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Flocculation potential of crude bio-flocculants from bacteria isolated in Oyun river, Ilorin, Kwara state

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ABSTRACT

This study aims to evaluate and optimize the flocculating activity of crude bio-flocculants from selected bacteria isolates in river Oyun, Ilorin Kwara state. Water samples were collected for bacteria isolation. Screening of the isolate for duplicate and bio-flocculant production before optimization of conditions and molecular identification was done. Seventeen (17) bacteria isolates were obtained after preliminary screening to remove duplicate organisms. Out of these, six (6) with bio-flocculant capability were selected for optimization via variation with respective pH range, carbon and cation sources. The isolates were identified via molecular analysis as *Comamonas aquatica* MTK09, *Pseudomonas aeruginosa* MTK10, *Aeromonas* sp. MTK11, *Aeromonas caviae* MTK12, *Pseudomonas* sp. MTK13 and *Aeromonas veronii* MTK14 respectively after sequence blast. Highest activity (82.6%) across all sources were recorded with *Aeromonas caviae* MTK12, followed by *Aeromonas* sp. MTK11 and *Pseudomonas* sp. MTK13 using lactose as the sole carbon source. However, starch yielded least flocculating rate with all the bacteria isolates. Although, use of divalent cation source ($FeSO_4$) recorded highest overall flocculating activity (298.6%) while highest activity yields of 74.2% was obtained with KCl for *Pseudomonas aeruginosa* MTK10. The bio-flocculant activities of the isolates were most active at pH 12 with overall activity of 488.9% and optimized result of 99.5% for *Aeromonas caviae* MTK12 while other pH ranges also had significant activities for other isolates. In conclusion, this study reveals that the activity of bio-flocculants can be improved via change of production conditions thereby obtaining better yield which in turn can improve the flocculating activity. Furthermore, the respective activities of the isolates recorded at varying pH acclaim its applicability in different environmental conditions.

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1.0 INTRODUCTION

Water is an essential factor for all living organisms nonetheless, this natural resource is ever more subjected to pollution due to population growth, food demand and increase in agricultural and industrial uses. Increase of industrial, agricultural and commercial chemicals been discharged into the aquatic environment have caused various harmfulness on aquatic organism and water bodies (Kolawole et al., 2011). One of the stages of water treatment process involves removal of debris resulting with the addition of inorganic flocculants like alum (poly-aluminum chloride) (Hubbard, 2004). Flocculants have been widely used in industrial processes aside water treatment including food processing and fermentation processes (Salehizadeh and Shojaosadati, 2001; 2003).

Flocculation is a process that removes colloidal particles from solution but the major problem with usage of inorganic flocculants is the adverse effects on both the environment and human health (Zhang et al., 2007; 2013). Health safety of conventional synthetic flocculants has necessitated the need for alternative flocculants from micro-organisms inhabiting many environments, particularly those from unusual environment such as open river. This study aimed to isolate micro-organism (bacteria) with probable flocculating activity from a river water in Ilorin, evaluate the influence of factors that can affects the flocculating activity and to molecularly identify the bio-flocculant producing isolates.

2.0 MATERIALS AND METHODS

Study Area

The study site was Oyun river located in Ilorin (Kwara State) on latitude and longitude 8º 29' 47.90" N; 4º 32' 31.70" E (Latitude.to, 2019). Ilorin is a metropolitan city made up of people from different ethnic groups (last population-814,192 in 2015) (WPR, 2019).

Sample collection and Material sterilization

Sample (Water) was collected in duplicate into sterile disposable bottles following procedure reported by Kolawole et al. (2011; 2019). The water sample was collected before the sun reached its peak at about 7am to 9am and transported within the hour to the Microbiology laboratory of the Central Research Laboratories, University of Ilorin. Collection of samples was according to the standard guidelines and methodology by WHO (1997). All materials and work bench were sterilized either by heat and 70% ethanol respectively.

Media Preparation and Medium Constitution

Nutrient Agar was prepared according to manufacturer's instruction (Sigma Aldrich; Germany). The bio-flocculant fermentation medium was composed using the following: "10 g of glucose, 1 g of peptone, 0.3 g of $MgSO_4.7H_2O$, 5 g of K_2HPO_4"in a litre of distilled water. The initial pH was adjusted to 7.0 with either "NaOH (0.1M) or HCl (0.1M)" (Busisiwe et al., 2016).

Isolation of Microorganism

Using the spread plate method, bacteria were isolated from the water samples on Nutrient Agar as colonies. Distinct colonies were sub-cultured and then incubated at 37°C for 24 hours. Pure isolates were kept as stock on agar slant and refrigerated at 4°C.

Preliminary Identification of Bacterial Isolates

To ensure varieties and avoid duplication of isolates, characterization and separation was based on colonial morphology, staining reactions and biochemical tests according to laboratory manual (Fawole and Oso, 2004;2007: Forbes et al., 2012).

Screening for Flocculant Producing Microorganisms

Each bacterial isolate was inoculated into 5 ml of sterile growth medium contained in a McCartney bottle and incubated at 25°C with shaking (120 rpm) for 7 days. The fermentation broth was centrifuged at 4000 × g for 30 min at 4°C to sediment the cells. The cell free culture supernatant (CFCS) was used to assay for flocculating activity.

Determination of Flocculating Activity

Using a suspension of kaolin clay, flocculating activity was measured according to the method described by Kurane et al. (1994) and Zhang et al. (2008). Three milliliters of 1% (w/v) $CaCl_2$ and 2.0 ml of CFCS was added into 100 ml of kaolin suspension (4.0 g/l) in a 250 ml conical flask, the mixture was vigorously stirred, poured into 100 ml of measuring cylinder and allowed to stand for 5 minutes at room temperature. The optical density (OD) of the clarifying solution was measured at 550nm using spectrophotometer (UV-5200; USA). Control was prepared using distilled water in place of the cell free culture supernatant (CFCS). The flocculating rate (FR) was determined by using the formula: FR = {(A – B)/A} X 100:

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

Effect of Culture Conditions on Bio-flocculants Production

Carbon Source: Using the description by Lachhwani (2005), the effects of glucose, lactose and starch on bio-flocculants production were assessed. The flocculating activity was measured following Zhang et al. (2008) procedure.

Cations: By substituting each selected cation ($CaCl_2$, KCl and $FeSO_4$) for each culture and maintaining other parameters at constant, the effect of cation was evaluated (He et al., 2010).

pH: The pH of the kaolin solutions was adjusted to 2, 4, 6, 8, 10 and 12 with HCl and NaOH respectively (Xiong et al., 2010). The flocculating activity was determined at each pH value while keeping a constant condition of the Carbon and Cation source that most favoured the flocculating activities across the isolates.

Molecular Identification of Bio-flocculants Producing Bacterium amplicon

The PCR was carried out in a reaction volume of small reaction tube in a thermal cycler. The thermal cylinder temperatures ranged from 60^{o}C to 95^{o}C. The reagents used and their concentration are given as follows: 0.8 µl of magnesium chloride, 0.8 µl of tween 20, 0.5 µl of the primer 1 (for the forward reaction), 0.5 µl of primer 2 (for backward reaction), 1.0 µl of 10x buffer, 0.5 µl of 2.5 dNTPs (Deoxynucleotide triphosphates), 0.06µl of the taq polymerase, 2.84 µl of nuclease free water and 3.0µl of the isolated DNA. These were all mixed in a reaction tube, vortexed and inserted into the PCR machine for the reaction. The primer used for the two reactions were the 16S primers (5'-AGAGTTTGATCCTGGCTCAG-3) (Kolawole et al., 2019). The product was purified and was sequenced to determine the respective species of the test organisms at the bioscience centre of the International Institute of Tropical Agriculture (IITA)

16S rRNA sequence analysis

As previously stated by Kolawole et al., 2019, the 16S rRNA sequence identification of the bio-flocculant producing bacteria were aligned and compared with the deposited sequences in the database of NCBI GenBank using BLAST engine.

Data Analysis

The respective data were presented with 2016 version of Microsoft excel package with defined standard error value significant to each parameter.

3. RESULTS AND DISCUSSION

Seventeen (17) bacteria isolates were obtained after preliminary screening to remove duplicate organisms. Out of these, six (6) with bio-flocculant capability were selected for optimization via variation with respective pH range, carbon and cation sources. Plate 1 shows the molecular result of the respective isolates on gel. It also shows the molecular marker of 1kb followed by the 850bp weight of the isolates which were later allotted unique identifier of "MTK" and accession numbers due to the distinctiveness of the strains to other deposited isolates in the Gene Bank. The respective strains and their accession numbers are *Comamonas aquatica MTK09*(MK288112), *Pseudomonas aeruginosa MTK10* (288113), *Aeromonas sp MTK11* (288114), *Aeromonas caviae MTK12* (288115), *Pseudomonas sp MTK13* (MK288116) and

Aeromonas veronii MTK14 (MK288117). In addition to the recorded flocculating activity of the flocculant from the isolates, their strains were also confirmed as new.

The activity recorded from the crude bio-flocculant from lactose culture was noticed to be most active, followed by glucose and least was with starch except with *C. aquatica MTK09* which had considerably lower activity with lactose when compared to other isolates (figure 1). The highest recorded with starch as carbon source was noticed with *Pseudomonas sp MTK13* while the least was with *P. aeruginosa MTK10*. This could be related to the difference in strains of the isolates which influences the flocculant production and activity. The lower activity recorded with starch is similar to the work of Kolawole et al., 2019 which was attributed to the complexity of the carbon source and thus its utilization is harder for the isolates compared to simple sugars such as glucose, lactose and sucrose (NHS, 2017). The order of increase of flocculating activity in this study is from starch to glucose and to lactose.

The result of figure 2 reveals the activity of the bio-flocculant with different cation sources where the highest activity was recorded with *P. aeruginosa MTK10* for KCl and $FeSO_4$, *Aeromonas* sp *MTK11* for $CaCl_2$and the least with *A. veroniiMTK12*for $CaCl_2$ and *Pseudomonas* sp *MTK13* for $FeSO_4$ and KCl respectively. The report on $CaCl_2$ is in line with report of Nontembiso et al., 2011 which also recorded lower FA. The divalent cation was noticed to significantly support the flocculating activities across all isolates. Cations are amongst the factors discovered to impact the flocculating rate of organisms and they are known to function by stabilizing and neutralizing the charges, forming bridges between particles thus enhancing bio-flocculation (Sobeck and Higgins, 2002; Kolawole et al., 2019). The overall activity of the cations across the isolates revealed $FeSO_4$to be most supportive, followed by $CaCl_2$ and KCl.

Previous reports have shown pH tolerance of bacteria species ranging from "4.5 to 9/10" (Claudia et al., 1999; Janda and Abbott, 2010; Igbinosa et al., 2012) with an optimum of 7.5. Likewise, an optimal pH range (6-8) for *Providencia* species, pH 6-9 for *Raoultella ornithinolytica*, pH 5-9 for *Alcaligenes* sp, pH 5-7.1 for *Klebsiella pneumoniae* and pH 5-10 for *Pseudomonas* sp (Bruce et al., 1981; Naqvi, 2007; Hossain et al., 2016; Lu et al., 2017; Sharma et al., 2017; Aleanizy et al., 2018; Kolawole et al., 2019) have been recorded. Amongst all the evaluated pH ranges for the flocculating activities in this study, pH 12 reveals to be supportive to all the flocculants compared to the irregular activities obtained from other range. *Pseudomonas* sp *MTK13, A. veronii MTK14*and *C. aquatica MTK09* were recorded to have spike activity at close to neutral pH except for *Aeromonas* sp *MTK11* and *P. aeruginosa MTK10* which had a decline of activity. *A. caviae MTK12* was however noticed to maintain a stable activity across the near neutral pH range. Highest FA was noticed with flocculant from *A. caviae MTK12* at pH12 while the least was at pH2 with crude flocculant from *Pseudomonas* sp *MTK13*.

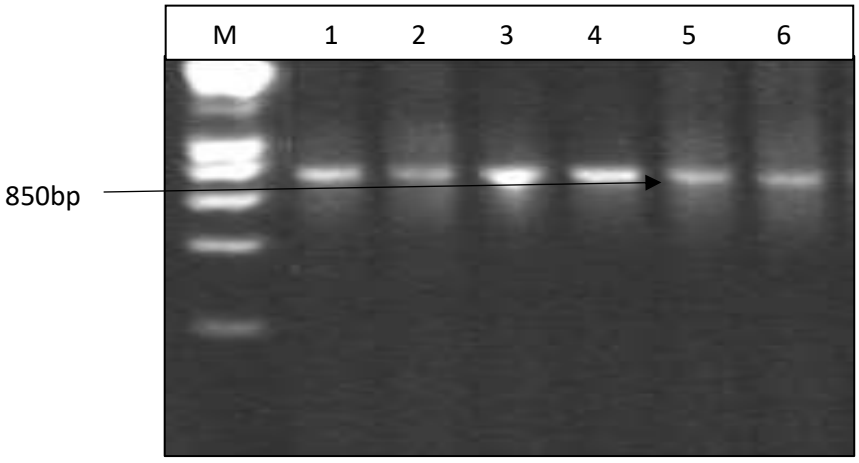

Plate 1: Bacteria isolates with bio-flocculating activity on agarose gel
(M= Ladder -1kb; 1-6= *Comamonas aquatica MTK09, Pseudomonas aeruginosa MTK10, Aeromonas sp MTK11, Aeromonas caviae MTK12, Pseudomonas sp MTK13, Aeromonas veronii MTK14*)

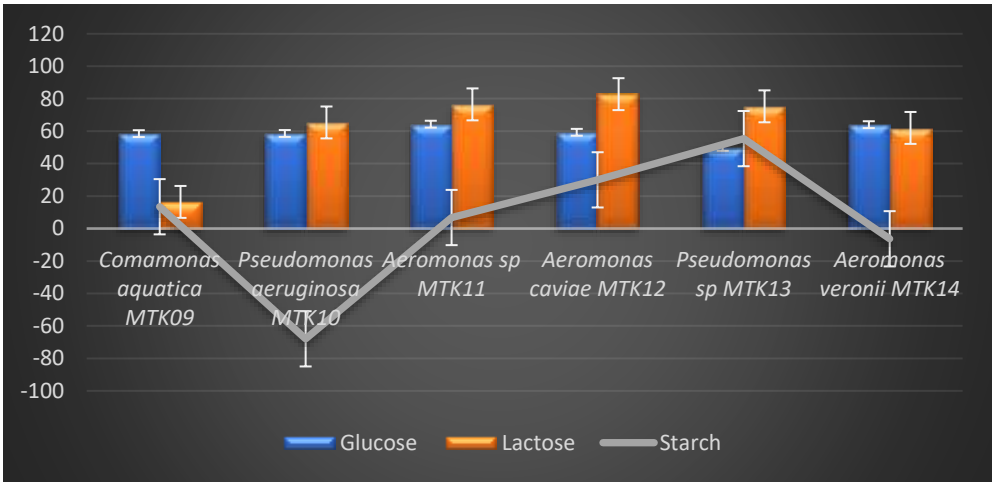

Figure 1: Effect of carbon sources on F.A.

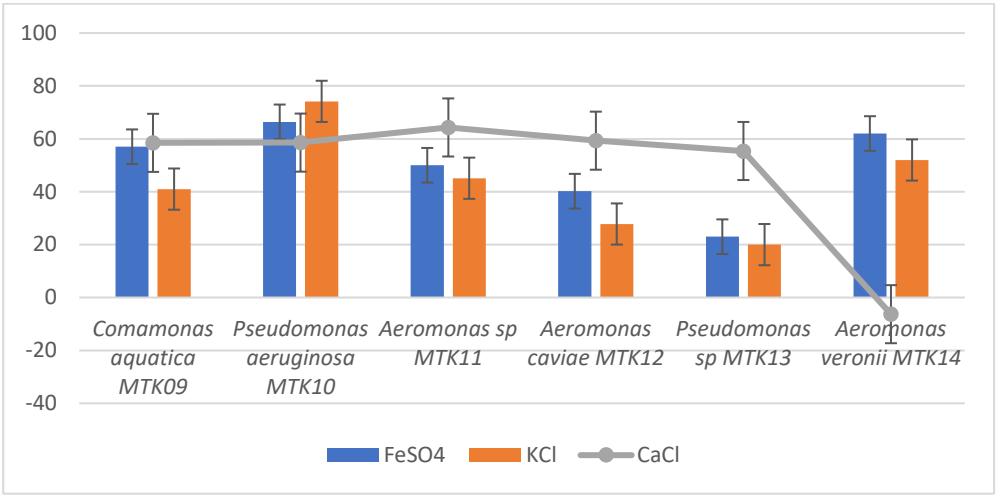

Figure 2: Effect of cations on F.A.

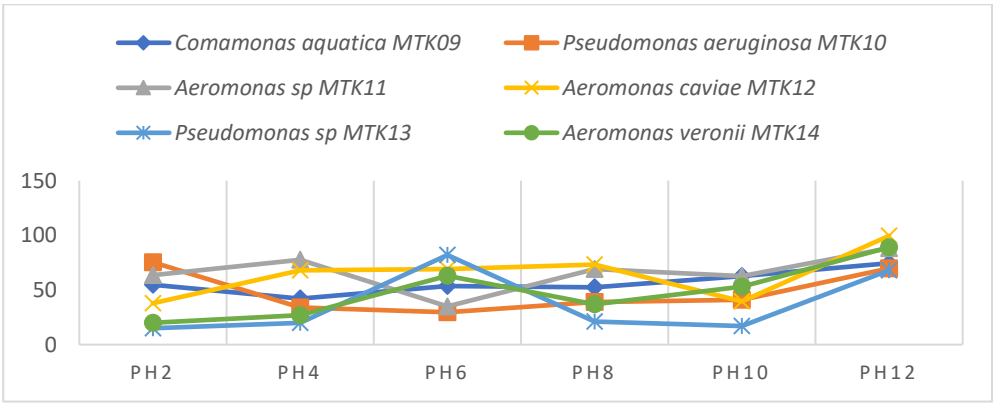

Figure 3: The effect of pH range on F.A.

Conclusion

This study has revealed new bacteria strains from our indigenous environment with flocculant producing capabilities. Additionally, it also showed that the activity of bio-flocculants can be improved via change of production conditions thereby obtaining better yield which in turn can improve the flocculating activity. Furthermore, the respective activities of the isolates recorded at varying pH range depicts its applicability in different environmental conditions for treatment purpose.

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