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EFFECT OF NUTRIENT SUPPLEMENTATION ON LACTIC ACID PRODUCTION BY LACTIC ACID BACTERIA

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ABSTRACT

In this research, five substrates; yam, cassava, corn, potatoes and rice were used for lactic acid fermentation. The microorganisms were isolated using de Mann Rogosa and Sharpe (MRS) medium using the spread plate technique. Ten pure colonies were identified and presumptively classified using standard morphological and biochemical identification processes. After optimizing culture conditions at 35^{o}C, pH 6.0 and fermentation time 122hrs, the effect of Tween 80 supplementation on lactic acid production was investigated. Supplementation of culture medium with Tween 80 was done at two different concentrations (3.0 g/L and 5.0 g/L) and the quantity of lactic acid produced for each concentration determined using total titratable acidity (TTA) method. Tween 80 (3.0 g/L) in the culture medium had dual effects on lactic acid production by the lactic acid bacteria. The highest quantity of lactic acid concentration was produced by Lactobacillus plantarum strain Z2 (0.62 ± 0.01 g/L) in yam fermentation, a 113.7% increase in lactic acid production capacity over that of the un-supplemented yam broth. A percentage increase of 34.5% in lactic acid production capacity was observed for same microorganism (Lactobacillus plantarum strain Z2) in supplemented cassava broth over that of the un-supplemented one. Lactobacillus pentosus strain BSR3 (SL2) produced the highest quantity of lactic acid on the supplemented potatoes broth, a percentage increase of 153% over that of the un-supplemented potatoes broth. For supplemented rice broth, the highest quantity of lactic acid (0.63 ± 0.02 g/L) was produced by Lactobacillus pentosus strain BSR3 (C3P), a percentage increase of 35.9% over that of the un-supplemented rice broth. There was increased lactic acid production by Lactobacillus pentosus strain BSR3 (C3P), Lactobacillus pentosus strain BSR3 (SL2), Lactobacillus plantarum strain FM02 in the Tween 80-supplemented corn broth. However, there were decreases in lactic acid production by Lactobacillus pentosus strain BSR3 (C3P) in cassava broth, by Lactobacillus plantarum strain Z2 in both corn and potato broths supplemented with the Tween 80 at 3.0 g/L. The addition of Tween 80 at 5.0 g/L resulted in decreased lactic acid production. The percentage reduction in descending order of lactic acid production in yam, cassava, corn, potatoes and rice broths by Lactobacillus plantarum strain Z2 were 79.3%, 83.6%, 90.9%, 70% by Lactobacillus pentosus strain BSR3 (SL2) and 86.9% (Lactobacillus pentosus strain BSR3 (C3P)) respectively. For un-supplemented broths, Lactobacillus plantarum strain Z2 produced the highest lactic acid (0.56 ± 0.35 g/L) from potatoes broth. With supplementation of broths with Tween 80 (at 3.0 g/L), lactic acid production was enhanced, with Lactobacillus pentosus strain BSR3 (SL2) producing the highest lactic acid concentration (0.76 ± 0.01 g/L) from potato broth. It is

recommended, for large-scale production of lactic acid, that potatoes broth be fermented with Lactobacillus pentosus strain BSR3 (SL2) under the optimized culture conditions.

Keywords: Isolation, pure colonies, supplementation, fermentation, lactic acid, concentration.

INTRODUCTION

Lactic acid is a versatile organic acid with broad applications across multiple industries, including food and food-related sectors (Sreenath *et al*., 2001; Naveena *et al*., 2004), pharmaceuticals, cosmetics, textiles, chemicals, and leather production (Mariano, 2015; Zhang, 2008; Gao *et al*., 2011). Global lactic acid production is estimated at 1.5 million metric tons, with a projected annual growth rate of 8.2% by 2030 (Ojo and de Smidt, 2023). The increasing demand is driven by its role as a food preservative (Aguirre-Garcia *et al*., 2024), its antibacterial and detergent properties in pharmaceutical and personal care products (Ruiz-Ruiz *et al*., 2017). Lactic acid is also used in the production of polylactic acid (PLA)—a biodegradable and compostable thermoplastic (Ahmad *et al*., 2024).

Lactic acid bacteria (LAB) are classified into homofermentative and heterofermentative groups based on their metabolic pathways. Homofermentative LAB primarily convert sugars into lactic acid as the sole product, whereas heterofermentative LAB produce lactic acid along with ethanol, acetic acid, formic acid, and carbon (iv) oxide (Eiteman and Ramalingam, 2015; Taskila and Ojamo, 2013). However, as Zaunmüller *et al*., (2006) noted, this classification is not absolute, as actual metabolic outcomes depend on carbon source type (e.g., hexose vs. pentose) and fermentation conditions such as growth rate and carbon/energy availability.

Efficient lactic acid production is influenced by multiple factors, including chemical parameters (pH, nutrient composition), physical conditions (temperature, mixing), and biological variables (biomass concentration) (Comparetti *et al*., 2013). Additionally, operational conditions such as substrate size, inoculum type and concentration, sugar and lactic acid levels, and the presence of inhibitory compounds can affect fermentation efficiency (Eiteman and Ramalingam, 2015). Optimizing these conditions—especially pH and temperature—is critical, as they directly impact cellular metabolism, microbial growth, substrate utilization, and lactic acid production (Rawoof *et al*., 2020).

Surfactants such as Tween 80 (polysorbate-80) have been recognized for their role in enhancing microbial growth. Tween 80 ($C_{64}H_{124}O_{26}$) is a non-ionic surfactant and emulsifier with a molar mass of 1,310 g/mol, a density of 1.06 g/cm³, and complete solubility in water. When added at appropriate concentrations, unsaturated fatty acids like Tween 80 act as essential growth factors for LAB, improving membrane permeability and metabolic activity (Partanen *et al*., 2001). However, excessive concentrations may have inhibitory effects, necessitating careful optimization of its use in fermentation processes.

This study investigates the effect of Tween 80 supplementation on lactic acid production from starch-based substrates, aiming to optimize conditions for improved yield and efficiency.

MATERIALS AND METHODS

Microbial Sample Collection

Soil samples were collected from six cassava processing plants in Ohuhu, Umuahia North Local Government Area, Abia State, Nigeria, where cassava processing had been conducted for a minimum of five years. Samples were taken from areas with the highest spillage of grated cassava, at a depth of 10–12 cm, using sterile trowels. The collected samples were placed in sterile

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containers and transported to the laboratory for analysis within two hours.

Serial dilution techniques were employed for microbial isolation. A 1.0 mL aliquot of the 10^{-4} dilution was inoculated onto de Man, Rogosa, and Sharpe (MRS) medium, a selective medium for lactic acid bacteria (LAB), for further microbial analysis.

Isolation and Characterization of Lactic Acid Bacteria

LAB isolation was conducted following the spread plate method described by Abd *et al*. (2010). Inoculated MRS agar plates were incubated anaerobically at 37°C for 48 hours, after which distinct colonies were randomly selected, sub-cultured, and purified through successive streaking on MRS agar. The pure cultures were maintained on MRS agar slants broths and stored at 4°C until further analysis.

Preliminary morphological examination and biochemical characterization of LAB isolates were performed. Additionally, growth tolerance in 4% and 6.5% NaCl was assessed following the protocol of Karnwal *et al*. (2016).

Molecular characterization was conducted based on 16S rRNA gene sequencing, followed by BLAST searches for phylogenetic identification.

Substrate Collection and Preparation

Five starch-based substrates (yam, cassava, corn, potatoes, and rice) were obtained from Nkwoegwu Market, Ohuhu, Umuahia North L.G.A., Abia State, Nigeria.

Treatment and Processing of Starch-Based Substrates

Substrate preparation followed the methodologies of Vishnu *et al*. (2002), Wakil and Ajayi (2013), and Odunfa and Adeyele (1985). Cereal grains (corn and rice) were manually sorted to remove stones, debris, and defective grains, then ground into fine powder using an electric blender (Scanfrost, Model SFKAB405, made in China) and sieved through a 150 µm mesh sieve. The resultant flour was used for fermentation. For cassava, yam, and potatoes, the tubers were peeled, washed with distilled water, and cut into smaller portions. The smaller pieces were then blended and placed in sterile muslin cloth, tied securely, and left in a funnel for liquid drainage. These unprocessed starch materials were used as natural fermentation substrates. A 10 g portion of each substrate was dissolved in 100 mL of distilled water to prepare starch solutions for fermentation.

Growth and Lactic Acid Production by Lactic Acid Bacteria

The synthetic medium, starch-based solutions, and inoculum were prepared following the methods of Mudaliyar and Kulkarni (2011), Mudaliyar *et al*. (2012), and Cheng *et al*. (1991). Each culture medium was inoculated with 5% bacterial inoculum as per the procedure of Karnwal *et al*. (2016). The inoculation was repeated for each LAB isolate and substrate.

Molecular Identification of Lactic Acid Bacteria

DNA Extraction

DNA extraction was performed at the Center for Molecular Biology and Biotechnology (CMBB), Michael Okpara University of Agriculture, Umudike (MOUAU), Nigeria. Genomic DNA was extracted using Zymo-Spin™ kits, following the manufacturer's instructions. The extracted DNA was separated using 1% agarose gel electrophoresis for integrity assessment.

PCR Amplification

The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using universal primers, 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1429R (5′-TACGGCTACCTTGTTACGAC-3′). PCR was conducted in a thermal cycler (T Gradient model, Biometra, Germany) under the following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, annealing at 50°C for 30 s, and

extension at 68°C for 1 min, with a final extension at 68°C for 10 min. PCR products were stored at 4°C, and their integrity was assessed using 1% agarose gel electrophoresis stained with EZVision® Bluelight DNA Dye.

PCR products were stored at 4°C, and their integrity was assessed using 1% agarose gel electrophoresis stained with EZVision® Bluelight DNA Dye.

Sequencing and Phylogenetic Analysis

PCR products were purified using ExoSAP Protocol and sequenced using the Applied Biosystems™ BigDye™ Terminator v3.1 Cycle Sequencing Kit on an ABI 3500XL Genetic Analyzer (Inqaba Biotec, South Africa). The obtained 16S rRNA sequences were analyzed using BLAST (NCBI GenBank database) to identify LAB strains.

Phylogenetic analysis was conducted using ClustalW for sequence alignment, and evolutionary relationships were inferred using the Neighbor-Joining method. Evolutionary distances were computed using the Maximum Composite Likelihood method, and all ambiguous positions were removed (pairwise deletion). Phylogenetic analyses were conducted using MEGA X software (Saitou and Nei, 1987; Kumar *et al*., 2018).

Lactic Acid Estimation

Lactic acid concentration was quantified using the total titratable acidity (TTA) method (A.O.A.C., 1990) as modified by Parimala and Muthusamy (2017). Fermented broth (50 mL) was centrifuged at 10,000 rpm for 5 min to pellet bacterial cells. The supernatant (25 mL) was transferred into a 100 mL flask, and three drops of phenolphthalein indicator were added.

Titration was performed using 0.1 M NaOH, with the endpoint determined by the first appearance of a pink colour. Each mL of 0.1 M NaOH corresponds to 90.08 mg of lactic acid. The lactic acid concentration was calculated as follows:

$$\text{Total titratable acidity of lactic acid } \left(\frac{\text{g}}{\text{L}}\right) = \frac{\text{mL NaOH} \times \text{N NaOH} \times 90.08 \times 100}{\text{Volume of sample used} \times 1000}$$

where:

N = Molarity of NaOH

mL = Volume of NaOH (titrant)

MW = Molecular weight of lactic acid (90.08 g/mol)

Lactic acid production was expressed as g/L of culture medium.

Effect of Tween 80 Supplementation on Lactic Acid Production

Tween 80 was supplemented into the fermentation medium at 3.0 g/L and 5.0 g/L to evaluate its effect on lactic acid production. The culture medium was incubated under optimized conditions (35°C, pH 6.0, and 122 hours). The total titratable acidity (TTA) method was used to quantify lactic acid concentration in both supplemented and un-supplemented media.

Statistical Analysis

All experiments were conducted in triplicate, and data were analyzed using IBM SPSS Statistics 25. Descriptive statistics (mean and standard deviation) were computed. Differences among treatments were evaluated using ANOVA, followed by Duncan's Multiple Range Test for post-hoc comparisons. Statistical significance was determined at $p < 0.05$. Values were reported as means ± standard deviation, and means with different superscripts across columns were considered significantly different ($p < 0.05$).

RESULTS

The morphological, biochemical and sugar fermentation tests as seen in Table 1 show the presence of seven (7) *Lactobacillus* sp, one (1) *Lactococcus* sp, one (1) *Leuconostoc* sp and one (1) *Streptococcus* sp. The result of effect of temperature on lactic acid production is

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shown in Table 2. The production of lactic acid production by the lactic acid bacteria in the temperature ranges was found to be statistically non-significant. The temperature range was within the range of incubating mesophilic lactic acid bacteria. The temperature of 35^{o}C was chosen for further studies because it aligned with that of many researchers in lactic acid production by lactic acid bacteria.

Table 1: Morphological and biochemical identification of lactic acid bacteria isolates

Isolate	SL1	SL2	SL3	SL4	SL5	SL6	SL7	SL8	C3P	C2Y
Colony morphology	Rounded ends, occurring singly.	Milky, single, pair, rounded.	Flat, Creamy, Mucoid.	Circular, Creamy.	Straight, Short-chain, Pair, rounded.	Creamy, circular, smooth, short-chain.	Circular, milky, slimy.	Circular, milky, slimy, long and short-chain.	Single, pair, rounded.	Straight, rounded ends, short-chains.
Gram stain	+	+	+	+	+	+	+	+	+	+
Catalase	–	–	–	–	–	–	–	–	–	–
Motility	–	–	–	–	–	–	–	–	–	–
Coagulase	–	–	–	–	–	–	–	–	–	–
Citrate	–	–	–	–	–	–	–	–	–	–
Gas production	–	–	–	–	–	–	–	–	–	–
4% NaCl	+	+	+	–	+	+	+	+	+	+
6.5% NaCl	+	–	–	+	–	+	+	–	–	–
Growth at 35ºC	+	+	+	+	+	+	+	+	+	+
Growth at 40ºC	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+
Fructose	+	–	–	+	+	+	+	–	+	+
Galactose	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+
Maltose	–	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+	+	+
Presumptive organism	*Lactobacillus* sp	*Lactobacillus* sp	*Lactobacillus* sp	*Lactococcus* sp	*Lactobacillus* sp	*Lactobacillus* sp	*Leuconostoc* sp	*Streptococcus* sp	*Lactobacillus* sp	*Lactobacillus* sp

Positive reaction (+), Negative reaction (-)

After optimization processes, the *Lactobacillus* sp. (SL1, SL2, C3P and C2Y) were selected for further studies because they showed the best lactic acid production potentials under the optimized conditions of temperature, pH and incubation time.

Molecular characterization of the bacterial species was done based on genotypic characteristics (16S rRNA gene sequences similarity with the type strains) during BLAST searches and identified as *Lactobacillus plantarum* strain FM02 (C2Y), *Lactobacillus pentosus* strain BSR3 (C3P), *Lactobacillus plantarum* strain Z2 (SL1) and *Lactobacillus pentosus* strain BSR3 (SL2) (Figure 1).

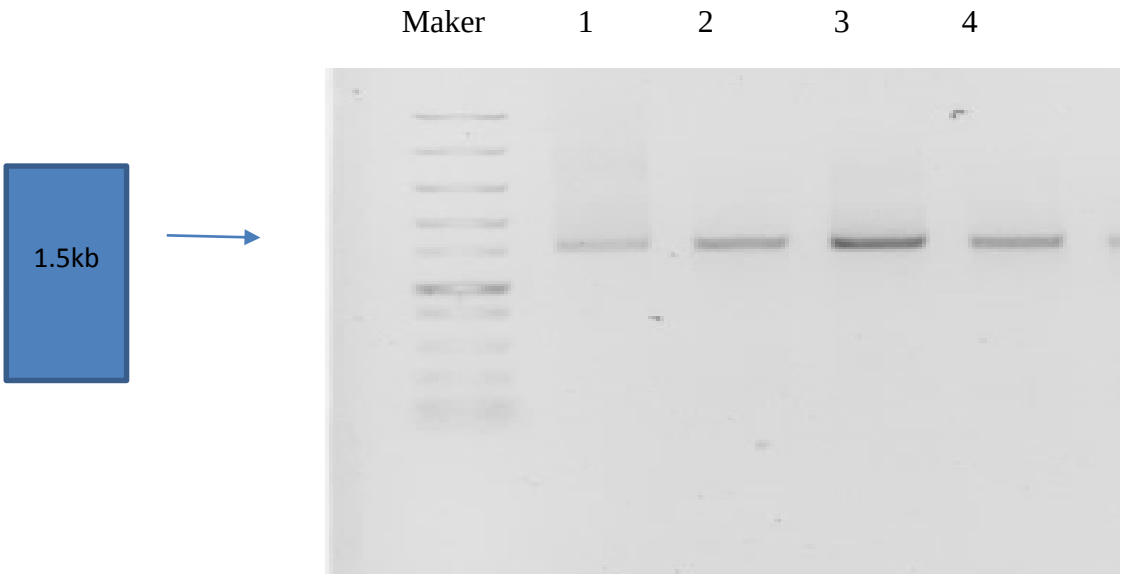

Figure 1: PCR gel image for the Amplicons (1= C2Y, 2 = C3P, 3 = SL1, 4 = SL2,).

C2Y = *Lactobacillus plantarum* strain FM02, C3P = *Lactobacillus pentosus* strain BSR3,

SL1 = *Lactobacillus plantarum* strain Z2, SL2 = *Lactobacillus pentosus* strain BSR3.

Table 2: Summary of Blast Prediction

S/N	Seq ID	Matched organism	% Identity	Accession number
1	C2Y	*Lactiplantibacillus plantarum* strain FM02	97.54%	MG913360.1
2	C3P	*Lactiplantibacillus pentosus* strain BSR3	100.00%	KY203913.1
3	SL1	*Lactiplantibacillus plantarum* strain Z2	99.86%	ON063304.1
4	SL2	*Lactiplantibacillus pentosus* strain BSR3	99.86%	KY203913.1

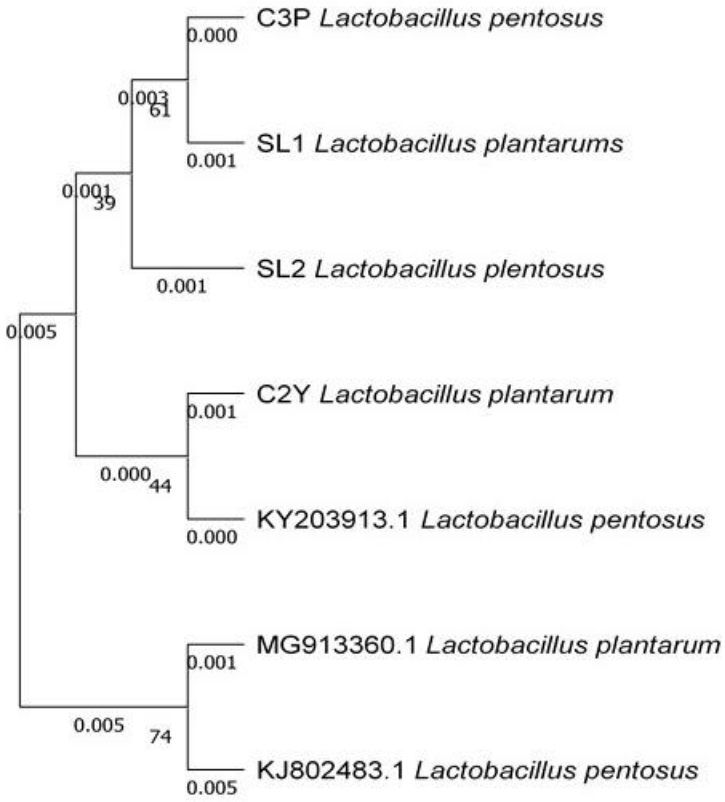

Figure 2: Phylogenetic tree showing the evolutionary distance between the bacterial isolates.

Lactic acid production without Tween-80 supplementation

Table 3 shows the concentrations of lactic acid produced from the starch-based substrates by the lactic acid bacteria. In yam broth, the highest lactic acid concentration (0.43 ± 0.10 g/L) was produced by *Lactobacillus pentosus* strain BSR3 (SL2) while the least concentration of lactic acid was produced by *Lactobacillus plantarum* strain Z2 (0.29 ± 0.25 g/L). Fermenting cassava broth, *Lactobacillus plantarum* strain Z2 produced the highest quantity of lactic acid (0.55 ± 0.12 g/L) while the least concentration (0.35 ± 0.24 g/L) of lactic acid was produced by *Lactobacillus*

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pentosus strain BSR3 (SL2). In corn broth, the highest concentration of lactic acid (0.55 ± 0.14 g/L) was produced by *Lactobacillus plantarum* strain Z2 while the least concentration (0.17± 0.06 g/L) of lactic acid was produced by *Lactobacillus plantarum* strain FM02. In potatoes broth, *Lactobacillus plantarum* strain Z2 produced the highest concentration (0.56 ± 0.35g/L) while the least concentration (0.30 ± 0.11 g/L) was produced by *Lactobacillus pentosus* strain BSR3 (SL2). The highest concentration of lactic acid (0.46 ± 0.40 g/L) was produced by *Lactobacillus pentosus* strain BSR3 (C3P) while *Lactobacillus plantarum* strain FM02 produced the least concentration (0.25 ± 0.21 g/L) were produced in rice broth. The overall highest concentration of lactic acid (0.56 ± 0.35g/L) was produced by *Lactobacillus plantarum* strain Z2 in potatoes broth.

Table 3: Lactic acid production (g/L) without Tween-80 supplementation

	Yam	Cassava	Corn	Potatoes	Rice
C3P	0.39 ± 0.09[a]	0.49 ± 0.20[a]	0.28 ± 0.13[b]	0.37 ± 0.37[a]	0.46 ±0.40[a]
SL1	0.29 ± 0.25[a]	0.55 ± 0.12[a]	0.55 ± 0.14[a]	0.56 ± 0.35[a]	0.42 ± 0.18[a]
SL2	0.43 ± 0.10[a]	0.35 ± 0.24[a]	0.23 ± 0.07[b]	0.30 ± 0.11[a]	0.32 ± 0.20[a]
C2Y	0.35 ± 0.10[a]	0.41 ± 0.26[a]	0.17 ± 0.06[b]	0.50 ± 0.31[a]	0.25 ± 0.21[a]
Fcal	.433	.530	7.547	.463	.398
p-value	.735	.674	.010	.716	.75
Remark	NS	NS	S	NS	NS

Lactic acid production with Tween-80 supplementation (at 3.0 g/L)

Table 4 shows the production of lactic acid when Tween 80 at concentration of 3.0 g/L was added to the culture medium. Tween 80 at concentration of 3.0 g/L improved lactic acid production generally but decreased production of the acid was also observed with certain bacteria in some broths. *Lactobacillus plantarum* strain Z2 produced the highest quantity of lactic acid (0.623 ± 0.013 g/L) from yam broth, a 113.7% increase in the lactic acid production capacity of the microorganism over its ability in the un-supplemented yam broth. A percentage increase of 34.5% was observed for same microorganism (*Lactobacillus plantarum* strain Z2) on lactic acid production from cassava broth compared with its lactic acid production in the un-supplemented cassava broth.

Lactobacillus pentosus strain BSR3 (SL2) produced the highest quantity of lactic acid on the supplemented potatoes broth, a percentage increase of 153% over that of the un-supplemented potatoes broth. For supplemented rice broth, the highest quantity of lactic acid (0.625 ± 0.022 g/L) was produced by *Lactobacillus pentosus* strain BSR3 (C3P), a percentage increase of 35.9% over that of the un-supplemented rice broth.

Decreased lactic acid production was observed for *Lactobacillus pentosus* strain BSR3 (C3P) in cassava broth. Also, despite increased lactic acid production by *Lactobacillus pentosus* strain BSR3 (C3P), *Lactobacillus pentosus* strain BSR3 (SL2)**,** *Lactobacillus plantarum* strain FM02 in supplemented corn broth, there was a slight drop in lactic acid production by *Lactobacillus plantarum* strain Z2 in same broth compared with the un-supplemented corn broth. Decreased lactic acid production was also observed for *Lactobacillus plantarum* strain Z2 in corn and potatoes broth.

Table 4: Effect of Tween 80 (3.0 g/L) supplementation on lactic acid production (g/mL)

	Yam	Cassava	Corn	Potatoes	Rice
C3P	0.43 ± 0.05^{c}	0.45 ± 0.04^{c}	0.44 ± 0.04^{b}	0.53 ± 0.01^{c}	0.63 ± 0.02^{a}
SL1	0.62 ± 0.01^{a}	0.74 ± 0.01^{a}	0.53 ± 0.03^{a}	0.45 ± 0.01^{d}	0.54 ± 0.02^{b}
SL2	0.56 ± 0.02^{b}	0.64 ± 0.01^{b}	0.54 ± 0.04^{a}	0.76 ± 0.01^{a}	0.45 ± 0.01^{c}
C2Y	0.42 ± 0.03^{c}	0.67 ± 0.06^{ab}	0.54 ± 0.04^{a}	0.62 ± 0.07^{b}	0.44 ± 0.025^{c}
F-cal	176.28300	171.77500	152.39600	150.64100	334.65600
p-value	<0.001	<0.001	<0.001	<0.001	<0.001
Remarks	S	S	S	S	S

Values presented are means of triplicates ± standard deviation. Means with different superscript across a column are significantly different ($p < 0.05$). S = Significant.
C3P = *Lactobacillus pentosus* strain BSR3; SL1 = *Lactobacillus plantarum* strain Z2;
SL2 = *Lactobacillus pentosus* strain BSR3**;** C2Y = *Lactobacillus plantarum* strain FM02.

Table 5 shows the effect of the addition Tween 80 at the concentration of 5.0 g/L on lactic acid production by Lactic acid bacteria. There was, generally, poor lactic acid production. All the lactic acid bacteria showed highly reduced capacity in lactic acid production. The percentage reduction in bacterial lactic acid producing capacity (in descending order of lactic acid producing capacity) in yam, cassava, corn, potatoes and rice broths were 79.3% (*Lactobacillus plantarum* strain Z2), 83.6% (*Lactobacillus plantarum* strain Z2), 90.9% (*Lactobacillus plantarum* strain Z2), 70% (*Lactobacillus pentosus* strain BSR3 (SL2)) and 86.9% (*Lactobacillus pentosus* strain BSR3 (C3P)) respectively.

Table 5: Effect of Tween 80 (5.0 g/L) supplementation on lactic acid production.

	Yam	Cassava	Corn	Potatoes	Rice
C3P	0.03 ± 0.01^{a}	0.03 ± 0.01^{b}	0.03 ± 0.02^{a}	0.04 ± 0.02^{b}	0.06 ± 0.03^{a}
SL1	0.06 ± 0.04^{a}	0.09 ± 0.01^{a}	0.05 ± 0.04^{a}	0.02 ± 0.01^{b}	0.05 ± 0.02^{ab}
SL2	0.05 ± 0.04^{a}	0.07 ± 0.04^{a}	0.05 ± 0.03^{a}	0.09 ± 0.01^{a}	0.02 ± 0.01^{bc}
C2Y	0.03 ± 0.02^{a}	0.08 ± 0.02^{a}	0.05 ± 0.03^{a}	0.07 ± 0.01^{a}	0.01 ± 0.01^{c}
Fcal	.627	4.583	.286	20.600	5.160
p-value	.618	.038	.835	<0.001	.028
Remark	NS	S	NS	S	S

Values presented are means of triplicates ± standard deviation. Means with different superscript across a column are significantly different ($p < 0.05$). NS = Not significant, S = Significant.C3P = *Lactobacillus pentosus* strain BSR3; SL1 = *Lactobacillus plantarum* strain Z2; SL2 = *Lactobacillus pentosus* strain BSR3**;** C2Y = *Lactobacillus plantarum* strain FMO2.

DISCUSSION

This study followed standard procedures for microbial sample collection, isolation, characterization of lactic acid bacteria (LAB), and substrate processing for lactic acid fermentation. The methodologies employed align with previous studies on starch-based lactic acid production (Karnwal *et al*., 2016; Vishnu *et al*., 2000; Wakil and Ajayi, 2013; Ray *et al*., 2009; Maheshwaran and Palaniswamy, 2017; Mudaliyar and Kulkarni, 2011).

Characterization and Identification of Lactic Acid Bacteria

The LAB strains identified in this study (*Lactobacillus pentosus* strain BSR3 (C3P, SL2), *Lactobacillus plantarum* strain Z2 (SL1), and *Lactobacillus plantarum* strain FM02 (C2Y)) were characterized based on morphological, biochemical, and genotypic analyses, with 16S rRNA sequencing confirming their identity. The detection of *Lactobacillus plantarum* in this study corroborates findings by Ray *et al*. (2009),

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Coelho *et al*. (2011), and Saavedra *et al*. (2021), while *Lactobacillus pentosus* has been previously reported as a key lactic acid producer by Garde *et al*. (2002), Tabacof *et al*. (2023), and Gonzalez-Leos *et al*. (2019).

Effect of Tween 80 Supplementation on Lactic Acid Production

Supplementing the fermentation medium with 3.0 g/L of Tween 80 generally enhanced lactic acid production. This agrees with Srivastava *et al*. (2015), who reported increased lactic acid yields upon Tween 80 supplementation. Additionally, Feng *et al*. (2006), Qi *et al*. (2009), and Naveena *et al*. (2005) observed that Tween 80 influences enzyme production and activation, supporting the role of this surfactant in microbial metabolism.

However, a reduction in lactic acid yield was observed in certain cases, particularly for *Lactobacillus pentosus* strain BSR3 (C3P) in cassava broth and *Lactobacillus plantarum* strain Z2 in corn and potato broths. These reductions may be attributed to substrate composition, particularly oil and bioactive compound content. Cassava, for instance, contains 23.75% oil (Falcao *et al*., 2022), while corn endosperm contains approximately 35% oil, rich in tocopherols, phenolic compounds, carotenoids, and anthocyanins (Muxin and Bingcan, 2023). Potatoes, despite their low fat content (0.3%–0.5%), contain significant levels of polyphenols, including chlorogenic acid, catechin, lutein, and glycoalkaloids (Hanjo *et al*., 2021; National Library of Medicine, 2020). Glycoalkaloids, in particular, are known for their antimicrobial properties (Camire *et al*., 2009), which could have contributed to the observed reduction in lactic acid yield in the affected substrates.

Possible Mechanism of Inhibition at Low Tween 80 Concentration

Studies by Zyuzina *et al*. (2021) suggest that Tween 80, when combined with oils, may exhibit toxicity at low concentrations. This could explain the reduced lactic acid production in corn, potato, and cassava broths supplemented with 3.0 g/L Tween 80. The ability of *Lactobacillus pentosus* strain BSR3 (SL2) and *Lactobacillus plantarum* strain FM02 to maintain high lactic acid yields in these substrates may be due to the presence of genes encoding detoxification mechanisms that degrade inhibitory compounds at lower Tween 80 concentrations. However, at higher concentrations (5.0 g/L), these protective mechanisms may become overwhelmed, leading to cellular stress and reduced metabolic activity.

Effect of High Tween 80 Concentration (5.0 g/L) on Lactic Acid Production

A significant decline in lactic acid yield was observed when Tween 80 was increased to 5.0 g/L, consistent with findings by Nagarjun *et al*., (2005), who reported that Tween 80 concentrations exceeding 4.0 g/L negatively impact microbial growth. The likely mechanism of inhibition is related to membrane destabilization, as Tween 80 has been shown to dissolve lipid bilayers, thereby increasing membrane permeability (Gonzalez *et al*., 2008; Liu *et al*., 2006; Qi *et al*., 2009; Silva *et al*., 2007; Zeng *et al*., 2006). This excessive permeability can lead to membrane rupture, loss of intracellular integrity, and ultimately cell death, explaining the sharp decline in lactic acid production observed at higher concentrations.

CONCLUSION

This study highlights the dual role of Tween 80 in lactic acid fermentation. At 3.0 g/L, Tween 80 generally enhances lactic acid production by improving microbial metabolism and enzyme activity, although its effect is substrate-dependent. In contrast, at 5.0 g/L, Tween 80 exerts a toxic effect on LAB, likely due to cell membrane destabilization. These findings underscore the importance of carefully optimizing Tween 80 concentration to maximize lactic acid yield without compromising microbial viability. Further research should explore the molecular mechanisms underlying Tween 80

interactions with different substrate components to refine its application in industrial lactic acid fermentation.

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