Original Research Article

ISOLATION AND CHARACTERISATION OF BIOSURFACTANT PRODUCING BACTERIA FROM DOMESTIC WASTES

ABSTRACT

Surfactants are surface active agents that have a great role in to reducing the interfacial tension between two phases. It is a complex mixture of several phospholipids, lipoproteins and ions. Surfactants are organic compounds contain hydrophobic and hydrophilic parts. Biosurfactants are the organic compounds that are synthesized from bacteria, yeast and fungi. They are structurally diverse compounds mainly produced by hydrocarbon utilizing microorganisms. Mainly biosurfactant producing bacteria are *Bacillus subtillis*, *Escherichia coli*, *Pseudomonas aeruginosa* etc. Biosurfactants also have properties like reduce the surface tension, used in cosmetics and also for pollution control. They are really biodegradable. Biosurfactant have little side effects than chemical surfactants.

Keywords: Biosurfactant, Bisorption, *Bacillus subtilis.*

1.INTRODUCTION

Surfactants are surface active agents that have a great role in to reducing the interfacial tension between two phases.It is a complex mixture of several phospholipids,lipoproteins and ions [1]. Surfactants are organic compounds contain hydrophobic and hydrophilic parts.Biosurfactants are

the organic compounds that synthesized from bacteria,yeast and fungi.Biosurfactants also have properties like reduce the surface tension,used in cosmetics and also for pollution control. They are really biodegradable.Biosurfactant have little side effects than chemical surfactants [2].

Nowadays the biosurfactants are mainly concerned as a type of remediation technology for pollution control [3].Biosurfactants are nontoxic and they act as emulsifiers. Biosurfactants are biologically produced from living organisms and they have advantageous characters like structural diversity,non toxic,higher biodegradability etc [4]. So the Biosurfactant are mainly used in cosmetics,pharmaceticules and pollution control. Biosurfactants are of different chemical composition such as Glycolipids,lipopolysaccharides,oligosaccharides and lipopeptides etc [5] .The substrates for production of microbes are domestic wastes.Domestic wastes are wastes that generated in day-to-day life.The main house hold wastes are frying oils,vegetable wastes,waste water from drainage etc[6] .In our project we are considering mainly the area that contaminated with soap water solution near washing area.

Microbial fermentation is the suitable method for the biosurfactant productions.Major steps involed in this work are sample collection,isolation of bacteria,screening and characterisation of isolated strains etc.Isolation is carried in Mineral Salt Medium.Screening is done by oil spreading method,Emulsification test,foaming activity etc.Biosurfactants are also many other advantages like easly biodegradable,low toxicity,biocompatibility and digestibility,speficity in their action.Biosurfactants can be efficiently used in handling industrial emulsion,control of oil spills,biodegradation and lowering the toxicity of industrial discharge and in bioremediation of polluted soil etc.

2.MATERIALS AND METHOD

Biosurfactants are produced by variety of microorganisms like bacteria, fungi and yeast.Variability of biosurfactants in their nature and chemical composition are depend on the type

of microorganism.Substrate for commercial microbial production are agro-industrial wastes such as rice water and water from processing of cereals, pules and molasses etc.Frying oil is the major thing that is used both in food industry and at the domestic scale.Several vegetable oil such as sunflower and soyabean oil.

2.1.Culture and Isolation of *Bacillus subtilis*

For the culturing of the B.subtilis LB medium, a culture medium was utilized. The significant fixings in the LB medium were Tryptone - 1g, Yeast – 0.5g, NaCl - 1g, and soil tests. To culture, the Bacillus bacteria 30g of soil test were gathered from the homegrown waste regions and warmed up to 90ºc. Then poured the warmed soil into a 50ml culture broth medium and shaken well in a shaker incubator and inoculated this culture in 48 hrs (Fig.1). After the incubation time frame, the bacterial strain was separated. For the segregation nutrient agar plates were prepared. Agar plates were set up by utilizing nutrient agar that was completely blended in 100ml of refined water. Agar was boiled and autoclaved then poured into Petri dishes and permitted to solidify. After the agar sets the bacterial culture was streaked on agar plates by disinfected inoculation loop and incubated the agar plates at 30ºc for 48hrs.

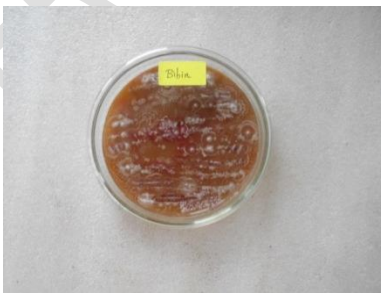

Fig. 1. Culture plate showing the colonies of Bacillus subtillis. This shows bacteria culture streaked on solidified agar. Each white spots denotes bacterial colonies.

3. IDENTIFICATION METHODS

3.1.Physical characterization

For the physical identification of the culture isolates, we used a simple staining method called Gram Staining developed by Hans Christain Gram. For that place, a slide with heat fixed smear on the staining tray. Gently flood smear with a primary stain called crystal violet then after some time it will rinse with distilled water and added Gram's iodine. After some time wash off the iodine and the smear will appear purple. Then added any decolourizing agent like 95% ethyl alcohol or acetone. Rinsed with water then added safranin to counter stain then view under the microscope. The gram-positive bacteria results in purple/blue color and the gram-negative bacteria shows pink/red color.

Gram negative-E.coli,Vibrio cholera,Salmonella.

Gram positive-Staphylococcus,Sterptococcus,Clostridium tetani,Bacillus subtillis.

3.2. Biochemical characterization

For the biochemical identification of the isolates here, two simple methods were used. The chemical methods are called Catalase Test and Indole Test.

3.2.1. Catalase Test:- It is mainly performed by adding the substrate hydrogen peroxide to an appropriately incubated culture broth. If catalase was produced by bacteria they will liberate free oxygen gas on reaction. Bubbles of oxygen represent the positive catalase test.

3.2.2. Indole Test:- This test determines the bacterial capability to break down the indole from the tryptophan molecule. For that twenty-four hour pure culture was inoculated in tryptone broth and incubated for again twenty-four hours at 30^{o}C. After twenty-four hours 2ml media was poured into a separate sterile test tube and added few drops of Kovac's reagent and agitate the tube was for a few minutes until a cherry red color will appear. This color indicates the positive indole test.

Figure 1:- Indole Test; Test tubes showing dark colour (cherry red) indicates indole positive and strain is E.coli and other tubes are negative for indole test.

Table: 1 Identification of bacterial strain

Sl.No	TEST	SAMPLES		
		S1- Nattika	**S2-Thrithaloor**	**S3- Kodungaloor**
1	Gram staining	G-ve	G+ve	G+ve
2	Catalase Test	Positive	Positive	Positive
3	Indole Test	Positive	Negative	Negative
4	Bacterial strains	*E.coli*	*B.subtillis*	*B.subtillis*

5. RESULT AND DISCUSSION

Bacterial isolates were isolated from contaminated soil samples by streak plate method.They were further identified by Gram staining and Biochemical methods.To culture the biosurfactant producing bacteria prepared required amount of LB medium [7].To inoculate the bacteria took 30g of collected soil samples from different locations.The soil samples were mainly collected from washing area.In order to inoculate the collected soil samples were warmed up to 60°C.After that the warmed soil samples were added in to prepared LB medium.Then it shaked well at 250rpm in 37°C thoroughly and incubated it in inoculation hood for 48hrs.After the 48hrs the culture was streaked on agar plates for the isolation of the bacterial strains.Agar plates were prepared by mixing bacteriological agar in a nutrient medium(LB medium).The nutrient agar medium was warmed up to when agar was boiled.After that the agar was autoclaved then poured in to clean petri dishes and allowed the petri plates to cool in order to solidify the agar in petri plates.When the agar gel gets solidify the bacterial culture was streaked.Sreaking was mainly carried out by using inoculation loop in laminar air flow [8].After the streaking the agar plates are incubate at 30°C for 48hrs. *Bacillus subtillis* and *E.coli* are the major bacteria were isolated from the collected samples.According to gram staining the *Bacillus subtillis* is Gram positive .It also show positive catalase test.Bacillus is rod shaped bacteria.These gram positive sporeforming rods produce colonies which are dry,flat and irregular,with lobate margins.*Escherichia coli* is a gram negative,facultatively anaerobic,rod shaped,coliform bacterium of genus Escherichia.The three soil samples were collected from Nattika,Engandiyoor and Kandashankadavu etc were isolated *Bacillus subtillis*.These samples were showed positive catalase test.The main aim of catalase test is to determine the ability of an organism to produce catalase.It is a common enzyme found in living organisms exposed to the oxygen.It is a protective enzyme i.e. able to the dangerous chemical hydrogen peroxide [9].A pure culture growth from the overnight was smeared on glass slide and then added 3% hydrogen peroxide and observed.The rising bubbles are observed.The soil samples that collectd from Nattika also isolates the gram negative bacteria *E.coli*.It was identified by using

Indole test.The aim of the ability of microbe to degrade the amino acid tryptophan. Development of cherry red colour at the interface of the reagent and the broth with in seconds after adding the Kovac's reagent indicates the presence of indole reagent and test is positive [10].

5.1 *Bacillus subtilis*

Bacillus subtilis is a gram positive bacteria.*Bacillus* strains also produced biosurfactant are confirmed by using oil spread assay [11]. This oil spreading method is rapid and easy to carry out. The oil spreading technique is a reliable method to detect biosurfactant production by different microorganisms[12]. Surfactin is the major biosurfactant screened from *bacillus subtillis* was a study conducted by [13]. Surfactin have the capability of oil biodegradation was also confirmed by testing the petroleum oil contaminated soil from North East India.Enhanced production of surfactin from *Bacillus subtillis* by continous product removal and metal cation addition was also studied. Surfactin is a crystalline peptide lipid surfactant produced by *Bacillus subtillis*. Lipopeptide is a another biosurfactant derived mainly from a marine *bacillus* and also described another anti fungal biosurfactant Iturin from Bacillus is also reported[14] .The culture of *Bacillus subtilis* were used for the introduction of antibiotics. Lipopeptide is used for insecticidal activity against fruit fly.*Bacillus subtilis* utilize crude oil and hydrocarbons as sole carbon sources used for oil spill cleans up[15].

5.2 *Escherichia coli*

E.coli is the gram negative bacteria.In this studies the E.coli was detected by indole test. They conducted study about the methods for E.coli identification in food and water. Rhamnolipids were the major biosurfactant isolated from *E.coli*[16].Production of rhamnolipids from *pseudomonas* and *E.coli* was also studied by [17]. Today E.coli have great role in biotechnology. Nowadays the *E.coli* were used to produce heterologus proteins,recombinant proteins etc [18]. The modified *E.coli* cells are used for the vaccine development,bioremediation and biofuels [19]. One of the major

application of biosirfactants is to control the environment pollution control and medical science. Bioremediation in general aims at providing cost effective contaminant specific treatments to reduce the concentration of individual or mixed environmental contaminants [20]

Microorganisms capable of hydrocarbon degradation have often been isolated from aquatic environments tested a biosurfactant from *Pseudomonas aeruginosa* for its ability to remove oil from contaminated Alaskan gravel samples under various conditions [21]. Biodetox (Germany) described a process to decontaminated soils,industrial sludge and waste water and investigated the effects of the effects of rhamnolipid biosurfactants on in situ biodegradation of hydrocarbon entrapped in a porous matrix and reported a mobilization of hydrocarbon entrapped with in the soil matrix at biosurfactant concentrations higher than critical micelle concentration (CMC)[22]. It is well known that microbial cells may chelate metals from solution[23]. There is also another study conducted to investigate the potential of rhamnolipid biosurfactants produced by *Pseudomonas aeruginosa* in the removal of metals from soil contaminated with cadmium reported[24].Another application of biosurfactants is oil storage tank cleaning. Surfactants have been studied for use in reducing the viscosity of heavy oils thereby facilitating recovery,transportation and pipelining [25].An area of considerable potential for biosurfactant applications is in the field of microbial enhanced oil recovery (MEOR).Biosurfactants can also aid oil emulsification and assist in the detachment of oil films rocks. Biosurfactants have some therapeutic applications.

Biosurfactants may be used for the dispersion of inorganic minerals in mining and manufacturing processes.Some other applications of biosurfactants are in the coal oil mixture coal water slurry as dispersants,emulsifiers in cosmetics,paints,additives for rolling oil.They also used as foaming agents in Toileteries,cosmatics,ore floatation,as metal sequestering agents in mining, as demulsifiers in waste treatment etc.Biosurfactants are beginning to acquire a status as potential performance effective molecules in various fields.

6. CONCLUSION

In conclusion the study represented surfactant activity of the bacterial strains isolated from industrial waste contaminated soils from the cloth washing areas.This confirms that environment has an influence on the metabolism of the tested microbes.This study suggests that,*Bacillus subtillis* and *E.coli* isolated from contaminated soil showed biosurfactant producing ability.We have also observed that the isolated bacterial strains have oil recovery property.It is mainly confirmed by oil spreading assay [26]. For this assay 10µl of crude oil is added to the surface of 40ml distilled water ina petri dish to form a thin layer of oil.If biosurfactant is present in the supernatant the oil is displaced and a clearing zone is formed.The diameter of this clearing zone on the oil surface correlates to surfactant activity,also called oil displacement activity.*E.coli* was firstly constructed to produce rhamnolipids.The novel rhamnolipids that showed the improved performances of interfacial tension and the potential different application in enhanced oil recovery were successfully produced by engineered *E.coli*.In genetic engineering the DNA sequence of prokaryotes or eucaryotes is introduced into a host organism.One of the most example of genetic engineering is cloning of rat insulin or human insulin genes in *E.coli*.As the cloned *E.coli* cells grow the insulin genes are expressed and insulin is synthesised. Biosurfactants have some therapeutic applications.Lipopeptides produced by *Bacillus subtillis* have antimicrobial activity and anti fungal activity.Surfactin and Iturin have a antifungal activity were mainly produced by *Bacillus subtillis*.The usefulness of biosurfactant in other field is especially in personal and health care and as a therapeutic agents.Concerns about pesticide pollution have promted global efforts to find alternative biological control technologies.Staghellini and Miller evaluated the biological control potential of rhamnolipid producing strain and concluded that biosurfactants have potential for the biological control of zoosporic plant pathogens. Surface active agents are needed for the hydrophilization of heavy soils to obtain good wettability and also to achieve equal distribution of fertilizers and pesticides in the soils.Biosurfactant have also been used in formulating poorly

soluble organophosphorous pesticides. The usefulness of biosurfactants in bioremediation is expected to gain more importance in coming year and it is the most versatile process chemicals for use in the near future.The usefulness of biosurfactant in other fields is emerging,especially in personal health care and as therapeutic agents.With increased efforts on developing improved application technologies strain improvement and production process biosurfactant are expected to be among the most versatile process,biosurfactants are expected to be among the most versatile process chemicals for use in the near future.

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Abbreviations:

Bacillus subtilis (B.subtilis), Luria Bertani (LB),