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# Antifungal Properties of Biosurfactant Produced by Bacterial Strains SR17 and JS29 Against Phytopathogens of Rubber

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***Abstract*—** Biosurfactants are microbial metabolites synthesized by certain microorganisms, yeast, and fungi. They possess characteristic features showing physiological properties especially antifungal and antibacterial behaviour. Among all derivatives of glycolipid particularly the species *Pseudomonas* that contains rhamnose biosurfactants proves to have the highest potential in inhibiting the flourishing range of phytopathogens. The practical examples of spraying of pesticides and fertilizers in the agricultural fields result in a hazardous impact in plant growth hence the application of biosurfactants have a promising future in different aspects of sustainable agriculture because of being biodegradable and cost effective. The paper focused on the inhibition by biosurfactants obtained from *Pseudomonas aeruginosa* SR17, *Pseudomonas aeruginosa* JS29, *Bacillus altitudinis* MS16 and V1 strain (not yet identified) against the rapid growth of phytopathogenic fungal cultures. In the initial stage, the inhibitory impact of Cell Free Supernatant (CFS) of each bacterial strains were homogenized with Potato Dextrose Agar (PDA) for analyzing upon phytopathogens. Later, the crude biosurfactant is extracted from centrifuged CFS at temperature 40°C under reduced pressure. Authors narrated that a 500mg/l concentration of crude is incorporated with PDA and analyzed for the inhibition. Paper concludes two noble strains SR17 and JS29, which demonstrate an average inhibition of 57% and 63% respectively. Biosurfactants of these two peculiar strains can be used effectively in the prevention of disease.

***Keywords***— Percentage Inhibition, Cell free supernatant, Surface tension and optical density, Phytopathogenic fungal culture.

## I. INTRODUCTION

Biosurfactants are a class of chemical compounds formed by the molecular structures which make it amphiphilic in nature, that are distributed at the interface of liquid phases with different degrees of polarity [1]. Several chemical compositions of biosurfactants are capable of showing their emphasis on physiological activities that are carried out by numerous prokaryotic and eukaryotic organisms. Based on the chemical composition and microbial origin surfactants are classified into glycolipids, lipopeptides, polysaccharide-protein complexes, protein-like substances, lipopolysaccharides, phospholipids, fatty acids, and neutral lipids [2]. Biosurfactants are effective in controlling the growth of plant pathogens and behaves as a perfect antibacterial and antifungal agent. Therefore, rhamnolipids falls under the category of best biosurfactants for commercial exploitation [3,4]. However, surfactants have the potentiality to reduce surface and interfacial tension resulting in enhancement of the capacity in detergency, emulsification, lubrication, solubilization, and phase dispersion [5,6,7]. Rhamnolipids, in addition, also have an enormous capacity to reduce the surface tension of pure water from 72mN/m to less than 30mN/m [8].

Remainder of the paper is sectioned in three sections: Section II provides a glimpse about the related work in reference to the current work. Section III provides an explanation on the preparation of nutrient medium, harvesting and extraction in production of biosurfactant. Section IV discusses the variations of surface tensions, optical densities and morphological features of phytopathogenic fungi. Section V provides the concluding remarks.

## II. RELATED WORK

In recent years, rhamnolipids produced by *Pseudomonas aeruginosa* have been convinced to be the most efficient biosurfactant for their antifungal and antibacterial activity. Biosurfactants have various implementations in the field of food production, pharmaceuticals, agriculture, and the environment. Microbial biosurfactants are more beneficial than their chemical counterparts in both their physical and chemical properties such as foaming, environmental compatibility, and higher biodegradability [9]. Chemical fertilizers and pesticides are extensively used for the high yield variety of crops resulting in harmful effects in biotic and abiotic components of the environment. These

chemotoxic materials enter the body of the living being through the food chain and cause serious health problems. The applications of biosurfactants have a promising future in several aspects of sustainable agriculture as they are biodegradable and environment-friendly.

Table 1. Optical densities (600 nm) and surface tensions of different bacterial strains.

| Bacterial Strains | Optical density | Surface tension (mN/m) |
|---|---|---|
| *Bacillus altitudinis* MS16 | 1.7150 | 34.85±0.45 |
| *Pseudomonas aeruginosa* JS29 | 2.2275 | 28.25±0.35 |
| *Pseudomonas aeruginosa* SR17 | 2.1935 | 27.25±0.15 |
| V1 strain | 2.3460 | 26.95±0.20 |

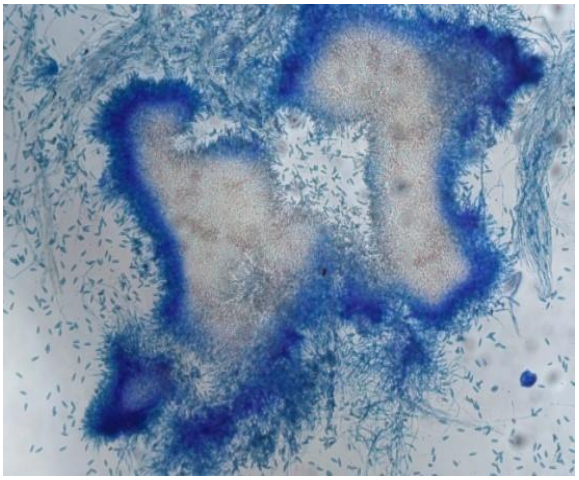


Figure 1. Spore structure of phytopathogenic fungi (culture 1) under 40X magnification.

## III. METHODOLOGY

Authors studied four bacterial strains viz., *Bacillus altitudinis* MS16, *Pseudomonas aeruginosa* JS29, *P. aeruginosa* SR17 and V1 strain that was cultured in Nutrient Broth and incubated in shaking at 200 rpm for a duration of 24 hours at a temperature of 35°C±2°C.

**Preparation of Nutrient Broth**

Procedure: 50 ml of sterilized Nutrient Broth was inoculated with loopful of *Bacillus altitudinis* MS16, *Pseudomonas aeruginosa* JS29, *P. aeruginosa* SR17 and V1 strain in four Erlenmeyers respectively. The flasks were incubated at temperature 35°C±2°C for 24 hours.

**Preparation of Mineral Salt Medium (MSM) I**

**Composition (g/L):** 4.0g of sodium nitrate, 0.1g of potassium chloride, 0.5g of potassium dihydrogen phosphate, 1g of dipotassium hydrogen phosphate, 0.01g calcium chloride, 0.1g of yeast extract, 0.5g of magnesium sulphate heptahydrate, 0.01g of ferrous sulphate heptahydrate, 2%(w/v) of D-(+)-Glucose and 10ml/L of trace element solution (TES: 0.26g of boric acid, 0.5g of copper sulphate pentahydrate, 0.5g of manganese sulphate monohydrate, 0.06g of ammonium heptamolybdate tetrahydrate and 0.7g of zinc sulphate heptahydrate).

**Procedure:** 5% (v/v) each of SR17, JS29 and V1 strains from 24 hours nutrient broth cultures was transferred to Erlenmeyers containing 200ml sterilized MSM I respectively. Two replicates for each strain was prepared and kept in shaking at 200 rpm for a duration of 24-48 hours at a temperature of 35°C±2°C.

**Preparation of Mineral Salt Medium (MSM) II**

**Composition (g/L):** 3.3g of sodium nitrate, 0.01g of sodium chloride, 2.2g of dipotassium hydrogen phosphate, 0.14g of potassium dihydrogen phosphate, 0.04g of calcium chloride dihydrate, 0.6g of magnesium sulfate heptahydrate, 0.2g of ferrous sulphate heptahydrate, 20g of D-(+)-Glucose and 500µl trace element solution (TES); 2.32g of zinc sulphate heptahydrate, 1.78g of manganese sulphate tetrahydrate, 0.56g of boric acid, 1g of copper sulfate pentahydrate, 0.39g of sodium molybdate dihydrate, 1g of disodium EDTA and 0.66g of potassium iodide.

**Procedure:** Each of the two Erlenmeyer flasks was filled with 200ml sterilized MSM II which was inoculated with 10% (v/v) MS16 strain. Later, these flasks were incubated in shaking at 200 rpm for 24- 48 hours at 35°C±2°C.

**Cell Growth Measurement**

After 24-48 hours of incubation, the absorbance (OD600) of the enumerated MSM cultures were tested on spectrophotometer (SHIMADZU UV-visible Spectrophotometer) [10].

**Estimation of Surface Tension**

After 24-48 hours in incubation, the surface tension of turbid MSM cultures were measured with the Tensiometer (Kruss).

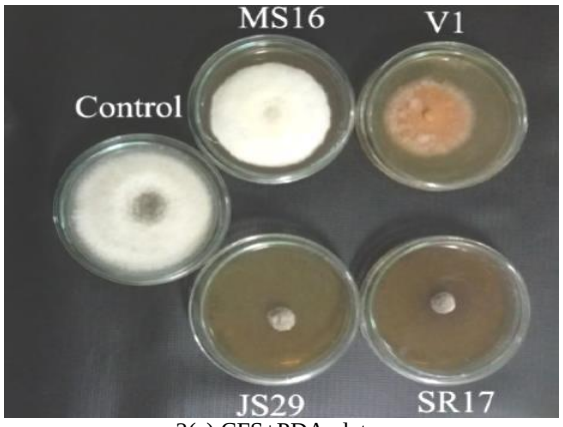
**Harvesting of Cell Free Supernatant (CFS) containing Biosurfactant**

Each MSM cultures were centrifuged separately at 10,000 rpm for 20 minutes at a temperature of 4°C. Successively, the supernatant obtained is stored in the refrigerator maintaining the same temperature.

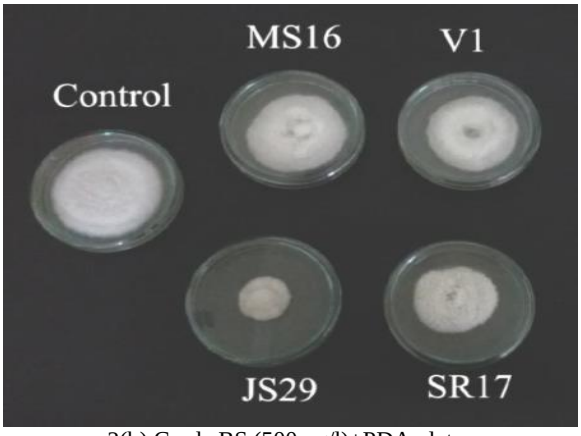
**Extraction and Yield Determination of Biosurfactant Production**

CFS obtained after centrifugation was deproteinized at a temperature of 110°C for 15 minutes and acidified to pH 2 by adding 6N HCl. The solution is allowed to precipitate overnight maintaining a temperature of 4°C. After overnight storage, in a separating funnel equal amount (1:1) of biosurfactant and ethyl acetate (as a solvent) is being added. The eluded biosurfactant was then made more concentrated by evaporating the solvent in rotary evaporator. The temperature was kept at 40°C under reduced pressure. Finally, the crude biosurfactant is quantified.




2(a) CFS+PDA plates




2(b) Crude BS (500mg/l)+PDA plates

Figure 2. Inhibition of phytopathogen of culture 1 by biosurfactant of each bacterial strains.

Table 2. Inhibition of fungal culture on CFS+PDA plates after 7 days incubation.

| Fungal cultures | PDA+CFS (SR17) | PDA+CFS (JS29) | PDA+CFS (V1) | PDA+CFS (MS16) |
|---|---|---|---|---|
| Culture 1 | 83.29% | 79.14% | 34.29% | 24.71% |
| Culture 3 | 80.75% | 85.62% | 61.25% | 35.50% |
| Culture 5 | 82.85% | 82.14% | 45% | 29.57% |
| Culture 6 | 84% | 83.75% | 55% | 13.12% |

Table 3. Inhibition of phytopathogen with 500 mg/L concentration of crude biosurfactant after 7 days incubation.

| Fungal cultures | P. aeruginosa SR17 (On PDA+ 500 mg/L Crude BS) | P. aeruginosa JS29 (On PDA+ 500 mg/L Crude BS)* | B. altitudinus MS16 (On PDA+ 500 mg/L Crude BS)* | V1 strain(On PDA+ 500 mg/L Crude BS)* |
|---|---|---|---|---|
| Culture 1 | 83.29% | 79.14% | 34.29% | 24.71% |
| Culture 3 | 80.75% | 85.62% | 61.25% | 35.50% |
| Culture 5 | 82.85% | 82.14% | 45% | 29.57% |
| Culture 6 | 84% | 83.75% | 55% | 13.12% |

**Antifungal Activity of Biosufactant (CFS) against Phytopathogenic Fungi**

Four phytopathogenic fungal cultures (obtained from germ plasm, IASST), isolated from rubber was plugged into and maintained in Potato Dextrose Agar (PDA). In a 250 ml Erlenmeyer flask, 150 ml of sterilized PDA was poured into 12 plates in order to make three replicates for each fungal strain. The fungal culture was plugged with a cork borer (6mm in diameter) and was inoculated into the PDA plates. Plates were then incubated at 25°C±1°C for 5-7 days.

Antifungal activity of the produced biosurfactant was studied against four different phytopathogenic fungal cultures. 200 ml CFS obtained from each bacterial strain after centrifugation is homogenized with 7.8g PDA and sterilized at 121°C for 15mins. Three replicates were made for each bacterial CFS incorporated with PDA. All the four fungal strains were inoculated in each CFS+PDA medium. Alongside, two positive control plates for each fungal strain were prepared in PDA. All the plates were incubated at 25°C±1°C for 5-7 days.

**Concentration based analysis of Crude Biosurfactant against Phytopathogens**

The antifungal activity of the fungal cultures by the extracted crude biosurfactant was determined based on a particular concentration. Four 250 ml Erlenmeyer flasks were added with each containing 200 ml PDA which was homogenized with 500 mg/l crude biosurfactant. This medium was autoclaved at 121°C for 15mins. The medium is poured into plates and the fungal plugs were inoculated. Plates were incubated for 5-7 days at 25 ±1°C. Three replicates were prepared for each bacterial strain. Two control plates for each fungal culture were also incubated along with the treated plates.

**Morphological Study of Phytopathogenic Fungi**

The fungal cultures were stained with Lactophenol Cotton Blue (LPCB) in glass slides and observed under microscope at 40X magnification. The structure of mycelium, spores and other features were specifically studied.

## IV. RESULTS AND DISCUSSION

**Surface Tension and Optical Density**

After 24 hours of incubation, nutrient broth inoculated with strains JS29 and SR17 appeared light green in colour due to the production of a toxin, pyocyanin ($PCN^-$) and Erlenmeyer flasks inoculated with strains MS16 and V1 appeared pale yellow in colour. MSM containing bacterial strains SR17, JS29, V1 and MS16 after 24-48 hours of incubation, formation of froth was observed which indicates the production of biosurfactant by the respective strains. The specimens were observed for their optical density after duration 24-48 hours of incubation (Table 1). Moreover, surfactant has the capability to lower surface tension of water from 75 to 35mN/m and the interfacial tension of water/hexadecane from 40 to 1mN/m [11]. Surfactin possess the ability to reduce the surface tension of water to 25 mN/M and the interfacial tension of water/hexadecane to < 1mN/M [1]. The media enumerated with bacterial strains showed a rapid decrease in their

surface tension from 74 N/m to 27 N/m (approximately) V1 strain showed the highest reduction in its surface tension (Table 1).

**Percentage of Inhibition of Phytopathogens by CFS**

Certain biosurfactants produced by bacterial strains are well known for predominantly showing antifungal activity. They sustain the capacity to inhibit the normal growth process of a fungus. Among all the CFS of each bacterial strain treated for antifungal activity, strains of *P. aeruginosa* SR17 and JS29 showed the highest percentage of inhibition. Species of *Pseudomonas* are generally known to produce rhamnolipid, which are effective against phytopathogens. Data for the percentage of inhibition by each of the bacterial strains' CFS has been showed in Table 2.

**Percentage Inhibition of Phytopathogen at 500 mg/L Concentration of Crude BS**

The inhibition of phytopathogens as a result of CFS was not based on a certain concentration. It was not so accurate to determine the inhibitory activity at varied unknown concentrations, so at a known concentration it became easier to evaluate the inhibitory activity. Henceforth, the antagonistic effect of crude biosurfactant at concentration 500 mg/L on phytopathogenic fungi was analysed. On this basis of inhibition at concentration 500 mg/L, strains of *P. aeruginosa* SR17 and *P. aeruginosa* JS29 showed the highest degree of inhibitory activity in comparison to the strains of *Bacillus altitudinus* MS16 and V1. The percentage inhibition has been elaborately depicted in Table 3.

**Morphological Characteristic of Phytopathogenic Fungi**

All the fungal cultures observed under microscope had aseptate and coenocytic hyphae. Spore formation was seen in culture 1 and culture 3. Morphology of the spores of culture 1 to some extent resembled genus *Colletotrichum*. Spores of culture 3 resembled exospores which were observed motile in nature. While in culture 5 and 6 spore formation was not observed.

## V. CONCLUSION

Biosurfactants has enormous capacity in inhibiting the multiplication of fungal plant pathogens. They on their synthesis by the bacterial strains can reduce the surface tension from 74mN/m to 25mN/m. Rhamnolipids are efficiently capable of showing antifungal property. In this paper, after investigating on the inhibition of the phytopathogenic fungal culture by the CFS of the bacterial strains, we can conclude that *Pseudomonas aeruginosa* SR17 and *P. aeruginosa* JS29 strains are the most efficient and effective against all the four phytopathogenic fungal cultures. While in contrast to these experimental analysis, on experimentation with a concentration of 500mg/L crude biosurfactant from each bacterial strains, we can conclude that *P.aeruginosa* JS29 was observed to be the best biosurfactant for inhibiting the growth of phytopathogens. Hence strains SR17 and JS29 are the best among all four bacterial strains for inhibiting the growth of phytopathogenic fungi. These biosurfactants can be used as a biocidal agent for treating against several plant diseases.

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