TÜBİTAK

Turkish Journal of Biology

http://journals.tubitak.gov.tr/biology/

Research Article

Turk J Biol
(2015) 39: 160-166

doi:10.3906/biy-1406-44

Biosurfactant production by *Pleurotus ostreatus* in submerged and solid-state fermentation systems

Zülfiye VELİOĞLU[1], Raziye ÖZTÜRK ÜREK[2,*]

[1]Department of Biotechnology, Graduate School of Natural and Applied Sciences, Dokuz Eylül University, Izmir, Turkey
[2]Department of Chemistry, Faculty of Science, Dokuz Eylül University, Izmir, Turkey

Received: 16.06.2014 • **Accepted:** 13.08.2014 • **Published Online:** 02.01.2015 • **Printed:** 30.01.2015

Abstract: Biosurfactants are surface active molecules that are produced by microorganisms. Their positive features attract the attention of many researchers compared to their chemically synthesized counterparts. This research studied the production of a biosurfactant by *Pleurotus ostreatus* in submerged fermentation (SmF) and solid-state fermentation (SSF), with and without shaking. Oil spreading efficiency, emulsification index, and surface tension were assessed during the study. The highest emulsification index (E_{24}) values of SmF, SSF with shaking, and SSF without shaking were 60 ± 5.0%, 29 ± 2.5%, and 42 ± 4.0%, respectively, and surface tension values were 40.7 ± 0.9, 30.8 ± 0.8, and 30.6 ± 0.8 mN m^{-1}, respectively. Based on these data, the produced biosurfactant in SSF with and without shaking was extracted and the amounts were determined as 4.69 ± 0.2 and 4.095 ± 0.2 g L^{-1}, respectively. In addition to these analyses, chemical composition of the extracted biosurfactant was determined and FT-IR spectroscopy was used to confirm the various functional groups. The obtained results of chemical composition and FT-IR indicated the probability of carbohydrate–peptide–lipid complex features of biosurfactants produced by *P. ostreatus*.

Key words: Biosurfactant production, *Pleurotus ostreatus*, submerged fermentation, solid-state fermentation, surface tension

1. Introduction

Surfactants are amphiphilic surface-active agents possessing both hydrophilic and hydrophobic moieties, which reduce surface and interfacial tensions between 2 immiscible fluids such as oil and water (Saharan et al., 2011). Biosurfactants are structurally diverse groups of surfactants synthesized by microorganisms. The major classes of biosurfactants are glycolipids, lipopeptides, phospholipids, and polymeric and particulate surfactants (Nitschke and Costa, 2007). They mainly reduce surface tension, critical micelle concentration, and interfacial tension between liquid–liquid/liquid–solid systems (Marchant and Banat, 2012). Biosurfactants have numerous advantages compared to their chemically synthesized counterparts: they can be produced from renewable resources, they are active under extreme conditions (pH and temperature), they are highly biodegradable, and they have low toxicity (Nott et al., 2013). They are widely used in the pharmaceutical, biomedical, cosmetic, petroleum, and food industries, as well as in environmental and agricultural applications. Considering these properties, the importance of biosurfactant production by different biological sources can be understood. In recent years, *Pseudomonas*, *Bacillus*, and *Candida* species have been particularly known as the predominant biosurfactant producers (Nitschke and Costa, 2007). Microbial production of biosurfactants is growth-associated and may possibly occur with the growth of microbial cells under growth-restrictive conditions (Saharan et al., 2011). Physicochemical properties and production efficiency of biosurfactants might be changed depending on the chemical compounds of the growth medium and the microorganism. Biosurfactants, produced from bacteria and yeast species, have been widely studied and their physicochemical properties have been determined. Although biosurfactant producer fungi species have not been sufficiently studied, as far as we know, *Aspergillus* fungus species are known as biosurfactant producers (Colla et al., 2010).

According to numerous studies, the culture conditions solid-state fermentation (SSF) and submerged fermentation (SmF) are the most effective parameters of biosurfactant production (Neto et al., 2008; Colla et al., 2010). SSF has some advantages, such as the use of low-cost substrates and simple equipment, low volumes of water, low energy demand, and higher concentration of

* Correspondence: raziye.urek@deu.edu.tr

products obtained, in comparison to SmF (Akpinar and Ozturk Urek, 2012). On the other hand, SmF has the advantage of great homogeneity of culture medium and maintenance of parameters such as temperature and pH (Colla et al., 2010). Using diverse microorganisms, growth media, and culture conditions enables the production of biosurfactants of different chemical structures and expands the fields they are used in. Different biological sources and substrates are also important in lowering the production cost and increasing the production yield.

The present study focused on biosurfactant production by *Pleurotus ostreatus* using 2 different production media and conditions (SmF and SSF). *P. ostreatus* is a type of white rot fungus and is known as active lignin degrader. Despite some well-known biosurfactants such as rhamnolipids (*Pseudomonas* sp.); sophorolipids (*Candida* sp.); surfactin, iturin, and fengycin (*Bacillus* sp.); and emulsan (*Acinetobacter* sp.), information on production yield and physicochemical properties of biosurfactant produced by *P. ostreatus* is limited (Chamanrokh et al., 2008; Abdel-Mawgoud et al., 2009; Chandran and Das, 2011; Pemmaraju et al., 2012). Biosurfactant production was screened by oil spreading technique, emulsification index, and surface tension assay. Moreover, biosurfactant produced in SSF, an effective and economical production approach using sunflower seed shell as the carbon source, was further characterized by Fourier transform infrared (FT-IR) spectroscopy, and the chemical composition of the extracted biosurfactant was determined.

2. Materials and methods

2.1. Chemicals

Potato dextrose agar (PDA) and peptone were purchased from Oxoid Microbiology Products (UK). All other medium chemicals were obtained from Sigma-Aldrich (Germany). Other reagents were of analytical grade and were obtained from Merck (Germany). KBr was of FT-IR grade and was also obtained from Merck.

2.2. Organism and culture conditions

P. ostreatus (Jacq.) Pleurotus Kumm. (MCC16) was grown on PDA (39 g L^{-1}) at 25 °C for 7 days (Stajic et al., 2006) and was stored at 4 °C. For SmF and SSF we used a basidiomycete-rich medium (pH 6.0) (g L^{-1}): NH_4NO_3, 0.724; KH_2PO_4, 1.0; $MgSO_4.7H_2O$, 1.0; KCl, 0.5; yeast extract, 0.5; $FeSO_4.7H_2O$, 0.001; $ZnSO_4.7H_2O$, 0.0028; $CaCl_2.2H_2O$, 0.033; and peptone, 10.0 (Bazalel et al., 1997). For SmF cultivations we added 10 g L^{-1} D-glucose and 10 mL L^{-1} sunflower seed oil; for SSF, we added 10 mL L^{-1} sunflower seed oil. For biosurfactant production in SmF we used agar plugs (1 cm^2) that were inoculated into 99 mL of medium and 1 mL of sunflower seed oil in 250-mL Erlenmeyer flasks after sterilization (at 121 °C for 20 min). Cultivation was carried out at 29 °C and 150 rpm of shaking for 20 days (Nikiforova et al., 2009). Cells were removed by centrifugation at 5000 rpm and 4 °C for 20 min, and supernatant was used for determining the analysis below. For SSF, biosurfactant production medium was prepared with 5 g of sunflower seed shell (obtained from Doğa Gıda, Izmir, Turkey) in 250-mL Erlenmeyer flasks. After sterilization, 9.9 mL of liquid medium and 0.1 mL of sunflower seed oil (humidity: 70%) was added to the solid medium, and agar plugs (1 cm^2) were inoculated. Flasks were incubated at 29 °C for 20 days with and without shaking (150 rpm). For extraction, each flask received 25 mL of distilled water and was agitated for 1 h at 150 rpm at 29 °C. The suspension was centrifuged at 5000 rpm and 4 °C for 15 min and supernatant was used in the analysis described below.

2.3. Emulsification index measurements

Emulsification index was measured according to Pinto et al. (2009) by combining 3.5 mL of extract and 2 mL of sunflower seed oil. The mixture was agitated in a vortex agitator at high speed for 1 min. Nonfermented culture media was used as the blank. The emulsion index was calculated after 24 h (E_{24}) and 48 h (E_{48}) by the height of the emulsion layer divided by total height and multiplied by 100, as shown in Eq. (1). Emulsifying activity (EA) was determined according to Eq. (2).

$$E_{sample} = (H_{emulsion\ layer} / H_{total}) \times 100;\ H = \text{height} \quad (1)$$

$$EA = (E_{sample} - E_{blank}) \times D;\ D = \text{dilution of sample in water} \quad (2)$$

2.4. Oil spreading test

Sunflower seed oil (200 µL) was added to distilled water in a 50-mL petri dish to form a layer on the surface, and 20 µL of cell-free supernatant was gradually added to the center of the oil layer. The diameter of the clear zone on the oil surface was measured in relation to the biosurfactant concentration. Distilled water was used as a negative control and standard surfactants, such as Tween 80 and Triton X-100, were used as positive controls (Youssef et al., 2004).

2.5. Measurement of surface tension

The extracts obtained during the biosurfactant production process in SmF and SSF that had the highest emulsification index values were used for the determination of surface tension using a Sigma 701 digital surface tensiometer (KSV Instruments LTD, Finland), working on the principle of the Du Nuoy ring method at room temperature (24 °C).

2.6. Determination of protein and reducing sugar concentrations

Protein concentration was measured according to Bradford and bovine serum albumin was used as the standard (Bradford, 1976). Concentration of reducing sugars was measured by 3,5-dinitrosalicylic acid reagent. A standard

curve was prepared using different glucose concentrations (Miller, 1959).

2.7. Extraction of biosurfactant

After *P. ostreatus* cells were removed from the cultivation medium by centrifugation, in order to precipitate the produced biosurfactant, the method of Jain et al. was used (2012). The resulting precipitation was collected by centrifugation at 5000 rpm and 4 °C for 30 min. The supernatant was removed and 10 mL of chloroform and methanol (2:1, v/v) was added to the precipitated pellet and incubated in a rotary shaker at 30 °C and 200 rpm for 20 min. The content was centrifuged at 5000 rpm and 4 °C for 30 min and the supernatant was evaporated by air drying (Chander et al., 2012).

2.8. Chemical composition of biosurfactant

After the extraction process, the chemical composition of crude biosurfactant was determined. Protein, total carbohydrate, and lipid levels were measured according to Bradford (1976), Dubois et al. (1956), and Bligh and Dyer (1959), respectively.

2.9. Fourier transform infrared spectroscopy

FT-IR spectroscopy is the most useful method for identifying types of functional groups such as alkyls, carbonyls, and esters. The FT-IR spectra were recorded on a PerkinElmer Spectrum BX spectrometer in the spectral region of 4000–400 cm^{-1}. The extracted and dried biosurfactant was analyzed and a KBr pellet was used as a background reference.

2.10. Statistical analysis

The Tukey test was used for statistical significance analyses. The values were the means of 3 separate experiments.

3. Results

This study aimed to produce biosurfactant by *P. ostreatus* using different production conditions and media. Nikiforova et al. (2009) showed production of an emulsifying agent by *P. ostreatus*, yet as far as we know, research on biosurfactant production by *P. ostreatus* is limited to this study. In our study, we investigated emulsification index after 24 and 48 h (E_{24} and E_{48}) during the incubation period. Studies on the emulsifying properties of SmF with sunflower oil are shown in Figure 1. In a similar way, the emulsification index of SSF (with and without shaking) was followed during the incubation period. According to our results, the highest and the most stable emulsification index of SSF (with and without shaking) was observed on the fifth day of incubation (Table 1). Therefore, the following analyses were applied on day 13 and day 5 of the samples of SmF and SSF, respectively.

The oil spreading technique measures the diameter of clear zones caused by a drop of biosurfactant solution on an oil–water surface (Morikawa et al., 2000). In the present

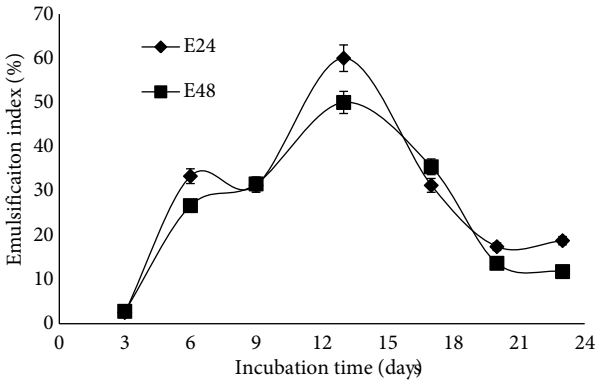

Figure 1. Variations in emulsification index of biosurfactant produced by *P. ostreatus* in SmF during the incubation period. The values are the mean ± SD of 3 separate experiments.

study, oil spreading ability was investigated in comparison to Tween 80 (0.1%, w/v) and Triton X-100 (0.1%, w/v) as positive controls and distilled water as a negative control (Table 2).

Youssef et al. (2004) reported that the oil spreading test is correlated with the ability of cultures to reduce surface tension, and the diameter of clear zones is linearly related to biosurfactant concentration. Molecules of a water droplet are held together by cohesion forces, and these intermolecular forces build surface tension. The surface tension of distilled water at room temperature is 72 mN m^{-1}, and when surfactant is added to it, the surface tension value is reduced (Satpute et al., 2010). In our study, the surface tensions of the samples on day 5 of SSF (with and without shaking) and on day 13 of SmF were assessed (Figure 2). The biosurfactant produced in SSF with and without shaking cultivations had similar surface tension values (30.8 ± 0.8 and 30.6 ± 0.8 mN m^{-1}, respectively; $P > 0.05$). These results were significantly lower than the surface tension of the biosurfactant produced in SmF (40.7 ± 0.9 mN m^{-1}) ($P < 0.05$). The data indicating that biosurfactants were produced in SSF and SmF culture media by *P. ostreatus* and surface tension measurement values were correlated to oil spreading values.

In addition, the reduction of sugar and protein levels of the samples on day 5 of the cultivation period for SSF

Table 1. Emulsification index (%) values of biosurfactant produced by *P. ostreatus* in SmF and SSF. The values are the mean ± SD of 3 separate experiments.

Samples	E_{24} (%)	E_{48} (%)
SSF with shaking	29 ± 2.5	21 ± 2.0
SSF without shaking	42 ± 4.0	39 ± 3.5
SmF	60 ± 5.0	50 ± 4.5

Table 2. Diameters of clear zones according to oil spreading technique for biosurfactant produced by *P. ostreatus*. The values are the mean ± SD of 3 separate experiments.

Samples	Diameter of clear zones (cm)
SSF with shaking	3.5 ± 0.2
SSF without shaking	4 ± 0.2
SmF	4 ± 0.2
Tween 80	9 ± 0.5
Triton X-100	9 ± 0.5
Distilled water	Negative

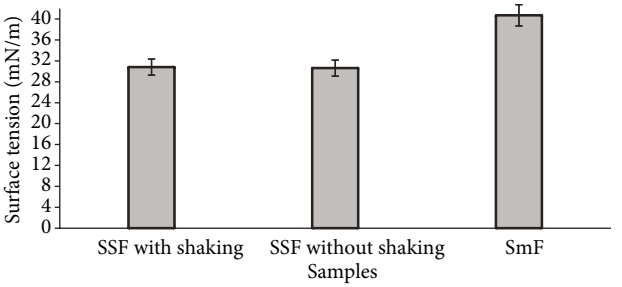

Figure 2. Surface tension values of biosurfactant produced by *P. ostreatus* in SmF and SSF. The values are the mean ± SD of 3 separate experiments.

with and without shaking was determined as 41.9 ± 2.3 and 42 ± 2.3 mM and 4787.7 ± 35 and 5070.76 ± 37 ppm, respectively. After the analysis we extracted the produced biosurfactant in SSF with and without shaking, and the amounts were determined as 4.69 ± 0.2 and 4.095 ± 0.2 g L^{-1}, respectively. The obtained results were comparable to those of Abouseoud et al. (2008), who extracted 2 g L^{-1} biosurfactant. After the extraction process, the color of the resultant precipitate gives information about the type of biosurfactant; generally, polymeric biosurfactants like emulsan have brown precipitate, while glycolipid-type biosurfactants such as rhamnolipid have gray-white precipitate (Patil and Chopade, 2001; Chander et al., 2012). In our study, we had brown precipitate from both SSF productions (with and without shaking). The chemical composition of extracted biosurfactant was determined as 12.46 ± 0.9% carbohydrate, 19.06 ± 1.1% protein, and 60.48 ± 4.5% lipid. It can be inferred from the results that the produced biosurfactant might be a complex-structured biosurfactant. The results obtained thus far show that biosurfactant produced in SSF was more active than biosurfactant produced in SmF. Therefore, molecular compositions of biosurfactant produced in SSF (with and without shaking) were extracted and evaluated by FT-IR spectroscopy (Figure 3).

The most important bands were located at 3297 and 3291 cm^{-1} (O-H stretching) and at 2929 cm^{-1} (C-H bands: CH_2-CH_3 stretching), associated with the stretching vibration of the C–H bond of constituent sugar residues. A peak at 1659 cm^{-1} (C=O stretching) suggested the presence of carbonyl functionality in carboxylate or amide moieties of protein and peptide amines. The peaks at 1530 and 1535 cm^{-1} (N-H bending) were also an indication of proteins. The peaks at 1200–1400 cm^{-1} were an indication of hydrocarbon chain. Furthermore, the peaks at 1077 and 1076 cm^{-1} (C-O stretching) implied the presence of uronic acid and O-acetyl ester (Jain et al., 2012). Since the peaks at 1659, 1530, and 1535 cm^{-1} showed the presence of protein, it might be concluded that the produced biosurfactants were protein-containing, such as lipopeptides or polymeric biosurfactants (Das and Mukherjee, 2007; Chamanrokh et al., 2010). The high protein concentrations of produced biosurfactants supported this result. The peaks at 2929, 1077, and 1076 cm^{-1} are found in the structure of polysaccharide, which contains biosurfactants such as glycolipids and polymeric biosurfactants (Chamanrokh et al., 2010; Chander et al., 2012; Jain et al., 2012; Pulate et al., 2013).

4. Discussion

The present study proposes that *P. ostreatus* has biosurfactant production potential for SSF. The highest emulsification index (E_{24}) value of SSF without shaking was 42 ± 4.0% and surface tension value was 30.6 ± 0.8 mN m^{-1}. The amount of produced biosurfactant was determined as 4.095 ± 0.2 g L^{-1}.

Emulsification is the dispersion of a liquid into another liquid, leading to the mixing of 2 immiscible liquids (Satpute et al., 2010). The emulsification index is reliable in detecting biosurfactant production. The emulsification index stability designates the strength of a surfactant. The emulsification index values indicated that the product exhibited good and stable emulsifications (E_{24} = 60 ± 5.0%; E_{48} = 50 ± 4.5%) on day 13 of incubation. Based on this result, it can be deduced that the highest biosurfactant production and activity in SmF was on day 13. Colla et al. (2010) obtained emulsification index values of biosurfactant produced by *Aspergillus* sp. of 42.67% and 2.85% in SmF and SSF, respectively. In our study, the values obtained with regard to emulsification index were higher in SmF than SSF, similar to the study of Colla et al. (2010). Our emulsification index values were also higher than theirs. In our study, biosurfactant produced in SmF conserved its emulsification index by 83.33 ± 5.4%, while biosurfactant produced in SSF with and without shaking conserved its index by 72.41 ± 4.9% and 92.86 ± 6.2%, respectively. Although E_{24} and E_{48} values of biosurfactant

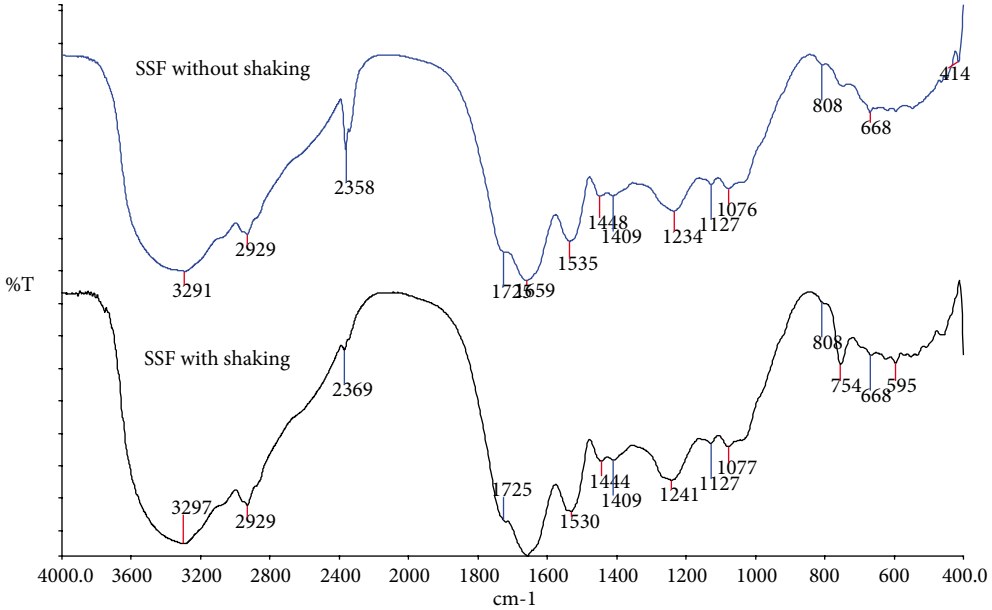

Figure 3. FT-IR spectra of biosurfactant produced by *P. ostreatus* in SSF (with and without shaking).

produced in SmF were higher than those produced in SSF with and without shaking, the most stable emulsion was formed in SSF without shaking. When choosing any application of biosurfactant, stability is as important as the emulsification index, as stable products facilitate long-term productivity.

Priya and Usharani (2009) applied oil spreading tests for 4 different oil types for rhamnolipid, which is produced by *Bacillus subtilis* and *Pseudomonas aeruginosa*. The highest clear area's diameter values were 2 and 2.2 cm in diesel oil, respectively. This shows that the biosurfactants that we produced by *P. ostreatus* have higher surface activity than rhamnolipid produced by *B. subtilis* and *P. aeruginosa*. Furthermore, while the biosurfactants produced in SmF and SSF without shaking had the same efficiency, the biosurfactant produced in SSF with shaking had lower oil spreading ability. Considering the results of this study, it can be suggested that the biosurfactant produced in SSF without shaking is as active as the biosurfactant produced in SmF. In this case, since the SSF technique is economical and easy, it is preferable to SmF in order to produce these compounds.

According to previous research, sophorolipid produced by *Candida bombicola* reduced the surface tension of water to 24 mN m^{-1} (Das and Mukherjee, 2007). Surfactin produced by *B. pumilus* was reduced to 27 mN m^{-1} (Slivinski et al., 2012), and rhamnolipid produced by *P. aeruginosa* and emulsan produced by *Autochthonous bacteria* were reduced to 33 and 30 mN m^{-1}, respectively (Chamanrokh et al., 2010; Kaya et al., 2014). In our study, biosurfactant produced in SSF with and without shaking had a significant surface tension value in comparison to the literature.

In terms of emulsification index, biosurfactant produced in SmF as a surface-active agent was far more effective than biosurfactant produced in SSF with and without shaking. However, surface tension values showed that biosurfactant produced in SSF with and without shaking was more effective in reducing surface tension. Generally, emulsification index and surface tension are correlated; biosurfactant that has a high emulsification index is expected to decrease surface tension to lower values. In our study, biosurfactant produced in SmF had the highest E_{24} and E_{48} values, despite reducing surface tension to levels lower than SSF (with and without shaking). In the study of Abouseoud et al. (2008), biosurfactants produced by *P. fluorescens* with 3 different carbon sources (hexadecane, olive oil, and glucose) had much the same surface tension value, although E_{24} values showed changes. According to these results, it can be deduced that surface tension and emulsification index are based on growth medium components and growth condition.

The produced biosurfactant was comparable to the literature findings in terms of emulsification index (E_{24}) and surface tension values of SmF, SSF with shaking, and SSF without shaking at 60 ± 5.0%, 29 ± 2.5%, and 42 ± 4.0% and at 40.7 ± 0.9, 30.8 ± 0.8, and 30.6 ± 0.8 mN m^{-1}, respectively. These results showed that SSF without shaking was more efficient than SSF with shaking and more efficient than SmF in producing biosurfactants from *P. ostreatus*. These data supported the use of SSF, which requires low-cost substrates, simple equipment, and low energy. Based

on these data, FT-IR spectroscopy was used for chemical characterization of the extracted biosurfactant from SSF with and without shaking (4.69 ± 0.2 and 4.095 ± 0.2 g L^{-1}, respectively). Both crude biosurfactants had fairly similar absorption bands. This means that shaking is not essential to produce an active biosurfactant in SSF. In addition, this result was supported by reduced sugar and protein levels. Both biosurfactants produced in SSF (with and without shaking) had almost the same level of reduced sugar and protein. Regarding the results of chemical composition analysis and FT-IR spectroscopy, it can be concluded that the produced biosurfactant is likely to be a carbohydrate–peptide–lipid complex biosurfactant.

In conclusion, our results suggest that there is great potential for biosurfactant production by *P. ostreatus* using SSF. The highest emulsification index (E_{24}) values of SSF without shaking were 42 ± 4.0% and the surface tension value was 30.6 ± 0.8 mN m^{-1}. The amount of produced biosurfactant was determined as 4.095 ± 0.2 g L^{-1}. According to the results of chemical composition and FT-IR analysis, the produced biosurfactant is a complex type of biosurfactant. Further investigation regarding structural characterization and applications of the biosurfactant obtained are in progress. Furthermore, sunflower seed shell was used as a solid substrate in SSF production; hence, it was not only an alternative way to reduce environmental pollution, but also a potential approach for production of valuable biotechnological products.

Acknowledgments

We thank Dr Ufuk Malayoğlu for surface tension analysis and Doğa Gıda, İzmir, Turkey, for providing the solid substrate. This work was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) under grant no. 113M801.

References

Abdel-Mawgoud AM, Aboulwafa MM, Hassouna NAH (2009). Characterization of rhamnolipid produced by *Pseudomonas aeruginosa* isolate Bs20. Appl Biochem Biotechnol 157: 329–345.

Abouseoud M, Maachi R, Amrane A, Boudergua S, Nabi A (2008). Evaluation of different carbon and nitrogen sources in production of biosurfactant by *Pseudomonas fluorescens*. Desalination 223: 143–151.

Akpinar M, Ozturk Urek R (2012). Production of ligninolytic enzymes by solid state fermentation using *Pleurotus eryngii*. Prep Biochem Biotech 42: 582–597.

Bazalel L, Hadar Y, Cerniglia C (1997). Enzymatic mechanisms involved in phenanthrene degradation by the white rot fungus *Pleurotus ostreatus*. Appl Environ Microbiol 63: 2495–2501.

Bligh EG, Dyer WJ (1959). A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911–917.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254.

Chamanrokh P, Mazaheri Assadi M, Amoabediny G, Rashedi H (2010). Cleaning oil-contaminated vessel by emulsan producers (*Autochthonous bacteria*). Iran J Environ Health Sci Eng 7: 209–222.

Chamanrokh P, Mazaheri Assadi M, Noohi A, Yahyai S (2008). Emulsan analysis produced by locally isolated bacteria and *Acinetobacter calcoaceticus* RAG-1. Iran J Environ Health Sci Eng 5: 101–108.

Chander CR, Lohitnath T, Mukesh Kumar DJ, Kalaichelvan PT (2012). Production and characterization of biosurfactant from *Bacillus subtilis* MTCC441 and its evaluation to use as bioemulsifier for food bio-preservative. Adv Appl Sci Res 3: 1827–1831.

Chandran P, Das N (2011). Characterization of sophorolipid biosurfactant produced by yeast species grown on diesel oil. Int J Sci Nat 2: 63–71.

Colla LM, Rizzardi J, Pinto MH, Reinehr CO, Bertolin TE, Costa JAV (2010). Simultaneous production of lipases and biosurfactants by submerged and solid-state bioprocesses. Bioresource Technol 101: 8308–8314.

Das K, Mukherjee AK (2007). Comparison of lipopeptide biosurfactants production by *Bacillus subtilis* strains in submerged and solid state fermentation systems using a cheap carbon source: some industrial applications of biosurfactants. Process Biochem 42: 1191–1199.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956). Colorimetric method for determination of sugars and related substances. Anal Chem 28: 350–356.

Jain RM, Mody K, Mishra A, Jha B (2012). Isolation and structural characterization of biosurfactant produced by an alkaliphilic bacterium *Cronobacter sakazakii* isolated from oil contaminated wastewater. Carbohyd Polym 87: 2320–2326.

Kaya T, Aslım B, Kariptaş E (2014). Production of biosurfactant by *Pseudomonas* spp. isolated from industrial waste in Turkey. Turk J Biol 38: 307–317.

Marchant R, Banat IM (2012). Microbial biosurfactants: challenges and opportunities for future exploitation. Trends Biotechnol 30: 558–565.

Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31: 426–428.

Morikawa M, Hirata Y, Imanaka T (2000). A study on the structure-function relationship of the lipopeptide biosurfactants. Biochim Biophys Acta 1488: 211–218.

Neto DC, Meira JA, Araújo JM, Mitchell DA, Krieger N (2008). Optimization of the production of rhamnolipids by *Pseudomonas aeruginosa* UFPEDA 614 in solid-state culture. Appl Microbiol Biotechnol 81: 441–448.

Nikiforova SV, Pozdnyakova NN, Turkovskaya OV (2009). Emulsifying agent production during PAHs degradation by the white rot fungus *Pleurotus ostreatus* D1. Curr Microbiol 58: 554–558.

Nitschke M, Costa SGVA (2007). Biosurfactants in food industry. Trends Food Sci Tech 18: 252–259.

Nott K, Richard G, Laurent P, Jérôme C, Blecker C, Wathelet JP, Paquot M, Deleu M (2013). Enzymatic synthesis and surface properties of novel rhamnolipids. Process Biochem 48: 133–143.

Patil JR, Chopade BA (2001). Studies on bioemulsifier production by *Acinetobacter* strains isolated from healthy human skin. J Appl Microbiol 91: 290–298.

Pemmaraju SC, Sharma D, Singh N, Panwar R, Cameotra SS, Pruthi V (2012). Production of microbial surfactants from oily sludge-contaminated soil by *Bacillus subtilis* DSVP23. Appl Biochem Biotechnol 167: 1119–1131.

Pinto MH, Martins RG, Costa JAV (2009). Bacteria biosurfactants production kinetic evaluation. Quím Nova 32: 2104–2108.

Priya T, Usharani G (2009). Comparative study for biosurfactant production by using *Bacillus subtilis* and *Pseudomonas aeruginosa*. Botany Research International 24: 284–287.

Pulate VD, Bhagwat S, Prabhune A (2013). Microbial oxidation of medium chain fatty alcohol in the synthesis of sophorolipids by *Candida bombicola* and its physicochemical characterization. J Surfact Deterg 16: 173–181.

Saharan BS, Sahu RK, Sharma D (2011). A review on biosurfactants: fermentation, current developments and perspectives. Genet Eng Biotechnol 29: 1–39.

Satpute SK, Banpurkar AG, Dhakephalkar PK, Banat IM, Chopade BA (2010). Methods for investigating biosurfactants and bioemulsifiers: a review. Crit Rev Biotechnol 30: 1–18.

Slivinski CT, Mallmann E, Araujo JM, Mitchell DA, Krieger N (2012). Production of surfactin by *Bacillus pumilus* UFPEDA 448 in solid-state fermentation using a medium based on okara with sugarcane bagasse as a bulking agent. Process Biochem 47: 1848–1855.

Stajic M, Persky L, Friesem D, Hadar Y, Wasser SP, Nevo E, Vukojevic J (2006). Effect of different carbon and nitrogen sources on laccase and peroxidases production by selected *Pleurotus* species. Enzyme Microb Tech 38: 65–73.

Youssef NH, Duncan KE, Nagle DP, Savage KN, Knapp RM, McInerney MJ (2004). Comparison of methods to detect biosurfactant production by diverse microorganisms. J Microbiol Meth 56: 339–347.