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Optimization of rhamnolipid biosurfactant production from *Serratia rubidaea* SNAU02 under solid-state fermentation and its biocontrol efficacy against Fusarium wilt of eggplant

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ABSTRACT

This work was aimed to explore rhamnolipid production under solid state fermentation using a potential substrate mahua oil cake and to evaluate the biocontrol efficacy of rhamnolipid against Fusarium wilt of eggplant. The combination of Response Surface Methodology and Central Composite Design was employed to optimize higher biosurfactant production. Therefore, four factors *viz.,* substrate concentration, inoculum size, pH and temperature were selected for optimization of rhamnolipid production. The results revealed that the optimum conditions for reduction of surface tension were mahua oil cake 7.78 g, 2.4 ml inoculum size (1×10^8 cells/ml), pH 7 and 30° C temperature. To evaluate the biocontrol efficacy the application of rhamnolipid at various concentrations (0, 100, 250 and 500 μg/ml) by soil and foliar application were employed in the pot culture assay. *In vitro* study indicated that rhamnolipid producing strain SNAU02 was the most effective antagonist against *Fusarium oxysporum* f. sp. *melongenae* and used for pot culture study. On the basis of economic analysis, treatment T_9 (*Fusarium oxysporum* f. sp. *Melongenae* ($\times 10^6$ spores/ml) + 50 ml of 250 μg biosurfactant/ml to soil + foliar spraying of biosurfactant (250 μg/ml) ranked among the efficacious treatments and was just as effective as a synthetic fungicide. In control treatment, occurrence of disease severity and disease incidence was observed from early stage of crop growth until harvest stage. The pot experiment results indicated that SNAU02 rhamnolipid could be a promising agent in the biocontrol of Fusarium wilt of eggplant, which might help to minimize the yield loss of eggplant.

Introduction

Biosurfactants are amphiphilic molecules, capable of reducing surface and interfacial tension between immiscible fluids [1]. Biosurfactants are generally categorized based on their chemical composition and microbial origin. They include lipopeptides, glycolipids, polysaccharide–protein complexes, lipopolysaccharides, protein-like substances, neutral lipids and phospholipids, fatty acids [2]. In recent years, chemical surfactants are extensively used in food, agriculture, cosmetics, industrial and pharmaceutical applications. The surfactants are mostly chemically synthesized which cause environmental hazards. The increasing environmental awareness has led to the isolation of biosurfactant producers due to their unique properties including lower toxicity, lower critical micelle concentration, higher biodegradability and ecofriendly nature.

Biosurfactant can be economically produced by various agro-industrial substrates. However, one of the major challenges could be the cost effective production of biosurfactant and the optimization of culture condition to achieve higher yield. So far, most of the biosurfactant production has been performed in submerged fermentation. However, solid state fermentation has several advantages over submerged fermentation as it requires less energy for cultivation, agitation unit is not required and utilization of very less solvent for extraction of product. Hence, SSF could be an alternate method for production of biosurfactant. In solid state fermentation, solid substrates supply all the essential nutrients to the microorganisms for their optimum function. Therefore, selection of the appropriate solid substrate plays a very important role

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for the development of efficient SSF process [3–5].

Biosurfactant producers can only be effective when they are maintained at their optimal ambient conditions, required for growth and its activity. One of the best methodologies for optimization experiment is Response Surface Methodology. RSM is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching for the optimum conditions [6].

The potential of biosurfactants for biological control was pioneered by Stanghellini and Miller (1997), who demonstrated that rhamnolipids can disrupt zoospore membranes and cause lysis of zoospores of many oomycete plant pathogens. The glycolipid type of biosurfactant is the most commonly used in the biological control experiments. Many literature have documented that biosurfactants produced by microorganisms have antifungal activity against the plant pathogens and they can also be seen as a promising biocontrol agent [7–10].

The Brinjal or eggplant (*Solanum melongena* L.) is one of the widely grown vegetables in India. This crop happens to be highly susceptible to Fusarium wilt disease. *Fusarium oxysporum* f. sp. *Melongenae* (Fomg) is the most destructive pathogen of Fusarium wilt of eggplant. The soil-borne fungus invades the vascular bundles, causes severe wilting and death of the above ground parts of plants by blocking the xylem transport system [11]. Several biosurfactants from microbes have antimicrobial activity against plant pathogens and therefore they are considered to be a promising biocontrol molecule in sustainable agriculture [12,13]. There are hardly reports demonstrating the biocontrol efficacy of rhamnolipid biosurfactant on growth parameters and yield attributes of the eggplant under pot culture study.

Presently, the efficient disease management option to control Fusarium wilt relies on use of chemical fungicides. However, the regular usage of chemical fungicides is hazardous to the environment and human health. Henceforth, it is very essential to determine new ecofriendly methods which will aid in reducing the use of chemical fungicides. Using Mahua oil cake, in the present study, we adopted RSM to optimize the biosurfactant production by *Serratia rubidaea* SNAU02 employing solid state fermentation. Furthermore, to evaluate the rhamnolipid biosurfactant efficacy to control Fusarium wilt disease on eggplant. In addition, the growth and yield parameters were assessed under pot culture condition.

Materials and method

Microorganism

The strain *S. rubidaea* SNAU02 (Accession number KC560769) was used in the present study [14]. The strain was grown on nutrient agar (NA), sub-cultured each month and stored at 4 °C. The fungal strain *Fusarium oxysporum* f. sp. *melongenae* was obtained from Department of Plant Pathology, Faculty of Agriculture, Annamalai University, India. The fungal culture was maintained on Potato Dextrose Agar (PDA) slants and Potato Dextrose Broth (PDB). The biocontrol strain *Pseudomonas aeruginosa* MTCC 2581 obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India was used as a reference strain.

Solid state fermentation

For the development of solid state fermentation (SSF), seven agro-industrial by-products were obtained from local market in Chidambaram *viz.* coconutoil cake, groundnut oil cake, castor oil cake, gingelly oil cake, sunflower oil cake, palm oil cake and mahua oil cake. All the aforementioned substrates were dried at 60 °C for 48 h, blended to fine powder and stored in air-tight polythene bags till use. All the solid substrates were directly used in the fermentation media without any pretreatment. To develop the solid state fermentation, 5.0 g substrate was transferred to 250 ml Erlenmeyer flask and to this a salt solution was added to maintain the final moisture level at 65%. The composition of the salt solution was as follows (%): NH_4NO_3 - 0.5, NaCl –0.9, $MgSO_4 \cdot 7H_2O$ - 0.1, pH-7.0. The fermentation media was sterilized at 121 °C for 15 min, cooled and inoculated with 2.0 ml inoculum of *S. rubidaea* SNAU02 and incubated at 30 °C for seven days. All the experiments were performed in triplicates.

Response surface methodology (RSM) for the optimization of biosurfactant production under SSF

To maximize the production of biosurfactant production, it is essential to standardize the fermentation media composition and growth conditions. One of the traditional method for media optimization is by changing level of one variable at a time holding the test variable constant but this method is laborious, time consuming method and high chances of error as it manually optimized. Therefore, there is a necessity to adapt response surface methodology for media optimization and biosurfactant production. Response Surface Methodology (RSM) is the most relevant multifactorial techniques used in analytical optimization; it has been used widely in optimization due to the decreases in the number of experiments and the cost of production [15].

In this regard, to enhance the biosurfactant production four factors *viz*., substrate concentration, pH, temperature and inoculum size were optimized by Response Surface Methodology under SSF. The experiment was performed using Central Composite Design (CCD) for which a total of 30 treatment combinations were generated using designer expert 7.0 software (Stat-Ease Inc. Minneapolis, USA). From the experimental data according to this design, a second-order polynomial regression model equation was derived as below:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 D + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{44} D^2 + \beta_{12} AB + \beta_{13} AC + \beta_{14} AD + \beta_{23} BC + \beta_{24} BD + \beta_{34} CD.$$

where Y: predicted response (Surface tension (mN/m), ST), β_0: intercept, A: Substrate concentration, B: Inoculum size, C: pH, D: temperature, β_1, β_2, β_3 and β_4 are the linear coefficients; β_{11}, β_{22}, β_{33}, and β_{44} are the squared coefficients; β_{12}, β_{13},β_{14}, β_{23}, β_{24}, β_{34} are the interaction coefficients; A^2, B^2, C^2, D^2, AB, AC, AD, BC, BD, CD are the interaction between the variables as significant terms.

Every level was included in the run matrix for the study on the effect of various independent variables in the production of biosurfactant by *S. rubidaea* SNAU02. Each experiment was done in three sets.

Determination of the biosurfactant production

For extraction of biosurfactant, 100 ml of distilled water was added to each SSF flask and contents were agitated at 200 rpm at 30 °C for 1 h on an orbital shaker. Then the contents were filtered using a cheese cloth, the filtrate was pooled and then centrifuged at 2822 G-force for 10 min. Surface tension was measured according to Velioglu and Urek [16]. The surface tension of extracted supernatant obtained from SSF process was measured by Du Nuoy ring method using Krüss-K6 tensiometer.

Characterization of rhamnolipid biosurfactant

To characterize the biosurfactant under solid state fermentation, the extract collected was acidified using conc.HCl to pH 2.0, and

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