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Optimization of acid production by *Pediococcus pentosaceus* through fermentation of various sugars

Tharshana,U Thavaranjit ,A.C

Department of Botany, Faculty of Science, University of Jaffna.

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ABSTRACT

Lactic acid bacteria (LAB), such as *Pediococcus pentosaceus*, play a crucial role in the fermentation of a wide range of food products utilizing carbohydrates as their primary carbon source. This study focused on enhancing acid production by *P. pentosaceus* through the use of various sugars as substrates. Fermentation was carried out using glucose, lactose, sucrose, and maltose, each at a concentration of 1%. The resulting acid production was measured by titration with 0.1 M NaOH and reported as a percentage of acid content. Statistical analysis, one-way ANOVA, $p \leq 0.05$ analysis, Tukey's test – indicated that lactose produced a significantly higher acid content compared to the other substrates. Optimization of fermentation parameters—including substrate concentration (1%, 3%, 5%, 7%, 9%, 11%, 13%), incubation temperature (10 °C, 30 °C, 37 °C, 44 °C, 60 °C), time (1 day, 2 days, 3 days, 4 days, 5 days, 6 days), NaCl concentration (0%, 0.1% , 0.3%, 0.5%, 0.7%, 0.9%) and pH (3, 4, 5, 6, 7, 8)—was performed using a one-factor-at-a-time approach. The optimal conditions for acid production were determined as 5% lactose, incubation at 37 °C for 48 hours, with 0.7% NaCl and a pH of 6. Under these conditions, acid production increased by 2.15 - fold compared to the initial values. This study emphasizes lactose as the most effective substrate for acid production by *P. pentosaceus* and determines the optimal fermentation conditions to enhance acid yield. These findings have the potential to advance the industrial utilization of *P. pentosaceus* in the food and fermentation sectors.

Keywords: Acid production, Lactose, Optimization, *Pediococcus pentosaceus,* Titration

1. INTRODUCTION

Lactic acid bacteria (LAB), such as *Pediococcus pentosaceus*, are essential microorganisms used in the fermentation of a wide variety of food products, including dairy, meat, and bakery items. These bacteria primarily derive energy through the fermentation of carbohydrates, producing organic acids as key metabolic by-products [1].

The genus *Pediococcus* is a gram positive, catalase-negative, facultatively aerobic and homofermentative *cocci* species that produces lactic acid as the major end product of glucose fermentation [2]. The efficiency of acid production depends on several factors, including the type of carbohydrate used, fermentation conditions, and the specific metabolic pathways of the microorganisms involved [3]. The common end products of bacterial fermentation include lactic acid, formic acid, acetic acid, butyric acid, acetone, ethyl alcohol, carbon dioxide and hydrogen [4].

While LAB can ferment various sugars like glucose, lactose, sucrose and maltose, the optimal conditions for maximizing acid production are not always well-defined. Understanding and optimizing these conditions is crucial for improving the yield of fermented products and enhancing industrial applications.

This study investigates the fermentation of different carbohydrates by *Pediococcus pentosaceus*, aiming to identify the most suitable substrate and optimize fermentation conditions for enhanced acid production. The findings are expected to significantly contribute to the development of more efficient fermentation strategies and broaden the industrial applications of LAB in food processing and biotechnology.

2. MATERIALS AND METHODOLOGY

2.1 Bacterial subculturing

Lactic acid bacteria were obtained from the culture collections (Stored at 4 ℃) in the Department of Botany, University of Jaffna, and streaked on a nutrient agar plate with 2% of lactose under aseptic conditions and incubated at 37 ℃ for 24 - 48 hours to obtain young cultures.

2.2 Identification of lactic acid bacteria

The lactic acid bacterium was subjected to Sanger DNA sequencing for molecular identification. The obtained nucleotide sequence was analyzed and compared with sequences available in the NCBI database using homology search tools. Based on the molecular sequence analysis and the high similarity observed with reference sequences, the isolate was identified as *Pediococcus pentosaceus*.

2.3 Standardization of lactic acid bacteria

Sterile saline solution (0.85%) was prepared. Bacterial culture was inoculated into a saline solution using a sterile inoculating loop. Then the inoculum was standardized using 0.5M Mc Farland reagent.

2.4 Preparation of fermentation broth

Bacteriological peptone (1%) and NaCl (0.5%) were dissolved in 100 mL of distilled water and dispensed into a 100 mL conical flask. Fermentation broths were prepared using 1% of substrate such as glucose, lactose, sucrose and maltose. The conical flasks were plugged using cotton wool and autoclaved at 121 ℃ at 15 lbs / inch^2 for 15-20 minutes.

2.5 Inoculation of lactic acid bacteria

1 mL of standardized bacterial inoculum was transferred into fermentation broths separately by using a sterile pipette and allowed to incubate at 37 ℃ for 24 - 48 hours.

2.6 Determination of acid production.

After the incubation, 25 mL of each fermentation broth with 3-5 drops of Phenolphthalein indicator was titrated against 0.1 M NaOH solution until the color of the solution turned pink. Each titration was repeated three times. The percentage of acid was calculated by using the following formula,

$$R = \frac{\{V_{(titr)} \times C_{(titr)} \times MW \times F \times 100\}}{1000 \times W_{(samp)}}$$

R = % of lactic acid
$V_{(titr)}$ = total volume of titrant needed to reach the endpoint in mL
$C_{(titr)}$ = Concentration of titrant
MW = Molecular weight of Lactic acid (90.08 g/mol)
F = dilution factor
$W_{(samp)}$ = sample amount in either gram or mL

2.7 Selection of the sugar that produced significantly higher acid

The following statistical analyses were performed to determine the sugar type that produced a significantly higher acid content.

a. One-way ANOVA
b. Significance testing at $p \leq 0.05$
c. Tukey's post-hoc test

2.8 Optimization of acid production

Lactose was selected as the most suitable substrate for acid production by *Pediococcus pentosaceus* due to its ability to yield significantly higher acid content. Optimization of acid production was conducted using a one-factor-at-a-time approach to evaluate the effects of various parameters. To study the effect of substrate concentration, fermentation broths containing lactose at concentrations of 1, 3, 5, 7, 9, 11, and 13 g/100 mL were prepared,

autoclaved at 121 °C under 15 lbs/in² pressure for 15–20 minutes, cooled, inoculated with 1 mL of standardized bacterial culture, and incubated at 37°C for one day. Acid production was quantified using a standard titration method. To evaluate the effect of incubation temperature, fermentation broths with 5% lactose were prepared similarly and incubated at 10 °C, 20 °C, 37 °C, 44 °C, and 60 °C for one day. For the effect of the incubation period, 5% lactose broths were inoculated and incubated at 37 °C for 1 to 5 days, with acid production measured daily. The effect of NaCl concentration was studied using broths containing 5% lactose and varying NaCl concentrations (0%, 0.1%, 0.3%, 0.5%, 0.7%, and 0.9%), following incubation at 37°C for 2 days, where optimum acid production was observed. Lastly, to assess the influence of pH, broths with 5% lactose and 0.3% NaCl were adjusted to pH 4, 5, 6, 7, and 8 before sterilization, inoculation, and incubation at 37 °C for 2 days. In all experiments, acid production was measured using the standard titration method. All titrations were conducted in triplicate to ensure accuracy and reproducibility. One-way ANOVA, $p \leq 0.05$ analysis and Tukey's test statistical analyses were performed for each tested parameter.

3. RESULTS AND DISCUSSION

3.1 Different sugar fermentation and acid production by *Pediococcus pentosaceus*

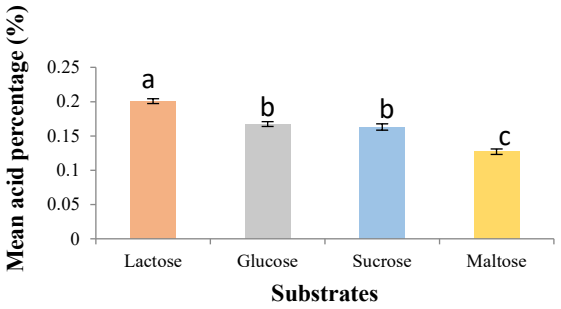

Figure 1. Acid content (%) in different sugar fermentation by *Pediococcus pentosaceus*. Different letters (a,b,c) denote a significant difference between the mean values.

Pediococcus pentosaceus can ferment various carbohydrates including glucose, lactose, maltose and sucrose. All the carbohydrates produced considerable amount of acid. Based on the statistical analysis, there was a significant difference in acid production among the four different substrates. Lactose produced a significantly high level of acid content (0.2006%) whereas maltose produced a significantly lower amount of acid content (0.1270%). Based on Turkey's method for multiple comparisons with a 95% confidence interval, it indicated that glucose and sucrose belong to the same group. There was no significant difference in acid production among glucose and sucrose. In lactose and maltose, there was a significant different in the production of acids.

Sucrose had the largest standard deviation (S=0.0045%). Therefore, the distribution is larger and the data more variable than the mean. Lactose and glucose had the smallest standard deviation (S= 0.0035%). It indicates the low distribution and the data are clumped around the mean. As lactose produced highest amount of acid content, it was selected as the substrate for optimization.

3.2 Optimization of the production of acids

3.2.1 Effect of substrate concentration on acid production

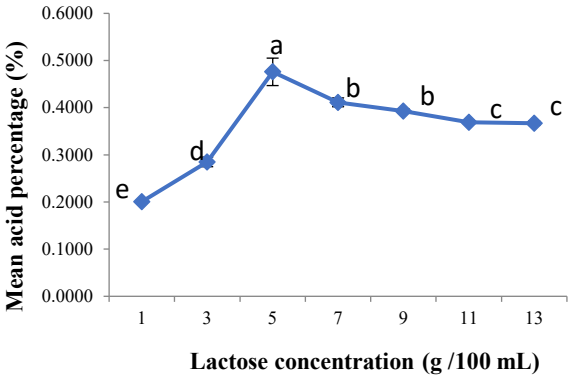

Figure 2. Effect of Lactose concentration on acid production of *Pediococcus pentosaceus.* Different letters (a,b,c,d,e) denote a significant difference between the mean values.

In *Pediococcus pentosaceus,* the highest acid content was obtained at 5% of lactose (0.4760%). The acid concentration was increased from 1% to 5% lactose concentration (W/V) and then it was slightly decreased. Based on the one-way ANOVA ($p \leq 0.05$), there was a significant difference in acid production among the different

lactose concentrations. As indicated by Tukey's test, 5 g/100 mL lactose produced the highest acid content among the tested concentrations, which was selected for further studies.

When the lactose concentration is low, the reaction rate increases proportionally with increasing lactose concentration. When the concentration of substrate reaches the optimum level, the enzyme becomes saturated with substrate, which prevents further increase of acid production and results in a slight reduction of acid production due to osmotic stress. The excess substrate can create osmotic imbalances, making it difficult for microorganisms to function effectively [5].

3.2.2 Effect of incubation temperature on acid production

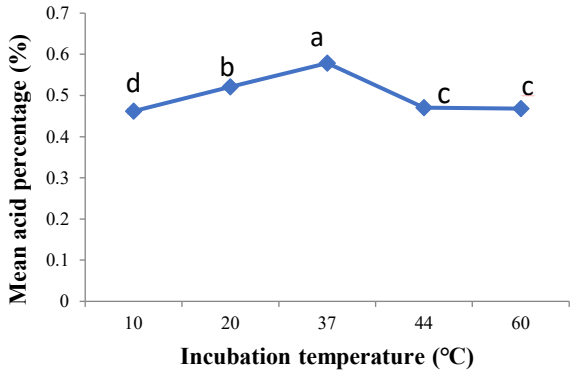

Figure 3. Effect of incubation temperature on acid production of *Pediococcus pentosaceus.* Different letters (a,b,c,d) denote a significant difference between the mean values. (Optimized lactose concentration – 5 g/ 100 mL)

Pediococcus pentosaceus showed significantly higher acid content (0.5786%) at 37 °C than at the other tested temperatures. Acid production increased as the temperature rose from 10 °C to 37 °C, but declined beyond 37 °C. This trend reflects the general principle that metabolic reaction rates are accelerated with increasing temperature up to an optimal point, after which they decrease due to thermal inhibition of enzymatic activity. Extremely high temperatures can denature an enzyme, causing it to lose its form and cease to function [6].

Temperature is also one of the important factors, influences the activity of metabolic cell enzymes, with each enzyme exhibiting maximum activity at its optimum temperature. In a previous study investigating [6] the optimum temperature for lactic acid production, whey was incorporated into the medium and the inoculated cultures were incubated at temperatures ranging from 25 to 50 °C. Lactic acid production increased significantly from 25 °C to 37 °C, reaching a maximum of 43.6 g/L at 37 °C. However, production declined to 34.2 g/L at 45 °C and further decreased to 20.2 g/L at 50 °C. At the optimum temperature, enzymatic reactions achieve their maximum velocity, whereas temperatures below or above this point slow the reaction rate, thereby affecting the overall cellular metabolic processes [6].

In this study, acid production increased as the temperature rose from 10 to 37 °C, with the maximum yield observed at 37 °C using 5% lactose. Beyond this point, acid production declined, reaching its lowest level at 60 °C. Therefore, 37 °C was selected as the optimal temperature for further optimization.

3.2.3 Effect of incubation time on acid production

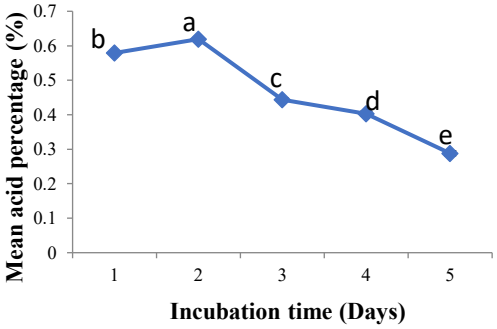

Figure 4. Effect of incubation time on acid production of *Pediococcus pentosaceus.* Different letters (a,b,c,d,e) denote a significant difference between the mean values.

Incubation time is a key factor influencing bacterial metabolic activity [7]. The highest acid content (0.6193%) was achieved by *Pediococcus pentosaceus* after 2 days of incubation at 37 °C with 5% lactose. One-way ANOVA ($p \leq 0.05$) revealed a significant difference in acid production among the different incubation periods. Acid content increased up to 2 days with 5% lactose,

after which it declined with further extension of incubation time. This is due to the growth of the culture entering to the stationary phase and, as a consequence, slowing down the metabolic activity of bacteria. Based on the results, two days incubation period was chosen for further optimization studies.

Incubation time has a significant impact on lactic acid production, with variations in duration directly affecting the acid production. Generally, extending the incubation period increases lactic acid yield as it allows more time for lactic acid bacteria to convert sugars, like lactose, into lactic acid. However, excessive incubation can lead to a decline in lactic acid production due to nutrient depletion and the accumulation of metabolic by-products, which inhibit the metabolic activity of bacteria [8].

An earlier study [8], evaluated the influence of incubation time on the physicochemical and microbial properties of yoghurt formulated with three starter cultures—*Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus*—and stabilized with taro (*Colocasia esculenta*) starch. The findings demonstrated that a 36-hour incubation period resulted in optimal yoghurt quality when using these cultures in combination with locally sourced taro starch from Indonesia [8].

3.2.4 Effect of NaCl concentration on acid production

In *Pediococcus pentosaceus*, NaCl concentration of 0.7% produced the highest acid content (0.6286%) when cultured with 5% lactose at 37 °C for 2 days. One-way ANOVA ($p \leq 0.05$) revealed a significant difference in acid production among the tested NaCl concentrations. According to Tukey's test, 0.7 g/100 mL NaCl was selected for further studies, as it yielded the highest mean value compared to 0.5 g/100 mL.

The physicochemical characteristics and quality of spontaneously fermented cabbage (*Brassica oleracea* L.) were evaluated at five NaCl concentrations (1.0%, 2%, 3%, 4%, and 5% w/w), with 0% (w/w) NaCl serving as the control, over a four-week storage period.

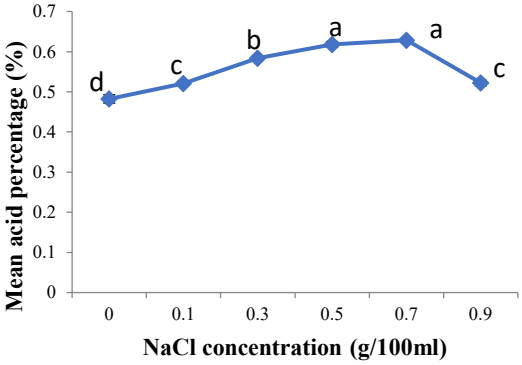

Figure 5. Effect of NaCl concentration on acid production of *Pediococcus pentosaceus.* Different alphabets (a,b,c,d) denote significant difference between the mean values.

At the end of the study, microbiological analysis and sensory evaluation revealed that treatments with 2–3% NaCl exhibited significantly higher overall acceptability in terms of color, aroma, and taste [9].

3.2.5 Effect of pH on acid production

In *Pediococcus pentosaceus*, the highest acid content (0.6310%) was observed at pH 6 with 5% of lactose and 0.7% of NaCl incubated at 37 °C for 2 days, after which it began to decline. This indicates that the optimal pH for acid production is species dependent.

One-way ANOVA ($p \leq 0.05$) revealed a significant difference in acid production among the tested pHs. An earlier study investigated the effect of pH on lactic acid (LA) production from food waste (FW) using three anaerobic inocula and a control containing only FW. The optimal condition for LA production was observed at pH < 4. Another study confirmed that reactor pH is a critical factor in promoting lactic acid fermentation and its accumulation. Under acidic conditions, LA accumulated rather than being converted to volatile fatty acids (VFAs), due to inhibition of the microbial community responsible for VFA production [10].

Effect of acidic pH (4, 5, 6 and uncontrolled) on lactic acid (LA) fermentation from food waste was investigated by batch fermentation experiments using methanogenic sludge, fresh food waste and anaerobic activated sludge as inocula.

Results showed that due to the increase of hydrolysis, substrate degradation rate and enzyme activity, the optimal LA concentration and yield were obtained at pH 5 [11].

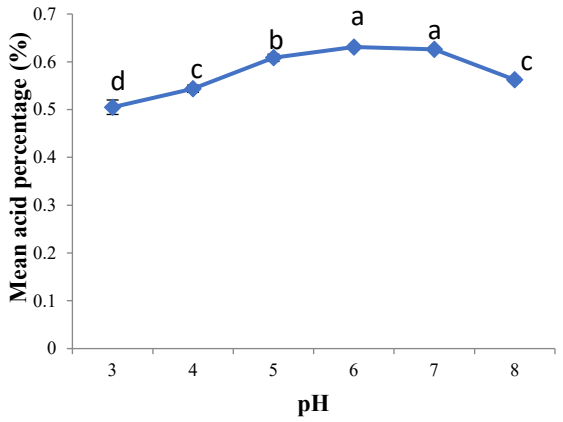

Figure 6. Effect of pH on acid production of *Pediococcus pentosaceus*. Different letters (a,b,c,d) denote a significant difference between the mean values.

In bio-wastes, pH adjustment had a significant effect on lactic acid production ($p < 0.01$). The impact of pH regulation without the addition of exogenous strains was examined, and results indicated that adjusting the pH at the onset of fermentation could sustain high lactic acid production (16.59 ± 0.4 g/L), suggesting that indigenous microorganisms are favored at a pH of 6.2 [12].

4. CONCLUSION

Glucose, lactose, maltose and sucrose produced a significant amount of acid during the fermentation. The highest acid content (0.2006%) was obtained using lactose as a substrate by *Pediococcus pentosaceus*. The optimal conditions for the acid production (0.6310%) by *P. pentosaceus* were obtained as 5% Lactose as substrate, incubation at 37°C for 48hours with 0.7% NaCl and a pH of 6. After optimization the acid content was increased by 2.15 – fold compared to the initial values. These findings can contribute to improving industrial applications of *P. pentosaceus* in food and fermentation industries.

5. REFERENCES

1. Gunkova, P & Buchilina, A & Maksimiuk, N & Bazarnova, Y & Girel, K. (2021). Carbohydrate Fermentation Test of Lactic Acid Starter Cultures. IOP Conference Series: *Earth and Environmental Science.* 852. 012035. 10.1088/1755-1315/852/1/012035.
2. Franz CM, Endo A, Abriouel H, Reenen CA, Gálvez A, Dicks LM. The genus Pediococcus. Lactic acid bacteria: Biodiversity and taxonomy. 2014 Apr 30:359-76.
3. Abedi E, Hashemi SMB. Lactic acid production - producing microorganisms and substrates sources-state of art. *Heliyon.* 2020 Oct 12;6(10):e04974. doi:10.1016/j.helyon.2020.e04974.
4. Reiner, K.(2012). Carbohydrate Fermentation Protocol, *American Society for Microbiology, 2012;11:12.*
5. Mussatto, Solange & Teixeira, José. (2010). Lignocellulose as raw material in fermentation processes, In *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, 11, 897-907.
6. Sarkar, Diptendu. (2019). A study on optimization of lactic acid production from whey by *Lactobacillus* sp. isolated from curd sample, 5:816-24.
7. Laure NN, Dawan J, Ahn J. Effects of Incubation Time and Inoculation Level on the Stabilities of Bacteriostatic and Bactericidal Antibiotics against *Salmonella* Typhimurium. Antibiotics (Basel). 2021 Aug 22; 10(8):1019. doi: 10.3390/antibiotics10081019.
8. Krisnaningsih A. T, Radiati L. E, Purwadi, Evanuarini H, Rosyidi D. The Effect of Incubation Time to The Physicochemical and Microbial Properties of Yoghurt with Local Taro (*Colocasia Esculenta*(L.) Schott) Starch as Stabilizer. *Curr Res Nutr Food Sci* 2019; 7(2).
9. Premakumar, Kanagarajah & Sahana, Srithara & Sabrana, M. (2021). Effects of Salt Concentration on Storage Ability of Sauerkraut. *International Journal of Agricultural Sciences.* 7(2):11-6.
10. Pau, Simone & Tan, Lea & Lens, P.N.L. (2021). Effect of pH on lactic acid fermentation of food waste using different mixed

culture inocula. *Journal of Chemical Technology & Biotechnology.* 97. 10.1002/jctb.6982.

11. Tang J, Wang XC, Hu Y, Zhang Y, Li Y. Effect of pH on lactic acid production from acidogenic fermentation of food waste with different types of inocula. *Bioresour Technol*. 2017 Jan;224:544-552.
12. Tsapekos Panagiotis, Alvarado-Morales Merlin, Baladi Samin, Bosma Elleke F., Angelidaki Irini, Fermentative Production of Lactic Acid as a Sustainable Approach to Valorize Household Bio-Waste, *Frontiers in Sustainability,* 2020, 1:4.