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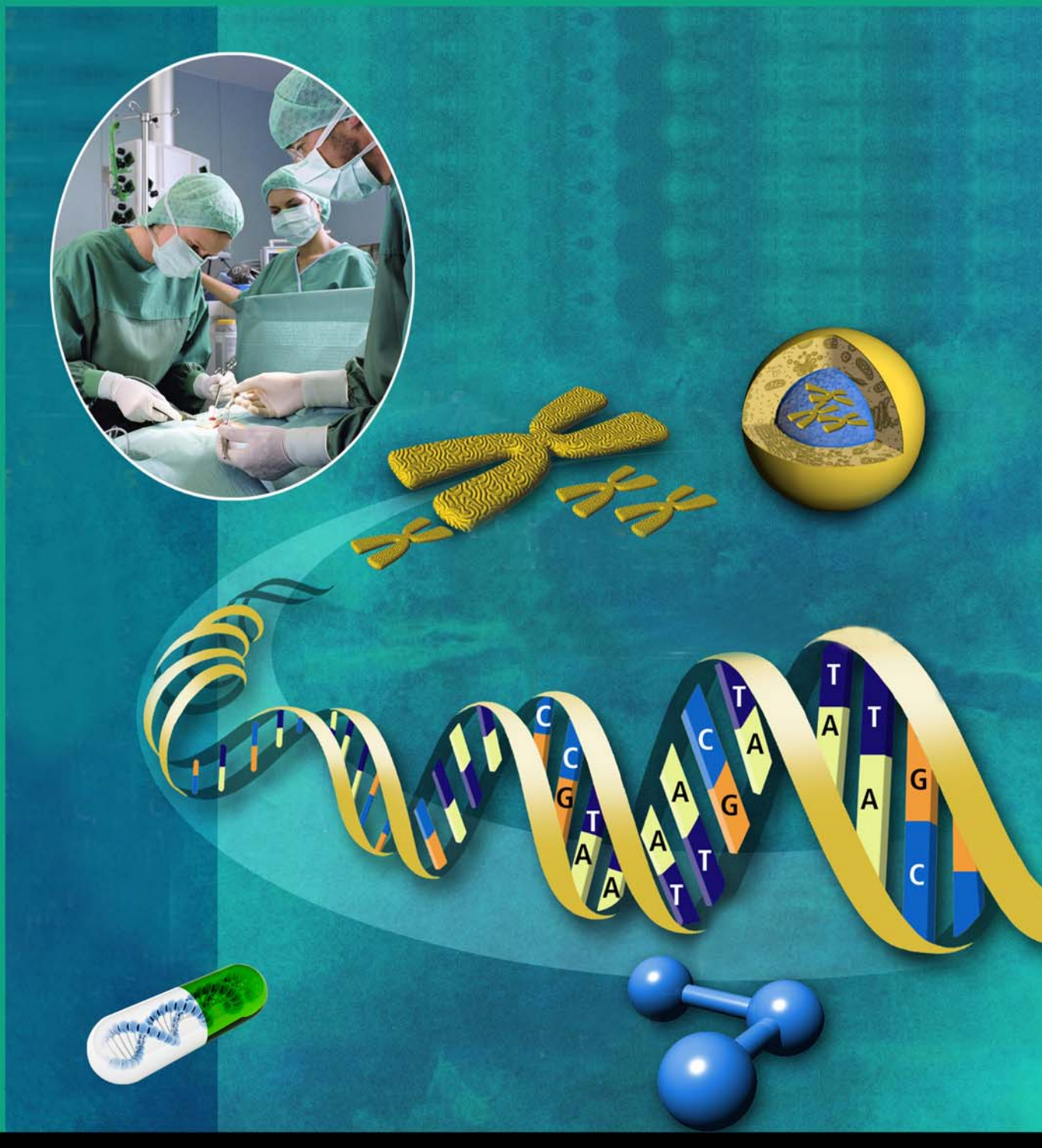

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Research Paper

ISOLATION AND CHARACTERIZATION OF BACTERIA THAT ARE ASSOCIATED WITH THE PRODUCTION AND SPOILAGE OF OGI (AKAMU)

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The fermented maize starch known as Ogi in Yoruba and *Akamu* in Igbo is a popular staple food. It is a traditional weaning food used mainly in West Africa. Bacteria were isolated from steep water and aqueous Ogi. Steep water and aqueous Ogi were labeled as Ogi 1 and Ogi 2 or sample 1 and sample 2, respectively. The bacteria assessment at the critical points of production of Ogi was aimed at establishing the sources of contamination. The steep water and the aqueous Ogi were screened for bacteria. The bacteria organisms were isolated from nutrient agar, cysteine-lactose-electrolyte-deficient (CLED) agar, de man, rogosa and sharpe (MRS) agar. The *leuconostoc* specie was isolated from nutrient agar and *Lacto bacillus* species were isolated from de man, Rogosa and sharpe (MRS) agar. The bacteria isolated from cysteine-lactose-electrolyte-deficient (CLED) agar were *Escherichia* species, *Pseudomonas* species and *Proteus* species. The critical points of contamination of ogi during production could be through water that were used for soaking, during grinding and the muslin clothes used for sieving. Appropriate safety measures and good manufacturing practices could ensure quality ogi that would be free from all these microbial contaminants.

Keywords: Ogi, Akamu, Bacteria, Contaminants, Steep water and Nutrient Agar

INTRODUCTION

Cereals are the major sources of energy and protein in the diets of most Africans. There are various types of cereals which include maize (*Zea mays*), sorghum (*Sorghum vulgar*) and millet (*perinisetum specatum*). In Nigeria, maize, sorghum and millet are grown mainly in southern part of the country (Banigo *et al*., 2002).

In Nigeria maize is used in the production of ogi (Yoruba) or Akamu (Igbo). Maize is an important source of carbohydrate, protein, Vitamins B and Minerals. Maize is deficient in two

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amino acids, lysine and tryptophan making it a poor proteinous food (Jay, 2005). The maize porridge (ogi) has become part of the staple diets for young adults, nursing mothers and weaning ration for infants between the ages of 1-2 yrs (Adebolu *et al*., 2007). Ogi is a choice meal for patients that are in need of soft and easily digestible foods. Ogi is a fermented non-alcoholic starch food that has sour taste. It turns into a semi-solid porridge when cooked. Ogi can be consumed with varieties of other food products like bread, fried bean cake (Akara), Moi-moi, fried yam, cooked beans and fried plantain. Ogi can also be consumed with milk, tea, sugar and honey to improve its taste and nutrients (Osungbara, 2009). This supplementation is also done to improve the low quality of maize protein and to replenish the substantial loss of nutrient at different stages of production.

Micro-organisms are involved in the processing of ogi especially during the fermentation process. Some of these micro-organisms could also be responsible for the spoilage when not properly stored.

The aim of this research was to isolate and characterize microorganisms that are associated with ogi's production and to identify the microorganisms that could cause ogi's spoilage during storage at wrong temperatures.

MATERIALS AND METHODS

Hard Wares, Incubator, Hot air oven, Autoclave, Bunsen burner, Beam weighing balance, Microscope, PH meter, Glass wares, Beaker, Conical flask, Durham's tube, Pipette, Slide, Cover slip and Petri-dish.

Reagents/Media

Nutrient agar, CLED agar, MRS agar, Distilled water, Normal saline, Aqueous crystal violet Lugol's Iodine, Aqueous safrannin, Immersion oil, Hydrogen peroxide, Peptone water, Kovac's reagent, Phenol red, Methyl red, Glucose, Lactose, Sucrose and mannitol.

Sterilization

The glass wares (Pipette, test tubes, beaker, etc.) were sterilized in the hot air oven at 180^{o}C for 2 hours. The media used were sterilized in the autoclave at 121^{o}C for 15 min.

Collection of Sample

Maize grains were collected from Abakpa market Enugu in a sterile container and was transferred to the laboratory (Microbiology Laboratory of Institute of Management and Technology Enugu) where the examination was carried out.

Preparation of Media

The media used were prepared according to the manufacturer's instruction. The media used includes nutrient agar, de man, rogosa and sharpe (MRS) agar and cystein-lactose-electrolyte-deficient (CLED) agar.

Preparation of Samples

The maize grains were cleaned and then steeped in water. 0.5 ml of steep water was pipetted into a sterile petri-dish and about 10 ml of freshly prepared media, cooled to 45^{o}C in a water bath was poured into the petri-dish containing the steep water. This was gently rotated both clockwise and anticlockwise to enhance even distribution. The petri-dish was allowed to solidify and incubated for 24 h at 37^{o}C. The numbers of microbial colonies were counted using a colony counter. The plates that had between 30-300 colonies were

counted and the plate below 30 colony forming unit was too few to be reliable (TFTR), while those that had more than 300 colonies were too numerous to be counted (TNTC) and plate with congested colony unit was discarded.

After steeping for 4 days, the grains were milled and sieved to get ogi. The ogi was divided into two which is sample I and sample II. The samples were soaked in water for 7 days. The sample I was the sample that its water was not changed whereas sample II was sample that its water was changed daily.

Five folds serial dilutions were prepared for each of the sample. After the dilution, the media prepared was poured into the petri-dish and was allowed to solidify before 0.5 ml of aliquot was pipetted into the medium and spread with a sterile hockey stick. The surface was allowed to dry and then incubated inverted at 37^0C for 24 h.

Isolation of Bacteria

The colonies were collected with a sterile wire loop and were streaked on already solidified medium to obtain pure culture. Each pure culture was then sub-cultured into agar slants in bijou bottles and kept as stock culture.

Characterization of Isolates

Gram Staining of the Isolates

The gram staining divides bacteria into two groups which are gram positive (Red or pinkish color) and gram negative (pink or reddish in color) bacteria. The gram staining was conducted as follows: A smear of culture was prepared on clean slide by emulsifying a little quantity of the growth on a drop of normal saline. The smear was allowed to air dry and was heat fixed. Crystal violet was then added as a primary stain for 30 s and then drained off with distilled water. Lugol's iodine was added and allowed to react for 30 s and immediately it was washed off with distilled water. Acetone alcohol was also added and immediately was equally washed off with distilled water. The smear was counter stained with safranin for 1 min and was washed with distilled water. The smear was allowed to dry. A drop of oil immersion was placed on the stained smear and viewed with a high objective microscope.

Biochemical Tests for Identification of Bacteria

Catalase Test

The test demonstrates the presence of catalase which is an enzyme that catalyses the release of oxygen from hydrogen peroxide (H_2O_2). A colony of 24 h old culture was picked using a sterile loop and then emulsified in a few drop of hydrogen peroxide on a clean slide. Presence of effervescence indicated catalase positive reaction whereas negative reaction showed no effervescence.

Motility Test

This test was carried out to determine the presence of or absence of flagella as organelle of movement in the bacteria isolates. Discrete colonies of overnight culture was placed on microscopic slide containing a drop of peptone water and covered with a cover slip after a minute. Then, it was viewed microscopically with high power objectives. Motile organisms were seen swimming around indicating a positive reaction while non motile organism indicated negative organisms.

Sugar Fermentation

10 ml of peptone water was introduced into 4 sterile test tubes respectively. 1 g of respective carbohydrate such as glucose, lactose and

mannitol were added into each of the test tubes that contain the peptone water and labelled accordingly. They were stirred to dissolve completely over a Bunsen burner after which 3 drops of phenol red were added into each of the test tubes. The tubes were plugged with cotton wool and sealed with foil before sterilization in autoclave at 115°C for 15 min. After the sterilization of medium, the cultural organisms were inoculated into each of the tubes respectively and durham's tubes were inserted in an inverted position into each of the tubes. Then, they were inserted in an inverted position into each of the tubes. They were also incubated at 37°C for 24 h. A change in the coloration of medium after 24 h from purple to yellow indicated acid production due to the fermentation of sugar by the organisms while retention of the purple color indicated a negative reaction. Gas production was shown by the presence of bubbles on the surface of the medium and on upward movement of the inverted durhams's tubes.

Indole Test

Some organisms have tryptophanese enzyme which helps them to hydrolyze the amino acid tryptophan. The sterile wire loop was used to inoculate organism in a test tube containing 5 ml of peptone water (medium) and incubated for 48 h at 37°C. After incubation, 0.5 ml of Kovac's reagent was added into the tube and allowed to stand for 15 min. A rose spank color indicated positive reaction.

Methyl Red Test

In carrying out this test, a test organism was inoculated in a test tube containing 5 ml of prepared peptone water and was incubated for 48 h. At 37°C after incubation, 0.5 ml of methyl red was added into the test tubes and allowed to stand for 15 min. Red color indicated positive result.

RESULTS AND DISCUSSION

The pH at the initial time of steeping during the production of ogi was 6.8 and at the end of the steeping the PH reduced to 4.7. Table 2 shows that pH value during the steeping of maize for ogi production. The decrease in pH may be as a result of the activities of lactic acid bacteria which is responsible for the production of lactic acid during the steeping of maize grains for ogi production (Odunfa and Adeyele, 2000).

The total plate count of bacteria from steeping water after 24 h of steeping was 70 and this increased to 150 counts at the end of steeping. Table 3 shows the total count of bacteria from steeping water. This increase could be as a result of increase in acidity of the steeping water that favors the growth of lactic acid bacteria. The total count from ogi I and ogi II at initial stage was 40. The bacteria in ogi I increased due to the microbial build up in ogi I as the water had not been changed.

The bacteria organisms isolated from both steep water and soaked ogi were *Lactobacillus* and *Leuconostoc* species. The exposure of water to direct rays from sun provides the required warmth and physical condition for the growth of *Escherichia* species (Ozoh and Kuyanbana, 2006). Ozoh and Kuyanbana (2006) also affirmed water as the source of *Escherichia* species in maize and other cereal porridge. On the other

Table 1: Change of pH Value During The Steeping of Maize for Ogi Production

Steeping days	pH
1	6.8
2	6.0
3	5.0
4	4.7

Table 2: The Total Plate Count of Bacteria From Steep Water

Days	Total Plate Count
1.	70
2.	90
3.	125
4.	150

hand, the growth of *Lactobacillus* species was unhindered at the different stages of ogi production. Relatedly, an exponential increase in

Table 3: Standard Plate Count of Different Colonies From Steep Water

Days	A	B	C
1	-	50	20
2	50	20	20
3	80	15	30
4	75	20	55

KEYS: A= Cream coloured of MRS agar; B = Green coloured on CLED agar; C = Small cream on NA.

growth of lactic acid bacteria was earlier reported by Kunele (1999) in both fermented and cooked porridge.

From this research work, it was observed that water is the major source of bacterial contamination of ogi. Omemu and Adeosun (2010) said that ogi could be contaminated through the muslin clothes used in sieving the shaft due to lack of sterilization or not being washed thoroughly before using it. The critical

Table 4: Morphological Characteristics of Bacterial Isolated From Steep Water

Code No	Morphological Characteristics	Gram Reaction	Presumptive Organisms
A	Cream coloured colonies on mrs	Gram positive rod in shape	*Lactobacillus* spp
B	Green coloured on CledLight yellow on Cled agar	Gram negative cocci in shape and Gram negative and spherical in shape	*Pseudomonas* spp*Escherichia* spp
C	Cream colour on nutrient agar	Gram positive rod in shape	*Lauconostoc* spp

Table 5: Biochemical Test on Bacteria Isolated From Steep Water

Code No.	Catalase	Motility	Indole	Methylated Red	Glucose	Lactose	Sucrose	Mannitol	Presumptive Organism
A	+	+	-	-	AG	AG	AG	-	*Lactobacillus* spp.
B Light yellow	-	+	+	+	AG	AG	AG	AG	*Escherichia* spp.
Green color	-	+	-	+	G	-	-	G	*Pseudomonas* spp.
C Small cream color	-	-	-	+	AG	-	-	AG	*Lauconostoc* spp.

Key: A= Acid; B = Gas; AG = Acid and Gas; + = Positive; - = Negative.

Table 6: Total Plate Count of Bacteria From Stored Ogi I and II

Days	Stored Ogi I	Stored Ogi II
1	40	40
2	45	38
3	50	35
4	59	30
5	64	27
6	72	24
7	82	20

points of contamination during the production of ogi included points of soaking the maize, milling and sieving.

Table 7: Standard Plate Count of Bacteria of Different Colonies From Stored Ogi I and II

	Ogi I			Ogi II		
Days	A	B	C	A	B	C
1	18	12	10	18	12	10
2	20	15	10	20	10	8
3	22	18	10	18	8	9
4	25	23	11	15	8	7
5	30	25	9	17	5	5
6	35	25	12	15	5	4
7	40	31	11	10	3	7

Keys: Ogi I = Ogi with unchanged water; Ogi II = Ogi with changed water; A = Cream coloured on MRS agar; B = Green, blue, and light yellow on CLED agar; C = Small cream on nutrient agar.

Table 8: Morphological characteristics of Bacteria Isolated from Stored Ogi

Code No	Morphological Characteristices	Gram Reaction	Presumptive Organism
A	Rod in shape	Gram positive rod	*Lactobacillus* spp.
B	Light yellow and spherical in shape	Gram negative	*Escherichia* spp.
	Green colour and coci	Gram negative	*Pseudomonas* spp.
	Blue growth and spherical in shape	Gram negative	*Proteus* spp.
C	Small cream colour and rod shape	Gram positive	*Lauconostoc* spp.

Table 9: Biochemical Test on Bacteria Isolated Form Store Ogi I and Ogi II

Code No.	Catalase	Motility	Indole	Methylated Red	Glucose	Lactose	Sucrose	Mannitol	Presumptive Organism
A	+	+	-	-	AG	AG	AG	-	*Lactobacillus* spp.
B Light yellow	-	+	+	+	AG	AG	AG	AG	*Escherichia* spp.
Green colour	-	+	-	+	G	-	-	G	*Pseudomonas* spp.
Blue colour	+	+	-	-	AG	AG	AG	-	*Proteus* spp.
C	-	-	-	+	AG	-	-	AG	*Lauconostoc* spp.

Key: A= Acid; B = Gas; AG = Acid and Gas; + = Positive; - = Negative.

CONCLUSION

In conclusion, water could be the major source of contamination of ogi as shown from the results of this research.

RECOMMENDATION

To increase the shelf life of ogi, good and personal hygienic practices should be maintained at every stages of production in other to avoid contamination. Again, appropriate training of attendants and vendors about the safety standards and good manufacturing practices are essential to maintaining contaminants free ogi and ensuring good health to the consumers.

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