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Single cell oil production from different agro industrial wastes by oleaginous bacteria

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ABSTRACT

Microbial lipophilic compounds called Single cell oils (SCO), has been the object of research and industrial interest for many years, due to their specific characteristics. Such SCO products are potential for using it as alternative sources of animal or plant oils. Most of the work for single cell oil production has, so far, been performed on yeasts mainly because of their ability to accumulate a large amount of lipids intracellularly. Since, this present study aimed to isolate lipid accumulating bacteria for the production of Single cell oil (SCO). This study also focuses on to produce SCO in higher amount using various carbon sources from agro-industrial wastes.

KEYWORDS

Liphophilic compounds;
Intracellular;
Amimal or plant oil;
Agro industial-wastes.

INTRODUCTION

Lipids are oily organic substances and are important structural components of membranes and in many organisms, play a crucial role in energy storage[7]. Natural sources of oils and fatty acids include plants, animals and microorganisms[3]. Plant oils account for the majority of the natural oils and fats on the world market and account for about 83×10^6 tonnes annually[7].

These sources alone cannot be able to meet the total requirement of lipids. The another drawback behind this is, from these sources, a complex mixture of fatty acids with varying lengths (Docosahexaenoic Acid) and degrees of unsaturation were obtained and it needs expensive lipid purification and also it gets contaminated by environmental factors, which leads to typical smell and unpleasant taste[7]. The demand for some unsaturated fatty acid such as Docosahexaenoic Acid (DHA), are rapidly increasing, due to a rapid increase in aquaculture and applications in food and pharmacy[1].

Microorganisms, will be, the suitable alternatives, as they have the ability to convert a number of waste materials into a series of value- added products[1,3]. The oils or lipids, thus produced from microorganisms are known as "SINGLE CELL OIL" and the microbes are called 'oleaginous microbes', since they accumulate more than 20% of their biomass as lipids[3]. These microbes provide an economically feasible source of poly unsaturated fatty acids, provided that most of the PUFAs occur in triacylglycerols (TAG), which is the preferred form to take lipids within the diet[6]. Microbial lipid production has been an object of research and industrial interest for more than 60 years.

FULL PAPER

Microbial oils are cheap and economically feasible source of some polyunsaturated fatty acids (PUFA) which are pharmaceutical and neutraceutical importance. Some essential fatty acids (EPA) produced by microorganism are a direct precursor for a number of biologically active compounds or eicosenoids[7]. Arachidonic acid, an EFA is necessary for the visual activity and cognitive development of infants[8]. Docosahexaenoic acid (DHA) reduces or inhibits risk factors involved in various diseases including cardiovascular diseases[7].

Oils containing the PUFA such as γ- linolenic acid, arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid are of current interest. Oils containing such fatty acids are found in a number of microorganisms. More than 40 natural or synthetic lipophilic compounds were screened for antimicrobial activity[4]. Several lipopeptides have potent antibiotic activity and have been the subject of several studies on the discovery of new antibiotics.

The exploitation of oleaginous microorganism for the production of single cell oil value added products has relevance and importance to our nation's economy. Most of the work has so far been, produced single cell oil mainly from yeasts. Prokaryotic microorganisms should now also consider as a source of lipids with potential application in the oil industry. In this view, the present study was aimed to isolate SCO producing bacteria and to produce single cell oil in maximum quantity using different agro-industrial wastes and based on the optimization of various factors.

MATERIALS AND METHODS

Isolation of oleaginous bacteria, that is, single cell oil producing bacteria were isolated from three different oil contaminated regions by using mineral salt medium.

Screening for SCO

All the bacterial isolates were sub cultured in Supplemented Nutrient broth which contain 500ml Nutrient broth and 500ml Mineral Salt Solution (it contains per liter distilled water, KH_2PO_4, 20g; K_2HPO_4, 5.0g, $(NH_4)_2SO_4$, 30g; NaCl, 0.1g; $FeSo_4$. $7H_2O$, 0.01; $MgSO_4$. $7H_2O$, 0.2; $CaCl_2.2H_2O$, 0.01; and $MnSO_4$. $7H_2O$, 0.2 g; $CaCl_2$. 2 H_2O, 0.01; and $MnSO_4$. H_2O, 0.002 g, glucose, 0.03%, yeast extract, 0.03% pH was adjusted to 7.2) and placed in a shaker at 200 rpm for 3 to 5 days at 30° C.

Total lipid extraction and cell dry matter

The broth was centrifuged (after three days of incubation) for 15 minutes at 5000 rpm and the pellet was dried overnight at 80° C and then weighed. Total lipid content of accumulating bacteria was extracted by Folch method[3]. A known weight of cell dry matter was extracted with chloroform: methanol (2:1), twice at room temperature and centrifuged. The lower chloroform layer containing lipid was obtained and allowed for solvent evaporation and the lipid weight was determined gravimetrically.

Maximum SCO production using agro industrial wastes

Different substrates such as potato infusion, orange wastes, sugarcane molasses and rice bran were prepared by autoclaving 100g of agro industrial wastes in 500ml of water ant the extracts were filtered and added to the supplemented nutrient broth in ratio of 50% (v/v). 2ml of 24 hours old SCO potential strains were added to the 100 ml of SNB medium containing agro-industrial wastes and incubated, and it was centrifuged and dried by Folch method[6] and the lipid weight was determined gravimetrically.

Optimization of SCO production

The maximum lipid accumulation on the microbial cells was optimized by using different environmental factors such as pH (at pH – 6, 7 and 8) and temperature (at 28°C, 37°C and 55°C), and incubation duration (24, 48, 72 and 96 hours) and at aerobic and anaerobic condition and at different nutritional factors such as nitrogen source and trace element solution in different proportions or concentrations, incorporated in the MUA media (Minimal unbalanced agar media contains $NH_2HPO_4.7H_2O$-6.7g, KH_2PO_4 1.5g, $(NH_4)2SO_4$- 0.5g, $MgSO_4.7H_2O$ – 0.2g, $CaCl_2.2H_2O$- 0.01g, Fe(III) NH_4 citrate- 0.06g, trace element solution- 1ml, glucose – 5g, agar- 15g, and distilled water – 1000ml). After the three days of incubation, lipid accumulation was detected by Sudan Black staining of the colonies[2].

Antimicrobial activity of lipids

The single cell oil or lipids extracted from the potential isolates were tested for antimicrobial activity using bacterial cultures and yeasts as the test organism.

RESULTS AND DISCUSSION

Microbial lipophilic compounds called Single cell oils (SCO), has been the object of research and industrial interest for many years, due to their specific characteristics. Such SCO products are potential for using it as alternative sources of animal or plant oils. In this study, a total of 21 different isolates were isolated from three different oil contaminated environments for the SCO production. From the total bacterial isolates, the potential SCO producers were screened by Sudan black staining and by determination of total lipid content on cell dry matter by Folch method. After extraction by Folch method, based on the percentage of total lipid content, five isolates were selected and named as EMS1, EMS2, EMS3, KPS3 and IOL2.

In this study, agricultural wastes such as potato infusion, orange wastes, sugarcane molasses and rice bran were used to produce maximum yield of single cell oil. Gouda *et al*.[3], suggested that *Rhodococcus* sp., and *Gordonia* sp., accumulated lipid more than 50% of the biomass with most tested agro-industrial wastes. In this study, sugarcane molasses and rice bran has showed more than 40% - 95% of SCO production in all the tested organisms. For the strain EMS1, sugarcane molasses is a better source, and for EMS2 potato infusion and sugarcane molasses has showed higher amount of SCO production (94%). For IOL2, 88% of SCO was obtained when rice bran is used as its sole carbon source (TABLE 1)

TABLE 1 : Maximum SCO production using different agro-industrial wastes

Strain	Potato infusion	Orange wastes	Sugarcane molasses	Rice bran
	Percentage of SCO			
EMS1	30	30	47	45
EMS2	94	55	94	88
EMS3	36	74	89	50
KPS3	85	35	34	43
IOL2	75	29	66	88

The potential to synthesize SCO or lipids was tested qualitatively by Sudan black staining of the colonies growing on different nutritional and environmental parameters. pH has a little influence in the bacterial isolates. EMS2 has showed a poor growth at pH 6 and 8 and all other strains showed good growth and lipid accumulation at pH 6, 7 and 8. Regarding temperature, 28°C was optimal for the growth and maximum lipid accumulation. At 37°C EMS 2 has showed poor growth and no growth was obtained at 50°C. All grows well under aerobic condition. The effect of incubation temperature was previously reported by Papanikoloau *et al*.[5]. In this present study, in an increasing hour of incubation time, all the bacteria showed a good growth and maximum lipid accumulation. Even after 96 hours of incubation, the lipid accumulation remained constant.

In Papanikoloau *et al*.[5] report, 0.5g/litre of ammonium sulphate has produced highest amount of lipid. In this study, 0.4 g/litre has showed maximum lipid accumulation. The trace element solution was also influenced the lipid accumulation when its concentration is altered. All these optimization studies showed that temperature, pH, and aeration has adversely affect the growth of the organisms and incubation hours and nitrogen concentration has also affect the lipid accumulation and not the growth of the bacteria. Optimization of SCO for temperature, pH, and nitrogen concentration is necessary for evaluation of optimum growth and maximum SCO production.

Antimicrobial activities of lipids

The lipids extracted from cell dry matter were analysed for their antimicrobial properties. Previously, it was reported that gram positive and yeasts only showed higher sensitivity to the lipid compounds[4]. But, in the present study, yeast strains, *Cryptococcus* sp.,

TABLE 2 : Antimicrobial activities of lipids

Strain / Test organisms	EMS1 (mm)	EMS2 (mm)	EMS3 (mm)	KPS3 (mm)	IOL2 (mm)
Bacillus sp.,	8	-	7	-	7
Staphylococcus sp.,	12	10	-	-	12
E. coli	-	8	10	-	9
Vibrio sp.,	18	10	9	10	7
Saccharomyces sp.,	-	8	-	-	11
Candida sp.,	22	9	-	-	14

and *Staphylococcus* sp., showed higher sensitive to the crude lipid extracted from those five isolates. The lipid compounds extracted from EMS1 and IOL2 are very active against *Saaccharomyces sp.*, for gram negative bacteria, all the lipid extracts showed bacteriostatic effect and EMS1 has a partial zone of inhibition of 18mm diameter for *Vibrio* sp., (TABLE 2)

The five potential single cell oil producers, EMS1, EMS2, EMS3, KPS3 and IOL2 were identified as *Enterobacter sp., Alcaligenes sp., Citrobacter sp., Derxia sp.*, and *Pseudomonas sp.*, respectively. In future, many unknown bacteria may result in the strains that are even better source of SCO producers than the ones we know. Efficient production techniques with detailed knowledge of lipid mechanism should be developed.

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