganisms exhibit the ability to grow at the limits of environmental factors—pH, temperature, salinity, and pressure—which critically influence growth. Among these organisms, the immense potential of alkaliphiles (syn. alkalophile) has been realized since the 1960s primarily due to the pioneering work of Horikoshi (1999).

From the industrial point of view, enzymes and many more products have been reported from alkaliphiles such as antibiotics and carotenoids. However, their potential for degradation of xenobiotics is not common. Alkaliphiles also found to be a major part of biogeocycling of important inorganic compounds (Sarethy *et al*., 2011). Products of industrial importance from alkaliphiles have been commercialized, the most successful of which have been in the food industries. It is worthy of note that industrial production of products from alkaliphiles is so far not enough to fulfil expectations (Sarethy *et al*., 2011). These authors reported that some alkaphilic microorganisms produce enzymes such as lactic acid bacteria involved in fermentation. The initiation of the fermentation of lactic acid begins when lactic acid bacteria grow in food raw materials. As a result, carbohydrates are converted into lactic acid, flavouring compounds (for example, diacetyl and acetoin), carbon dioxide, and other substances (Islam *et al*., 2020). Everyone agrees that lactic acid bacteria predominate in the fermentation processes of starchy food products and that these processes are beneficial in Sub-Saharan Africa prepared either from cereals or cassava (Sanni *et al*., 2002). Lactic acid bacteria are a group of Gram-positive bacteria, non-respiring, non-spore forming, cocci or rods that produce lactic acid during fermentation and are important for food manufacturing, especially in the milk, vegetables and meat industries (González *et al*., 2010). A large number of pathogenic microbes discovered in food cannot live in low pH, hence, lactic acid fermentation of food has been discovered to lower the risk of having pathogenic organisms grow in the food (Abdel and Dardir, 2009). A large number of lactic acid bacteria are generally regarded as safe (GRAS) and since ancient times discovered to be starter cultures to produce fermented foods through traditional means, having the ability to produce lactic acid and bacteriocins that act as natural preservatives and thus can extend the shelf life of fermented food (Perez *et al*., 2014). Lactic acid bacteria (LAB) largely discovered in foods that are being fermented are a diverse group of bacteria which phylogenetically belongs to the order Lactobacillales (Adesulu-Dahunsi *et al*., 2022). This diverse order includes 6 families, over 30 genera and over 300 species. LAB have a long history of application in fermented foods because of their beneficial effects on the shelf life, organoleptic and nutritional characteristics of food. With regard to food processing, *Lactobacillus* genera is the most important member of the group. (Pasolli *et al*., 2020). These microorganisms give lactate as the main end products from metabolism of glucose, and certain species also produce ethanol, CO_2, and acetate. Lactate metabolism is rare amongst organisms (Makoto, 2019). Soil has been reported as source microbes capable of producing metabolites of industrial relevance including enzymes (Nimisha *et al*., 2019). The use of different bacteria in different product development especially regarding pH range is time consuming and expensive, hence the need to involve bacteria with varying pH range adaptation in industrial sectors.

MATERIALS AND METHODS

Collection of Samples

Twenty- seven (27) samples were collected aseptically from different locations in Ekiti State specifically in Ado Ekiti, Ifaki Ekiti and Ilupeju Ekiti. Cassava mash, ogi and soil samples from cassava dumpsite were collected. De Man-Rogosa-Sharpe agar (MRS) was prepared, sterilized and used for bacterial isolation. The chemical reagents (potassium iodide, iodine crystals,

Potassium nitrate, ammonium nitrate, urea powder, yeast extract, tryptone, and peptone, sodium chloride) used were of analytical grade. Decimal solutions of 10^{-1} to 10^{-6} of samples were prepared by transferring 1 mL of the solutions into 9 mL of distilled water in test tubes and aliquot of 0.1 mL of 10^{-4}diluent from each sample was dispensed into already prepared and sterilized plates of MRS agar (Rhaiem *et al*., 2016). The plates were incubated at 30 ^{0}C for 48 hrs anaerobically to observe the growth of LAB. Colonies were severally sub-cultured to obtain pure cultures. The pure cultures were screened for amylase production on modified MRS agar with its carbon source substituted with soluble starch. Fourteen isolates with zone of starch hydrolysis, when flooded with iodine solution (potassium iodide and iodine crystals), beyond colony diameter (Díaz-Ruiz *et al*., 2003) were morphologically (Gram positive) and biochemically (catalase, urease test, indole, citrate test etc. and sugar fermentation tests**)** Sneath, 1986 characterized while four of them were molecularly characterized and identified.

Molecular Identification and Sequencing

DNA Extraction and PCR Amplification

Genomic DNA was extracted from the samples using the Quick-DNA Fungal/Bacterial Miniprep Kit by following the manufacturer`s instructions. The target region was amplified using OneTaq® Quick-Load® 2X Master Mix (NEB, Catalogue No. M0486) following the conditions presented below.

Both ITS-1 and ITS-4 primer pairs were used for molecular identification of the organisms tested positive to amylase production. A 12.5 µL High fidelity PCR amplification system containing 6.25 µL standard buffer, 1 µL DNTP, 4.75 µL H_2O, 0.5 and 10 µM each of the primers. The 35 cycles of amplification was carried out in a PCR mastercycler (Eppendorf) as follows: 94 °C for 5 min; annealing at 50 °C for 1 min and extension at 68 °C for 1.30 min; and a final extension step at 68 °C for 30 min. The PCR products were thereafter sent for sequencing. The full-length sequences obtained were matched with previously published sequence available in NCBI using BLAST. Multiple sequence analysis was carried out using CLUSTALX and further MP (Maximum parsimony) plot was constructed using MEGA 6.2.2. The sequencing data were mapped into the GeneBank (https://www.ncbi.nlm.nih.gov/genbank) for their accession numbers.

List of Primers

Primers	Oligosaccharides
ITS1	TCCGTAGGTGAACCTGCGG
ITS4	TCCTCCGCTTATTGATATGC

Growth and Culture Conditions

Stock cultures were maintained on sterilized MRS slants. A standard inoculum was prepared by 0.5 mL adjusted to O. D. 0.3 at 540 nm with physiological saline solution and was added to 100 mL of growth medium. The cultures were incubated at appropriate conditions. Samples were removed and the growth were estimated at O. D. 540 nm.

Effect of Carbon Sources on the Growth of Bacterial Isolates

Effect of various carbon sources on the growth of the isolates was studied using fructose, starch, lactose, galactose, and maltrose. A concentration of 0.5% NaCl, 1% of peptone and 1% of each sugar was prepared. 5 mL of the concentration was dispensed into each of the test tube in duplicate and the test tubes were sterilized, allowed to cool, inoculated with the isolates and incubated for 48hrs at 35 ^{o}C. Bacteria growth was determined at 540 nm using spectrophotometer (Model No 721) Health Medical Equipment, England.

Effect of Nitrogen Sources on the Growth of Bacterial Isolates

Potassium nitrate, ammonium nitrate, urea powder, yeast extract, tryptone, and peptone were used as nitrogen sources. MRS medium was compounded by substituting its nitrogen with desired nitrogen compound. 5 mL of the medium was dispensed into each of the test tubes in duplicate and treated as stated in the effect of carbon sources on the growth of bacterial isolates. Bacteria growth was determined at 540 nm using spectrophotometer (Simair., *et al*., 2018).

Effect of pH on the Growth of Bacterial Isolates

Effect of pH on the growth of the bacterial isolates was carried out at various pH levels of 2, 3, 4, 5. 6, 7, 8, 9. MRS broth was prepared and sterilized as stated earlier. 5 mL of the medium was dispensed into each of the test tubes in duplicate and treated as stated in the effect of carbon sources on the growth of bacterial isolates. Adjustment of the pH was done by the addition of hydrochloric acid at (0.1N) and sodium hydroxide at (0.1N) to achieve acidity and alkalinity respectively. Bacteria growth was determined at 540 nm using spectrophotometer (Simair., *et al*., 2018).

Effect of Temperature on the Growth of Bacterial Isolates

Effect of initial temperature on the growth of organism was carried out at different temperature ranges 30, 35, 40, 45, 50, 55, 60. Broth media were prepared and treated as stated earlier. The growth was measured at 540 nm using spectrophotometer.

RESULTS

Molecular Identification of Isolates

The results correspond to the similarity between the sequence queried and biological sequences within the NCBI database.

Codes of Isolates	Percentage ID	Predicted organism	GeneBank Accession
A3	100.00	*Lactobacillus brevis*	FJ476121.1
A7	99.48	*Pediococcus acidilactici*	CP096031.1
A8	99.93	*Pediococcus pentasaceus*	AB362605.1
A10	99.61	*Pediococcus acidilactici*	CP053421.1

Effect of Starch Concentrations on the Growth of Bacterial Isolates

Different starch concentrations were observed to affect the growth of the bacterial isolates in this study. Except for *Pediococcus acidilactici* (A10) that had its best growth at 0.5 % w/v, the growth of other four isolates increased as the starch concentrations increased up to 2.0% w/v after which the growth declined (Figure 1).

Effect of Metal Ions on the Growth of Bacterial Isolates

Metal ions indicated that the ions had varying growth effect on each of the isolates (Figure 2). Cu^{2+} stimulated the highest growth on all the isolates, though with different values (Figure 2). Na^{+} gave the least growth values for all the isolates.

Effect of Carbon Sources on the Growth of Bacterial Isolates

The results in table 1 reveal that for the medium containing galactose, the highest value of growth was produced by *Pediococcus acidilactici* (A7), followed by *Lactobacillus brevis*(A3) and then *Pediococcus acidilactici*(A10) while the least growth value was recorded for *Pediococcus pentasaceus*(A8). Fructose was observed to give the best growth for all the isolates apart from *Lactobacillus brevis*(A3) with maltose as the best carbon source for the growth. The least value of growth (0.075±0.035 mg/mL) was noticed with *Pediococcus acidilactici*(A10) in the presence

of starch. For the medium containing maltriose, growth pattern of the isolates are *Pediococcus acidilactici* > *Pediococcus pentasaceus* > *Pediococcus acidilactici* > *Lactobacillus brevis* with 0.495±0.024, 0.315±0.019 ,0.285±0.025 and 0.185±0.024 mg/mL respectively. For the medium containing maltose, *Lactobacillus brevis* (A3) and *Pediococcus acidilactici* (A10) had different growth values but are not significantly ($p \leq 0.05$) different from each other.

Effect of Nitrogen Sources on the Growth of Bacterial Isolates

Results in Table 2 depict how different nitrogen sources influenced the bacterial growth in this study. It revealed that for the medium containing peptone, the highest value of growth was produced by *Pediococcus pentasaceus* (A8) followed by *Pediococcus acidilactici*(A7) and then *Pediococcus acidilactici*(A10) while the least growth value was recorded for *Lactobacillus brevis*(A3). *Pediococcus acidilactici* (A10) had the highest growth in medium with urea followed by *Lactobacillus brevis* (A3), *Pediococcus pentasaceus* (A8) and then *Pediococcus acidilactici* (A7). In the medium containing yeast extract, the growth of the isolates were *Pediococcus acidilactici* > *Lactobacillus brevis* > *Pediococcus acidilactici* > *Pediococcus pentasaceus* with 0.215±0.007, 0.195±0.005, 0.185±0.035, 0.085±0.000 mg/mL respectively. Different variations in growth values were observed for all the isolates in media containing tryptone, Ammonium trioxonitrate and Potassium trioxonitrate.

Effect of pH Values on the Growth of Bacterial Isolates

Results in Table 3 show that *Lactobacillus brevis* (A3) and *Pediococcus acidilactici* (A7) had their highest growth values of 1.225±0.007 and 1.100±0.004 mg/mL respectively at pHs 6 and 5 respectively. For isolates *Lactobacillus brevis* and *Pediococcus pentasaceus* there was general increase in the growth values from pHs 2 to 6 and the growth then decreased down to pH 9 while for isolates *Pediococcus acidilactici strains* the increase in growth was observed between pHs 2 and 5 and then decreased down to pH 9.

Effect of Temperature on the Growth of Bacterial Isolates

Except for *Lactobacillus brevis* (A3) that had its maximum growth at 45 °C, other isolates grew best at 35 °C. In all, there was gradual increment from 30 °C to the optimum when the growth began to decline down to 60 °C (Table 4).

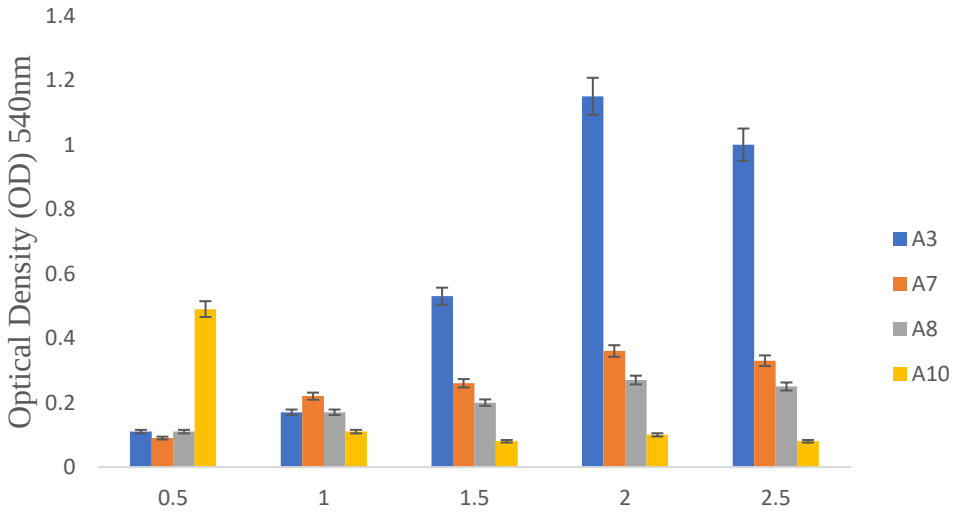

Figure 1: Effect of Conentrations (%) of Starch on the Growth of the Isolates

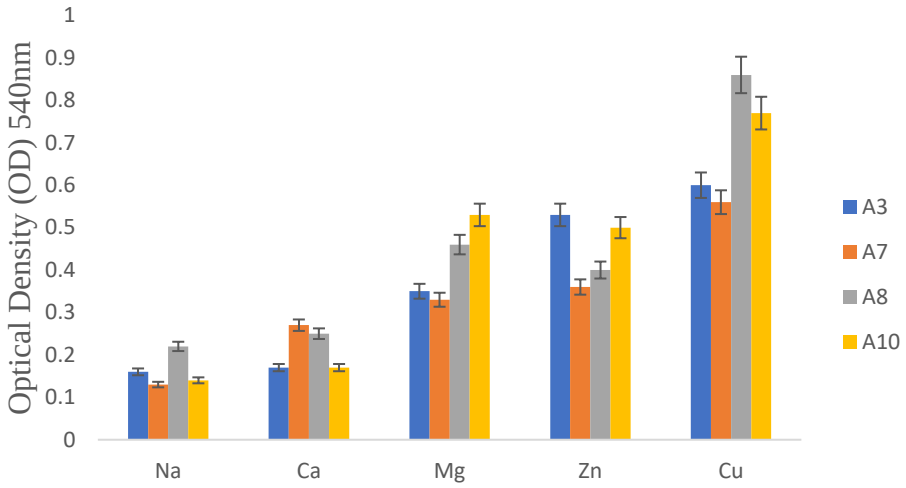

Figure 2: Effec of Metal ions on the Growth of Isolates

Table 1. Effect of Carbon Sources on the Growth of Bacterial Isolates

Microorganisms	Maltose	Fructose	Lactose	Maltrose	Galactose	Soluble starch
Lactobacillus brevis (A3)	0.395±0.025^{b}	0.175±0.022^{b}	0.265±0.012^{b}	0.185±0.024^{d}	0.235±0.024^{b}	0.105±0.022^{b}
Pediococcus acidilactici (A7)	0.155±0.022^{c}	1.255±0.022^{a}	0.305±0.023^{a}	0.285±0.025^{c}	0.285±0.022^{a}	0.115±0.024^{b}
Pediococcus pentasaceus (A8)	0.425±0.014^{a}	1.250±0.036^{a}	0.255±0.024^{b}	0.315±0.019^{b}	0.015±0.023^{c}	0.155±0.045^{a}
Pediococcus acidilactici (A10)	0.355±0.024^{b}	1.275±0.012^{a}	0.250±0.035^{b}	0.495±0.024^{a}	0.215±0.054^{b}	0.075±0.035^{c}

Values are presented in mean±SD. Values with the same superscript across the column are not significantly different at p≤0.05 DMRT

Table 2.Effect of Nitrogen Sources on the Growth of Bacterial Isolates

Microorganism	PEP	UREA	TRY	YST EXT	NH_4NO_3	KNO_3
Lactobacillus brevis(A3)	0.105±0.000^{d}	0.135±0.004^{b}	0.185±0.007^{a}	0.195±0.005^{b}	0.325±0.004^{c}	0.305±0.007^{a}
Pediococcusaci dilactici(A7)	0.175±0.002^{b}	0.065±0.006^{d}	0.175±0.008^{b}	0.215±0.007^{a}	0.535±0.005^{a}	0.265±0.007^{c}
Pediococcus pentasaceus(A8)	0.185±0.004^{a}	0.120±0.004^{c}	0.145±0.008^{c}	0.085±0.000^{c}	0.010±0.000^{d}	0.285±0.025^{b}
Pediococcusaci dilactici(A10)	0.165±0.004^{c}	0.155±0.04^{a}	0.175±0.007^{b}	0.185±0.035^{b}	0.355±0.024^{b}	0.315±0.032^{a}

Values are presented in mean±SD. Values with the same superscript across the column are not significantly different at p≤0.05 DMRT
Legend; PEP- Peptone, TRY- tryptone, YST EXT- yeast extract

Table 3. Effect of pH Values on the Growth of Bacterial Isolates

Microorganisms	pH2	pH3	pH4	pH5	pH6	pH7	pH8	pH9
Lactobacillus Brevis(A3)	0.135±0.01^{a}	0.155±0.02^{a}	0.165±0.007^{c}	0.245±0.031^{b}	1.225±0.07^{a}	0.405±0.00^{b}	0.280±0.00^{a}	0.285±0.04^{a}
Pediococcus acidilactici(A7)	0.010±0.00^{b}	0.155±0.01^{a}	0.270±0.007^{a}	1.100±0.04^{b}	0.435±0.04^{a}	0.245±0.01^{c}	0.235±0.04^{b}	0.185±0.04^{c}
Pediococcus pentasaceus(A8)	0.045±0.01^{c}	0.115±0.06^{b}	0.215±0.04^{b}	0.250±0.07^{c}	0.510±0.04^{a}	0.315±0.00^{d}	0.180±0.07^{c}	0.165±0.02^{c}
Pediococcus acidilactici (A10)	0.083±0.00^{c}	0.177±0.05^{ab}	0.211±0.07^{b}	0.706±0.04^{b}	0.382±0.04^{c}	0.273±0.02^{b}	0.213±0.00^{b}	0.198±0.04^{c}

Values are presented in mean±SD. Values with the same superscript across the column are not significantly different at p≤0.05 DMRT

Table 4. Effect of Temperature on the Growth of Bacterial Isolates

Microorganism	30 °C	35 °C	40 °C	45 °C	50 °C	55 °C	60 °C
Lactobacillus brevis(A3)	0.235±.007^{c}	0.325±.07^{a}	0.665±0.04^{a}	1.115±0.01^{a}	0.625±0.008^{a}	0.435±0.07^{b}	0.365±0.00^{b}
Pediococcus acidilactici(A7)	0.215±0.00^{b}	0.815±0.05^{bc}	0.400±0.01^{b}	0.250±0.02^{c}	0.235±0.02^{b}	0.105±0.01^{d}	0.085±0.04^{d}
Pediococcus pentasaceus(A8)	0.305±0.03^{a}	0.855±0.04^{b}	0.665±0.04^{b}	0.435±0.05^{d}	0.305±0.04^{c}	0.275±0.04^{c}	0.185±0.03^{c}
Pediococcus acidilactici(A10)	0.635±0.02^{d}	0.945±0.02^{d}	0.650±0.04^{ad}	0.535±0.05^{b}	0.430±0.02^{d}	0.365±0.06^{c}	0.320±0.02^{a}

Each values are presented in mean±SD. Values with the same superscript across the column are not significantly different at p≤0.05 DMRT

DISCUSSION

A number of different starch hydrolysing bacteria have been isolated from different environments, including starch containing and dumpsites. Akinyele *et al*. (2004) reported the presence of *Lactobacillus brevis* from fermenting cassava mash. Oyedeji *et al*. (2013) also identified the involvement of several lactic acid bacteria in starch fermentation and these include homofermentative, heterofermentative *Lactobacillus* species and heterofermentative *Leuconostoc* species. In this study, LAB; *Lactobacillus brevis*, *Pediococcus acidilactici*, *Pediococcus pentasaceus*, and *Pediococcus* were isolated from starchy materials and dumpsites. As recorded in this study, *Lactobacillus brevis, Pediococcus acidilactici, Pediococcus pentasaceus* and *Pediococcus acidilactici* bacteria, among other bacteria, had been reportedly found in silage fermented vegetables, fermented food and dairy products (Teixeira *et al*., 2023).

Different carbon sources have various stimulatory effects on the growth of microorganisms as shown in this study. This shows that different isolates have vary preferences and abilities when it comes to utilizing different carbon sources. This variation could be due to differences in metabolic pathways and enzyme production capabilities. Andrea *et al*. (2023) observed similar case in their study on feeding LAB with different sugars: effect on exopolysaccharides (EPS) production and their molecular characteristics. They reported that the LAB showed different growth patterns depending on the sugar type and conditions. The type of carbon mostly utilized by specific microorganism might also be related to environment from which the organisms are isolated since organisms thrive on sugars that are plentiful in their natural environment where they play unique roles in the cycling of nutrients and interactions among communities (Ankit *et al*., 2017). Based on their capacity to exploit the different carbon sources, certain isolates may predominate at various ecological habitats and the isolates with a greater spectrum of carbon source use can have a competitive advantage (Bonnet *et al*., 2020; Tse *et al*., 2021; Sobika *et al*., 2021).

The influence of various nitrogen sources on the growth of bacterial isolates was examined, and similar to how the isolates exhibit a substantial difference in growth with different carbon sources, similar relationships were observed with nitrogen sources. The growth of the isolates is negatively impacted by urea, a straightforward nitrogen molecule, as seen by the poor growth rates of all the isolates. This demonstrates that the isolates reduced ability to use urea as a major nitrogen source may be the cause of their poor growth. Isabela *et al*. (2011), had earlier noticed that LAB cannot use urea as a source of nitrogen because they lack the enzymes needed to break urea down into its usable components. However, some LAB might be able to use urea in an indirect manner by degrading other amino acids that have urea as a structural component. Also certain lactic acid bacteria might have received the required genetic tools via horizontal gene transfer to directly use urea as a nitrogen source (Abdullah, 2010). However, in this study, *Pediococcus pentasaceus* showed the maximum growth when peptone was used among the organic nitrogen sources compare with other isolates, demonstrating that isolates metabolize nitrogen sources differently and may have varying abilities to digest and absorb

the complex peptide combination. In this study, *Pediococcus pentasaceus* grew better with peptone as a nitrogen source. This observation had earlier been reported by Katepogu *et al*. (2022). However, when tryptone was employed as a nitrogen source, *Lactobacillus brevis* grew better demonstrating their capacity to utilize the peptides and amino acids present in tryptone for growth. In this study, it was detected that *Pediococcus acidilactic* and *Lactobacillus brevis* grew substantially more than the other isolates when ammonium trioxonitrate was used as nitrogen source. In all, inorganic nitrogen sources were more preferred by all the isolates. This is contrary to the observation of Amira *et al*. (2020) where peptone was reported to be more preferred by *Lactobacillus reuteri* depending on the source. Apart from *Pediococcus pentasaceus*, other isolates utilized yeast extract for better growth values. This observation was noticed by Torre *et al*. (2018) in lactic acid production by *Lactobacillus delbrueckii* from orange peel waste. Different interactions of the isolates to various nitrogen sources reveal their predilection for particular nitrogen compounds. This might be a reflection of their natural habitats and the kinds of nitrogen sources that are present there (Tse *et al*., 2021).

In the present study, we found 35 °C as the optimum growth temperature for the 75% of the isolates and 45 °C for 25% of the isolates. Although the growth continued down to 60 °C, the values decreased as the temperature increased. The optimal temperature of three of the isolates subjected to optimization in this study was consistent with the 35 ^{o}C falling in line with the recommended optimal temperature suggested by Ahmed *et al*. (2016), except for *Lactobacillus brevis* that had its optimal temperature at 45 ^{o}C. This may be due to the mesophilic nature of the species. As per earlier report of Akinyele *et al*. (2004), the high temperature may inactivate the expression of gene responsible for the utilization of the substrates in the medium. As the isolates' growth responses to temperature changes are indicative of their enzymatic and metabolic flexibility, the ideal temperature of the isolate may be influenced by that of the enzyme. Enzymes are extremely temperature-sensitive, and they can have a big impact on cellular functions (Kok *et al*., 2012) The changes in enzyme kinetics and metabolic pathways between the isolates are probably what caused the growth pattern discrepancies.

This results in this study showed isolates with varying degrees of pH adaptation due to their ability to grow at various degree of acidity. The best growth pHs for all the isolates fell between 5.0 and 6.0. Mehmet *et al*. (2023) stated that while most LAB grow best at pH levels close to neutral, some bacterial species, including *Lactobacillus*, which is one of the isolates used in this study, are different and grow well at pH 3 and this is reflective of our results in this study. Song *et al*. (2021), also reported the tolerance of *Pediococcus pentasaceus* and *Pediococcus acidilactic* at pH values from 2.5 to 4. 0. Additionally, it is likely that the isolates' responses to various pH levels were influenced by their enzyme capacities. Enzymes that function best at particular pH levels are said to be important for their metabolic activities, and variations in enzyme profiles may be a factor in the observed variation in growth responses (Kaarel *et al*., 2003). The presence of enzymes, whose optimum activity was reported in the pH range of 4.5 to 7 by Yadav *et al*. (2020), is similar to that of *Pediococcus pentasaceus* observed in this study.

CONCLUSION

Nutritional and environmental factors have major influence on the growth and activities of microorganisms in any habitat. This study was able to establish that our LAB were able to transverse the verge of acidic medium to alkali region in their activities which make them applicable in fermentation range of pH 4.0 to 9.0 conveniently. The composition and concentration of media greatly affected the growth of the isolates. We have also discovered that, maximum growth occurred in presence of cupper ion and the bacteria had significant metal tolerance as well.

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