Trop J Nat Prod Res, December 2023; 7(12):5699-5709

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Effects of Some Physicochemical Conditions on the Growth and Histamine Production by *Enterococcus* Isolates from Fermented *Pentaclethra macrophylla* (Oil Beans) in Nsukka, Nigeria

James N. Ezema[1*],Olaedo E. I. Imanyikwa[1], Emmanuel A. Eze[2]

[1]*Department of Medical Microbiology, Faculty of Basic Clinical Sciences, College of Medicine, Enugu State University of Science and Technology, Enugu, Nigeria.*
[2]*Department of Microbiology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.*

ARTICLE INFO

Article history:
Received 05 October2023
Revised 07 November2023
Accepted 30 November2023
Published online 01 January 2024

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ABSTRACT

Enterococci are among the most common bacterial contaminants of fermented oil beans. Their activities result in undesired products such as histamine, the accumulation of which leads to food poisoning. This research aimed to determine the effects of physicochemical environments for growth and histamine production by *Enterococcus* species and ways of preventing histamine accumulation through modification of such environments. Histamine-producing *Enterococcus* species isolated from fermented oil beans were cultured in Niven's broths with different pH and Sodium Chloride concentrations at different temperatures. Growth rates in each broth were determined by direct counts of bacterial cells. Histamine productions were measured indirectly by measuring pH changes with time in each broth. Statistical significances of the effects of these parameters were determined at 95% confidence interval. All the species grew at different pH used, with optimum growth observed between pH of 5 and 6. Histamine production was favoured by lower acidic pH. Different growth rates were recorded at different temperatures with optimum growth occurring at about 37°C. Growths were more rapid at Sodium Chloride concentrations of 0.5% and 1% and slowed down as concentration increased to 5% and 10%. These growth rates also had direct correlations with histamine production, with highest levels of histamine detected in the media with highest bacterial counts. The activation of histidine decarboxylase gene was a function of pH. The resulting enzyme activities were influenced by temperature and pH while bacterial biomass determined the enzyme concentration. Therefore, growth and histamine production were influenced by the interdependence of these biological factors.

Keywords: Oil bean seed, *Enterococcus*, Fermentation, Histamine production, Physicochemical environments

Introduction

Fermented Oil bean seed is an indigenous delicacy in many parts of Eastern Nigeria due to its nutritional and organoleptic values. The nutritional qualities improve due to the activities of microbial fermenters, which break down its undigestible and antinutritional contents.[1,2] Enterococci are among the most common bacterial contaminants of oil bean seeds. They are ubiquitous in the environments as inhabitants of soils, water, plants, as well as commensals in the intestines of humans, animals and insects. [3, 4, 5]Although certain species have continued to gain prominence as the major cause of nosocomial infections, causing such diseases as urinary tract infections, bacteraemia, intra-abdominal infections, and endocarditis,[6-10] many species are known probiotics that are involved in the fermentation of dairy products and traditionally fermented foods, adapting to several food environment, especially as non starter fermenters.[11,12]

*Corresponding author. E mail: nnabuike.ezema@esut.edu.ng
Tel: 08067587489

Citation: Ezema JN, Imanyikwa OEI, Eze EA. Effects of Some Physicochemical Conditions on The Growth and Histamine Production by *Enterococcus* Isolates from Fermented*Pentaclethramacrophylla* (Oil Beans) in Nsukka, Nigeria.. Trop J Nat Prod Res. 2023; 7(12):5699-5709. http://www.doi.org/10.26538/tjnpr/v7i12.49

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Enterococcihave been widely implicated in fermentation and spoilage of various foods, carrying out proteolitic and lypolytic activities, and producing high levels of lactic acid and biogenic amines.[13-15] Fermentation of traditional condiments involves both starter probiotics and environmental contaminants.[16, 17] In addition to lactic acid production, various kinds of biogenic amines could be produced depending on the amino acid contents of the food. Histamine production is as a result of decarboxylation of histidine in the food, and this is controlled by two major factors: the presence of histidine decarboxylase *(hdc)* gene, and food environments including pH, temperature and salt concentration.[18,-21] Accumulation of histamine in foods often leads to condition generally referred to as histamine intolerance. This manifests as cough, respiratory distress, asthma, sneezing, rhinorrhea, nasal obstruction and phlegm, hives, itching, redness, pruritis, urticaria, tongue swellings, dysmenorrheal etc. [22] These symptoms result from excessive absorption of histamine accumulated in the food during fermentation. This research aimed to investigate the effects physicochemical environments on growths and histamine production by the organism, and possible ways of preventing histamine accumulation and its consequent health implication through modification of such environments. The steps and parameters used reflect the natural course of local oil bean processing in the study area such that the results will serve as a valuable guide towards standardizing such processing.

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Materials and Methods

Materials:MRS broth (Titma Biotech, India), Glycerol (IDH, China), Nutrient agar (IDH, China), Niven's agar (Oxoid, United Kingdom), Conc. Hydrochloric acid (Sigma Aldrich, Germany), Conc. Sodium hydroxide (Molychem, India), Distilled water (Lion Tablw Water, UNN), 95% Ethanol (Sigma Aldrich, Germany), Buffer solution,1:1(Labochem, India), pH meter (Hanna, Italy), Glass rod (Pyrex, England), Micropipette (Alpha Surgicare, China), Petri dishes (Alpha Surgicare, China), Cotton wool (Alpha Surgicare, China), Bijou bottles (Lab Tech, India), Litmus paper (Whatman, England). The stock organisms were species of *Enterococcus* from Microbiology laboratory, Faculty of Biological Sciences, UNN, previously isolated from fermented Oil bean seeds and confirmed for histamine production. They include *E. faecalis, E. faecium, E. gallinarum*and *E. gilvus.* The isolates were preserved in a cryoprotectant containing De Man Rogosa and Sharpe and glycerol (80%MRS broth/20% Glycerol) and stored in the freezer. The viabilities of the organisms were tested by plate count before use and they grew viably on MRS agar and tested for histamine production on Niven's agar (Appendix IV).

Modification of Niven's broth to obtain various pH and NaCl concentrations

A standard Niven's broth was prepared in replicates and each modifiedto reflect differences in pH and Sodium Chloride concentrations, [23] as shown in Table 1.

Histamine production assay at different initial pH

From the stock cultures, 0.5 ml of each of the cell suspension was transferred into MRS broth and incubated for 48 h at 37°C to revive the cells. About 0.1 mL of each of the sub-cultures was then transferred into duplicate bottles containing 20 mL of Niven's broth with initial pH of 4. The broth was incubated for at 37°C for six days. The pH of the broth was tested with pH meter at 24 h intervals to ascertain the level of histamine produced with time [23] which corresponds to the quantity of histamine produced in the broth with time, since histidine was the only amino acid in the growth medium and the only source of alkaline substance. The alkanility levels correspond with the quantity of histamine produced. The procedure was repeated in broths with different initial pH of 5, 6 and 7 as modified in Table 1.

Histamine production assay at different temperatures

From the stock cultures, 0.5 ml of each of the cell suspension was transferred into MRS broth and incubated for 48 h at 37°C to revive the cells. About 0.1 mL of each of the sub-cultures was then transferred into duplicate bottles containing 20mL of Niven's broth (pH of 5.3). The broths were incubated at 8°C for a period of six days. The pH of the culture was tested using pH meter at intervals of 24 h (ie 24, 48, 72, 96, 120 and 144 h) post-inoculation. [23] This was to ascertain the alkalinity levels of the medium which corresponds with the quantity of histamine produced in the broth with time since histidine was the only amino acid in the growth medium and the only source of alkaline substance. This procedure was repeated with incubation temperatures of 25°C, 37°C and 42°C.

Histamine production assay at different NaCl concentrations

From the stock cultures, 0.5 mL of each of the cell suspension was transferred into MRS broth and incubated for 48 h at 37°C to revive the cells. 0.1 ml of each of the sub-cultures was then transferred into duplicate bottles containing 20mL of Niven's broth with 0.5% NaCl concentration. The broth was incubated at 37°C for six days. The pH of the broth was tested with pH meter at 24 h intervals to ascertain the level of histamine produced with time [23]. The alkalinity levels correspond with the quantity of histamine produced. The procedure was repeated with broths containing 1%, 5% and 10% sodium chloride concentrations as modified in Table 1.

Determination of growth rates of the organisms at different physicochemical conditions

From the cell suspension of the revived organisms, 0.1 mL each of the broths was transferred to 0.9ml MRS broth to obtain a 10-fold dilution. Then 0.1ml of the dilution was transferred to duplicate MRS agar, spread with glass rod and incubated at 37°C. Viable counts of the cells were carried out by direct plate count of colony-forming units (cfu) at 24 h intervals to determine the corresponding cell growth rates.[24]

Statistical analysis

The effects of pH, temperature and salt concentration on growth and histamine production by the organisms were analyzed using univariate analysis of variance (ANOVA). The correlation between bacterial growths and histamine productions were analyzed using Pearson's correlation model.

Table 1: Modification of pH values and NaCl concentrations of the Niven's broth

Standard Niven's Broth		**pH modification**				**NaCl conc. modification**			
		A	B	C	D	A	B	C	D
Bacto-Tryptone	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Yeast Extract	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
L- histidine	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Sodium Chloride	0.5	0.5	0.5	0.5	0.5	0.5	1.0	5.0	10
Calcium carbonate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Bromocresol purple	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006
pH	5.3	4	5	6	7	5.3	5.3	5.3	5.3

Bold numbers show modified values

Results and Discussion

The effects of histidine and histidine decarboxylase gene on the growth and histamine production by non histamine-producing *Enterococcus* specie

The result shows a normal growth curve with little or no lag phase (Figure 1a). There was a steep exponential growth between the 2nd and the 3rd day post-inoculation. A near stationary phase was observed from 3rd to 4th day, followed by a decline phase for the next 48h. There was no production of histamine by this specie for the period. This was indicated by decline in pH level (Figure 1b). The first 24 h witnessed a uniform pH value. However, this was followed by a gentle and steady decline in pH for 48 h. A steady pH was observed again between 3rd and 4th day, which was followed by a sharp decline in pH values from 4th to 6th day post-inoculation. The presence or absence of histidine decarboxylase (hdc) gene in the cells as well as presence of histidine in the growth media were the major determinant of histamine production.[26] Non histamine-producing *Enterococcus* specie grew well in the histidine-rich medium but could not produce histamine (Figure 1b). This is obviously due to the absence of *hdc* gene in the cell which is responsible for histidine decarboxylation.[24]Consequently, the pH of the medium decreased due probably to production of lactic acid.

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ISSN 2616-0684 (Print)
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Growth and histamine production of histamine-producing enterococcus strains in histidine-negative medium

There were normal growths of all the representative strains used in the growth assay when grown in histidine-negative medium. *E. faecium* recorded the highest count while *E. gallinarum* showed the lowest growth. Despite the absence of amino acid in the medium the organism could be sustained for up to a week from other nutrients (Figure 2a). There was no histamine production by any of the organisms as the average changes in pH, as can be observed in Figure 2b, are all negative. *Enterococcus faecalis* recorded the highest net negative value in pH while *E. gallinarum* recorded the least. There were drastic drop in pH in the growth media within the first 48 h. Thereafter, fluctuations were observed. Histamine-producing species grew well in the medium lacking histidine due to its ability to utilize other nitrogen sources but could not produce histamine due to absence of the requisite raw (histidine) material for histamine production.

Effects of initial pH on the growth and histamine production of Enterococcus isolates

The *Enterococcus* isolates were found to grow across different pH. The most favourable pH varied from one specie to another. However, the growths were favoured as pH increased in all the species. In all the pH values, there were up to 24 h of lag phases followed by exponential phases that climaxed at about the third or fourth day. While growth balance was fairly maintained in pH of 5 for up to 5th day, a sharp decline was seen in other pH from the fifth day (Figures 3a, 4a, 5a, and 6a). Histamine productions, as indicated by changes in pH, were activated faster at lower initial pH. The production level decreased as initial pH increased.

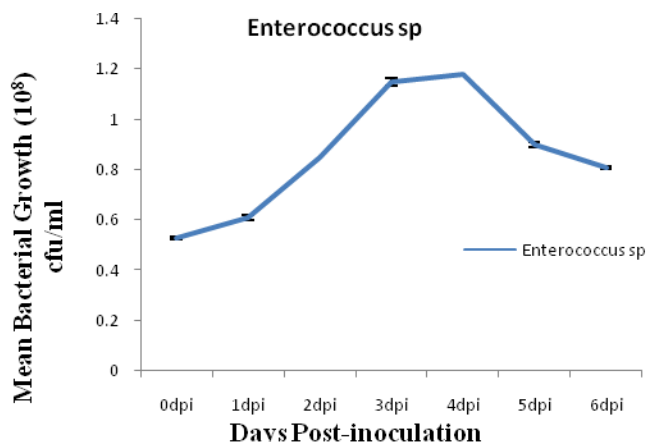

Figure 1a: Growth of Non Histamine-producing *Enterococcus* sp in a standard Niven's Broth (Positive Control).
Key:cfu = Colony forming unit, dpi = Days Post-inoculation

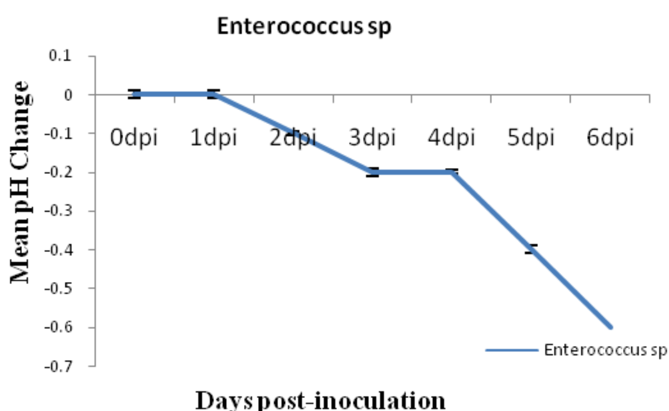

Figure 1b:pH Change in standard Niven's broth growing Non Histamine-producing *Enterococcus* specie (Control).
Key: dpi = days post-inoculation

The histamine level at a particular initial pH also increased steadily with time except at neutral pH (pH of 7) where the histamine level fluctuated with time. It was also observed that all the organisms took up to 24 h or more to initiate histamine production at all pH levels except pH of 4 where it commenced immediately after inoculation (Figures 3b, 4b, 5b and 6b).

Initial pH of growth media played significant roles in the bacterial growths (Figures 3a, 4a, 5a & 6a). All the species adapted to different pH values, with higher bacterial counts at acidic than at neutral pH. This seems to represent the natural environment from where the species are mostly isolated. The mechanisms of adaptation of *Enterococcus* species in an acidic environment have been attributed to several physiologic factors including its ability to synthesize amines from amino acids to counteract acid stress. [27, 28]The longer lag phases observed at lower pH values were due to initial acid stress experienced by the cells before it was circumvented, as indicated by the rapid growth after about 48 h. [29] The readiness by the cells to produce histamine increased with decreasing pH values. This followed from the fact that the *hdc* enzyme responsible for histamine production is activated by acidic pH, with optimum enzyme activity around pH of less than 4.8-6.0.[30] However, cells in the media with higher pH values commenced histamine production in the subsequent days due to the synthesis of lactic acid by the cells which brings the pH of the media low enough to activate *hdc* enzyme (Figures 3b, 4b, 5b, & 6b).

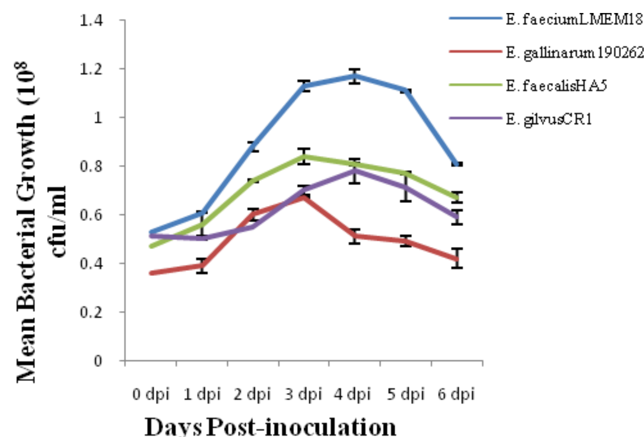

Figure 2a: Growth of *Enterococcus* in a standard Niven's Broth lacking histidine (Control).
Key:cfu = Colony forming unit, dpi = Days Post-inoculation

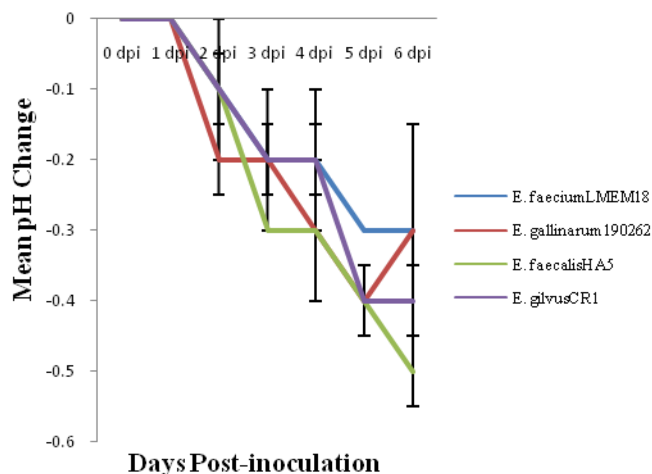

Figure 2b:pH Change in standard Niven's broth lacking histidine (Control).
Key: dpi = days post-inoculation

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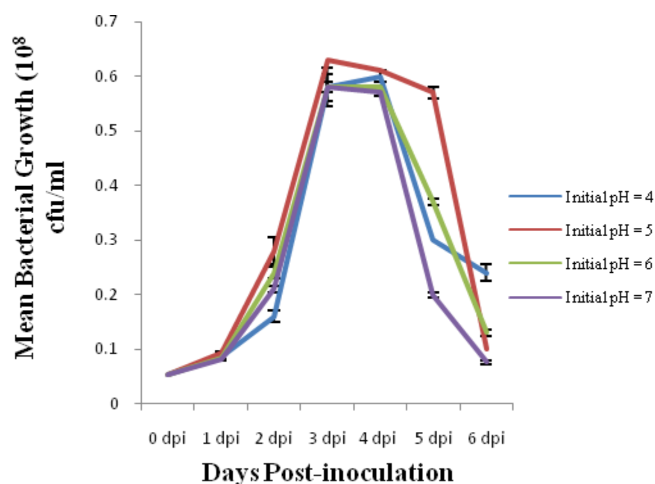

Figure 3a: Effects of Initial pH on the Growth of *Enterococcus faecium*
Key:cfu = Colony forming unit, dpi = Days Post-inoculation

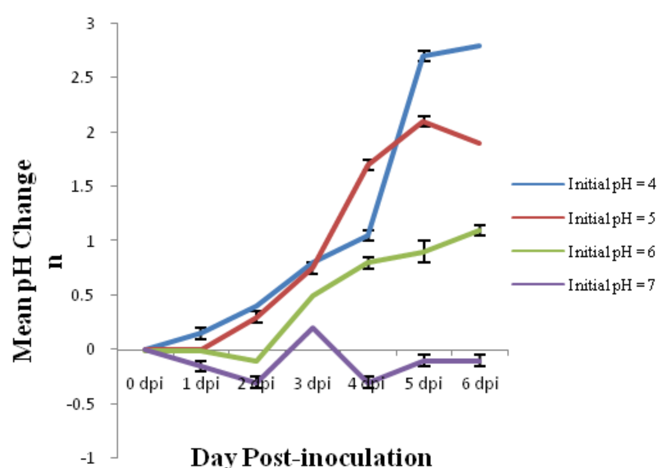

Figure 3b: Effect of Initial pH on Histamine Production by *Enterococcus faecium*LMEM18
Key: dpi = days post-inoculation

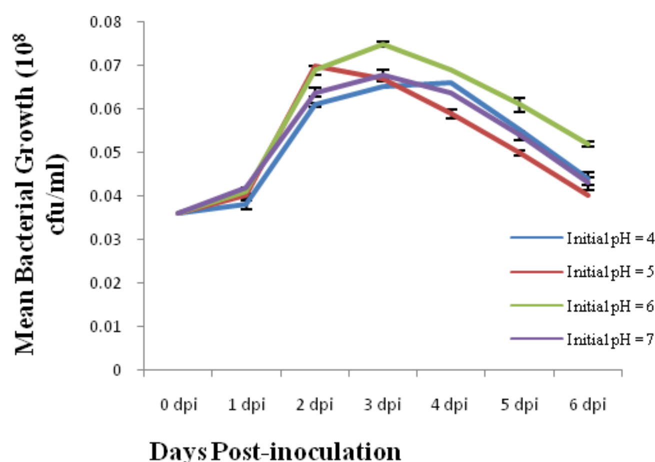

Figure 4a: Effects of Initial pH on the Growth of *Enterococcus gallinarum.*
Key:cfu = Colony forming unit, dpi = Days Post-inoculation

Effects of temperature on the growth and histamine production by enterococcus species

The effect of temperature on the growth of *E. faecium* is shown in Fig. 6(a). Temperatures of 10°C, 25°C, 37°C and 42°C were selected to represent processing and storage conditions of Oil bean seed. These temperatures supported the growth of *Enterococcus* species but at different rates. The optimum growth temperature for all the *Enterococcus* species was 37°C. The organisms also grew well at 25°C and 42°C, the effects of which were almost on the growths of all the species. Growths occurred at 10°C but at a very slow rate. The maximum growth at all temperatures was seen at about the third day, followed by a steady decline (Figures 7a, 8a, 9a, 10a). Temperatures of 37°c and 10°C yielded the highest level of histamine in all the organisms with fluctuations, especially from the third day of incubation. The highest histamine level was attained at a temperature of 37°C while the lowest was seen at 25°C (Figures 7b, 8b, 9b, 10b).

Enzyme mediated metabolic activities are largely temperature-dependent. All the species were observed to grow in a wide range of temperatures but at a remarkably different rate (Figures 7a, 8a, 9a and 10a). The temperature values used: 10, 25, 37 and 42°C represent the processing and storage temperatures of oil bean seed from where the bacteria were isolated. At 10°C, which represents refrigeration temperature, growth rates were very slow due to reduced enzyme activities. The extracellular activities of any secreted enzyme could be going on but at much slower rate too. This temperature is for post-fermentation storage which does not permit a serious metabolic activities and further fermentation, as the enzyme activities are already stalled. The growth rates of the bacteria were also assayed at 25°C, representing the natural indoor storage temperature of the fermented oil bean seed. There were significant growths at this temperature; hence fermentation goes on appreciably at this temperature. Enzymatic activities for both growth and metabolism are more at this temperature than at 10°C. Both intracellular and extracellular digestions of nutrients for growth are more at 25°C than at the refrigeration temperature, thus there were increased growth rates. Optimum growth rates were observed at 37°C. This is the temperature for peak enzyme activities. The log phases were faster at this temperature, yielding the overall highest bacterial. Steady decline in growth rates were later observed due to nutrient depletion and waste intoxication. At 42°C, there were decreases in the growth rates of the organisms. These were probably due to reduced enzyme activities following temperature distortion. However, there was obvious better adaptation of *E. gilvus* to lower temperature than other *Enterococcus species*. The reason for this is not clear but it is believed to be due to other factors not yet known.

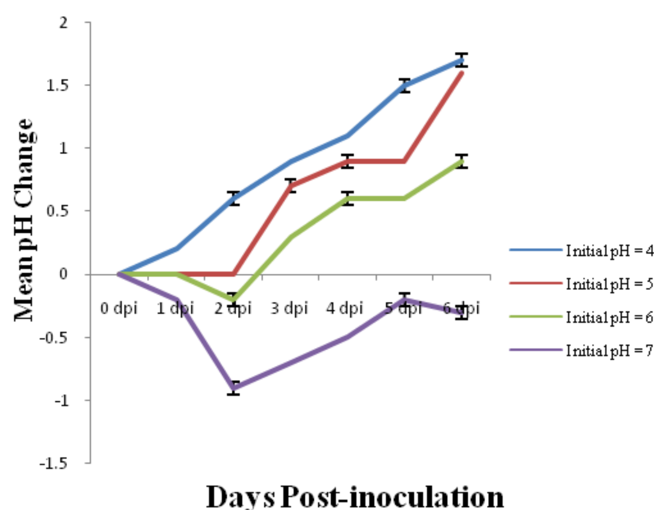

Figure 4b: Effect of Initial pH on Histamine Production by *Enterococcus gallinarum.*
Key: dpi = days post-inoculation

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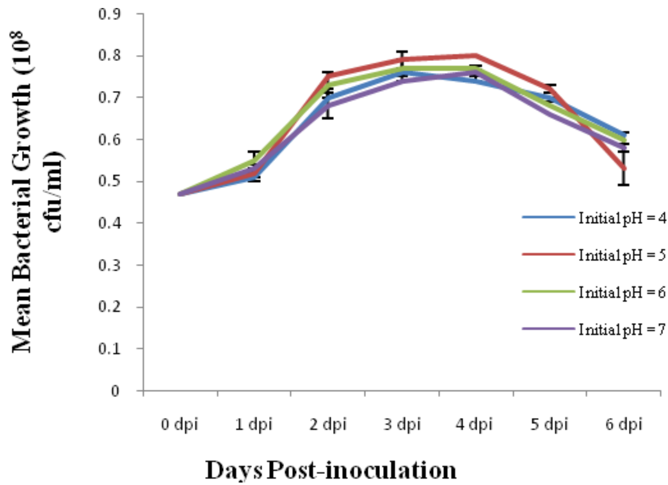

Figure 5a: Effects of Initial pH on the Growth of *Enterococcus faecalis*.
Key:cfu = Colony forming unit, dpi = Days Post-inoculation

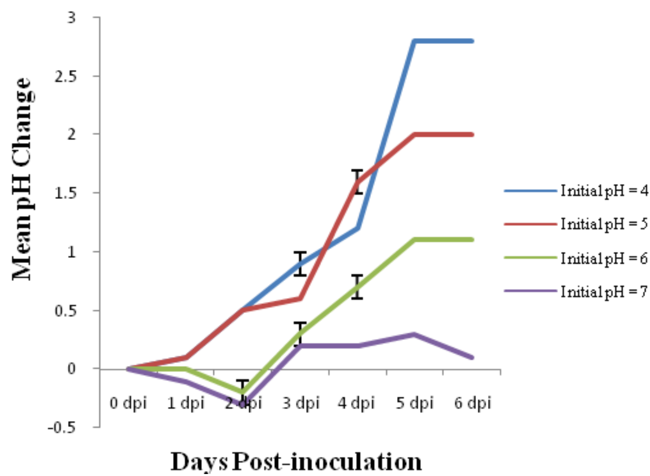

Figure 5b: Effect of Initial pH on the Production of Histamine by *Enterococcus faecalis*.
Key: dpi = days post-inoculation

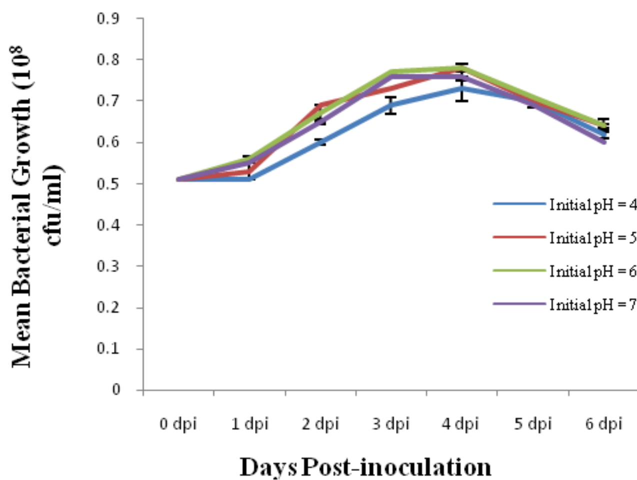

Figure 6a: Effects of Initial pH on the Growth of *Enterococcus gilvus.*
Key:cfu = Colony forming unit, dpi = Days Post-inoculation

Histamine production by lactic acid bacteria including the *Enterococci* is mediated by *hdc* enzyme. It is therefore expected that histamine levels at all times would correspond with temperatures to reflect the enzyme activities, with the highest histamine level occurring at the optimum temperature for enzyme activities. This expected trend was not consistently observed among these isolates. All the species began histamine production at all temperatures from the point of inoculation reaching peak levels at different times (7b, 8b, 9b &10b). The highest level of histamine was produced at 37°C. The histamine levels at different temperatures do not follow a sequential pattern, giving the impression that it is not temperature-dependent as against the reports on how temperature had affected histamine production in some spoilage organisms.[31, 32] The histamine levels dwindle with time throughout the period probably due to the neutralizing effect of lactic acid being produced in the media at the same time. This may have played a more important role than temperature in histamine production by the isolates.

Effects of Sodium Chloride concentration on the growth and histamine production by Enterococcus species

The cells grew best at 0.5% and 1% NaCl with little or no lag phase and with 1% NaCl concentration yielding the highest bacterial counts. As the salt concentration becomes higher at 5% and 10%, there was initial cell death before growth commences.

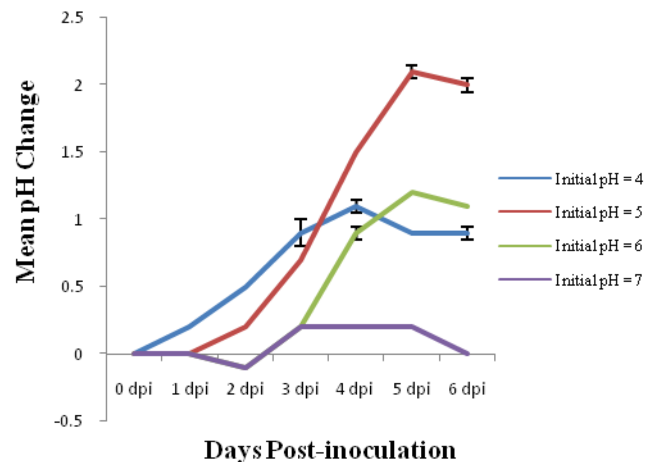

Figure 6b: Effect of Initial pH on the of Histamine Production by *Enterococcus gilvus*.
Key: dpi = days post-inoculation

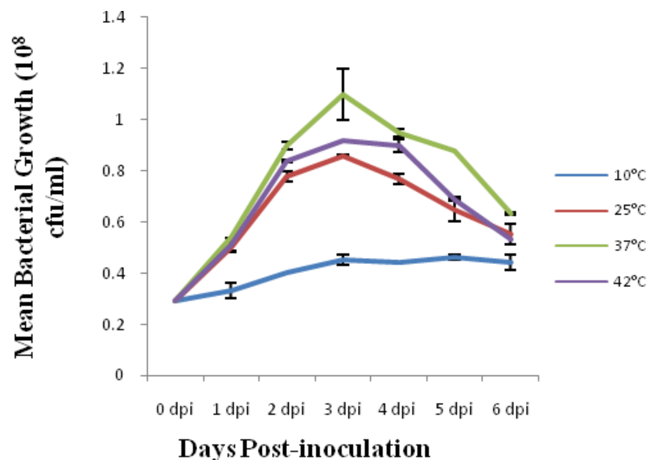

Figure 7a: Effects of Temperature on the Growth of *Enterococcus faecium*L.
Key: cfu= Colony forming unit, dpi = days post-inoculation

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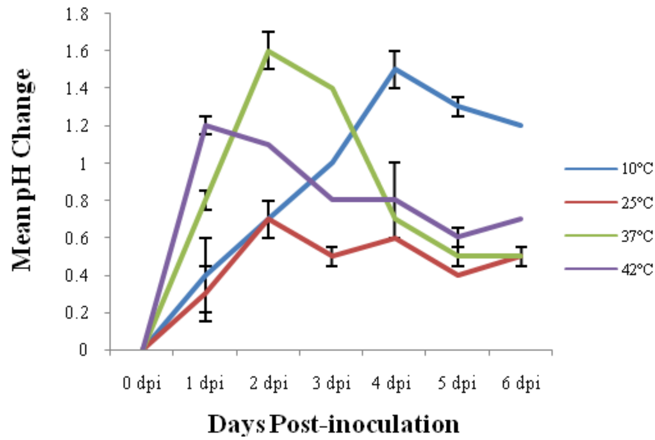

Figure 7b: Effect of Temperature on Histamine Production by *E. faecium*.
Key: dpi = days post-inoculation

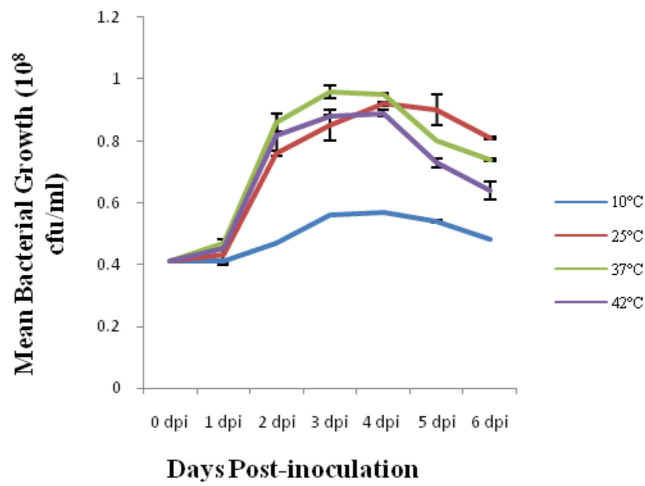

Figure 8a: Effects of Temperature on the Growth of *Enterococcus gallinarum*.
Key:cfu = Colony forming unit, dpi = Days Posy-inoculation

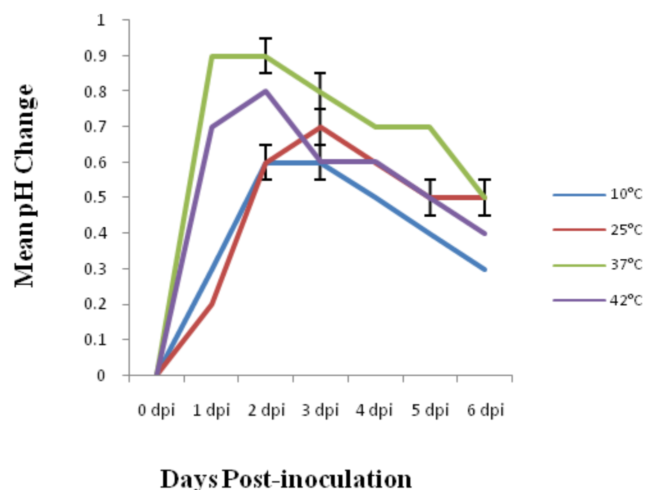

Figure 8b: Effect of Temperature on Histamine Production by *Enterococcus gallinarum*.
Key: dpi =Days Post-inoculation

This occurred for up to 24 h in the medium with 5% NaCl and 48 h in the medium with 10% NaCl concentration, except *E. faecalis* which began growth in broths with 5% and 10% NaCl concentrations 24h post-inoculation. All the growths reached peak level between 4-5 days of incubation Figures 11a, 12a, 13a and 14a. Histamine production at different concentration of NaCl is shown in Figures 11b, 12b, 13b and 14b. Highest level of histamine production was observed in broth with 0.5% salt concentration, followed by 1% concentration. Histamine production began within the first few hours in these concentrations and rose steadily up till the 5th day. In the broths with 5% and 10% salt concentrations, histamine production was delayed; production began 24h post-inoculation in 5% NaCl broth and 48h post-inoculation in 10% NaCl broth.
Growths were conspicuously influenced by the concentrations of Sodium Chloride in the media (11a, 12a, 13a & 14a). For the four species of *Enterococcus,* there were normal growths in the media containing 0.5% and 1% salt concentrations. The growths witnessed little or no lag phases before entering exponential phases in each of the curves. These concentrations were probably isotonic or just normal enough for the cells to tolerate. The observed growths from the point of inoculation indicate that the cells have already acclimatized to this range of salt concentrations in their previous environments. Adjustment to other environmental factors such as nutrients and pH was however evident in the little lag phases observed. On the contrary, higher concentrations of NaCl (5% and 10%) had initial lethal effects on the organisms.

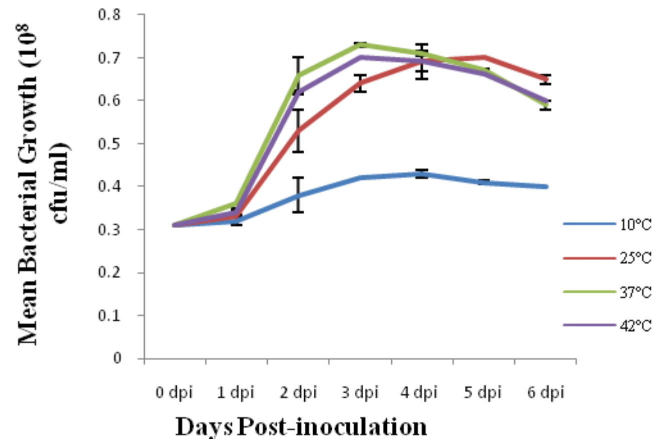

Figure 9a: Effects of Temperature on the Growth of *Enterococcus faecalis*HA5.
Key:cfu = Colony forming unit, dpi = Days Posy-inoculation

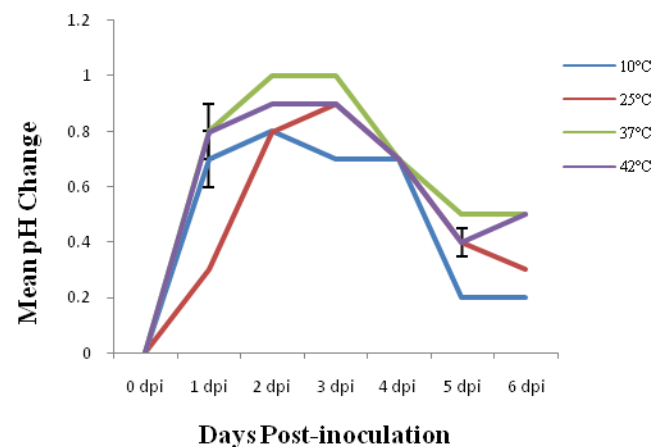

Figure 9b: Effect of Temperature on the Production of Histamine by *Enterococcus faecalis.*
Key: dpi = Days Post-inoculation

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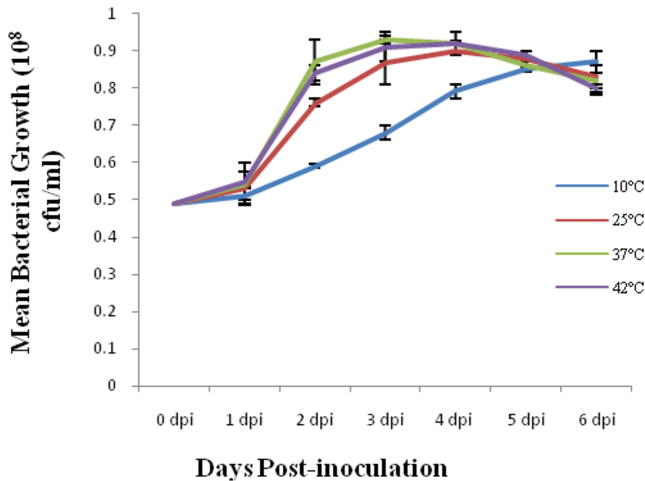

Figure 10a: Effects of Temperature on the Growth of *Enterococcus gilvus.*
Key: cfu = Colony forming unit, dpi = Days Post-inoculation

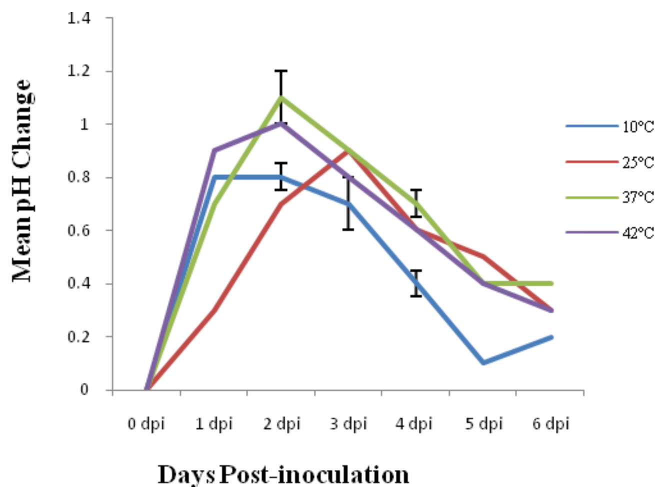

Figure 10b: Effect of Temperature on Histamine Production by *Enterococcus gilvus*CR1.
Key: dpi = Days Post-inoculation

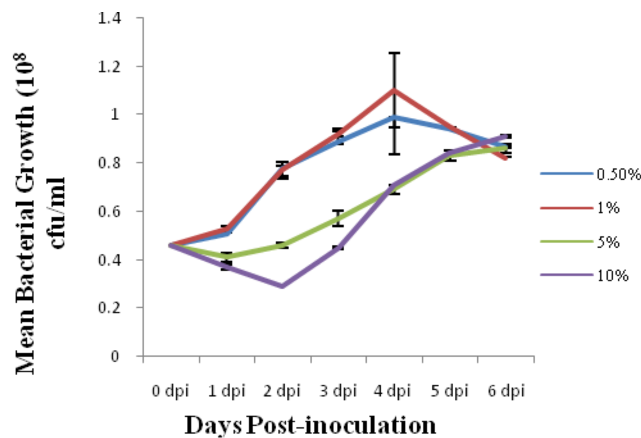

Figure 11a: Effects of NaCl Concentration on the Growth of *Enterococcus faecium.*
Key:cfu = Colony forming unit, dpi = Days Post-inoculation

The bacterial cell counts had to decrease as a result of cell death occasioned by high salinity. These initial cell deaths are more in the medium with 10% NaCl where it took the bacteria up to 48 hrs to get acclimatized to salty environment except in *E. faecalis* that adapted to both concentrations after 48 hrs. This can be seen in the initial downward movement of the growth curves. It is less severe with 5% NaCl where the decreasing cell counts reversed after 24 hrs. Both 5% and 10% Sodium Chloride are hypertonic to the *Enterococcus*. Once exposed, the cells began to plasmolyse and die. This buttresses the earlier studies reporting the ability of some lactic acid bacteria such as *Lactobacillus* to survive high salt environments.[33, 34]

Histamine production began almost immediately after inoculation in the media with 0.5% and 1% NaCl except in *E. gallinarum*where the production of histamine began after 24 hrs (11b, 12b, 13b &14b). The most favourable salt concentration happens to be 1% NaCl in almost all the isolates. The reason for this can be attributed to osmotic and ionic equilibrium between the bacteria's intracellular and extracellular environments.[33]This can also be correlated with immediate growth response of the organisms at these same concentrations. In both concentrations also, the pH rose by as high as 1.4 corresponding to the bacterial counts in the growth media. Conversely, histamine production in the media with salt concentrations of 5% and 10% did not commence till 24 to 48 hrs of inoculation. This is obviously due to osmotic stress experienced by the cells. There were initial cell deaths due to this osmotic stress which resulted in apparent decrease in the level of histamine across the species. Other lactic acid bacteria have also been reported to reduce its yield at high salt concentration and consequently their histamine production levels due to this osmotic stress.[33]

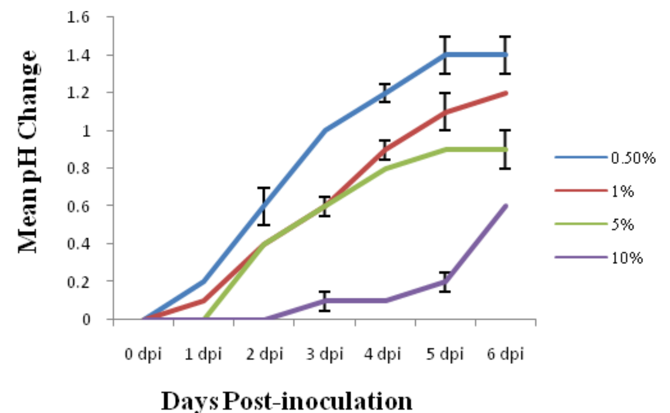

Figure 11b: Effect of NaCl Concentration on Histamine Production by *Enterococcus faecium*.
Key: dpi = Days Post-inoculation

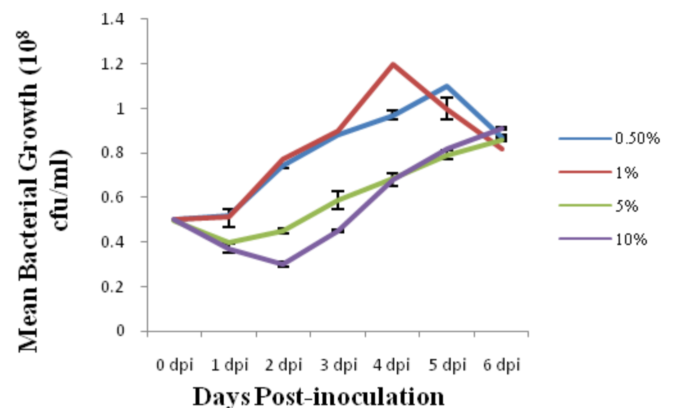

Figure 12a: Effects of NaCl Concentration on the Growth of *Enterococcus gallinarum*.
Key:cfu = Colony forming unit, dpi = Days Post-inoculation

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Trop J Nat Prod Res, December 2023; 7(12):5699-5709 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

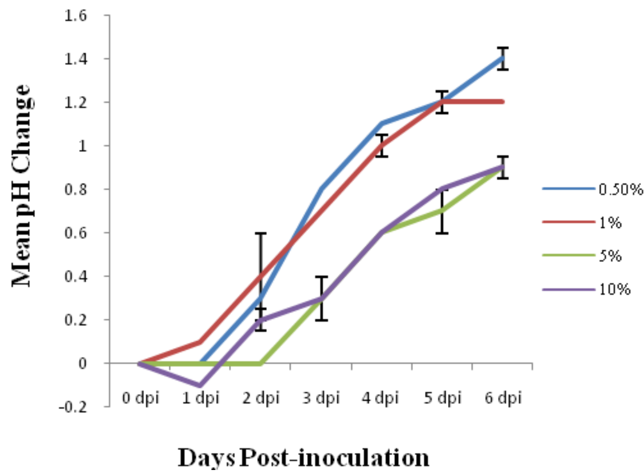

Figure 12b: Effect of NaCl Concentration on the Production of Histamine by *Enterococcus gallinarum*.
Key: dpi = Days Post-inoculation

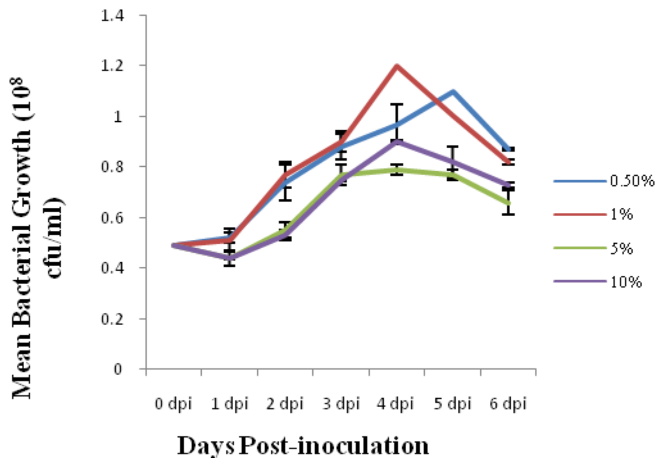

Figure 13a: Effects of NaCl Concentration on the Growth of *Enterococcus faecalis*.
Key:cfu = Colony forming unit, dpi = Days Post-inoculation

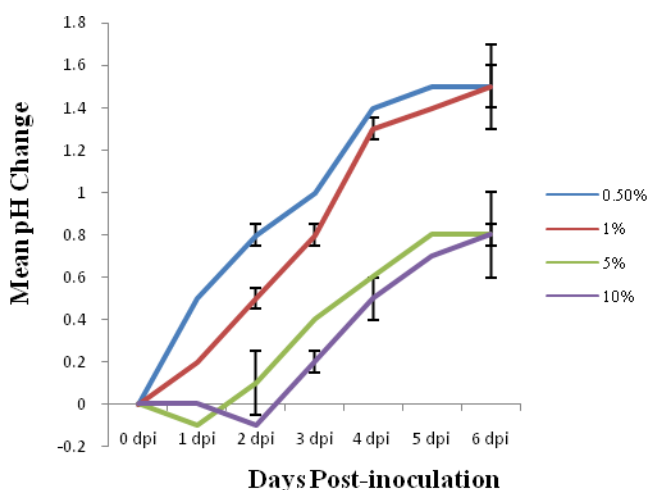

Figure 13b: Effect of NaCl Concentration on the Production of Histamine by *Enterococcus faecalis.*
Key: dpi = Days Post-inoculation

There were however possibilities of continued synthesis of lactic acid by the cells which increased the acidity of the media, thus reducing the pH levels. Histamine levels in the media with 5% and 10% NaCl began to increase from 24 and 48 h respectively. This was as a result of adaptation of the organisms to high salt environment and subsequent increase in the bacterial biomass.[31] Histamine levels continued to rise in all the media with different salt concentrations in all the species. This was because the *hdc* enzyme continued its extracellular activities even as the bacterial growth had started declining. This is evident when the growth curves are compared with the histamine production curves. While the growth curves peak at 4th and 5th days as the case may be, the production of histamine continued even beyond the 5th day.

Significant levels of the effects of the physicochemical conditions
The individual effects of the parameters (pH, temperatures and salt concentration) on the growth and histamine production by the bacterial isolates were analyzed for statistical significance (Tables 2 and 3). All statistical significances were tested. None of the parameters had significant ($p < 0.05$) effects on the growth of the *Enterococcus* species (*E. faecium, E. gallinarum, E. faecalis*and *E. gilvus*) except *E. faecium* which was significantly affected by temperature. The parameters, however, influenced histamine production by the bacteria with pH and salt concentration having significant effect on some species. There were positive correlations between the mean bacteria growth and the histamine levels in all the parameters and in all the species (Table 4).

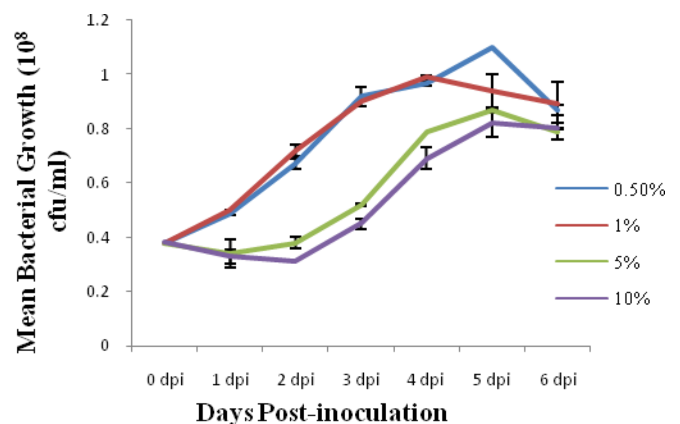

Figure 14a: Effects of NaCl Concentration on the Growth of *Enterococcus gilvus.*
Key: cfu = Colony forming unit, dpi = Days Post-inoculation

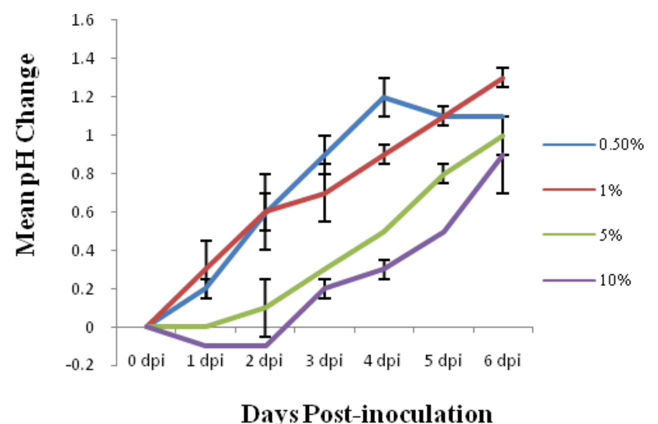

Figure 14b: Effect of NaCl Concentration on Histamine Production by *Enterococcus gilvus.*
Key: dpi = Days Post-inoculation

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Statistical analysis of the effects of ph, temperature and salt concentration on the growth of *enterococcus* species

Parameter	Organism	Level of Significance	Remarks
pH	*E. faecium*	0.92	Not significant
	E. gallinarum	0.86	Not significant
	E. faecalis	0.98	Not significant
	E. gilvus	0.88	Not significant
Temperature	*E. faecium*	0.03	Significant
	E. gallinarum	0.07	Not significant
	E. faecalis	0.07	Not significant
	E. gilvus	0.73	Not significant
Sodium Chloride Concentration	*E.faecium*	0.17	Not significant
	E. gallinarum	0.12	Not significant
	E. faecalis	0.30	Not significant
	E. gilvus	0.18	Not significant

Table 3: Statistical analysis of the effects of pH, temperature and salt concentration on histamine production by *enterococcus* species

Parameter	Organism	Level of Significance	Remarks
pH	*E. faecium*	0.03	Significant
	E. gallinarum	0.00	Significant
	E. faecalis	0.05	Significant
	E. gilvus	0.07	Not significant
Temperature	*E. faecium*	0.30	Not significant
	E. gallinarum	0.31	Not significant
	E. faecalis	0.72	Not significant
	E. gilvus	0.76	Not significant
Sodium Chloride Concentration	*E.faecium*	0.05	Significant
	E. gallinarum	0.43	Not significant
	E. faecalis	0.05	Significant
	E. gilvus	0.11	Not significant

Table 4: Correlation analysis between mean bacterial growth and mean histamine production under different physicochemical parameters

Parameter	Organism	Person's Correlation	Level of Significance	Remarks
pH	*E. faecium*	1	0.09	Not significant
	E. gallinarum	1	0.85	Not significant
	E. faecalis	1	0.17	Not significant
	E. gilvus	1	0.06	Not significant
Temperature	*E. faecium*	1	0.03	Significant
	E. gallinarum	1	0.00	Significant
	E. faecalis	1	0.01	Significant
	E. gilvus	1	0.07	Not significant
Sodium Chloride Concentration	*E.faecium*	1	0.00	Significant
	E. gallinarum	1	0.00	Significant
	E. faecalis	1	0.00	Significant
	E. gilvus	1	0.00	Significant

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However, while there was significant ($p < 0.05$) correlation between mean growth and histamine levels in the media with varied temperature and salt concentrations, there were no significant ($p < 0.05$) correlations in the media with varied pH.
Enterococcus species, just like other LAB, tolerate a wide range of pH and temperature and adapt in slightly salt environment.[28, 30, 34] This accounts for the inability of the parameters to exert significant effects on the growths of the species. On the other hand, pH and salt concentration had significant ($p < 0.05$) effect on histamine production. This is obviously due to the direct relationship between pH and level of activation of *hdc* gene in the cells.[18, 25]The bacterial biomass also determined the quantity of histamine produced as each cell is expected to be producing histamine, and this accounted for the observed statistical significance.

Conclusion

Physicochemical parameters such as pH, temperature and salt concentration exert significant influence on the growth and metabolism of microbial fermenters. The ability of *Enterococcus* species to decarboxylatehistidine amino acid to produce histamine is largely determined by combined effects of pH, temperature and salt concentration. Histamine accumulation in fermented foods is a leading cause of food allergy. This had constituted serious public health hazard in processed foods. Regulation of these parameters is key to good processing of oil bean seeds and other fermented food items. The research will be a valuable guide in developing a standard scientific method of oil bean processing. A standard practice in oil bean processing will eliminate health hazards associated with crude processing being practiced in the rural communities.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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