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Short Communication

Increase in production of biosurfactant from *Oceanobacillus* sp. BRI 10 using low cost substrates

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Abstract

The Antarctic isolate *Oceanobacillus* sp. BRI10 producing biosurfactant(BS) was cultivated in media containing different low cost carbon and nitrogen sources. Initially glucose in basal salt medium was replaced individually with sugarcane juice, whey and local commercial table sugar. Maximum emulsification index (E24) of 67.4% was obtained with sugarcane juice. Further, effect of various nitrogen sources was examined on BS production. Among them sodium nitrate was found to be the most suitable compound. E24 increased to 68.74% in the presence of sugarcane juice and sodium nitrate. The yield of biosurfactant in this medium was 14.25g l^{-1}. The chemical characterization of biosurfactant revealed its glycolipoprotein nature consisting of lipids, carbohydrates, and proteins in the ratio of 4:94:2. Our results indicate a 14-fold increase in the yield and eight times decrease in the cost of production without major difference in the chemical nature of the biosurfactant, with respect to control. This is a significant result with regards to scale-up studies, recovery, and application.

Keywords: antarctic, biosurfactant, cost effective, glycolipoprotein, sugarcane juice, yield

1. Introduction

Biosurfactants are surface active agents of biological origin. They have a wide range of applications in various sectors due to their characteristic properties and advantages over their chemical counterparts (Jadhav *et al.*, 2013). However, the use of biosurfactants is limited due to i) high cost production, ii) low yields, and iii) high cost of recovery or downstream processing. In order to make them economically competitive it is necessary to i) reduce substrate cost, ii) optimize culture conditions, iii) improve recovery process, and iv) use overproducing mutant and/or recombinant strains for high yields. Among these approaches first strategy involves the use of agro based substrates, industrial or municipal wastes. This has helped significantly in lowering the production cost.

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Thus, enhanced yields and decrease in cost of biosurfactants would make them a suitable alternative to synthetic compounds, thereby making the processes more ecofriendly. With this view, the present work deals with studies on effect of low cost substrates on biosurfactant production from the Antarctic isolate *Oceanobacillus* sp. BRI 10. The paper also gives a comparative account of yield and chemical characteristics of biosurfactant under already optimized conditions (Jadhav *et al.*, 2013) and in a cost effective medium.

2. Materials and Methods

2.1 Effect of carbon sources

BRI 10 was grown in MSM (marine salt medium) which was used as inoculum at the 5% (v/v) level for further experiments.BRI 10 was cultivated in basal salt medium (BSM) supplemented with various carbon and nitrogen sources for production of biosurfactant. Basal salt medium (gl^{-1}) com-

position was as following, 0.87g K_2HPO_4, 0.6g $MgSO_4.7H_2O$, 0.1g NaCl, 0.2g KCl, 6.5g tris (hydroxylmethyl) aminomethane, 0.05g yeast extract, 10g glucose, and 1ml of mineral salt (g l^{-1}) [$ZnSO_4.7H_2O$ (2.3), $MnSO_4.4H_2O$ (1.78), $CuSO_4.5H_2O$ (1.0), $Na_2MoO_4.2H_2O$ (0.39), $Co.Cl_2.6H_2O$ (0.42), EDTA(1.0) and KI (0.66)]. The pH of medium was adjusted to 7.0± 0.2. Experiments were performed in 250 ml flasks containing 50 ml medium by varying one parameter at a time keeping other parameters constant. Glucose in the medium was substituted with low cost substrates such as sugarcane juice, whey and local commercial table sugar (LCTS) individually at same concentration. BSM containing glucose was used as a control. BRI 10 was incubated at room temperature for 48 hrs at 120 rpm. Biosurfactant production was measured by calculating an emulsification index (E24) as described below.

2.2 Effect of nitrogen sources

BSM containing optimized carbon source was used to study the effect of various low cost nitrogen sources. Yeast extract in the medium was replaced by different nitrogen sources like soyabean meal, casein hydrosylate, casein protein, corn barn and sodium nitrate individually and used t 0.05 g l^{-1} concentration. BRI 10 was incubated as mentioned above. Biosurfactant production was measured by calculating an emulsification index (E24).

2.3 Measurement of emulsification activity (E24)

The emulsification activity of biosurfactant was determined by measuring the emulsification index (E24). It was evaluated by adding 2 ml kerosene and 2ml cell-free broth (obtained by centrifugation of culture broth at 10,000 rpm for 10 min) in a test tube, vortexed at high speed for 2 min and allowed to stand for 24 hrs. The percentage of emulsification index was calculated by using the following equation:

$$E24 = \text{height of emulsion} / \text{total height of the mixture} \times 100 \quad (1)$$

2.4 Biosurfactant recovery

BRI 10 was cultivated under optimized conditions. The cell free broth was collected and acidified to pH 2.0 with HCl. The biosurfactant was extracted twice using equal volume of chloroform: methanol (2:1) solution in a separatory funnel. The bottom layer was extracted and collected. The solvent was removed from the biosurfactant by rotary evaporation (Vacuum Rotary Evaporator: Kemi Science, Germany) at a temperature of below 40°C. The quantification of dried extract was carried out gravimetrically and was used for further studies.

2.5 Characterization of biosurfactant

Preliminary characterization of biosurfactant was carried out by thin layer chromatography (TLC). A portion of crude biosurfactant was separated on silica plates (Si 60F254, 0.25mm, Merck) using chloroform:methanol:water (65:25:4) as developing solvent system. Detection reagents used were iodine vapors for lipid, ninhydrin reagent for amino acids and alkaline permanganate (1% $KMnO_4$ and 2% Na_2CO_3) solution for sugars, respectively. Protein, carbohydrate and lipid content were estimated by Lowry's method (Lowry *et al.*, 1951), phenol-sulphuric acid method (Dubois *et al.*, 1956) and sulfo-phospho-vanillin test (Floch *et al.*, 1956), respectively. Fourier transform infrared spectroscopy (FTIR) of the sample was carried out as described by Jadhav *et al.* (2013).

3. Results and Discussion

The effect of low cost substrates on the production of biosurfactant from *Oceanobacillus* sp. BRI 10 was studied. As shown in Figure 1, maximum E24 of 67.4% was obtained using sugarcane juice in place of glucose while in control experiment, it was 55%.Almost 18% increase in E24 was observed in this medium (considering 67.4% as 100% E24 activity). Thus, sugarcane juice was found to be the most suitable substrate among the compounds tested. E24 of 68.74% was obtained using sodium nitrate as nitrogen source (Figure 2) indicating that the medium containing sugarcane

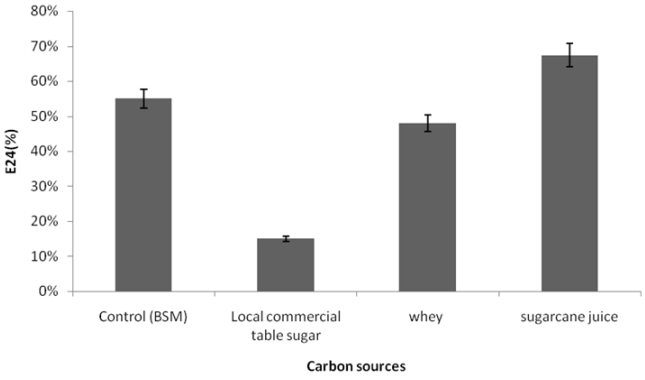

Figure 1. Effect of carbon source on biosurfactant production. In the experiment, BSM was used as control medium.

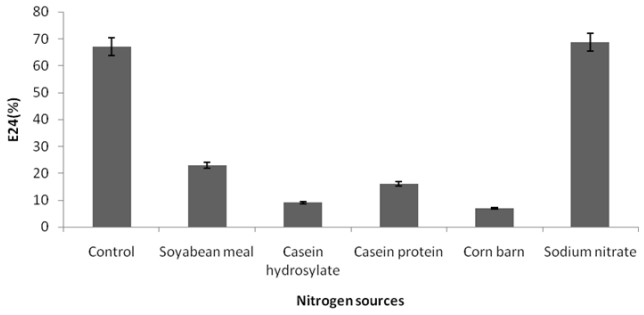

Figure 2. Effect of nitrogen source on biosurfactant production. In this experiment, BSM containing sugarcane juice in place of glucose was used as control medium.

juice and sodium nitrate was the best medium. Our literature survey revealed the use of sugar beet molasses (Onbasli and Aslim, 2009), soya bean oil, palm oil (Oliveira *et al.*, 2009), ground nut oil (Rufino *et al.*, 2008), frying oil (Haba *et al.*, 2000), soy molasses (Rashedi *et al.*, 2005), whey (Dubey and Juwarkar, 2001), potato substrate (Fox and Bala, 2000), cassava waste water (Nitschke and Pastore, 2006), orange fruit peels (George and Jayachandran, 2009), as well as cashew apple juice (Rocha *et al.*, 2007) as low cost substrates for the production of biosurfactant. Many reports are available on sugarcane molasses (Sarin and Sarin,2008; Panesar *et al.*, 2011) with an emulsification index in the range of 60-70%. However, very few papers were published describing the use of sugarcane juice for biosurfactant production. Reis *et al.* (2004) have observed an E24 value of 57% using *Bacillus subtilis*, in the medium containing sugarcane juice and cane molasses. Optimization of biosurfactant was reported for *Pseudomonas fluorescens* (Abouseoud *et al.*, 2007) and *Oleomonassagaranesis* AT18 (Saimmai *et al.*, 2012). The maximum emulsification activity (56%) or yield (5.30 g l^{-1}) was supported in the medium with sodium nitrate, using olive oil and molasses as carbon sources respectively. In the view of this our results are significant.

We have recorded 14.25 g l^{-1} of biosurfactant in the cost effective medium. It indicates almost a 14 fold increase in the yield as compared to that of control. Other studies usingvarious low cost substrates for biosurfactant production reported its yield in the range of 0.18-9.18 g l^{-1} (Dubey and Juwarkar, 2001; Rashedi *et al.*, 2005; Nitschke and Pastore, 2006; Rutino *et al.*, 2008; Onbasli and Aslim, 2009; George and Jayachandran, 2009; Praveesh *et al.*, 2011). The carbon and nitrogen sources include beet molasses, orange fruit peel, sugarcane molasses, curd whey waste, and others as mentioned above. Probably this is the first report on biosurfactant production by using low cost substrates using Antarctic isolate. Studies on biosurfactant BSUC from *Candida antarctica* have been reported by Hua *et al.* (2003). The authors indicated the potential of BS-UC in bioremediation of petroleum contamination.

Preliminary analysis of biosurfactant by TLC suggested the presence of carbohydrates, lipids and amino acids. Results of quantitative estimation indicated that they were present in the ratio of 4:94:2 respectively. The glycolipoprotein nature of biosurfactant was confirmed by FTIR analysis (Figure 3). The FTIR analysis of the biosurfactant (Figure 3) exhibited strong and broad band covered a wide range of 2,800-3,500 cm^{-1} (for OH stretch). A prominent and stake shaped band was located near 1,700 cm^{-1} (for C=O ester bond). C–H stretching bands of CH_2 and CH_3 groups were observed in the region 2,850-2,960 cm^{-1}. CH_2 and CH_3 bends were confirmed at 1,465 and 1,377 cm^{-1}. Wave numbers 3,282 and 3,358 cm^{-1} inferred the presence of N–H/C–H bonds of protein. This was confirmed with wave numbers 1,531 and 1,625 cm^{-1} indicating NH bend in protein. This data confirmed the glycolipoprotein nature of the biosurfactant. According to Mulligan *et al.* (2014) reports on GLPs (glycolipoprotein, glycolipopeptides and glycoprotein) contribute to 19% of total publications. Their applications have been documented in emulsification of crude oil, diesel, lubricant oil, and others and are found to be better than chemical surfactants.

Comparisons of the results of chemical analysis with that of the controlsuggested that the chemical nature of biosurfactant remains almost unaltered when produced in low cost medium. Interestingly, very few reports are available on the chemical characteristics of biosurfactant produced by using low cost media (Rufino *et al.*, 2008) and also on biosurfactant of glycolipoprotein nature (Jadhav *et al.*, 2013). Earlier studies on rhamnolipid type of biosurfactant revealed

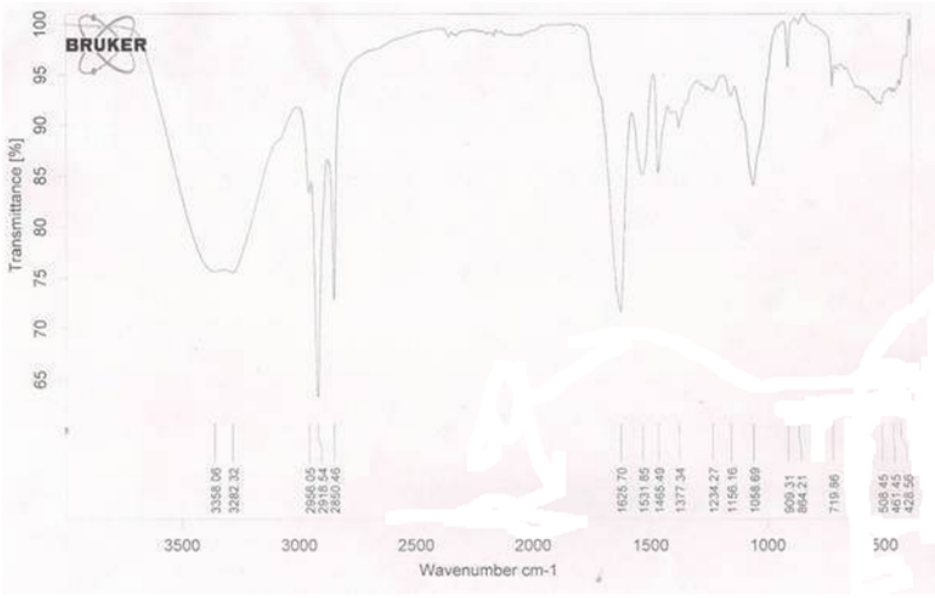

Figure 3. Fourier transform infrared spectrum of the biosurfactant produced by *Oceanobacillu* sp. BRI10

its application in environmental remediation, enhanced oil recovery (EOR) (Nguyen *et al.*, 2008) and also as dewatering agent of industrial oily sludge (Long *et al.*, 2013). Our observation of increased and cost effective production of biosurfactant from *Oceanobacillus* sp. BRI10 (present work) may prove to be first step towards its application for bioremediation purpose.

Comparing the cost of medium with that of control, we found that the medium containing glucose is 8.3 times costlier to that containing sugarcane juice (keeping other components constant).

4. Conclusions

Thus, a 14 fold increase in the yield and 8 times decrease in the cost of production without any major difference in chemical nature of biosurfactant is a significant result with regards to scale up studies, recovery, and application.

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