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Optimization of biosurfactant production from Yarrowia lipolytica

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Abstract

A *Yarrowia lipolytica* strain identified on the basis of 18S rDNA analysis was isolated from a crude oil contaminated site and screened for biosurfactant production using Palm Oil Mill Effluent (POME) as substrate. The yeast was screened for biosurfactant-producing ability using such methods as drop collapse assay and Oil Displacement (O.D) test. The crude extracts were tested for ability to emulsify 20 different hydrocarbons. In order to improve biosurfactant production by the isolate, an optimization study was carried out using various Carbon (Glucose, Sucrose, Lactose, Maltose and POME), Nitrogen (Ammonium nitrate, Sodium nitrate, POME, Yeast Extract and Peptone), pH and Salinity. Production of biosurfactant was estimated in terms of emulsification) index (% $E.I_{24}$), biomass and Oil Displacement (O.D) test. Glucose and yeast extract were the best carbon and nitrogen studied with the isolate. Environmental factors such as pH 8 and salinity 7.5g/l were found to be optimum for the biosurfactant production. Among the hydrocarbons tested, crude oil gave the highest emulsification index of 62%.

Introduction

Biosurfactants are amphiphilic molecules mainly produced by microorganisms as a secondary metabolite [1]. They possess both hydrophilic and hydrophobic moieties and are able to display a variety of surface activities that helps to solubilize hydrophobic substrates [2]. The enormous market demand for surfactants is currently met by numerous synthetic, mainly petroleum based, chemical surfactants. These compounds are usually toxic to the environment and non-biodegradable. Strict environmental regulations and increasing awareness for the need to protect the ecosystem have efficiently resulted in an increasing interest in biosurfactants as possible alternatives to chemical biosurfactants [3]. Although biosurfactants exhibit such important advantages, they have not yet been employed extensively in industry because of relatively high production costs. One possible strategy for reducing costs is the utilization of alternative substrates such as agroindustrial wastes [4]. Biosurfactant productions are strongly influenced by medium compositions such as carbon sources, nitrogen sources, salinity, growth factors. Environmental factors and growth conditions such as pH, temperature, agitation and oxygen availability also affect biosurfactant production through their effects on cellular growth or activity. The classical method of medium optimization involves changing one variable at a time while keeping the others at fixed levels [5]. This study involves the optimization of various parameters and its effect on biosurfactant yield.

Materials and methods

Sample collection

The POME samples were aseptically collected in sterile containers from the largest palm oil processing mill in Aluu community (Umu-oda), Rivers State, Nigeria, while the soil samples were collected from Bodo, Ogoni land, Rivers state, Nigeria. The samples were transported to the laboratory in an ice jackets and analyzed for microbiological and physicochemical properties within 4h of collection [6].

Isolation of *Y. lipolytica*

The soil samples were processed by first carrying out 1:10 serial dilution using sterile Ringer's solution followed by the spread plate method on Yeast Peptone Dextrose Agar (YPDA). The media were prepared aseptically following manufacturer's instructions. The YPDA plates were supplemented with Chloramphenicol (250mg/250ml) to prevent the growth of bacteria and incubated at 28^0C for 3-5days. Pure colonies of the isolates were stored on slants at 4^0C until needed [6].

Identification

Preliminary identification of *Y. lipolytica* was done according to the procedures of Kurtzman [7] followed by Molecular biological method identification using 18S rDNA gene sequencing and BLAST analysis, http://www.ncbi.nlm.nih.govt/BLAST

Screening of biosurfactant activity

A loopful of the isolate was transferred to test tubes containing 10ml of POME. The cultures were grown on a rotary incubator shaker for 5days, 180rpm at 28^0C. The cultures

were centrifuged at 4000rpm for 30min and the supernatant were used for screening biosurfactant activity by the following methods; Drop Collapse assay, Oil spreading test and Emulsification assay.

Drop Collapse Assay

About 10 µl of the culture supernatant was added to the surface of the oil. After 1min, the shape of the drop on the surface of the oil was observed with a magnifying glass [8]. The results were interpreted as (+++) to (++) corresponding to highly positive to moderate positive collapse on the oil surface. Those cultures that gave rounded drops were scored negative (-ve) indicating the lack of biosurfactant production.

Oil spreading test

10 µl of crude oil was added to the surface of 40 ml of distilled water in a Petri dish to form a thin oil layer. Then, 10 µl of culture supernatant was gently placed on the centre of the oil layer. If biosurfactant is present in the supernatant, the oil is displaced and a clear zone is formed [9]. The diameter of this clear zone on the oil surface was measured with a vernier caliper.

Emulsification assay

The emulsifying capacity was evaluated by an emulsification index (E_{24}). The E_{24} of culture samples was determined by adding 2ml of the carbon sources to 2ml of the culture supernatant in a test tube, vortexed at high speed for 2min and allowed to stand for 24h. The E_{24} index is given as percentage of the height of the emulsified layer (cm) divided by the total height of the liquid column (cm) [10]. The results were compared with Tween 80 as positive control.

$$E_{24} = \frac{\text{Height of emulsion formed}}{\text{Total height of solution}} \times 100$$

Optimization Process

Optimization of biosurfactant production involved changing the following parameters like pH, Salinity, Carbon and Nitrogen based on the one-factor at a time approach.

Optimization of Carbon source

The effect of carbon source was studied with different carbon sources like Glucose, Sucrose, Lactose, Maltose and POME at different concentrations of 2.5g/l, 5g/l, 7.5g/l, and 10g/l and 12.5g/l. All other parameters were kept unaltered.

Optimization of Nitrogen source

The effect of Nitrogen source was studied with different nitrogen sources like Ammonium nitrate, Sodium nitrate, POME, Yeast Extract, Peptone, at different concentrations of 0.625g/l, 1.25g/l, 2.5g/l and 5.0g/l and 7.5g/l. The optimum nitrogen for maximum activity was selected by varying the nitrogen in the medium using glucose as carbon source. All other parameters were kept unaltered.

Optimization of pH

The important characteristics of most organisms are their strong dependence on pH for cell growth and production of metabolites [11]. The optimum pH for maximum activity was selected by varying the pH of the medium at (2 -12) with 1N HCL or 1N NaOH using glucose as carbon and yeast extract as nitrogen source. All other parameters were kept unaltered.

Optimization of Salinity

The effect of salinity was studied with various concentrations; 2.5g/l, 5.0g/l, 7.5g/l, 10g/l, 12.5g/l and 15g/l. The optimum salinity for maximum activity was selected by varying the salt concentration of the medium using glucose, yeast extract and pH (8).

Result and discussion

The physicochemical parameters of the POME sample showed that it contained essential minerals and metals required for the growth of the yeasts such as nitrate, phosphate, calcium, magnesium and sodium as shown in Table 1;

Table 1: Physicochemical parameters of POME

Parameter	Concentration
pH	4.62
Temperature (^{0}C)	26.2
Conductivity(µS/cm)	8740
TOC (mg/l)	67,120
Nitrate(mg/l)	4.64
Phosphate(mg/l)	68
Calcium(mg/l)	208
Magnesium(mg/l)	703
Potassium(mg/l)	1589
Sodium(mg/l)	1.499
COD(mg/l)	96,500
BOD(mg/l)	29,520

In oil displacement test the supernatant from the isolate produced clear zone of 5.0 cm while Tween 80 a synthetic emulsifier produced 3.8cm and in the drop collapse test both supernatant and tween 80 were assigned +++ indicating highly positive collapse. This clearly indicated that the organisms produced biosurfactant.

Reports by [12] has illustrated that the type and concentration of biosurfactant depends especially on the composition of important nutrients and growth condition. In this study, optimization condition of the biosurfactant production by *Y. lipolytica* was evaluated. Out of all the carbon sources used, glucose (7.5g/l) exhibited maximum biosurfactant production measured by the Oil displacement (1.9cm), biomass

(2.31g) and E.I (65%); table 1. [13] in a similar work produced a bioemulsifier from *Y. lipolytica* in Yeast Peptone Dextrose Agar medium containing glucose as carbon source. Glucose (4%) was also reported by [14] to be the best medium for biosurfactant production by *Y. lipolytica*. Different nitrogen sources were evaluated on biosurfactant production by *Y. lipolytica*. In the production medium, a nitrogen source is needed for cell growth, with great importance for proteins and enzymes synthesis [14]. In the present study both organic and inorganic Nitrogen sources were also tested. Optimum growth was observed with Yeast extract (0.625g/l) as nitrogen source with O.D (3.80cm), biomass (2.085g) and E.I (62%). [15] produced biosurfactant from *Y.lipolytica* using yeast extract (0.5g/l) as nitrogen source. [14] pointed out that when nitrogen source is in excess biosurfactant production decreases because carbon source is used for yeast growth. [16] also stated that organic nitrogen was preferentially utilized for cell growth rather instead of inorganic nitrogen for biosurfactant production.

Table 1: Optimization of carbon source

Carbon (7.5g/l)	O.D (cm)	Biomass (g)	E.I (%)
Glucose	1.9	2.310	65
Sucrose	1.2	1.190	54
Lactose	0.6	0.665	48
Maltose	1.1	0.932	51
POME	1.1	0.985	52

Table 2: Optimization of Nitrogen source

Nitrogen (1.25g/l)	O.D (cm)	Biomass (g)	E.I (%)
Yeast extract	3.80	2.085	62
Peptone	1.90	1.987	58
NH_4NO_3	0.80	0.901	44
$NaNO_3$	0.50	0.754	40
POME	1.20	1.886	50

pH 8 was also observed to enhance biosurfactant production with O.D (3.5cm), biomass (1.520g) and E.I (65%). [14] reported biosurfactant production from *Y. lipolytica* using a combination of canola oil and glucose and reported stability in the pH range of 3- 9; table 3. Salinity of 7.5g/l was observed to give the best yield for biosurfactant production with O.D (5.01cm), biomass (2.30g) and E.I of 70%, table 4. [16] also reported isolation of *Y. lipolytica* from marine environments, implying that this yeast may play an important role in such environments.

Table 3: Optimization of pH

pH	O.D (cm)	Biomass (g)	E.I (%)
2	1.40	0.300	40
4	1.90	0.612	50
6	2.850	1.215	62
8	3.50	1.520	65
10	2.40	0.898	54
12	1.60	0.454	45

Table 4: Optimization of salinity.

Salinity (g/l)	O.D(cm)	Biomass (g)	E.I (%)
2.50	1.80	0.237	45
5.00	2.90	0.908	62
7.50	5.010	2.300	70
10.00	4.10	1.265	65
12.50	1.70	0.876	62
15.00	1.50	0.498	58

Similarly the organism was able to form stable emulsions for 24 h. 90% of all the carbon sources achieved considerable emulsification activity with the supernatants, with the highest emulsification index of 62% for Crude oil, while BTEXs (Benzene, Toluene, Ethylene, Xylene), Naphthalene, Hexane, Hexadecane, Diesel, Engine oil, Gasoline, Kerosine, Diethylether and Petroleum Ether had emulsification indices above 50%; fig.1. Of the emulsions formed 72% were thermally stable at 40^{o}C while 85% were stable at 37^{o}C and 64% were stable at 70^{o}C after 30 days. From previous data this is the first report on a putative biosurfactant from *Y. lipolytica* been tested on as many as twenty carbon sources. The emulsification index of the test biosurfactant, when compared with that of synthetic surfactant (Tween 80) was found to be 65% higher (p=0.05). These emulsification results showed that biosurfactant produced from a substrate can emulsify different hydrocarbons to varying degrees which confirmed its applicability against different hydrocarbon pollution [17]. The notable thermal stability demonstrated by the crude biosurfactants produced by the *Y. lipolytica* indicates their potential applications in enhancing bioavailability and bioremediation of recalcitrant and hydrophobic environmental contaminants.

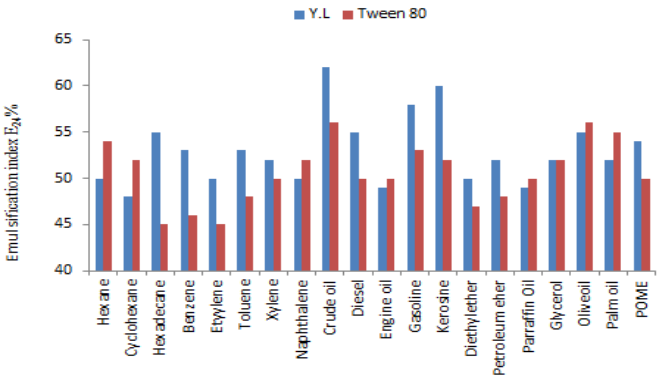

Fig. 1: Emulsification Profile of *Yarrowia lipolytica* on different carbon sources

Conclusion

These results show that *Yarrowia lipolytica* have the ability to secrete biosurfactant. The optimum biosurfactant production was observed when the test isolate was grown in POME supplemented with glucose 7.5g/l, Yeast extract 0.625g/l, Salinity 7.5g/l and pH of 8. In further study Response Surface Methodology (RSM) will be used in studying the interaction between the variables.

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