African Journal of Microbiology Research Vol. 4(24) pp. 2811-2821, 18 December, 2010
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 ©2010 Academic Journals

Full Length Research Paper

Bioconversion of soy processing waste for production of surfactants

Mahmoud Z. Sitohy[1], Mona M. Rashad[2]*, Samy F. Sharobeem[1], Abeer E. Mahmoud[2], Mohamed U. Nooman[2] and Amr S. Al Kashef[2]

[1]Department of Agricultural Biochemistry, Faculty of Agriculture, El Zagazeg University, Egypt.
[2]Department of Biochemistry, National Research Centre, Dokki, Cairo, Egypt.

Accepted 2 December, 2010

The production and properties of biosurfactants synthesized by *Candida guilliermondii* NRRL Y-2075 and *Bacillus subtilis* NRRL B-94 using soy processing waste (okara) as substrate were investigated under different fermentation techniques. Higher oil displacement activities for the isolated biosurfactants were achieved by using submerged fermentation technique (SMF) for both organisms. Preliminary chemical characterization and IR spectroscopy for *C. guilliermondii* biosurfactant revealed that, it is mainly a glycolipid complex, while that of *B. subtilis* biosurfactant was found to be a complex mixture of lipoprotein and glycolipid. Both biosurfactants formed stable water-in-oil emulsions with motor oil and sunflower oil, while soybean oil was only emulsified by *C. guilliermondii* biosurfactant. The surface tension of water was reduced to 51 mN/m by *C. guilliermondii* biosurfactant at a critical micelle dilution (CMD) of 8% concentration, while *B. subtilis* biosurfactant showed a minimum surface tension of 48 mN/m at CMD value of 10% concentration. Both biosurfactants exhibited good antimicrobial activity against the tested bacteria and yeast strains with different values of minimum inhibitory concentrations (MIC).

Key words: Surfactant, *Candida guilliermondii*, *Bacillus subtilis*, soy processing waste, submerged fermentation.

INTRODUCTION

Surfactants are amphipathic molecules which reduced the surface tension between water and hydrocarbon interface (Kitamoto et al., 2002). They have a great variety of applications in medicine, households, agriculture, and petroleum industry, including oil extraction and processing; cleaning of vessels from oil fractions, acceleration of well drilling and enhancement of oil recovery; they can also be used for ecosystem bioremediation, (Rozanova et al., 1987; Ganitkevich, 1988; Banat, 1995; de Gusmao et al., 2010). Currently, almost all the industrially produced surfactants are chemically derived from petroleum (Banat et al., 2000) and require both synthesis and several purification steps, rendering the process costly and liable for contamination with unknown hazards.

As a result of increasing environmental awareness and emphasis on a sustainable society in harmony with the global environment, during the recent years, natural surfactants of microbial origin (biosurfactants) may be recommended to replace the presently used chemically-synthesized surface active agents (Koma et al., 2001; Hua et al., 2003; Bednarski et al., 2004; Das and Mukherjee, 2007; de Gusmao et al., 2010). They have been attracting attention as they offer several advantages over chemical surfactants e.g. low toxicity, inherent good biodegradability and ecological acceptability. They may also be used at extreme temperatures, acidity and salt concentrations (Banat, 1995), beside their ability to be produced from renewable and cheap substrates (Desai and Banat, 1997; Nitschke and Pastore, 2003, 2006). Several studies have been reported on the production of biosurfactants by bacteria specially *Bacillus* sp. (Ohno et al., 1995; Kim et al., 1997; Koma et al., 2001;

*Corresponding author. E-mail: monarashad122@hotmail.com. Fax: 00202-33370931.

Roongsawang et al., 2002). Between yeasts, species of *Candida* have been widely used in the production of biosurfactants from soluble and insoluble carbon sources (Sarubbo et al., 1999, 2006; de Gusmao et al., 2010; de Luna et al., 2009). The biosurfactant presence could be mainly observed during microbial growth in medium containing lipid substrates (Desai and Banat, 1997; de Gusmao et al., 2010; de Luna et al., 2009).

The key factor governing the success of biosurfactant production is the development of an economical process that uses low-cost materials and gives high productivity (Rufino et al., 2007). To enhance the efficiency of biosurfactant production by microorganisms, inexpensive medium components such as food industry by-products or waste should be explored, since they represent about 50% of the total production costs (Daniel and Otto, 1999; Haba et al., 2000). The food industries generate large quantities of polluting organic wastes and residues (Malnou et al., 1987) which can be used for the production of biosurfactants (O'Toole, 1999; Makkar and Cameotra, 2002). Soybean has played important roles in food industries as a rich and cheap source of high quality protein and oil (Ahn et al., 2008). Okara is soy pulp produced from soybean processing. About 1.1 kg of fresh okara is produced from every kilogram of soybean processed for soymilk (Khare et al., 1995).It is usually discarded causing putrefaction or disposal problems (Erickson, 1995; O'Toole, 1999) or used as a fertilizer or as a substrate for production of useful metabolites (O'Toole, 1999; Rashad and Nooman, 2008; Rashad et al., 2010).

Consequently, the objective of this work was to produce and characterize the biosurfactants from *Candida guilliermondii* NRRL Y-2075 and *Bacillus subtilis* NRRL B-94 cultivated on soy processing waste (okara) aiming for the development of a more effective fermentation medium with low cost production, and to comparatively assess the obtained products as potential biosurfactants and bioemulsifiers.

MATERIALS AND METHODS

Microorganisms

C. guilliermondii NRRL Y-2075; *B. subtilis* NRRL B-94; *Escherichia coli* NRRL B-3703; *B. subtilis* NRRL B-4219; *Saccharomyces cerevisiae* NRRL Y-12632 and *Candida albicans* NRRL Y-12 were obtained from the Agricultural Research Service, Peoria, Illinois, USA. The cultures were maintained on a stock slant medium (Wickerman, 1951) for yeast and on nutrient agar (Difco) for bacteria.

Substrate

Okara (soybean residue, a by-product from the manufacture of soybean milk) was obtained from Food Technology Research Institute, Soy Processing Centre, Agricultural Research Centre, Giza, Egypt and frozen until used. All chemicals and reagents used were of analytical grade.

Inoculum preparation

The yeast isolates were streaked on YME medium with agar (Wickerman, 1951), while the bacterial isolates were streaked on a nutrient agar slants and all cultures were incubated for 24 h at 30℃. A loop of each culture of *C. guilliermondii* and *B. subtilis* was inoculated in 50 ml of sterile inoculum medium (composed of the stock medium without agar), then incubated at 30℃, 120 rpm for 24 h.

Cultivation conditions

Different media were used for growing both *C. guilliermondii* NRRL Y-2075 and *B. subtilis* NRRL B-94 to produce biosurfactant (Table 1). An aliquot of 1 ml of each inoculum was transferred to 50 ml of each production medium in an Erlenmeyer flask (250 ml) and incubated for 72 h, 150 rpm on controlled incubator shaker (New Brunswick Scientific, USA) at 30℃ for yeast and 37℃ for bacteria. At the end of the fermentation period, (the remaining oil was discarded) and the cultures were centrifuged in a cooling centrifuge at 15000 rpm for 15 min at 4℃ to obtain the culture filtrates.

For solid state fermentation (SSF), the pre-culture was mixed for seeding thoroughly with a sterile stainless steel spatula at a ratio of 1 ml to every 15 g of okara. Then, the cultures were grown for 72 h in a static incubator at 30℃ for yeast and 37℃ for bacteria. At the end of the fermentation period, 45ml of methanol was added to the fermented product of 15 g of intact okara and the mixture was shaken at 92 strokes/min for 60 min with reciprocal shaker, and then filtered to obtain the culture filtrate (Ohno et al., 1995).

Isolation of biosurfactant

For Liquid and submerged cultures, biosurfactant produced by *C. guilliermondii* was isolated according to Kim et al. (1999) as follows; the cell free extracellular fluid was twice extracted with 2 volumes ethyl acetate then the ethyl acetate phase was treated with anhydrous Na_2SO_4 to remove the residual water. Ethyl acetate was evaporated and the remaining material was dissolved in one volume of methanol and the oily residue in methanol was discarded. The oily syrupy crude biosurfactant was obtained after methanol evaporation. While in case of using the bacterial culture, biosurfactant was isolated from the *B. subtilis* cultures according to Roongsawang et al. (2002) as follows; the culture filtrate was subjected to acid precipitation by adding concentrated HCl to achieve a final pH of 2.0 and allowing the precipitate to form at 4℃ overnight. The precipitate was collected by centrifugation and washed three times with diluted HCl (pH 2.0). The biosurfactant was extracted three times with methanol and the solvent was removed using a rotary evaporator under vacuum conditions forming a white precipitate.

In case of solid state cultures, biosurfactants produced by both organisms were isolated according to Ohno et al. (1995) as follows: the solid crude extract of surfactant was isolated by adding 3 volumes of methanol to one volume of the solid media and the mixture was shaken at 92 strokes/min for 60 min with reciprocal shaker (New Brunswick Scientific, USA), the crude extract was filtered through a 0.20 µm membrane filter (GELMAN sciences , USA), white precipitate of biosurfactant was obtained after successive washing with methanol (Roongswang et al., 2002).

Functional properties

The oil displacement test was carried out slowly by dropping of 15 µl of crude oil onto the surface of 40 ml of distilled water layer contained in a Petri dish (15 cm in diameter) that spread all over the

Table 1. Media composition used for biosurfactant production by yeast and bacteria.

Medium	Composition	Microorganism	References
Control-1	It contains (g/l) Glucose,20 ; Glutamate, 5.0 ; $MgSO_4$, 0.5 ; KCl, 0.5 ; K_2HPO_4, 1.0; $FeSO_4.7H_2O$, 0.15; $MnSO_4$, 0.005;$CuSO_4.5H_2O$, 0.16; Yeast extract, 1.0, final pH 7.0.	*B. subtilis*	Nakano et al. (1988)
Control-2	It contains (g/l) Soybean oil, 100; NH_4NO_3, 1.0; K_2HPO_4, 2.55; NaH_2PO_4, 0.15; $MgSO_4.7H_20$,0.5 ; $CaCl_2.2H_2$ 0,0.1; $MnSO_4.H_2O$, 0.02; peptone, 1.0, final pH 7.8.	*C. guilliermondii* and *B. subtilis*	Kim et al. (1999)
A	As control-2 except that 100 g soybean oil was replaced by 100 g fresh okara.	*C. guilliermondii* and *B. subtilis*	
B	As control-2 supplemented with a mixture of soybean oil and fresh okara equivalent to 100 g oil.	*C. guilliermondii*	
Control-3	It contains 15 g of fresh okara; 833 µl aqueous solution of 0.45g/ml glucose, 75 µl of 1M KH_2PO_4, 225 µl of 1M $MgSO_4$ and 1ml H_2O to adjust the moisture to 82%.	*C. guilliermondii* and *B. subtilis*	Ohno et al. (1993).
C	As control-3 except that 10 g crude soybean oil was added to the medium	*C. guilliermondii* and *B. subtilis*	

water surface area. This was followed with the addition of 10 µl of an aqueous solution containing the surfactant (0.1%) onto the surface of the oil layer. The average value of the diameters of the clear zones of triplicate experiments was measured and recorded then calculated as percentage of the Petri dish diameter (Ohno et al., 1993).

Surface tension and critical micelle dilution (CMD^{-1}) were determined with Du-Nouy tensiometer (Kruss type 8451). CMD^{-1} is defined as the reciprocal of the biosurfactant dilution at which a sharp increase in surface tension is observed. To determine the CMD^{-1} different serial dilutions of the biosurfactants from *C. guilliermondii* or *B. subtilis* were tested (McInerney et al., 2004). The emulsification index was measured using the method described by Cooper and Goldenberg (1987) where 2 ml of hydrocarbons (hexane, hexadecane and motor oil) or vegetable oils (sunflower oil and soybean oil) were added to 2 ml of the biosurfactants solution (0.1%) from *C. guilliermondii* or *B. subtilis* in a graduated screw cap test tube, then mixed and vortexed at high speed for 2 min. Negative control of emulsification was carried out using water and motor oil. The emulsification index (E) was calculated by dividing the measured height of the emulsion layer by the total mixture height and multiplying by 100. The emulsion stability was determined after 24 h and was observed during consecutive 7 days.

Biosurfactants composition

Protein was estimated according to Lowry et al. (1951), using bovine serum albumin as a standard. Total carbohydrates were determined and calculated as glucose (Dubois et al., 1956). Lipid content was estimated either by total lipid test kit (Biodiagnostic BD) as described by Zollner and Kirsch (1962) in case of *C. guilliermondii* biosurfactant and according to Pedersen (1962) in case of *B. subtilis* biosurfactant.

Fourier transform infrared spectroscopy (FTIR)

The infrared (IR) spectrum (from 400 to 4000 wave numbers,cm^{-1}) of both biosurfactants were recorded using KBr pellet in Nicolet Impact 6100 FTIR spectrophotometer JASCO, USA.

Antimicrobial activity

Antimicrobial activity was determined on the basis of minimum inhibitory concentration (MIC) values, defined as the lowest concentration of antimicrobial agent needed to inhibit the development of visible growth after incubation for the required time. Serial dilutions of the biosurfactants were prepared in DMSO (dimethyl sulfoxide) in case of *C. guilliermondii* biosurfactant and in distilled water in case of *B. subtilis* biosurfactant. Control plates (zero concentration of biosurfactant) containing only nutrient agar for bacteria, and stock medium (Wickerman, 1951) for yeast were prepared.

The antimicrobial activities of the tested concentration (15 µl biosurfactant per disc) were determined by disc diffusion technique (British pharmacopoeia, 1968). The test microorganisms (*E. coli*, *B. subtilis*, *S. cerevisiae* and *C. albicans*) were spot-inoculated on each plate (15 µl per spot). The inoculated plates were incubated at 37°C for 24 h (bacteria) and 30°C for 48 h (yeast). The zone of inhibition was measured in mm, and mean values of inhibition zone were calculated from triple reading in each test.

Table 2. Oil displacement activity of biosurfactants produced from different media by *C. guilliermondii* and *B. subtilis*.

Medium		Oil displacement (%)	
Status	**Treatment**	***C. guilliermondii***	***B. subtilis***
Liquid	Control-1	----	37 ± 1.6
	Control-2	27 ± 1.1	5 ± 0.8
Submerged	A	70 ± 2.4	74 ± 2.7
	B	100 ± 0.2	58 ± 2.2
Solid	Control-3	57 ± 2.2	65 ± 2.1
	C	54 ± 1.8	48 ± 1.9

The values are average of 3 experiments and standard deviation.

RESULTS AND DISCUSSION

In this study, the yeast *C. guilliermondii* and the bacterium *B. subtilis* were examined for the production of biosurfactant in different media using different fermentation techniques of okara. Analysis of dry okara used in our previous work from the same source, revealed that it contains 10% fat, 38.8% total carbohydrate, 12.2% fiber and 28% protein. The moisture and ash contents were found to be 71.6 and 4.31% respectively (Rashad et al., 2010). Oil displacement activity was the criteria adopted for the selection of the best medium, producing high activity of the surfactant by both organisms, as it is directly proportional to the concentration and activity of the biosurfactants (Morikawa et al., 2000; Rodrigues et al., 2006). Three media status (liquid, submerged and solid) were used, utilizing okara as a substrate for the production of biosurfactant by *C. guilliermondii* NRRL Y-2075 and *B. subtilis* NRRL B-94 (Table 2). The submerged media (A & B), derived from the liquid media (Control-2) of kim et al. (1999) after modifying the carbon source, showed the highest oil displacement activities either for *C. guilliermondii* (100±0.2 and 70±2.4%) or *B. subtilis* (74±2.7 and 58±2.2%), respectively. Modification of the carbon source consisted of adding okara at the expense of or in addition to original soybean oil ingredient.

These values were much higher than the corresponding values obtained with the original liquid media (Control-2) for *C. guilliermondii* and *B. subtilis* (27±1.1% and 5±0.8%, respectively) and that of the synthetic media (Control-1) of Nakano et al. (1988), which was used in the current study with *B. subtilis* (37±1.6%). Hence, the difference in the productivity is not only related to the medium status but more relevantly to the carbon source. Substituting soy bean oil by okara in the medium of Kim et al. (1999) has effectively increased the production of the biosurfactant to 70±2.4 and 74± 2.7 in case of *C. guilliermondii* and *B. subtilis*, respectively. On the other hand, supplementing okara to the soybean oil in control (2) medium, maximized the biosurfactant activity in case of *C. guilliermondii* (100±0.2%) but negatively affected it in case of *B. subtilis* (58±2.2 %).

These results indicate that the utilization of okara as industrial by-product with soybean oil in the medium, provided good additional sources of carbon and nitrogen for growth stimulation and biosurfactant production from *C. guilliermondii* NRRL Y-2075. These results agree with Kim et al. (1999), Vance-Harrop et al. (2003), Rufino et al. (2007) and Sobrinho et al. (2008) who stated that a glycolipid-type biosurfactant can be produced from different species of Candida by using a medium consists of different oils as substrates (soybean oil, babassu oil, vegetable oil refinery residue and ground-nut oil refinery residue, respectively) supplemented with some nutrients in liquid state fermentation. The production of biosurfactant on a solid medium was conducted using the medium of Ohno et al. (1993) which used okara as the sole carbon source (Cotrol-3). Both *C. guilliermondii* and *B. subtilis* gave moderate oil-displacement based productivity, 57±2.2 and 65±2.1%, respectively, indicating relatively higher preference for the latter microorganism for such medium state. Modifying the carbon sources in the media (control-3) by adding additional carbon source of soybean oil (10 g) has negatively affected the oil displacement based productivity of the two microorganisms especially in case of the bacteria (48±1.9%). Hence, the addition of soybean oil in case of solid media has a different action from that observed on the submerged media.

The results also indicated that the activity of biosurfactant produced by microorganisms not only depends on the strain and medium composition, but it also depends on the fermentation type. Production of surfactin in solid state fermentation (SSF) using okara as a solid substrate by *B. subtilis* MI 113 was reported by Ohno et al. (1995). Also potato substrate and cassava waste water were found to be suitable carbon sources for surfactant production by *B. subtilis* ATCC 21332 and LB5a strains respectively (Fox and Bala, 2000; Nitschke and Pastore, 2006). The results illustrated in Table 2 indicated that, the submerged fermentation of okara with

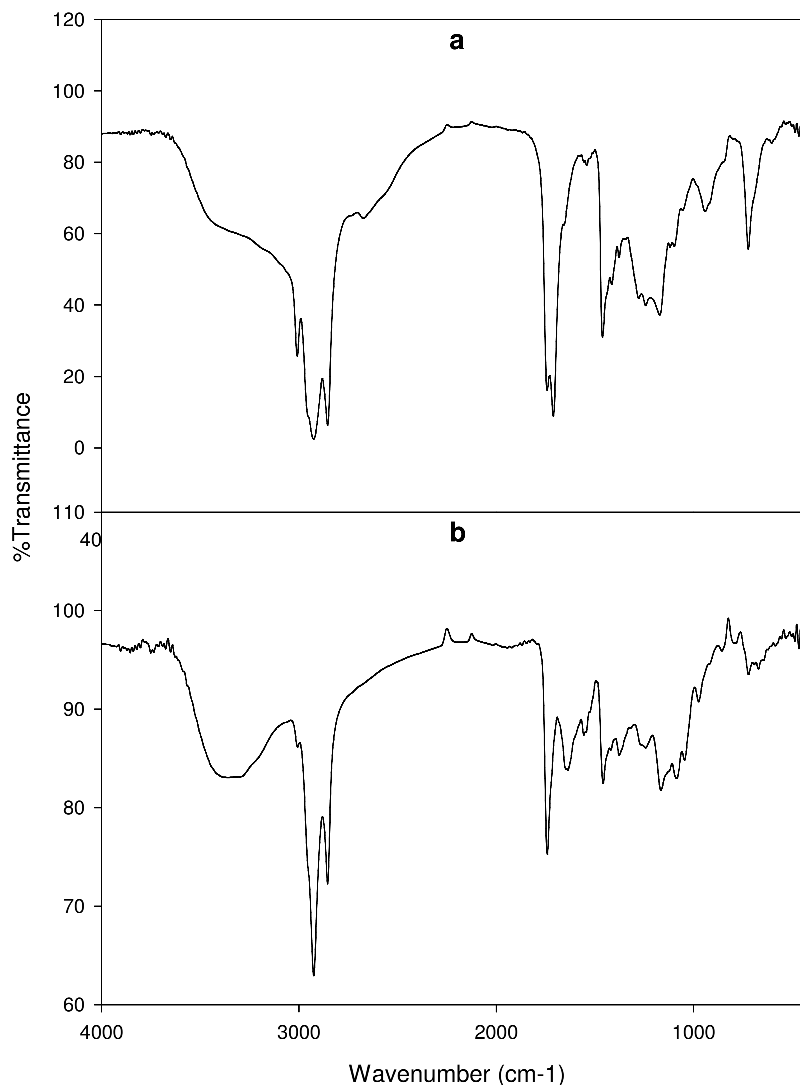

Figure 1. FTIR spectrum of two biosurfactants produced by *C. guilliermondii* (a) and *B. subtilis* (b).

media A and B by *B. subtilis* and *C. guilliermondii* respectively, was selected as the best conditions for biosurfactants production and the produced biosurfactants were subjected for further characterization.

Chemical characterization

The chemical analysis of *C. guilliermondii* biosurfactant revealed that, it is a complex of lipids (54 g%), carbohydrate (34 g%) and protein (2.3 g%) referring to its most probable glycolipid nature. These results agree with those observed by Batrakov et al. (2003) and Amaral et al. (2006). While that of *B. subtilis* was found to contain lipid, carbohydrate and protein content at ratios of 62.3, 7.6 and 18.6 g%, respectively. Hence, the bacterial biosurfactant may be a complex mixture of lipoprotein and glycolipids. So the chemical compositions and natures of the two biosurfactants are widely divergent and dependent mainly on the fermenting organism.

These data were confirmed by infrared spectroscopy. The IR spectrum of *C. guilliermondii* biosurfactant (Figure 1a) exhibited a band at 3673 cm^{-1} which is an indicator of significant water and O–H content, typical of polysaccharides. The spectrum also showed strong bands at 1711.51 and 1415.49 cm^{-1}, which is attributed to carboxylic carbonyls and C-N amide, respectively. An intense band at 1542.77 cm^{-1}, indicating the presence of carbonyl bond to amide. Also, the inclusion of the aliphatic chains (CH_3-CH_2-) was supported by the presence of bands at 2925, 2854 and 2673 cm^{-1}. These results confirm the previous primary conclusion based on approximate chemical composition that *C. guilliermondii* biosurfactant belongs mainly to glycolipid class in spite of

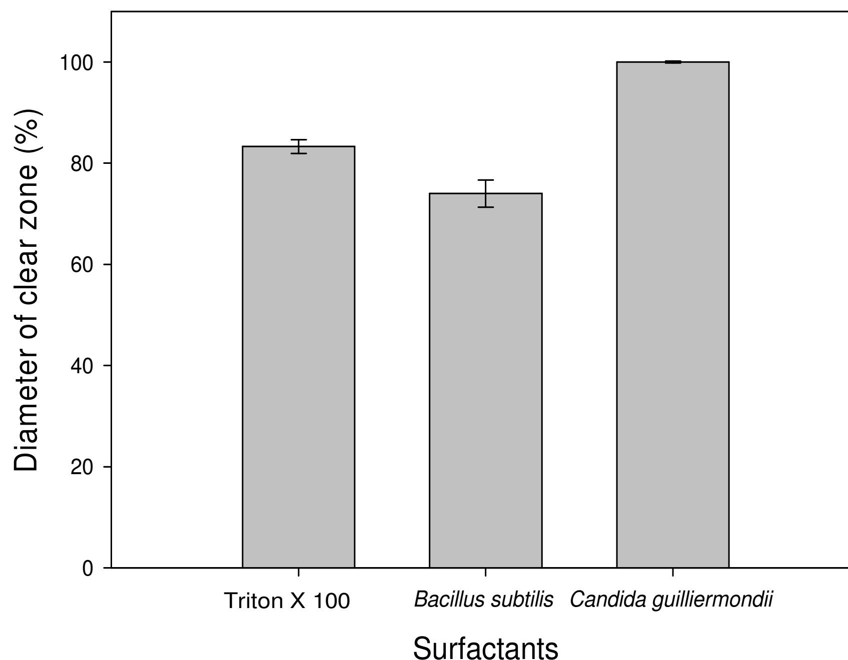

Figure 2. Percentage of the clear zone on the oil surface deduced from oil displacement testing with the biosurfactants produced by *B. subtilis* and *C. guilliermondii* compared with Triton X100. Error bars indicate standard deviations from triplicate measurements.

the indicators of the presence of few lipoprotein moieties in this complex biosurfactant. On the other hand, the IR spectrum (Figure 1b) of *B. subtilis* NRRL B-94 biosurfactant showed strong bands, indicating the presence of a peptide component at 3360 cm^{-1} resulting from the N-H stretching mode, and at 1637 cm^{-1} (stretching mode of the CO-N band). The bands at 2925 – 2855 cm^{-1} and at 1458 - 1376 cm^{-1} reflect aliphatic chains (CH_3 – CH_2 -), while the band at 1741 cm^{-1} refer to the presence of ester carbonyl group.

These results were strong evidence that the *B. subtilis* biosurfactant is a surfactin containing aliphatic and peptide-like moieties (cyclic lipopeptide) which agrees with the previous studies on the biosurfactins produced by *Bacillus* sp. (Nakayama et al., 1997; Joshi et al., 2008). It has been reported that different species of *Candida* genus produce lipid-carbohydrate complexes (Desai and Desai, 1992; Sobrinho et al., 2008), protein-carbohydrate complexes (Cirigliano and Carman, 1985), and protein-carbohydrate-lipid complexes (Pareilleux, 1979; Zinjarde et al., 1997; Sarubbo et al., 1999; Rufino et al., 2007). Other biopolymers produced by *Candida* species seem to be long fatty acids (Kappeli et al., 1978) and other complexes (Desai and Desai, 1992). Earlier reports on the chemical composition of *Bacillus* sp. biosurfactants were lipopeptides (Arima et al., 1968; Marahiel et al., 1977; Mukherjee and Das, 2005; Nitschke and Pastore, 2006) ,although Thavasi et al. (2008) found that the biosurfactant produced by *Bacillus megaterium* was classified as a glycolipid with carbohydrate and lipid combination.

Oil displacement efficiency

The data in Figure 2 illustrate the comparative oil displacement efficiency of the two prepared biosurfactants as compared to a well known industrial surfactant (Triton X-100). It is evident that the biosurfactant produced by *C. guilliermondii* NRRL Y-2075 (0.1%) gave the highest oil spreading efficiency (100% oil displacement). This is more effective than Triton X-100 (80% oil displacement) and *B. subtilis* NRRL B-94 biosurfactant (57% oil displacement) at the same concentration.

Surface tension

One of the important characterization properties of a potent surfactant is its ability to lower the surface tension in aqueous solutions. The surface tension and critical micelle dilution (CMD) were determined to evaluate the surface activity of the produced biosurfactants (Figure 3). CMD is an indirect indication of surfactant concentration (Makkar and Cameotra, 1997). As shown in Figure 3, it is

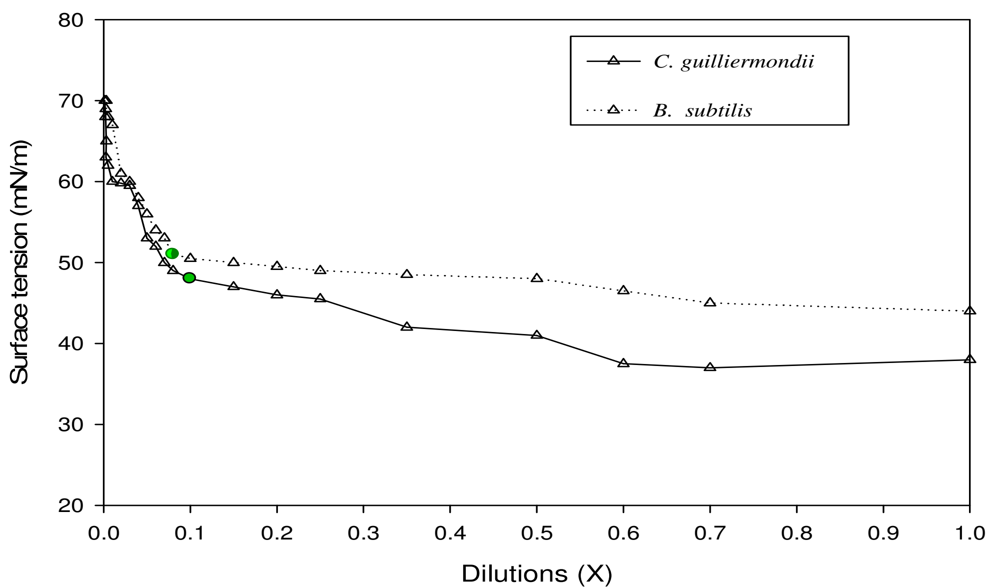

Figure 3. Surface tension of different dilutions of *C. guilliermondii* and *B. subtilis* biosurfactants. The critical micelle dilutions points (CMD-1) are marked in circles.

clear that the *C. guilliermondii* biosurfactant exhibited good surface tension reducing activity and the S.T. of water decreased from 70 to 51 mN/m when the biosurfactant was diluted to 0.08X (8% concentration) which corresponds to CMD. Also, the *B. subtilis* biosurfactant exhibited a good surface tension reducing activity when the S.T. of water decreased to 48 mN/m at a dilution of 0.1X (10% concentration) which corresponds to its CMD.

Generally, the CMD values of the two biosurfactants are in the same range of efficiency. The surface tension reducing effects of the prepared biosurfactants are similar to the results of Amaral et al. (2006), who found that the *Yarrowia lipolytica* biosurfactant has the ability to reduce S.T. of water up to 50 mN/m. Our results also illustrated that both biosurfactants produced, exhibited a higher minimum surface tension than that of biosurfactants produced from many *Candida* and *Bacillus* species, which ranged from 26 to 32 mN/m (Kim et al., 1997; Makkar and Cameotra, 1999; Nitschke and Pastore, 2006; Sarubbo et al., 2006; Rufino et al., 2007; Sobrinho et al., 2008).

Emulsification activity

Emulsification activities of both biosurfactants produced were measured with various water-immiscible substrates (Figure 4). Highest emulsion values for both biosurfactants were obtained using motor oil followed by sunflower oil. Soybean oil gave a good emulsification with *C. guilliermondii* biosurfactant but not as effective with *B. subtilis* biosurfactant. Hexane and hexadecane were not effectively emulsified by both biosurfactants. These results indicated that the two biosurfactants produced have high emulsification specificity towards both hydrocarbon and vegetable oils. The emulsification activities were more observed with long chain hydrocarbons or triglycerides than with short chain ones hydrocarbons.

In addition, the emulsification capacity favored the mineral oil over the vegetable oil, while it was not equal among all vegetable oils. The emulsification preference towards hydrocarbons (especially in case of *C. guilliermondii* biosurfactant) support the conclusion of the mostly glycolipid nature of this product. Similar results obtained by Pornsunthorntawee et al. (2008) with the crude *Pseudomonas aeruginosa* biosurfactant. The influence of time on emulsion stability of both biosurfactants was also studied (Figure 5). Most emulsions formed with motor oil or vegetable oils showed considerable stability during 7 days.

However, the emulsions formed under the action of *C. guilliermondii* biosurfactant were more stable with motor oil or vegetable oils than those formed under the action of *B. subtilis* biosurfactant. Prominent stability was particularly observed for emulsion formed with motor oil under the action of the two biosurfactants especially in case of *C. guilliermondii* one. The high emulsion stability of the two biosurfactants with motor oil may refer to the potential action of these two biosurfactants in removing oil pollution. The emulsification activity towards motor oil was also reported for biosurfactants from *Rhodococcus* bacteria (Abu-Ruwaida et al., 1991) and *Candida*

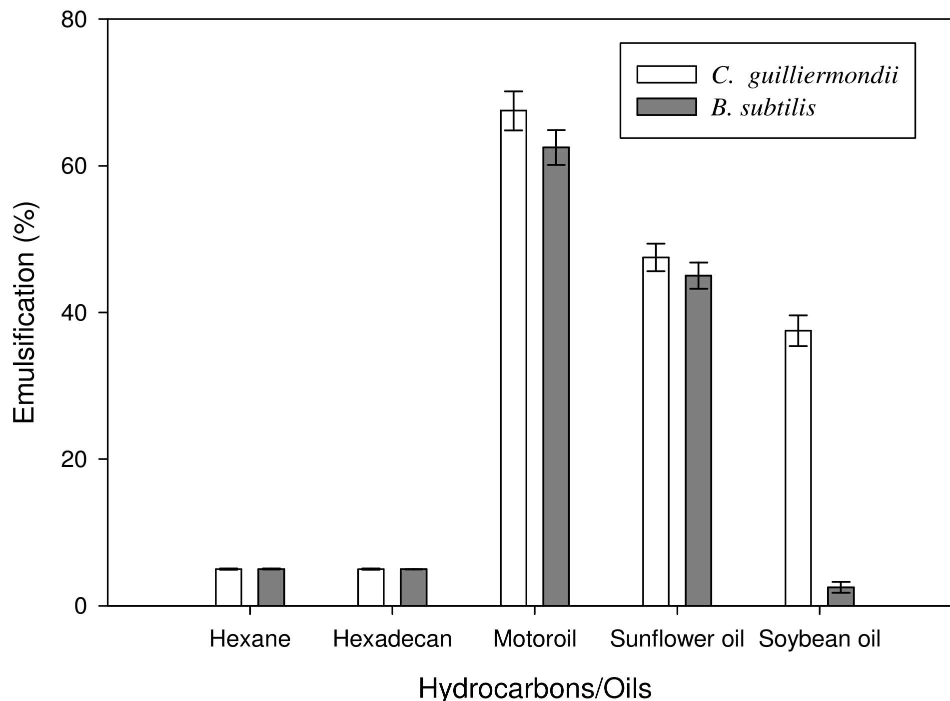

Figure 4. Emulsification activity after 24 h (E24) of two biosurfactants from *C. guilliermondii* and *B. subtilis* against different hydrocarbons and vegetable oils. Error bars indicate standard deviations from triplicate measurements.

lipolytica (Rufino et al., 2007).

Antimicrobial activity

Antimicrobial activity of different concentrations of both produced biosurfactants has been assessed against yeasts and bacteria (Gram +ve and -ve strains) based on the degree of growth inhibition of the tested organism. The results in Table 3 illustrate that, *C. guilliermondii* biosurfactant exhibited high antimicrobial activity against both Gram +ve and -ve bacteria. The lower minimum inhibitory concentration (MIC) value was found against *B. subtilis* (18.75 µg/ml) followed by *E. coli* (75 µg/ml). The growth inhibition of the tested yeast strains (*S. cerevisiae* and *C. albicans*) by *C. guilliermondii* biosurfactant was observed at concentrations >300 µg/ml. Table 3 also revealed that the *B. subtilis* biosurfactant was active against bacteria (Gram +ve and -ve) at MIC of 150 and 300 µg/ml against *E. coli* and *B. subtilis,* respectively.

It was also active against *S. cerevisiae* at MIC value of 150 µg/ml, but it was inactive towards *C. albicans* at concentration less than 300 µg/ml. Kitamoto et al. (1993) reported that both types of *Candida antarctica* biosurfactants (MEL-A and B) exhibited antimicrobial activity particularly against Gram-positive bacteria and their MIC ranged from 3.1 to 25 µg/ml but it was inactive towards the tested yeast strains at concentrations less than 400 µg/ml. Das and Mukherjee (2005) found that *P. aeruginosa* biosurfactant exhibited significant antimicrobial activity against Gram +ve, Gram -ve bacteria and yeasts at a concentration of 25 µg/ml. Das et al. (2009) also found that the extracellular biosurfactants produced by a marine bacterium using different carbon sources were highly active against a few bacterial and fungal strains and the MIC ranged from 50 to 450 µg/ml, according to the carbon sources used in the media.

Conclusion

The present study indicates that okara is a viable substrate for surfactant production by *C. guilliermondii* and *B. subtilis*, and its utilization in this production could be useful in the soybean processing industries waste management while addressing the economic issues related to surfactant production costs. The isolated biosurfactants have attractive properties as emulsifiers for both hydrocarbons and vegetable oils which suggest their potential use as emulsifying agents especially in the problems relating to pollution with petroleum and food industries as emulsifiers. Also, antimicrobial activity of the produced biosurfactants against bacteria (Gram +ve and -ve) and yeasts suggested the future use of these valuable molecules in pharmaceutical industry as

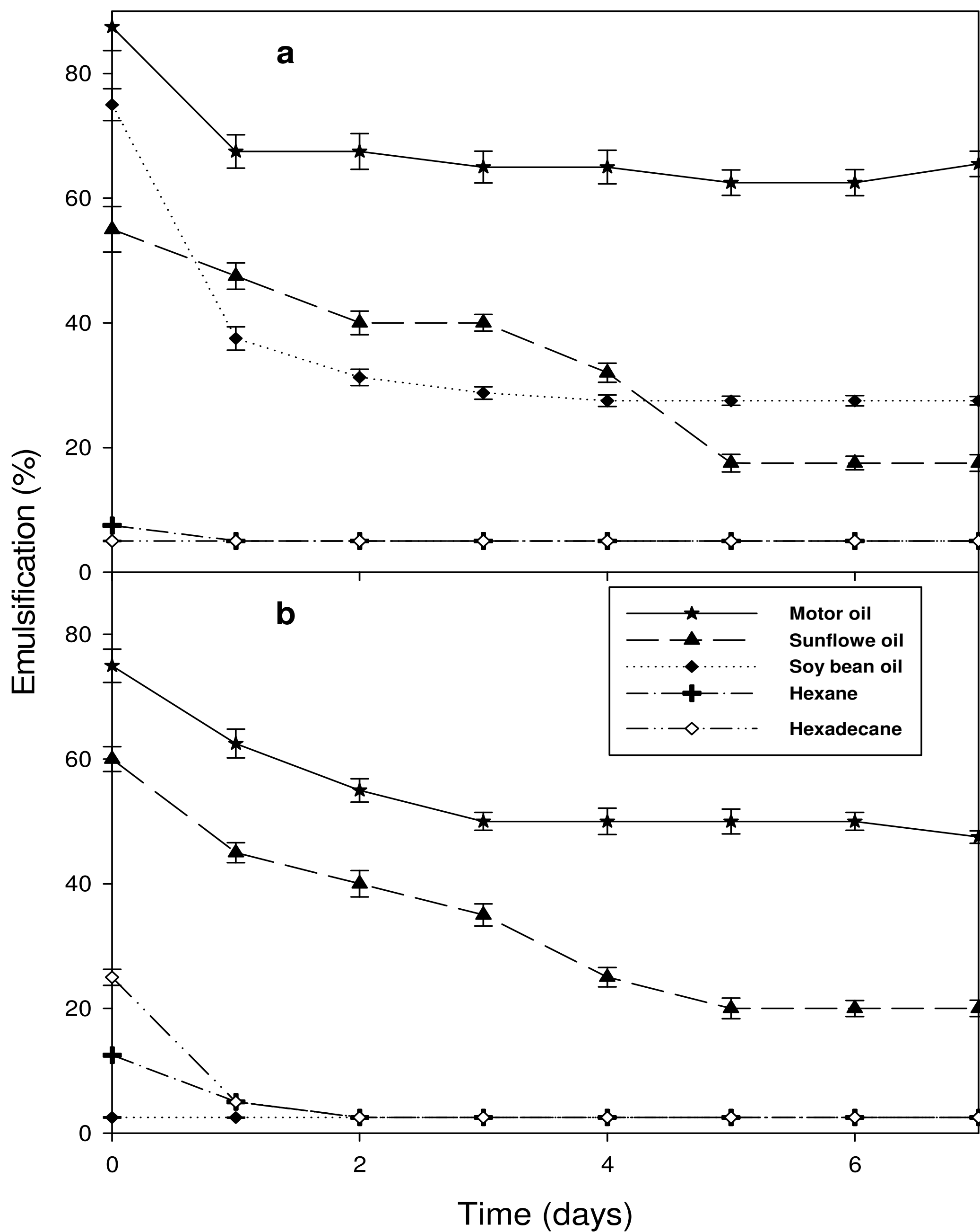

Figure 5. Emulsification stability of two biosurfactants from *C. guilliermondii* (a) and *B. subtilis* (b) during 7 days at room temperature. Error bars indicate standard deviations from triplicate measurements.

Table 3. Antimicrobial activity of biosurfactants obtained from okara under the action of *C. guilliermondii* and *B. subtilis*.

Test microorganisms	Minimum inhibitory concentrations (MIC)* (µg/ml)	
	C. guilliermondii **biosurfactant**	***B. subtilis*** **biosurfactant**
E. coli	75.0	150
B. subtilis	18.75	300
S. cerevisiae	> 300	150
C. albicans	> 300	>300

Results represent mean of three independent experiments. *MIC determined by disc diffusion technique.

antibiotics or food industries as preservatives.

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