# RAPID IDENTIFICATION OF MICROBIAL BIOFILMS - A PHENOTYPIC AND BIOCHEMICAL CHARACTERIZATION OF ISOLATED AND POTENTIAL MICROBES FROM ESTURINA, GOSTANI RIVER AT BAY OF BENGAL.

Y. Gayatri Devi* and Y.V.K. Durga Prasad[1].
[1]Head of the Department, Department of Microbiology, Dr. V. S. Krishna Govt Degree & P.G. College, Visakhapatnam.
*Research scholar, Department of Biotechnology,Center for Reasearch, Dr. V.S.Krishna Govt Degree & P.G. College, Visakhapatnam

**ABSTRACT**

Biofilms can be defined as communities of microorganisms attached to a surface. It is clear that microorganisms abide abstruse changes during their alteration from planktonic (free-swimming) organisms to cells that are part of a complex,, surface-attached community. These changes are reflected in the new phenotypic characteristics developed by biofilm bacteria and occur in response to a variety of ecology signals. The main aim of the present study to isolate the potential microbes from the esturina, Gostani River at Bay of Bengal. Out of 310 bacterial isolates G3A, G3L and G3D shows distinctbiochemical characters and antimicrobial activity.

**Keywords:** Biofilm, Gostani River, Isolation, Biochemical and antimicrobial.

## INTRODUCTION

Biofilms are the prevailing lifestyle of bacteria in most natural environments. They consist of microbial communities that usually accumulate at solid–liquid interfaces and are entrapped in a matrix of highly hydrated extracellular polymeric substances(Fleming 2010).The term “biofilm” is used to describe a layer of micro-organisms in an aquatic environment held together in a polymetric matrix attached to a substratum such as pipes, tubercules or sediment deposits. Attachment is a first step in the process of microbial colonisation of any surface and may initially limit the rate of the process (Escher and Characklis, 1988). Biofilm development is a result of successful attachment and subsequent growth of micro-organisms on a surface. Under suitable conditions a biofilm develops, initially through the accumulation of organic matter on the metal surface, which is then colonised by bacteria (Wolfaardt and Archibald 1990).Many bacteria isolated from natural environments possess an important ecological quality, namely that of resis tance to antibiotics, which can be picked up in the course of selective processes (Nair et.al. 1992 silva et.al. 1995)Biofilms protect bacteria from host defense mechanisms, antimicrobial activity, and adverse conditions (Fux *et.al.* 2005, Gotz 2002). The isolation may begin with pretreatment of samples which favour the survival of the preferred organism. This is followed by growth on selective or non-selective media and often associated with batch or continuous enrichment. Classical methods of screening to obtain suitable organisms are very time consuming expensive and often without any

guarantee. Screening may be defined as the use of highly selective procedures to allow the detection and isolation of only those microorganisms of interest from among a large microbial population. The screening procedure saves a lot of time and labor without necessity of extensive studies to be carried out on each individual organism.

## MATERIALS AND METHODS

### Study Area:

Studies were carried out in esturina river Gostani at Bhimili region, Visakhapatnam district, Andhra Pradesh, India. The Gosthani rises in the Ananthagiri Hills of the Eastern Ghats and flows through the Borra Caves which lie near its source. It flows for 120 km before joining the Bay of Bengal through an estuary near Bheemunipatnam. The river basin drains the two coastal districts of Vizianagaram and Visakhapatnam. Bheemunipatnam or Bhimli, located at the confluence of the river with the Bay of Bengal was one of the earliest outposts of the Dutch East India Company in India and there are many monuments from that era in the town.Ancient Buddhist settlements at Pavuralakonda, near Bhimli and at Gudiwada have been excavated. The river is thought to have provided the inhabitants with drinking water and the estuary at Bhimli facilitated sea-borne trade. The Gosthani's waters are diverted for agricultural and industrial purposes and the river is the chief source of drinking water to the cities of Vizianagaram and Visakhapatnam. The depth in the deepest portion of the river is variable from place to place. It is 2 meter in the inlet area at the low tide, 1 - 1.5 meter in the backwaters and 1.5 - 2 meters in the middle section of the river. (Ganesh et.al.2013)

### Sampling:

Biofilms from water sources were collected from three stations. Which represents three environmental conditions Station 1 (marine water zone) Station 2 (Fresh water and Sea water zone) Station 3 (Fresh water zone). The samples were collected in three seasons at these stations: Season1(summer), Season 2 (Rainy) and Season 3 (winter). In each Station we use 5 sampling points by using iron plates (8X10 cm) for the formation of biofilms and collection of samples.

**Numbering of the Samples:**

| | **Season 1** | **Season 2** | **Season 3** |
|---|---|---|---|
| **Station 1** | A1, A2, A3, A4, A5. | B1, B2, B3, B4, B5. | C1, C2, C3, C4, C5. |
| **Station 2** | A6, A7, A8, A9, A10. | B6, B7, B8, B9, B10. | C6, C7, C8, C9, C10. |
| **Station 3** | A11, A12, A13, A14, A15. | B11, B12, B13, B14, B15. | C11, C12, C13, C14, C15. |

### Isolation of Microbes:

For the isolation of the microbes from each sample, SCDA media was used for primiary screening. Composition of SCDA (Tryptone 15 g, soya peptone 5g, sodium chloride 5g, agar 15g) the media was maintained 7.3±2 pH.

**Catalase test**

A Loop ful of the bacterial culture were placed on the glass slide for the identification of the catalase test. 3% hydrogen peroxide was used for the mixing of the culture and an immediate bubbling indicated a positive catalase test.

**Oxidase test**

Nutrient broth was used for this experiment. Each strain was grown on 10 ml nutrient broth and kept for 24h of incubation, each test tube was added with ready to use oxidase discs (Himedia, India) and observed for color changes. Strains which produce oxidase, made the disc color changes to blue within 15 to 30 sec. Strains were delayed oxidase positive, when the color changes to purple within 2 to 3 min. Microorganisms were oxidase negative if the color did not change.

**Starch Hydrolysis Test:**

The isolated strains were individually streaked on to the agar plates and incubated for 24 – 48 hours at 35ºC. For assimilation starch for energy and catabolic reactions, it must be degraded into basic glucose units by amylase. These enzymes are secreted by the microorganisms into the medium, which degrade starch primarily to glucose. After completion of the incubation period, the plates were flooded with Gram's iodine solution. A clear zone around the colonies was resulted as positive.

**Casein Hydrolysis Test:**

Skim milk agar media was used for the hydrolysis of casein. The plates were streaked with isolates and incubated for 24h at 35ºC. After completion of the incubation period, a clear zone was formed around the colonies, resulted for hydrolysis of casein by extra cellular protease.

**Gelatin Hydrolysis Test:**

The test is used for determinationof the ability of an organism to produce proteolytic enzymes (gelatinases) that liquefy gelatin. Nutrient broth was used for the identification of gelatin hydrolysis test, contains 12% gelatin, converting it into a semisolid medium. Then the test strains were inoculated individually in aseptic condition to a sterile tube of gelatin medium and incubated at 35ºC for 24h. The tubes were placed into a refrigerator for few minutes, which cause undigested gelatin to resolidify. If the gelatin has been digested, the medium in the tube will fail to solidify after refrigeration. If gelatinase is present, the liquid medium will fail to
solidify upon refrigeration.

**Antibacterial Activity:**

Broth dilution technique was used for antibiotic susceptibility assay, the technique to be relatively simple for manual testing of small numbers of cultures (James and Lizzie. 1977).Well diffusion technique was used for the identification of inhibition of the zones for the isolates G3A, G3L and G3D.

## RESULTS AND DISCUSSION:

The process of the method used for the biofilm production was quantitatively investigated using the method of adherence to polystyrene microtiter plates proposed by Christensen *et al.* (1985).The organisms in biofilms tend to become more resistant to antibiotics and disinfectants there by become a reservoir for subsequent spread of pathogenic organisms. (Ashoka *et.al.* 2015). A total of 310 bacterial strains were randomly isolated from the media plates. After primary and secondary screening of the isolates each pure strains were examined for the biochemical and morphological characters. Inside that the strains G3A, G3L, G3D showing different characters.

| **Character** | **Isolate G3A** | **Isolate G3L** | **Isolate G3D** |
|---|---|---|---|
| Colony shape | Circular | Circular | Circular |
| Gram's stain | + | + | + |
| Oxidase test | Negative | Negative | Negative |
| Catalase test | Positive | Positive | Positive |
| Gelatin hydrolysis | Positive | Negative | Positive |
| Casein hydrolysis | Positive | Positive | Positive |
| Starch hydrolysis | Positive | Positive | Positive |
| Antibacterial activity (Zone of inhibition in mm) | | | |
| Ampicillin | 8.0 | 12.8 | 9.5 |
| Rifampin | 7.8 | 10.5 | 9.8 |
| amoxicillin | 6.5 | 8.5 | 6.5 |
| Neomycin | 7.8 | 12.9 | 10.5 |

All the three isolates are showing gram's positive result. The strain G3L showing highest antibacterial activity comparing with G3A and G3D. G3A and G3D shows the activity for the hydrolysis of starch, casein and Gelatin. The strain G3L, gram's positive bacteria showing the activity for the hydrolysis for casein and starch, it was not able to hydolyse the proteolytic enzymes..

## REFERENCES

Ashoka M, Nupur P, Dharitri M, Mamta B, Debasish S, Swetlina J, Debabrata D, Nirupama C. 2015.Study of biofilms in bacteria from water pipelines. *Journal of Clinical and Diagnostic Research.* V9(3): 09-11

Christensen, G.,Simpson, W. and Yonger, J. 1985.Adherence of coagulase negative staphylococci to plastic tissue cultures: a quantitative model for the adherence of staphylococci to medical device. *J. Clin. Microbiol*. 22. 996-1006.

Escher AR and Characklis WG. 1988. Microbial colonization of asmooth substratum: A kinetic analysis using Image Analysis. WaterWastewater Microbiol. Water. *Sci. Tech*.20. 277-283.

Fux, C. A., J. W. Costerton, P. S. Stewart, and P. Stoodley.2005. Survival strategies of infectious biofilms. *Trends Microbiol*.13**:**34-40.

Flemming H-C, Wingender J. 2010. The biofilm matrix.*Nat Rev Microbiol*.8: 623–633.

Ganesh B, A.G.S.S. Naidu, M. Jagannadha Rao, T. Karuna Karudu and P. Avatharam 2013. Studies on textural characteristics of sediments from Gosthani River Estuary - Bheemunipatnam, A.P., East Coast of India.*J. Ind. Geophys. Union*. V.17(2). 139-151

Gotz, F. 2002. Staphylococcus and biofilms.*Mol. Microbiol*.43. 1367-1378.

James B. Evans and Lizzie J. Harrell. 1977. Agar shake tube technique for simultaneous determination of aerobic and anaerobic susceptibility to antibiotics. *Antimicrob. Agentschemother*. 12(4). 534 – 536.

Nair S, Chandramohan D., Loka Bharathi P.A. 1992. Differential sensitivity of pigmented and non-pigmented marine bacteria to metals and antibiotics. *Water Res.* 26. 431.

Silva A. L., Hofer E. 1995. *Escherichia coli* isolated from salt-water fish: resistance to drug and colicinogeny. *Biomed. Lett.* 51.175.

Wolfaardt GM and Archibald R. 1990. Microbially inducedcorrosion or biocorrosion in industrial water systems. *Tech. SA.* 1-7.