Production, Purification And Antimicrobial Activity Of Biosurfactants From Saccharomyces Cerevisiae And Pseudomonas Aeruginosa

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ABSTRACT
Chemical surfactants are highly toxic to the environment so the Biosurfactants have paying attention by many scientists because of their low toxicity, biodegradability and ecological acceptability. The aim of this present study is to produce the Biosurfactants from Saccharomyces cerevisiae and Pseudomonas aeruginosa and to check its antimicrobial activity against several bacterial (Such as Escherichia coli, Salmonella sp., Shigella sp., Listeria sp., Vibrio sp., and Staphylococcus aureus) and fungal (Such as, Aspergillus niger, Aspergillus flavus, Aspergillus nidulans, Aspergillus fumigates and Candida albicans) pathogens. The present study clearly demonstrated the antimicrobial effect of Biosurfactants synthesized by Saccharomyces cerevisiae and Pseudomonas aeruginosa by their inhibition zone against pathogens. The Biosurfactants synthesized by Pseudomonas aeruginosa have the highest ability of antimicrobial activity against all the tested microbial pathogens.

KEYWORDS: Biosurfactants, Production, Purification, Antimicrobial Activity

INTRODUCTION
Surface active agents like surfactants have an important role in our daily life. Surfactants are amphipathic molecules that build up at interfaces and decrease the interfacial tensions and form collective structures. The huge market demand for surfactants is currently met by many synthetic and mainly by petroleum-based, chemical surfactants. Currently, there are more than 54% of the total surfactant production is utilized in laundry detergents and remaining was intended for industrial uses (Van Hamme et al., 2006; Banat et al., 2000). Based on the anionic, non-ionic, cationic and amphoteric charges the chemical substances were classified. Among that, the cationic surfactants are the most toxic and have historically been used as antimicrobials, while anionics are less toxic and are more active against gram positive than gram negative bacteria, and non-ionics are often considered as nontoxic (Develder and Lauryssen, 2010). Almost all these surfactants were mostly produced or derived from petroleum products. These synthetic surfactants are very hard to
degrade by microbes and also it cause pollution and damage to the environment (Urum and Pekdemir, 2004). So, there is an emerging need in the industry to alternate these chemical surfactants by the use of renewable based products (Vaz et al., 2012).

Biosurfactants are a chemically diverse group of surface active molecules produced by various groups of microorganisms on fermentation process by using low cost or cheaper agro based materials and waste materials (Rodrigues et al., 2006). Due to its, low toxicity and environmental friendly nature it have wide range of industrial applications such as, bioremediation, health care, oil, food processing and pharmaceutical industries. Thus the present study has carried out to check the antimicrobial activity effectiveness of two different Biosurfactants derived from *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*.

**MATERIALS AND METHODS**

**Isolation of Bacterial Strains**
The bacterial strains such as *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa* were isolated from the petroleum contaminated soil samples and they were screened by using oil spreading method (Youssef et al., 2004).

**Production of Intracellular Biosurfactants**
For the production of biosurfactant 1000ml of Mineral Salt Medium and Yeast Extract Peptone Dextrose broth were used for *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa* respectively. The broth was prepared with optimum pH and then the substrate with optimum concentration was added into the broth and the both cultures were added in to the specific broth and it was incubated at 37°C for 48 hours. After the incubation, the broth cultures were centrifuged at 10,000 rpm for 10 minutes and the pellets were washed with PUM buffer and then used for further purification (Sarubbo, et al., 2006).

**Determination of Biosurfactants**
The biosurfactant from the both sample was estimated by using orcinol assay method. In this method, the orcinol assay was used for the direct assessment of the amount of glycolipids in the sample. The 100 μl of each sample was added with 900 μl of a solution containing 0.19% orcinol (in 53% H2SO4). After heating for 30 min at 80°C, the samples were cooled at room temperature and the OD values were measured. Control was prepared with distilled water. The rhamnolipid concentrations were calculated from a standard curve prepared with L-rhamnose and expressed as rhamnose equivalents (Saravanan and Vijayakumar, 2012).

**Purification and Characterization of Biosurfactants**
The pellets were resuspended in a 40ml of dichloromethane in a separating funnel and shaken vigorously and the allowed surfactant was recovered in the organic layer at the top. The extraction was performed twice and the organic layers were pooled and evaporated. The organic layers were collected in separate conical flasks (Abushady et al., 2005; Yalcin and Ergene, 2010). It was characterized by using thin layer chromatography (TLC).

**Antimicrobial Activity of Biosurfactants:**
Mueller Hinton agar was prepared and poured in a sterile petriplate. The plates were allowed to solidify. After solidification the pathogenic bacteria (Such as *Escherichia coli*, *Salmonella* sp., *Shigella* sp., *Listeria* sp., *Vibrio* sp., and *Staphylococcus aureus*) and fungi (Such as, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus fumigates* and *Candida albicans*) were isolated from contaminated foods using specific medium and they were maintained at specific broth. 24 hours broth culture of each pathogen was swabbed with sterile cotton swabs. Then 30 μL
of purified biosurfactants, synthesized from *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* were dropped onto 10 mm in diameter sterile discs. The plates were incubated at 37°C for 24 hours. After the incubation period the zone of inhibition was observed around the disc and it was measured (Cao et al., 2009).

**RESULTS AND DISCUSSION**

Normally, microbial produced compounds are easily degraded (Mohan et al., 2006) and mostly suited for the environmental applications compared to the synthetic compounds (Mulligan, 2005). The microbial derived Biosurfactants have some of the potential applications of in pollution and environmental control, hydrocarbon degradation heavy metal removal, hexa-chloro cyclohexane degradation and antimicrobial activity (Singh, et al., 2007). In this present study both the bacterial and yeast strains such as, *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* (Fig 1 & 2) were isolated from the petroleum contaminated soil samples. Both the strains were showed a maximum zone in oil spreading method. The Biosurfactants were partially purified from both the microbes by using separating funnel and it was purified by using dichloromethane. Then the partially purified bio surfactants were analyzed by using thin layer chromatography (Fig 3 & 4).

![Pseudomonas aeruginosa](image1)

Fig 1: *Pseudomonas aeruginosa*

![Saccharomyces cerevisiae](image2)

Fig 2: *Saccharomyces cerevisiae*

![Purification of Biosurfactants from Pseudomonas aeruginosa](image3)

Fig 3: Purification of Biosurfactants from *Pseudomonas aeruginosa* by using
Thin Layer Chromatography

Fig 4: Purification of Biosurfactants from *Saccharomyces cerevisiae* by using Thin Layer Chromatography

The antimicrobial activity of Biosurfactants was evaluated against several bacterial and fungal pathogens. The Biosurfactants synthesized by *Pseudomonas aeruginosa* have the highest ability of antimicrobial activity against all the tested microbial pathogens. The results were shown in (Table 1 & 2, Figures 5 & 6). Govindammal and Parthasarathy, 2013, also reported the bio surfactant synthesized by *Pseudomonas fluorescence* MFSO3 have showed a maximum and minimum zone of inhibition towards many pathogenic microbes, the results of our study were similar to them. Cao et al., 2009 also stated that, in their studies the lipopeptide biosurfactant from *Bacillus natto*TK-1 also exhibited both the bacterial and fungal pathogens.

<table>
<thead>
<tr>
<th>Bacterial Pathogens</th>
<th>Biosurfactants from <em>Pseudomonas aeruginosa</em></th>
<th>Biosurfactants from <em>Saccharomyces cerevisiae</em></th>
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<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>+++</td>
<td>+++</td>
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<tr>
<td><em>Salmonella sp.</em></td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td><em>Shigella sp.</em></td>
<td>+++</td>
<td>+</td>
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<tr>
<td><em>Listeria sp.</em></td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td><em>Vibrio sp.</em></td>
<td>+++</td>
<td>+</td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

− : No Inhibition Zone; +: ZOI up to 4 mm;  
− ++: : ZOI up to 8 mm;  +++: ZOI up to 12 mm
Fig 5: Zone of Inhibition (ZOI) of two different Biosurfactants against different bacterial pathogens

Table 1: Antimicrobial activity of two different Biosurfactants against different fungal pathogens

<table>
<thead>
<tr>
<th>Fungal Pathogens</th>
<th>Biosurfactants from <em>Pseudomonas aeruginosa</em></th>
<th>Biosurfactants from <em>Saccharomyces cerevisiae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus niger</em></td>
<td>+++</td>
<td>++</td>
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<tr>
<td><em>Aspergillus flavus</em></td>
<td>+++</td>
<td>+++</td>
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<tr>
<td><em>Aspergillus nidulans</em></td>
<td>+++</td>
<td>+++</td>
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<tr>
<td><em>Aspergillus fumigates</em></td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

−: No Inhibition Zone; +: ZOI up to 4 mm; ++: ZOI up to 8 mm; +++: ZOI up to 12 mm

Fig 6: Zone of Inhibition (ZOI) of two different Biosurfactants against different fungal pathogens
The maximum antimicrobial effect of biosurfactant produced by *Pseudomonas aeruginosa* may be due to the presence of Rhamnolipids (Toribio et al., 2010). Generally, the Rhamnolipids molecules of rhamnose are linked to β-hydroxy-decanoic acid and they have many industrial applications. Rahman et al., 2007 and Da rosa et al., 2010 also reported that the biosurfactant production by *P. aeruginosa* DS10-129 and *P. aeruginosa* LBM10 have rhamnolipid type biosurfactant. Abalos et al., 2001 reported that, the rhamnolipid mixture obtained from *P. aeruginosa* showed inhibitory activity against the bacteria (*Escherichia coli*, *Micrococcus luteus* and *Alcaligenes faecalis*, *Serratia arcescens*, *Mycobacterium phlei* and *Staphylococcus epidermidis*) and fungi (*Aspergillus niger*, *Chaetomium globosum*, *Enicillium crysogenum*, *Aureobasidium pullulans*, *Botrytis cinerea* and *Rhizoctonia solani*) in various concentrations. The present study also clearly demonstrated the antimicrobial effect of both the bacterial and yeast strains such as, *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* against several microbial pathogens. It is suggested to use this compounds or Biosurfactants in pharmaceutical and cosmetics for dermal and other applications like antifungal agents against plant and seed pathogenic fungi.

REFERENCES


