



Exploration of Probiotic Potential of Lactic Acid Bacteria from *Pentaclethra macrophylla* in Okigwe, Imo State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Lactic acid bacteria, commonly found in fermented foods, are renowned for their potential health benefits, attracting significant attention for their possible contributions to human well-being. This study focuses on evaluating the probiotic potential of lactic acid bacteria isolated from *Pentaclethra macrophylla* in Okigwe, Imo State.

Methods: *Pentaclethra macrophylla*, a fermented food product known locally as Ugba, was sourced from vendors at Eke Okigwe Market, Okigwe, Imo State.

Results: Three distinct lactic acid bacterial: *Escherichia coli*, *Lactobacillus* species and *Pediococcus* species were identified and characterized based on morphological and biochemical features, confirming their classification as lactic acid bacteria and *Lactobacillus* species. The isolates exhibited Gram-positive, non-motile, and catalase-negative characteristics consistent with established criteria. Regarding probiotic traits, cholesterol assimilation varied among the isolates, with B2 demonstrating the highest assimilation potential - 75% at 10% and an impressive 85% at 20%. Bile salt assimilation showed concentration-dependent responses, with B2 displaying superior tolerance - 51.6% growth at 20% bile concentration. Moreover, all isolates exhibited robust growth across acidic pH levels (3.0, 4.0, 5.0, and 7.0), underscoring their resilience to acidic environments.

Conclusion: This study underscores the promising probiotic characteristics of the isolated lactic acid bacteria strains, positioning them as potential candidates for developing functional food supplements. Further investigation into their interactions with other microorganisms and intestinal epithelial cells promises valuable insights into their viability and effectiveness for practical applications in functional foods. This research contributes to ongoing exploration of indigenous probiotic strains, suggesting avenues for integration into the food and health sectors.

Keywords: Probiotics; lactic acid bacteria; *Pentaclethra macrophylla*.

1. INTRODUCTION

Probiotics, those tiny yet powerful microorganisms found in foods like yogurt, hold a significant role in promoting health and nutrition, benefiting both humans and animals alike.

In the human context, probiotics, exemplified by strains such as *Lactobacillus* and *Bifidobacterium*, function as harmonious partners with our bodies. They bolster our immune systems, contribute to lowering blood pressure, facilitate lactose digestion (a particularly welcome relief for those with lactose intolerance), and have even shown promise in reducing cholesterol levels [1,2,3].

In the sphere of animal nutrition, the effects of probiotics are equally noteworthy. They establish and maintain a balanced and healthy environment within the animal gut, resulting in improved growth rates, reduced mortality, and enhanced efficiency in utilizing feed resources [4].

Furthermore, probiotics exert their influence on the intricate ecosystem of microorganisms residing in our guts, collectively known as the gut microbiota. Here, they play a role in shaping this microbial community, thereby impacting human health positively. Their effects encompass

modulating the responsiveness of our immune systems, enhancing nutrient absorption, and acting as guardians against potentially harmful pathogens [5].

The applications of probiotics extend beyond these realms, with emerging research revealing their potential in treating an array of health conditions. From gastrointestinal issues to urinary tract infections, acne, and even cancer, probiotics offer a diverse range of therapeutic possibilities. Their mechanisms of action include competitive exclusion of harmful bacteria, the production of inhibitory substances that thwart pathogens, and the regulation of intestinal transit.

In summary, probiotics stand as champions in the pursuit of improved health and nutrition, benefiting both human and animal populations. As our understanding of these beneficial microorganisms deepens, they continue to offer promising avenues for a healthier and more resilient future.

2. MATERIALS AND METHODS

Four samples of Ugba were randomly collected from food stuff vendors at Eke Okigwe market in Okigwe, Imo State. The samples were transported to the Microbiology Laboratory Unit of Alex Ekwueme Federal University Ndufu- Alike Ebonyi state for analysis.

2.1 Media Preparation

All media used were aseptically prepared according to manufacturer's instruction, weighed and mixed in a distilled water in a conical flask. The content of the flask was shake for proper dissolution of dehydration media. The flask was covered with cotton wool, wrapped firmly with aluminum foil and autoclaved at 121°C for 15 minutes. It was allowed to cool at 45° C before it was aseptically dispensed in 20ml volume Petri dishes and allowed to gel.

2.2 Isolation and Identification of Lactic Acid Bacteria from Fermented Food

Tenfold serial dilutions of each sample was done, Gram staining and the following biochemical test was carried out (Citrate utilization test, oxidase test, indole test, methyl test, agar fermentation test (fructose). Catalase test, Voges Proskauer (VP) test).

2.3 Probiotic Potentials of Lactic Acid Bacteria Isolates

Cholesterol assimilation and Bile salt assimilation was carried out from the culture medium and its acid tolerance by lactic acid bacteria isolates was done by applying [6].

3. RESULTS

The result shows four distinct lactic acid bacterial isolates and their percentage assimilation of bile and cholesterol which are presented in the tables and figures below.

4. DISCUSSION

The isolated colonies were named from A1, A2, B1, and B2. The isolates were from samples from different food vendors in Eke Okigwe Market. Table 1 showed the morphology and biochemical characteristics of the isolates and it showed that all four isolates were rod shaped (bacilli). All the isolates were Gram positive but except Isolate UgB2 and all catalase negative. This conforms to the characteristic properties of lactic acid bacteria, as equally isolated and characterized in the work of [7]. Thus, both the isolates were Gram positive, non-motile, catalase negative and exhibited negative pattern of citrate utilization, H₂S formation, indole production, oxidase test, urease activity and VP reaction. These were the common characteristics of lactic acid bacteria and *Lactobacillus* species. These findings were also similar to those reported by

[8]. All the isolates were positive to sugar fermentation-fructose. From the results, all isolate was *Lactococcus* sp, *Streptococcus* sp, *pedicoccus* sp.

The cholesterol assimilation capabilities of the bacterial isolates were assessed at 10% and 20% concentrations. Isolate A1 demonstrated limited cholesterol utilization, with growth percentages of 11.02% and 50.9% at 10% and 20%, respectively. A2 exhibited moderate assimilation, increasing from 18.0% at 10% to 21% at 20%. B1 displayed a moderate ability to assimilate cholesterol, reaching 48.8% at 10% but showing a possible saturation effect at 20% with a growth of 20.1%. Significantly, B2 exhibited high cholesterol assimilation, showing substantial growth at both concentrations, with percentages of 75% at 10% and an impressive 85% at 20%. In descending order of cholesterol assimilation potential, the bacterial isolates are as follows: B2> B1> A2> A1. These results corroborate the work of [9] who reported an *In vitro* characterization of probiotic strains in Colombia whose cholesterol assimilation decreased as the concentration of the cholesterol increased.

The bile salt assimilation, in isolate A1 at 10% bile, showed the highest growth at 31.5%, indicating some resistance to bile at this concentration, A2 also, exhibited good growth at 25%, suggesting a certain level of tolerance, B1 Displayed moderate growth at 12%, indicating lower tolerance to bile and B2 was similar to B1, with 14.4% growth. At 20% bile, Growth significantly decreases to 20.6% for A1, indicating reduced tolerance to higher bile concentrations. For A2, it showed decreased growth at 36.9%, suggesting sensitivity to elevated bile levels. For B1, growth is lower at 20.6%, but there was a significant increase compared to 10%, suggesting some adaptation and B2 it exhibited a substantial increase in growth to 51.6%, indicating a higher tolerance to 20% bile. In descending order of bile assimilation potential, the bacterial isolates at 10% are as follows: B2 > A1> A2> B1 at 20% descending order of bile assimilation are as follows B2 >A2 >B1>A1. The acid tolerance level of the isolates was done at pH 3.0, 4.0, 5.0 and 7.0. All the isolates were positive to these pH variations; this implies that they were able to grow under the different pH values they were subjected to. When lactic acid bacteria enters the body, the first contact is gastric acid, with a very low pH of approximately 2-3 [10].

Table 1. Morphological and biochemical characteristics of lactic acid bacteria (LAB) species isolated from *Pentaclethra macrophylla*

Isolates	Cultural features	Gram reaction	Cell shape	Cell arrange	Cat. test	Cit. test	Slant (Lact.)	Butt (Glu.)	H ₂ S prod.	Gas prod.	Ind. test	MR test	VP test	Oxi. test	Probable Species
Ug A1	R, T, NLF, D	-ve	Rod	Single	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	<i>Escherichia coli</i>
Ug A2	R, T, NLF, D	+ve	Rod	Single	-ve	+ve	+ve	+ve	+ve	+ve	ve	-ve	+ve	-ve	<i>Lactobacillus</i> species
Ug B1	R, T, NLF, D	+ve	Rod	Single	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	<i>Pediococcus</i> species
Ug B2	R, T, NLF, D	-ve	Rod	Single	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	<i>Escherichia coli</i>

Key Interpretations

Ug= Ugba, R= Round

T= Tiny, NLF= Non-lactose fermenter

D= Drizzling, Cat.= Catalase

Cit.= Citrate, Lact. = Lactose

Glu.= Glucose

H₂S= Hydrogen sulphide

Ind.= Indole

MR= Methyl red

VP= Vogues proskour

Oxi.= Oxidase

-ve= Negative result

+ve= Positive result

The morphological and biochemical characteristics of three lactic acid bacteria isolates *Escherichia coli*, *Lactobacillus* species and *Pediococcus* species obtained from *Pentaclethra macrophylla* were carried out. All isolates exhibited rod-shaped cells, were Gram-positive, non-lactose fermenters, and catalase-negative. They displayed variations in traits such as citrate utilization, hydrogen sulfide (H₂S) production, gas production, indole production, and reactions in different biochemical tests (MR, VP, oxidase). These characteristics align with properties of lactic acid bacteria and *Lactobacillus* species

Table 2. Result of fermentation of Isolate in sugar (Fructose)

Isolates	Fermentation result of Fructose
UgA1	+ve
UgA2	+ve
UgB1	+ve
UgB2	+ve

Key Interpretation

Ug= Ugba

+ve= Positive fermentation

This showed that there were three (3) different lactic acid bacteria isolates which could ferment the given sugar.

The presence of the "+" sign across all isolates signifies their ability to ferment fructose, showcasing a commonality in their sugar utilization capabilities

Table 3. The percentage of assimilated cholesterol by isolates in different concentrations

Isolates	10% Conc	20% Conc
Ug A1	11.02%	50.90%
Ug A2	18.00%	21%
Ug B1	48.80%	20.10%
Ug B2	75%	85%

Key Interpretation

Ug= Ugba

%= Percentage

The percentage of cholesterol assimilated varied among the isolates at different concentrations. All the isolates demonstrated significant increases in assimilation from 10% to 20%, except for UgB1, which showed a reduction in assimilation. Specifically, UgB2 exhibited a maximum assimilation of 75% at 10% cholesterol, reaching an impressive 85% at 20% concentration. The other isolates (UgA1, UgA2, UgB1) also showed varying degrees of cholesterol assimilation at both concentrations

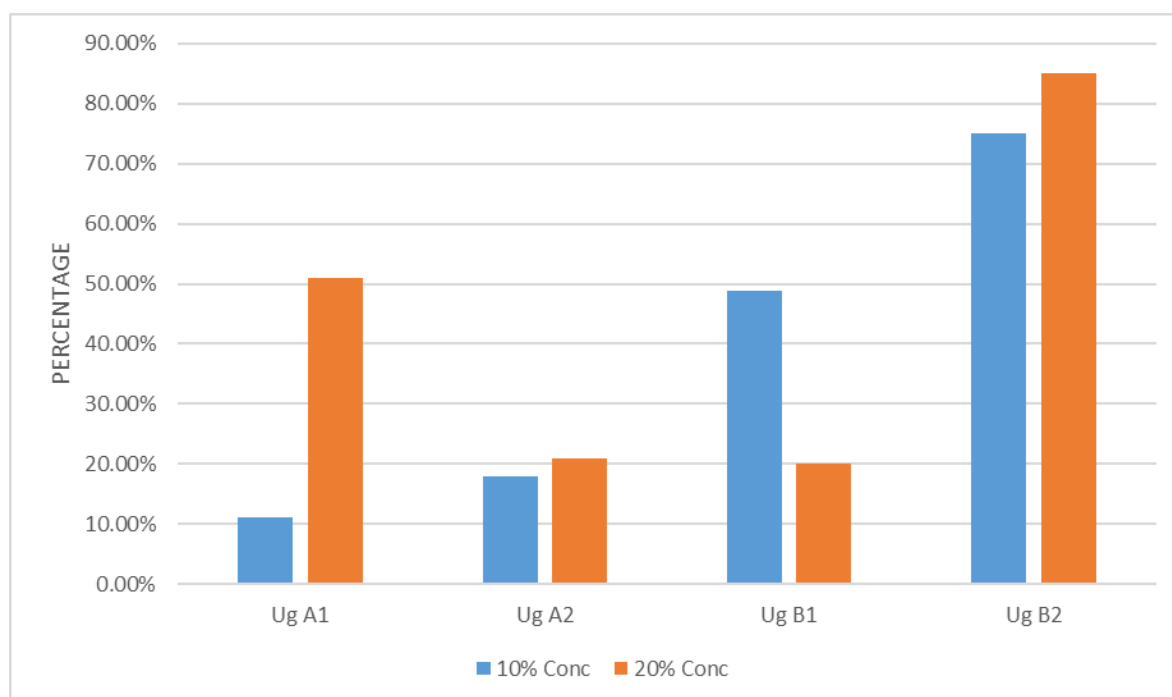


Fig. 1. Percentage of assimilated cholesterol

Table 4. The percentage of assimilated biles by isolates in different concentrations

Isolates	10% Conc	20% Conc
UgA1	31.50%	55.90%
UgA2	25%	36.90%
UgB1	12%	20.60%
UgB2	14%	51.60%

Key Interpretation: Ug= Ugba
%= Percentage

The results highlight the bile assimilation capabilities of the lactic acid bacteria isolates (UgA1, UgA2, UgB1, UgB2) at different concentrations. The bile assimilated had a maximum value of 31.5% for UgA1 at 10% bile and substantial increase to 55.9% for UgA1 at 20% bile. The other isolates also exhibited varying degrees of bile assimilation at both concentrations

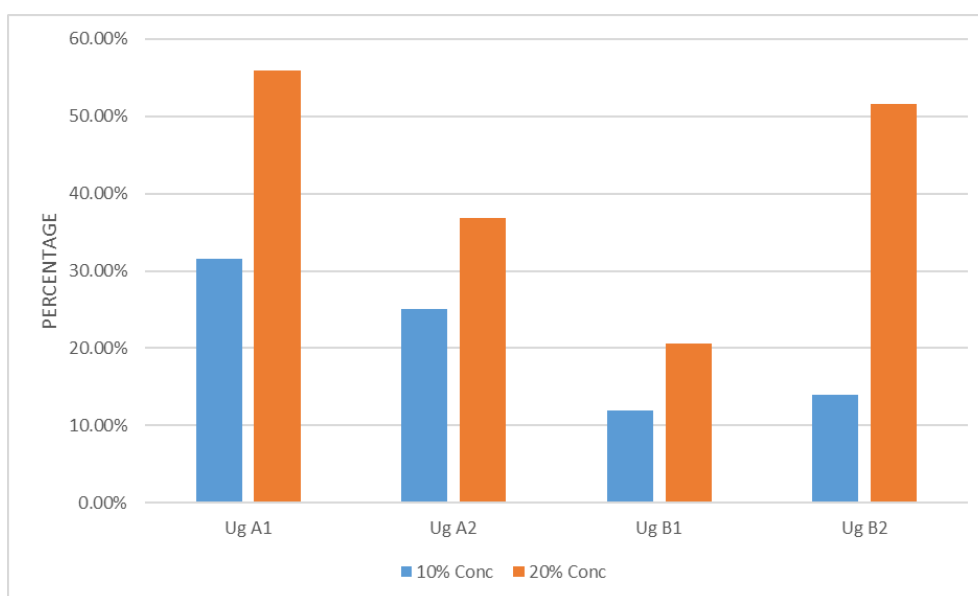


Fig. 2. Percentage of assimilated bile

Table 5. Acid tolerance by lactic acid bacteria isolates

Isolates	pH 3	pH 4	pH 5	pH 7
UgA1	+	+	+	+
UgA2	+	+	+	+
UgB1	+	+	+	+
UgB2	+	+	+	+

Key Interpretation: Ug= Ugba
+ = Positive (Growth occurred)

The lactic acid bacteria isolates were tolerant to acidic pH, thereby displayed robust growth cross all pH levels (3, 4, 5, 7). The presence of the "+" sign for each isolate at all pH levels indicates positive growth, highlighting their adaptability and viability under varying acidic conditions

5. CONCLUSION

In conclusion, the lactic acid bacteria isolated from *Pentaclethra macrophylla* in Okigwe, Imo State, exhibit promising probiotic potential. The isolates demonstrated typical characteristics of lactic acid bacteria, including Gram-positive staining, non-motility, and catalase negativity. They also displayed a range of beneficial traits such as positive results in sugar fermentation-

fructose tests and varying degrees of cholesterol assimilation ability.

These findings suggest that the lactic acid bacteria from *Pentaclethra macrophylla* could potentially be developed as probiotic candidates for human health applications. Further research is warranted to explore their full spectrum of probiotic properties, safety, and efficacy in order to harness their potential benefits for both human and animal health.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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