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Genomic Sequencing of the Bacteria involved in the Fermentation of Ogiri and Okpehe

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Abstract

This research focuses on the genomic sequencing of the bacteria involved in the fermentation of Okpehe and Ogiri. Isolation of the bacteria was done on the fermented samples by inoculating the samples on Nutrient Agar and MacConkey Agar and Biochemical tests (Gram staining, citrate, Oxidase and Catalase) was carried out on the organisms obtained. DNA extraction, Polymerase Chain Reaction, Gel electrophoresis and Genomic sequencing was done on the predominant bacteria in both Ogiri and Okpehe.

Introduction

Ogiri-eguisi is an indigenous fermented soup condiment which is used as a flavoring agent whose character and organoleptic properties depend on microbial activities [1].

It has an oily gray pasty consistency with a very strong pungent smell when it is in its raw state. It is produced by traditional methods of uncontrolled solid substrate fermentation of melon seeds. The raw seeds are boiled, the water drained and allowed to ferment naturally for four days in clay pots. The fermented seeds are then mashed into pastes, wrapped in leaves and kept over a fire place to dry. In Nigeria, melon is mostly cultivated in the southeastern part and is usually interplanted with yam and cassava where it serves as a cover crop [2]. Ogiri eguisi is known to contribute to the caloric and protein intake and are generally added to soups as low-cost meat substitute by low income families in parts of Nigeria [3]. Melon seeds contain good levels of crude protein which range from 24.3 - 41.6% dw while fat content range from 42.9 - 57.3% [4].

Okpehe is used as a food condiment in Nigeria by the Idoma and Igala people of the middle belt region and some parts of the Eastern and Southern Nigeria. It adds variety and pleasure to the otherwise monotonous traditional diet. It serves not only as a seasoning agent but also as a low-cost source of protein in the diet [5]. Okpehe can serve as a substitute for meat for low-income earners and can reduce protein-calorie malnutrition and essential fatty acid deficiencies [5].

Okpehe is consumed as a food supplement eaten mixed with other foods like tapioca, boiled yam, boiled maize, fish and meat. These are usually mixed with palm oil, pepper and other additions like salt and akanwu (trona-sodium sesquicarbonate or potash) [6]. Okpehe is generously applied to soups as a flavor enhancer and consumed as a meat analogue especially by the poor people [7]. Fermented *Prosopis africana* seeds are rich in protein and may be used in exceptional cases as condiment for bread production [8].

Materials and Method

Study Area

This study was carried out at the laboratory of the Department of Microbiology and department of chemistry at the

Sample Collection

Fresh samples of *Citrullus colocynthis* and *Prosopis africana* seeds (unfermented) were purchased from Gwagwalada market in FCT, Abuja and placed in a clean plastic container and transported to the laboratory.

Preparation of Sample Using Traditional Method

African mesquite condiment and Ogiri were produced traditionally. Raw mesquite seeds (1000g) were cleaned to remove extraneous materials. The seeds were washed in clean water, drained and boiled for 12 hours. The boiled seeds were de-hulled and cotyledons were boiled in enough quantity of water for one hour and drained. Dehulled melon seeds were also boiled for four hours. The boiled mesquite and melon seeds were allowed to cool, ground to form paste, wrapped and left to ferment for five days, moulded into shapes and stored in the refrigerator.

Microbiological Analysis of Fermented Sample

One gram of sample was diluted serially in seven folds dilution. 10^{-2} Diluted sample was streaked into sterile molten agar medium plate MacConkey Agar (MA) and Nutrient Agar (NA) were used to Isolate bacteria. Then the plates were incubated at 37°C.

Biochemical Tests

Biochemical tests was carried out according to the method described by Ogbu J. C. et al., (2024)

Molecular Characterisation of the Isolate

DNA extraction, Polymerase Chain Reaction, Gel electrophoresis and Genomic sequencing was done on the bacteria.

Results

Biochemical Test Result

Gram Rxn	Citrate	Catalase	Oxidase	Motility	Probable Organism
-	-	+	-	+	Klebsiella spp

Morphological Characteristics

Probable Organism	No of Colony	Size	Shape	Optical property	Elevation	Margin	Colour	Media
Klebsiella spp	14	Large	Irregular	Translucent	Raised	Entire	Pink	Mac
	18	Large	Irregular	Translucent	Raised	Entire	Pink	Mac

Sequencing result

CTCCCMGGGCGGGKCGATTAAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCWAAT
 CGACATCGTTTACGGCGTGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTCGACCTGAGC
 GTCAGTCTTTGKCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTACCGCTACA
 CCTGGAATTCTACCCCCCTCTACMAGACTCTAGCYTGCCAGTTTCGAATGCAGTTCCAGGTTGAGCCCG
 GGGATTTACATCCGACTTGACARACCGCCTGCGTGCGCTTTACGCCAGTAATTCCGATTAACGCTTGC
 ACCCTCCGTATTACCGCGGTGCTGGCACGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATCGM
 YRAGGKTATTAACCTYAWSGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCCGAAGGCCTTCTTCAYACAC
 GCGGCATGGCTGCATCAGGCTTGCGCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGG
 ACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCC
 RTTACCYCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCRTGAGGCCCGAAGGTCCCCWCTTT
 GGTCTTGCGACRTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGWTTCCCA
 GACATTACTACCCGTCCGCCGCTCGTCACCCGAGARMAAGCTCTCTGTGCTAYCGCTCKACWTGYRTGT
 GTKAKGCCTGCCGCCAGCRATCTATMTSAKMCAYGAAWCWMWACWCTAYARA

Reverse sequence

AYCAACTCTACGGGKACCTGTGYTACGACTTGYTGAGCTAYSTTCTWACTCTAGAACCCACTCCCATGG
WGTGACGGGSGGTGTGTACAAGGCCCGGGAACGTATTCACCGTAGCATTCTGATCTACGATTACTAGC
GATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACATACTTTATGAGGTCCGCTTG
CTCTCGCGAGGTCGCTTCTCTTTGTATATGCCATTGTAGCACGTGTGTAGCCCTGGTCGTAAGGGCCATG
ATGACTTGACGTCATCCCCACCTTCCTCCAGTTTATCACTGGCAGTCTCCTTTGAGTTCCCGGCCAACCG
CTGGCAACAAAGGAWAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATTTACAACACGAGCTGAC
GACAGCCATGCAGCACCTGTCTCACARTTCCCGAAGGSACCAATCCATCTCTGGAAAGTTCTGTGGATGT
CAAGASCAGGTAAGGTTCTTCGCGTTGCATCGAATTAACCACATGCTCCACCGCTTGKGCGGGCCCCCG
TCAATTCATTTGAGTTTAACTTGCGGCCGTACTCCCCAGGCGGTCGAYTTAACGCGTTAGCTCCGGAA
GCCACGCCTCAAGGGCACAACCTCCAAATCGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCT
GTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTMTTGTCCAGGGGGCCGCCTTCGCCACCGGTATTC
CTCCAGATCTCTACGCATTTACCGCTACACCTGGAATTCTACCCCCCTCTACAAGACTCTAGCCTGCCAG
TTTCGAATGCAGTTCCCAAGTTGAGCCCGGGGATTTACATCCGACTTGACAGACCGCCTGCGTGCGCTT
TACGCCCAGTAATTCCGATTAACGCTTGACCCCTCCGTATTACCGCGGCTGCTGGCACGARTTAGCGGTG
CTTCTTCTGCGGGTAACGTCAATCGACGAGTATTACCTCAWCGCCTTCCTCCCCGCTGAAGTGCTTACAA
CCGAAGGCCTTCTTCMMCMCCGCATGGCTGCATMAGGCTTGCGCCATTGTCATATGCCCACTGCTGCT
CCGGTAGGGCTGAACCGTTYAGTCATGTGCTGGCATCCTTCAGAACAGCTAGAATCTGGCTAGTAGCGT
ACCACCTMTAGCTAGCCATTCTRGACATCTKWAWGGCSTA

Conclusion

The number of Organisms isolated is limited to only Organisms that can grow in MacConkey agar. According to the genomic sequencing result, the organism isolated is *Klebsiella pneumoniae*. *Klebsiella pneumoniae* is the common bacteria involved in the fermentation of Ogiri and Okpehe.

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