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Assessment of Surfactant Degrading Potential of Fungal Isolates from Detergent Contaminated Soil in Ondo State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author DJA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AKO and AY managed the analyses of the study. Author AY managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To isolate and identify fungal flora from the detergent contaminated soil in Ondo State, Nigeria and also to evaluate biodegrading potentials of the potent isolates by comparing and quantifying their enzyme activity.
Place and Duration of Study: Ondo State, Nigeria, between June and October, 2017.
Methodology: Detergent degrading fungi were isolated from detergent contaminated soil by supplementing culture media with test surfactant. The isolated fungi were subjected to enzyme analysis to study the alkylsulphatase enzyme production/activity.
Results: Six fungal isolates showed remarkable potential for alkylsulphatase production. In the enzyme study, *Aspergillus clavatus* (1.48 mM/min), *Aspergillus flavus* (1.46 mM/min) and *Geotrichum candidum* (1.40 mM/min) showed better enzymatic action in the enzyme study as compared to others.
Conclusion: It can be concluded that *Aspergillus clavatus*, *Aspergillus flavus* and *Geotrichum candidum* can be found in soil environment polluted with detergent. They possess the mechanism

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involved in the degradation of detergent thus they are capable of surviving the toxic effect of the pollutant. They can efficiently produce alkylsulphatase; thus can be employed in enzyme production and utilised in the bioremediation of environments contaminated with surfactants.

Keywords: Alkylsulphatase; bioremediation; detergent; enzyme; soil.

1. INTRODUCTION

Detergents are chemical substances majorly used for cleaning purposes such as laundry and dish washing. The main component of the detergent is the surfactant which aids cleaning processes [1]. They can be found in both solid and liquid forms. Surfactants are organic substances which enhances the cleaning, rinsing and/ or fabric softening process due to their surface-active properties and are discharged into the environment either directly or indirectly by wastewater pathway where no treatment is available or from a waste water treatment plant [1]. They can thus act on biological waste water treatment processes and hinder the aeration and treatment facilities due to their high foaming and low oxygenation capacity [2]. Because of their large consumption world wide, surfactants have the potential for wide disposal into the aquatic and terrestrial environment. Large consumption of these substances in the world together with their adverse effects on living organisms makes these chemical substances one of the major environmental concerns [3]. Biodegradation of surfactants is being performed by soil or aquatic microorganisms leading to the generation of water, biomass, salts and carbon (iv) oxide gas [3]. Surfactants drastically affect the different trophic levels of the food chain including microbes, invertebrates, fish, plants and higher vertebrates including man [4]. These substances are toxic to microbes, plants, animals and man. Such toxicity can lead to stunted growth and eventually death in plants. It has a lethal effect on soil microbes [5]. It could be carcinogenic and lead to low sperm count when ingested in man [6]. These substances could lead to developmental disorder in frog and toad, snails, worm and fish. Detergent as a pollutant when present in the soil environment can disrupt the soil microbial ecosystem, and affect soil fertility thereby resulting in the ecological imbalance. Surfactants may enter the terrestrial environment through several routes; one of such is majorly the use of sewage sludge as fertiliser on agricultural land. High concentrations of surfactants and their degradation products may affect the biota. On the other hand, due to their amphiphilic nature, surfactants may interact with both inorganic as well as organic contaminants affecting their bioavailability [7]. The alkylsulphatase enzyme produced by some microorganism is involved in the biodegradation of detergents, which hydrolyses inorganic sulphate from its ester linkage with alcohols, the later being readily assimilated through normal metabolic pathways [8].

This research therefore, evaluates the biodegrading capability of some fungal flora isolated from soil contaminated with detergent on surfactant, in Ondo State, Nigeria by comparing the alkylsulphatase production activities of the fungal isolates.

2. METHODOLOGY

2.1 Collection of Samples

Soil samples were collected from carwash parks, in selected major towns (with high availability of active carwash parks) in Ondo State; Akure, Owo, Idanre, Ikare, Ondo and Ore. The soil samples were collected (within the depths of 5 cm to 15 cm) and kept in sterile containers. The containers were labelled and transported to the laboratory for analysis.

2.2 Isolation of Detergent Degrading Fungi

Isolation of detergent degrading fungi from the soil samples was done by collecting the soil samples in sterile containers from the carwash parks; where the waste water effluent is being deposited. Serial dilutions were carried out. The diluted soil sample was inoculated onto potato dextrose agar supplemented with test surfactant. Incubation was done at room temperature for 5 days and observations were made for fungal identification and characterisation [9]. The isolated fungi were characterised by macroscopic and microscopic techniques [9,10].

2.3 Determination of Alkylsulphatase Production

2.3.1 Preparation of enzyme extract

Fungal broth culture was incubated at 23°C for a period of 72 hours in an orbital shaker at 150

rpm. Some certain quantity (Fifty millilitres) of the broth culture was collected at the end of specific time intervals (twelve hours) and it was centrifuged for 15 minutes at 4°C. The supernatant was decanted off [11]. Cell pellets were collected with one millilitre (1 ml) of tris buffer and it was later subjected to homogenisation for a period of 15 minutes. The homogenised pellets were collected and centrifuged for 15 minutes at 4°C. The supernatant was collected and kept for the enzyme assay [11].

2.4 Alkylsulphatase Enzyme Assay

Four hundred and fifty micro litres (450 µl) of fifty millimolar (50 mM) Tris-hydrochloric acid (pH 7.5) and five hundred micro litres (500 µl) of one hundred millimolar (100 mM) SDS was pipette into a container of one hundred micro litres (100 µl) of the enzyme. It was then incubated for a period of time (15 minutes). One hundred micro litres (100 µl) of the mixture, 9.9 ml of distilled water, two and a half millilitres (2.5 ml) of methylene blue solution and one millilitre (1 ml) of chloroform was pipette into a separating funnel and shaken vigorously for 40 seconds. A chloroform layer was formed. The chloroform layer formed was collected into a tube by carefully releasing the separating funnel tap and the absorbance which indicates the quantity of enzyme produced was read at 600 nm. Enzyme activity was determined by evaluating the rate of SDS (sodium dodecyl sulphate) elimination with respect to quantity of enzyme used and time of incubation [11].

2.5 Analysis of Data

Data obtained were subjected to descriptive one way analysis of variance, using SPSS version 16 and treatment means were separated with Duncan's Multiple Range Test.

3. RESULTS AND DISCUSSION

The detergent degrading fungi isolated from the contaminated soil were *Articulospora inflate*, *Penicillium italicum*, *Aspergillus clavatus*, *Aspergillus flavus*, *Mucor mucedo* and *Geotrichum candidum*. Some of which were isolated in other related research [9]. The isolates were majorly from soils collected in Akure town, Owo town, Idanre town and Ore town. Fig. 1 depicts the enzyme activity of *Articulospora inflate* having its highest enzyme activity as 1.35 mM/min, while its optical density was 1.0 at this point. Fig. 2 illustrates the enzyme activity of *Penicillium italicum* having its highest enzyme activity as 1.23 mM/min, its optical density was 1.62 at this point. Fig. 3 shows the enzyme activity of *Aspergillus clavatus*, the highest enzyme activity of *Aspergillus clavatus* was 1.48 mM/min and its optical density was 1.34 at this point. Fig. 4 depicts the enzyme activity of *Aspergillus flavus* having its highest activity as 1.46 mM/min at an optical density of 0.96. Fig. 5 depicts the enzyme activity of *Mucor mucedo*, it was able to produce an highest enzyme activity of 1.35 mM/min at an optical density of 1.00. Fig. 6 illustrates the enzyme activity of *Geotrichum candidum*, its highest enzyme activity was 1.04 mM/min at an optical density of 1.12.

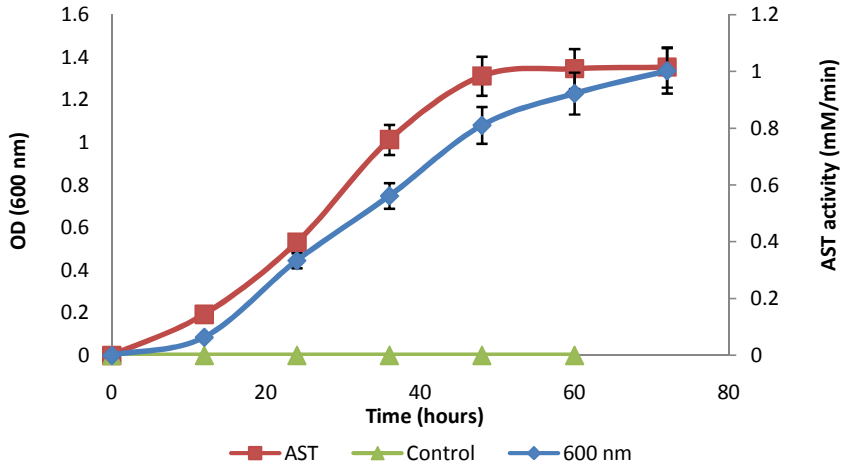

Fig. 1. Alkylsulphatase activity (AST) of *Articulospora inflate*

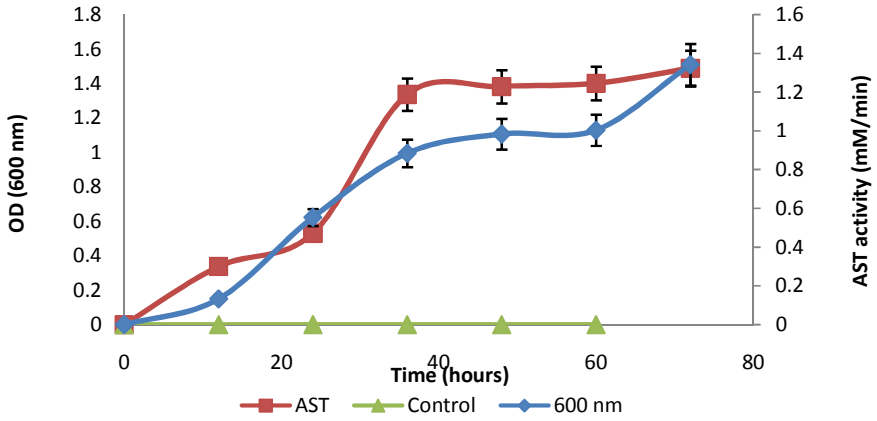

Fig. 2. Alkylsulphatase activity (AST) of *Penicillum italicum*

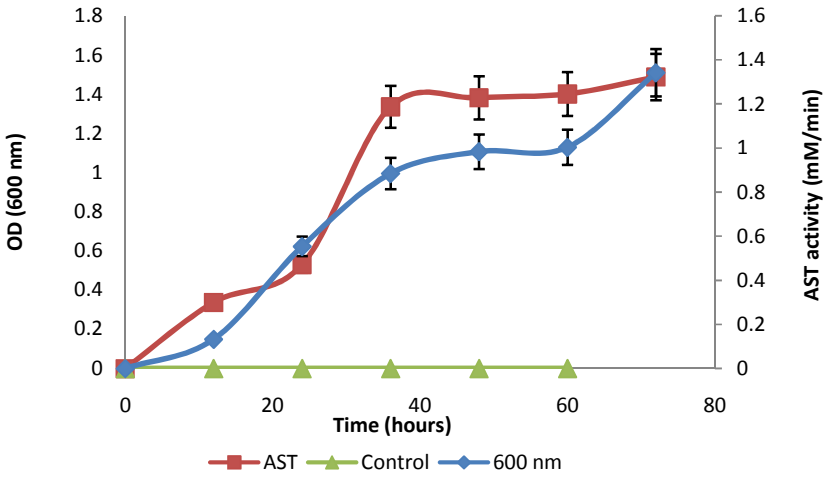

Fig. 3. Alkylsulphatase activity (AST) of *Aspergillus clavatus*

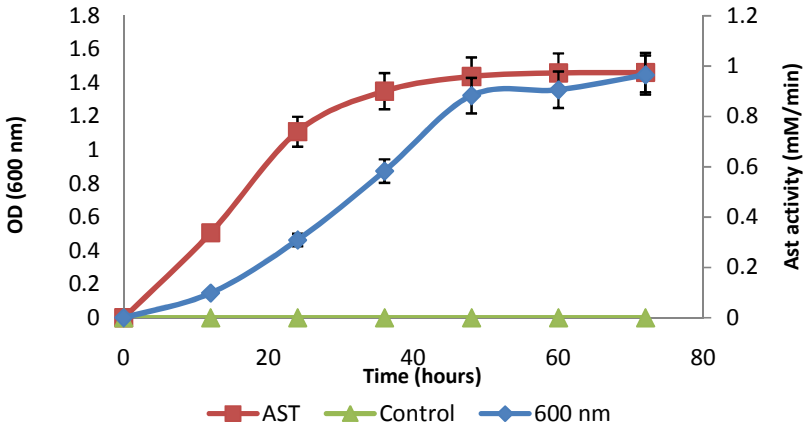

Fig. 4. Alkylsulphatase activity (AST) of *Aspergillus flavus*

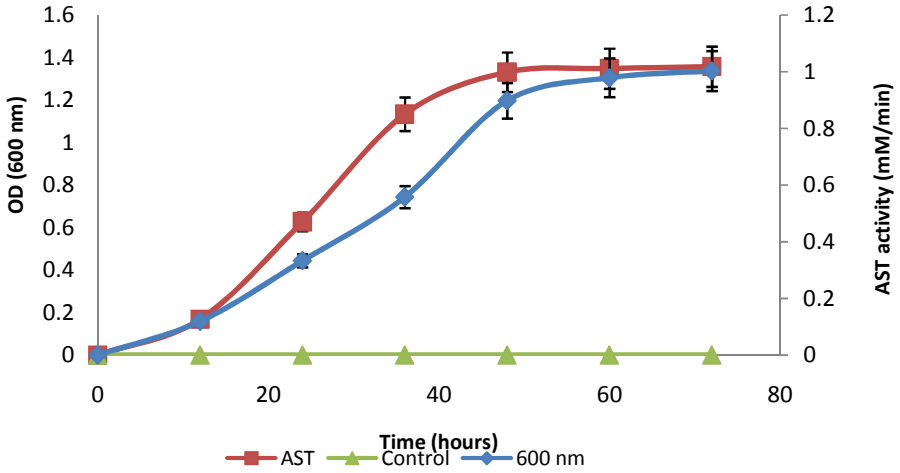

Fig. 5. Alkylsulphatase activity of *Mucor mucedo*

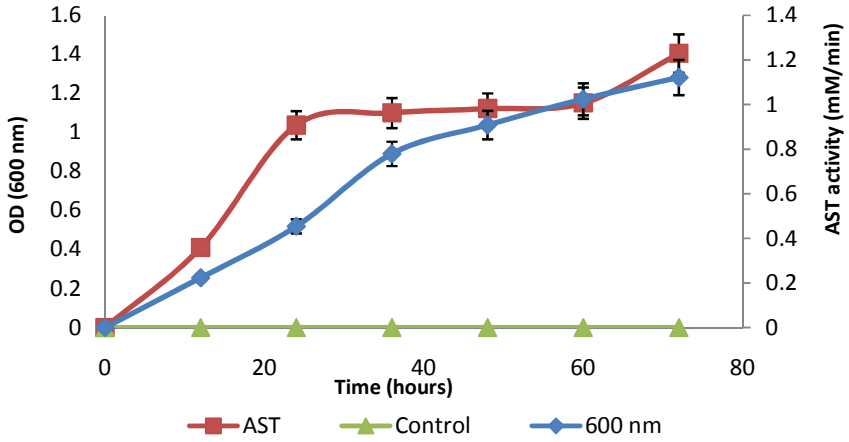

Fig. 6. Alkylsulphatase activity (AST) of *Geotrichum candidum*

In the enzyme studies, the fungal flora isolated from the polluted soil samples were able to produce the alkylsulphatase enzyme. Fungi act as potential degraders by excreting enzymes from vesicles on top of their hyphae to their environment to break down pollutants to simpler forms; which are being absorbed for growth and biomass accumulation [12]. Variations in the alkylsulphatase enzyme production capacities of the various fungal isolates could be as a result of their genetic makeup [11]. There were variations in the quantity of alkylsulphatase enzyme activities produced by the fungal isolates and this could be as a result of molecular mass of alkylsulfatase; which is found to vary in different bacterial species and genera [13]. Some of these fungal isolates were also observed to posses the ability to produce the alkylsulphatase enzyme in other related research [14]. The better adapted fungi will produce more of the enzyme. The results suggest that bioremediation by the fungal isolates are promising for the biodegradation of surfactants as pollutants in the soil environment.

4. CONCLUSION

The study illustrates the pattern of alkylsulphatase enzyme production and activities of the various fungal isolates in relation to time and microbial growth. It can be concluded that *Aspergillus clavatus*, *Aspergillus flavus* and *Geotrichum candidum* can be found in soil environment polluted with detergent. They possess the mechanism involved in the degradation of detergent thus they are capable of surviving the toxic effect of the pollutant. They

can efficiently produce alkylsulphatase; thus can be employed in enzyme production and utilised in the bioremediation of environments contaminated with surfactants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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