Production and Partial Characterization of a Glycoprotein Bioemulsifier Produced by *Lactobacillus plantarum* subsp. *plantarum* PTCC 1896

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ABSTRACT

Given the growing interest in the production of new and low cost bioemulsifiers, the rice and wheat bran and straw were investigated in this study for the production of bioemulsifier by *Lactobacillus plantarum* subsp. *plantarum* PTCC 1896 (probiotic). The strain produced bioemulsifier only in the rice bran hydrolysate medium. The bioemulsifier amount reached around 0.7 g L⁻¹ for 72 hours of fermentation. The new biomolecule was extracted, purified, and its structural and thermal properties were evaluated. The functional groups and the structure of the molecule were revealed by GPC, FT-IR, ¹HNMR and ¹³CNMR techniques. The bioemulsifier was a water soluble extracellular high molecular weight (>10⁷ Da) α-glucan (81.74%) bound with protein (18.18%). Thermal behavior was studied using DSC and TG analysis. Thermal analysis showed the bioemulsifier broke down above 211.74°C, and the melting point was 182.0°C with the enthalpy value of 101.7 J g⁻¹. These results might provide incentives for the industrial production of the biodegradable and safe bioemulsifier introduced in this study, which seems to offer potential applications in the food and medical industries.

Keywords: Probiotic, Rice bran hydrolysate, Structural properties, Thermal analysis.

INTRODUCTION

Microbial Surface Active Compounds (MSACs) are amphiphilic compounds produced by different microorganisms which either adhere to cell surface or excrete extracellularly (Beltrani et al., 2015). MSACs can be divided into the following two main classes: low-molecular-weight compounds called biosurfactants, such as lipopeptides, glycolipids and glycopeptides, and high-molecular-weight compounds known as bioemulsifiers, which are polymers of proteins, polysaccharides and lipid, such as glycoprotein, lipopolysaccharides and lipoproteins. The major criterion used for choosing an effective biosurfactant is its ability to reduce surface tension. Cooper (1986) considered a culture as promising if it could reduce the surface tension of a liquid medium to 40 mN m⁻¹ or less. Willumsen and Karlson (1996) provided a similar definition: a good biosurfactant producer is defined as the one being able to reduce the surface tension of the growth medium by ≥ 20 mN m⁻¹, as compared with distilled water. Bioemulsifiers are more effective in forming and stabilizing water in oil or oil in water emulsions, but do not necessarily decrease surface or interfacial tension (Portilla-Rivera et al., 2010; Beltrani et al., 2015). Usually, different microbial genera produce structurally different MSACs, whereas

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strains belonging to the same genera produce structurally similar MSACs. However, small differences in the molecular structures of MSACs can have major impact on their functions and potential industrial applications (Saimmai et al., 2012).

MSACs are preferred to the synthetic ones due to environmental compatibility, biodegradability, lower toxicity, higher selectivity, and effectiveness at a wide range of temperature, pH, and salinity levels (Jain et al., 2013). Due to their diverse functional properties including wetting, emulsification, foaming, antimicrobial and anti-adhesive activities, they find wide applications in many industrial areas (Markande et al., 2013; Campos et al., 2014).

MSACs obtained from microorganisms like lactobacilli, which are Generally Recognized As Safe (GRAS), offer a great promise for medical and food applications. In recent years, production of MSACs by lactobacilli has attracted much attention due to their antimicrobial and anti-adhesive activities (Gudina et al., 2010).

In spite of numerous advantages of MSACs over synthetic chemical surfactants, their large scale and economical production is strongly associated with cost-effective bioprocesses and the use of renewable and low-priced raw materials. While it is advantageous, this process faces its own limitations when it comes to selecting proper wastes for use as culture media with the right balance of nutrients to allow cell growth and MSAC accumulation by lactobacilli. Moreover, the properties of the final product largely depend on the composition of the culture media and/or conditions. Therefore, designing a low-cost and at the same time efficient culture medium should be a priority for bioemulsifier production by lactobacilli (Portilla-Rivera et al., 2010; Thavasi et al., 2011).

Lignocellulosic by-products is highly recommended (Li et al., 2010; Portilla-Rivera et al., 2010). Wheat and rice comprise a major portion of human food not only on a global scale, but more specifically in Asia, where they are widely grown and used (FAOSTATE, 2012). This large amount of wheat and rice could be beneficially exploited to produce significant amounts of by-products such as bran and straw as the potential sources of nutrients for microorganisms employed in generating value-added products (Li et al., 2010).

The main objective of the present work was to investigate the efficacy of using low-cost agricultural by-products as sole nutrient sources in producing a biodegradable and safe bioemulsifier for application in food and pharmaceutical industries. For this purpose, rice and wheat bran and straw were screened as carbon sources for extracellular bioemulsifier production by Lactobacillus plantarum subsp. plantarum PTCC 1896. In the second stage, such properties as bioemulsifier amount, structure and thermal properties of bioemulsifier produced on the selected carbon source were investigated.

MATERIALS AND METHODS

Microorganism

Lactobacillus plantarum subsp. plantarum PTCC 1896 (Lp PTCC 1896) used in this study was previously isolated from Iranian infants’ fecal flora in our laboratory (Mirlohi et al., 2009). The strain has reportedly exhibited probiotic activity in MRS (de Man Rogosa and Sharpe, Merck, Germany) medium (Mirlohi et al., 2009) and has been successfully used as a biocontrol agent in suppressing gray mold rot (Botrytis infections) in strawberries (Zamani-Zadeh et al., 2013). The isolated strain was kept frozen at -80°C in MRS broth containing 30% (v v⁻¹) glycerol solution. For further experiments, the microbial culture was activated twice in MRS broth at 37°C for 18 hours under microaerobic conditions (10%
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CO$_2$ in a CO$_2$ incubator (Binder TM, Germany).

Screening Agricultural Byproducts for Bioemulsifier Production

Preparation of Media

Agricultural byproducts, namely, Rice Bran (RB), Rice Straw (RS), Wheat Bran (WB), and Wheat Straw (WS) were used as the feedstock for fermentative bioemulsifier production. They were obtained locally (Isfahan, Iran) and milled using a ball-mill hammer and then they were passed over a 60-mesh sieve. Then, 100 g of each of the RB and WB powders and 50 g of each of the RS and WS powders were transferred into a 2-L flask containing 400 mL of 1.5% H$_2$SO$_4$. Hydrolysis process was carried out at 80°C for 20 hours at 220 rpm (IKA®KS4000 I control, Germany) (Li et al., 2010). Hydrolysates produced from the agricultural byproducts were neutralized with powdered CaCO$_3$ to a final pH of 6.0 and the CaSO$_4$ (neutralizing product) was separated from the hydrolysates supernatant by centrifugation (7,500×g, 5 minutes at 25°C) (Sigma k-16, Germany). The clarified liquors were sterilized at 121°C for 15 minutes to be used as the fermentation media (Portilla-Rivera et al., 2010). Total sugar, reducing sugar, and protein contents of each hyrdolysate were determined using the methods suggested by Dubois et al. (1956), Miller (1959) and Bradford (1976), respectively.

Fermentation Process

Once the substrates had been prepared, fermentation was performed in 250 mL Erlenmeyer flasks containing 100 mL of each hydrolysate diluted with distilled water up to 20 g L$^{-1}$ of the reducing sugar. Each medium was inoculated with 1 mL of the 18 h inoculum of the strain grown in MRS broth (a starting cell count of 7.31±0.07 Log Colony Forming Unit (CFU) mL$^{-1}$ in all flasks). The media were incubated in a shaking incubator (Jaltajhiz, Iran) at 37°C and at 120 rpm for 72 hours. The cells were precipitated by centrifugation (10,000×g, 5 minutes at 10°C) (Sigma k-16, Germany) and emulsification activities of cell-free culture supernatants were determined according to the procedure described by Paraszkiewicz et al. (2002). Briefly, canola oil was added to the cell-free culture supernatant (1:1) and vortexed for 2 min. Emulsification Index (EI) values were determined after 24 and 72 hours of Emulsion formation (EI$_{24}$ and EI$_{72}$, respectively) using the following formula:

$EI= (HEL/HL) \times 100$

Where, HEL is the height of the emulsified layer, and HL is the height of the total liquid. Emulsion Stability (ES, %) corresponds to the ratio of EI$_{72}$ to EI$_{24}$.

Time-course study of bioemulsifier production in rice bran hydrolysate medium was performed by determination of the bacterial cell dry weight and EI values (EI$_{24}$ and EI$_{72}$) of the cell-free culture supernatant at different time intervals. All fermentation experiments were carried out in triplicate.

Extraction and Purification of Bioemulsifier

The chloroform-methanol (2:1) was added to the cell-free culture supernatant and stored overnight at 4°C. The bioemulsifier was subsequently separated by centrifugation (7,500×g, 5 minutes, and 10°C) (Sigma k-16, Germany). The crude bioemulsifier was dialyzed against distilled water at 4°C in a Cellu-Sep® membrane (molecular weight cut-off 6,000–8,000 Da, Membrane Filtration Products, Seguin, USA). Then, the dialyzed solution of bioemulsifier was purified by ultrafiltration using Amicon Ultra-15 Centrifugal Filter Unit with Ultra-100 membrane (Merck Millipore, USA), freeze-dried (Christ alpha-1-4, Germany), weighted and stored at −20°C.
Estimation of Weight Average Molecular Weight (Mw)

The Mw of the freeze-dried bioemulsifier was determined by a Gel Permeation Chromatography (GPC) system (Shao et al., 2014). A GPC system equipped with a PL Aquagel OH mixed-H 8 μm column (Agilent1100 series, Santa Clara, USA) and a Refractive Index (RI) detector was used. Sample (1 mg mL\(^{-1}\)) was eluted with water with a flow rate of 1 mL min\(^{-1}\) at 30°C and acquisition interval of 0.43 seconds.

Chemical Composition

Purified bioemulsifier was subjected to chemical analysis for estimation of total sugars (Dubois et al., 1956) and protein (Bradford, 1976).

Proteinase K Treatment

To investigate the role of proteinous fraction in emulsification activity, supernatant was treated with Proteinase K. The cell-free culture supernatant was treated with Proteinase K (1mg mL\(^{-1}\); 30 U mL\(^{-1}\), Roche TM, Germany) at 37°C for 2 hours and the emulsification activity was studied as described above (Markande et al., 2013).

Monosaccharide Characterization

The monosaccharide composition of the freeze-dried bioemulsifier was estimated by hydrolyzing in 4 M HCl at 121°C for 4 hours followed by using NaOH to adjust the pH to neutral. The reducing sugar content was then estimated by High Performance Liquid Chromatography (HPLC) system (Navon-Venezia et al., 1995). Hydrolysis products and standard sugars (glucose, xylose, galactose, arabinose and mannose) were applied to a HPLC (Jasco International Co., Tokyo, Japan) equipped with a Aminex HPX-87H column (300×7.8 mm, Bio-Rad, Richmond, CA, USA). The injected quantity was 20 μL. The column was eluted with H\(_2\)SO\(_4\) (0.005M) at a flow rate of 0.6 mL min\(^{-1}\) and detected using a Refractive Index (RI) detector.

Fourier Transform Infrared (FT-IR) Analysis

The freeze-dried bioemulsifier was mixed with potassium bromide (1:100 w w\(^{-1}\)). The mixtures were then pressed to obtain translucent pellets. The infrared absorption spectrum of the bioemulsifier was recorded using a FT-IR spectrophotometer in the frequency range of 4,000–400 cm\(^{-1}\) (Jasco International Co., Tokyo, Japan) to identify the functional groups.

Nuclear Magnetic Resonance (NMR) Spectroscopy

The spectra of \(^1\)H and \(^13\)C NMR were obtained to identify the freeze-dried bioemulsifier chemical structures (Shao et al., 2014). The sample was recorded as solution in Deuterium Oxide (D\(_2\)O) at 60°C on an Ultrasound 400 MHz spectrometer (Bruker, Germany).

Differential Scanning Calorimetric (DSC) Analysis

DSC analysis was carried out to investigate the thermal properties of the bioemulsifier. DSC analysis of the bioemulsifier was carried out with a differential scanning calorimeter (Sanaf, Iran). About 10 mg of freeze-dried sample was loaded on a platinum pan and scanning was performed in the range of 30-300°C with a temperature gradient of 6.2 °C min\(^{-1}\) under a nitrogen atmosphere (Miao et al., 2015a).

Thermal Gravimetric (TG) Analysis

TG analysis was carried out to investigate the thermal degradation behavior of the
bioemulsifier. TG analysis of the bioemulsifier was carried out with STA 449F3 Jupiter system (Netzsch, Germany). About 8 mg of freeze-dried sample was loaded on a platinum pan and scanning was performed in the range of 30–500°C with a temperature gradient of 5 °C min⁻¹ under a nitrogen atmosphere. The Derivative ThermoGravimetry (DTG) trace was also obtained by taking the first derivative of the TG curve (Miao et al., 2015a).

RESULTS AND DISCUSSION

Screening Agricultural Byproducts for Bioemulsifier Production

It is known that the functional properties and chemical structures of bioemulsifiers are strongly influenced by both the producing microorganism and certain parameters of the culture medium (Thavasi et al., 2011). In this context, different agricultural byproducts (Table 1) were screened for bioemulsifier production by Lp PTCC 1896, based on the emulsification index of the culture supernatant. The established criterion for emulsion-stabilizing capacity is the ability of a bioemulsifier to preserve at least 50% of the original emulsion volume 24 hours after its formation (Beltrani et al., 2015).

Among the hydrolysates used in this study, the rice bran hydrolysate was the only substrate which showed emulsification activity, probably because of its low C:N ratio (1.1:1) (Table 1), which is known to be a critical factor implicated in the production of bioemulsifiers (Toledo et al., 2008).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Total sugars (g L⁻¹)</th>
<th>Reducing sugar (g L⁻¹)</th>
<th>Protein (g L⁻¹)</th>
<th>C:N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice bran hydrolysate</td>
<td>56.45±1.77</td>
<td>41.90±0.98</td>
<td>51.15±0.75</td>
<td>1.1:1</td>
</tr>
<tr>
<td>Rice straw hydrolysate</td>
<td>39.66±3.72</td>
<td>27.47±1.86</td>
<td>26.49±1.17</td>
<td>1.5:1</td>
</tr>
<tr>
<td>Wheat bran hydrolysate</td>
<td>144.99±1.96</td>
<td>78.90±3.65</td>
<td>59.40±1.60</td>
<td>2.4:1</td>
</tr>
<tr>
<td>Wheat straw hydrolysate</td>
<td>41.36±0.82</td>
<td>26.90±4.92</td>
<td>12.69±1.18</td>
<td>3.3:1</td>
</tr>
</tbody>
</table>

* Data are presented as means±SD (n =3).
temperature, and nutrient media components. The optimization of culture conditions for bioemulsifier from \textit{Lp} PTCC 1896 will be addressed in our future study.

**Extraction and Quantification of Bioemulsifier**

The use of chloroform-methanol (2:1) resulted in forming a yellowish water soluble bioemulsifier precipitated at the interface, which was separated and dialyzed. Almost 100\% of the emulsification activity that was present in the culture supernatant was recovered in the precipitate. The dialyzed solution of the crude bioemulsifier was separated by GPC (Figure 2). One sharp (I) (80.11\%) and two small peaks (II and III) (19.89\%, supposed as contaminants) were separated on the column. Thus, for further purification, the dialyzed sample was ultra-filtered (100 kDa) and freeze-dried. The total amount of the purified bioemulsifier extracted was 0.70±0.05 g L$^{-1}$, which was within the range described by other researchers (between 0.1 and 3.15 g L$^{-1}$) (Navon-Venezia \textit{et al.}, 1995; Toledo \textit{et al.}, 2008; Jain \textit{et al.}, 2012; Beltrani \textit{et al.}, 2015). Many studies have reported the production of biosurfactants/bioemulsifiers by lactobacilli under nutrient-rich media. In laboratory studies, yeast extract, peptone, and corn steep liquor, mainly as nitrogen sources, along with minerals, are used to supplement agricultural waste hydrolysates in media. Also, detoxification of acid hydrolysate was performed to further reduce toxic compounds. The supplementation and detoxification steps could lead to higher finished price of microbial products (Moldes \textit{et al.}, 2007; Portilla-Rivera \textit{et al.}, 2010). This study is the first report on the production of bioemulsifier from a \textit{Lactobacillus} strain grown in a waste based medium, without any supplementation and detoxification. Thus, this new carbon source could be considered for the industrial production of bioemulsifier.

**Chemical Composition of Bioemulsifier**

The bioemulsifier exhibited high M\(_W\) exceeding the exclusion limit of the column (10\(^7\) Da), as indicated by early elution at 3.81 minutes of retention time and confirmed by calculating the exclusion limit through the extrapolation of dextran standards retention times (Figure 2). The purified bioemulsifier consisted of 81.74±3.96\% polysaccharide and 18.18±1.26\% protein. No emulsification activity was observed after Proteinase \(K\) treatment of the bioemulsifier produced, indicating that the proteinous fraction played a major role in the emulsification activity. The acid hydrolysated bioemulsifier resulted in single sugar; the monomer was identified as glucose, showing that the bioemulsifier was composed of a glucan. Our present

\[\text{Figure 1.} \ (a) \text{ Growth and (b) Bioemulsifier production by} \ Lp \ PTCC \ 1896 \text{ cultivated in rice bran hydrolysate medium at 37°C and 120 rpm for 72 hours. Emulsification Index (EI) values at different time intervals were determined after 24 and 72 hours of emulsion formation (EI}\_24 \text{ and EI}\_72, \text{ respectively) using cell-free culture supernatant and canola oil. Data are presented as means±SD (n= 3).}\]
Production and Characterization of a Glycoprotein

This work is the first report on the production and characterization of the extracellular polysaccharide-protein bioemulsifier from a *Lactobacillus* strain. Alasan, the extracellular polysaccharide and proteins containing emulsifier of *A. radioresistens* KA53, has been studied extensively. It consists of protein (25%) and heteropolysaccharide (75%) containing four major sugars, viz. glucosamine, galactosamine, glucose (minor), and galactose, with the molecular weight of approximately 10^6 Da (Navon-Venezia *et al.*, 1995). Liposan, an extracellular bioemulsifier produced by *Candida lipolytica*, is composed of 83% carbohydrates and 17% proteins (Cirigliano and Carman, 1985). *Curvularia lunata* IM2901 produced a bioemulsifier consisting of a complex of polysaccharide (48%) and protein (25%). It is also identified as a polymer of D-glucose (Paraszkiewicz *et al.*, 2002). Bioemulsifiers produced from *Bacillus subtilis*, *Alcaligenes faecalis*, *Enterobacter sp.*, *Halomonas* sp., and *Cronobacter sakazakii* are comprised of carbohydrate, protein, uronic acid and sulfate (Calvo *et al.*, 2002; Toledo *et al.*, 2008; Jain *et al.*, 2012). Proteins present in bioemulsifiers have been proved to play major roles in emulsification with hydrocarbons and oils, both as enhancers in a polysaccharide-protein complex and as standalone emulsifiers (Navon-Venezia *et al.*, 1995; Yadav *et al.*, 2012; Markande *et al.*, 2013).

**FT-IR Characterization**

For the primary identification of the main functional groups present in the bioemulsifier, it was submitted to FT-IR analysis (Figure 3). The FT-IR spectrum of bioemulsifier exhibited a variety of typical absorption peaks of polysaccharides and proteins. The obvious absorption band at 3,420.14 cm\(^{-1}\) was due to the stretching frequency of the hydroxyl groups (O-H), which was typical of polysaccharides (Shao *et al.*, 2014; Beltrani *et al.*, 2015). The weak band at 2,925.48 cm\(^{-1}\) was attributed to C-H stretching, whilst the one at 1,458.89 cm\(^{-1}\) was ascribed to the C-H bending vibrations (Shao *et al.*, 2014). The absorption at 1,654.62 and 1,542.77 cm\(^{-1}\) indicated the C=O stretching of Amides I and N-H bending vibration in Amides II, respectively (Dikit *et al.*, 2010). Each particular polysaccharide showed specific bands in the 1,200–1,000 cm\(^{-1}\) region, and the location and intensity of these bands

![Figure 2](image-url)  
**Figure 2.** Gel Permeation Chromatography (GPC) analysis of the bioemulsifier synthesised by the strain *Lactobacillus plantarum* A. A GPC system equipped with a PL Aquagel OH mixed-H 8 μm column (Agilent1100 series, Santa Clara, USA) and a Refractive Index (RI) detector was used. Sample (1 mg mL\(^{-1}\)) was eluted with water with a flow rate of 1 mL min\(^{-1}\) at 30°C and acquisition interval of 0.43 seconds.
were also specific for each polysaccharide. The intense band at 1,043.30 cm\(^{-1}\) was associated with the bioemulsifier polysaccharide fraction. The band at 1,155.15 and 1,043.30 cm\(^{-1}\) corresponded to the C—OH stretching vibrations and the (C—O—C) glycosidic band vibration, thereby showing the pyranose configuration of the sugar residues. Also, the bands at 858.16 cm\(^{-1}\) were associated with the stretching of the α-isomeric carbon, which was further confirmed by the results of \(^1\)H NMR characterization (Shao et al., 2014).

NMR Characterizations

Figure 4 shows \(^1\)H NMR and \(^{13}\)C NMR spectra of the bioemulsifier dissolved in D\(_2\)O. In the \(^1\)H NMR spectrum (Figure 4-a), chemical shifts of an anomeric region were observed at 5.14-5.55 ppm, corresponding to α-configuration evidenced by the FT-IR spectrum as well (Shao et al., 2014). The chemical shifts at 5.55 and 5.14 ppm were attributed to the anomeric proton of the α-1, 4-linked D-glucopyranose and α-1, 6 D-glucopyranose, respectively. Moreover, the broad peak of α-1, 4 linkage was split into two overlapping peaks (5.55 and 5.54 ppm) which corresponded to the α-1, 4, 6-linked D-glucopyranose residues and non-reducing terminal residue, respectively. The chemical shifts from 3.59 to 4.33 ppm were assigned to the protons of carbons C2–C6 of the sugar ring (Miao et al., 2015b). The protein groups related to the glucan–protein structure seemed to be present, as indicated by the signal at 1.03-1.51 ppm (Dikit et al., 2010; Jain et al., 2013). These attributions were considered once the groups N–CH\(_3\) and N–H were generally observed at 0.5–3.0 ppm, respectively (Gonzaga et al., 2005).

In the \(^{13}\)C NMR spectrum (Figure 4-b), the chemical shifts at around 99.74 ppm were identified as the C1 of α-1, 4-linked D-glucopyranose, α-1, 6-linked D-glucopyranose and α-1, 4, 6-linked D-glucopyranose, respectively. Whereas the signals at around 77.22 ppm corresponded to the C4 of α-1, 4-linked D-glucopyranose and α-1, 4, 6-linked D-glucopyranose, and those at 60.75 ppm were attributed to the C6 of non-reducing terminal residues and α-1, 4-linked D-glucopyranose. Some major signals in the non-anomeric region (69.43–73.23 ppm) were attributed to the C2, C3, C4 and C5 substituted glucose residues (Miao et al., 2015b). The presence of additional peaks at 19.89 ppm may suggest the consideration of the presence of glucan–protein structure (Gonzaga et al., 2005). The data suggested that polysaccharide fraction of the
bioemulsifier from *Lp* PTCC 1896 was a glucan that comprised mainly of α-1, 4 bonds, with fewer α-1, 6 linkages and α-1, 4, 6 branching points (Miao et al., 2015b). Similar characteristic spectral peaks of NMR were also observed in the bioemulsifier (polysaccharide-protein complex) obtained from *Cronobacter sakazakii* (Jain et al., 2012).

Thermal Properties (DSC and TG Analysis)

DSC analysis was conducted in order to study the melting point and changes in enthalpy (H) values of the bioemulsifier (Figure 5-a). The first transition at 167.8°C was associated with the crystallization point, and the second one represented melting (182.0°C) with an enthalpy of transition of 101.7 J g⁻¹. The melting temperature and enthalpy of transition of the bioemulsifier isolated in this study were higher than those of the α-D-glucan from *L. reuteri* SK24.003 (147.7 °C and 78.4 J g⁻¹) (Miao et al., 2015a).

Thermal stability of bioemulsifier is an important characteristic determining its commercial utilization. Degradation of bioemulsifier took place by two well differentiated steps as observed in TG
Figure 5. (a) DSC and (b) TG and DTG analysis of the bioemulsifier from \( Lp \) PTCC 1896. About 8-10 mg of the freeze-dried bioemulsifier was loaded on a platinum pan and scanning was performed under a nitrogen atmosphere. Temperature gradients of 6.2 and 5 °C min\(^{-1} \), respectively, were used for the DSC and TG analysis.

analysis (Figure 5-b). An initial weight loss (9.60 %) was recorded from 30 to 113.74°C due to the loss of water molecules, which was followed by second phase degradation (211.74-500°C). The bioemulsifier was broken down above 211.74°C and the weight was dramatically lost (52.84%) for the second degradation process, with the maximum degradation at 312.74°C, which was due to the depolymerization of exopolysaccharide-protein. A similar degradation pattern was reported for other polysaccharide-protein complexes such as bioemulsifier produced by \( Cronobacter sakazakii \) and the natural hydrocolloide Arabic gum (Jain et al., 2012; Miao et al., 2015a). Due to the high degradation temperature of the bioemulsifier, it would be safe for use in food processing, where, in most processes, temperature rarely overpasses 150°C.

CONCLUSIONS

Probiotic lactobacilli culture would be a valuable alternative to produce bioemulsifiers for food applications if the lactobacilli could be grown in low-cost and safe culture media. The results of screening agricultural byproducts for bioemulsifier production by probiotic \( Lp \) PTCC 1896
offered excellent opportunities for the discovery of a new molecule with distinctive properties. The strain was found to produce a water soluble extracellular high molecular weight α-glucan bound with protein. The emulsification activity together with high thermo-stability (high melting point and high degradation temperature) could have promising prospects for food applications. The rice bran is inexpensive and easy to obtain, showing satisfactory results for the production of bioemulsifier. In view of its applications, the process optimization for its production, and the structure–function relationship in food systems should be further investigated.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Isfahan University of Technology for their financial support. The authors declare that no conflict of interest exists.

REFERENCES


تولید و شناسایی جزئی بیومولسیفایر گلیکوبروتينی تولیدی توسط لاکتوویسوس PTCC 1896 روي عصاره هیدرولیزی سبوس برنج

ن. بخشی، م. شیخ زین الدین، و س. سلیمانیان زاد

چکیده

با توجه به علاقه روزافزون به تولید بیومولسیفایرهای جدید و ارازن قیمت، در این مطالعه سبوس و ساقه برنج و گندم برای تولید بیومولسیفایر توسط لاکتوویسوس PTCC 1896 (پروئویک) ارزیابی شدند. این سویه فقط در محیط عصاره هیدرولیزی سبوس برنج بیومولسیفایر تولید گردید. بازه تولید بیومولسیفایر در حدود 70/170 g L⁻¹ بعد از ۷۲ ساعت تخمیر به دست آمد. مولکول زیستی جدید، استخراج، خاصیت‌های و خصوصیات ساختاری و حرارتی آن بررسی شد. گروه‌های عاملی و ساختار مولکول با استفاده از روش‌های ¹HNMR, FTIR, GPC, DSC و TG محول به آپ و با وزن مولکولی بالا (۱۰/۰ دا) یزد. رفتار حرارتی می‌تواند به استفاده از ۱۱۷/۶۷ C تجزیه می‌شود و نقطه ذوب آن ۱۸۲/۰ ۰ با آنتالپی ذوب ۱ J g⁻¹ مقرن به صرفه و ایمن فراهم می‌کند که به نظر می‌رسد می‌تواند در مصارف غذایی و دارویی مورد استفاده قرار گیرد.