World Journal of Pharmaceutical and Life Sciences

www.wjpls.org

Impact Factor: 7.409 Coden USA: WJPLA7

LACTIC ACID PRODUCTION BY MUTAGENIZED *ENTEROBACTER HORMAECHEI* SUBSP. *XIANGFANGENSIS* ISOLATED FROM PROCESSED MILK

Michael Bamitale Osho*, Precious Jesuferanmi Awosile, Gbemisola Mary Alaba, Oluwasegun Benjamin Koleoso

Department of Microbiology and Biotechnology, College of Natural and Applied Sciences, McPherson University, Seriki Sotayo, P.M.B. 2094, Sapon, Abeokuta, Nigeria.

***Corresponding Author: Michael Bamitale Osho**

Department of Microbiology & Biotechnology, College of Natural and Applied Sciences, McPherson University, Seriki Sotayo, P.M.B. 2094, Sapon, Abeokuta, Nigeria. **DOI:** https://doi.org/10.5281/zenodo.18150361

How to cite this Article: Michael Bamitale Osho*, Precious Jesuferanmi Awosile, Gbemisola Mary Alaba, Oluwasegun Benjamin Koleoso. (2026). Lactic Acid Production by Mutagenized Enterobacter Hormaechei Subsp. Xiangfangensis Isolated from Processed Milk. World Journal of Pharmaceutical and Life Science, 12(1), 105–115.

Article Received on 05/12/2025 **Article Revised on 25/12/2025** **Article Published on 01/01/2026**

ABSTRACT

Lactic acid (LA), a valuable weak natural organic acid extensively utilized in various industries has been conventionally synthesized through chemical processes. However, the growing demand for sustainable and eco-friendly production methods has stimulated interest in microbial synthesis. The study was to explore the potential of multi-strain lactic acid bacteria isolated from processed liquid milk to synthesize lactic acid. Microorganisms were isolated and screened on deMan Rogosa Sharpe (MRS) agar and were identified according to morphological characterization and 16S rRNA sequencing. Mutagenesis of positive isolate using chemical and physical means, quantitative and qualitative production using High-Performance Liquid Chromatography (HPLC) were investigated. The isolate was discovered to have a clear zone on MRS agar, which allowed for its molecular characterization as *Enterobacter hormaechei* subsp. *xiangfangensis* with accession number OP492057.1. It was then chosen for mutagenesis using ethidium bromide (EB) and UV light. Thirteen mutants were identified, and three of those were examined to see if they could generate LA using various substrates. HPLC confirmed mutants and parent strain to produce hetero-fermentative lactic acid using cheese whey substrate. The UV mutant strain showed a significantly higher yield (2008.50 µg/mL) than both the EB mutant (1451.25 µg/mL) and the parent strain (233.75 µg/mL) at 37 °C, pH 4.5, and 150 rpm 16 h fermentation period. This study showed that mutagenesis greatly influences optimum LA production due to mutants developing enhanced cell membrane integrity and pH tolerance, thus permitting strains to synthesize LA even at inhibitory concentrations.

KEYWORDS: Lactic Acid; *Enterobacter hormaechei* subsp. *xiangfangensis;* Processed Milk: Cheese Whey; Corn Steep Liquor.

1.0 INTRODUCTION

Lactic acid (2-hydroxypropanoic acid, CH_3-CH(OH)-COOH), a valuable organic acid, has gained significant attention for its various industrial applications. Traditionally, lactic acid has been produced through chemical processes, which are associated with environmental concerns and limited sustainability. As a result, there is a growing interest in microbial synthesis of lactic acid using lactic acid bacteria (LAB) (Sun *et al*., 2017). Lactic acid bacteria are known for their ability to convert sugars into lactic acid through fermentation. Isolation and screening of LAB from different sources, including bovine milk, have been carried out to identify strains with high lactic acid production capabilities. Bovine milk is a promising source of LAB due to its abundance and the natural presence of lactic acid bacteria which possess the unique capability to convert sugars into lactic acid through fermentation (Sun *et al*., 2017; Johansen *et al*., 2020).

Genetic engineering techniques, such as targeted gene disruption and gene knockout, have been employed to introduce specific genetic modifications in LAB. These techniques utilize recombinant DNA technology to

manipulate LAB genomes. For instance, the disruption of lactate dehydrogenase genes using genetic engineering tools has been reported to enhance lactic acid production in LAB strains (Zhang *et al*., 2022).

In summary, mutagenesis in LAB involves the use of chemical mutagens, physical mutagens, and genetic engineering techniques to induce genetic mutations and improve lactic acid production. These approaches have been successfully employed to modify LAB strains and enhance their capabilities in various industrial applications.

Genetic engineering techniques have been employed to enhance the metabolic efficiency of lactic acid bacteria for increased lactic acid production. Genetic modifications targeting key enzymes and metabolic pathways involved in lactic acid synthesis have shown promising results. These modifications can lead to improved substrate utilization and lactic acid yield (Yadav *et al*., 2021). Multi-strain lactic acid bacteria consortia have demonstrated the potential for enhanced lactic acid production. Chen *et al*. (2019) investigated the utilization of strain combinations, including *Lactobacillus plantarum*, for effective utilization of rice straw hydrolysates and observed improved lactic acid production compared to individual strains. The selection and improvement of multi-strain lactic acid bacteria involve various strategies, such as genetic engineering, strain combination, and optimization of fermentation conditions. Genetic engineering techniques can be employed to enhance the metabolic pathways of LAB, thereby increasing lactic acid production (Yadav *et al*., 2021). Combining different strains of LAB can lead to synergistic effects, resulting in higher productivity (Chen *et a*l., 2019). Additionally, optimizing fermentation conditions, including temperature, pH, and nutrient availability, can further enhance lactic acid synthesis (Dong *et al*., 2018).

Optimization of fermentation conditions is crucial for maximizing lactic acid yield. Factors such as temperature, pH, and nutrient availability play significant roles in the efficiency of lactic acid fermentation. Dong *et al*., (2018) optimized fermentation conditions for lactic acid production from Jerusalem artichoke tuber hydrolysate by *Lactobacillus paracasei* LA104, resulting in increased lactic acid yield. The industrial applications of lactic acid have further emphasized the need for sustainable and efficient production methods. Lactic acid serves as a food additive, pH regulator, and precursor for biodegradable polymers. Nguyen *et al*., (2022) highlighted the industrial applications of lactic acid and the importance of optimizing fermentation conditions for improved production.

Overall, the literature demonstrates the significance of microbial synthesis of lactic acid using LAB. Isolation of LAB from bovine milk, genetic engineering techniques, utilization of multi-strain consortia, and optimization of fermentation conditions all contribute to improving lactic acid production. These approaches promote sustainable and eco-friendly production methods and offer practical solutions for industrial-scale lactic acid production.

The study on microbial synthesis of lactic acid by improved multi-strain lactic acid bacteria from processed liquid milk is justified by its potential to provide sustainable and environmentally friendly methods for lactic acid production, optimize resource utilization, improve process efficiency and yield, and cater to industrial applications. The most effective technique to obtain genetically stable and practical strains of microbes for industrially significant products has always been to isolate and screen them from natural sources. Furthermore, because fermentation uses renewable raw resources as opposed to petrochemicals, it is becoming a more significant activity. The findings from this study contributed to advancing the field of microbial synthesis and provide practical solutions for the production of lactic acid.

The objective of this study was to explore the potential of multi-strain bacteria isolated from processed liquid milk and screened as *Enterobacter hormaechei* subsp. *xiangfangensis* for desirable traits and production of pure isomeric form of lactic acid. Processed liquid milk serves as an abundant source of LAB, and these bacteria have evolved to efficiently ferment lactose into lactic acid. By enhancing the performance of these natural lactic acid producers, overall lactic acid production yield and efficiency can be improved (Johansen *et al*., 2020).

2.0 MATERIALS AND METHODS

2.1 Sample collection

Three brands of commercial evaporated tin milk samples were obtained from supermarket for the study and composite of these samples was used. For the production of lactic acid, two fermentation broth, corn steep liquor and cheese whey were obtained commercially from Iwo Road market in Ibadan North West Local Government, Oyo State, Nigeria.

2.2 Isolation and screening of isolates

For the enrichment and isolation of bacteria, aliquots of the commercial milk were serially diluted to a dilution factor of 10^{-6} and 0.1mL was then spread evenly on MRS agar plates, LAPT medium and MacConkey agar plates. The plates were incubated for 44 h at 37 ˚C. For those that developed growth on the agar plates, they were then sub-cultured into MRS agar plates, LAPT medium and MacConkey agar plates to obtain a pure culture and then incubated for 24 h at 37^{o}C. It was then kept on nutrient agar slant. Screening of LAB was carried out as bacterial colonies were picked and streaked on fresh MRS agar plates and supplemented with sodium azide (0.02 gL^{-1}), bromocresol purple (0.012 gL^{-1}) and 2% (w/v) sucrose, placed in anaerobic jar and then incubated for 44 h at 37 ^{o}C (Yoganand *et al.*, 2012).

2.3 Development of Mutant Variants via UV Radiation and Ethidium Bromide Treatment in MRS Culture

Bacteria in the logarithmic growth phase were diluted and plated onto MRS agar supplemented with 4 g of sucrose and 0.012 g of bromocresol purple. For 30, 60, and 90 s, UV light at a distance of 2 cm was applied to the Petri plates. There was also a control group that included 10^{-1}, 10^{-2}, and 10^{-3} dilution cultures in it, but they were not subjected to UV light. All cultures were incubated at 37 ˚C for 60 h while being completely darkened by wrapping in aluminium foil. A modified version of the Gawel *et al*., (2002) protocol was employed. The logarithmic growth phase (17 h culture) of bacteria was extracted from MRS broth and centrifuged at 10,000 rpm for 15 mins at 37 °C in a cold centrifuge. The bacteria were then twice washed with a cold, sterile 0.9 g NaCl solution using a vortex. The procedure was done twice. Two millilitres of each cell suspension containing 0.25, 0.5, and 0.75 $mgmL^{-1}$ of ethidium bromide were added, and the mixture was agitated for 30 mins at 200 rpm on an orbital shaker set at 37˚C. After centrifuging the treated cells for 15 mins at 10,000 rpm for 15 min at 37 ˚C, they were twice washed with MRS broth and 0.9% NaCl solution. MRS broth was added to the washed and treated cell suspension and 100μl (0.1 mL) of the cell was dispensed on a fresh MRS agar plate and spread on the agar using a spreader then incubated at 37 ˚C for 60 h. Control culture was not exposed to ethidium bromide.

2.4 Molecular Characterization of potential isolate

Genomic DNA isolation was carried out using Zymo Research Quick-DNA Bacterial Kit (USA). Amplification of polymerase chain reaction (PCR) of 16S rRNA isolate was done using 16S rRNA gene fragments of ~1.5 KB using universal primers (27F and 1492R). Initial denaturation at 94 °C for 5 minutes with 30 cycles followed by denaturation at 94°C for 1 min, then annealing at 55 °C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 10 mins. Purification was carried out using a fast gene gel/PCR extraction kit (Zymo Research Quick, USA), and the resulting sequences were analyzed using the BLAST program in the NCBI gene bank database which was obtained at http://blast.ncbi.nlm.nih.gov/Blast.cgi.

2.5 Effect of Incubation Period on Lactic Acid Production

The time course of lactic acid production by wild strain isolate (WS), physical mutant and chemical mutant isolates were evaluated by taking the concentration value at every 6 h interval for 36 h incubation. Incubation periods under consideration were between 12 h and 18 h. The organisms were incubated for 36 h at 37 ^{o}C.

2.6 Optimized production of lactic acid from fermentation broth

For the production of lactic acid, two fermentation broth, corn steep liquor and cheese whey were used. The pH of each broth was taken using the pH meter. Broths were acidic pH 3.5-4.0 and were therefore adjusted to 4.5 with 0.1 N NaOH. Each broth (280 mL) were prepared in a conical flask and a mixture of the two broths in equal proportion and then 40 ml of each broth were dispensed into100 mL conical flasks with broths in seven conical flasks each and then sterilized before it was inoculated with 200 μl of isolates which include a wild strain, three physical mutagen (30 s, 60 s and 90 s) and three chemical mutagen (0.25, 0.5 and 0.75$mgmL^{-1}$) from nutrient broth. After inoculation, 0 h titration was done and the titre value was obtained. The samples were then placed in a water bath shaker at 37 ^{o}C for 36 h at a speed of 150 rpm and at every 6 h interval, the titre value was obtained and this process was repeated at each interval. From the result we calculated the optimum value from all the broth and the kind of sample inoculated into the broth.

The whole process was only repeated for cheese whey substrate with only 3 samples (wild strain, 90 s as physical mutagen and 0.5 $mgmL^{-1}$ as chemical mutagen). In this process, the samples were incubated in the water bath shaker for 10 h at 37 ^{o}C at a speed of 150 rpm and at every 2 h interval the titre value was obtained and recorded.

Using the formula 0.9 V where 'V' denotes the volume in mL of 0.1 M NaOH needed to neutralize 10 mL of lactic acid solution, the concentration of lactic acid produced by the LAB was estimated using the volume of NaOH.

2.7 Qualitative analysis of lactic acid using high performance liquid chromatography

A modified version of the Ramanjooloo *et al.*, (2014) procedure was applied to the qualitative determination process. In order to ascertain whether a homolactate bacteria was indeed isolated and the impact of various mutagenesis techniques on the lactic acid output, the fermentation end-products were qualitatively determined. At the sixth hour which had the optimum and suitable production, a sample was taken from each to be centrifuged for qualitative analysis. After centrifuging, the supernatant was dispensed in a sterile cryoviale and stored in a refrigerator before it was sent for the analysis.

HPLC Conditions

Mobile Phase A: 10 mM KH_2PO_4 in water pH: 3.0 (98% v/v)
Mobile Phase B: Acetonitrile (2% v/v)
FR: 1.0 mL/min
SP: Waters X bridge Shield RP (150 mm x 4.6 mm I.D, 3.5 um)
Column Temp: 40 degC
Injection Vol.: 50 uL
P = ~120 Bar

Standard Preparation of 2000 $ugmL^{-1}$ Stock solution: Equivalent of 50 mg (58 mg of Lactic Acid solution) was

weighed and diluted with mobile phase A in a 25 mL volumetric flask to the mark with same diluent.

Calibration standards of 20-1000 ugmL^{-1} were prepared from the stock solution for HPLC analysis.

Sample Preparation: Samples were centrifuged at 4000 rpm for 10 mins. The supernatant was diluted by 4 using mobile phase A.

HPLC - Agilent 1100 series with an online degasser, quaternary pump, auto liquid sampler, thermostated column compartment and a variable UV wavelength detector running on Chemstation Software.

3.0 RESULTS

3.1 Isolation and identification of isolates

It was discovered through the isolation and identification of the isolates (Plate 1) that the processed liquid milk sample showed a yellow zone on the MRS agar plate supplemented with 2% (w/v) sucrose, bromocresol purple (0.012 g/L), and sodium azide (0.02 g/L). Colonies on MRS agar plates enriched were chosen following 44 h of incubation at 37˚C.

Plate 2: Morphological growth of *Enterobacter hormaechei* subsp. *xiangfangensis* on MRS agar supplemented with sodium azide, bromocresol purple and sucrose

3.2 Effect of physical & chemical mutagens on isolates

The effect of the UV light exposure on the isolate showed change in the morphology and growth of the organism but still exhibited a little yellow zone on the plate. It also reduced the survival rate of the isolate such that there was no much growth on the plate. Further analysis showed that it inhibited the ability of the organism to hydrolyze protein and also produce or stop the activity of the enzyme lactate dehydrogenase. The effect of the ethidium bromide exposure on the isolate showed the isolate struggling to survive on the plates after each exposure at different concentration such that the colonies that grew were few. There was no visible change in the morphology but still exhibited a yellow/cream zone. Further analysis showed that the chemical inhibited the production of enzyme lactate dehydrogenase by the organism and also stops the enzyme from performing its activity.

3.3 Identification of the PCR-Amplified DNA

The bacterial isolates were identified through sequencing of the PCR-amplified 16S rRNA gene, and the resulting sequences were compared against the non-redundant nucleotide database of the National Center for Biotechnology Information (NCBI). The isolate with accession number OP492057.1 showed the highest sequence similarity to *Enterobacter hormaechei* subsp. *xiangfangensis*, although its closest match in the database was reported as *Enterobacter cloacae* based on 16S rRNA similarity Table 1. A partial genome shotgun sequence of *E. hormaechei* subsp. *xiangfangensis* (approximately 1002 bp) was obtained. Plate 2 shows the agarose gel electrophoresis profile of the amplified DNA, demonstrating that the isolate produced a PCR product of approximately 2000 bp, consistent with the expected size of the 16S rRNA gene. Figure 1 presents the phylogenetic tree depicting the relationship between the isolate and other closely related species.

Table 1: Identity of bacteria isolate by sequence of the genomic DNA.

Accession No NCBI	Nucleotide Number	Reference NCBI database	Identity	Query cover
OP492057.1	1002	*Enterobacter* h*ormaechei* subsp. x*iangfangensis*	90.68%	85%

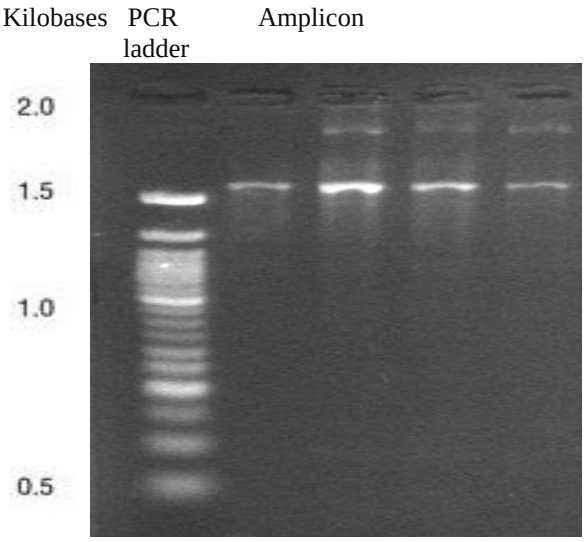


Plate 2: Agarose Gel Electrophoresis of DNA fragment amplified by Polymerase Chain Reaction (PCR) Prepared from positive isolate Amplicon contains PCR product of DNA of *Enterobacter hormaechei* subsp. x*iangfangensis.*

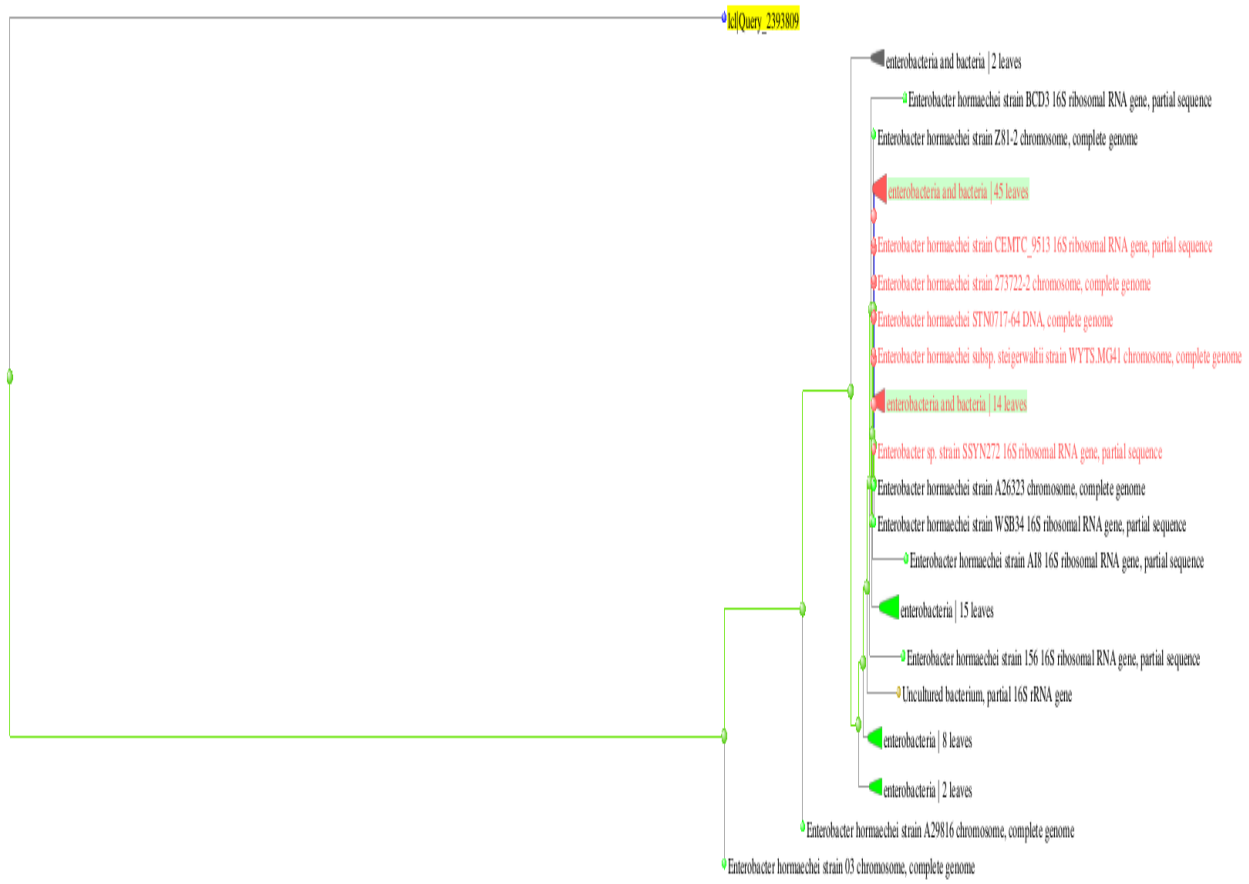


Fig. 1: Phylogenetic tree of the identified *E. hormaechei* subsp. *xiangfangensis*

3.4 Effect of incubation period on lactic acid production

Fig 2 - Fig 4 revealed that the optimum lactic acid production was at 18 h at 36 h incubation period. At 36 h incubation period, there were increments in lactic acid production in cheese whey from 12 h up to 18 h of incubation. Activity decreased slowly up thereafter till 36 h. So, the maximal incubation period of the studied organism for lactic acid production was 18 h.

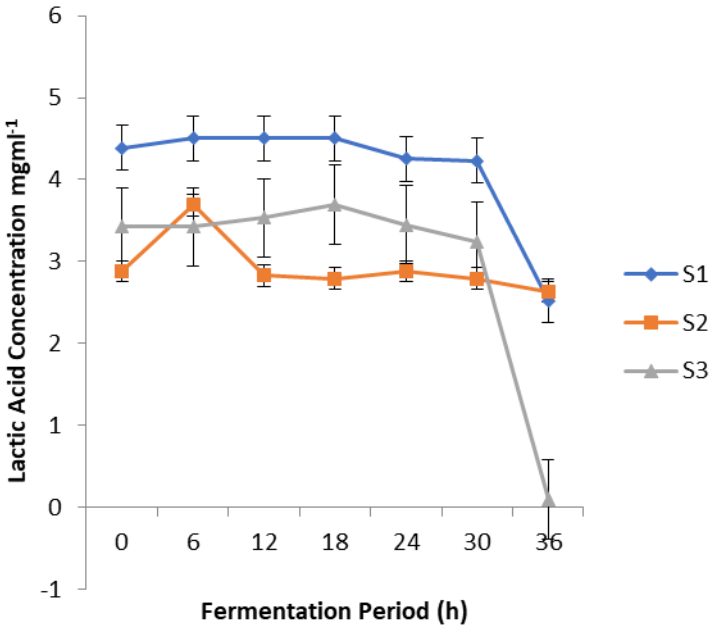


Figure 1: Effect of wild strain isolate (WS) in cheese whey (S1), corn step liquor (S2) and mixture (S3) in the production of lactic acid mutants, U30 had the highest production of lactic acid (3.78 mgml^{-1}) at incubation period of 12 h.

3.5 Optimization production of lactic acid from wild strain and mutant isolates

After the 36-h incubation of the Wild Strain (WS) in cheese whey, corn steep liquor and the mixture, the production of lactic acid was proven to be relatively stable and high (4.5 mgmL^{-1}) in cheese whey (S1) between 6-18 h before it declined at 18 h while in corn steep liquor (S2) it increased at 6 h with a concentration of 3.69 mgmL^{-1} and declined at the remaining period of incubation (Fig.2). For the mixture (S3), it was relatively stable at the incubation period from 6-18 h and increased at the 18 h (3.69 mgmL^{-1}) before it became stable again and finally declined at 30 h incubation period.

For physical mutant in cheese whey, U30 had a relatively stable production of lactic acid between 6 h to 24 h and declined at 30 h- 36 h. U60 had a stable production of lactic acid within the incubation period of 6 h to 12 h and then declined from incubation period of 12 h to 36 h. At incubation period of 12 h, U90 had a very high production of lactic acid (4.76 mgmL^{-1}). According to the graph, U90 had the most lactic acid generation in the cheese whey. U30 produced more lactic acid in corn steep liquor over the first 18 h of incubation (from 2.52 and 2.97 to 3.06 mgmL^{-1}), but this production subsequently decreased over the course of the next 18 h (Fig 3). U60 had a concentration of 2.79 mgmL^{-1} at incubation period of 6 h and declined or reduced for the rest of the incubation period. U90 also had a little increase in the production from 1.87 mgmL^{-1} at 6 h to 2.7 mgmL^{-1} at 12 h and later reduced for the rest of the incubation period. Therefore, from the values, U30 had the highest production of lactic acid in corn steep liquor. In the mixture, among the three mutants, U30 had the highest production of lactic acid (3.78 mgml^{-1}) at incubation period of 12 h.

For the chemical mutants in cheese whey, E50 had the highest production of lactic acid (5.04 mgml^{-1}) among the three mutants at 18 h incubation period and reduced for the rest of the incubation period. The other two mutants were relatively stable and low. In corn steep liquor, E50 produced the highest concentration of lactic acid (3.24 mgml^{-1}) among the three mutants at 18 h incubation period and decreased for the remaining period. E25 increased in its production (2.88 mgml^{-1}) a little at 6 h incubation period and kept reducing and increasing for the remaining period while E75 also had an increase in the production of lactic acid (3.06 mgml^{-1}) at 18 h incubation period and reduced after 18 h till 36 h incubation period (Fig 4). In mixture, the three mutants produced lactic acid at a relative stable concentration of 3.6 mgml^{-1} and reduced or declined after 18 h incubation period. Among the three, E25 produced the highest concentration of lactic acid 3.70 mgml^{-1} at 12 h of 36 h incubation period. In conclusion, during the 36 h incubation period, only cheese whey enabled optimum production of lactic acid with the following isolates; WS, U90 and E50.

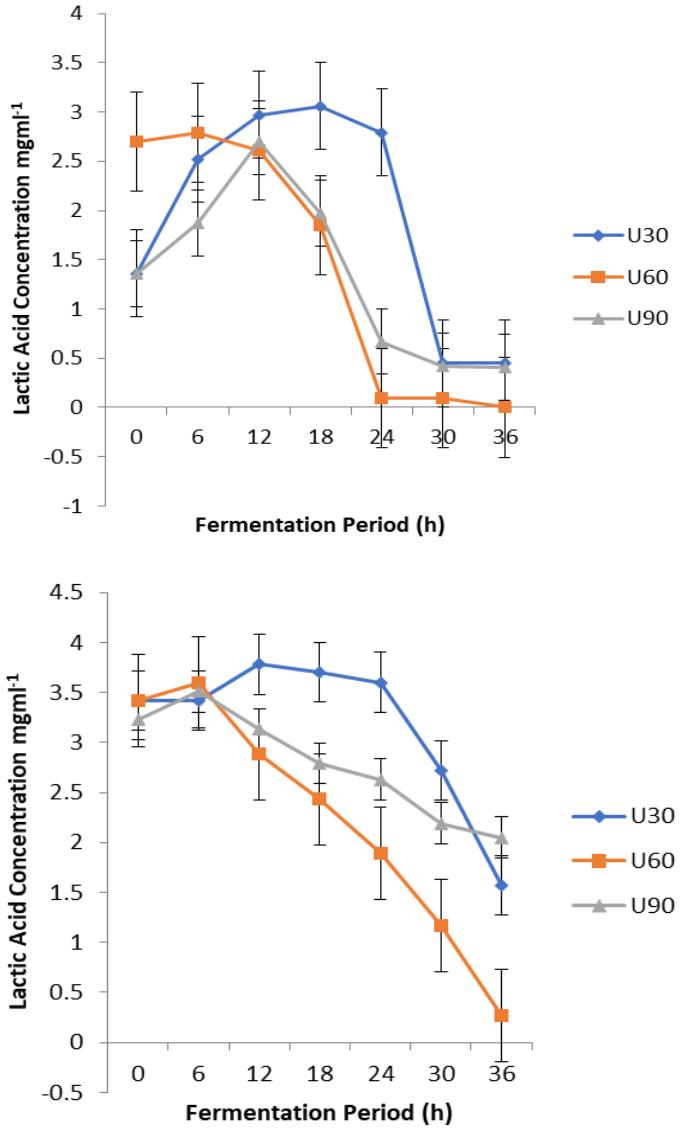
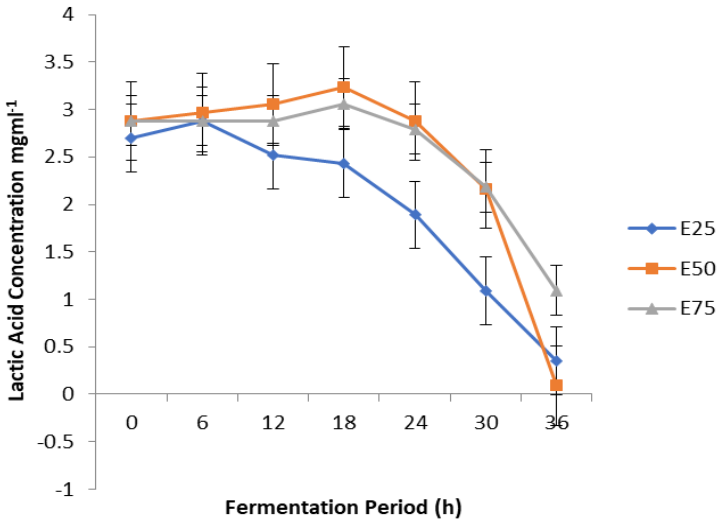
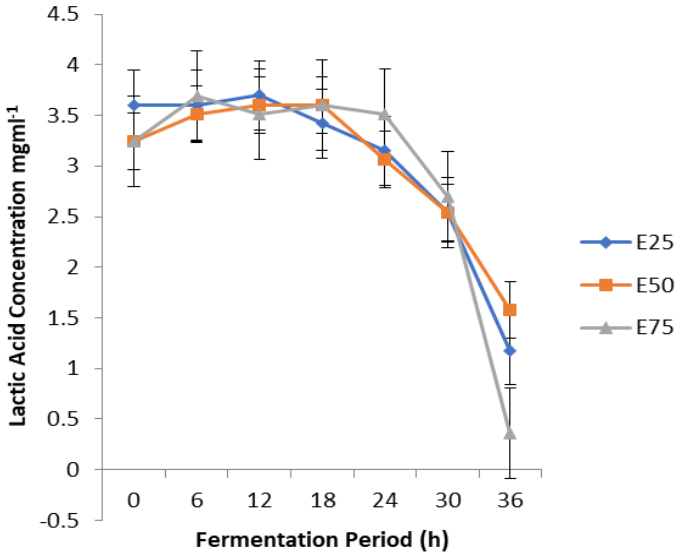


Figure 2: Effects of physical mutants in (a) corn steep liquor and (b) mixture on lactic acid production. ***The error bars represent the SD of three samples.***

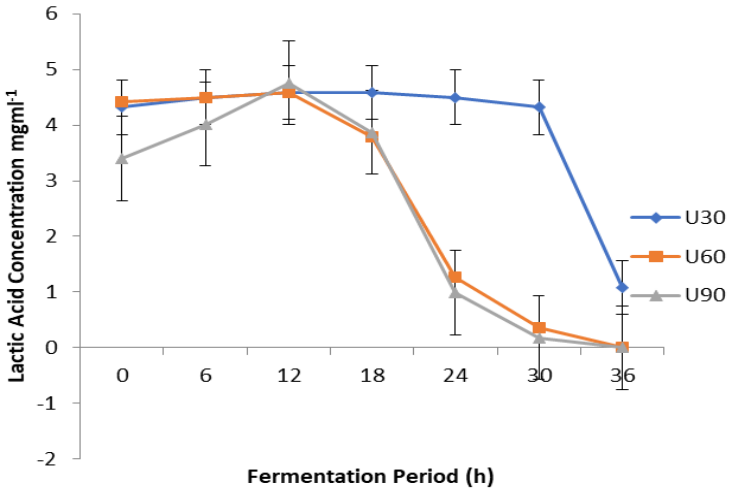


(a)

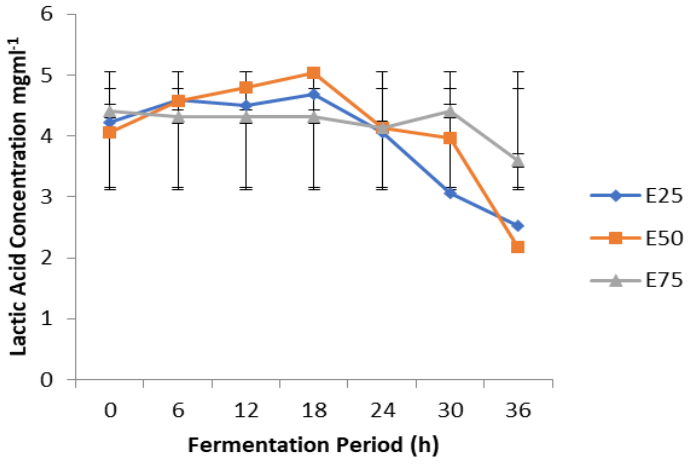


(b)

Figure 3: Effects of chemical mutants in (a) corn steep liquor and (b) cheese whey on lactic acid production. ***The error bars represent the SD of three samples.***



(a)



(b)

Figure 4: Effect of (a) physical mutants (b) chemical mutants in cheese whey on lactic acid Production. ***The error bars represent the SD of three samples.***

3.7 Qualitative determination of lactic acid using HPLC

From the cheese whey enabled optimum lactic acid production, these isolates WS, UV90 and EB50 were subjected to HPLC analysis to confirm the presence and the amount of lactic acid. The HPLC analysis on the isolate; Wild strain isolate, Physical mutant (at 90 s exposure) and chemical mutant (0.5 $mgmL^{-1}$) (WS, U90 and E50) respectively showed that WS yielded 233.75 $ugmL^{1}$ which was the optimum lactic acid production with a retention time of 2.622 min and area cover of 364.3 5 mAU. UV90 yielded 2008.50 μgmL^{-1} lactic acid with a retention time of 2.64 min and area cover of 263.53 mAU. EB50 yielded 1451.25 μgmL^{-1} lactic acid production with a retention time of 2.53 min and area cover of 39.01 mAU which turned out to be the lowest production. Conclusively, the UV mutant strain showed a significantly higher yield than both the EB mutant and the parent strain. Similarly, the EB mutant produced a significantly greater yield than the parent strain Figure 5 & 6). Overall, both mutagenesis treatments resulted in statistically meaningful increases in yield relative to the control (parent) strain (Table 2).

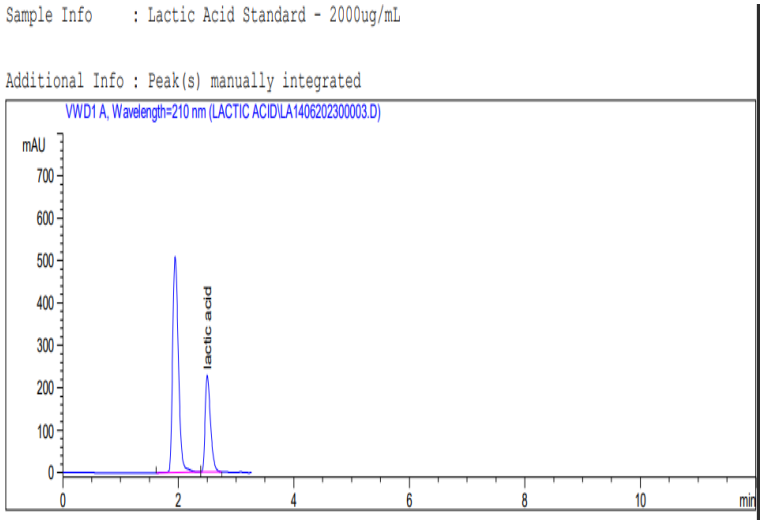
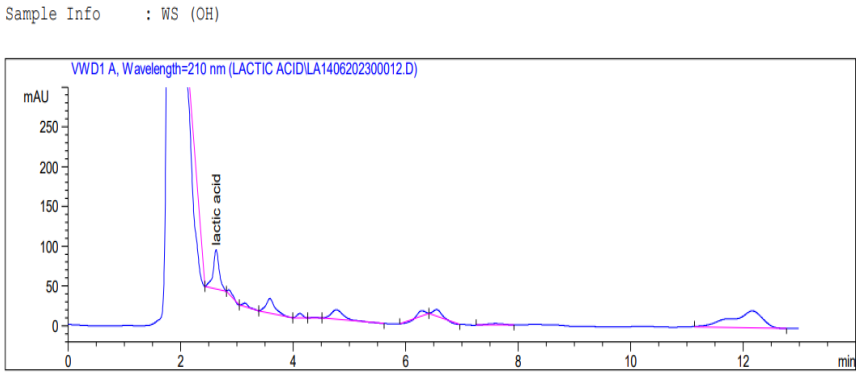
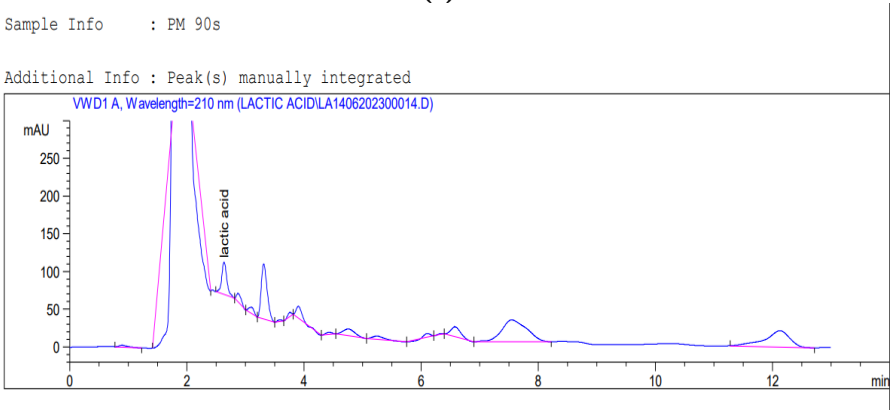


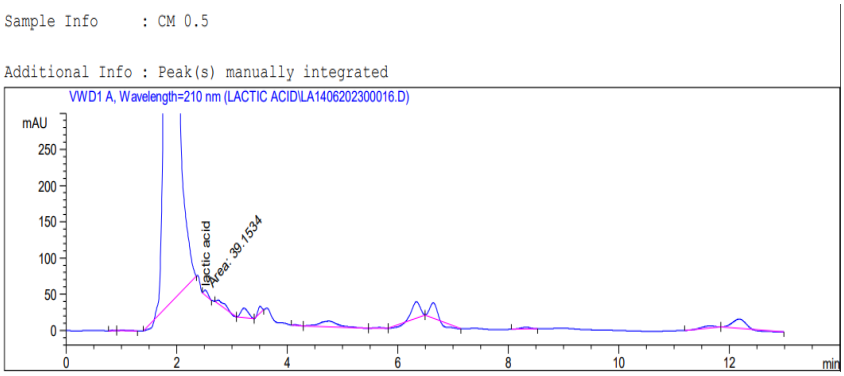
Figure 5: Chromatogram of lactic acid standard assay.



(a)



(b)



(c)

Figure 6: Chromatogram of lactic acid assay of (a) wild strains (b) physical mutants (c) chemical mutants isolate from Cheese Whey.

Table 2: Summary of One Way ANOVA.

Source	SS	df	MS	F
Between Strains	4,953,909.56	2	2,476,954.78	8229.09
Within Strains	1,806.00	6	301.00	
Total	4,955,715.56	8		

There is a highly significant difference among the three strains ($p \ll 0.001$). Mutant strains (UV and EB) produced dramatically higher yields compared to the parent strain.

Isolates	n	Mean	SD
Parent strains	3	226.67	14.01
UV Mutant Strain	3	2000.33	12.01
EB Mutant Strains	3	1456.33	23.71

Interpretation

The F-value = 8229.09, which is extremely high. This indicates highly significant differences among the Parent, UV Mutant, and EB Mutant strains.

4.0 DISCUSSION

Since sodium azide is a strong inhibitor of iron-porphyrin, it effectively stopped the growth of most fungi and non-LAB organisms. Because LAB are not iron-porphyrin syntheses, they can develop when sodium azide is present. The negative catalase test result is also due to this Lactobacillus trait. The most successful method for visual detection of LAB was determined to be bromocresol purple (0.012 gL^{-1}). Bromocresol blue is a pH indicator that turns yellow when lactic acid is produced. The amount of lactic acid that a bacterial colony produces is indicated by the yellow circle surrounding it. Adnan and Tan (2007) state that mesophilic bacteria reach their highest reproductive and activity temperature at 37˚C, which is why this temperature was selected for the incubation period. According to Kotzamanidis *et al.* (2002), bacterial cells undergo autolysis as a result of rising lactate concentrations, which lowers cell biomass. The reason the lactate content kept rising even soon after is that the released lactate dehydrogenase enzymes are still active. This study showed that mutagenesis of the isolate by ultraviolet ray and ethidium bromide resulted in the increase of lactic acid production compared to the production from parental strain which supported Banjo *et al.*, (2018) that the mutagenesis of *A. flavus* by ultraviolet ray and ethidium bromide resulted in an increased ascorbic acid yield of 6.99 gL^{-1} and 7.28 gL^{-1} production respectively compared to a yield of 3.92 gL^{-1} from the parental strain of *A. flavus.* Inoue *et al.*, (2025) generated a mutant yeast strain, *DLac_Mut2_221*, through UV mutagenesis and produced about 1.52-fold higher D-lactic acid from methanol compared to its parent strain. Ishrat *et al.*, (2025) reviewed summarizing progress in utilizing lignocellulosic biomass to produce lactic acid with genetically engineered lactic acid bacteria (LAB) reported that the engineered strains *Bacillus* or LAB can reliably yield high titers 110 g/L under optimized fermentation conditions.

The HPLC analysis also showed the effect of the exposure of the strain chemically and physically on their ability to produce more lactic acid. For the effect of the UV light on the strain which was the physical mutagenesis, there are two possible things that could have occurred such that the exposure led to the inhibition of production of the enzyme lactate dehydrogenase or the enzyme was produced and it inhibited the ability of the organism to hydrolyze the protein or possibly killed the organism during the process. For the effect of the ethidium bromide on the strain which was the chemical mutagenesis, there are also two possible actions that could have occurred during the production such that the chemical stopped the production of the enzyme lactate dehydrogenase from the organism which aids the lactic acid production or the enzyme was produced but was inhibited by the chemical from performing its activity. If

it was said that the organism died, there will be no production of the enzyme or lactic acid. The simultaneous saccharification and fermentation process allow to use lignocellulosic biomass as a cost-effective and high-yield strategy. *Escherichia coli* JU15 strain was metabolically engineered to metabolize C5 and C6 sugars at its optimal growth temperature (37℃) and gave a high yield of d-lactic acid production (Perez-Morales *et al.*, 2024). Jolien *et al.* (2025) explored the potential of 168 genetically diverse acid-tolerant yeast *K. marxianus* strains to identify the best candidate strains Km3, best candidate strain was subjected to adaptive laboratory evolution, and yielded an 18% increase in LA production (0.81 g-1) from titers of 120 g-1 while requiring less neutralization agent and showing capacity to efficiently ferment xylose-containing feedstocks. Total genome sequencing identified general transcription factor gene *SUA7* as the cause that resulted in increased performance of the evolved clone.

5.0 CONCLUSION

The result of this study showed that the mutant strains yielded the optimum production of lactic acid than the parent/wild mutant strain. The result also revealed that cheese whey was a more favourable substrate for the production of lactic acid than corn steep liquor in fermentation. However, low productivity of parent strain might be due to high concentration of lactic acid to its cell, hence mutants might have developed enhanced cell membrane integrity and improved pH tolerance, thus permitting strains to continue synthesizing lactic acid even at inhibitory concentrations. Mutants often show improved resistance to temperature fluctuations, osmotic stress, inhibitors in the fermentation medium. This leads to more efficient production and stable under fermentation conditions.

REFERENCES

1. Adnan AFM, Tan IKP. Bios. Tech, 2027; 98: 1380-1385.
2. Banjo, TT, Kareem SO, Banjo TO Abayomi OS. Nig Jour Biotech, 2019; 35(2): 8–15.
3. Chen S, Lin J, Zheng J. Biochem Eng Jour, 2019; 151: 107-330.
4. Chen S, Tong Q, Ding J, Ren D, Zhang L, Jiang M, Zhang C. Appl Environ Microb, 2019; 85(6): 02-18.
5. Dong Y, Hu J, Fan L, Wang J, Jour Microb Biotech, 2018; 28(5): 815-821.
6. Gawel D, Maliszewska-Tkacyk M, Jonczyk P, Schaaper RM, Fijalkowska IJ, Bacteriology, 2002; 184: 4449-4454.
7. Hofvendahl K, Hahn-Hägerdal B, Enzy Microb Technol, 2000; 26: 87-107.
8. Harris DC. Quantitative Chemical Analysis (8th ed.). New York, NY: W.H. Freeman and Company.
9. Inoue Y, Nakamura K, Yamada R, Matsumoto T, Ogino H. Metab Eng Commun, 2025; e00262.
10. Ishrat P, Quratulain SH, Ayyub A, Mehboob NA, Yasar SA, Mumtaz N, Koser F, Akram H, Sultan HY, Zubaria S, Mazhar S, Nawaz S, Riaz A, Abbas S, Imam HA. Discov Appl Sci., 2025; **7**: 1350.
11. Johansen E, Nærdal I, Skåra T, Axelsson L, Rudi K. Front Microbiol, 2020; 11: 73.
12. Jolien S, Héctor E, Godoy J, Goossenaerts, EVB, Quinten D. Jeroen B, Raúl AO, Eugenio M, Alexander D, Kevin JV; Microb Cell Fact, 24: 179.
13. Khanam R, Prasuna GR, Jour Sci Ind Res., 2014; 73: 331-337.
14. Kotzamanidis C, Roukas T, Skaracis G, World Journ Microb Biotechnol, 2002; 18: 441-448.
15. Mahwish S, Shazia AB, Muhammad S, Tanzila S, Shazia N, Turk Jour Biochem, 2018; 43(3): 323–332.
16. Nelson DL, Cox MM, Lehninger Principles of Biochemistry (7th ed.). New York, NY: W.H. Freeman and Company, 2017.
17. Nguyen TTM, Vu TTT, Tran TM, Le TT. Biotechnology Reports, 2022; 33: 00658.
18. Oshiro M, Shinto H, Tashiro Y, Miwa N, Sekiguchi T, Okamoto M, Ishizaki A, Sonomoto K, Bios Bioeng, 2009; 108: 376-384.
19. Osho MB, Sobande OE, Nigerian Journal of Biotechnology, 2019; 36(1): 186-193.
20. Perez-Morale G, Caspeta L, Merino E, Cevallos MA, Gosset G. Martinez A, Biotech Biof Biop., 2024; **17(**132): 2731-3654.
21. Singhvi M, Joshi D, Adsul M, Varma A, Gokhale D, Green Chemistry, 2010; 12: 1106-1109.
22. Sobrun Y, Bhaw-Luximon A, Jhurry D, Puchooa D, Advanced Bios Biotech, 2012; 3: 398–407.
23. Sun J, Qiu T, Liu H, Zhang W, Yu L, 2017.
24. Tashiro Y, Kaneko W, Sun Y, Shibata K, Inokuma K, Zendo T, Sonomoto K, Appl Microb Biotech, 2011; 9: 1741-1750.
25. Wang G, Ma M, Zhang Y, Liu L, Liu X, Jiang X. Jiang C, Appl Microb Biotech, 2018; 102(7): 3269-3281.
26. Wang Q, Lu Z, Du R, Xu Y, Zhang C, Appl Biochem Biotechnol, 2020; 190(1): 217-230
27. Yadav V, Kundu S, Sen R, A review. Proc Biochem, 2021; 111: 4-18.
28. Yoganand S, Archana B, Dhanjay J, Daneshwar P, Adv Bios Biotechno, 2012; 3: 398-407.
29. Zhao J, Zhang B, Meng, Y., Han Y, Wang Y, Jour Biotech, 2020; 10(5): 1-11.