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Investigation of Bioflocculant Producing Bacteria from Ogba River, Benin City, Edo State, Nigeria

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Abstract: The use of synthetic flocculants for chemical and industrial treatment of water and wastewater is very popular. However, they have shown to be non-degradable and possesses carcinogenic and neurotoxic capabilities hence the need to explore new and safer alternatives such as bioflocculants. Bio-flocculation is a dynamic process which results from the manufacture of extracellular polymer by living organisms. This study was aimed at investigating the possibility of freshwater bacteria isolated from water samples collected from Ogba River, Benin City, Nigeria to have flocculating ability. Screening for flocculating bacteria was done on water samples collected from different sampling points on the Ogba River using bioflocculant production media (BPM), and identification of isolates was carried out using molecular procedures. Kaolin clay suspension was used to test the flocculating potential of the bacterial isolates. Physicochemical examination of water samples revealed significant turbidity, with variable distinctions in pH (5.58- 5.78), temperature (25.660C - 26.400C) and conductivity (34µS cm-1- 254 µS cm-1). Nine isolates were identified by polymerase chain reaction (PCR) were; *Staphylococcus aureus, Proteus mirabilis, Providencia rettgeri, Proteus vulgaris, Bacillus subtillis, Shigella* sp., *Salmonella* sp., *Citrobacter freundii* and *Enterobacter aerogenes*. For flocculating potential, *Staphylococcus aureus* had the highest flocculating activity of 70.73±0.05% while *Bacillus subtilis* had the least flocculating potential of 7.89±0.21 %.

1. Introduction

Flocculation is used widely to remove very fine particles in water and wastewater treatment. Chemical floc-agents are commonly used for their high flocculating efficiency and cost-effectiveness (Dkhissi *et al.*, 2018; Wang *et al.*, 2011). However, they have been reported to possess properties that are of great hazard and is been found to be associated with some serious health issues, such as brain disorder, and also difficult to control in the environment. Hence, the necessity for safe and environmentally friendly flocculants has become crucial. The beneficial effects microbial flocculants such as biodegradability, safety and harmlessness to the environment and humans, have made them potential alternatives in water treatment, fermentation and downstream processes. Although bioflocculants possess these desirable characteristics, there is a need to find species with the ability to give bioflocculants with high flocculating activity at low cost (Suopajarvi *et al.*, 2013).

Bioflocculation is as a result of sticking together of materials secreted by bacteria, algae, fungi, actinomycetes forming a polymeric network which cause particles to coalesce (Piyo *et al*., 2011). The process of flocculation is widely applied in wastewater treatment and achieved with the addition of flocculants that facilitate the process (Lee *et al*., 2014). Clarifying agents are substances of synthetic, natural or biological origin applied to bring about separation via flocculation, having wide range of applications in petroleum industry, sewage waste, chemical industry, treatment of drinking water.

The aquatic community represents an environment with large undiscovered microorganisms having the ability to produce biological metabolites (Zhang *et al*., 2007; Li *et al*., 2013). It is complex and contains different life forms having ability to survive at extreme conditions of pressure, salinity, and temperature thus endowing freshwater microbes with unique features to produce different novel bioactive materials (Li *et al*., 2013).

There have been various reports on bio-flocculants produced from marine environments extracted from seaweeds with very scanty information on bioflocculants produced by freshwater bacteria (Kumar *et al*., 2004), hence, the need to explore bacteria diversities from freshwater environment for bioflocculant producing abilities. Ogba River is very necessary to the lives of the surrounding inhabitants. Activities such as farming, fishing, washing of clothes and cars, disposal of domestic and industrial wastes and religious activities are carried out within and around this river (Anyanwu, 2012). This objective of this study is to investigate bioflocculant-producing bacteria from Ogba River in Benin City, Nigeria.

2. Methodology

2.1 Location of Sampling Area

The samples analyzed were sourced from Ogba River located in Benin City, Edo state. The river arises from the highland area of Ekehuan-Ugbiyokho area of Benin City between latitude 6.20oN and Longitude 5.34^{o}E, flowing southwest for about 12 km as a sub-tributary of the Ossiomo River, occupying an area of 40 km^{2} it is about 1-3 m in width and has a depth of 1 - 2 m (Ikhile, 2018). Samples were collected at three different point of Ogba River; point 1 located at the zoo was open to a number of macrophytes within the water. Point 2 located by the bridge, 0.5 km downstream of point 1 where domestic and recreational activities as well as idol worshipping and baptism take place, and point 3 located at the community square, 1km downstream of point 2, where no human activity was observed at the time of sampling.

2.2 Physicochemical and Bacteriological Test for Water Sample

Physicochemical parameters such as pH, conductivity and temperature, were measured in-situ. The enumerations of total viable bacterial counts were conducted using the pour plate method (Zhang *et al*., 2013). Water samples were serially diluted with sterile physiological saline as diluent. Aliquots of 1ml of the undiluted water samples of 10^{-1}, 10^{-2} and 10^{-3} were pipetted onto sterilized petri dishes in triplicate, upon which approximately 20 ml of cool molten nutrient agar (for bacterial count) was poured onto each of the plates and swirled gently to ensure even mixing. The plates with nutrient agar were incubated at 37 ^{0}C for 24 hrs. Incubated plates were inspected for visible microbial colonies and those with microbial colonies were separated and counted and their cultural characteristics were examined. Sub-culturing of the bacterial colonies was done using the streaking method and then incubated at 37^{0}C for 24 hrs for the bacterial isolates.

2.3 Bioflocculant Production Medium (BPM)

The bioflocculant (BPM) medium used composed of 10 g glucose, 0.5 g Mono-Potassium Phosphate (KH_2PO), 2 g Di-Potassium Phosphate (K_2HPO_4), 0.1g Calcium Chloride ($CaCl_2$), 0.5 g Magnesium sulphate heptahydrate ($MgSO_4.7HO$), 1.0 g peptone, mixed in 1litre sterile water with pH adapted to 7.0 using Hydrogen chloride and Sodium hydroxide (Zhao *et al.*, 2013). The preparation was sterilized using autoclave at 121 ^{0}C for 15 mins.

2.4 Determination of Bioflocculant Production Activity

The assay of bioflocculant production potential was carried out using Kaolin clay suspension. About 1.5 ml of the culture supernatant and 2 ml of 1% $CaCl^2$ were put into 75 ml of kaolin clay suspension in 100 ml conical flask. The mixture was carefully stirred for 60 secs and left to stand still on the work bench for 7 min. The blank serving as control was prepared also; however, a fresh broth was used to replace the bioflocculant (Okaiyeto *et al.*, 2013). The turbidity in the uppermost portion supernatant was determined at 500 nm with the aid of a Thermoscientific GENESYS 10S UV-Vis Spectrophotometer.

The flocculating potential was estimated as;

$$\text{Flocculating Activity (\%)} = \{(B-A)/B\} \times 100 \quad \text{- Equa. 1.}$$

Where: A is the turbidity (OD) of the sample at 500nm; B is the turbidity (OD) of the blank (control) experiment at 500nm. All investigations were done in three places in order to get average values.

2.5 Molecular Identification of Bacterial Isolates

The bacterial isolates were identified molecularly based on 16S rRNA gene amplification by polymerase chain reaction (PCR) accompanied by sequencing. DNA templates of bacterial isolates were prepared using boiling method (Ahmed and Dablool, 2017).

2.6 Statistical Analysis

All experimental analyses were done in triplicates and the results were expressed as the average ± standard deviations.

3. Results and Discussion

3.1 Result for physicochemical analysis of water samples

The result for physicochemical analysis of water samples from the various sampling sites is presented in Table1.

Table1: Mean physicochemical parameter of water samples from Ogba River, Benin City, Edo State at a depth of 3.43 m

Sampling area	PH	Temperature (^{0}C)	Conductivity (μScm^{-1})
Point 1	5.59±0.85	26.40±0.62	234±16.45
Point 2	5.78±0.58	25.66±0.50	254±21.55
Point 3	5.58±0.44	327.20±0.22	34±18.22

3.2 Mean viable bacteria counts of the water samples

The mean viable bacteria count of the water samples collected on a monthly interval over a period of five months are depicted on Table 2.

Table 2: Mean viable bacteria counts of water samples

Sampling area	November	December	January	February	March
Point 1	2.78±1.22	2.50±7.57	2.45±2.00	2.66±2.51	2.12±1.11
Point 2	2.33±2.20	1.35±1.52	1.06±3.42	1.40±5.55	1.88±2.01
Point 3	1.78±6.61	1.50±5.03	0.74±1.44	0.65±3.27	0.66±1.01

3.3 Flocculating Potentials of the bacterial isolates

The flocculating potential of the various bacterial isolates using kaolin clay suspension as the test medium is shown in Figure 1.

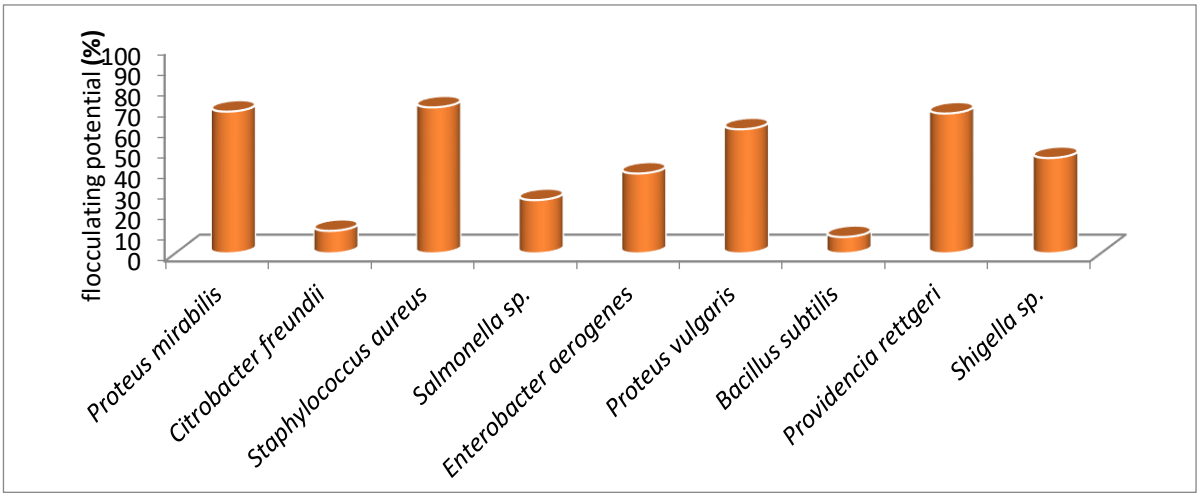

Figure 1: Flocculating potential of bioflocculant-producing bacteria isolated from Ogba River

DISCUSSION

Nine bacterial isolates identified as *Staphylococcus aureus, Proteus mirabilis, Providencia rettgeri, Proteus vulgaris, Bacillus subtilis, Shigella* sp., *Salmonella* sp. *Citrobacter freundii* and *Enterobacter aerogenes* were isolated from Ogba River and reported to be producers of bioflocculants. This report is in agreement with findings by Makapela *et al.* (2016) (*Bacillus* sp.), Fujita *et al.* (2001) (*Citrobacter* spp. TKF04), Lu *et al.* (2005) (*Enterobacter aerogenes*), Deng *et al.* (2005) (*Bacillus subtilis*) which documented the isolation of bacteria with flocculating ability from marine and freshwater domain.

The highest flocculating activity of 70.73 % was obtained from *S. aureus*, with *Bacillus subtilis* having least flocculating activity of 7.78 %. This finding is in contrast to a study by Zaki *et al.* (2011) where *Pseudomonas aeruginosa* and *Bacillus* sp. had highest flocculating activity of 97.59 % and 96.03 % respectively. Activities such as open defecation and wastes disposal carried out at the sampling points may have contributed to the observed difference in the flocculating activity of the bacterial isolates. It can be observed that least flocculating potential from *Bacillus subtilis* was isolated from point 3 where no human activities occurred during sampling time.

Staphylococcus aureus (70.73 %), *P. rettgeri* (67. 75%), *P. mirabilis* (68.68 %), *Proteus vulgaris* (60.12 %), with high flocculating activity were all isolated from point 1 and 2 where open defecation, idol worshipping, baptism, car washing and recreational activities is been carried out. This finding is in agreement with a finding by Yang *et al.* (2012) where bioflocculant producing bacteria with high flocculating activity were isolated from water samples with faecal materials. It has been reported that slimes like faecal materials and debris settling at the bottom sediments can alter the interactions of organisms at all levels of biodiversity thus increasing potentials for organisms to produce bioflocculants (Yang *et al*., 2012).

From this study, *Proteus* spp. was mostly isolated from Ogba River with high flocculating potential. This is in accordance with a report by Xia *et al.* (2008) who isolated *Proteus mirabilis* TJ-1 with high flocculating ability. Bacterial isolates with least flocculating activity may be as result of stirring of the production medium and factors such as pH and temperature affecting the bioflocculant production (Deborah *et al*., 2017). The physicochemical parameters of the water samples had no significant effect on the production of bioflocculant. Piyo *et al.* (2011) also carried out a study on bioflocculant potential of bacterial isolates where the physicochemical parameters had no effect on bioflocculant production potential. There is need to carry out more research work on Ogba River in order to isolate bacteria with high bioflocculant potential.

Conclusion

It has been revealed that Ogba River harbours bioflocculant-producing bacteria with various level of flocculating potential due to various activities that takes place. S. aureus isolated from point where effluents are been disposed and open defecation takes place has the highest flocculating potential of 70.73 %. Other bacteria such as *P. mirabilis*, *P. rettgeri* and *Proteus vulgaris* isolated from same point also showed high flocculating potential of 68.68 % 67.75 % and 60.12 % respectively making them potentially viable for industrial use.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.

Compliance with Ethical Standards*:* This article does not contain any studies involving human or animal subjects.

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