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Quest

Editorial

T*he Quest* has always striven to report on the most exciting discoveries and trends across the life science spectrum, with the goal of offering researchers in academia an engaging glimpse of what's happening both in and outside their own disciplines. we aim to further that dialogue by offering highly readable articles about new directions and discoveries in the life sciences.

Piroxicam is a non-steroidal anti-inflammatory drug (NSAID) of the oxicam class used to relieve the symptoms of painful, inflammatory conditions like arthritis. *Hibiscus rosa-sinensis* alcoholic leaf extract (AEH) were given to piroxicam induced toxicity in mice and based on the results of the study it can be concluded that AEH exhibited a protective action against piroxicam toxicity and effective in combating oxidative stress-induced hepatic damage. Glycomics is the comprehensive study of glycomes including genetic, physiologic, pathologic, and other aspects. Glycomics is the systematic study of all glycan structures of a given cell type or organism and is a subset of glycobiology. Recently Glycomics can be used for the detection of ovarian cancer and liver sclerosis, discovery of new glycodiagnostics, identification of serum glycoprotein biomarkers helping in disease detection, etc.

Dopamine is currently used to treat heart, vascular and kidney disorders. It can be safely used in cancer treatment to curb the growth of blood vessels in tumors. And also secondary plant metabolites are used in signalling and regulation of primary metabolic pathways. The authors do the review on used of plant secondary metabolic to cure dental caries. Plants active against dental organisms has been listed and this review highlight the role of medicinal plants in the treatment of dental caries and infections associated with dental care.

Biosurfactants are valuable microbial amphiphilic molecules with effective surface-active and biological properties. Microbes synthesize them, especially during growth on hydrocarbons, providing an alternative to conventional chemical surfactants. Authors isolate bacteria from the oil contaminated sites, on medium containing oil as sole carbon source, were screened for biosurfactant production.

Here by all ARIBAS members are invited to read and contribute to ''QUEST'' to propagate the idea of knowledge gaining by sharing.

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NEWS AND VIEWS:-

REVIEW ARTICLE:-

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Manuscripts submitted to Quest should adhere to below mentioned criteria.
Research News: About 400 words (1 page)
Research Article: About 2000 words (4 pages)

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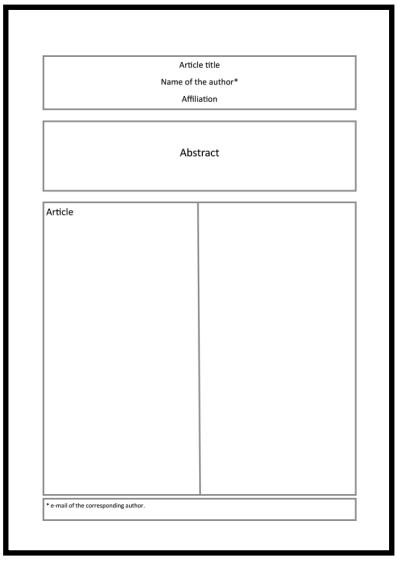
Font: Calibri
Font Size: 14
Columns: 2
Line Spacing: 1
Margin: Narrow
References: 1) In text citing, S No, Superscript.
2) Author's name (s), *Journal name*, **Volume No**, Page No**,** (year).
3) Maximum number of references should not exceed than 25.



Mechanisms Involved in Toxicity of Liver Caused by Piroxicam in Mice and Protective Effects of Leaf Extract of *Hibiscus rosa-sinensis*

Piroxicam is one of the important therapeutic nonsteroidal anti-inflammatory class of drugs used mainly to suppress pain and inflammation in arthritis and other musculoskeletal disorders. Besides being anti-inflammatory, these drugs are analgesic and antipyretic often used for the relief of nonspecific fever condition. Recently, piroxicam has also gained attention as an effective therapy for tumors, colorectal, and invasive bladder cancers. The objective of the current study is to evaluate the protective effects of the alcoholic leaf extract of Hibiscus rosa-sinensis (AEH), Malvaceae, against piroxicaminduced toxicity in mice. Sixty adult Swiss albino mice (Musmusculus) were divided into four groups (n = 10), which included a control group, a group treated orally with AEH (30 mg kg−1 b.w.) for 15 days, a group treated orally with piroxicam (6.6 mg kg−1 b.w.) for 15 days, and another group treated orally with piroxicam and AEH for 15 days. The results indicated that treatment with piroxicam alone resulted in a significant increase in the activities of serum marker enzymes, namely, aspartate transaminase, alanine transaminase, and alkaline phosphatase with profound hepatic lipid peroxidation as evidenced by a marked increment in the level of thoibarbituric acid reactive substances along with a distinct diminution in reduced glutathoine content and various antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase in the liver. However, treatment with AEH during piroxicam treatment retrieved or partially antagonized the effects induced by piroxicam toward the normal values of controls. Histopathological observations also corroborate with the above findings. It can be concluded that AEH exhibited a protective action against piroxicam toxicity and effective in combating oxidative stress-induced hepatic damage.

-Contributed by Srushti Modi
IGBT II

Glycomics - An Overview of the Carbohydrate Study

Glycomics not only involves the study of carbohydrates alone, but also deals with the study of carbohydrates combining or being associated with other macromolecules - lectins, glycoproteins, glycolipids, enzymes specific for carbohydrates, cell receptors specific for carbohydrates, sugar molecules present in nucleic acids, etc. .."

The term "glycome" is used to refer to the entire glycan (carbohydrate) component - free as well as combined - present in the living cells of organisms. Glycomics is thus defined as the systematic study of the glycan structures present in any living cell. Glycomics not only involves the study of carbohydrates alone, but also deals with the study of carbohydrates combining or being associated with other macromolecules - lectins, glycoproteins, glycolipids, enzymes specific for carbohydrates, cell receptors specific for carbohydrates, sugar molecules present in nucleic acids, etc.Glycomics is regarded as one of the most complex entities to be studied in the living cell.

Carbohydrates are one the most abundant macromolecules present in living organisms. They are extremely complex biomolecules present in the following forms - unbound, chain, branched, bound, conjugated, etc. Glycan molecules are extremely critical for the proper functioning of the living cell.

In medical field development of glycotherapeutic compounds through post-translational modifications, as well as therapy for other processes such as inflammation, infection, neurodegeneration, etc.

Glycomics can also be used for the detection of ovarian cancer and liver sclerosis, discovery of new glycodiagnostics, identification of serum glycoprotein biomarkers helping in disease detection, etc.

It can be also used in metabolic oligosaccharide labeling and engineering for studying complex glycan structures in bacteria .
istinction in the levels of serum glycan can be used as a means of detecting gastric cancer. It implies that glycomics may be used as a marker for the detection of the disease. Marine glycans obtained from red seaweed can be used in skincare products as they act against adverse effects of excessive sunlight exposure to the skin.

*-contributed by Aksh*ay
IGBT-X

Dopamine -- an inexpensive drug

Dopamine, a compound present in the body as a neurotransmitter and a precursor of other substances including adrenaline. It is currently used to treat heart, vascular and kidney disorders. It can be safely used in cancer treatment to curb the growth of blood vessels in tumors.

Reporting in the *International Journal of Cancer*, the researchers show that dopamine prevented the growth of blood vessels in two animal models without causing many of the serious side effects of the far-more expensive anti-angiogenic drugs currently used in cancer therapy.

Furthermore, the agent prevented the drop in the number of neutrophils (i.e., neutropenia) found in the blood that is typically caused by 5-fluorouracil, a chemotherapy agent commonly used in the treatment of gastrointestinal and other tumors, such as colon, stomach, pancreas and breast cancers.

The Principal investigator Sujit Basu, MD, PhD, professor of pathology and of medical oncology at the OSUCCC -- James said that in this study, they demonstrate for the first time that the inexpensive drug dopamine lacks the serious side toxicities commonly seen with the anti-angiogenesis drugs presently used in the clinic said . Furthermore, dopamine can prevent the low-neutrophil count that is often induced by a very common anti-cancer drug used for the treatment of gastrointestinal cancers.

Finally, because dopamine is being used in the clinics for other disorders, our findings can be rapidly transferred to the clinic for the treatment of cancer patients.

Earlier studies by Basu and others have shown that dopamine blocks the growth of new blood vessels in tumors by inhibiting the action of vascular endothelial growth factor-A (VEGF-A).

VEGF-A-induced angiogenesis plays a critical role in the initiation, growth and progression of solid tumors, and the majority of the anti-angiogenic drugs currently used in the clinics have anti-VEGF-A actions. This study will help to rapidly translate the use of this inexpensive but effective anti-angiogenic drug, dopamine, for the treatment of cancer in the clinics.

Basu and his colleagues conducted this study using an animal model of human colon tumors transplanted into mice and a mouse model of lung cancer. The technical findings included:

- Dopamine did not cause hypertension or affect liver functions (i.e., levels of alanine aminotransferase and aspartate aminotransferase were not elevated, as can happen with currently available anti-VEGF drugs)
- Renal function was unaffected by dopamine treatment; serum blood urea nitrogen (BUN) levels remained normal in both animal models and in normal animals, while animals treated with the anti-angiogenic inhibitor sunitinib showed increased levels

- Dopamine administration did not affect platelet or neutrophil counts, although both were decreased by treatment with sunitinib. Dopamine prevented neutropenia (low neutrophil count) induced by 5-FU, an anti-cancer drug commonly used to treat gastrointestinal cancers

Source: Ohio State University Wexner Medical Center.

-Contributed by Dipika Patel

Role of Plant Secondary Metabolites in Dental Caries

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Abstract: Dental caries is an ancients disease. Dental caries is a disease in which minerals of the tooth are dissolved into surrounding bacterial plaques and to saliva. Dental caries is one of the globally affecting diseases of the oral cavity. Medicinal plants are useful in many diseases dates back from the history. Plants contain phytochemicals, which have pronounced antimicrobial activity and other properties. The phytoconstituents present in the plants exhibit anti-cariogenic effects through various modes of action, including bactericidal effects on oral bacteria, prevention of adherence of bacteria to the tooth surfaces. Plants active against dental organisms has been listed and this review highlight the role of medicinal plants and phytochemicals like flavanoids, polyphenols, terpenes, alkaloids in the treatment of dental caries and infections associated with dental care.

Introduction

Oral diseases continue to be a major health problem worldwide[1]. Tooth decay and periodontal disease are among the most important global oral health problems, although conditions such as oral and pharyngeal cancers and oral tissue lesions are also significant health concerns. Despite general advances in the overall health status of the people living in industrialized countries, including oral and dental health, the prevalence of dental caries in school aged children is up to 90% and the majority of adults are also affected[1]. There is considerable evidence linking poor oral health to chronic conditions, for example, there is a strong association between severe periodontal diseases and diabetes[2]. Tooth loss, caused by poor periodontal health (which affects up to 20% of the adult population worldwide) can lead to significant morbidity and premature death. The economic impact of oral diseases is an important consideration with up to 10% of public health expenditure in developed countries related to curative dental care[1]. In most developing countries, expenditure in oral health care is low; access to dental healthcare is limited and is generally restricted to emergency dental care or pain relief. While there has been a marked improvement in oral health in most developed countries worldwide, populations of dentally disadvantaged individuals exist in these countries, often indigenous child populations and those people of low socioeconomic status, where or Many modern drugs have been isolated from natural sources based on their use in traditional medicine.

Many bacteria and fungi produce diseases which are manifested in or about the oral cavity. Some of these diseases or lesions are of a specific nature and are produced by a specific contribution to the problem of caries etiology[3]. The highest caries susceptibility is in the age group of 20-40 years, also the females are more susceptible to dental caries as compared to males[4]. Dental decay is a chemical parasitic process consisting of two stages, the decalcification of enamel or its total destruction and the decalcification of dentine (dissolution of the softened residue[5]. The cariogenic Streptococci is critical to the development of pathogenic plaque. A large number of Streptococcus, Actinomyces and Lactobacillus species are involved in root caries and periodontal diseases[6]. Antibiotic resistance has increased substantially in the recent years and is posing an ever increasing therapeutic problem one of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants[7,8,9]. Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. It is expected that plant extracts showing target sites other than those

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used by antibiotics will be active against drug resistant pathogens[10]. The human oral cavity is a habitat for about 500 cultivable and non-cultivable bacterial species. Up to 100 species can be present in a particular oral cavity. While the majority of these species are commensals, a subset is opportunistic pathogens. Their key role of in the etiology of periodontitis and dental caries, the most prevalent diseases in the world, is well established[11]. Dental caries causes symptoms and treatment based on the etiology of periodontitis (Figure 1). They have also been implicated in the etiology of a number of systemic diseases like infective endocarditis respiratory infections[12,13] cardiovascular diseases[14] and brain abscess[15] .

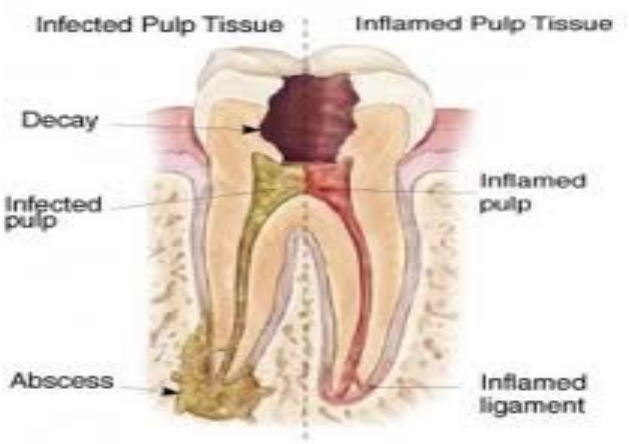

Figure 1: Dental caries causes symptoms
(Source:www.south waterf rontdental.com)

Despite several agents being commercially available, these chemicals can alter oral microbiota and have undesirable side-effects such as vomiting, diarrhea and tooth staining[16]. For example, bacterial resistance to most (if not all) of the antibiotics commonly used to treat oral infections (Penicillins and cephalosporins, erythromycin, tetracycline and derivatives and metronidazole) has been documented[17]. Other antibacterial agents used in the prevention and treatment of oral diseases including cetylpyridinium chloride, chlorhexidine, amine fluorides or products containing such agents, are reported to exhibit reported to exhibit toxicity, cause staining of teeth or in the case of ethanol (commonly found in mouthwashes) have been linked to oral cancer. Hence, the search for alternative products continues and natural phytochemicals isolated from plants used in traditional medicine are considered as good alternatives to synthetic chemicals[18].

The bacteria that produce the acids fall into the category of acidogenic bacteria and are also aciduric, which means that they can live preferentially under acid conditions. In normal dental plaque, these acidogenic bacteria occupy less than 1% of the total flora.

The mechanisms of plaque formation include

- Absorption of proteins and bacteria to form a film on the tooth surface.
  The effect of van der waals and electrostatic forces between microbial surfaces and the film to create reversible adhesion to the teeth.
- Irreversible adhesion due to intermolecular interactions between cell surfaces and the pellicle.

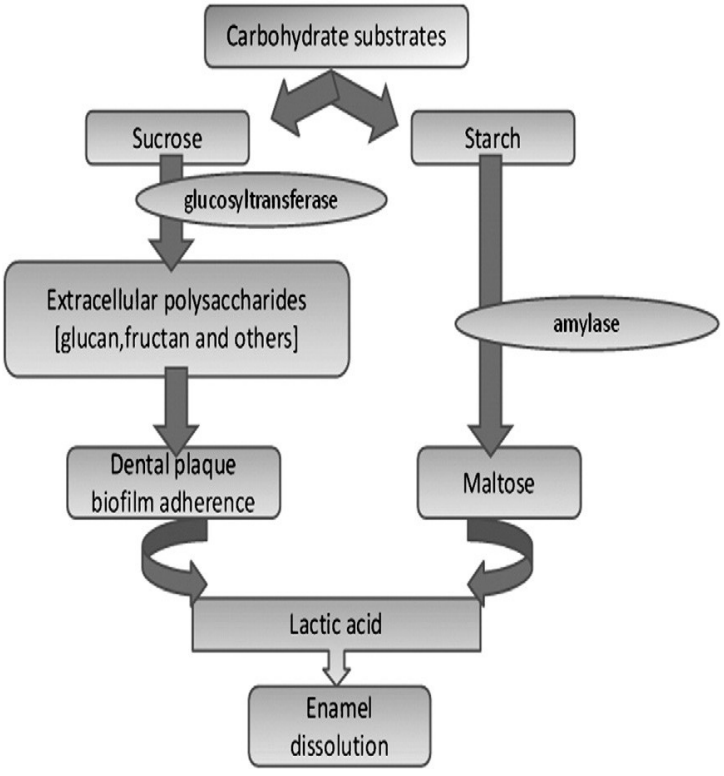

Figure 2: Mode of action of dental caries (Source:www.shockmd.com)

- Secondary colonisers attach to primary colonisers by intermolecular interaction.

The cells divide and generate a biofilm. Any drugs commonly used today are of herbal origin. Indeed, about 25 percent of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Chemical compounds such as amino acids, carbohydrates and proteins, are products of primary metabolism and are vital for the maintenance of life processes, while others like alkaloids, phenolics, steroids, terpenoids, are products of secondary metabolism and have toxicological, pharmacological and ecological importance[19]. However, the main classes of bioactive compounds from plants include flavonoids, terpenes, alkaloids, saponins, and coumarins[20]. Some are made from plant extracts; others are synthesized to mimic a natural plant compound. Herbal medicinal products are defined as any medicinal product, exclusively containing one or more active substances[21].

Bioactive molecules found in seed extracts like Phenolic compound, tannins, saponins etc are present[20]. Maximum more bioactive compound present the poly phenol and Phenolic compound in seed extracts. The rich antimicrobial effects of polyphenols have also been widely reported as has their ability to inactivate bacterial toxins, and there is an increasing interest in this topic because plant polyphenols could represent a source of new anti-infective agents against antibiotic-resistant human pathogens. Moreover, grape seed extracts inhibit the growth of anaerobic bacteria, such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, associated with periodontal diseases. Extracts from *Perilla frutescens* var. *japonica* seeds have shown inhibitory activity against oral cariogenic Streptococci and periodontopathic *Porphyromonas gingivalis*. *Perilla* seed polyphenols were isolated and their activity was tested. The flavonoid luteolin was the phenol that was most active against bacterial growth[22].

Neem (Azadirachta indica) belonging to Meliaceae family with 'azadirachtin' as an active ingredient has been used in India and south Asia for thousands of years to clean teeth and fight oral infections. Studies suggest that 'azadirachtin' is appropriate for treating gingivitis and oral infections because it inhibits bacterial growth[23]. The efficacy of Soluneem (3%) on the inhibition of Mutans streptococci which is relatively similar to that of standard antimicrobial agent Chlorhexidine.CHX has been used as a therapeutic agent due to its antimicrobial activity. However, its long term use is under scrutiny due to its various undesirable adverse effects. Soluneem could be a promising alternative to other antimicrobial agents for the prevention of dental caries. Modern science validates that neem products are ecofriendly, economical and effective alternative to synthetic drugs. It is also tempting to speculate that Soluneem can also be used in combination with other various naturally occurring antimicrobial agents which has a wide range of antimicrobial action[24].

Two secondary metabolite compounds of steroid and triterpenoid derivates had been extracted from the leaf of *Eclipta alba* L. Hassk. The extraction of the compounds was carried out by maceration and then isolated by column chromatography. Structural elucidation was established using GC-MS. Both of the compounds showed antibacterial activity against S. mutans, A. viscosus, and L. kaesal at the concentration of 5%, 10%, and 25%[25]. The results of anti-bacterial activity were found that acetone extract of *S. trilobatum* was most effective against ability to inhibit the growth of dental (bacterial) pathogens (*Staphylococcus aureus, Streptococcus mutans, Streptococcus salivarius, Streptococcus sanguinis and Lactobacillus acidophilus*). Maximum antibacterial activity was observed against *S.*

salivarius (23 mm) and lowest activity against *S. sanguinis* (9 mm). The phytochemical analysis revealed the presence of alkaloids, flavonoids, steroids, terpenoids, tannins and saponins which might be accountable for its anti-bacterial potentiality. The results validate the traditional uses of *S. trilobatum* in treatment of dental diseases .

The antimicrobial activity of *Psidium guajava* was checked with ethanol, acetone, chloroform, methanol and water extracts against selected bacterial isolates. The acetone and methanol extract showed maximum inhibitory activity but ethanol extract inhibit only the growth of *Pseudomonas aeruginosa* and the water extract revealed high activity against both *Streptococcus viridians* and *Bacillus megaterium*. In phytochemical screening, the acetone and ethanol extracts gave positive results for steroids, terpenoids and flavornoids. Phenolic compounds were there only in acetone extract. Saponins were absent in ethanol extract and tannins simply present in acetone and methanol extract[27]. Licorice roots extract contains Glycyrrhizol A, a compound that has strong anti-microbial activity against cariogenic bacteria

Chemically, the air dried stem bark of *S Persica* through chemical studies showed that it is composed of trimethyl amine, salvadorin, chlorides, high amounts of fluoride and silica, sulphur vitamin C, small amounts of tannins, saponins, flavonoids and sterols. It has been shown that vitamin C and sit sterol content of this plant have great roles in strengthening the gum capillaries and preventing gum inflammation. Similarly, calcium salts and fluoride are quite effective in preventing dental caries[29]

Conclusion

Plant have great source as anticariogenic compound against oral pathogenic microorganisms, which can be used to treat infectious diseases. The very good activity of plant based secondary metabolites will be successful in the future development of effective for toothpaste or mouth washer against oral microorganisms. It indicates that plants have the potential to generate herbal metabolites. The plant secondary metabolites demonstrating anticariogenic activity and dental related problem could result in the discovery of new chemical classes of antibiotics that could serve as selective agents for the maintenance of human health and provide biochemical tools for the study of infectious diseases. Further phytochemical studies are required to establish the types of compounds responsible for the anticariogenic effects of these medicinal plants.

References

1. Petersen, PE., Bourgeois, D., Ogawa, H., Estupinan-Day, S., Ndiaye, C. Bull World Health Organ*,* **83***, 6*61–9 (2005).
2. Petersen, PE. Commun Dentist Oral Epidemiol*,* **31**, 3–24 (2003).
3. Paster, BJ, Boches, SK, Galvin, JL, Ericson, RE, Lau, CN, Levanos, VA, *et al*. J Bacteriol, **183**, 3770-3783 (2001).
4. Nigam, P. & Srivastava, A.B. *Fed. Oper. Dent*. **1 (1)**, 36-44 (1990).
5. Marsh, P.D. Journal of dental resistance.**71:** 1431 – 1438 (1992).
6. Sachipach, P., Osterkwalder, V. & Guggenheim, B. *Caries Res.,* **29**, 382-395 (1995).
7. Guillemot, D. Current opinion in Microbiology*,* **2**, 494 – 498 (1999).
8. Jones, R.N. Microbiology & Infectious Diseases, **31**, 461 – 466 (1998).
9. Kim, H., Park. S. W., Park J. M., Moon, K.H., & Lee. C.K. Natural Product Science*,* **1**, 50 – 54 (1995).
10. Ahmad, I & Beg, A.Z. Journal of Ethnopharmacology, **74**, 113-123 (2001).
11. Meyer, DH, Fives-Taylor, PM. Curr Opin Microbiol, **1**, 88-95(1998).
12. **.** Mojon, P and Bourbeau, J. Curr Opin Pulm Med, **9**, 166-170 (2003).
13. Scannapieco, FA, Wang, B, Shiau, HJ. Ann Periodontol, **6,** 8-86 (2001).

17. Bidault, P., Chandad, F., Grenier, D. *J Can Dent Assoc*, **73**, 721–5 (2007).
18. Prabu,GR., Gnanamani, A., Sadulla, S. J Appl Microbiol**, 101**, 487–95(2006).
19. Bandaranayake WM. Wetlands Ecology and Management. **10,** 421 – 52 (2002).
20. Cowan, M. Clinical Microbiology Reviews. **12**, 564 – 82 (1999).
21. Margret Chandira, B.Jayakar. International Journal of Pharmaceutical Sciences Review and Research. **3(1)**, 01 – 110 (2010).
22. Sato, M., Fujiwara, S., Tsuchiya, H., Fujii, T., Linuma, M., Tosa, H., et al., J ethnopharmacol, **54**, 71-6 (1996).
23. Pai MR, Acharya LD, Udupa N. Journal of Ethnopharmacology.**2(4)**, 99-103 (2004).
24. Mitesh Kathariya, Anantharaj Adhimuthu, Rahul Kathariya, Praveen Prasanna, Renuka Kathariya, Kalyani Bhate. OHDM, **13(4)**, 1086-1990(2014).
25. Kartini Hasballah, Murniana, and Nurdin Saidi. Biosciences Conference Banda Aceh, **22-24 2(1),** 411-415 (2012).
26. Sai Durga Prasad. Sugnanam, Mallikarjuna. Kunjam and Venumadhav. Rajanala. Asian Journal of Plant Science and Research. **5(2)**, 34-37 (2012).
27. Shafi Thompson T, Arunima Ashok, Sukesh K. Journal of Pharmacy and Pharmaceutical Sciences. **4(2)**, 400-401(2012).
28. Mohammad Y, Mohammad I, Assem NM, Mohammad Z. RJPBCS,**1(2)**, 202-206 (2010).
29. Rajeshwar digra, C Rao,idhi Gupta, Shelja Vas. Journal of Orofacial Research, **4(1)**, 41-45(2014).

Biosurfactant production and oil degradation by some indigenously isolated bacteria

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Abstract: Biosurfactants are valuable microbial amphiphilic molecules with effective surface-active and biological properties applicable to several industries and processes. Microbes synthesize them, especially during growth on hydrocarbons, providing an alternative to conventional chemical surfactants. Bacteria isolated from the oil contaminated sites, on medium containing oil as sole carbon source, were screened for biosurfactant production using various methods like blood hemolysis, CTAB agar, oil spreading assays and emulsification index. The selected isolates, GAC, PEC 1 and ONGC 2 were further compared for extent of crude oil degradation in presence of medium supplements. Among the 03 isolates, ONGC 2 was most efficient in crude oil degradation as evident by Gas chromatography analysis.

INTRODUCTION

Oil spills have been a major issue since decades. Used engine oil can be considered as one of the source responsible for polluting the soil with hydrocarbons. Used engine oil consists of Petroleum ether or Benzine ,Gasoline, Naptha, Mineral spirits, Kerosene, Fuel oil, Lubricating oil, Paraffin wax, Asphalt or Tar. Accidental release of hydrocarbons into the environment and its attendant detriments is not restricted to oil producing regions alone, but other areas which are also prone to the increasing risks and possibility of spills due to tanker accidents and leakage from ruptured pipelines networked across such areas [1].

Soil is a rich source of micro-organisms which promote the microbial degradation of hydrocarbons and residual oil[2]. It has been found that soils receiving hydrocarbons like the area of oil sludge, oil fields etc. have significant higher population of hydrocarbon degrading micro-organisms. Several methods of isolating and enumerating petroleum degrading bacteria have been reported including plating on oil agar, silica gel oil media and inoculating liquid media containing hydrocarbons by the most probable number method.

The principle of enrichment culture is to provide growth conditions that are very favorable for the organisms of interest and as unfavorable as possible for competing organisms. Hence, the microbes of interest are selected and enriched[3].

Naturally occurring surface-active compounds derived from microorganisms are called biosurfactants. Biosurfactants are amphiphilic biological compounds produced extracellularly or as part of the cell membrane by a variety of yeast, bacteria and filamentous fungi[4]. The biosurfactants are complex molecules covering a wide-range of chemical types including peptides, fatty acids, phospholipids, glycolipids, antibiotics, lipopeptides, etc. Biosurfactants have several advantages, including low toxicity, high biodegradability, low irritancy and compatibility with human skin[5]. Biosurfactants have gained importance in the fields of enhanced oil recovery, environment bioremediation, food processing and pharmaceuticals[6].

MATERIALS AND METHODS

Collection of soil samples

The soil sample were collected from 4 different sites like Garden, Garage, petrol pump from

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Anand and ONGC Chand Kheda in pre sterilized bag. The soil sample was collected from 5 cm depth. All the soil sample was carefully transferred to lab and stored in refrigerator at 4°C.

Crude oil sample

The crude oil was collected from ONGC Chand Kheda, Ahmedabad.

Isolation and screening of oil degrading bacteria

Samples from various sites contaminated with oil were inoculated in Bushnell and Haas Medium Hi-Media (BHM) in 250 ml Erlenmeyer flasks with 2% crude oil as sole source of carbon. The flasks were incubated on rotary shaker at 100 rpm for 7 days at room temperature, after which culture was transferred to fresh media with crude oil as the sole carbon source. After three to four such transfers, the suspensions from each flask were streaked on Bushnell Hass Agar plates. Isolated colonies were obtained on plates spread with crude oil as sole source of carbon.

Growth determination

Bacterial growth was determined by two methods

1) By taking O.D .of culture at different time periods
2) By measuring wet and dry weight periodically

To measure O.D. of bacterial culture, bacteria were grown in Bushnell and Hass medium containing 2% crude oil as a carbon source. OD at 620 nm was measured at different time interval that is 24, 48 and 72 h.

To measure wet weight, first culture was grown for different time intervals (24,48 and 72hrs), then samples were drawn in pre-weighed 2.0 ml microfuge tubes and centrifuged it at 10000 rpm. The supernatant was discarded and the weight of tube + pellet was recorded, this gave the wet weight of the biomass. Then tube was kept in the oven at 70°C and weight was taken till two constant weights were obtained.

The isolated culture was identified on the basis of biochemical tests and 16S rDNA sequence.

Gas chromatography

The extent of crude oil degradation by the bacteria was measured by gas chromatography. For gas chromatography the culture was grown in presence of different carbon sources like crude oil, glucose, and tween 80. Centrifuged the culture at 10,000 rpm for 15 minutes and supernatant was extracted with hexane in separating funnel, 50 ml hexane, 30 ml culture supernatant.

Screening of biosurfactant producing bacteria

The isolated colonies were tested for their biosurfactant production by several methods

(1) Blood agar method
(2) CTAB agar method
(3) Oil spread technique
(4) Emulsification index

(1) Blood Agar Method

The test culture was streaked on the blood agar plate and incubated at 37°C for 24-48 hrs. After incubation, looked for the zone of hemolysis surrounding the colony [7].

(2) CTAB agar method

The test culture was cultivated on a light blue mineral salts agar plate containing the cationic surfactant cetyltrimethyl ammonium bromide and the basic dye ethylene blue. Anionic surfactants were secreted by the microbes growing on the plate; they form a dark blue insoluble pair with cetyltrimethyl ammonium bromide and ethylene blue. Biosurfactant producing colonies were surrounded by dark blue halos.

(3) Oil spread technique

30 ml of distilled water was taken in the pertiplate, 1 ml of crude oil was added in the centre

of the plate containing distilled water. Then added 20µl of the supernatant of the culture isolated from the soil in the centre of the plate. The biosurfactant producing organism can displace the oil and spread in the water.

(4) Emulsification index

Hydrocarbon was added to culture broth (1:2 v/v), vortexed for 2 min and allowed to stand for 24 h. The height of emulsion was measured by measuring the layer formed in between aqueous and kerosene layer. EI was calculated by measurement of emulsion height [8].

Extraction and purification of biosurfactant

For extraction of biosurfactants culture was grown till 10 days, after that centrifuged at 10000 rpm for 10 min at 4°C. Supernatant was collected and adjusted the pH to 2.0 with 6 N HCl. The supernatant kept at 4°C over night. The precipitate was collected by centrifuging it at 10000 rpm for 30 min.Collected the pellet and dissolved in distilled water. Adjusted the pH to 7.0 with NaOH.

RESULTS

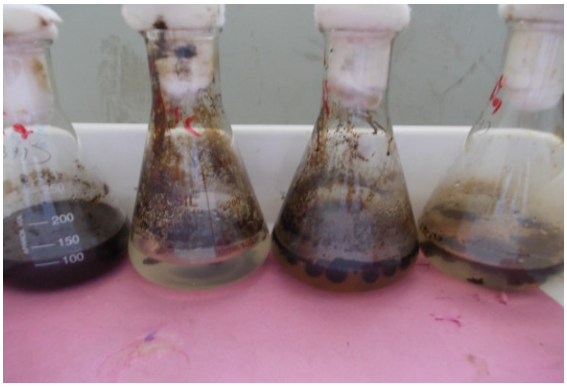
The soil samples were inoculated in BH medium with crude oil as the carbon source followed by three successive transfers in fresh medium containing crude oil as the sole carbon source after an interval of two weeks. Finally the culture was transferred to BH agar plate containing crude oil for isolation and screening of HC degrading microorganism. Total 16 isolates were obtained from different samples with maximum 05 isolates from oil well of ONGC soil followed by 04 each from Petrol pump and Garage and 3 isolates from Garden. From 16 isolates 8 was selected on the bases of their maximum growth capacity (Fig 1a and b).

Morphological Characterization

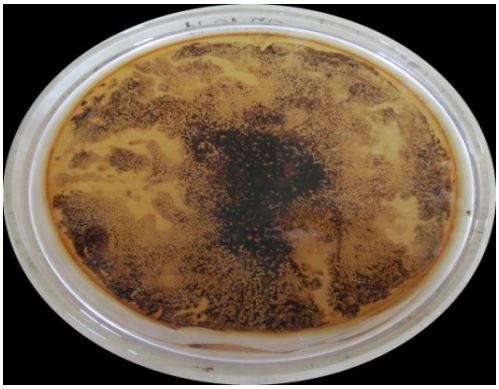
Out of the 08 isolates selected, 03 were Gram's positive and 05 were Gram's negative. All the Gram's negative isolates were rods except for one which was cocci. Similarly, 02 Gram's positive were cocci and only 01 was Gram's positive rod (Table 1).

Table 1 Gram's nature and shape of bacterial isolates

S.N.	SAMPLE	GRAM'S NATURE	SHAPE
1	GRC 1	Negative	Short Rod
2	GRC 2	Negative	Big Rod
3	PEC 1	Negative	Small Cocci
4	PEC 2	Negative	Big Rod
5	GAC 1	Positive	Small Cocci
6	GAC 2	Positive	Small Cocci
7	ONGC 1	Positive	Short Rod
8	ONGC 2	Negative	Short Rod



(a)



(b)

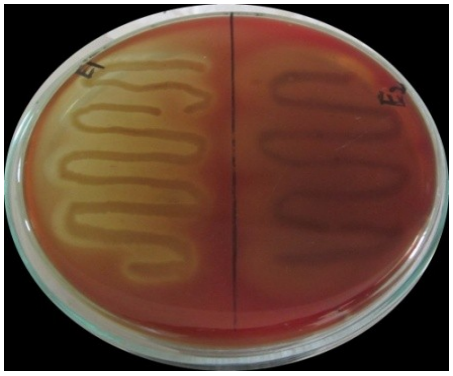
Fig 1. (a) Control (Oil containing medium without bacteria) and Test flasks (b) Growth of bacteria on agar

Table 2 Biochemical Tests

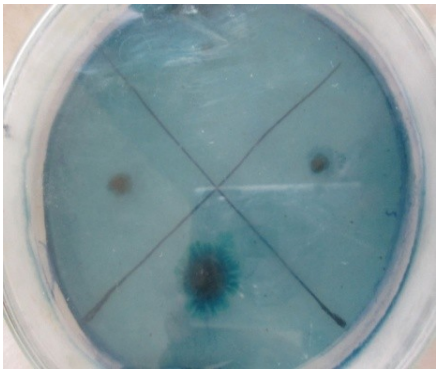
S.N.	TEST	PEC 1	GAC	ONGC 2
1	C.H TEST GLUCOSE SUCROSE DEXTROSE MALTOSE	 +VE +VE +VE +VE	 +VE +VE +VE +VE	 +VE +VE +VE +VE
2	METHYL RED	-	-	_
3	VP	-	-	_
4	SIMMON CITRATE	+VE	+VE	+VE
5	INDOLE PRODUCTION	-	+VE	+VE
6	NITRATE REDUCTION	+VE	-	+VE
7	CASEIN HYDROLYSATE	-	-	+VE
8	DEHYDROGENASE	+VE	+VE	-
9	AMMONIA PRODUCTION	-	-	-
10	HYDROGEN SULPHIDE	+VE	-	+VE
11	GELATIN HYDROGENASE	-	-	-
12	UREA HYDROLYSIS	-	-	-
13	LEAD ACETATE	-	-	-
14	TRIPLE SUGAR IRON	-	+VE	+VE

Table 3 Various Tests for Biosurfactant Production

SAM-PLE	DRY WEIGHT mg	OD nm	BLOOD AGAR TEST	BLUE AGAR TEST	ACETONE PESIPITATION	OIL SPREAD IN LIQUID cm	OIL SPREAD IM SOLID cm
GAC	0.066	1.941	β Hemolysin	+ve	+++	2.0	0.6
PEC 1	0.061	0.730	β Hemolysin	+ve	+++	2.0	0.9
ONGC 2	0.15	0.854	β Hemolysin	+ve	++++	Complete spread	1



(a)



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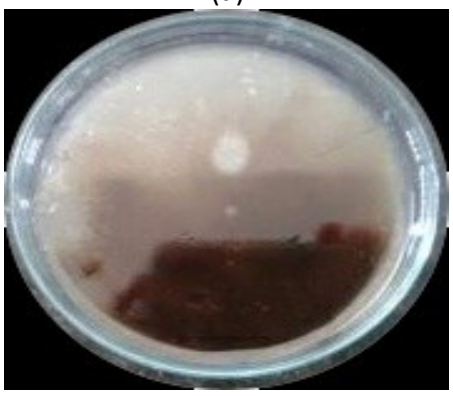
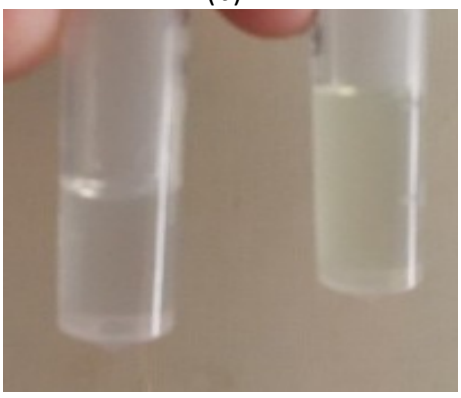


(c)



(d)

Fig 2 (a)Blood Agar Assay (b) CTAB Agar Method (c) Oil spread method d)Acetone precipitation

Table 4 Mesaurement of emulsification Index using different types of oil

SAMPLE	KEROSIN	BTEX	SUNFLOWER OIL	CRUDE OIL
PEC 1	2.5 cm	3.2 cm	1.0 cm	1.2 cm
GAC	2.0 cm	3.5 cm	1.5 cm	1.6 cm
ONGC 2	3.0 cm	4.0 cm	2.0 cm	1.8 cm

Three isolates were selected for further study, namely PEC1, GAC and ONGC 2. The biochemical tests performed on three isolates are shown in Table 2.

The 03 bacterial isolated showing good growth in medium with crude oil as sole source of carbon were further screened for biosurfactant production. Results are summarized in Table 3.

Hemolysis on blood agar

The identification of biosurfactant by hemolysis method is based on the fact that surfactant interacts strongly with cellular membranes and proteins. Exotoxin like hemolysin cause lysis of RBCs. Earlier only those isolates that showed hemolysis were considered to be potential producer of biosurfactant[9]. All the 03isolates showed positive -hemolytic activity (Fig .2a). [10] scored the hemolytic activity during kinetic study of fermentative biosurfactant production by *Lactobacillu*s strains.

Blue Agar Method

The CTAB agar method is a semiquatitative assay for the deteciton of extracellular glycolipids or other anionic surfactants. All the 03 isolated showed dark blue color halo around their colonies indicating the production of anioinc biosurfactant by the isolates, out of which the biggest halo is formed by ONGC 2 (Fig 2b). In the CTAB test designed by Siegmund and Wagner, two isolates showed greenish halos around the colonies on CTAB methylene blue agar medium [11].

Oil spread Method

Soild surface

The diameter of the clearing zone on the oil surface correlates to surfactant activity, also called oil displacement activity. Pure biosurfactant has a linear correlation between quantity of biosurfactant and diameter of the clear zone. In the oil spreading technique, [12] showed that the extent of oil displacement is directly proportional to the concentration of the biosurfactant produced. As shown in the table maximum clearing zone was observed with ONGC 2 followed by PEC 1 and GAC (Table 3).

Liquid surface

It is one of the best methid to detect the presence of biosurfacnt producters. Colony surrounded b an emulsified halo is considered positive for biosurfactant production. ONGC 2 showed complete spread of the oil on the surface within minutes follwoed by PEC 1 and GAC.

Acetone precipitation test

As hown in Table 3, upon addition of chilled acetone to culture supernatant, maximum amount of precipitate was formed by ONGC 2 follwed equally by GAC and PEC 1.

Emulsification Index (EI)

The EI height and stability indicates concentraiton and strenght of biosurfactant.Evaluating the emulsifiaction capacity is a simple screening method suitbale for the a first screening of biosurfactant producing microbes [13]. Table 4 shows the emulsificaiton index observed when different types of oil are used. It can be concluded that ONGC 2 is the best biosurfacnt producing strain among the three isolates studied here.

Crude oil degradation by ONGC 2

A comparison of GC chromatogram of control and tests clearly indicate significant degradation of crude oil by bacterial isolates. A total of 79 peaks were observed in control sample containing un-inoculated flask, which got significantly reduced in reduced in height and number in the sample from BH+ Crude Oil. Two and just one when peak were observed in the samples from BH + crude Oil + Glucose and BH + crude Oil + Tween 80 respectively.

Sample from BH+ Crude Oil. Two and just one when peak were observed in the samples from BH + crude Oil + Glucose and BH + crude Oil + Tween 80 respectively. The information regarding co-metabolism in

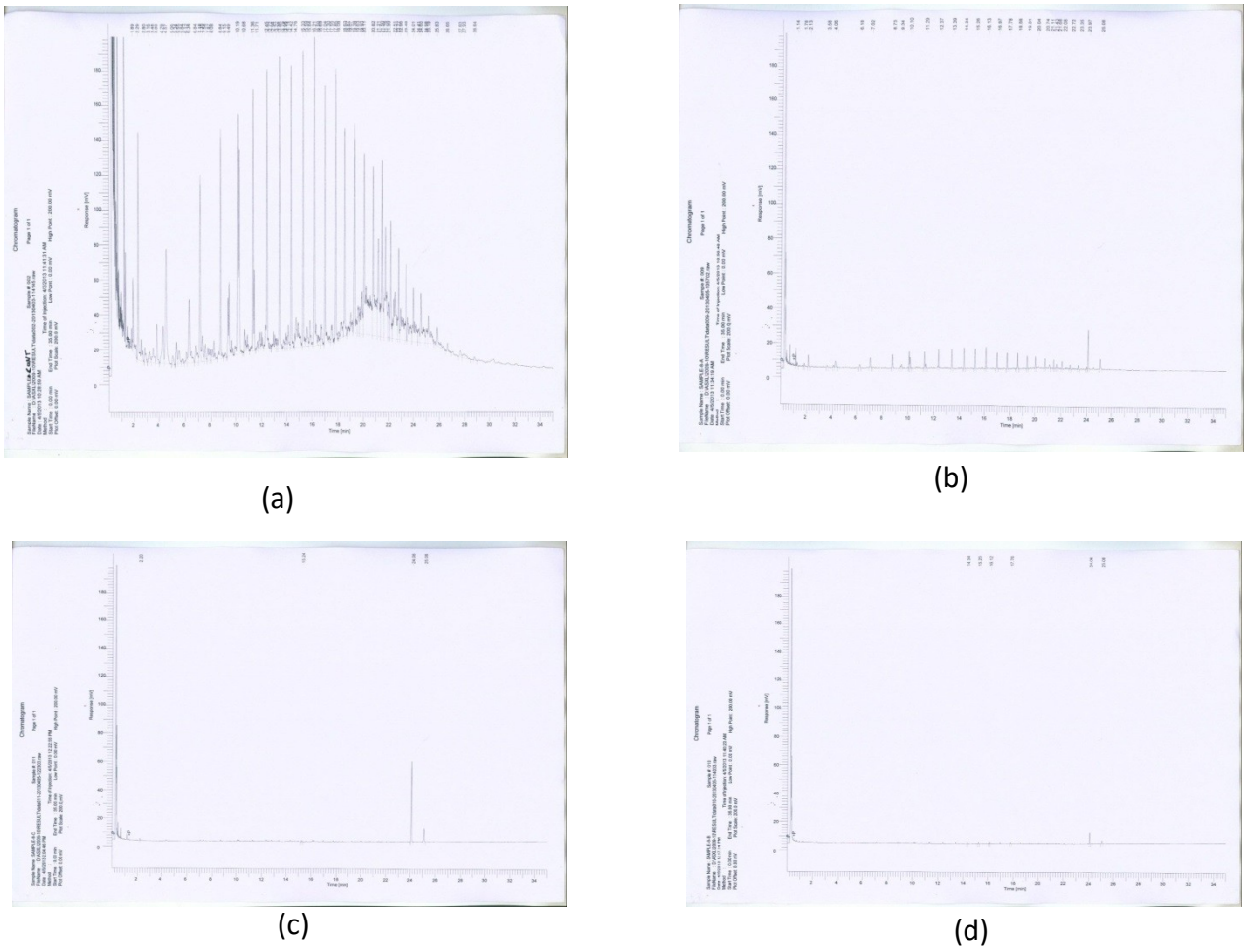


Fig 3 Gas chromatogram showing extent of crude oil degradation by ONGC 2 in presence of different carbon sources (a) Control (un-inoculated) (b) BH+ Crude Oil (c) BH + crude Oil + Glucose (d) BH + crude Oil + Tween 80

the presence of glucose is not available but people have used this phenomenon for the degradation of hydrocarbons. Various strategies were published for the aerobic co-metabolism of chlorinated solvents [14]. In one of the study, medium supplemented with 2% (v/v) crude oil as the sole carbon substrate to study biosurfactant production by a thermophilic *Bacillus subtilis* strain was done [5]

Conclusion

The bacterial isolate ONGC 2, showed maximum growth, crude oil degradation and biosurfactant productionas comapred to other two isolates. The isolate has been identified as *Enterobacter cloacae* based on 16S rDNA sequence. This strain can be used for cleaning of oil spills or biosurfact production after further optimization studies.

Rreferences

1. Vincent, A.O. , Egharevba, F., Medjor O. W., Osaro K. I., Enyemike, E. D. (2011) Microbial Degradation and its Kinetics on Crude Oil Polluted Soil. Res. J. Chem. Sci., **1(6)**, 8–14 .
2. Atlas, R. M., and Bartha, R. (1993) Microbial Ecology-Fundamentals and Applications. Benjamin-Cummings, Redwood City, CA.

1. Huy,N., Jin, S. Amada, K. Haruki, M., Huu, N.B., Hang, D.T., Ha, D.T.C., Imanaka, T., Morikawa, M. and Kanaya, S. (1999) Characterization of petroleum-degrading bacteria from oil-contaminated sites in Vietnam J. Biosci. Bioeng., **88**, 100–102 .
2. Chen,S.Y., Wei, Y.H. and Chang J.S. (2007) Repeated pH- stat fed-batch fermentation for rhamnolipid production with indigenous *Pseudomonas aeruginosa* S2. Appl. Microbiol. Biotechnol., **76** (1):67-74.
3. Makkar, R.S., Cameotra, S.S. (1997) Biosurfactant production by a thermophilic *Bacillus subtilis*strain. *J. Ind. Microbiol. Biotechnol* 1997;**18** (1):37–42.
4. Muthusamy, K., Gopalakrishnan, S., Ravi, T.K., Sivachidambaram, P. (2008) Biosurfactants: properties, commercial production and application, Curr. Sci. **94**, 736-746.
5. Rashedi, H., Jamshidi, E. Mazaheri M. A. and Bonakdarpour, B. (2005) Isolation and production of biosurfactant from *Pseudomonas aeruginosa* isolated from Iranian southern wells oil Int. J. Environ. Sci. Tech. **2(2**): 121-127.
6. Ellaiah, P, Prabhakar, T, Sreekanth, M, Taleb, A.T., Raju, P.B., Saisha, V. (2002) Production of glycolipids containing biosurfactant by *Pseudomonas* species. Indian J Exp Biol **40:** 1083–1086 .
9. Carrillo, P.G., Mardaraz, C., Pitta-Alvarez, S.I., Giulietti, A.M. (1996) Isolation and selection of biosurfactant-producing bacteria. W. J. Microbiol. Biotechnol. 12(**1**):82–84.
10. Rodrigues, L, Moldes, A, Teixeira, J, Oliveira, R. (2006) Kinetic study of fermentative biosurfactant production by Lactobacillus strains. Biochem. Eng. J. 28(**2**):109–116.
11. Siegmund I, Wagner F. (1991) New method for detecting rhamnolipids excreted by *Pseudomonas* species during growth on mineral agar. Biotechnol. Tech. 5(**4**):265–268.
12. Morikawa, M., Hirata, Y., Imanaka T. (2000) A study on the structure-function relationship of lipopeptide biosurfactants. Biochimica,Biophysica Acta. 1488(**3**): 211–218.
13. Walter, V., Syldatk, C., Hausmann, R. (2010) Screening concepts for the isolation of biosurfactant producing microorganisms. Adv Exp Med Biol. **672:**1–13 .
14. Semprini, L. (1997) Strategies for the aerobic cometabolism of chlorinated solvents Curr. Opin. Biotechnol. **8**:296-308.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