ISSN No:-2456-2165

Isolation and Production of Biosurfactants from Marine Actinomycetes

P. Bhagya Sri *, T. Deepthi, V. Naga Anjani, Dr. ALT Kalyani, Dr. Y. A Chowdary
NRI college of pharmacy

Abstract:-This article describes about the isolation and production of biosurfactants from marine actinomycetes. Few microbes produced biosurfactants has certain chemical properties with high molecular weights which was obtained from living surface of microbial cells exhibited as amphiphiles. Consists of hydrophilic and hydrophobic moieties reduces surface tension. They were used in pharmaceutical applications. TREHALIPIDS, SOPHOROLIPIDS, RHAMNOLIPIDS, PHOSPHOLIPIDS; are the complex compounds present in the biosurfactants and degradation was also studied in In-vitro, produced from the marine organisms of actinomycetes shows antibiotic and probiotic activity. Actinomycetes are a GRAM POSTIVE BACTERIA, comes under the prokaryotic organisms and exhibited as single protein which is a marine sponge collected from south east region for isolation washed with sea water to remove any bacteria to form homogenate, incubated at 400c. colonies, isolated, and by keeping the temperature desired for 24 hours. marine actinomycetes were identified by using their Bio-surfactant properties were investigated using statistical data, and screening methods for specific operations were developed.

***Keywords*:-** *Amphiphiles, Trehalipids, Sophorolipids, Rhamnolipids, Phospholipids, Gram Positive Bacteria.*

I. INTRODUCTION OF BIOSURFACTANT AND ACTINOMYCETES

A wide range of microorganisms produce potent surface-active agents, known as biosurfactants. While glycolipids are the most common low molecular weight surfactants, high molecular weight surfactants are generally either poly anionic hetero polysaccharides with covalently-linked hydrophobic side chains or complexes containing both polysaccharides and proteins. The nutritional environment of the growing organism has a significant impact on biosurfactant yield. Biosurfactants are an interesting group of materials for application in many areas such as agriculture, public health, food, health care, waste utilisation, and environmental pollution control such as hydrocarbon degradation in soil.

Biosurfactants (BS) are amphiphile compounds that are produced in living surfaces, primarily microbial cell surfaces, or excreted extracellularly. They contain hydrophobic and hydrophilic moieties that reduce surface tension (ST) and interfacial tensions between individual molecules at the surface and interface, respectively. Because BS and bio emulsifiers both have emulsification properties, bio emulsifiers are frequently classified as BS, even though emulsifiers may not lower surface tension. Mycolic acid, glycolipids, polysaccharide-lipid complex, lipoprotein or lipopeptide, phospho lipid, or the microbial cell surface itself can all be biosurfactants [1].

Laboratory attempts to elicit secondary metabolite production do not fully reflect the genetic diversity discovered by genome sequencing. This relative scarcity of secondary metabolites is due, in part, to cryptic gene clusters that only activate under specific conditions [2]. Because marine actinomycetes frequently coexist with other microorganisms, they may have secondary metabolites for chemical defence that are only expressed in the presence of competing microbial strains [3].

Probiotics are defined as "a live microbial feed supplement. Which beneficially affect the host animal by improving its industrial microbial balance." . In the case of aquatic animals, not only the digestive tract but also the surrounding water is important. So, [4] broadened the definition by removing the restriction to the improvement to the intestine as "a live microbial supplement, which beneficially affects the host animal by improving its microbial balance".

Single cell proteins (SCPs) are isolated as dried cells and/or purified proteins from the cells of microorganisms with high protein content. SCPs have a high protein content with a wide amino acid spectrum, a low fat content, and a higher protein:carbohydrate ratio than forages, making them an appealing nutrient supplement for humans [5]. SCPs contain vitamins such as thiamine, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, folic acid, biotin, cyanocobalamin, ascorbic acid, -carotene, and -tocopherol, as well as minerals, nucleic acids, and lipids [6,7]. SCPs have previously been used for a variety of applications ranging from food (aroma carriers, vitamin carriers, emulsifying acids, etc.) and feed (pigs, poultry, cattle, fish) production to the paper and lead industries [7,8].

Actinomycetes are Gram-positive bacteria with a filamentous structure formed by asexual spores and branching filaments. From over 22,000 known secondary metabolites, actinomycetes produce approximately 70% of available secondary metabolites [9]. These bacteria are widely distributed in soil and aquatic environments, and they contain 50% uncultivable soil microorganisms, making them the most dominant and important group of soil microbes. Secondary metabolites produced by actinomycetes include antibiotics, enzymes, nutritional

ISSN No:-2456-2165

materials, cosmetics, antitumor, immune modulators, and enzyme inhibitors [10].

Biosurfactants are surface-active biomolecules produced by microorganisms that have a wide range of applications. Surface active biomolecules have sparked widespread interest in recent years due to their unique properties such as specificity, low toxicity, and relative ease of preparation. It has both hydrophilic and hydrophobic regions, which cause it to aggregate at interfaces between fluids with different polarities, such as hydrocarbons and water [11,12], lowering interfacial surface tension [13]. There was also evidence that it improved nutrient transport across membranes and had an effect on various host-microbe interactions.

Biosurfactants, as opposed to chemical or synthetic surfactants, have several advantages, including biodegradability, biocompatibility, and digestibility. By biodegrading and detoxifying industrial effluent and bioremediating contaminated soil, biosurfactants can help to clean up the environment. Because of their specificity and the availability of raw materials, they are the most preferred surfactants [14].

II. MATERIALS AND METHODS

A. Sponge Associated Actinomycetes:

Sponges (Porifera) are the most ancient multicellular phylum, with fossils dating back to the Precambrian period [15]. Porifera is divided into three major classes: Hexactinellida (glass sponges), Calcarea (calcareous sponges), and Demospongiae (demosponges), with the latter accounting for 85% of all living sponges [16]. Sponges are abundant not only on tropical reefs, but also in polar latitudes, fresh water lakes, and rivers [17,18]. Sponges have formed close relationships with a wide range of microorganisms, including viruses, bacteria, archaea, fungi, protozoa, and single-celled algae, and the nature of the sponge-microbe interaction is diverse [19]. Most sponges have a general pattern of microbial distribution, with photosynthetically active microorganisms such as Cyanobacteria located in the outer light exposed layers and heterotrophic and possibly The inner core is inhabited by autotrophic bacteria [20]. So far, at least 32 bacterial phyla and candidate phyla were described from marine sponges by both cultivation-dependent and cultivation-independent techniques; with the most common phyla being Acidobacteria, Actinobacteria, Chloroflexi, Cyanobacteria, Gemmatimonadetes, Nitrospira, Planctomycetes, Proteobacteria, (Alpha, Delta, Gamma subclasses) and Spirochaetes [15,17].

III. SCREENING METHODS FOR POTENTIAL PROCEDURES

A. Haemolytic Activity :

Surfactants cause haemolysis at a given concentration due to their amphiphilic nature. A primary screening method for biosurfactant production is haemolytic activity in blood agar plate (peptone 5gm, yeast extract 3gm, Nacl2 5gm, sheep blood 5ml). Blood agar plates containing 5% (v/v) human blood were used to detect hemolytic activity. After 24 hours of incubation at 370°C, the plates were examined for haemolysis. The plates were checked for clearance from the colony. The presence of a clearing zone indicated the presence of a biosurfactant producing microorganism [21].

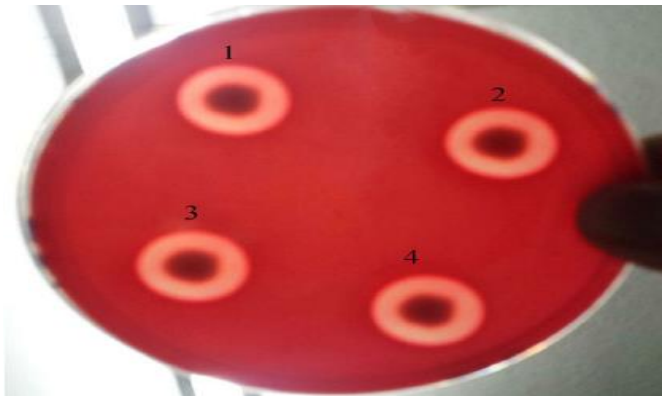

Fig 1 Heamolytic Activity

B. Drop Collapsing Method :

The qualitative drop collapse test described by yourself et al. was used to screen biosurfactants. Mineral oil (2l) was added to 96-well microliter plates in this method. After 1 hour of equilibration at 370C, 5l of culture supernatant was added to the surface of the oil. After 1 minute, the shape of the drop on the oil surface was observed. The culture supernatant that causes the drop to collapse was scored as positive, while the drops that remained beaded were scored as negative, when compared to distilled water as a control [22].

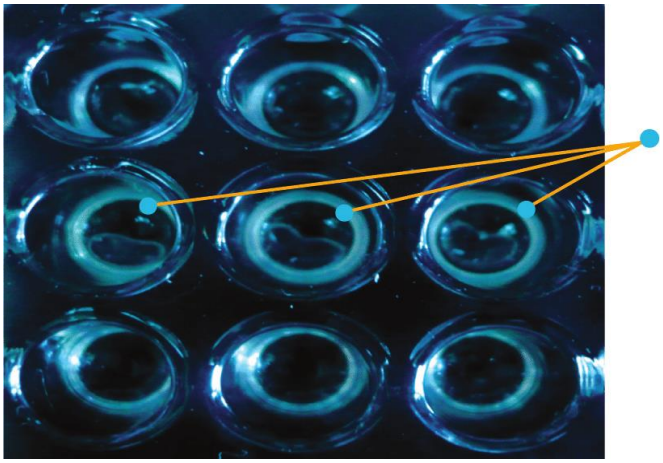
Fig 2 Drop Collapsing Method

C. Oil Displacement Method :

The oil displacement assay was carried out using the methodology of Morikawa et al. [23]. In an asterile Petri plate, 0.015% (v/v) weathered crude oil was laid on 40 l of Milli Q water. Following that, 10 l of culture supernatant was gently applied to the surface of the oil film. After 1 minute, the diameter and area of a clear halo visible under visible light were measured.

ISSN No:-2456-2165

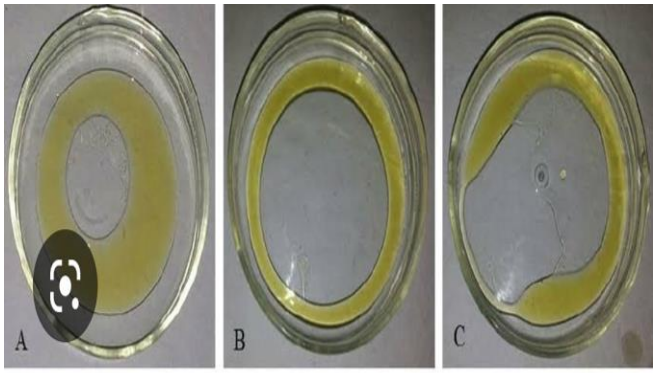

Fig 3 Oil Displacement Method

D. Screening For Lipase Product :

Actinomycetes isolation agar was supplemented with tributyrin 1 percent to screen for lipase-producing isolates. Using 0.1N NaOH, the pH of the medium was brought down to 7.3–7.4. The tribyulin agar plates were streaked with an inoculum loopful. The establishment of a clean zone around the colonies was checked on the plates [24].

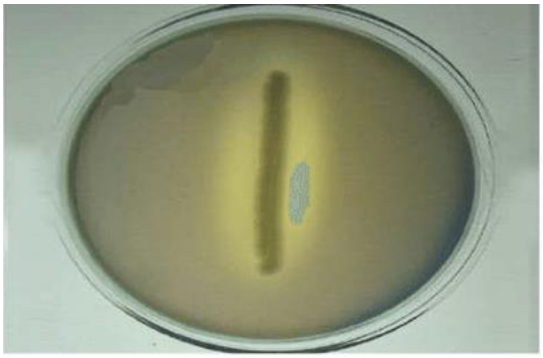
Fig 4 Screening for Lipase Product

E. Emulsification Method :

The method outlined by Plaza et al. [25] was used to measure the emulsification activity. A test tube was inoculated with 2 mL of crude olive oil and 2 mL of cell-free media, and the mixture was then vortexed rapidly for 2 min to homogenise it. Following 24 hours, the emulsification activity was determined using the formula: Total height of the emulsified layer divided by total height of the liquid layer gives E24 (%) [26].

Fig 5: Emulsification Method

IV. MATERIALS AND METHODS OF MARINE ACTINOMYCETES STRAIN

A. Time Course of Biosurfactant Production :

Batch culture was used to track the kinetics of biosurfactant formation under ideal circumstances. Under submerged culture circumstances, the experiment lasted twelve days, moving from the log phase to the stationary phase. Filtration was used to extract the resulting cell-free supernatant, which was then removed by cold centrifugation at 10,000 rpm for 20 minutes at 40 C. The generation of biosurfactants was examined in the supernatant [27,28].

B. Determination of Critical Micellar Concentration :

Composition of the biosurfactant chemical Using bovine serum albumin as a standard, Lowry's technique was utilised to evaluate the concentration of protein from separated biosurfactant [29]. The amount of carbohydrates was determined using the phenol sulfuric acid technique [30].

C. Antimicrobial Activity :

The area zone was estimated and the crude biosurfactant's antibacterial activity was assessed using the well diffusion method. Human pathogens such Escherichia coli, B. subtilis, pseudomonas aeruginosa, staphylococcus, and Candida albicans were evaluated against extracted active chemicals. [31]

V. RESULTS AND DISCUSSIONS

A. Sponge Associated Actinomycetes :

➢ Screening for Biosurfactant Production

In the study, MSA10 was identified as a potent biosurfactant producer using all four tests—haemolytic activity, oil displacement method, drop collapsing test, and lipase activity—used to screen for the production of biosurfactants (see figures 1 and 2).

Table 1 Screening for Biosurfactant Production

TEMPERATURE	O.S.T
10	0
20	2
30	4
40	6
50	8

Fig 6 : Screening for Biosurfactant Production

- How temperature affects the biomass and biosurfactant production of N. alba in ALA medium. The emulsification index E24 was used to calculate emulsification activity. Utilizing the clear zone diameter as determined by OST, surfactant activity was evaluated.

B. Critical Micellar Concentration of Biosurfactant :

Surface tension of various biosurfactant concentrations (log of mg/l) was separately measured in order to estimate the CMC value of the biosurfactant (FIG. 7). The surface tension of the Milli Q distilled water was measured to be (72 Mn/m), and the addition of biosurfactant decreased it up to 30 Mn/m. It's important to note that the CMC forms near the conclusion of the exponential phase. Even though surfactant synthesis continued after it reached an exponential phase, its properties, like surface tensions, remained constant. The circumstances under which CMC is utilised allow for the observation of this phenomena. Streptomyces strain biosurfactant has a cmc of 110 mg/l, it was discovered. The results obtained in the present experiment were found to be similar to those of Rashedi et al. [32].

C. Anti Microbial Activity of Biosurfactant :

Streptomyces species' biosurfactant isolates displayed a wide range of resistance to pathogenic strains. The biosurfactant was partially purified and demonstrated activity against the bacteria B. subtilis, p. aeruginosa, S. aureus, and E. coli as well as high activity toward the yeast C. albicans (Fig2) Ysuge et al. claim that lipopeptide surfactants, primarily the surfaction, streptofin, and gramicidin produced by microbes, are powerful antibiotics with a wider antibacterial activity than glycolipid-producing strains. A c. antartica glycolipid surfactant has shown antimicrobial action against gram-positive bacteria [33–35].

Table : 2 Anti Microbial Activity of Biosurfactant

S NO.	BIOSURFACTANT	SDS
1	1.02	0.4
2	1	0.6
3	1.2	0.9
4	1.2	1

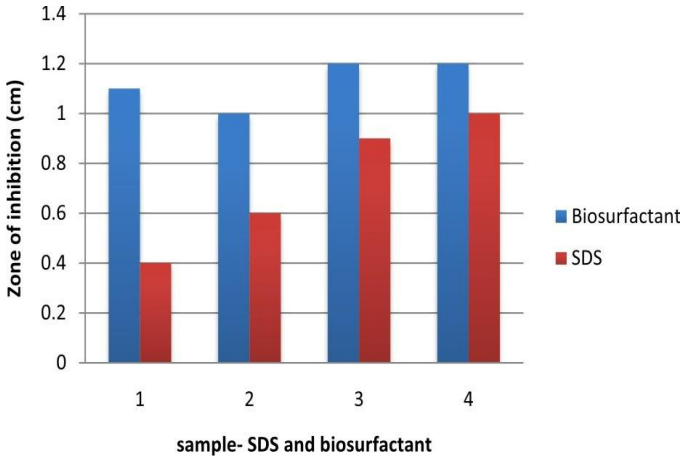

Fig 7 : Anti Microbial Activity of Biosurfactant

VI. CONCLUSION

A marine strain of Streptomyces B3 created a complex biosurfactant made of lipids, proteins, and carbohydrates. The biosurfactant produced by Streptomyces B3 using olive oil as the substrate and having a high lipid content may offer a viable focus for further research on its use as a substance with effective biological activity for increased oil recovery. The findings underline the value of controlling physiological variables and how they affect the generation of biosurfactants, as well as the distinctive biochemical characteristics of these sorts of microbial natural products.

Studying several physical characteristics, such as critical micelle concentration, surface and interfacial tension, emulsification activity, and its high tolerance to environmental conditions including temperature, pH, and salinity, demonstrated the significance of biosurfactants for industrial usage. Water's surface tension was lowered by the isolated biosurfactant from 72 mN/M to 29 mN/M. The biosurfactant has been refined, and critical micelle concentrations were 110 mg/l. The separated biosurfactant's functional analysis revealed that the produced biosurfactant was a glycolipid by nature. The inexpensive manufacture of these useful compounds for therapeutic uses, such as biosurfactant as a substitute antibacterial or anticancer agent in the medical area for applications against microorganisms responsible for diseases, may prefer the production of the biologically active portion. This study's findings can be Studying many physical characteristics, including essential micelle concentration, surface and interfacial tension, emulsification activity, and its high stability allowed researchers to draw the conclusion that biosurfactants are crucial for the bioremediation of spills in marine environments.

REFERENCES

[1]. Ramana, K. V. and Karanth, N. G. K., J. Microbial. Biotechnol., 1988, 3, 66–69.
[2]. Genomics-inspired discovery of natural products. Curr. Opin. Chem. Biol. Winter J.M., Behnken S., Hertweck C. 2011;15:22–31.
[3]. Elicitation of secondary metabolism in actinomycetes. Biotechnol. Adv. Abdelmohsen U.R., Grkovic T., Balasubramanian S., Kamel M.S., Quinn R.J., Hentschel U. 2015;33:798–811.
[4]. Inhibition of Vibrio anguillarum by Pseudomonas fluorescens AH2, a possible probiotics treatment of fish. Appl. Environ.Microbiol Gram, L., Melchiorsen, J., Spanggaard, B., Huber, I. and Nielsen, T.F. (1999), 65: 969-973.
[5]. P.G. Single Cell Protein- A Review. Int. J. Pharm. Res. Sch Srividya, A.R.; Vishnuvarthan, V.J.; Murugappan, M.; Dahake, 2013, 2, 472–485.
[6]. Single Cell Protein Production: A Review. Int. J. Curr. Microbiol. Appl. Sci Suman, G.; Nupur, M.; Anuradha, S.; Pradeep, B. 2015, 4, 251–262.
[7]. Recycling of orange waste for single cell protein production and the synergistic and antagonistic effects on production quality. J. Clean. Prod Zhou,

Y.-M.; Chen, Y.-P.; Guo, J.-S.; Shen, Y.; Yan, P.; Yang, J.-X. 2019, 213, 384–392.

[8]. In Situ Gelling System: Smart Carriers for Ophthalmic Drug Delivery. Int. J. Pharm. Res. Sch Nagare, B.; Bhambere, S.; Kumar, S.; Kakad, K.; Nagare, N. 2015, 4, 10–23.

[9]. antiviral antibiotics produced by an actinomycete. Uyeda, M.J.A., Fattiviracins, 2003. 17(2): p. 57-66.

[10]. Bio-Prospecting for Broad Spectrum Antibiotic Producing Actinomycetes Isolated from Virgin Soils in Kericho County Rotich, M.C., 2018, 67-69

[11]. Biosurfactants production and possible uses in microbial enhanced oil recovery and oil pollution remediation: A review. Bioresour. Technol., 51: Banat, I.M., 1995 1-12.

[12]. Microbial production of biosurfactants and their importance. Curr. Sci., Karanth, N.G.K., P.G. Deo and N.K. Veenanadig, 1999, 77: 116-126.

[13]. Microbiological aspects of surfactant use for biological soil remediation. Biodegradation, Volkering, F., A.M. Breure and W.H. Rulkens, 1998. 8: 401-417.

[14]. Isolation and characterization of biosurfactant-producing Alcanivorax strains: Hydrocarbon accession strategies and alkane hydroxylase gene analysis. Res. Microbiol., Olivera, N.L., M.L. Nievas, M. Lozada, G. del Prado, H.M. Dionisi and F. Sineriz, 2009. 160: 19-26.

[15]. Hentschel U., Piel J., Degnan S.M., Taylor M.W. Genomic insights into the marine sponge microbiome. *Nature*. 2012;**10**:641–654.

[16]. Van Soest R.W., Boury-Esnault N., Vacelet J., Dohrmann M., Erpenbeck D., de Voogd N.J., Santodomingo N., Vanhoorne B., Kelly M., Hooper J.N. Global diversity ofsponges (*Porifera*) *PLoS One*. 2012;**7**:11-12.

[17]. Schmitt S., Tsai P., Bell J., Fromont J., Ilan M., Lindquist N., Perez T., Rodrigo A., Schupp P.J., Vacelet J., et al. Assessing the complex sponge microbiota: Core, variable and species-specific bacterial communities in marine sponges. *ISME J*. 2012;**6**:564–576.

[18]. Belarbi el H., Contreras Gomez A., Chisti Y., Garcia Camacho F., Molina Grima E. Producing drugs from marine sponges. *Biotechnol. Adv*. 2003;**21**:585–598.

[19]. Webster N.S., Taylor M.W. Marine sponges and their microbial symbionts: Love and other relationships. *Environ. Microbiol*. 2012;**14**:335–346.

[20]. 6. Hentschel U., Fieseler L., Wehrl A., Gernert C., Steinert M., Hacker J., Horn M. Microbial diversity of marine sponges. In: Müller W.E.G., editor. *Sponges (Porifera)* Springer; Berlin, Germany: 2003. pp. 59–88.

[21]. isolationandselection of biosurfactant producing bacteria. World Journals of and Biotechnol., Callo,PC.MardarzandS.Pitta-Alvarez,1996.12:82-84.

[22]. Comparison of methods to detect biosurfactant production bydiverse microorganism. Journal of MicrobiologicalProduction and characterization of lipopeptide, Methods, Youssef, N.H.,K.E .Dunacn, D.P.Nagle,K.N.Savage,R.M.KnappandM.J.McInerey , 2004. 56: 339-347.

[23]. a sponge associated marine actinomycetes Nocardiopsis alba MSA10.Bioprocess Biosystems Engineering, A newlipopeptide biosurfactant produced by Arthrobacter sp. strain MIS 38.J Bacteriol Morikawa M, Daido H, Takao T, Marato S, Shimonishi Y, Imanaka T: 1993, 175:6459–6466

[24]. The potential of bacterial isolates for emulsification with a range of hydrocarbons. Acta Biotechnol Rahman .S.M.,RahmanT.J., Lakshmanaperumalsamy P. Marchant R., Banat I.M. 2003;23:335–345.

[25]. Biosurfactant-enhanced removal of total petroleum hydrocarbons from contaminated soil. J Hazard Mater. Lai C.C., Huang Y.C., Wei Y.H., Chang J.S. 2009;167:609–614.

[26]. L. Rodrigues, A. Moldes, J. Teixeira, R. Oliveira, Biochem. Eng. J. 28 (2006) 109

[27]. M. Abouseoud, R. Maachi, A. Amrane, S. Boudergua, A. Nabi, Desalination 223 (2008) 143.

[28]. Protein measure-ment with the Folin phenol reagent. J. Biol. Chem. Lowry O, Rosebrough N, Farr A, Randall R. 1951. 193:23.. 265-275

[29]. Phenolsulphuric acid method for total carbohydrate. Anal. Chem. Dubois M, Gilles K, Hamilton J, Rebers P, Smith F. 1956. 26:350.

[30]. Colloids Surf., B: Bio interfaces G.S. Kiran, T.A. Hema, R. Gandhimathi, J. Selvin, T. Anto Thomas, T.R. Ravji, K. Natarajaseenivasan, (2009)73: 250.

[31]. H. Rashedi, E. Jamshidi, M.M. Assadi, B. Bonakdarpour, Int. J. Environ. Sci. Technol. 2 (2005) 121.

[32]. K. Tsuge, T. Ano, M. Shado, Arch. Microbiol. (1996) 243.

[33]. Peypoux, J.M. Bonmatin, J. Wallach, Appl. Microbiol. Biotechnol (1999) 51:553.

[34]. M. Richter, M. Willey, R. Suessmuth, G. Jung, H.P. Fielder, FEMS Microbiol. Lett. 163 (1998) 165.

[35]. G.S. Kiran, T.A. Hema, R. Gandhimathi, J. Selvin, T. Anto Thomas, T.R. Ravji, K. Natarajaseenivasan, Colloids Surf., B: Biointerfaces (2009) 73: 250.