



Bacteria Associated with Selected Rivers in Akure, Nigeria and their Alkylsulphatase Activity/Production

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To isolate, characterize and identify surfactant degrading bacteria from selected rivers in Akure, Nigeria and also to compare and quantify the biodegrading potentials of each of the bacterial isolates.

Place and Duration of Study: Akure metropolis, Ondo state, Nigeria, between June and November, 2013.

Methodology: Surfactant degrading bacteria were isolated from the water samples by supplementing culture media with test surfactant. The bacteria isolated were later subjected to the alkylsulphatase enzyme assay to quantify their various enzyme production/activity.

Results: The total bacterial load of the water samples range from $7.20 \pm 0.69 \times 10^3$ cfu/ml to $40.0 \pm 2.31 \times 10^3$ cfu/ml, while the surfactant degrading bacteria counts was within the range of $3.30 \pm 0.02 \times 10^2$ cfu/ml to $5.37 \pm 2.3 \times 10^3$ cfu/ml. *Pseudomonas putida* and *Exiguobacterium profundum* were able to produce more of the alkylsulphatase enzyme amongst the isolated surfactant degrading bacteria.

Conclusion: It can be concluded that the set of bacteria isolated from the selected aquatic environments are capable of carrying out biodegradation of surfactants and that they are abundant in the selected environments. *Pseudomonas putida* and *Exiguobacterium profundum* have higher biodegrading potentials and they can be exploited in the bioremediation of water bodies polluted with surfactants.

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1. INTRODUCTION

Inspired by the observation that the bacterium *Pseudomonas* sp is able to grow on the common surfactant sodium dodecyl sulfate (SDS) in the 1960s [1], sulfatase research turned into a hot topic due to potential applications in bioremediation. Ultimately, sulfur is incorporated into the essential amino acids cysteine and methionine. In case inorganic sulfate is unavailable, microorganisms are forced to express other sulphur metabolizing enzymes, such as alkylsulphatases. The most common indicator for sulfatase activity is the ability of microorganisms to grow on detergent-contaminated soil or wastewater and several bacteria have been isolated from such sources [2,1]. Forcing an organism to express the desired sulfatase activity is usually achieved by limiting the available sulphur to organically bound sources, such as Sodium dodecyl sulphate (SDS).

Biodegradation of surfactants is initiated by alkylsulphatase enzymes, which hydrolyse inorganic sulphate from its ester linkage with the liberation of alcohols. The retaining pathway cleaves the S–O ester bond, releasing the product alcohol [1]. This is followed by the oxidation of the liberated alcohols by the appropriate alcohol dehydrogenase and assimilation through normal metabolic pathways [1]. Surfactants such as detergents and soaps are usually found as contaminants in water bodies after being used mostly in laundry processes. Surfactants are routinely deposited in numerous ways on land and into water systems, whether as part of an intended process or as industrial and household waste causing pollution [3]. Some of them are known to be toxic to animals, ecosystems and humans, and can increase the diffusion of other environmental contaminants [3]. This research therefore, evaluates the biodegrading potentials of some bacteria isolated from selected water bodies on surfactants, in Akure, Nigeria by comparing the alkylsulphatase activities of each bacterial isolate.

2. METHODOLOGY

2.1 Collection of Samples

Water samples were collected from five different rivers in Akure; namely, Ala, Majo, Ero, Owena

and Otere. Each sample was collected using sterile containers, labelled and transported to the laboratory for Analysis.

2.2 Isolation of Bacterial Surfactants Degraders

Isolation of surfactants degrading bacteria (SDB) from the water samples was done by collecting water samples in sterile containers from the selected rivers, serial dilutions was carried out. The serial diluted sample was inoculated onto Nutrient agar supplemented with the test surfactant at 0.01%. The inoculated plates were incubated aerobically at 28°C for 48 hrs. At the end of the period of incubation, the plates were checked for growth [4]. The cultural characteristics of pure culture were noted for bacterial characterization [5]. The bacterial isolates were subjected to Gram's reaction and biochemical tests (Voges proskaeur, citrate, indole, methyl red, catalase and oxidase) to identify the isolates [6].

2.3 Determination of Alkylsulphatase Production

2.3.1 Preparation of enzyme extract

Nutrient broth was prepared, supplemented with SDS at 0.01%, and inoculated with the bacterial isolates. The culture broth was incubated in an orbital shaker at 150 rpm. At the end of twelve hours, fifty millilitre of the broth culture was collected and centrifuged for 15 minutes at 4°C. The supernatant was decanted off. One millilitre (1 ml) of tris buffer was used to collect the cell pellets at the base of the centrifugation tube. The pellets were homogenized for 15 minutes. The homogenized pellets were then centrifuged for 15 minutes at 4°C. The supernatant was decanted and kept for the enzyme assay.

2.3.2 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 containing fifty micro litres (50 µl) of the enzyme. It was then incubated for 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. 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 652 nm.

2.3.3 Determination of protein content in enzyme extract

Four different reagents were prepared for the protein analysis, the first reagent (A) was prepared by mixing 2 g of sodium carbonate in 0.1 M of sodium hydroxide. The second reagent (B) was prepared by mixing 2% sodium potassium tatarate and 1% copper sulphate. The third reagent was prepared by mixing one millilitre (1 ml) of reagent B and fifty millilitres (50 ml) of reagent A. The fourth reagent (D) was folin solution. Fifty micro litres (50 μ l) of the enzyme extract was then collected in a container, then four hundred and fifty micro litres (450 μ l) of reagent B and two and half micro litres (2.5 μ l) of reagent C were then added to the enzyme extract and it was left to stand for 10 minutes. Then a quarter micro litres (0.25 μ l) of reagent D was then added and left to stand for 30 minutes. The absorbance of the mixture which indicates the concentration of protein in the extract was read on a Colorimeter (Corning, 2232, 253) at a wave length of 660 nm.

2.4 Analysis of Data

Data obtained were subjected to descriptive one way analysis of variance, using SPSS version 16 Microsoft windows 7 and means were separated with Duncan's Multiple Range Test.

3. RESULTS AND DISCUSSION

Table 1 represents the bacterial population of the selected rivers; total bacterial counts for the water samples were generally high exceeding the limit of 1.0×10^2 cfu/ml which is the standard limit of heterotrophic count for unpolluted water [7]. The high total heterotrophic count is indicative of the presence of high organic matter in the rivers sampled. The sources of bacterial contamination might be as a result of surface runoff, effluent carrying animal wastes and natural soil/plant bacteria [8].

The surfactant degrading bacteria isolated from the river water samples spiked with test surfactant were *Escherichia coli*, *Klebsiella* sp, *Enterobacter* sp, *Bacillus subtilis*,

Exiguobacterium profundum, *Citrobacter* sp, *Pseudomonas putida*, *Proteus* sp and *Klebsiella oxytoca*. Some of which were isolated in other related research [9] and [4]. Total plate count of surfactant degrading bacteria was low compared to total bacteria count, which may be as a result of the surfactant toxicity. Fig. 1 depicts the enzyme activity of *Klebsiella oxytoca*. The highest enzyme activity of *Klebsiella oxytoca* was 0.06 mM/min/ml with a protein concentration of 32.55 mg/ml and optical density of 0.16 at the end of the 30 hours incubation period. From Fig. 2, the highest enzyme activity of *Escherichia coli* (0.01 mM/min/ml) was detected after the 18 hours incubation period; it remained stable till the end of the 30 hours incubation period. The protein concentration was progressive from the end of the 12 hours incubation period starting from 3.59 mg/ml (12 hours incubation period) to 6.90 mg/ml as the highest protein concentration. Fig. 3 illustrates the enzyme activity of *Exiguobacterium profundum*, the highest enzyme activity for *Exiguobacterium profundum* after the 30 hours incubation period was 0.36 mM/min/ml with protein concentration of 70.07 mg/ml. From Fig. 4, *Bacillus subtilis* was able to produce an enzyme activity of 0.22 mM/min/ml, which was its highest with protein concentration of 36.41 mg/ml at the end of the 30 hours incubation period. Fig. 5 illustrates the enzyme activity of *Proteus* sp. The highest enzyme activity of *Proteus* sp was 0.24 mM/min/ml (30 hours incubation period) with protein concentration of 36.97 mg/ml. Fig. 6 shows the enzyme activity for *Pseudomonas putida*. Enzyme activity for *pseudomonas putida* was at its peak (0.44 mM/min/ml), with protein concentration of 36.41 mg/ml at the end of the 30 hours incubation period. From Fig. 7, *Citrobacter* sp was able to produce the highest enzyme activity of 0.05 mM/min/ml with a protein concentration of 32.83 mg/ml at the end of the 30 hours incubation period. From Fig. 8, the highest enzyme activity of *Enterobacter* sp was 0.04 mM/min/ml, with protein concentration of 30.62 mg/ml and at an optical density of 0.12 at the end of the 30 hours incubation period.

Fig. 9 shows the enzyme activity of *Klebsiella* sp. *Klebsiella* sp was able to produce an enzyme activity of 0.133 mM/min/ml, which was the highest, with protein concentration of 30.34 mg/ml at the end of the 30 hours incubation period. *Pseudomonas putida* and *Exiguobacterium profundum* were able to carry out the degradations efficiently as compared to the other isolates, as they were able to produce more of the alkylsulphatase enzyme. The

variation in the alkylsulphatase activity is related to differences in the amount of enzyme produced. This could also be related to the genetic makeup of the various bacterial isolates.

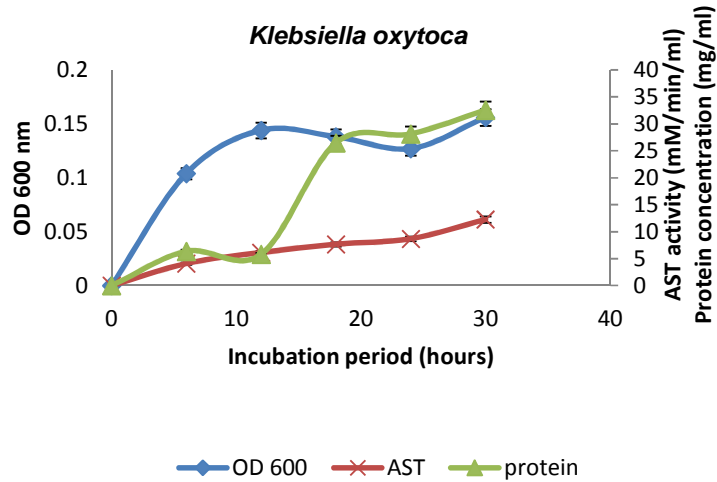


Fig. 1. Alkylsulphatase (AST) activity and protein concentration of *Klebsiella oxytoca*

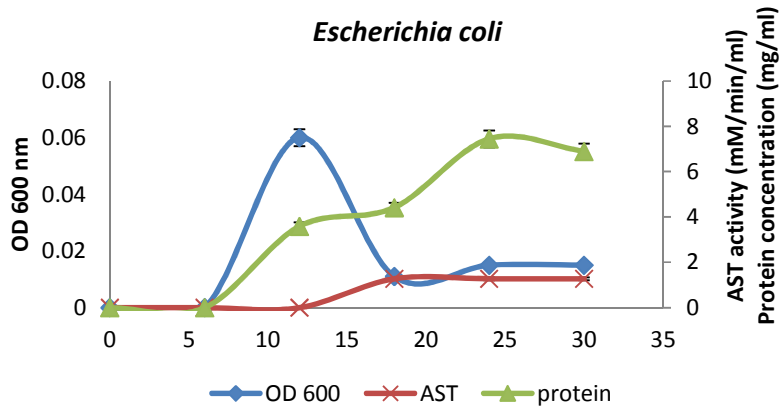


Fig. 2. Alkylsulphatase (AST) activity and protein concentration of *Escherichia coli*

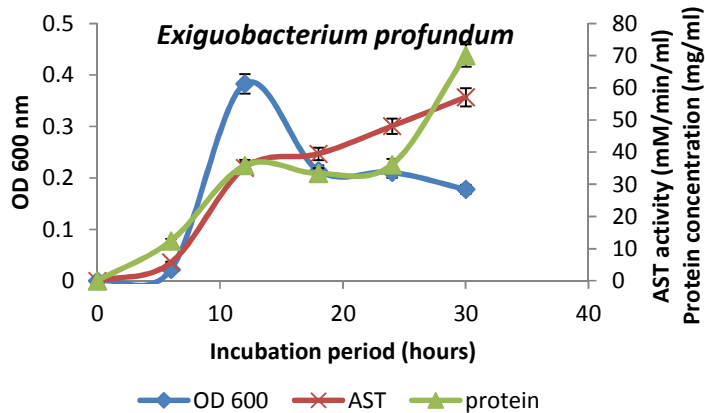


Fig. 3. Alkylsulphatase activity (AST) and protein concentration of *Exiguobacterium profundum*

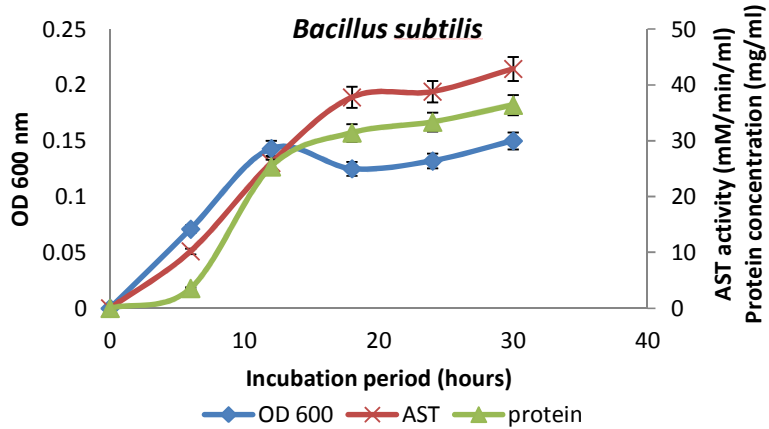


Fig. 4. Alkylsulphatase activity (AST) and protein concentration of *Bacillus subtilis*

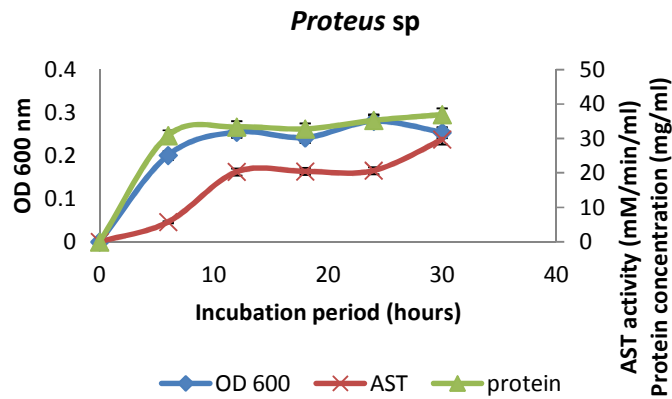


Fig. 5. Alkylsulphatase activity (AST) and protein concentration of *Proteus sp*

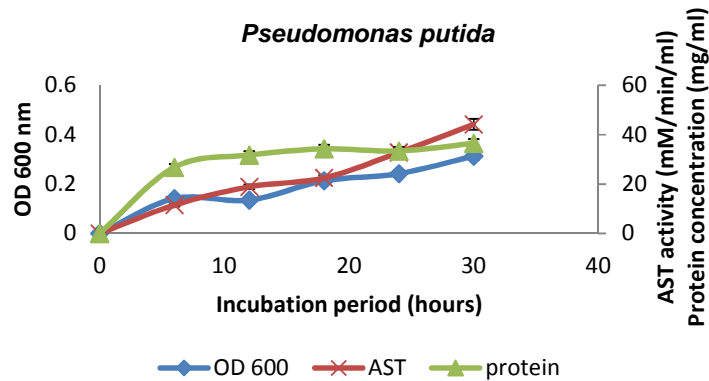


Fig. 6. Alkylsulphatase activity (AST) and protein concentration of *Pseudomonas putida*

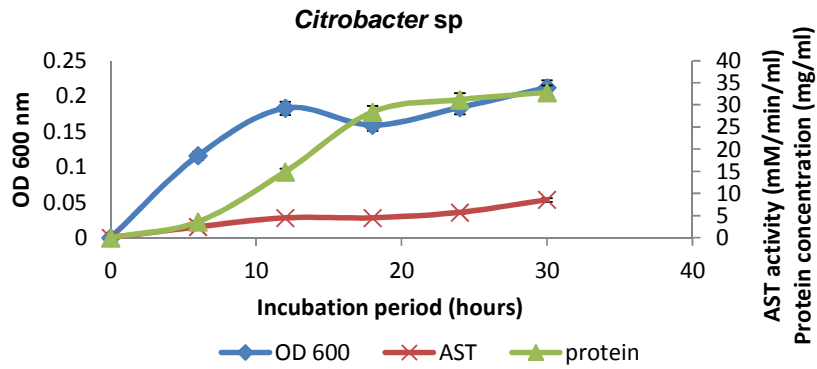


Fig. 7. Alkylsulphatase activity (AST) and protein concentration of *Citrobacter sp*

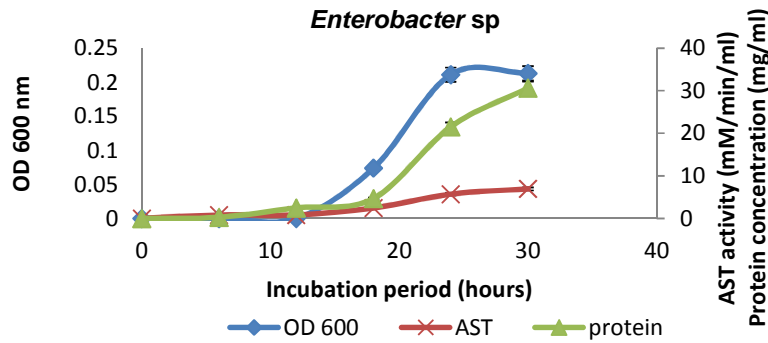


Fig. 8. Alkylsulphatase activity (AST) and protein concentration of *Enterobacter sp*

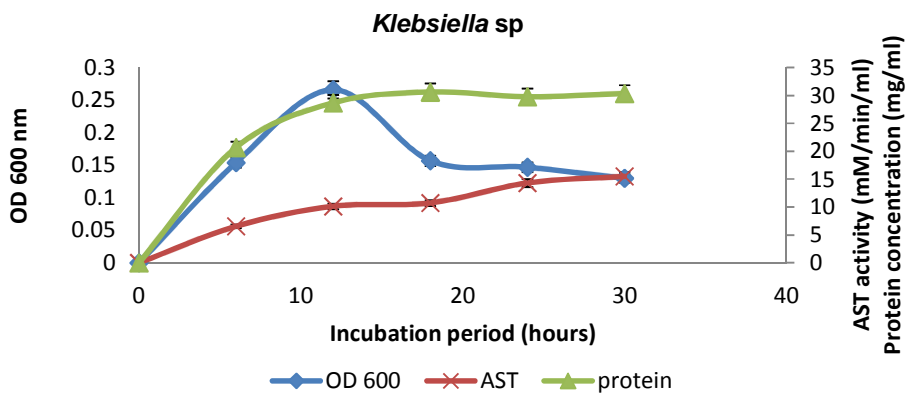


Fig. 9. Alkylsulphatase activity (AST) and protein concentration of *Klebsiella sp*

Table 1. Total bacterial count and surfactant degrading bacterial count of the river water sample

Sample	TBC (cfu/ml)	SDBC (cfu/ml)
A	8.40x10 ³ ±0.61 ^b	3.30x10 ² ±0.02 ^a
B	40.0x10 ³ ±2.31 ^e	3.20x10 ³ ±0.23 ^b
C	38.67x10 ³ ±4.81 ^d	2.50x10 ³ ±0.15 ^b
D	7.20 x10 ³ ±0.69 ^a	4.50x10 ² ±0.04 ^a
E	13.67 x10 ³ ±4.53 ^c	5.37x10 ³ ±2.30 ^c

Values of means ± Standard error, values with dissimilar alphabets are significantly different from each other at $P \leq .05$ and vice versa.

Key: A = River Ala; B = River Majo; C = River Ero; D = River Otere and E = River Owena; TBC = total bacterial count; SDBC = Surfactant degrading bacterial count

4. CONCLUSION

The study was able to illustrate the pattern of enzyme production and activity of the various isolates with respect to time, microbial growth and protein concentration of each isolates. The study indicates an array of bacteria that could be selected for the remediation of water body containing surfactants as contaminants. The study indicates that enzyme activity increases with time, microbial growth and protein concentration. It could be concluded that the set of bacteria isolated can be exploited in the bioremediation of aquatic environments polluted with surfactants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Toesch M, Schober M, Faber K. Microbial alkyl- and aryl- sulphatase: Mechanism, occurrence, screening and stereo-

- selectivities. Applied Microbiology and Biotechnology. 2014;98:1487.
- Knaus T, Schober M, Kepplinger B, Faccinelli M, Pitzer J, Faber K, Macheroux P, Wagner U. Structure and mechanism of an inverting alkylsulfatase from *Pseudomonas* sp DSM6611 specific for secondary alkylsulfatase. FEBS J. 2012; 279:4374-4384.
- Emmanuel E, Hanna K, Bazin C, Keck G, Clement B, Perrodin Y. Fate of glutaraldehyde in hospital wastewater and combined effects of glutaraldehyde and surfactants on aquatic organisms. Environment International. 2005;31(3): 399-406.
- Sushma P, Anvita A, Nismitha S, Melwyn S. Degradation of anionic surfactants by *Bacillus subtilis* and *Bacillus cereus*. Journal of Pharmacy and Biological Science. 2012;3(1):42-45.
- Okpokwasili GO, Nwabuzor CN. Primary biodegradation of anionic surfactants in laundry detergents. Chemosphere. 1998; 17:2175-2182.
- Fawole MO, Oso BA. Laboratory manual of microbiology. Spectrum Books Ltd, Nigeria. 2008;127.
- United States Environmental Protection Agency (USEPA). Ecoregional nutrient criteria as water quality standards, office of health and environmental assessment, US Environmental Protection Agency, Washington, D.C; 2000.
- United States Environmental Protection Agency (USEPA). Protecting water quality from agricultural runoff. Fact Sheet No: EPA-841-F-05-001. US Environmental Protection Agency, Washington, D.C; 2005.
- Ojo OA, Oso BA. Isolation and characterization of synthetic detergent-degraders from wastewater. African Journal of Biotechnology. 2008;7:3753-3760.

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