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Screening for Bioflocculant-Producing Bacteria from the Marine Environment of Sodwana Bay, South Africa

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Abstract

Flocculants are chemicals that mediate flocculation process, by aggregating colloids from suspension to form floc. Chemical flocculants are hazardous to the environment, which inform the search for safer and eco-friendly alternatives from microorganisms. Bacterial strains were isolated from water and sediment samples collected from Sodwana Bay, South Africa, and physiological properties of the bacterial strains were observed. Flocculation test using kaolin clay suspension was done on all isolates and the ones that showed flocculating activity were identified molecularly using 16 rRNA gene sequence analysis. Forty marine bacteria isolates were gotten from sediments and water samples collected from Sodwana Bay. Most of the isolates exhibited a range of colony pigmentation (pink, creamy, yellow, and white). After purification of individual isolates, they were screened for their potential to produce bioflocculant. The result revealed that isolates marked SOD3, SOD10, SOD12, SOD26, SOD27, SOD28, SOD32, SOD33 and SOD34 produced bioflocculants as shown by the flocculating activities from their crude extract. All these isolates showed good flocculation of kaolin clay suspension above 60% (flocculating activity) except SOD12. These bioflocculant producing isolates were identified as *Pseudoalteromonas sp*, *Alcaligenes faecalis*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus stratosphericus*. The results showed Sodwana Bay, South Africa as a reservoir of bacteria with potential to produce flocculants. However, further studies on the optimisation of culture conditions for bioflocculant production, extraction, characterisation and application of isolates is on the way to underscore the biotechnological importance of these microbes as producers of substitutes to harmful chemical flocculants commonly used in water and wastewater treatment.

Keywords: Marine; Bacteria; Bioflocculant; Sediment; Sodwana Bay.

1.0 Introduction

The marine environment could be seen as a repository of many novel microorganisms that have potential to produce secondary metabolites (Bredholt *et al.*, 2008). The marine realm covers enormous amount of the earth's surface and high volume of its crust (70% and 90% respectively), thus providing the largest inhabitable space for microorganisms (Lam, 2006; Fenical, 1993). It is regarded as heavily complex and contains various assemblage of life forms that have adapted to extreme environmental conditions such as temperature, pressure and salinity. Marine environmental conditions usually differ from terrestrial, which could confer unique characteristics to marine microorganisms that enables them produce different new biologically active compounds (Stach *et al.*, 2003; Lam, 2006). An important characteristic for taxonomy and identification of products and functions, is in the isolation of new microorganisms. (Alain and Querellou, 2009).

In the field of microbiology, microorganisms of interest are usually isolated using general and selective media. In the last two decades, the presence of microbes which could not be cultivated in the environment is now made possible by a culture- independent method (Christen, 2008). There are new technologies for isolation of microorganisms, which includes micro-encapsulation techniques, fluorescence-activated cell sorting, micromanipulation techniques, diffusion chambers, floating filter method, and high throughput extinction-culturing methods (Alain and Querellou, 2009; Brehm-Stecher and Johnson, 2004). Pure culture isolates of bacterial species from different environmental sources is crucial in all the various branches of the field of microbiology since bacteria are found almost everywhere and live in consortium with other microbial communities.

Bioflocculation is the process whereby flocculation is mediated in the presence of microorganisms and/or their metabolite products (bioflocculants) (Tyagiet *et al*., 2009). It involves the removal of colloidal particles from solution by these metabolites products of the microorganisms, with or without the subsequent biodegradation of the colloidal particles (Tyagiet *et al.*, 2009). The production and usage of bioflocculants has gained much attention and research recently because of its biodegradability, harmlessness and lack of secondary pollution (Gong *et al.*, 2008). When used in dewatering and downstream processing it is inert and safe compared to chemical flocculants that are not readily biodegradable and some of their monomeric products are neurotoxic and even show strong human carcinogenic potential (Shih *et al.*, 2001). Due to the neurotoxic, carcinogenic and non-biodegradability of chemical flocculants, attention is shifting toward safer and more eco-friendly alternative derived from microorganisms. This has led researchers to isolate, screen and identify microorganisms with potential to produce bioflocculant.

The present study aimed to isolate microorganisms from water and sediment samples collected from Sodwana Bay, South Africa and screen them for bioflocculant producing potential. After screening, nine out of the forty isolated strains produced bioflocculants and showed very good flocculation of kaolin clay. These nine isolate where then selected and identified using 16S rRNA gene sequence.

2.0 Materials and Methods

2.1 Collections of Samples

Six different Seawater and sediment samples were randomly collected from approximately 0 to 20 m off shore of the Sodwana Bay using water and sediment samplers. The collected samples were transported in ice to the Hydrology and Microbiology laboratories of the University at Zululand, KwaDlangezwa, South Africa. Sediment samples were dried overnight at 30 ^{o}C.

2.2 Isolation and screening of microorganism

Microorganisms were isolated using an agar plate culture containing medium with composition of meat extract-1.0 g, peptone- 5.0 g, yeast extract-2.0 g, NaCl-8.0 g and agar-15.0 g per litre of filtered seawater according to the methods described by Jensen et al. (1991). Microorganisms were originally screened based on colony morphology. Two loopfuls of bacterial colonies were then grown in 50 ml of screening medium according to Zhang et al. (2007) and Ugbenyen et al (2012) with slight modifications on a rotary shaker (160 rpm) at 30℃ for 72h. The medium contained (g/L of filtered natural sea water): glucose-20; casein-0.7; yeast extract-0.5; KH_2PO_4-2; K_2HPO_4-5; NaCl-0.1 and $MgSO_4 \cdot 7H_2O$-0.2. At the end of incubation period, 2 mL of the fermentation broth was centrifuged (8.0 g, 30 min) to separate the cells, and the cell free culture supernatant was analysed for flocculating activity. Finally, isolates with good flocculating activity for kaolin was selected for further study. The pre-culture was stored at 4 ℃ and used for subsequent inoculations.

2.3 Flocculating activity experiment

Flocculating activity was measured according to the method described elsewhere (Kurane *et al.*, 1986; Zhang *et al.*, 2007; Cosa *et al.*, 2011; Ugbenyen *et al.*, 2012) with slight modifications. Briefly, 3 mL of 1% $CaCl_2$ and 2 mL of cell free supernatant were added to 100 mL kaolin suspended solution (4 g/L) in 250 mL flask. The mixture was stirred vigorously, poured into a 100 mL cylinder and allowed to stand for 5 min. The absorbance of the clarifying solution (at 550 nm) was measured spectrophotometrically. A control experiment was performed using the same method, but with fresh culture medium replacing the cell-free supernatant. The flocculating activity was calculated according to the equation:

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

where A is the absorbance at 550 nm of the sample and B is the absorbance at 550 nm of the control experiment.

2.4 Identification of Bioflocculant Producing Microorganisms

2.4.1 DNA Extraction

DNA extraction was done for selected isolates showing good potential for the production of bioflocculant using ZR fungal/bacterial DNA MiniPrep™ Kit from Zymo Research, Inc. The extraction protocol was performed according to the instruction of the manufacturer.

2.4.2 Polymerase Chain Reaction (PCR) Amplification of 16S rRNA Gene

PCR amplification was carried out in 50 µL reaction volume of Dream Taq™ Green PCR Master Mix containing 0.4 mM of each dNTP, 4 mM

$MgCl_2$, 0.2 µL Dream Taq™ polymerase, 1 µM of each forward and reverse universal primer (F:5'-ATGCCATAGCATTTTTATCC-3'), (R:5'-GATTTAATCTGTATCAGG-3') and 1 µg template DNA. The PCR condition include an initial denaturation (95 ºC for 3 min), 25 cycles of denaturation (95 ºC for 30 sec), annealing (56 ºC for 30 sec) and extension (72 ºC for 1 min), and a final extension (72 ºC for 5 min).

2.4.3 PCR Products Sequence

The PCR product sequence was done at the facilities of Inqaba Biotech Laboratories, Pretoria, South Africa using a sequencer. The 16S rRNA gene sequences obtained was compared with others in the NCBI genebank database with Basic Alignment Search Tool (BLAST) according to Stephen *et al.*, (1997).

Table 1: Morphology of Bacteria Isolates from Sodwana Bay

Isolate No	Type of Sample	Morphology/Colour	Pigmentation
SOD1	Sediment	Glistening pink colour,	Pinkish
SOD2	Sediment	Creamy serrated edges	Creamy
SOD3	Sediment	Moderate cream	Creamy white
SOD4	Sediment	Creamy white swarming colony	Creamy
SOD5	Sediment	Creamy tiny small colonies	Creamy
SOD6	Sediment	Translucent creamy colour	Creamy
SOD7	Sediment	Flat whitish serrated colony	Creamy
SOD8	Sediment	Whitish creamy swarming edged colony	Creamy
SOD9	Sediment	Creamy white swarming colony	Creamy
SOD10	Sediment	Light brown smooth and glistering colony	Clear
SOD11	Sediment	Flat creamy swarming colony	Creamy
SOD12	Sediment	Creamy branching mycelia colony	Creamy
SOD13	Sediment	Pink, smooth glistening colony	Pinkish
SOD14	Sediment	Creamy	Creamy
SOD15	Water	Creamy white	Creamy
SOD16	Water	Creamy white	No pigmentation
SOD17	Water	Creamy branching leaf like colony	Creamy
SOD18	Water	Whitish swarming and branching edge colony	Creamy white
SOD19	Water	Light creamy smooth edges	Creamy
SOD20	Water	Creamy white serrated edge colony	Creamy
SOD21	Estuary Water	Creamy serrated edge colony	Creamy white
SOD22	Water	Creamy serrated edge colony	No pigmentation
SOD23	Water	Whitish creamy serrated edge colony	Creamy
SOD24	Water	Creamy white branching edge colony	Creamy white
SOD25	Water	Dry whitish raised round colony	Creamy
SOD26	Water	Creamy, moderate edge colony	Creamy
SOD27	Estuary water	Creamy serrated edge colony	Creamy
SOD28	Water	Whitish creamy serrated edge	Creamy
SOD29	Water	White swarming edge colony	Creamy
SOD30	Water	Lightish creamy colony	No pigment
SOD31	Water	Yellow glistering rough edge colony	Yellowish
SOD32	Estuary	Whitish creamy, swarming colony	Creamy
SOD33	Estuary	Whitish cream serrated edge colony	Creamy
SOD34	Estuary	Creamy swarming edge colony	Creamy
SOD35	Water	Creamy	Creamy
SOD36	Water	Yellow glistering round edge colony	yellowish
SOD37	Estuary	Pink, smooth glistering colony	Pink
SOD38	Water	Creamy white swarming colony	Whitish creamy
SOD39	Water	Translucent creamy serrated edge colony	No pigment
SOD40	Water	Whitish serrated colony	Whitish

3.0 Results and Discussion

It is well documented that soil, water and activated sludge samples collected from terrestrial habitats have been found to be good sources for isolating flocculant-producing microorganisms (Fujita *et al.*, 2000; Lu *et al.*, 2005; Gao *et al.*, 2006; Yim *et al.*, 2007; Gong *et al.*, 2008). It has also been reported that there is a decrease in the rate of new metabolite isolated from microorganism of terrestrial origin. Rather what have been observed is an increases re-isolation of already known compounds (Fenical *et al.*, 1999; Ugbenyen *et al.*, 2012). This suggests that more research should focus on exploring under-exploited habitat like the marine environment for isolation of novel microorganisms for new bioactive compounds. The present study explores the marine environment of Sodwana Bay, South Africa, as a source of bacterial isolates with potentials to produce bioflocculant.

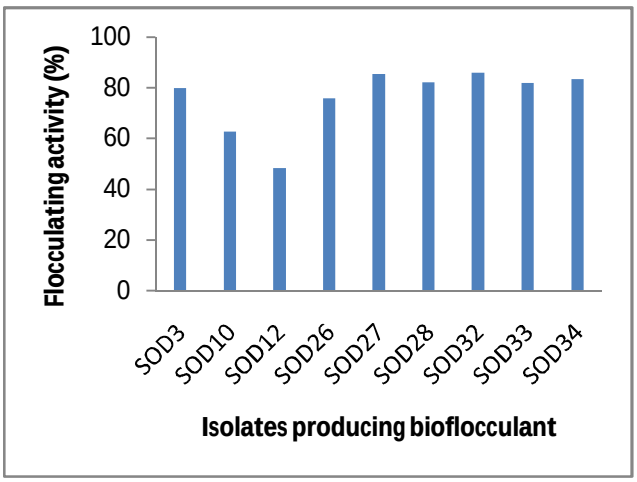

Figure 1: *Flocculating activities of bioflocculant-producing isolates from Sodwana bay. FA is the flocculating activity in percentage. SOD3-Pseudoalteromonassp; SOD10-Alcaligenesfaecalis; SOD12-Alcaligenesfaecalis; SOD26- Bacillus subtilis; SOD27- Bacillus cereus; SOD28- Bacillus cereus; SOD32- Bacillus cereus; SOD33- Bacillus cereus; SOD34- Bacillus stratosphericus.*

In this study, after screening forty marine isolates (Table 1), only nine where found to produce bioflocculants (Figure 1). All bioflocculant-producing isolates SOD 3, SOD 10, SOD 26, SOD 27, SOD 28, SOD 32, SOD 33 and SOD 34, showed good flocculating activities above 60%, except SOD 12. The advantages bioflocculant possess as an inert and greener flocculant over chemical flocculants which have been implicated in health problems (Dearfield and Abermathy, 1988; Kowall *et al.*, 1989) is the main aim for screening these isolates. Approximately, 25% of the total isolate, produces bioflocculants indicating that Sodwana Bay is a reservoir of bacteria with bioflocculant potential.

Identification by 16S rRNA gene revealed that of the nine (9) bioflocculant-producing isolates, *Bacillus* species were more predominant followed by *Alcaligenes* and *Pseudoalteromonas* species (Table 2). The genus *Bacillus* comprises of about 60 species which are gram positive rod-shaped bacteria that have been implicated in bioflocculant production (Salehizadeh and Shojaosadati, 2002; Ugbenyen *et al.*, 2013; Ugbenyen *et al.*, 2014). From identification results of bioflocculant-producing strains, the following *Bacillus* species were identified: *Bacillus subtilis*, *Bacillus cereus*, *Bacillus stratosphericus*, confirming these genera as producers of bioflocculant.

Table 2: Molecular Identification of Bacteria Isolates with Bioflocculant-Potential

Isolate No	Location	Identification by 16S rRNA
SOD3	Marine sediment	*Pseudoalteromonas* sp
SOD 10	Marine sediment	*Alcaligenes faecalis*
SOD12	Marine sediment	*Alcaligenes faecalis*
SOD 26	Marine water	*Bacillus subtilis*
SOD27	Estuary	*Bacillus cereus*
SOD 28	Marine water	*Bacillus cereus*
SOD 32	Estuary	*Bacillus cereus*
SOD 33	Estuary	*Bacillus cereus*
SOD 34	Estuary	*Bacillus stratosphericus*

On the other hand, *Alcaligenes faecalis* a gram-negative rod-like bacterium was also isolated from the marine environment of Sodwana Bay as a producer of bioflocculant. This further confirmed the findings of Shimiziu (1985), who earlier reported that *Alcaligenes faecalis* produces bioflocculant. *Pseudoalteromonas* sp. was another bacterium identified as a producer of bioflocculant from this study; it is a rod-shaped, gram-negative bacteria. It is interesting to note, that Dufourcq *et al.*, (2013), also isolated *Pseudoalteromonas* sp. from a marine coastal environment and found them to be a rich source of bioactive compounds. This suggests that the bacterium is predominant in the marine environment.

4.0 Conclusion

The present study indicate that, Sodwana Bay, South Africa is a reservoir of bacteria with potential to produce bioflocculants. However, further studies on the optimization of culture conditions for bioflocculant production, extraction, characterisation and application of the bioflocculants produced by these isolates will be done to underscore the biotechnological importance of these microbes as producers of alternative substitutes to the harmful chemical flocculants commonly used in water and wastewater treatment.

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Conflict of Interest

Authors declare no conflict of interest.

Authors Contribution

Conception: AMU, JJS and AKB; Design: AMU; Execution: AMU
Interpretation: AMU, JJS and AKB; Writing the paper: AMU
Proofreading the paper: JJS and AKB

References

Alain, K., and Querellou, J., 2009. Cultivating the uncultured: limits, advances and future challenges. *Extremophiles,* **13**, 583–594

Bredholt, H., Fjaervik, E., Johsen, G., and Ztchev, S.B., 2008. Actinomycetes from sediments in Trondheim Fjord Norway: Diversity and Biological Activity. *Mar. Drugs* **6**(1), 12–24.

Brehm-Stecher, B.F., and Johnson E.A., 2004. Single Cell Microbiology: Tools, Technologies and Applications. *Microbiol. Mol. Biol. Rev.* **68**, 538-559.

Cosa, S., Mabinya, L.V., Olaniran, A.O., Okoh, O.O., Bernard, K., Deyzel, S., and Okoh, A.I., 2011. Bioflocculant production by *Virgibacillus* sp. Rob Isolated from the Bottom Sediment of Algoa Bay in the Eastern Cape, South Africa. *Molecule,* **16**, 2431-2442

Christen, R. 2008, Global sequencing: a review of current molecular data and new methods available to assess microbial diversity. *Microbes Environ.,* **23**, 253–268

Dearfield, K.L., and Abermathy, C.O., 1988. Acrylamide: its metabolism developmental and reproductive effects, genotoxicity and carcinogenicity. *Mutat. Res.,* **195**, 45-77

Dufourcq, R., Chalkiadakis, E., Fauchon, M., Deslandes, E., Kerjean, V., Chanteau, S., Petit, E., Guezennec, J., and Dupont-Rouzeyrol, M., 2013. Isolation and partial characterization fbacteria (*Pseudoalteromonas* sp.) with potential antibacterial activity from a marine costal environment from New Caledonia. *Lett. Appl. Microbiol.,* **58**, 102-108.

Fenical, W., 1993. Chemical studies of marine bacteria: developing a new resource. *Chem. Rev.* **93**, 1673–1683.

Fenical, W., Baden, D., Burg, M., de Goyet, C.V., Grimes, J.D., Katz, M., Marcus, N.H., Pomponi, S., Rhines, P., Tester P., and Vena, J., 1999. Marine-derived pharmaceuticals and related bioactive compounds. In: From Monsoons to microbes: understanding the Ocean's Role in Human Health. Edited by Fenical W.National Academics Press. pp. 71-86.

Fujita, M., Ike, M., Tachibana, S., Kitada, G., Kim, S.M., and Inoue, Z., 2000. Characterization of a bioflocculant produced by *Citrobacter*sp. TKF04 from acetic acid and propionic acids. *J. Biosci. Bioeng.,* **89**, 40–46.

Gao, J., Bao, H., Xin, M., Liu, Y., Li, Q., and Zhang, Y., 2006. Characterization of a bioflocculant from a newly isolated *Vagococcus* sp. W31. *J Zhejiang Univ. Sci. B.* **7**, 186-192.

Gong, W., Wang, S., Sun, X., Liu, X., Yue, Q., and Gao, B., 2008. Bioflocculant production by culture of *Serratiaficaria* and its application in wastewater treatment. *Bioresour. Technol.,* **99**, 4668–4674.

Jensen, P.R., Dwight, R., and Fenical, W., 1991. Distribution of actinomycetes in near-shore tropical marine sediments. *Appl. Environ. Microbiol.,* **57**, 1102-1108.

Kowall, N.W., Pendleury, W.W., and Kessler, J.B., 1989. Aluminium-induced neurofibrillary degeneration affects a subset of neurons in rabbit cerebral cortex, basal forebrain and upper brain stem. *Neurosci.,* **29**, 329-337.

Kurane, R., Takeda, K., and Suzuki, T., 1986. Screening for characteristics of microbial flocculants. *Agric. Biol. Chem.,* **50**, 2301–2307.

Lam, K.S., 2006. Discovery of novel metabolites from marine actinomycetes. *Ind. Microbiol.,* **9**, 245-251.

Lu, W.Y., Zhang, T., Zhang, D.Y., Li, C.H., Wen, J.P., and Du, L.X., 2005. A novel bioflocculantproduced by *Enterobacteraerogenes*and its use in defecating the trona suspension. *Biochem. Eng. J.,* **27**, 1–7.

Salehizadeh, H., and Shojaosadati, S.A., 2002. Isolation and characterisation of a bioflocculant produced by *Bacillus firmus. Biotechnol. Lett.,* **24**, 35–40.

Shih, I. L., Van, Y. T., Yeh, L. C., Lin, H. G., and Chang, Y. N., 2001. Production of a biopolymer flocculant from *Bacillus licheniformis* and its flocculation properties. *Bioresour. Technol.,* **78**, 267–272.

Shimiziu, N., 1985. Floc-forming bacteria isolated from activated sludge in high BOD loading treatment. *J. Ferment. Technol.* **63**, 67–71.

Stach, J.E., Maldonado, L.A., Masson, D.G., Ward, A.C., Goodfellow, M., and Bull, A., 2003. Statistical approaches for estimating actinobacterial diversity in marine sediments. *Appl. Environ. Microbiol.,* **69**, 6189-6200.

Stephen, F.A., Thomas, L.M., Alejandro, A.S., Jinghui, Z., Zheng, Z., Webb, M., and David, J.L., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database searchprograms. *Nucleic Acids Res.,* **25**, 3389-3402.

Tyagi, R.D., Surampalli, R.Y., Zhang, T.C., Kao, C.M., Lohani, B.N., 2009. Sustainable Sludge Management: Production of Added Products. ASCE. Reston, Virginia pp. 146-156

Ugbenyen, A., Cosa, S., Mabinya, L., Babalola, O.O., Aghdasi, F., and Okoh, A., 2012. Thermostable Bacterial Bioflocculant Produced by *Cobetia* Spp. Isolated from Algoa Bay (SouthAfrica). *Int. J. Environl Res. Public Health,* **9**(6), 2108-2120.

Ugbenyen, A.M., Cosa, S., Mabinya, L.V., and Okoh, A.I., 2014. Bioflocculant Production by *Bacillus* sp. Gilbert Isolated from a Marine Environment in South Africa. *Appl. Biochem. Micro.,* **50**, 49–54.

Ugbenyen, A.M., and Okoh, A.I., 2013. Flocculating Properties of a Bioflocculant Produced by *Bacillus* sp. isolated from a marine environment in South Africa. *Chem. Biochem. Eng. Q.,* **27**(4)**,** 511-518.

Yim, J.H., Kim, S.J., Ahn, S.H., and Lee, H.K., 2007. Characterization of novel bioflocculant, p-KG03, from a marine dinoflagellate, *Gyrodiumimpudicum* KG03. *Bioresour. Technol.,* **98**, 361–367.

Zhang, Z., Lin,B., Xia, S., Wang, X., and Yang, A., 2007. Production and application of a novel bioflocculant by multiple-microorganism consortia using brewery wastewater as carbon source. *J. Environ. Sci.,* **19**, 667- 673.

