BIODEGRADATION OF OCHRATOXIN A IN SELECTED FOOD SPICES USING LACCASE OBTAINED FROM FUNGI ISOLATED IN OTA

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc) DEGREE IN MICROBIOLOGY IN THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF SCIENCE AND TECHNOLOGY, COVENANT UNIVERSITY, OTA, OGUN STATE, NIGERIA.

AUGUST, 2024

ACCEPTANCE

This is to attest that this dissertation is accepted in partial fulfilment of the requirements for the award of the degree of Master of Science in Microbiology in the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Ogun state, Nigeria.

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Prof. Akan B. Williams
(Dean, School of Postgraduate Studies) **Signature and Date**

DECLARATION

I, **AKINYELE, AYOMIDE FAITH (22PCQ0455)** declare that this research was carried out by me under the supervision of Dr. Eze F. Ahuekwe and Dr. Benson E. Adeola of the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Nigeria. 1 attest that the dissertation has not been presented either wholly or partially for the award of any degree elsewhere. All sources of data and scholarly information used in this dissertation are duly acknowledged.

AKINYELE, AYOMIDE FAITH

Signature and Date

CERTIFICATION

We certify that this dissertation titled " **BIODEGRADATION OF OCHRATOXIN A IN SELECTED FOOD SPICES USING LACCASE OBTAINED FROM FUNGI ISOLATED IN OTA**" is an original research work carried out by **AKINYELE, AYOMIDE FAITH (22PCQ0455)** in the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria under the supervision of Dr. Eze F. Ahuekwe. We have examined and found this work acceptable as part of the requirements for the award of Master of Science in Microbiology.

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Dr. Isaac O. Ayanda
(Head of Department) **Signature and Date**

Prof. Anthonia O. Oluduro
(External Examiner) **Signature and Date**

Prof. Akan B. Williams
(Dean, School of Postgraduate Studies) **Signature and Date**

DEDICATION

This work is dedicated to the GOD, who is the Father of all research and wisdom, and the source of strength and inspiration behind this research.

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ABSTRACT

The carcinogenicity, hepatotoxicity, and nephrotoxicity associated with the ingestion of ochratoxin A (OTA)-contaminated food spices demand an effective biodegradation approach that ensures food safety and sustainability. The aim of this research was to produce and characterise laccase from selected fungal specie and to determine its effectiveness in degrading OTA present in selected food spices. Fungal species were isolated from soil samples and screened for laccase production. Selected laccase-producing fungi was induced for quantitative laccase production by solid-state fermentation using a laccase basal medium supplemented with pre-treated corncobs. The produced laccase was extracted, characterised and enzyme activity analysed. The OTA in the selected food spices was detected and quantified using High-Performance Liquid Chromatography (HPLC). Using computational tools, insights were also provided on the mechanisms of biodegradation of OTA using obtained laccase. Eight fungi species were isolated from the soil sample, of which three screened positive for laccase production. The most efficient laccase-producing fungi (identified by intensity of the brown colouration on the screening medium) was selected and identified as *Pleurotus* sp. The laccase obtained from the selected fungi isolate had an activity of 51.93±1.8 U/mL. Following characterization, the optimum condition of temperature and pH were 70^{o}C and 7 respectively. HPLC chromatogram showed the presence of OTA at peak area of 132.55 and 132.16 for both chilli and turmeric respectively. There was a 29.5% and 29.9% reduction in OTA extracted from the selected chilli and turmeric spices respectively after incubation with the obtained laccase. Statistically, there were significant differences between the levels of detected OTA in the spices before and after incubation with laccase ($p<0.05$). The laccase was computationally predicted to bind to the phenol of the OTA which bears the chlorine atom responsible for the toxicity of the OTA. This binding results in a cleavage of the phenol group resulting in the breakdown of the OTA into OTα and L-β-phenylalanine. Therefore, treatment with laccase serves as an effective method for the degradation of OTA in food spices.

Keywords: Biodegradation, Computational Analysis, Laccase, Ochratoxin A, Spices