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Isolation and Characterization of Bioflocculant-Producing Bacteria from Wastewater at Jimeta, Adamawa State

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Authors' contributions

This work was carried out in collaboration between all authors. Author SDH designed the study and wrote the protocol. Authors DM and GAI wrote the first draft of the manuscript. Author GAI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study is to isolate, identify and screen for bacteria capable of producing bioflocculant from three wastewater disposal sites; Jimeta Abattoir, Jimeta Modern Market and the Gwari Market waste disposal site located within the Jimeta metropolis, Adamawa State. Nigeria.
Study Design: The samples were prepared by diluting 200 ml of distilled water in 50 ml of the wastewater samples for each.
Place and Duration of Study: The research was carried out at the Chevron Biotechnology Centre Modibbo Adamawa University of Yola, Nigeria which lasted for about two months.
Sample Collection: The wastewater samples were collected in sterile containers and were taken to Chevron Biotechnology Centre, Modibbo Adamawa University of Yola for further analysis.
Methodology: Screening of bioflocculant-producing bacteria was carried out using the three wastewater samples collected from Jimeta metropolis. Growth media for bioflocculant production was prepared and identification of the isolate was done using techniques such as gram staining, biochemical tests like detection of urease and catalase production, IMViC tests etc. Each of the bacterial isolates were tested for its ability to ferment carbohydrate, screened for bioflocculant

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production using bioflocculant production broth medium and lastly the flocculating activity of the isolates was determined.

Results: By the examination of physical parameters of waste water samples had revealed that, all the samples were turbid with distinction in colour, pH and temperature change. Six bacteria were isolated and identified based on their morphological, cultural and biochemical characteristics. From the biochemical characteristics, the organisms were confirmed as *Escherichia coli (ISO1), Pseudomonas aeruginosa (ISO2), Staphylococcus aureus (ISO3), Klebsiella spp. (ISO4), Salmonella spp. (ISO5) and Bacillus spp. (ISO6)* in the wastewater samples*. P. aeruginosa* has the highest flocculation activity with 87.32%, while *Salmonella spp.* has the least flocculating activity with 13.5%, while the flocculating activity of *E. coli, S. aureus, Klebsiella spp and Bacillus spp* are 35.76%, 47.87%, 56.6% and 69.54% respectively

Conclusion: This study has shown that bacterial bioflocculants are capable of removing/flocculating suspension particle such as kaolin clay simultaneously and effectively. *P. aeruginosa and Bacillus spp* can be explored for bioflocculant-production.

Keywords: Bioflocculant; bacteria; flocculating activity and wastewater.

1. INTRODUCTION

Bioflocculation can be referred to as the process where microorganism mediates the flocculation activity, where the biodegradable bioflocculant are secreted by the microorganisms [1]. The bioflocculants are made up of macromolecules like polysaccharides, proteins and lipids [2,3]. The most prominent places for the screening of bioflocculant producing microorganisms are soil, waste water, industrial effluents and activated sludge samples. The group of microbes found to produce bioflocculants are bacteria, yeast and fungi [4,5]. Recently bioflocculants producing microbes from human saliva and sputum which is quite an unusual environment has been discovered [6,7].

Bioflocculant has many advantages over chemical flocculants which include: biodegradability, nontoxic to human health, environmentally friendly and no risk of secondary pollutants [8,9,10]. Chemical flocculants such as plyaluminium chloride (PAC) and polyacrylamide are popularly used. Chemical flocculants have many associated health problems which ranges from being carcinogenic and result to diseases such as Alzheimer's disease [11,12,13]. This has raised concern over the use of chemical flocculants due to human health and the need to investigate wide variety of microbes for flocculant producing potentiality that has become necessary [8,14], especially in developing countries where research has been limited by many factors [15,16].

In this study the authors isolated and identified the bioflocculant producing microorganisms from three wastewater disposal sites; Jimeta Abattoir, Jimeta Modern Market and the Gwari Market waste disposal site located within the Jimeta metropolis, Adamawa State. Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Processing

Wastewater samples were collected from three different location in Jimeta; Jimeta Abattoir wastewater; Jimeta Modern Market Wastewater and Gwari Market wastewater using a sterile container. The water samples were taken to the Chevron Biotechnology Center, Moddibo Adama University of Technology, Yola. The samples were prepared by diluting 200 ml of distilled water in 50 ml of the wastewater sample for each [17].

2.2 Growth Media for Bioflocculation Production

The growth media for bioflocculation production was compose of glucose (20 g/L), (NH4)2SO4 (0.2 g/L), K2HPO4 (5 g/L), urea (0.5 g), MgSO4•7H2O (0.2 g), Yeast extract (0.5 g/L) and KH2PO4 (2 g/L) in distilled water and sterilized by autoclaving at 121℃ for 15 min prepared according to [18].

2.3 Isolation of Bioflocculating Producing Bacteria

Screening and enumeration of microorganisms in the waste water samples was carried out using serial dilution technique [19].

2.4 Identification of Isolates

The organisms were provisionally identified by colonial morphology in various culture media and

grams staining and they were subjected to various biochemical and biological tests for confirmation [17,20,21]. The tests include carbohydrate fermentation tests with glucose, lactose, sucrose, xylose and mannitol. The organisms were also tested for Indole production, Methyl red test, Voges-proskauer test, Citrate utilization, Urease test, Oxidase test, Catalase test etc.

2.5 Sugar Fermentation

Each of the isolates were tested for its ability to ferment a given sugar (lactose and sucrose) with the production of acid and gas or acid only and the growth medium used is peptone water as described by [22].

2.6 Screening of Isolates for Bioflocculant Production

The bacterial isolates were screened for bioflocculant production using Bioflocculant Production Broth medium (BPB) according to the method of [23]. The pH of the medium was adjusted according to [24].

2.7 Determination of Flocculating Activity

The flocculating activity of the isolates were determined according to the method described [1]. In which a suspension of kaolin clay was used as test material for flocculating activity determination. The kaolin clay was suspended in distilled water at a concentration of 5 g/L and used as a stock solution for the subsequent assays. The following solutions were mixed in a test tube: kaolin clay suspension (9 mL), culture supernatant (0.1 mL) and 1% $CaCl_2$ (0.25 mL). A reference tube, in which the culture supernatant was replaced with distilled water, was also included and measured under the same conditions. The solutions were gently mixed and allowed to settle for 5 minutes at room temperature. The optical density (OD) of the clarifying upper phase solution was then measured at 550 nm with a UV spectrophotometer and the flocculating activity determined as follows:

$$\text{Flocculating activity (\%)} = \frac{[(B - A)]}{B} \times 100\%$$

Where A and B are optical densities at 550 nm of the sample and control respectively.

3. RESULTS AND DISCUSSION

In the present investigation, several parameters were examined which include, gram staining, colony characterization of the isolates, biochemical characteristics of the isolates and flocculation activity of the bioflocculant-producing bacteria.

The result presented in Table 1 showed that the physical parameters of samples. The samples were turbid with distinct colour, pH and temperature.

The result presented in Table 2 showed that six bacterial isolates which were sub-cultured on a freshly prepared nutrient agar plates. The results of the gram staining reaction and colony characteristics of the isolates were also reported.

Table 1. Physical parameters of the wastewater samples

Collection site	Colour	pH	Temperature (°C)	Appearance
Jimeta Abattoir	Yellowish red	7.22	29	Turbid
Jimeta Modern Market	Whitish brown	6.70	36	Turbid
Gwari Market	Dark and brownish	6.89	33	Turbid

Table 2. Gram Staining and colony characterization of recovered isolates

Isolates	Gram staining reaction	Colony/Morphological Characteristics
ISO1	-	Rod shaped
ISO2	-	Short bacilli, pigment-like colonies
ISO3	+	Cocci, small colonies, yellowish-golden colour colony
ISO4	-	Rod shaped, bluish green pigment
ISO5	-	Rod shaped, short bacilli, dark colonies
ISO6	+	Bacilli shape, large, branched whitish colonies

+ = Gram Positive; - = Gram Negative

Table 3. Biochemical characteristics of the isolates

Isolate	Citrate test	Oxidase test	Catalase test	Coagulase test	Urease test	Indole test	Voges-Proskeur test	Methly-Red test	Lactose test	Sucrose test	Organism test
ISO1	-	-	+	-	-	+	-	+	*	-	*E. coli*
ISO2	+	+	+	NA	+	-	-	-	-	-	*P. aeruginosa*
ISO3	+	-	+	+	+	-	-	NA	-	+	*S. aureus*
ISO4	+	-	-	NA	+	-	+	-	*	+	*Klebsiella spp*
ISO5	-	-	NA	NA	-	-	-	+	-	-	*Salmonella spp*
ISO6	+	+	+	-	+	-	-	-	-	+	*Bacillus spp*

*NA = Not applicable + = Positive reaction, - = Negative reaction, * = positive and gas production*

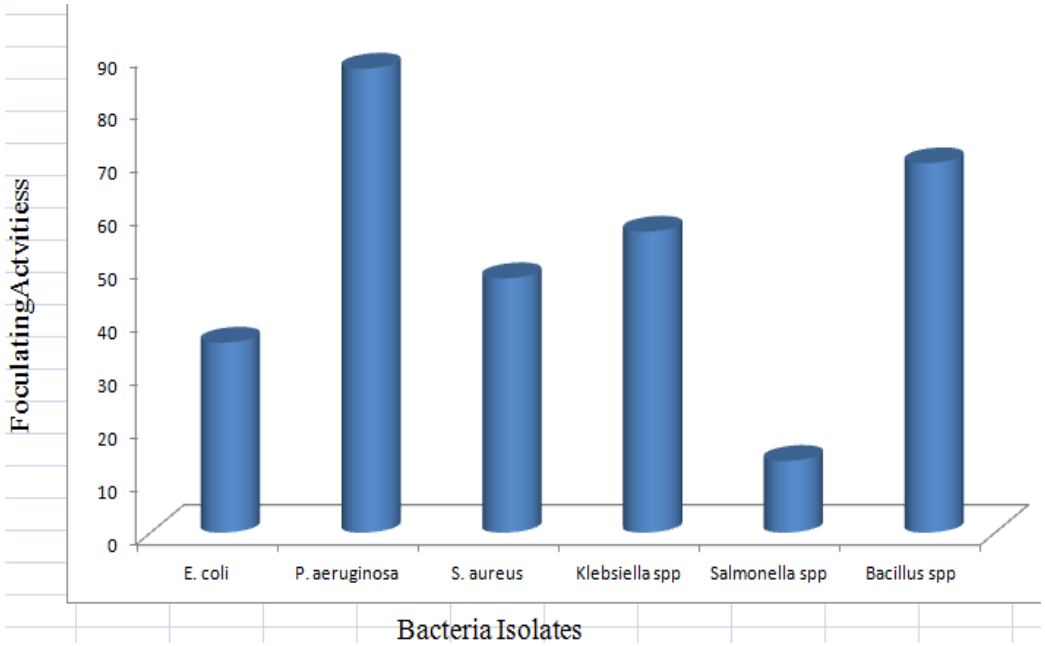

Fig. 1. Flocculating activity of the bioflocculant-producing bacteria

Table 4. Flocculation activity of the bioflocculant-producing bacteria

Isolate	Identified bioflocculant-producing bacteria	Flocculating activity (%)
ISO1	*E. coli*	35.76
ISO2	*P. aeruginosa*	87.32
ISO3	*S. aureus*	47.87
ISO4	*Klebsiella spp*	56.65
ISO5	*Salmonella spp*	13.5
ISO6	*Bacillus spp*	69.54

The results presented in Table 3 showed the biochemical characteristics of the isolates. The organisms identified were *Escherichia coli (ISO1), Pseudomonas aeruginosa (ISO2), Staphylococcus aureus (ISO3), Klebsiella spp. (ISO4), Salmonella spp. (ISO5) and* (ISO6) *Bacillus spp.*

The result presented in Table 4 showed the flocculating activity of each of the six bioflocculant-producing bacteria isolates which was determined after culture on a bioflocculant-production broth (BPB) in which their flocculating activity was tested using kaolin clay solution.

3.1 Discussion

In this study, Six bacterial cultures were identified which were *E. coli (ISO1), P. aeruginosa (ISO2), S. aureus (ISO3), Klebsiella spp. (ISO4), Salmonella spp. (ISO5) and Bacillus spp. (ISO6).* These bacteria have been known to be found in wastewater sites which is in agreement with the findings of [25] in which they identified and isolatd bacteria from abattoir wastewater. [26] reported the presence of *Klebsiella spp*, *Serratia sp*, among many microorganisms in raw textile effluents. According to International standards, any water contaminated to this level could pose a hazard/threat to the environment as it will be a favourable habitat for water-borne diseases [27]. *P. aeruginosa* has the highest flocculation activity with 87.32%, while *Salmonella spp*. has the least flocculating activity of 13.5% while the flocculating activity of *E. coli*, *S. aureus*, *Klebsiella spp* and *Bacillus spp*. are 35.76%, 47.87%, 56.6% and 69.54% respectively. These observations and results were similar with the finding of [28], they use strains of *S. aureus, P. plecoglossicida, P. pseudoalcaligenes, E. acetylicum, B. subtilis, K. terrigena with relatively high flocculating activity to produce bioflocculants*. The ability of the bioflocculant-producing bacteria isolated from wastewater samples to flocculate suspensions shows an efficient flocculating activity of the organisms after testing and determining their capability of flocculating kaolin clay in solution. *Salmonella spp.* having the least flocculating activity of 13.5% may be as a result of agitation of the culture medium and other factors affecting bioflocculant production, this report was similar to the report of [29]. Though, there has not been any known report on studies of *Salmonella spp*. for bioflocculant production. The agitation of the medium, temperature and others factors may go a long way in affecting the flocculating activity of the bacterial bioflocculant during screening of the bacterial isolates for bioflocculant production capabilities.

This similar phenomenon was reported in the cultivation of *Rhodococcus erythropolis* for the production of bioflocculant [30]. This study revealed that *P. aeruginosa* and *Bacillus sp*. produce the best flocculation activity of 87.32% and 69.54% respectively, this result agrees with the work of [28]. Hence there is a need to screen for more bacterial which has biofloccuting properties so as to reduce the use of chemical flocculants which are harmful to our human health especially in developing countries and in places that lack portable drinking water.

4. CONCLUSION

This research revealed that the bacteria *P. aeruginosa* and *Bacillus sp*. gave the highest flocculating activity. The other bacterial isolates: *E. coli*, *S. aureus* and *Klebsiella spp*. are also potential bioflocculant-producing bacteria with flocculating activity of 35.76%, 47.87% and 56.6% respectively.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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