TANNASE PRODUCTION BY BACTERIAL ISOLATES FROM DETERIORATED BAMBARA (*Vigna subterranean* (L) *Verde)* NUTS

BY

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (MSc.) DEGREE IN MICROBIOLOGY

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CERTIFICATION

I certify that this project work was carried out by EGUNJOBI ADEYINKA KAMORU, 15PCQ01216 in the Microbiology programme of the Department of Biological Science, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria.

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DECLARATION

I, EGUNJOBI ADEYINKA KAMORU, hereby declare that this project work was carried out by me in the Department of Biological Sciences, College of Science and Technology under the supervision of Professor A. A. Ajayi

The project work has not been submitted anywhere else for a degree award. The ideas are the products of a research conducted by me. All ideas from other authors have been duly acknowledged.

EGUNJOBI ADEYINKA KAMORU ----------

Researcher Signature and Date

DEDICATION

I dedicate this project work to God Almighty, the giver of life, my sustainer, provider of all resources and most of all the supplier of my strength, without whom this work would not become a reality.

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To God be the glory, this is great and marvelous in my sight.

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ABSTRACT

Tannase are hydrolytic enzymes that can be obtained from fungal and bacterial sources. They are used in the preparation of wine, beer, instant tea, coffee flavoured soft drinks and for clarification of juice. They are also used for treatment of waste water contaminated with polyphenolic compounds such as tannins which has been associated with environmental pollution. The immense potential of tannase has made it very important to optimize the production process to achieve maximum yields. Thirty-five grammes of Bambara nuts were thoroughly sorted and cleaned. Serial dilutions and subculturing of samples was carried out to obtain pure bacteria isolates. The isolates were screened for tannase production. Tannase producing bacterial isolates were identified based on clear zones of hydrolysis on tannin supplemented agar plates. They were further characterized by subjecting the isolates to various biochemical and molecular tests. The activity of the tannase was determined after termination using bovine serum albumin solution. Partial purification was carried out by ammonium sulphate precipitation. The optimum conditions of the tannase were determined using three parameters of temperature, pH and substrate concentration**.** Seven bacterial isolates (Six Gram positive and One Gram negative) were obtained. Two of the bacterial isolates (Isolate code G and C) were tannase producing bacteria with the highest clear zones of hydrolysis. Optimum conditions were ascertained at pH 6.0, temperature of 37^{o}C and substrate concentration of 1.0% for isolate G while isolate code C had pH 6.0, temperature of 35^{o}C and substrate concentration of 1.2% (w/v). Molecular identification of the tannase producing bacterial isolates revealed isolate code G as *Bacillus pumilus* strain AJ-2 (Accession number MF083685) and isolate code C has having 94% similarity with *Aeromonas sanarellii* strain A2-67**.** The Michaelis-mentens constant (Km) which is also known as the dissociation constant is the substrate concentration at half maximum velocity. Calculated from the

Lineweaver-Burk plot, the apparent Km for the hydrolysis of tannic acid by tannase producing bacteria isolate code C and G were approximately 0.18mg/ml and 0.06mg/ml respectively. The results of this study established that Bambara nuts are host to tannase producing bacteria. This is the first report of tannase from the strains of bacterial isolates studied.