PROBIOTIC ASSESSMENT OF LACTIC ACID BACTERIA ISOLATED FROM *PENTACLETHRA MA CROPHYLLA* IN OKIGWE, IMO STATE.

ABSTRACT

INTRODUCTION: Lactic acid bacteria, commonly found in fermented foods, have been associated with various health benefits and have garnered considerable attention due to their potential to contribute to human health. This study aims at assessing probiotic potential of lactic acid bacteria isolated from *Pentaclethra macrophylla* in okigwe, Imo state.

METHODS: *Pentaclethra macrophylla*, a fermented food product known as Ugba, obtained from food vendors in Eke Okigwe Market, Okigwe, Imo State.

RESULTS: Four distinct lactic acid bacterial isolates (UgA1, UgA2, UgB1, UgB2) were identified and characterized based on morphological and biochemical features, confirming their alignment with lactic acid bacteria and Lactobacillus species. The isolates exhibited Gram-positive, non-motile, and catalase-negative test, consistent with established characteristics. The probiotic characteristics of the isolates like cholesterol assimilation varied among the isolates, with B2 demonstrating the highest assimilation potential, with percentages of 75% at 10% and an impressive 85% at 20%. Bile salt assimilation results indicated concentration-dependent responses, with B2 exhibiting superior tolerance in growth with percentage of 51.6% at 20% bile concentration. Additionally, all isolates

displayed positive growth across varying acidic pH levels of (3.0, 4.0, 5.0, and 7.0), highlighting their resilience to acidic environments.

CONCLUSION: The outcomes of this study suggest that the isolated lactic acid bacteria strains harbor promising probiotic characteristics, positioning them as potential contenders for the development of functional food supplements. Further exploration into the interactions of these isolates with other microorganisms and epithelial cells of the intestine promises valuable insights into their viability and efficacy for practical applications for functional foods. This research contributes to the ongoing exploration of indigenous probiotic strains, offering potential avenues for integration into the food and health industries.

Keywords: Probiotics, Lactic Acid Bacteria, *Pentaclethra macropyhlla*.

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.

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.

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.

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).

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].

RESULT

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_2S prod.	Gas prod.	Ind. test	MR test	VP test	Oxi. test
UgA1	R, T, NLF, D	-ve	Rod	Single	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
UgA2	R, T, NLF, D	+ve	Rod	Single	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve
UgB1	R, T, NLF, D	+ve	Rod	Single	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
UgB2	R, T, NLF, D	-ve	Rod	Single	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve

Key Interpretations

Ug= Ugba, R= Round

T= Tiny, NLF= Non-lactose fermenter

D= Drizzling, Cat.= Catalase

Cit.= Citrate, Lact. = Lactose

Glu.= Glucose

H_2S= Hydrogen sulphide

Ind.= Indole

MR= Methyl red

VP= Vogues proskeur

Oxi.= Oxidase

-ve= Negative result

+ve= Positive result

The morphological and biochemical characteristics of four lactic acid bacteria isolates (UgA1, UgA2, UgB1, UgB2) 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 (H_2S) 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 four (4) 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.

FIGURE 1: PERCENTAGE OF ASSIMLATED CHOLESTEROL

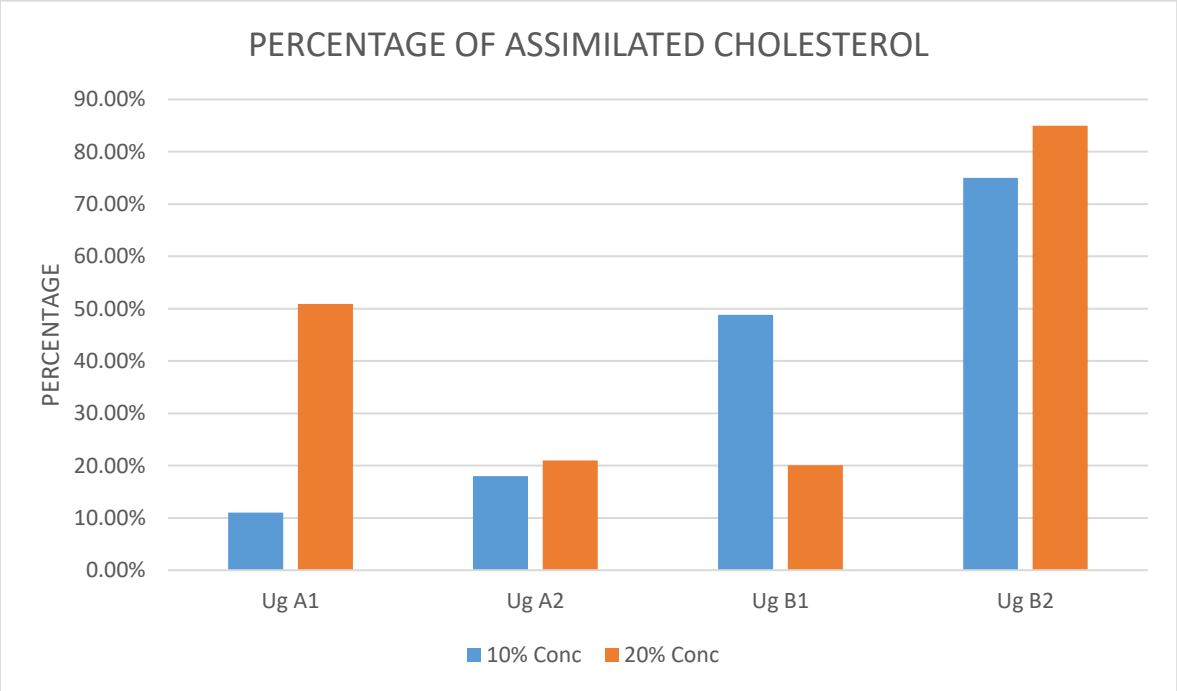

TABLE 4: The percentage of assimilated biles by isolates in different concentrations.

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.

FIGURE 2: PERCENTAGE OF ASSIMILATED BILE

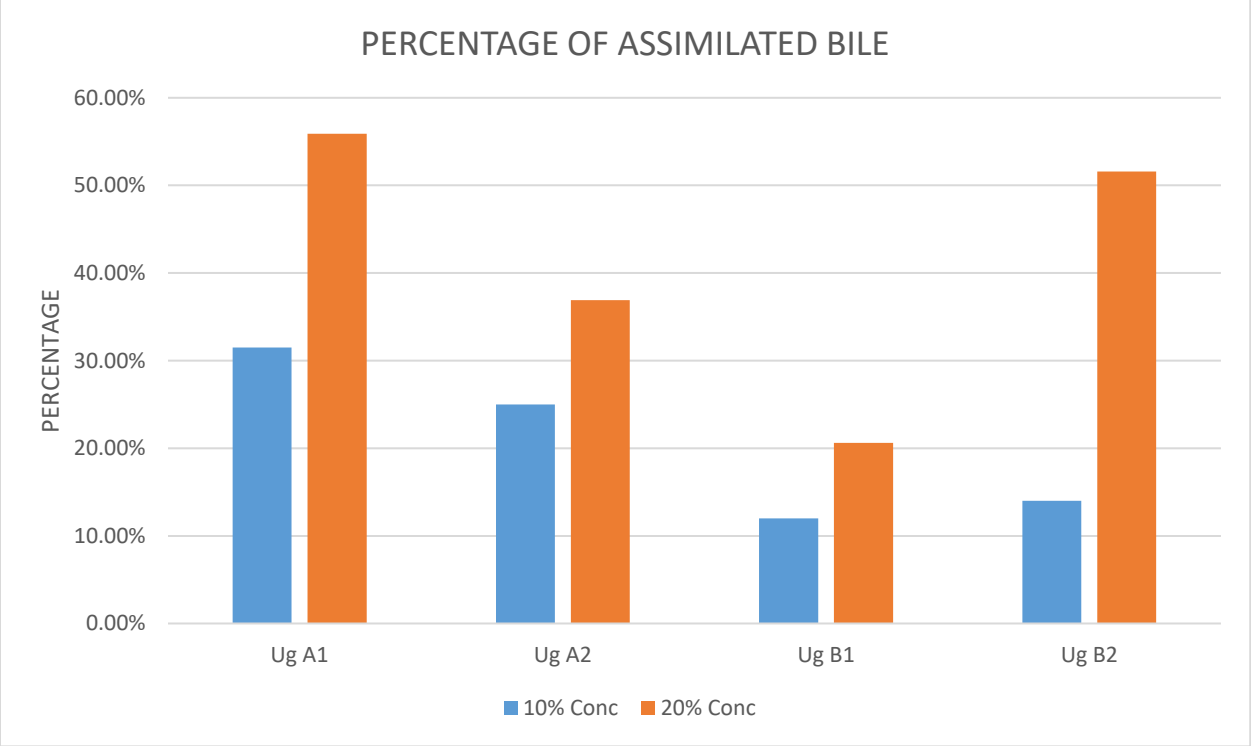

TABLE 5: Acid tolerance by lactic acid bacteria isolates

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.

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.

CONCLUSION

In conclusion, the bacterial isolates (A1, A2, B1, B2) obtained from different food vendors in Eke Okigwe Market demonstrated consistent characteristics typical of lactic acid bacteria and Lactobacillus species. The isolates were Gram-positive, non-motile, and catalase-negative, with negative results for various biochemical tests, aligning with the properties outlined in previous studies by [7, 8]. Additionally, all isolates exhibited positive sugar fermentation-fructose. Cholesterol assimilation capabilities varied among the isolates at 10% and 20% concentrations, with B2 exhibiting the highest assimilation potential, followed by B1, A2, and A1. These findings are in line with the work of [9], indicating a species-specific pattern of cholesterol assimilation.

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