NJBAS

Available online at http://www.ajol.info/index.php/njbas/index
Nigerian Journal of Basic and Applied Science (December, 2016), 24(2): 45-52
DOI: http://dx.doi.org/10.4314/njbas.v24i2.7

ISSN 0794-5698

Full Length Research Article

Production of Biosurfactants by *Actinomycetes* Isolated from Hydrocarbon Contaminated Soils and Ikpoba River Sediments in Benin-City, Nigeria

[1]E.I Atuanya [1]A. Dunkwu-Okafor and [2*]U. Udochukwu
[1]Department of Microbiology, University of Benin, P.M.B 1154, Benin City, Nigeria.
[2]Department of Biosciences, Salem University, Lokoja, Kogi State, Nigeria
[*Corresponding author: rev.dr.ud@gmail.com]

ABSTRACT

The production of biosurfactants by *Actinomycetes* isolated from hydrocarbon contaminated soils and Ikpoba river sediments were evaluated. Soil samples were collected from mechanic workshops located in various parts of Benin City and Ikpoba river sediments. Physico-chemical analyses were performed. Isolation of *Actnomycetes* was done using starch casein agar incorporation with antibiotics incubated for 7 – 10 days at 30^{o}C. Growth on mineral salt medium initiated the production of biosurfactants which was extracted by centrifugation and filtration followed by liquid extraction using chloroform: methanol (2:1v/v). Characterization and stability studies were conducted. The pH of the contaminated soil was 4.92 +/- 0.049 while that of Ikpoba river sediments was 6.62+/-0.056. The hydrocarbon contaminated soils had a higher concentration of surphur, nitrogen, potassium, sodium, magnesium and manganese compared to Ikpoba River, but only chlorine concentration was higher in Ikpoba River. Aerobic Gram positive rods with extensive branching were observed confirming growth of *Streptomyces* sp. The result showed stability across different temperature ranges with no significant difference observed in the two sites in emulsification activity ($P>0.05$). There was significant difference observed in the mean surface tension of the biosurfactants produced from the two sites across different temperature ranges with hydrocarbon contaminated soil having higher values ($P>0.05$). There was also significant surface tension difference between pH2 and pH8 ($P<0.05$) suggesting higher activity within those ranges. The result also show stability across different salt concentrations and had foaming characteristics. Actinomycetes have complex enzymatic mechanism that aids hydrocarbon mineralization and thus increases the potential for biosurfactant production. These biosurfactants are stable across temperature ranges and are not majorly affected by salt concentration; this property aids its potential usage in decontamination of oil contaminated areas in the Niger Delta region of Nigeria and other countries.

Keywords: Biosurfactants, Actinomycetes, hydrocarbon contaminated soils and Ikpoba river sediments

INTRODUCTION

The contamination of soil and groundwater with petroleum hydrocarbons is unfortunately a common phenomenon and has been causing serious environmental problems in Nigeria. The release of these contaminants to the environment including petroleum and petroleum based/derived products is one of the main causes of global contamination (Rahman *et al*., 2003). It is also a risk for human and animal health, since many of these contaminants are toxic and carcinogenic (Prabhu *et al.*, 2003). Hydrocarbon molecules that are released into the environment are difficult to remove since they are absorbed to surfaces and are trapped in the water immiscible phase. Bioremediation has been considered and proven to be one of the best approaches and a suitable alternative to diminish the effects caused by hydrocarbon contamination of soil and water. This can be accomplished by using the metabolic capacity of microorganisms that can use hydrocarbon as source of carbon and energy, or that can be modified by co-metabolism and also for restoration of soil because the technology is cost effective and environmentally safe (Menezes *et al.*, 2005). The efficiency of removal is directly related to the compounds chemical structure, bioavailability (concentration, toxicity, mobility

and access) and physico-chemical conditions present in the environment (Cooper and Gondenenberg, 1987).

Actinomycetes are Gram positive bacteria with a distinctive feature of possessing filamentous hyphae that do not normally undergo fragmentation and produce asexual spore. They degrade enormous number and variety of organic compounds and are extremely important in the mineralization of organic matter (Margesin and Schinner, 2001). The success of bioremediation technology is also dependent upon a microbial ability to access the complex hydrocarbon mixture (Margesin and Schinner, 2001) which are compounds with low water solubility and thus not readily available to microorganisms. Because of this, bacteria consortia display a wide array of metabolic mechanisms for coping with the breakdown of oil components including the production of surface active agents and emulsifiers (Willumsen and Karlson, 1965). These agents are small surfactant molecules with hydrophilic and hydrophobic portions thereby providing amphipathic properties which enhances the bacterial growth and bioremediation rate.

Biosurfactants are structurally diverse group of surface active molecules synthesized by microorganisms. Virtually all surfactants are chemically synthesized (Banat, 1995). Much attention has been directed towards biosurfactants owing to their advantages such as low toxicity, high biodegradability, better environmental compatibility, high foaming capacity/capability, higher selectivity, specific activity at extreme temperature, pH, salinity and ability to be synthesized them from removable food stocks (Bodour and Miller-Maier; Cameotra and Makkar, 1998). Surfactants accumulate at interphase between immiscible phases and can reduce surface and interfacial tensions. The significant reduction of interfacial tension caused by biosurfactant increases the solubility and emulsification of the immiscible phases and bioavailability of the insoluble substrate for the microorganisms (Banat *et al.*, 2010). Biosurfactant with such surface properties make good candidates for enhanced oil recovery (EOR) (Banat *et al.*, 2010). The most effective biosurfactant reduce surface tension (si) of water from 72dyness/cm to values in the range of 25-30 dyness/cm (Ron and Rosenberg, 2001). They are also known to have therapeutic application (Rodrigues *et al.,* 2006). Biosurfactants can also be used in bioremediation hydrocarbon contaminated soil and groundwater (Cameotra and Prothi, 2003). Recently, biosurfactant have been widely used in environmental protection including EOR, oil spill control, biogradation and detoxification of oil contaminated industrial effluents and soil (Cameotra and Prothi, 2003). Another most important application of biosurfactants is the stimulation of oil production in marginal wells that have approached the economic limitation of operation (Banat *et al.*, 2010). Among the many classes of biosurfactants, lipopeptides represent a class of microbial surfactants with remarkable surface properties and biological activities such as surplus crude oil recovery.

MATERIALS AND METHODS

Soil samples were collected from hydrocarbon contaminated soils in mechanic workshops located in various parts of Benin City and river sediments were collected from Ikpoba River in Edo state. The samples were collected and stored in accordance with (Karthik *et al.*, 2010). The physico-chemical parameters of the soil samples were analysed which include: pH, temperature, total organic carbon, silt and sand composition, nitrate, Phosphorus, calcium, magnesium, sulphate, potassium, vanadium and moisture content of the soil samples (Kalra and Maynard, 1991; Adelekan and Abegunde, 2011; Maniyar *et al*., 2011). Isolation and enumeration of *actinomycetes* was done using Starch-Casein Agar. Soil samples were serially diluted (10^{-6}) and 1ml of sample from each plated on starch casein agar. The colonies which showed morphological difference were selected and purified. The plates were incubated for 7 – 10

days at 30°C (Zaki *et al.,* 2013; Lakshmipathy *et al.*, 2010). The ability of biosurfactant producing *actinomycetes* to utilize hydrocarbon was determined by measuring the turbidity of the *actinomycetes* inoculation into the Bushnell Hass broth containing crude oil as carbon source of mineral salt medium (Lakshmipathy *et al.*, 2010).

Production and Extraction of Biosurfactants

The samples selected were inoculated in a 250ml Erlenmyer flask containing 250ml sterile mineral salts medium consisting of (Na_2HPO_4 6g, KH_2PO_4 3g, NH_4CL 1g, $MgSO_4$. $7H_2O$ 0.24g and $CaCL_2$ 0.01g, Daeto-agar 15g, distilled water (l) with 20g glucose pH 7 soybean oil 0.19g as carbon source, 5.0g glutamic acid. Trace elements solution containing (g/l): $ZnSO_4$. $7H_2O$ 2.32; $MnSO_4$. $4H_2O$ 1.78; H_3BO_3 0.56; $CySO_4$. $5H_2O$ 1.0; Na_2MO_4. $2H_2O$ 0.39; $CaCL_2$. $6H_2O$ 0.42; EDTA 1.0; $NiCL_2$. $6H_2O$ 0.004 and KI 0.66; and Bushnell Hass medium with 3% forcados blend crude oil as carbon source. The broth cultures were incubated on a reciprocal shaker at 120rpm for 5-7 days at 30°C (Sabina et al., 2010; Moliterni *et al.*, 2012). The biosurfactant was extracted from the culture by centrifugation and filtration. The cell free broth was extracted by liquid – liquid extraction from the cell–free supernatant using mixture of chloroform: methanol (2:1v/v). The extracts was divided in rotating evaporator, weighed and quantified (Jain *et al.*, 1991).

Biosurfactant Characterization

Surface tension measurement: The surface tension of the biosurfactant was measured by the ring method using the Du–Nony tension meter (Kruss type 8451) at room temperature equipment with plate and samples with cell culture broths. A measurement of surface tension from distilled water was used as negative control. The concentration at which micelles began to form was represented as the CMC. At the CMC, a sudden change in surface tension was observed. The CMC was determined by plotting the surface tension as a function of biosurfactant concentration and surface tension at this point was designated as 7cmc (Khopade *et al.,* 2012).

Emulsification activity and foaming properties: The foaming properties were evaluated by monitoring the stability of the foam formed by hand shaking of the crude biosurfactant for 2 hours. Emulsification index of culture sample was determined by adding 2ml of a hydrocarbon to the same amount of culture/same volume of biosurfactant in a graduated screw cap test tube and then vortexed for 2min and left 24hours. The E_{24} index was given as a percentage of height of emulsified layer (mm) divided by the height of the liquid column (mm) (Khopade *et al.,* 2012).

$$E_{24}(\%) = \frac{\text{Total height of the emulsified layer} \times 100\%}{\text{Height of total evolution}}$$

Stability Characterization

To determine the thermal stability of the biosurfactant, about 4ml of the culture supernatant were stored at 4 and 25°C heated at 70°C, 100°C and 21°C for 15mins and then cooled to room temperature. The surface and E_{24} value of each treatment will be performed as described (Techaoei *et al.,* 2007). The pH change effect was determined by adjusting the culture supernatural with acid (IN HCL) or (IN NaOH) to pH values ranging from 1-10 prior to filter sterilization to monitor the surface tension and E_{24} are measured and determined (Techaoei *et al.,* 2007). The effect of salinity was measured by adding 5-20% NaCL then the sample was subjected to surface tension test measured at E_{24}.

Biochemical and Antimicrobial Activity

The protein content of surfactant was estimated using biuret and ninhydrin method and lipid

content estimate by isolated and purification method, total carbohydrates were calculated using molish test. The crude biosurfactant was tested for antimicrobial activity using diffusion method and area of zone was calculated. Active compounds were tested against *Escherichia coli*, *Bacillus stubtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albican.*

RESULTS

The results from this study showed a wide range of difference in the physic-chemical parameters of the hydrocarbon contaminated soil and Ikpoba River sediment which influenced the production of biosurfactant by actinomycetes (Table 1). The emulsification activity of the biosurfactants for pH, salt concentration and temperature were observed and recorded in (Table 2, 3, and 4). The surface tension of the biosurfactants for temperature, salt concentration and pH were recorded in (Table 5, 6, and 7). Table 8, shows the result of the biochemical test for the biosurfactant and the cultural, morphological and biochemical test for *Streptomyces* sp. was shown in Table 9.

DISCUSSION

There was extensive branching of the mycelium observed in the Petri dish which was observed to be Gram positive rod, non motile, oxidase negative and catalase positive. The organism grew on mineral salt medium incorporated with crude oil. The organism was identified as *Streptomyces* sp. (Karthik *et al.*, 2010). Biosurfactant production was indicated by the presence of foam while on the rotator shaker (Moussa *et al.,* 2006; Khopade *et al.,* 2012). Biosurfactant production was independent on growth phase (Chakraborty *et al.,* 2009). There was no significant difference in the mean emulsification activity of the biosurfactants produced from the two sites across different pH ($p>0.05$), but with increase in pH between 2 and 8, there was significant difference in the emulsification activity from the extracted biosurfactants from the two sites (Table 2). A significant difference was observed in the mean pH surface tension of the biosurfactants produced from the two sites with the hydrocarbon contaminated soil from the mechanic workshop having a higher surface tension (Table 7).

Table 1: Physico-chemical qualities of Hydrocarbon contaminated soil and Ikpoba River

Parameters	Mechanic Workshop soil	Ikpoba River Sediment
pH	6.62±0.056	4.92±0.049
CL^- mg/kg	15.89±0.131	6.98±0.038
SO_4^{2-} mg/kg	1.35±0.01	3.717±0.023
NO_3^- mg/kg	3.03±0.021	8.34±0.436
PO_4^{3-} mg/kg	0.13±0	0.36±0
Na^+ mg/kg	1.58±0.01	4.35±0.02
K^+ mg/kg	2.97±0.025	8.16±0.044
Ca^{2+} mg/kg	3.95±0.031	9.97±0.055
Mg^{2+} mg/kg	1.02±0.01	2.81±0.017
Fe^{3+} mg/kg	29.97±0.025	26.38±0.142
Zn^{2+} mg/kg	4.97±0.040	9.16±0.041
Mn^{2+} mg/kg	1.22±0.01	3.35±0.02
Cu^{2+} mg/kg	0.56±0	1.54±0
Ni^{2+} mg/kg	1.35±0.01	3.72±0.02
Cd^{2+} mg/kg	0.1±0	0.72±0.006
V^{2+} mg/kg	0.13±0	0.47±0
Cr^{6+} mg/kg	0.26±0.	0.73±0.
Pb^{2+} mg/kg	3.23±0.025	8.89±0.049
Hg^+ mg/kg	<0.001	<0.001
As mg/kg	<0.001	0.11±0.025
EC us/cm	66.91±0.541	40.85±0.026
TDS ppm	33.95±0.273	20.92±0.116
T-Carbon %	0.58±0.058	0.33±0.038
T-Nitrogen %	0.06±0	0.74±0.056

Temperature majorly affects the biosurfactant surface tension. This thermal studies show that the biosurfactant is thermally stable across the temperature range studied (Zaki *et al.*, 2013). Khopade *et al.* (2012) reported that the applicability of surfactants in several fields depends on its stability at different temperature and pH. Heating of the biosurfactant to 100^{o}C caused no significant effect on the biosurfactant performance. This activity indicates its possible usefulness in food industries and pharmaceuticals where heating to stability is of paramount importance (Abouseoud *et al.*, 2008; Mulligan and Gibbs, 1989). Ikpoba River had a slightly higher temperature compared to the hydrocarbon contaminated site. There was no significant difference in emulsification activity of the biosurfactant, although there was an increase from 70^{o}C to 100^{o}C and slightly decreased when the temperature reach 121^{o}C (Table 4). Boisurfactants are generally stable across salt concentration ranges of 2-20% and that salt concentration does not affect biosurfactant emulsification (Khopade *et al.,* 2012; Sarubbo *et al*., 2007). The biosurfactant has stability at an alkaline pH and slightly higher makes it a useful tool in the bioremediation of marine crude oil spill (Prieto *et al*., 2008). The produced boisurfactant in this research possessed foaming ability, amino acids, peptides, fats and oil without carbohydrate.

Table 2: Emulsification Activity change on pH

	Emulsification Activity (E_{24}) (%)	
pH	**Mechanic Workshop soil**	**Ikpoba River Sediment**
2	23	30
4	28	34
7	34	36
8	36	46
11	32	38

Table 3: Emulsification Activity change on salt concentration

	Emulsification Activity (E_{24}) (%)	
Salt Content (%)	**Mechanic Workshop soil**	**Ikpoba River Sediment**
2	39	35
8	35	38
15	34	36

Table 4: Emulsification Activity on Temperature

	Emulsification Activity (E_{24}) (%)	
Temperature	**Mechanic Workshop soil**	**Ikpoba River Sediment**
70	30	32
100	27	30
121	25	27

Table 5: Surface Tension change on Temperature

	Surface Tension (dynes/cm)	
Temperature (^{o}C)	**Mechanic Workshop soil**	**Ikpoba River Sediment**
70	117	81
100	105	70
121	99	65

Table 6: Surface Tension change on Salt concentration

	Surface Tension (dynes/cm)	
Salt content (%)	**Mechanic Workshop soil**	**Ikpoba River Sediment**
2	111	83
8	104	76
15	107	79

Table 7: Surface Tension change on pH

pH	Surface Tension (dynes/cm) Mechanic Workshop soil	Ikpoba River Sediment
2	119.5	99.1
4	125.4	103.7
7	139.9	110.4
8	151.6	114.95
10	160.4	117.2

Table 8: Results of Biochemical Test for Biosurfactant

Test	Remarks
Molisch test	_
Buiret test	_
Ninhydrin test	+
Fats and oils	+

Table 9: Cultural, Morphological and Biochemical Test of *Streptomyces* sp. isolated from Ikpoba River sediment and Mechanic Workshop soil in Benin City.

Cultural	
Arial spore colour	white
Spore surface	Smooth
Mycelium Colony	Extensive branching
Colony	Power, granular and velvety
Mophology	
Motility	Non motile
Oxygen requirement	Aerobic
Biochemical	
Gram staining	Positive
Catalase test	Positive
Hydrocarbon Utilization	Negative
Arabinose, fructose	Positive
Probable isolate	*Streotomyces* sp.

CONCLUSION

Actinomycetes have the potentials of producing a wide range of beneficial compounds. Actinomycetes have complex enzymatic mechanism that aids hydrocarbon mineralization and thus increases the potential for biosurfactant production. The biosurfactants produced are stable across temperature ranges and are not majorly affected by salt concentration. This property may aid its potential use in decontamination of oil contaminated areas in the Niger Delta region of Nigeria and other countries.

REFERENCE

Abouseoud, M., Maachi, R., Amrane, A., Boudergua, D. and Nabi, A. (2008). Evaluation of different carbon and nitrogen sources in production of biosurfactant by *Pseudomonas fluorescens*. *Desalination*. **233**: 143-151

Adelekan, B. A. and Abegunde, K.D. (2011). Heavy Metals Contamination of Soil and Groundwater at Automobile Mechanic Villages in Ibadan, Nigeria. *International Journal of The Physical Sciences*. **6(5):** 1045-1058

Banat, I. M. (1995). Characterization of Biosurfactants and their use in Pollution removal: State of the Art. *Acta Biotechnologyca*. **15**: 251-267

Banat, I. M., Franzetti, A., Gandolfi, I., Bestetti, G., Martinotti, M. G., Fracchia, L., Smyth, T. J and Marchant, R. (2010). Microbial Biosurfactants Production, Applications and Future Potential. *Applied Microbiology and Biotechnology*. **87(2):** 427-444

Bodour, A.A and Miller-Maier, R. (1998). Application of a Modified Drop-Collapse Technique for Surfactant Quantification

and Screening of Biosurfactant-Producing Microorganisms. *Journal of Microbiology Methods*. **32**: 273-280

Cameotra, S. S. and Makkar, R. S. (1998). Synthesis of Biosurfactants in Extreme Conditions. *Applied Microbiology And Biotechnology.* **53**:495-508

Cameotra, S.S and Prothi, V. (2003). Effects of Nutrients on Optimal Production of Biosurfactants by *Pseudomonas putida-a gujarat* Oil Field Isolate. *Journal of Surfactants And Detergents*. **6:** 65-68

Chakraborty, S. Khopade, A., Kokare, C., Mahadik, K. and Chopade, B. (2009). Isolation and characterization of noval α-amylase from marine streptomyces sp. D1. *Journal of Molecular Catalysis B*. **58**: 17-23

Cooper, D.G and Gondenenberg, B. G. (1987). Surface-Active Agents from two *Bacillus* species. *Applied Environmental Microbiology.* **53:** 224-229

De Oliveira Camargo, F. A., Okeke, B. C. and Frankebberger, W. T. (2005). Diversity of Biosurfactant production during Diesel Biodegradation by mixed Microbial Consortia selected from Polluted Soils. *International Journal of Environmental Research*. **160**: 249-255.

Jain, D.K., Collins-Thomson, D. L., Lee, H. and Trevors, J.T. (1991). A drop collapsing test for screening surfactant producing microorganism. *Journal of Microbiological Methods.* **13:** 271-279.

Karthik, L., Kumar, G., Venkata, K. and Rao, B. (2010). Comparison of Methods and Screening of Biosurfactant Producing Marine Actinobacteria Islated from Nicobar Marine Sediment. *The LLOAM Journal.* **1(2):** 34-38.

Kalra, Y.P. and Maynard, D. G. (1991). Methods Manual for Forest Soil and Plant Analysis. Northwest Region, USA. pp125.

Khopade, A., Ren, B., Liu, X., Mahadik., Nzhang, L. and Kokare, C. (2012). Production of biosurfactant from marine *Streptomyces species* B3. *Journal of Colloid And Interface Science*. **367**: 311-318

Lakshmipathy, T. D., Prasad, A. and Kannabiran, K. (2010). Production of Biosurfactant and Heavy Metal resistance activity of *Streptomyces* sp. VITDDK3-a novel halo tolerant *Actinomycetes* isolated from Saltpan Soil. *Advances in Biological Research*. **4(2):** 108-115

Margesin, R. And Schinner, F. (2001). Biodegradation and Bioremediation of Hydrocarbon in Extreme Environment. *Apllied Microbiology And Biotechnology*. **56**:650-663

Maniyar, J. P., Doshi, V. D., Bhuyan, S.S. and Mujumdar, S.S. (2011). Bioemulsifer Production by *Streptomyces* sp. S22 Isolated from Garden Soil. *Indian Journal of Experimental Biology*. **49(4):** 293-297.

Moliterni, E., Gomez, R., Rodriguez, L., Fernandez, F. S. and Villaseenor, J. (2012). Biosurfactant production during diesel biodegradation by mixed microbial consortia isolated from a polluted soil. *International Journal of Environmental Research*. **6(3**): 751-760.

Moussa, T. A.A., Ahmed, G.M. and Abdel-Hamid, S.S. (2006). Optimization of cultural conditions for biosurfactant production from *Norcadia amarae*. *Journal of Applied Science Research*. **2(11):** 844-850.

Mulligan, C.N. and Gibbs, B. F. (1989). Correlation of nitrogen metabolism with biosurfactant production by *Pseudomonas aeruginosa*. *Applied Environmental Microbiology*. **55**: 3016-3019.

Prieto, L. M., Michelon, M., Burkert, J. F. M., Kalil, S. J. and Burkert, C. A. V. (2008). The production of rhamnolipid by a *Pseudomonas aeruginosa* strain isolated from a southern coastal zone in brazil. *Chemosphere*. **71**: 1781-1785.

Prabhu, Y. And Phale, P. (2003). Biodegradation of Phenanthrene by *Pseudomonas* sp. Strain PP2: Novel Metabolic Pathway,

Role of Biosurfactant and Cell Surface Hydrophobicity in Hydrocarbon Assimilation. *Applied Microbiology and Biotechnology*. **61**: 342-351.

Rahman, K., Rahman, T., Korkoutas, Y., Petsas, I., Marchant, R., and Banat, I. (2003). Enhanced Bioremediation of n-alcane in Petroleum Sluge using Bacteria Consortium amended with Rhamnolip and Micronutrients. *Bioresource Technology*. **90**: 159-168.

Ron, E. Z. and Rosenberg, E. (2001). Natural Roles of Biosurfactants. *Environmental Microbiology*. **3(4):** 229-236.

Rodrigues, L. R., Teixeira, J.A., Varder Mei, H. C. and Oliviera, R. (2006). Physiochemical and Functional Characterization of A Biosurfactant Produced by Lactococcus Lactis 53. *Colloids Surfactants B*. **49**: 79-86.

Sabina, V. R., Portillo-Ruiz, M. C., Ballinas-Casarrubias, M.L., Torres-Munoz, J.V., Rivera-Chavira, B.F. and Nevarez-Moorillion, G. V. (2010). Selection of biosurfactant/bioemulsifier producing Bacteria from Hydrocarbon Contaminated Soil. *Brazilian Journal of Microbiology*. **41**: 668-675.

Sarubbo, L.A., Farias, C. B.B. and Campos Takaki, E. M. (2007). Co-utilization of canole oil and glucose on production of a surfactant by *Candida lipolytica*. *Brazilian Journal of Microbiology.* **54**: 68-73.

Techaoei, S., Leelapornpisid, P., Santiarwarn, D. and Lumyong, S. (2007). Preliminary screening of biosurfactant producing microorganisms isolated from hot spring and garges in northern Thailand. *KMITL. Science Technology Journal*. **7**:38-43.

Willumsen, P. A. and Karlson, F. L. (1965). Use of Antibiotics for Selective Isolation and Enumeration of *Actinomycetes* in Soil. *Journal of General Microbiology*. **7:** 415-423

Zaki, S.A., Elkady, M. F., S. and Abd-El-Haleem, (2013). D. Characterization and Flocculation Properties of a Carbohydrate Bioflocculant from a newly Isolated *Bacillus velezensis* 40B. *Journal of Environmental Biology.* **34**:51-58.