Biosurfactant Production with Various Optimization Parameters

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ABSTRACT: Biosurfactants are amphiphilic compounds produced on living surfaces, mostly on microbial cell surfaces, or sometimes excreted extracellularly. They contain hydrophobic and hydrophilic moieties that reduce surface tension, and interfacial tensions between individual molecules at the surface and the interface respectively. Pseudomonas aeruginosa was grown on the basal medium and inspected for rhamnolipid production. Different carbon sources and various conditions of pH, temperature, agitation conditions and time of incubation were varied to find out optimum growing conditions. The optimum production of 131.2gm/350ml was found on the 12th day from the carbon source glucose at pH 7, temperature 370C, and 150 rpm.

Key Words: Pseudomonas aeruginosa, rhamnolipid, biosurfactant, glucose, optimization.

1 INTRODUCTION

Biosurfactants are important surface active substances that are produced by microorganisms. These compounds are capable of reducing surface and interfacial tension at the interfaces between liquids, solids and gases. They are of great interest because of their indispensable properties and are widely used in many industries [1]. Biosurfactants have many advantages such as biological availability, ecological acceptability, variability, low toxicity, biodegradability, ability to be produced from renewable and cheaper substances. In addition, biosurfactants are ecologically safe and used for bioremediation, decontamination of oil contaminated areas, tank cleaning and microbial enhanced oil recovery [2]. Some other different industrial applications are in personal care, mining, paper industry, agricultural applications, textiles, food industry and therapeutic applications [2,3,4]. These compounds are receiving considerable attention because they are more effective than chemical surfactants in increasing the bioavailability of hydrophobic compounds [5]. In spite of these advantages, biosurfactants have to compete with chemical surfactants for their high production cost. Some bacteria, yeasts and fungi are able to produce biosurfactants. Although most of them are bacteria [6-8] and yeasts [9-11] there are some strains of fungi [12] known to synthesize biosurfactants. There are five major classes of biosurfactants, i) glycolipids, ii) phospholipids and fatty acids, iii) lipopeptides lipoproteins, iv) polymeric surfactants, v) particulate surfactants[13]. Rhamnolipids are glycolipids produced by Pseudomonas aeruginosa, which are among the most effective surfactants known today [4]. Rhamnolipids are also used for the source of rhamnose [14]. The sugar rhamnose is a potential material as a fine chemical in scientific and industrial studies, as a component in chemical reactions and as a starting material in the synthesis of organic compounds. In this paper, we determined the conditions of rhamnolipid synthesis by isolated P. aeruginosa in detail.

2 MATERIALS AND METHODS

2.1 Microorganisms

The microorganism used in this study was Pseudomonas aeruginosa which was readily available in the Biotechnology department's culture collection, SIES GST, Nerul, Mumbai, India. They were maintained on nutrient agar slants and were kept at 4^{o}C. Transfers were made at 1 month intervals. Inocula were prepared by growing cells at 30^{o}C in nutrient broth in an incubator at 150 revmin^{-1} for 24 hours.

2.2 Media

The basal medium consisted of carbon source 7g/350ml, KH2PO4 0.244g/350 ml, Na2HPO4 0.7g/350ml, MgSO4.7H2O 0.139g/350ml, CaCl2.2H2O 3.49 x 10^{-3} g/350ml, FeSO4.7H20 3.49 x 10^{-4} g/350ml. The media was supplemented with Potassium nitrate as the sole nitrogen source.

The optimization of parameters like carbon source, pH, time of incubation and temperature were done and the rhamnolipid yield was compared.

The basal medium was inoculated with 2.0 ml of seed culture of P. aeruginosa [16] and was incubated in a rotary shaker.

3 ANALYTICAL METHODS

3.1 Estimation of Rhamnolipid

Blue agar plate method

The mineral salt medium composition per 100ml was NaNO3 1.5g, KCl 0.11g, NaCl 0.11g, FeSO4.7H2O 0.000028g, KH2PO4 0.34g, K2HPO4 0.44g, MgSO4.7H2O 0.05g, yeast extract 0.05g, glucose 2g, cetyl trimethyl ammonium bromide 0.05g, methylene blue 0.02g and agar.

CTAB plates were prepared with wells for injection of the samples. 30 microlitres of the sample was injected into each well and kept for incubation at 37^0 C until dark blue halos were found around the wells.

Thin Layer Chromatography

Normal phase silica gel coated glass sheets were used as the stationary phase in Thin Layer Chromatography. The samples were loaded on the sheets and eluted with Chloroform Methanol and Water mixture. Acetic acid and Sulphuric acid mixture was used as spraying agent to observe mono and di rhamnolipid.

3.2 Extraction of rhamnolipid

Solvent Extraction

The cells from the culture were removed by centrifugation at 1000xg at 4^0C for 10 minutes. The supernatant was then mixed with Methanol:Chloroform:1-Butanol mixture in the ration 1:1:1 and the flask was kept at 200rpm at 30^0C for 5 hours. Two layers were formed out of which the upper layer was discarded and the lower layer was kept for evaporation until brown coloured residue was obtained.

Ammonium Sulphate Precipitation

The biomass from the culture media were removed by centrifugation at 1000xg at 4^0C for 10 minutes. The supernatant was mixed with 40% (w/v) Ammonium Sulphate and kept for overnight incubation at 4^0C.

3.3 Quantification of rhamnolipid

To determine the rhamnolipid concentration, cultures were centrifuged at 6000 x g for 10 minutes. The cell pellet was washed with distilled water and centrifuged again. Rhamnolipid concentration in terms of rhamnose sugar was estimated by orcinol assay[6]. Rhamnolipid concentrations were calculated from standard curves prepared with 1 mg rhamnose corresponding approximately to 2.5 mg of rhamnolipids [6]

4 CHARACTERIZATION OF RHAMNOLIPID

FTIR analysis

The FTIR analysis of the brown residue cultivated on different carbon sources, were recorded on an FT-IR spectrometer in the spectral region 4000–450 cm^{-1} and compared with the FTIR of rhamnolipid from the library.

5 RESULTS AND DISCUSSIONS

Rhamnolipid production was examined at different parameters like carbon source, pH, temperature, agitation conditions and incubation time to determine the optimum conditions.

Rhamnolipid production was observed when the strain was grown in basal medium each containing a different carbon source. P. aeruginosa is well known for its production of rhamnolipid. In this study, the rhamnolipid production was studied using 8 different carbon sources including glucose, mannitol, sugarcane bagasse, orange peel, waste frying oil, olive oil, groundnut oil and soybean oil. The production was relatively high when glucose was used as the carbon source. A considerable amount of production was also observed in media substituted with soybean oil. The amount of rhamnolipid produced by other carbon sources was below detectable level. A better yield of production was noted when the media was supplemented with Potassium nitrate as the nitrogen source.

Production of rhamnolipid was carried out at varied temperatures viz; 30, 35, 37, 40°C. The maximum yield of rhamnolipid was obtained at 37° C. This was followed by optimization of pH. The pH was varied as 6.7, 7, 7.5 and 8 in which maximum rhamnolipid production was observed at pH 7. This was in turn followed by optimization of agitation condition at 130, 150, 170. Of these maximum yield of rhamnolipid was found at 150 rpm. The production of rhamnolipid carried out using glucose as carbon source, at 37° C, pH 7 and 150 rpm was sampled at an interval of 4 days out of which the maximum production of 131.2gm/350 ml was observed on the 12th day after which there was no increase in production. The statistical results indicated that the rhamnolipid production increased with increasing number of days with no significant increase in the amount of production after 12 days.

The characteristic functional groups were identified by the FT-IR spectra of rhamnolipid , in the spectral region of 4000–450cm^{-1} and compared with the peaks of standard FTIR of rhamnolipid obtained from FTIR library. Several C-H stretching bands of -CH2- and -CH3 groups were observed in the region 3500–3000 cm^{-1}. The carbonyl stretching peak was observed at 1635 cm^{-1}, which is characteristic of ester compounds. The ester carbonyl group was also confirmed from the peak at 1029 cm^{-1}, which corresponds to C-O stretching vibration.

6 CONCLUSIONS

Based on the experimental results it can be concluded that Rhamnolipid was successfully produced using *Psuedomonas aeruginosa* when glucose, soybean and olive oil were used as carbon sources, highest production being obtained from glucose. pH 7, Temperature of 37^0C and agitation at 150

rpm were found to be optimum for rhamnolipid production. The maximum quantity of rhamnolipid produced at optimized parameters was 131.2 mg/350ml. Production of rhamnolipid increases with increasing number of days.

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