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Research Article

EXTRACELLULAR GLYCOLIPIDS FROM SACCHAROMYCES CEREVISIAE AS BIOEMULSIFYING AGENTS IN FOOD PROCESSING

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ABSTRACT

The development of food processing sector and increasing diversity in consumers'taste hascontinuously putforth different demands in the interdisciplinary research. In this research study, a demand for bio-based emulsifying agent to improve the oil-aqueous based food has been taken as a predominant destination. Extracellular glycolipids were produced using *Saccharomyces cerevisiae MTCC* 181 strainand basic characterization studies were performed.Further, the scope of the partially purified bioemulsifier in food processing was examined using different experiments and found to be more promising.

Keywords: Bioemulsifier; *Saccharomyces cerevisiae*;Glycolipid;Emulsions; Mango pickle

INTRODUCTION

An emulsifying agent is a substance that enhances uniform mixing of two immiscible liquids, such as water and oil [1, 2]. Usually the emulsifying agents are derived from both chemical and biological sources and also they are being reported to be produced by micro-organisms.The emulsifying agents derived from biological sourcesare found to be more advantageous than the synthetic emulsifiers [3] since they have wide range of applications and are used in many industries world-wide, most notably infood, agriculture,leather, cosmetics, paper, medical, industry, waste utilization and textile industries [4,5].Bioemulsifiers are a type of biosurfactants, which are very surface-active molecules and capable of reducing surface and interfacial tension in aqueous as well as hydrocarbon mixtures. The advantages of the bioemulsifiersover synthetic emulsifiers are lower toxicity, higher biodegradability, improved foaming, stabilizing properties and temperature, pH andsalinity tolerance [5].

In this research work,efforts were put forth to produce extracellular bioemulsifierfrom non-pathogenic eukaryotic micro-organism,*Saccharomyces cerevisiae*and its ability in oil emulsification was tested, and the partially purified emulsifier was experimentally applied in the food products to identify its functional activity.

MATERIALS AND METHODS

Materials

Chemicals and reagents used in the study were obtained from Hi-media and Merck Chemicals, India.

Organism

The yeast culture *Saccharomyces cerevisiae*MTCC 181 used in the study was obtained from Microbial Type Culture Collection, Chandigarh, India.

Saccharomycescerevisiae

Saccharomyces cerevisiae was cultured and subcultured in Yeast Peptone Dextrose agar (0.5 g Yeast extract, 1g peptone, 1g Dextrose, 1g Agar, 50ml of Distilled water, pH-6.5). The culture plates were incubated for 24 hours in a bacteriological incubator at 37°C. After incubation, the plates were sealed and refrigerated for further use [].

Morphological analysis of yeast culture using simple staining

Yeast culture obtained after 24 hours of growth in YPD medium was smeared in a grease-free slide, heat fixed and stained with methylene blue for one minute. After staining, the slide was washed with sterile distilled water, air dried and placed under light microscope for morphological observation [7].

Screening for bioemulsifier production

The bioemulsifier production media (0.4g sodium nitrate; 0.1g Sodium chloride; 0.1g Potassium chloride;0.01g Calcium chloride; 0.3g Potassium di-hydrogen phosphate;0.3g Sodium dibasic hydrogen phosphate; 0.02g Magnesium sulphate;0.1g Ferrous sulphate; 0.08g Cobalt chloride;0.07g Copper sulphatepentahydrate; 0.07g Manganese sulphate; 0.001g Boric acid power; 0.07g Ferrous sulphate; 0.07g Zinc sulphate; 1ml Olive oil as carbon source; 1g of Ammonium nitrate as nitrogen source in 100ml of distilled water; pH adjusted to 6.8.)was prepared, sterilized and allowed tocool. 2 ml of liquid broth culture of *Saccharomyces cerevisiae*was transferred aseptically into the screening medium and incubated in an orbital shaker at 27°C for 5 days at 150 rpm. After incubation, the broth was aseptically transferred into the centrifuge tubes and centrifuged at 3500rpm for 20 minutes for the removal of micro-organisms. The culture supernatant was then subjected to screening tests for emulsification activity [8].

Oil spreading test

In oil spreading method, 10ml of distilled water was poured into a Petriplatefollowed by the addition of 50µl of groundnut oil on the surface of water. 10µlof culture supernatant of *Saccharomyces cerevisiae*was then added to the surface of oil. Observation was made for the appearance of clear halo zone [9].

Emulsification test

2 ml ofmustard oil(oil phase) and 2 ml ofculture supernatant (aqueous)were taken in a clean test tube and vortexed at high speed for 2 minutes and allowed to stand for an hour.As control, 2ml of oil was mixed with 2ml of distilled water and mixed well in vortex. After an hour, the sample tubes were observed for emulsified layer and compared with the control [9].

Mass production and purification of bioemulsifier

500 ml of bioemulsifier production media was prepared, sterilized, cooled and inoculated with 10 ml of *Saccharomyces cerevisiae*motherculture and incubated in an orbital shaker for 5 days at150 rpm for mass production of bioemulsifier. After incubation, the broth was centrifuged at 3500 rpm for 20 minutes. After centrifugation process, the supernatant was transferred into a fresh glass beaker. Pre-chilled acetone was then added to the supernatant in 1:4 ratio.The beakerwas then incubated at 5°C for 24hours for precipitation of biosurfactants. The acetone precipitated supernatant was again subjected to centrifugation at 8000 rpm for 25 minutes. The supernatant was discarded and the pellet containing bioemulsifier was dried in hot air oven at 60°C for 30 min for complete evaporation of acetone. The pellet was re-suspended in 10ml of double distilled water and stored in a vial at-20°C [10].

CHEMICAL ANALYSIS OF THE BIOEMULSIFIER

Qualitative analysis: The purified bioemulsifer was subjected to Benedict's test, biuret test and foam test for the qualitativeidentification of the presence of carbohydrates, protein and lipid respectively.

Benedict's test: 0.2 ml of purified bioemulsifier was taken and mixed with 0.8 ml of distilled water, and 2ml of Benedict's reagent was added and kept on boiling water bath at 95°C for 10 minutes. The tube was observed for coloured precipitation [11].

Biuret test: 0.2 ml of purified bioemulsifier was taken and mixed with 0.8 ml of distilled water and 2ml of biuret reagent was added and incubated at room temperature for 20 minutes. The tube was observed for colour change [12].

Foam test: 0.2 ml of purified bioemulsifier was taken and mixed with 0.8 ml of distilled water and shaken vigorously, and the tube was observed for foaming [12].

Quantitative analysis

1ml of partially purified bioemulsifier was taken in a test tube and 5ml of 2.5N HCl was added and kept in boiling water bath for 15 minutes. After hydrolysis, the pH was neutralised using NaOH. The hydrolysed sample was further subjected to quantification.

Carbohydrate estimation by anthrone

The quantitative estimation of carbohydrates was performed by anthrone estimation method. 1ml of hydrolysed material was taken and added with 4 ml of anthrone reagent and kept in water bath for 8 minutes. Then the mixture was allowed to cool and the absorbance was measured at 630nm in spectrophotometer. Amount of carbohydrates present was calculated by plotting the values in the glucose standard curve [13].

Lipid estimation by vanillin–phosphoric acid assay method

The estimation of lipids was performed by vanillin-phosphoric acid assay. 1 ml of the hydrolysed material was added with 3ml of vanillin reagent and incubatedat 40°C for 15 minutes. The absorbance of the mixture was then measured at 536nm in spectrophotometer. Amount of lipid present was calculated using the formula and oleic acid as the standard [14].

CALCULATION

$$\textbf{Total lipid in unknown } \left(\frac{g}{ml}\right) = \frac{A(unknown)}{A\ (standard)} \times \textbf{Concentration of standard } \left(\frac{g}{ml}\right)$$

APPLICATION OF THE BIOEMULSIFIER IN FOOD PROCESSING

Application in emulsifying different food grade oils

The emulsification index (E24) of the partially purified bioemulsifier was determined by adding 2 ml of different grade oils (Ricebran oil,determined by adding 2 ml of different grade oils (Ricebran oil,Mustard oil, Castor oil, Sunflower oil, Coconut oil, Olive oil, Groundnut oil, Neem oil, Gingelly oil) and 2 ml of culture supernatant in the test tube and vortexed at high speed for 2 minutes and allowed to stand for 24 hours. The E24 index is given as percentage of the height of emulsified layer (cm) divided by the total height of the liquid column (cm). The percentage of emulsification index is calculated by using the following equation:

$$E24 = Height\ of\ emulsion\ formed \div total\ height\ of\ solution \times 100$$

Application in mango pickle formulation

1 ml of partially purified bioemulsifier was mixed with5 g of mango pickle in a test tube. Mango pickle mixed with 1ml of water was taken as the control. The tubes were kept undisturbed. The emulsified layer was measured at 0 hours and 24 hours. The emulsification index was calculated.

Application in butter emulsion

1 ml of partially purified bioemulsifier was mixed with 2 g of butter and 2 ml of water in a test tube. Butter with 3ml of water was taken as the control. The tubes were kept undisturbed. The emulsified layer was measured at 0 hours and 24 hours. The emulsification index was calculated [15].

RESULTS AND DISCUSSION

Food processing industry is one of the ever growing industries in world and it is increasing its hands day-by-day. Many productive researches are being carried out in both the front and back ends of food processing, and as a result many promising food products are hitting the market consistently. One major theme among the productive research is to improve the quality of the food products in the form of sensory and nutrition. This current research work also focuses on developing a scopefulbioproduct for the food processing sector. The research majorly concentrated on producing and purifying microbial-based surfactants for food emulsification.

Morphological observation

The microscopical examination of the simple stained culture hadshownblue color spherical and oval shaped cells along with budding cells (Fig. 1). This confirms the nature of the yeast cells [16]. No other organisms were observed in the slides and hence the culture found to be contamination-free.

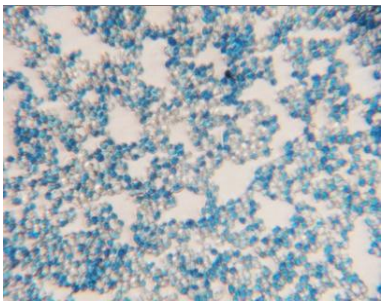


Fig. 1. Morphology of *Saccharomyces cerevisiae* Screening

The yeast culture was grown in bioemulsifier production media and the supernatant was tested for the emulsifier activity. A clear halo zone was observed in the oil spreading test (Fig. 2.i.), which confirms the presence of emulsifying compounds in the culture supernatant [17].Previously,biosurfactant production from *Saccharomyces cerevisiae* was reported by Alcantara VA in 2012. In this current work, persistent emulsified layer (Fig. 2.ii.) obtained as a result o emulsification testcan be considered as a positive result for the presence of emulsifier in the supernatant [18].

Emulsification in mango pickle	**0 hours** 96%	**24 hours** 96%

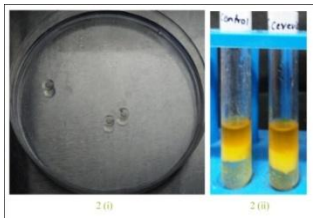


Fig. 2. Results of screening tests

Production, purification and chemical analysis

Following the positive results on screening, *S.cerevisiae*culture was subjected to mass production of bioemulsifier in the production media and was partially purified using acetone precipitation. Organic solventlike acetone precipitate the macromolecules by decreasing the dielectric constant of the medium, and low temperature wass maintained in order to reduce the denaturation process of the molecules. Previous work by Abouseoud, 2007 [19]has reported that, acetone has precipitated the macromoleculesthat indirectly confirms the purification of bioemulsifier.The partially purified bioemulsifer was tested for its major chemical composition using the qualitative tests, such as Benedict's test, biuret test and foaming test, among which Benedict's and foaming test gavethe positive results.The reducing sugar turns to enediols when heated and that reduces the cupric ions present in the Benedict's reagent to cuprous ions, which getprecipitated as red copper oxide [20]. From the results of the screening, the nature of the bioemulsifier could be sensed as a glycolipid, which is one of the categories of amphiphilic compound identified to be produced by the yeast cells [21].

Following the qualitative tests, analysis was performed to quantify the carbohydrates and lipids present in the bioemulsifer.The estimation of carbohydrates was performed by anthrone method. The carbohydrate was hydrolyzed into monomer using dilute acid. It was further dehydrated to hydroxymethyl furfural, which reacts with anthrone.This complex was found to be 109mg/ml of partially purified bioemulsifier solution [22]. The estimation of lipid was performed by vanillin-phosphoric acid assay wherelipids react with acid to form carbonium ions subsequently react with vanillin phosphate ester the complex was measuredspectrophotometrically and it was found to be 50 mg/ml of the partially purified bioemulsifier solution [23]. This shows the nature of the extracellular emulsifying agent [24] produced by *S. cerevisiae* to be glycolipid with the major composition of carbohydrate followed by lipid.

APPLICATION OF THE BIOEMULSIFIER IN FOOD PROCESSING

The research on application part of the partially purified bioemulsifier was classified into three sections which are as follows:

Application in emulsifying different food grade oils

The partially purified bioemulsifier was subjected for emulsification analysis against different food grade oils, such as Rice bran oil, Mustard oil, Castor oil, Sunflower oil, Coconut oil, Olive oil, Groundnut oil, Neem oil and Gingelly oil. The emulsification index was found to be the maximum for gingelly, rice bran, mustard and sunflower oil. It was found to be moderate in the case of neem and coconut, and little poor in the case of castor and ground nut oils (Fig. 3 &Graph 1). However in all the cases,bioemulsifying glycolipids from yeast have shown emulsification property in a wide range.The emulsion formed by the bioemulsifier was found to be stable even after an incubation period of 24 hours.

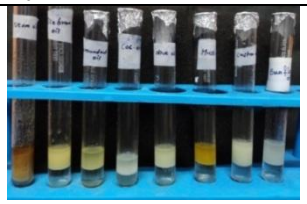
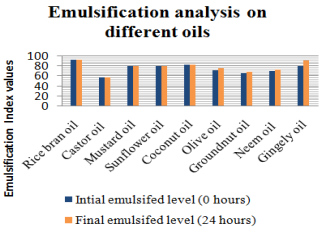


Fig.3. Emulsification test on different food grade oils



Graph 1. Emulsification analysis on different oils

Application in emulsifying mango pickle formulation

Pickle is one of the oil containing food products. Usually after every session of storage period, the oil layer gets separated and forms a separate layer above the product. This is due to the immiscible property of oil substances in aqueous media. Hence, the bioemulsifier was tested to identify its effect on forming strong emulsion in mango pickle,and it was found to be effective (Fig. 4). The initial emulsification index value of the bioemulsifer in mango pickle (aqueous – oil) emulsion was 96% and it was found to be the same after 24 hours.

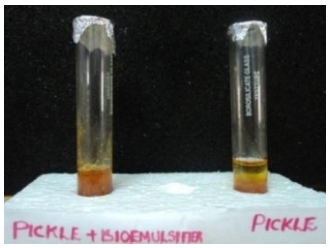


Fig. 3. Application in mango pickle

Application in emulsifying butter emulsion

Butter is one of the dairy products rich in fat and some milk proteins. Due to the high fat content, butter shows immiscibility in aqueous solution. Hence, the partially purified bioemulsifer was tested to find its ability in forming strong butter emulsions, and it was also found to be effective (Fig. 5). The initial emulsification index value of the bioemulsifer in mango pickle (aqueous – oil) emulsion was 98% and it was found to be 97% after 24 hours.

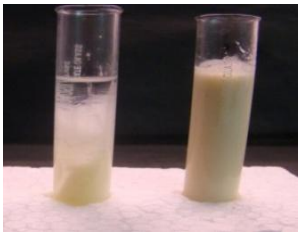


Fig. 3. Application in butter emulsion

Emulsification in mango pickle	**0 hours**	**24 hours**
	98%	97%

CONCLUSION

The research identifies *Saccharomyces cerevisiae* MTCC 181 as a good producer of extracellular bioemulsifer. The bioemulsifer produced by the strain was a glycolipid and it was found to emulsify a wide range of food grade oils. The emulsifier was also found to be a promising agent for its potential applications in food products, such as mango pickle and butter emulsion. As a future aspect, shelf life, nutritional study, sensory analysis, toxicity analysis can be performed to make the research commercially successful, which could support the food processing sector as well as the consumers with yet another biofriendly product.

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