Original Research Article

COMPARATIVE STUDY OF THE ALKYLSULPHATASE ACTIVITIES OF BACTERIA FOUND IN SOIL CONTAMINATED WITH DETERGENT IN ONDO STATE, NIGERIA

ABSTRACT

Aim: To isolate, characterize and identify detergent degrading bacteria from detergent contaminated soil in Ondo State, Nigeria and also to compare and quantify enzyme production and biodegrading potentials of each of the bacterial isolate.

Place and Duration of Study: Ondo state, Nigeria, between February and July, 2017.

Methodology: Detergent degrading bacteria were isolated from detergent contaminated soil samples by supplementing minimal salt media with test surfactant. The bacteria isolated were subjected to enzyme analysis to study the alkylsulphatase enzyme production/activity in relation to growth pattern.

Results: Some bacterial isolates showed remarkable potential for alkylsulphatase production. In the enzyme study, *Bacillus subtilis* (1.53 mM/min), *Pseudomonas putida* (1.36 mM/min) and *Pseudomonas fluorescens* (1.33 mM/min) showed better enzymatic activity than the other isolates. *Bacillus subtilis* showed the highest enzymatic activity of 1.53 mM/min.

Conclusion: It can be concluded that *Bacillus subtilis*, *Pseudomonas putida* and *Pseudomonas fluorescens* can be found in soil environment polluted with detergent. They are capable of surviving the toxic effect of the pollutant and efficiently producing alkylsulphatase; thus can be employed in enzyme production. They are capable of degrading detergent as a pollutant; thus can be utilized in the bioremediation of soil environments contaminated with surfactants

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Keywords: Alkylsulphatase, bioremediation, detergent, enzyme, *soil*.

INTRODUCTION

Soil is a mixture of minerals, organic matter, gases, liquids and countless organisms that support life on earth. Soil continually undergoes development by way of numerous physical, chemical and biological processes, which include weathering with associated erosion. Soil functions as a medium for plant growth [1]. It purifies, stores and supplies water [2], and influences distribution of plant species and provides a habitat for a wide range of organisms [3]. Soil is fundamental to human life on earth. Most plants require a soil substrate to provide water and nutrients, and whether we cultivate the plants directly or consume animals that feed on the plants; we don't eat without soil [3]. Soil pollution is typically caused by industrial activity, chemicals used in agriculture and improper disposal of waste. Contaminants in the soil have major consequences on human health [3]. Long term exposure to polluted soil affects the genetic makeup of the body and may cause congenital illness and chronic health diseases. Detergents are one of the major pollutants found in the soil after being used mostly in laundry processes [3]. Surfactants are routinely deposited in numerous ways on land and into water systems, whether as part of an intended process or as industrial and house hold waste causing pollution [4]. They are known to be toxic to animals, ecosystems and humans, and can increase the diffusion of other environmental contaminants [4]. Large quantities of surfactants are deposited in sediments and soils via sewage sludge used as fertilizers on land for farming. These surfactants drastically affect different trophic levels of the food chain including microbes, invertebrates, fish, plants and higher invertebrates including man [14]. Biodegradation of surfactants is performed by soil or aquatic microorganisms leading to the generation of water, biomass, salts and carbon (iv) oxide gas [5]. The alkylsulphatase enzyme produced by some microorganisms is involved in the biodegradation of detergents, which hydrolyses inorganic sulphate from its ester linkage with alcohols, the latter being readily assimilated through normal metabolic pathways [6].

This research therefore, assesses the biodegrading capabilities of bacteria isolated from soil contaminated with detergents on surfactants, in Ondo State, Nigeria by comparing the alkylsulphatase activities of each bacterial isolate.

METHODOLOGY

Collection of Samples

Soil samples were collected in replicates from five carwash parks; this was done in the six major towns in Ondo State; Akure, Owo, Idanre, Ikare, Ondo and Ore. The samples were collected in sterile containers, labelled and transported to the laboratory for Analysis.

Isolation of Detergent Degrading Bacteria

Serial dilutions were carried out on the soil samples. The serial diluted samples were inoculated onto minimal salt composition media (containing Dipotassium hydrogen phosphate, Potassium dihydrogen phosphate, sodium chloride, magnesium sulphate, ammonium dihydrogen phosphate, ferrous sulphate and nutrient broth) supplemented with test surfactant (sodium dodecyl sulfate) at 0.01%. The inoculated plates were incubated aerobically at 28^{o}C for 48 hours. At the end of the period of incubation, the plates were checked for growth [7]. The cultural characteristics of pure culture were noted for bacterial characterization [8]. The bacterial isolates were subjected to Gram's reaction and biochemical tests (Voges proskaeur, citrate, Indole, methyl red, oxidase and catalase) to identify the isolates [9].

Determination of Alkylsulphatase Production

Preparation of Enzyme Extract

Minimal salt composition media was prepared in broth form and supplemented with SDS at 0.01%, and it was inoculated with the bacterial isolates. The culture broth was incubated in an orbital shaker at 150 rpm. Fifty millilitres of the broth culture was collected at the end of six hours, increase in optical density which is an index of growth indicating the surfactant (sodium dodecyl sulphate) degradation was measured by taking absorbance reading at 600nm and it was centrifuged at 5,000 rpm for 15 minutes at 4^{o}C. The supernatant was decanted off. The cell pellets at the base of the centrifugation tube were collected using one millilitre (1ml) of tris buffer. The pellets were homogenized for 15 minutes. The homogenized pellets were then centrifuged for 15 minutes at 4^{o}C. The supernatant was decanted and kept for the enzyme assay. The enzyme extraction process was repeated at the end of every six hours [10].

Alkylsulphatase Enzyme Assay

The methylene blue active substance assay was employed here. SDS (sodium dodecyl sulphate) is anionic in nature, and thus, they get detected by the methylene blue active substance assay. Enzyme activity was assayed from the rates of SDS (sodium dodecyl sulphate) elimination [10].

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 pipetted into a container of fifty micro litres (50 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 pipetted into a separating funnel and shaken vigorously for 40 seconds. A chloroform layer was formed. The chloroform layer formed was carefully collected and the absorbance which indicates the quantity of surfactant degraded was read at 600 nm.

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.

RESULTS AND DISCUSSION

The detergent degrading bacteria isolated from the contaminated soil were *Xanthomonas campetris*, *Bacillus subtilis*, *Pseudomonas putida*, *Bacillus panthoteticus*, *Bacillus funiculus*, *Escherichia coli*, *Pseudomonas haloplanktis*, *Bacillus cereus*, *Pseudomonas fluorescens* and *Bacillus anthracis*. Some of which were isolated in other related research [11] [4]. Figure 1 depicts the enzyme activity of *Xanthomonas campetris* having its highest enzyme activity as 1.12 mM/min, while its optical density was 1.45 at this point. Figure 2 illustrates the enzyme activity of *Pseudomonas putida* having its highest enzyme activity as 1.36 mM/min, its optical density was 0.15 at this point. Figure 3 shows the

enzyme activity of *Escherichia coli*, the highest enzyme activity of *Escherichia coli* was 0.70 mM/min and its optical density was 0.99 at this point. Figure 4 depicts the enzyme activity of *Bacillus subtilis* having its highest activity as 1.53 mM/min at an optical density of 1.56. Figure 5 depicts the enzyme activity of *Klebsiella oxytoca*, it was able to produce a maximum enzyme activity of 0.95 mM/min at an optical density of 0.83.

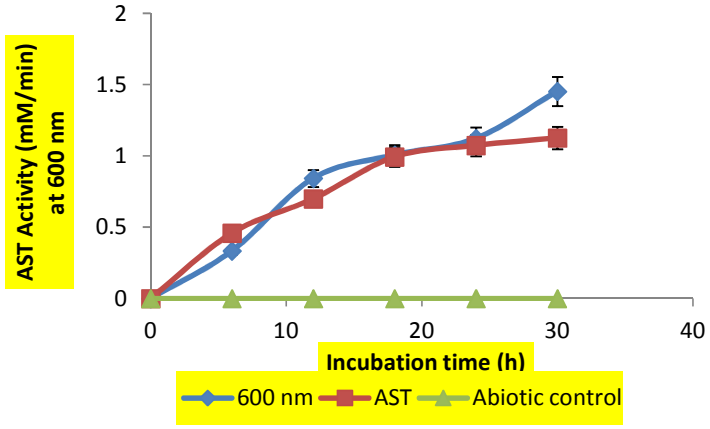


Fig. 1. Alkylsulphatase activity (AST) of *Xanthomonas campetris*

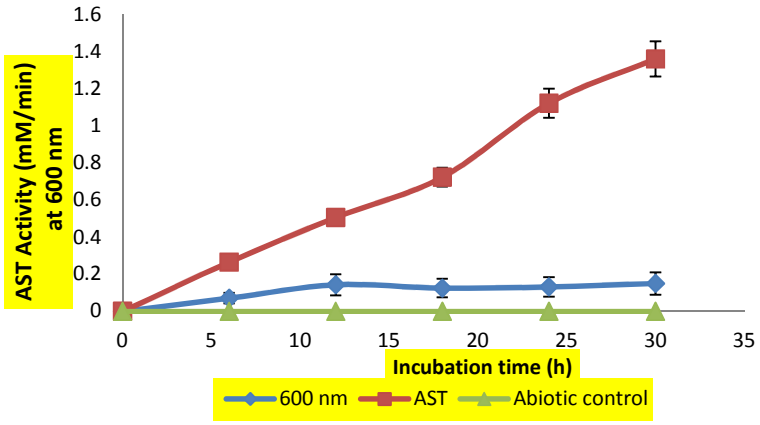


Fig. 2. Alkylsulphatase activity (AST) of *Pseudomonas putida*

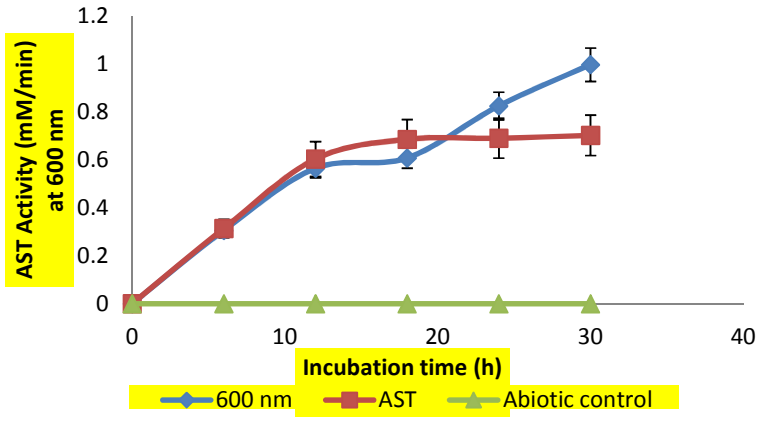


Fig. 3. Alkylsulphatase activity (AST) of *Escherichia coli*

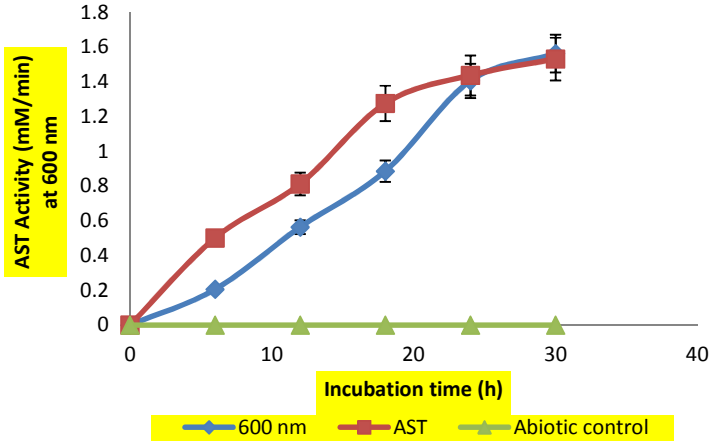


Fig. 4. Alkylsulphatase activity (AST) of *Bacillus subtilis*

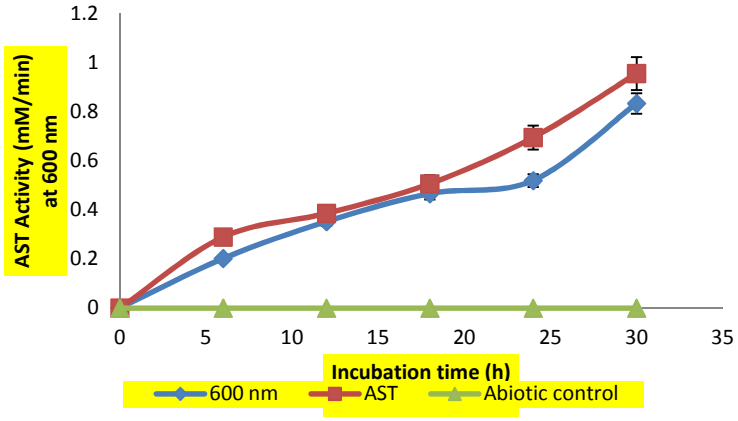
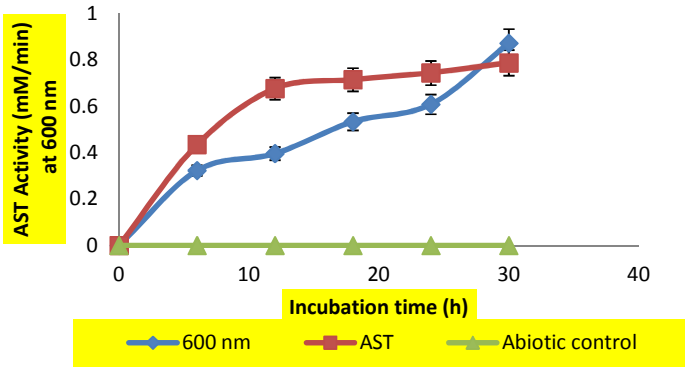


Fig. 5. Alkylsulphatase activity (AST) of *Klebsiella oxytoca*

From Figure 6, *Proteus mirabilis* was able to produce an enzyme activity of 0.78 mM/min, which was the highest. The optical density at this point was 0.87. Figure 7 depicts the enzyme activity of *Bacillus cereus*, it was able to produce an enzyme activity of 1.05 mM/min, which was the highest. Its optical density at this point was 0.90. From Figure 8, *Pseudomonas fluorescens* produced an enzyme activity of 1.33 mM/min, which was its highest. The optical density was 1.68 at this point. From figure 9, *Bacillus anthracis* was able to produce an enzyme activity of 0.92 mM/min, which was its highest, while its optical density at this point was 0.60. The detergent degrading bacterial counts observed at the various specific time intervals of enzyme production are presented in tables 1 and 2. The bacterial load of the individual isolate culture was observed to increase as their various enzyme activity increases at the specific time intervals. The following colony counts were observed when the bacterial isolates were at the peak of their enzyme activity. *Pseudomonas putida* ($73.33 \pm 0.66 \times 10^2$ cfu/ml), *Escherichia coli* ($39.33 \pm 0.33 \times 10^2$ cfu/ml), *Klebsiella oxytoca* ($54.00 \pm 0.58 \times 10^2$ cfu/ml), *Bacillus subtilis* ($81.88 \pm 0.33 \times 10^2$ cfu/ml), *Proteus mirabilis* ($56.33 \pm 0.33 \times 10^2$ cfu/ml), *Bacillus cereus* ($63.00 \pm 0.57 \times 10^2$ cfu/ml), *Pseudomonas fluorescence* ($74.33 \pm 0.88 \times 10^2$ cfu/ml), *Bacillus anthracis* ($53.33 \pm 0.33 \times 10^2$ cfu/ml) and *Xanthomonas campetris* ($68.33 \pm 0.33 \times 10^2$ cfu/ml).



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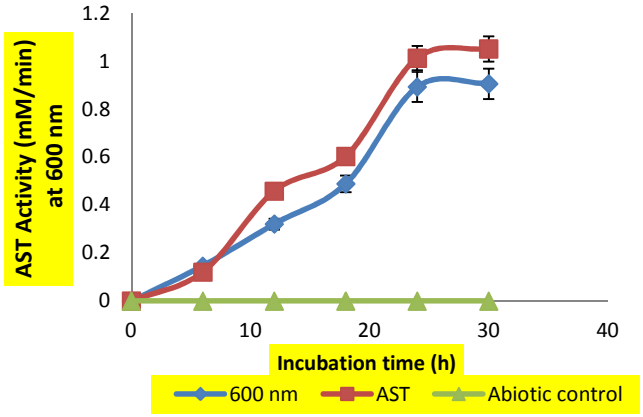
140 **Fig. 6. Alkylsulphatase activity (AST) of *Proteus mirabilis***



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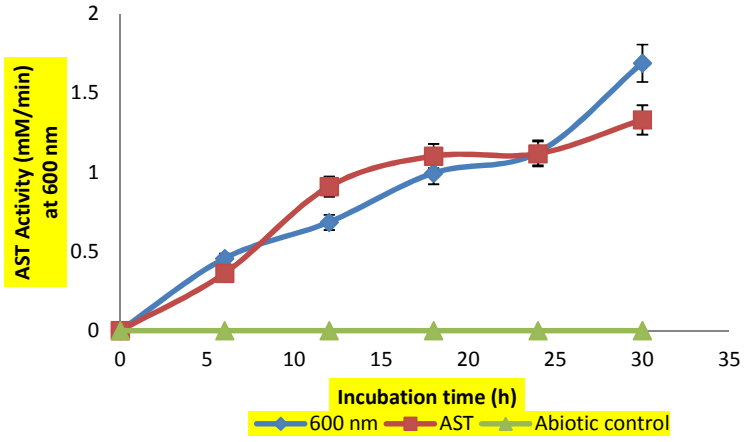
142 **Fig. 7. Alkylsulphatase activity (AST) of *Bacillus cereus***



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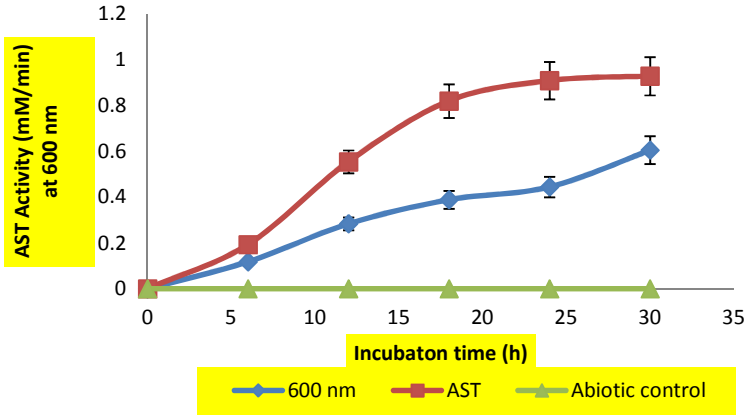
144 **Fig. 8. Alkylsulphatase activity (AST) of *Pseudomonas fluorescens***



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146 **Fig. 9. Alkylsulphatase activity (AST) of *Bacillus anthracis***

147 **Table 1. Detergent degrading bacterial cell growth during enzyme production**

Incubation time (hours)	*Pseudomonas putida* ($x10^2$ cfu/ml)	*Escherichia Coli* ($x10^2$ cfu/ml)	*Klebsiella Oxytoca* ($x10^2$ cfu/ml)	*Bacillus subtilis* ($x10^2$ cfu/ml)	*Proteus mirabilis* ($x10^2$ cfu/ml)
6	16.00 ± 0.58	19.66 ± 0.33	18.66 ± 0.33	20.66 ± 0.33	21.67 ± 0.33
12	23.00 ± 0.58	24.00 ± 0.57	20.33 ± 0.33	43.00 ± 0.58	31.33 ± 0.66
18	35.67 ± 0.33	30.33 ± 0.33	22.66 ± 0.33	71.57 ± 0.67	38.33 ± 0.33
24	67.66 ± 0.33	33.33 ± 0.33	33.33 ± 0.33	75.57 ± 0.66	51.00 ± 0.57
30	73.33 ± 0.66	39.33 ± 0.33	54.00 ± 0.58	81.88 ± 0.33	56.33 ± 0.33

148 *Values are means±Standard error*

149 **Table 2. Detergent degrading bacterial cell growth during enzyme production**

Incubation Time (hours)	*Bacillus cereus* ($x10^2$ cfu/ml)	*Pseudomonas fluorescence* ($x10^2$ cfu/ml)	*Bacillus anthracis* ($x10^2$ cfu/ml)	*Xanthomonas campetris* ($x10^2$ cfu/ml)
6	12.33 ± 0.33	20.33 ± 0.33	13.66 ± 0.33	20.66 ± 0.33
12	21.33 ± 0.33	53.33 ± 0.32	25.00 ± 0.58	31.67 ± 0.88
18	33.66 ± 0.88	66.33 ± 0.33	43.67 ± 0.33	58.66 ± 0.33
24	59.67 ± 0.33	66.67 ± 0.21	53.00 ± 0.57	63.33 ± 0.33
30	63.00 ± 0.57	74.33 ± 0.88	53.33 ± 0.33	68.33 ± 0.33

150 *Values are means*±Standard error

151 The bacterial isolates were able to produce the alkysulphatase enzyme; possessing the mechanisms
152 to carry out biodegradation of surfactants. *Bacillus subtilis*, *Pseudomonas putida* and *Pseudomons*
153 *fluorescens* were able to produce a substantial amount of the enzyme and carry out profound
154 degradation. In a related research, *Bacillus subtilis and Bacillus cereus* were analysed for their
155 capacity to degrade laundry and dish washing detergents. *Bacillus subtilis* showed better degradation
156 [7]. Several *Pseudomonas* sp have been reported as potent SDS (sodium dodecyl sulphate)
157 degrading isolates [12] [13]. There were variations in the quantity of alkylsulphatase enzyme
158 produced by the bacterial isolates and this could be as a result of molecular mass of alkylsulfatase;

which is found to vary in different bacterial species and genera [14]. Some of the bacteria showed better biodegrading potentials, and this could be as a result of the genetic makeup of the microorganisms [10]. Biodegradation of sodium dodecyl sulphate is initiated by primary or secondary alkylsulphatase enzymes; which converts it to dodecanol and finally to carbon (iv) oxide and water [15]. Increase in optical density was an index of microbial growth. The bacterial isolates were able to survive the biocide effect of SDS (sodium dodecyl sulphate) present in the growth medium due to their ability to form biofilms as a survival strategy to overcome the stress of the biocide [12]. The growth pattern increased with increase in enzyme production. The results suggest that bioremediation by the bacterial isolates are promising for the biodegradation of surfactants as pollutants in the soil environment.

CONCLUSION

The study was able to illustrate the pattern of enzyme production and activity of the various isolates with respect to time and microbial growth. The study indicates an array of bacteria that could be selected for the remediation of soil environment contaminated with detergent. The study indicates that enzyme activity increases with time and microbial growth. It can be concluded that *Bacillus subtilis*, *Pseudomonas putida* and *Pseudomonas fluorescens* can be found in soil environment polluted with detergent. They are capable of producing alkylsulphatase; thus can be employed in enzyme production. They are capable of surviving the toxic effect of the pollutant, being able to break down the surfactant molecule and utilize it for their own growth; thus they can be applied in the bioremediation of environments contaminated with detergent.

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