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ISOLATION OF BACTERIA THAT ARE ASSOCIATED WITH THE FERMENTED PRODUCT OF MESQUITE SEED LOCUST BEANS (PROSOPIS AFRICANA) "OKPEYE"

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ABSTRACT

Diverse bacterial were isolated from Okpeye, fermented product of mesquite seed. From the results of morphological and biochemical tests carried out on the isolated organisms, Lactobacillus plantarum, Bacillus subtilis, Micrococcus spp., Escherichia coli and Staphylococcus aureus were found to be present. The results also, indicated the presence of pathogenic and non-pathogenic bacteria Escherichia coli and Staphylococcus aureus which might have resulted from the use of contaminated water or lack of proper hygiene in the production of the food condiment.

Keywords: Fermented product, Mesquite seed, Bacteria and Isolated organisms

INTRODUCTION

Natural fermentation precedes human history. Since ancient times, however, humans have been controlling the fermentation process. Fermented foods are food produced or preserved by the action of micro-organism. "Okpeye" is a seasoning made from fermented mesquite seed; Prosopis africana (Sanni et al., 2000).

The prevailing population in Nigeria, as in other less developed countries, has resulted in an increasing demand for wild-under-exploited nutritious plant product with aesthetic and organoleptic appeal in the daily diet. The common edible portions of most underutilized plants are the seeds, which is in some cases are cooked or roasted, while some are cooked and fermented for use as soup and sauce ingredients, e.g., "Okpeye" from mesquite seed; Prosopis africana (Enujiugha, 2005). Supplementing our diet with fermented foods offer us these health benefits; it can help to reduce high cholesterol level in our blood, it strengthens and supports our digestive and immune systems helping our bodies to fight off and prevent diseases, like cancer (Ljungh and Wadstrom, 2009).

"Okpeye", a fermented condiment from Prosopis Africana seed that was produced using the traditional method contains only bacteria (Agboola, 2004).

The fermented products, namely: ugba and okpeye, respectively are mainly used as condiments in soups, sauces and porridges among consuming populations in Nigeria. The isolates were used to ferment freshly prepared oil seed sample, with subsequent evaluation of the desirable quality characteristics of textures, color and aroma. B. subtilis was found to give the products with acceptable quality attributes (Enijiugha, 2005).

When food ferments, certain waste products are produced by bacteria which help in breakdown of the food material. Examples of the waste products produced are alcohol (though it is usually in small quantity in fermented foods; unless the fermented food happens to be wine or beer), vinegar (in the form of acetic acid), lactic acid, carbonic acid. These acids are the wastes produced by the bacteria which are feeding on the decomposing "fermented" foods and can cause some side effects to the consumer, such as: affect the cells of the body, prevent the digestion of foods, stiffness or soreness in the muscles when harder work is made than usual (Enujiugha, and Badejo, 2002).

Then need to standardize the processing techniques and obtain hygienic and safe products, has led to the search for diverse groups of bacteria present in "Okpeye" a fermented condiment from Prosopis africana seed.

STATEMENT OF THE PROBLEM

The poor sanitary condition in most preparation and the environment being highly polluted with spoilage and pathogenic flora is highly to be the source of contamination to food items sold in the market. Over fermentation is also a source of contaminations, it makes the aroma of finished product much more offensive. The medical sciences have been in fight to cure people affected by food poison (Sofowara, 2000). However, the need to standardize the processing techniques and to obtain hygienic and safe products, has led to the search for diverse bacteria present in "Okpeye", a fermented condiment from Prosopis Africana.

OBJECTIVES OF THE STUDY

- **i.** To isolate and determine the bacteria associated with fermented product of mesquite locust beans seed (Okpeye) (Prosopis africana)
- **ii.** To identify the non-pathogenic bacteria
- **iii.** To identify the pathogenic bacteria

MATERIALS AND METHODS

Materials

Conical flasks, pipettes, petri-dishes, test tubes, glass rods, agar slant bottles, durhams tube, wire loops, cavity slide, microscopic slides, filter papers, weighing balance, autoclave, hot-air oven, microscope and incubator.

METHODS

Sample Collection

The sample (Okpeye) was collected from different sellers in different markets in Enugu State Nigeria under aseptic condition.

Sterilization

Properly washed material like beaker, conical flasks, etc were sterilized in hot air oven at 180°C for one hour and stored at 4°C.

Preparation of Culture Media

The media for culturing were aseptically prepared when needed according to the manufacturer's instruction and autoclaved at 121°C for 15mins. It was allowed to cool to 45°C.

Preparation of Samples

Okpeye being a solid food was blended and mixed with sterile diluents to obtain a 1.10 dilution (1g of sample in 100ml of distilled water). The suspension was serially diluted and samples of each dilution were plated.

Plating Technique

Pour plate method was used. After the serial dilutions, 1ml from each tube was poured to their respective plate containing molten agar. The plates were allowed to solidify, then inverted and incubated for 24h at room temperature for colonies to develop.

IDENTIFICATION TECHNIQUES

Cultural Characteristics

Cultural characteristics that contributed to identification were sizes, shapes, texture of the colonies and pigmentation.

Gram Staining

The slide containing the heat-fixed smear was led across a staining rack and placed over a staining dish. The smear was flooded with 0.5% aqueous crystal violet for 30 seconds. Excess of lugol's iodine was poured on the smear and allowed to react for 30 seconds to wash the residual stain on the smear; the iodine is carefully rinsed out with distilled water. Then the smear was rinsed briefly within 3 seconds with 50:50 acetone-alcohols until blue colour ceases to come off. The smear was quickly rinsed out with distilled water to avoid excess decolourization. The smear was again flooded with 1% aqueous safranin for 60 seconds after which the stain was washed off with distilled water. The back of the slide was dried between folds of filter paper. The slide was allowed to dry in air. A drop of immersion oil was placed on the smear and examined under the oil immersion objective of the microscope. Gram positive bacteria will be stained blue black or purple while gram negative bacteria will be stained red or pink.

BIOCHEMICAL TESTS

A wide range of microbial activities can be determined by biochemical test. These characteristics can be used to distinguish between microbes that appear morphologically identical. In addition, microbes produce biochemical's such as acids, alcohols, gases or specific enzymes, the detection of which may aid in identification of an organism. Biochemical tests were used.

(a) Catalase Test

A small amount of the culture was picked from the agar slopes using a clean sterile wire loop. This was indented in drops of H_2O_2 on a clean microscopic slide. Production of gas bubbles indicated a positive reaction.

(b) Oxidase Test

Three (3) drops of freshly prepared oxidase reagent was placed on a piece of filter paper in a Petri dish. A colony of the test organism was removed and smear of it was made on a filter paper using a glass rod. A blue-purple colour within 10 seconds indicated positive oxidase test.

(c) Indole Test

The peptone water medium was inoculated and incubated for 48 h at 37°C. The tubes were further allowed to stay for another 24 h in the incubator for the accumulation of indole. After this period, 2 drops of xylene and indole reagents were added respectively and swirled gently. A pink colour indicated a positive test.

(d) Methyl-red Test

The sterile glucose phosphate peptone water medium was slightly inculcated from a young agar slop culture and incubated for 27°C for 48 h. Then, 5 drops of the methyl red reagent was added into each tube. Cream irregular and pinkish-red colonies indicated Positive and negative results respectively.

(e) Citrate Test

The sterile medium was inoculated from a saline suspension of the test organism and incubated for 96 h at 37°C. A blue colour and streak growth indicated a positive reaction.

(f) Motility Test

About 2-3 drops of peptone water with growth of the organism was placed on a clean slide with a loop. The cover slip was place over the slide. The slide was left for some times and then examined microscopically with the high power objective. Motile organisms would be seen swimming around.

(g) Sugar Fermentation Test

Fermentation tests were carried out using the following sugars: glucose, lactose, sucrose, and mannitol. To each 10ml of peptone water in test tubes, 1.5g of each sugar was separately dissolved into it and labeled. 3 drops of 0.01% phenol red was added. Durham tubes were inserted in an invested position into the tubes for detection of gas production. The tubes were plugged with non-absorbent cotton wool and sealed with aluminium foil before being incubated for 24 h at 37°C. Acid production was indicated by a change in colour from orange to yellow while gas was indicated by the presence of air space at the bottom end of the inverted Durham tubes.

RESULTS

Sample No	Total Count
1	56
2	83
3	47
4	37
5	70
6	84
7	61
8	57

Table 1: Total Plate Count of Bacteria Isolated from Prosopis africana (Okpeye).

Sample No.	Morphological Characteristics				
	A	**B**	**C**	**D**	**E**
1	7	20	11	11	7
2	10	30	17	11	15
3	3	24	10	5	5
4	4	16	10	3	4
5	10	23	14	11	12
6	9	30	17	13	15
7	8	21	13	10	9
8	7	20	11	8	11

Table 2: Standard Plate Count of Isolated Bacteria

A: punctiform, convex with an entire margin

B: large colonies with undulated margin, circular form and flat elevation

C: pinkish irregular dusters

D: Medium sized colonies with a regular margin and convex elevation

E: Golden yellow colonies, clusters forming cocci

Morphological Characteristics	**Gram Reaction**	**Probable Organisms**
A	Gram positive rods	Lactobacillus spp.
B	Gram positive rods	Bacillus spp.
C	Gram positive cocci	Micrococcus spp.
D	Gram positive rods	Escherichia coli
E	Gram positive cocci	Staphylococcus spp.

Table 3: Cultural Characteristics of the Isolated Bacteria

Code No	**Catalase**	**Oxidase**	**Indole**	**Methyl-red**	**Citrate**	**Motility**	**Glucose**	**Lactose**	**Sucrose**	**Mannitol**	**Organisms**
A	-	-	-	-	+	-	+	+	+	+	Lactobacillus spp.
B	+	+	-	-	+	+	-	-	+	+	Bacillus spp.
C	+	+	-	-	-	-	+	+	-	-	Micrococcus spp.
D	+	-	+	+	-	+	+	+	+	+	Escherichia coli
E	+	-	-	-	-	+	+	+	+	+	Staphylococcus spp.

Table 4: Biochemical Test on the Isolates

Key: + = positive, - = negative

Organisms	**Mean Plate Count**	**Percentage Distribution**
A	7	12.29%
B	21.5	37.74%
C	12	21.06%
D	7.8	13.69%
E	8.67	15.22%
Total	**56.97**	**100%**

Table 5: Percentage Distribution of Each Bacterium

Key

A: Lactobacillus spp.

B: Bacillus spp.

C: Micrococcus spp.

D: Escherichia coli

E: Staphylococcus spp.

DISCUSSION

The bacteria isolation on Prosopis africana Okpeye that were collected from different markets in Enugu Nigeria revealed a high bacteria growth that consisted of beneficial and pathogenic bacteria.

From this, it can be deduced that the beneficial bacterial provided the condiment with quality that offers the consumers its health benefits. The pathogenic bacteria isolated can produce and also cause some side effects to the consumer.

The pathogenic bacteria could result from poor handling of the condiments during processing, poor hygiene, contamination from the environment and the use of contaminated water, etc. The beneficial bacteria could be as a result of natural fermentation that was characterized by the growth of microorganisms.

CONCLUSION

Pathogenic bacteria were found in Prosopis Africana okpeye that were analyzed. Proper care should be taken during the production and selling of Prosopis Africana okpeye in order to remove and reduce the health problems that might result from the intake of pathogenic bacteria contaminated Prosopis Africana okpeye.

RECOMMENDATION

The regulatory agencies such as NAFDAC, should help in the enlightenment of the producers especially local producers on the need for good sanitary condition during the production of such condiments; the effects of these pathogenic organisms should be made known to them. Lukewarm water offers an ideal heat for bacteria to grow in. washing up must not take place in warm water as bacteria are not killed and the conditions are ideal for their growth (Soforowa, 2000). Hot water must be used for washing up.

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