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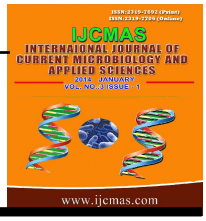

Original Research Article

Isolation and Identification of Bacterial Organisms that are Associated with the Spoilage of African Breadfruit

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A B S T R A C T

This research work was carried out to examine the bacterial organisms that are associated with the spoilage of African breadfruits (*Treculia africana*) (Ukwa). Samples of the spoilt breadfruits were analyzed using five fold serial dilution method and the last three test tubes were used for the inoculation on MacConkey and nutrient agar. After incubation for 24-48h, the bacterial organisms isolated from the sample were 30.4% of *Bacillus spp*, 23.25 of *Escherichia coli*, 14.5% of *Proteus spp*, 18.8% of *Staphylococcus spp* and 13.0% of *Streptococcus spp*. The *Bacillus spp* had the highest colony count of bacteria followed by *Escherichia coli*. This may be due to different ways of preservation or handling during dehulling. Consequently, consumption of African Breadfruit spoiled by these organisms poses a great danger to the consumers therefore, individuals should avoid eating spoilt breadfruits.

Keywords

African Breadfruit; Bacterial organisms; Consumers and Ukwa.

Introduction

African breadfruit (*Treculia africana*) Ukwa is the highest yielding food plants with a single tree producing up to 200 or more fruits per season. It is native to many tropical countries like West Indies, Ghana, Sierra Leone, Nigeria and Jamaica. *Treculia africana* is a tropical permanent crop among the large number of trees growing naturally in the high rain forest of moracee and th efains. Treculia africana has a height of about 24m to 36m and 6m width, propagation is by seed and vegetative by budding.

Its seed, commonly called "afon" and Ukwa by by Yoruba and Igbo's of Nigeria is popularly known as a tradition food item (Adeparusi, 2001).

There are many varieties of African breadfruit some are seedless while some are with seeds. Among these varieties only three have been recognized, namely *Mollis, var, Africana* and intermediate. Their taxonomic differences are mainly a function of size of fruit heads, hairiness of branch and shining March providing

good shade, the fruits are produced between March and August; the seed consist of two cotyledons in the internal structure which is creamy to whitish in colour enclosed by fibrous brown seed coasts. The head of Treculia intermediates are some times as big as a football or even bigger and the leaves are usually broad. *Treculia mollis* has small head and as mostly found in Edo and Delta state of Nigeria (Ejiofor *et al.*, 2008).

Some other varieties are *Treculia var aravu* which has ellipsoidal fruit, 8-12 inch, 10-30cm long and 15-22cm wide. It has yellowish green with brown spots on the sunny side, rough with sharp points which are shed on maturity. The pulp is light yellow and takes little time to cook. *Treculia var pei* has broad ellipsoidal fruit, rind light yellow and flaky when cooked, ripens earlier than other varieties and cook quickly when the breadfruit crop is scant the fruits of this cultivar are stored by burying in the ground until needed up to a year (Pearson, 2007).

Aim of the study

The aim of this research was to isolate and identify the bacteria that are associated with the spoilage of African breadfruit.

Objectives of the study

To determine the microbial load of the bacteria

To identify the source of contamination

Limitation of the study

This research work was limited to the isolation of bacterial organism associated with the spoilage of African breadfruit only, due to high cost of media and reagents, non availability of some materials that should be used in the analysis and lack of fund. It was difficult to get enough money and information needed to make these work more attractive, which made these research work to be carried out within a limited period of time.

Materials and Methods

Collection of sample

African breadfruit (*Treculia africana)* seeds were bought from Ogbete main Market Enugu, Nigeria. It was kept at room temperature for about 2-3 days where it was allowed to spoil. Then, the sample was homogenized and then put into a sterilized universal bottle containing distilled water.

Isolation of microbe using plating technique (spread plate methods)

The media was poured into 6 different Petri-dishes and then allowed to solidified, from the test tubes of the serial dilutions 10^{-3}, 10^{-4} and 10^{-5}, 0.1ml of the dilution was dropped on one end of each of the two different agar media (MacConkey and nutrient). And glass rod or huskstick was used to spread it. Then it was inverted and incubated at 37^{0}C for 24-48h and the plates were observed for growth.

Plate count

After the 24h of incubation, the number of colonies on each plate was counted and recorded. Duplicated plates were prepared for each dilution; therefore the total plate count for each dilution was

taken by average count of the three. The concentration of bacteria in the sample was calculated by multiplying the number of colonies on the plates by the dilution factor and calculating for 1g of the original samples.

Identification of bacteria

Identification of bacteria was based on the morphological and biochemical characteristics. The morphological characteristics of bacteria include; shape of colonies, colonial outline, colonial evaluation colour, consistency and size etc. Gram staining and motility test were also involved in morphological identification of bacteria isolates: catalase, methyl red, indole, coagulase and sugar fermentation test. Sugar fermentation test were carried out using the following sugars; glucose, sucrose, lactose and mannitol. To each 5ml of peptone water in test tube, 1.0g of each sugar was separately dissolved into the tube containing the peptone water and labelled. Then three (3) drops of phenol red was added to each of the test tube. The test organisms were then inoculated into the tubes using a sterilized inoculing wire. Durham tubes were inserted into the tubes in an inverted position for the detection of gas production. The tubes were plugged with non absorbent cotton wool and sealed with aluminium foil before being incubated for 24h at 37^0C. After incubation, the tubes were observed. Rising of the Durham tube in the tubes indicates the production of gas in the tube (that is a positive result).

Gram staining

A drop of sterile water placed in the middle of a grease free slide, with a sterile wire loop, small portion of the bacteria colony was put on it, and thoroughly emulsified and the mixture was spread evenly on the slide to make a thin smear. The resulting film was heat-fixed on a Bunsen burner flame. The smear flooded with crystal violet for 30 seconds. Then the smear was washed off with lugol's iodine, which was allowed to react for 30 seconds while the slide was laid across the staining rack, the iodine was carefully rinsed with distilled water and the smear was washed with acetone alcohol for 3 seconds and washed off immediately with distilled water to avoid excess decolouration. The slide was laid again across the staining rack and flooded with aqueous safranin for 60 seconds and washed off with distilled water. Then the back of the slide was dried between folds of filter paper. The slide was allowed to air dry.

The smear was examined under X 100 (oil immersion) objection. Gram positive organism appeared purple, while gram negative organism appeared pink or red.

Motility test

A clean grease free cavity slide was placed on a bench with the cavity uppermost. Then, a molten vaseline was placed round the edge of the depression of the cavity slide. A loop full of the test organism was transferred to the center of a clean dry cover slip placed on a flat sheet of paper.

The cavity slide was pressed down so that the vaseline seals the cover slip in position. Then the slide was quickly inverted so that the culture drops appear hanging. The motility was first examined using the low power objective to find the edge of the drop and then high power objectives to check for motility.

Table.1 Bacterial Morphological Characteristics

Nutrient agar	**MacConkey agar**	**Suspected organism**
Small smooth circular, creamy in colour	Smooth, pink colonies	*Escherichia coli*
Smooth and circular, shinning and plumond	Cocci, appeared in chains some in clustered	*Streptococcus spp.*
Irregular shape with dull surface	Cocci some occurs in chains. Arrange in chain and squarer end.	*Bacillus spp.*
Cocci appeared in clustered form	Blue grey translucent colonies	*Proteus spp.*

Table.2 Bacteriological Count

Nutrient agar			**MacConkey agar**		
10^{-3}	10^{-4}	10^{-5}	10^{-5}	10^{-4}	10^{-5}
30	17	9	21	9	2
25	10	6	15	7	-
14	5	2	10	3	-
23.3	10.7	5.7	15.3	6.3	0.7

Table.3 Number of Bacterial Count and Percentage Distribution

Bacterial organisms	**No. of organisms**	**Percentage distribution**
Bacillus spp.	16	30.4%
Escherichia coli	21	23.2%
Proteus spp.	10	14.5%
Staphylococcus spp.	13	18.8%
Streptococcus spp.	9	13.0%

Table.4 Biochemical test and gram reaction

Gram reaction	Motility	Catalase	Methyl red	Indole	Coagulase	Lactose	Gluscose	Sucrose	Mannitol	Identified bacterial
+	-	+	-	-	+	AG	A	AG	AG	*Staphylococcus spp.*
+	-	-	+	-	-	-	AG	A	-	*Streptococcus spp.*
-	+	-	+	+	-	G	A	AG	A	*Escherichia Coli*
+	-	+	-	-	-	-	AG	AG	G	*Bacillus spp.*
-	+	-	-	-	-	AG	G	A	-	*Proteus spp.*

+ = Positive ; AG= Acid and Gas - = Negative ; A = Acid ; AG= Acid and Gas; G = Gas

Biochemical identification

Catalase test

1-2 drop of 3% hydrogen peroxide was placed on a clean grease free slide, and then with a sterile glass rod, the organism was collected and mixed with the hydrogen peroxide. Bubbles or effervescence indicate that catalase is present (catalase positive).

Coagulase test

A drop of normal saline was placed on each ends of a slide. A colony of the test organism was emulsified in each of the drops to make two thick suspensions. A drop of plasma was added to one of the suspensions end and mixed gently clumping within 5-10 minutes indicate positive result while no clumping signifies negative result.

Methyl red test

2 ml of peptone water was inoculated with the bacteria organism and incubated at 37^0C for 48 h and added a drop of methyl red reagent (prepared by dissolving 0.04 grams of methyl red powder in 100 ml of ethyl alcohol) was added to the tube. Development of red colour implies that the result is positive. In isolated that do not produce acid during glucose fermentation, the methyl red changed to yellow showing a negative result.

Indole test

2 ml of peptone water was incubated with the bacteria organism and incubated at 37^0C for 48h. 0.5ml of Kovac's reagent was added; development of red colour indicates positive.

Results and Discussion

Several colonies were observed from the samples of spoilt African breadfruits obtained from Ogbete main market Enugu. The suspected bacterial organisms isolated were the *Bacillus spp., Escherichia coli, Proteus spp., Staphylococcus spp.* and *streptococcus spp.* At the end of the practical work, the results were tabulated.

Bacterial organism had long known to involve in spoilage of African breadfruit. From the present investigation, several species of bacterial organisms were isolated; they include *Bacillus spp, proteus spp, Escherichia coli, Staphylococcus spp* and *Streptococcus spp.* This study is completely in agreement with other experimental survey, which reports that bacterial organisms like *Bacillus spp, Streptococcus spp, Proteus spp, Escherichia coli and staphylococcus spp* are involved in the spoilage of African breadfruit (Buchanan, 2006).

It had been found that *Bacillus spp, Staphylococcus spp, Streptococcus spp, Proteus spp and Escherichia coli* were all present in different percentage of the spoilt breadfruits analysed so, consumption of these spoilt breadfruits are hereby discouraged from the results of this research.

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