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The yeast-like fungus *Aureobasidium thailandense* LB01 produces a new biosurfactant using olive oil mill wastewater as an inducer

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ABSTRACT

In this study, the biosurfactant production by an *Aureobasidium thailandense* LB01 was reported for the first time. Different agro-industrial by-products (corn steep liquor, sugarcane molasses, and olive oil mill wastewater) were evaluated as alternative low-cost substrates. The composition of the culture medium was optimized through response surface methodology. The highest biosurfactant production (139 ± 16 mg/L) was achieved using a culture medium containing yeast extract (2 g/L); olive oil mill wastewater (1.5%, w/w); glucose (6 g/L) and KH_2PO_4 (1 g/L) after 48 h of fermentation. The partially purified biosurfactant exhibited a critical micelle concentration of 550 mg/L, reducing the surface tension of water up to 31.2 mN/m. Its molecular structure was found to be similar to a lauric acid ester. The biosurfactant exhibited a better performance than the chemical surfactant sodium dodecyl sulfate (SDS) in oil dispersion assays, thus suggesting its potential application in bioremediation.

1. Introduction

The vast majority of the commercially available surfactants are of petrochemical origin. As such, their production contributes to an increased environmental pollution (Patel et al., 2003). Moreover, due to the low selectivity of petrochemical surfactants, significant amounts are usually required in the majority of the applications (Pacwa-Płociniczak et al., 2011; Sachdev and Cameotra, 2013). Microbial surfactants (so-called biosurfactants) are a promising alternative to the chemical surfactants; they exhibit a higher biodegradability, are less toxic and more stable at extreme temperature, salinity, and pH conditions when compared with their synthetic counterparts (Pacwa-Płociniczak al. 2011). Biosurfactants are as effective as the synthetic surfactants since they can also reduce the surface and interfacial tensions, stabilize oil-in-water and water-in-oil emulsions, and act as detergents, solubilizers, and lubricants (Makkar et al., 2011). These compounds are applied in the food, pharmaceutical and petrochemical industries (Gudiña et al., 2013b; Noparat et al., 2014a) and are usually present in detergents (Sajna et al., 2013), agriculture products (Sachdev and Cameotra, 2013) and cosmetics (Morita et al., 2013; Vecino et al., 2017). However, despite their potential applicability, the production of biosurfactants is still expensive when compared with synthetic surfactants, being about 60% of the production costs due to the culture medium (Gudiña et al., 2016a,b; Makkar et al., 2011). Therefore, the study of alternative substrates for the production of biosurfactants is expected to have a great impact on the sustainability of their production processes.

In this sense, the use of agro-industrial wastes as substrates for the production of different biomolecules has increased in the last years. Agro-industrial wastes are an excellent source of carbohydrates, lipids, and nitrogen that may be very useful in a number of bioprocesses, including the production of biosurfactants. Some examples of residues used for the biosurfactants production include animal fat, molasses, glycerol, corn steep liquor (CSL), wastes from olive oil production and from other vegetable oils extraction, distilleries residues, cheese whey, potato processing effluents and cassava wastewater (Gudiña et al., 2016a,b; Gudiña et al., 2015a; Makkar et al., 2011; Moya Ramírez et al., 2016; Noparat et al., 2014b; Saimmai et al., 2012; Santos et al., 2013). Olive oil mill wastewater (OMW), an agricultural waste generated during the extraction of olive oil, is widely available in the Mediterranean countries such as Spain, Italy, Greece, and Portugal. This residue contains sugars, lipids and aromatic compounds (Dermeche et al., 2013). OMW can be used in a number of biotechnological applications, as in the production of microbial enzymes (Brozzoli et al., 2009; Salihu et al., 2012), antioxidants (El-Abbassi et al., 2013;

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Rahmanian et al., 2014) and biosurfactants (Gudiña et al., 2016a,b; Mousavi et al., 2015 Ramírez et al., 2016). Another agro-industrial residue used as a source of amino acids, vitamins, and polypeptides is CSL, which is considered a good alternative source of organic nitrogen (Liu et al., 2015). Molasses, a by-product of the sugar refinery, which contains about 50% of sucrose besides other compounds, such as salts and organic acids, can also be used as an alternative carbon source (Mirończuk et al., 2015).

In addition to the composition of the culture medium, the C/N ratio and operational conditions (pH, temperature, agitation and aeration) greatly affect the production of biosurfactants and are commonly used in optimization strategies to increase productivity and reduce costs aiming at the development of sustainable bioprocesses (Ekpenyong et al., 2017; Henkel et al., 2012; Santos et al., 2016; Varjani and Upasani, 2017).

Several microorganisms can produce biosurfactants, including bacteria, yeasts, and some filamentous fungi. The genera *Pseudomonas* and *Bacillus* are the most extensively reported as biosurfactant producers (Gudiña et al., 2016a,b; Gudiña et al., 2015a; Sharma et al., 2015). The search for new surface active molecules has driven the study of microorganisms unexplored for this purpose, mainly from oil reservoirs and marine environments (Gudiña et al., 2016a,b; Gudiña et al., 2015b, 2013a; Manivasagan et al., 2014; Najafi et al., 2011; Saimmai et al., 2012; Sharma et al., 2015; Vilela et al., 2014). As a result, other microorganisms such as *Lachancea thermotolerans* (Mousavi et al., 2015), *Planococcusjake* (Kavitha et al., 2015), *Brevibacterium luteolum* (Vilela et al., 2014), *Sphingobacterium detergens* (Marques et al., 2012) and *Paenibacillus* sp. (Gudiña et al., 2015b) were recently reported as surface active molecules producers. Some studies suggested that certain *Aureobasidum* species play a major role in the degradation of petroleum (Xue et al., 2015). However, this role was not related to the production of biosurfactants by these isolates. Among the *Aureobasidium* species, the most studied is *Aureobasidium pullulans*, which produces pullulan (a polymer that can be used in microbial enhanced oil recovery to plug high permeability channels) and enzymes (Duan et al., 2008; Price et al., 2013). As far as we know, the production of biosurfactants by *Aureobasidium* species has only been reported for one *Auresobasidium pullulans* strain (Kim et al., 2015).

In this study, the agro-industrial by-products CSL, OMW, and sugarcane molasses were evaluated to develop a sustainable culture medium for the production of a new biosurfactant by an *Aureobasidium thailandense* strain isolated from the cashew (*Anacardium occidentale* L.) apple peduncle.

2. Materials and methods

2.1. Microorganism

The microorganism *A. thailandense* LB01 was isolated from cashew (*Anacardium occidentale* L.) apple peduncle at the Biotechnology Laboratory – LABIOTEC (Federal University of Ceará – UFC, Brazil). The microorganism was identified by molecular biology techniques (*data not shown*). The strain was stored at −80 °C in YPD medium supplemented with 20% (v/v) of glycerol. The inoculant was prepared in 250 mL Erlenmeyer flasks containing 100 mL of YPD broth that were incubated in an orbital shaker at 28 °C and 200 rpm for 12 h. The concentration of cells in the inoculum to start the fermentation was adjusted to 1×10^7 cells/mL, counted using a Neubauer improved cell chamber (Marienfeld GmbH, Germany). The inoculum size corresponded to 5% (v/v) of the final volume. The composition of YPD medium was: peptone 20 (g/L); yeast extract 10 (g/L); glucose 20 (g/L) at pH 6.5.

Table 1
Different culture media evaluated for the production of biosurfactant by *Aureobasidium thailandense* LB01.

	Synthetic medium	Medium 1	Medium 2	Medium 3	Medium 4
Glucose (g/L)	6.0	–	6.0	6.0	–
Molasses (%, w/w)	–	10.0	–	–	10.0
Yeast extract (g/L)	3.0	3.0	–	3.0	–
CSL (g/L)	–	–	3.0	–	3.0
Olive oil (%, w/w)	2.0	2.0	2.0	–	–
OMW (%, w/w)	–	–	–	2.0	2.0
KH_2PO_4(g/L)	1.0	1.0	1.0	1.0	1.0

2.2. Evaluation of carbon source, nitrogen source, and inducer for biosurfactant production

The strain *A. thailandense* LB01 was cultured using different carbon sources (sugarcane molasses and glucose) and nitrogen (yeast extract and CSL) sources, as well as two substrates that have been reported to induce biosurfactant production in different microorganisms, olive oil and OMW (Gudiña et al., 2016a,b). The composition of the different culture media studied is shown in Table 1. All media pH were adjusted to 5.5.

Sugarcane molasses and CSL were kindly donated by RAR (Refinarias de Açúcar Reunidas, S.A.; Portugal) and COPAM (Companhia Portuguesa de Amidos, S.A.; Portugal), respectively. The total sugars and total soluble protein concentrations present in molasses and CLS were determined using the phenol-sulfuric (Dubois et al., 1956) and Lowry methods (Lowry et al., 1951), respectively. Sugarcane molasses contained 490 g/L of carbohydrates and 0.6 g/L of protein, while CSL contained 75 g/L of carbohydrates and 5 g/L of protein. Moreover, sugarcane molasses contained 85 g/L of sugars, according to the DNS method (Miller, 1959). The concentration of molasses added to the different culture media was adjusted to correspond to 6 g/L of reducing sugars.

OMW was obtained from an olive oil mill located in the north of Portugal. OMW is mainly composed of water and residual oil. Its fatty acid composition, determined by GC/MS, was as follows (% (v/v)):11.9% palmitic acid (C16:0); 3.0% stearic acid (C18:0); 78.5% oleic acid (C18:1); 6.6% linoleic acid (C18:2).

The submerged fermentations were conducted in an orbital shaker for 144 h at 200 rpm and 28 °C, using 500 mL Erlenmeyer flasks containing 200 mL of culture medium. Samples (5 mL) were collected at 24 h intervals and centrifuged (2700*g*, 15 min) to obtain the cell-free supernatants for further analysis. Biosurfactant production was determined by measuring the surface tension of the cell-free supernatants, as described further on.

2.3. Optimization of the culture medium composition

After a preliminary evaluation of the effect of the carbon source, the nitrogen source and the inducer on biosurfactant production by *A. thailandense* LB01, three variables were selected to further optimize the composition of the culture medium, namely the concentrations of yeast extract, CSL and OMW. The carbon source and salt concentrations were kept constant at 6 g/L and 1 g/L, respectively. A fractional factorial design (2^{3-1}) with 3 repetitions at the central point, composed of a total of 7 trials, was carried out to determine which nitrogen source significantly affected the biosurfactant production. The production of biosurfactant was evaluated by the reduction of the surface tension in the cell-free culture broth as compared to the initial time point (0 h, i.e. no biosurfactant produced). *Statistica software v 13* was used for the

Table 2
Fractional factorial design (2^{3-1}) used to select the variables that affect the production of biosurfactant by *Aureobasidium thailandense* LB01, as inferred by the reduction of the surface tension of the culture medium after 48 h ST0: surface tension at 0 h ST48: surface tension at 48 h. (C): central point. Results correspond to the average of three measurements ± standard deviation.

Assays	Yeastextract (g/L)	OMW (% w/w)	CSL (g/L)	ST_0 (mN/m)	ST_{48} (mN/m)
1	1.0	1.0	2.0	37.0 ± 0.0	33.0 ± 0.0
2	2.0	1.0	1.0	49.0 ± 0.0	30.0 ± 0.0
3	1.0	3.0	1.0	52.0 ± 0.0	30.0 ± 1.0
4	2.0	3.0	2.0	47.0 ± 0.0	34.0 ± 1.0
5 (C)	1.5	2.0	1.5	49.0 ± 0.0	33.0 ± 0.0
6 (C)	1.5	2.0	1.5	50.0 ± 0.0	32.0 ± 0.0
7 (C)	1.5	2.0	1.5	48.0 ± 0.0	30.0 ± 0.0

experimental planning and analysis (Table 2).

As yeast extract and OMW were found to be the significant variables according to the fractional factorial design, a central composite rotated design (CCRD) was performed to optimize their concentrations towards a maximum biosurfactant production. The design was conducted with two variables at two levels (2^2), including 4 trials in the axial conditions and 3 repetitions at the central point, with a total of 11 trials. *Statistica software v 13* was used for the experimental planning and analysis (Table 3).

2.4. Surface tension measurement

The surface tension was measured using a KRÜSS K6 Tensiometer (KRÜSS GmbH, Hamburg, Germany) according to the Ring method (Rodrigues et al., 2006). The surface tension of the cell-free supernatants obtained after centrifuging the different samples taken along the fermentations was measured at 25 °C. The results were expressed as the average ± standard deviation, using the results obtained from each fermentation and each sample measured in triplicate.

2.5. Biosurfactant recovery

The biosurfactant was extracted from the cell-free supernatant by the Folch method (Folch et al., 1957). Briefly, one volume of cell-free culture broth was mixed with one volume of chloroform. After the mixture homogenization for 30 min, the organic phase was recovered using a separation funnel after resting for 24 h. Subsequently, chloroform was evaporated using a rotary evaporator at 450 mmHg and 40 °C for 40 min. After the solvent evaporation, the partially purified biosurfactant was freeze-dried for further characterization.

Table 3
Central composite design (2^2) used for the optimization of biosurfactant production by *Aureobasidium thailandense* LB01.ST_0: surface tension at 0 h. ST_{24}: surface tension at 24 h. ST_{48}: surface tension at 48 h. (C): central point. Results correspond to the average of triplicate measurements ± standard deviation.

Assays	Yeastextract (g/L)	OMW (% w/w)	ST_0	ST_{24}	ST_{48}
1	1.50	1.00	60.0 ± 0.5	33.0 ± 0.5	31.0 ± 0.5
2	4.50	1.00	53.0 ± 0.5	32.0 ± 0.0	30.0 ± 0.0
3	1.50	1.00	53.0 ± 2.0	31.0 ± 0.0	30.0 ± 0.0
4	4.50	3.00	50.0 ± 0.0	31.0 ± 0.0	29.0 ± 0.0
5	3.00	0.58	55.0 ± 0.0	31.0 ± 0.0	30.0 ± 0.0
6	3.00	3.40	50.0 ± 0.0	30.0 ± 0.0	29.0 ± 0.0
7	0.87	2.00	55.0 ± 1.0	31.0 ± 0.0	30.0 ± 0.0
8	5.10	2.00	51.0 ± 0.0	32.0 ± 0.0	30.0 ± 0.0
9 (C)	3.00	2.00	55.0 ± 0.0	31.0 ± 0.0	29.0 ± 0.0
10 (C)	3.00	2.00	56.0 ± 0.0	29.0 ± 0.0	29.0 ± 0.0
11 (C)	3.00	2.00	58.0 ± 0.0	31.0 ± 0.0	30.0 ± 0.0

2.6. Critical micelle concentration (CMC)

The critical micelle concentration (CMC) of the partially purified biosurfactant was calculated by plotting the surface tension against the logarithm of biosurfactant concentration. Biosurfactant concentrations ranging from 1 to 5000 mg/L were prepared in distilled water. The surface tension of each sample was measured in triplicate at 25 °C as described above. The CMC was found at the point of intersection between the two lines that best fit the pre- and post-CMC values (Gudiña et al., 2010).

2.7. Fourier transformed infrared Spectroscopy(FTIR)

The functional groups of the partially purified biosurfactant produced by *A. thailandense* LB01 were identified using a Cary 630 FTIR Spectrometer (Agilent Technologies), at wavelengths from 700 to 4000 cm^{-1}, with a spectral resolution of 1 cm^{-1}. The equipment allows the direct application of the lyophilized biosurfactant sample on the device cell without any sample preparation.

2.8. Mass spectrometry

The lyophilized biosurfactant (10 mg) was added to a mixture containing 5 mL of methanol and 15 µL of H_2SO_4. The mixture was heated at 60 °C for 1 h to esterify the sample. The analysis was performed in an ISQ single quadrupole Thermus GC/MS. The temperature was set to the range between 29.8 °C and 300 °C with an OV-1 capillary column (30 m × 0.25 mm ID × 0.25 mm film) using helium at 1.0 mL/min for the separation. The identification of the methyl esters was done by mass comparison with the equipment library compounds.

2.9. Oil displacement assays

The ability of the biosurfactant produced by *A. thailandense* LB01 to displace crude oil was evaluated in Petri dishes (50 mm diameter) containing 10 mL of distilled water. 700 µL of crude oil were dropped on the water surface to form a film covering the entire water surface area. The biosurfactant solution was prepared weighing the adequate amount of lyophilized biosurfactant and dissolving it in distilled water. After that, 40 µL of a biosurfactant solution (at a concentration of 10 mg/mL) were added in the middle of the crude oil layer, and the diameter of the clear zone formed was measured. Sodium dodecyl sulfate (SDS) was used as a reference surface active compound at the same concentration. Considering that the CMC of SDS is approximately 2332 mg/L, in order to keep the same ratio as the one used for the biosurfactant (18 X CMC), a second SDS concentration at 42 mg/mL (SDS-C2) was also tested. The negative control was performed using distilled water. All assays were performed in triplicate (Rodrigues et al., 2006).

3. Results and discussion

3.1. Study of biosurfactant production by A. thailandense LB01

When *A. thailandense* LB01 was grown in the synthetic medium (containing glucose and yeast extract as carbon and nitrogen sources, respectively), the surface tension of the culture medium was reduced up to 33.0 mN/m after 48 h (Table 4). As the surface tension was reduced below 40 mN/m, which is the widely accepted criterion to consider a microorganism as biosurfactant producer (Dzięgielewska and Adamczak, 2013; Gudiña et al., 2010; Vilela et al., 2014), it can be concluded that *A. thailandense* LB01 produced extracellular biosurfactants.

Subsequently, different culture media were prepared (as summarized in Table 1) to evaluate the potential use of low-cost alternative substrates (CSL, OMW and sugarcane molasses) for the production of

Table 4
Surface tension values (mN/m) obtained with *Aureobasidium thailandese* LB01 grown in different culture media at different time intervals. The composition of the different culture media is provided in Table 1. Results represent the average ± standard deviation of three independent experiments.

Time (h)	Surface Tension (mN/m)				
	Synthetic medium	Medium 1	Medium 2	Medium 3	Medium 4
0	48.0 ± 0.5	50.0 ± 0.0	49.5 ± 0.7	50.5 ± 0.7	50.0 ± 1.4
24	38.0 ± 1.0	35.0 ± 0.0	39.0 ± 0.0	35.5 ± 0.7	43.5 ± 2.1
48	33.0 ± 0.0	33.0 ± 1.4	37.0 ± 2.5	32.0 ± 1.4	35.0 ± 0.0
72	33.0 ± 0.0	33.0 ± 1.4	41.0 ± 2.5	32.5 ± 2.1	34.0 ± 0.0
96	33.0 ± 0.5	32.5 ± 0.7	43.0 ± 0.0	32.5 ± 0.7	36.5 ± 0.7
120	34.1 ± 0.5	32.5 ± 0.7	42.0 ± 0.0	32.0 ± 0.0	34.0 ± 1.4
144	33.0 ± 0.5	32.5 ± 0.7	42.0 ± 0.0	32.0 ± 0.0	34.0 ± 1.4

biosurfactants by *A. thailandense* LB01. According to the results obtained (Table 4), the replacement of glucose by sugarcane molasses (Medium 1) favored the production of biosurfactants by *A. thailandense* LB01, as the surface tension of the culture medium was reduced by 17.0 ± 1.4 mN/m after 48 h of fermentation. However, the use of CSL as the only nitrogen source (Medium 2) led to worst results when compared with the synthetic medium. The replacement of olive oil by OMW (Medium 3) also favored the production of biosurfactants by *A. thailandense* LB01, with a surface tension reduction of 18.0 ± 2.0 mN/m after 48 h of growth, similar to the result obtained with the synthetic medium. Finally, using a culture medium containing the three alternative substrates simultaneously (Medium 4) resulted in the same surface tension reduction achieved with the synthetic medium (Table 4).

The results obtained in the current study clearly demonstrate the ability of the newly isolated *Aureobasidium* strain to produce surface active compounds within 48 h of fermentation using different agricultural residues. It is important to highlight that this is the first report of a biosurfactant-producing *A. thailandense* strain. To the best of our knowledge, biosurfactant production by members of the genus *Aureobasidium* was only previously reported for an *A. pullulans* strain (Kim et al., 2015).

To assess the effect of OMW, yeast extract and CSL on biosurfactant production by *A. thailandense* LB01, a fractional experimental design (2^{3-1}) was carried out as shown in Table 2. Glucose and KH_2PO_4 were kept constant at the same concentrations given in Table 1 for the synthetic medium (i.e. 6 g/L for glucose and 1 g/L for KH_2PO_4).

As the fractional experimental designs are used only for the selection of variables and not for optimization, the confidence interval was set to 90%. The Pareto chart of the obtained effects is illustrated in Fig. 1. CSL showed a significant and adverse effect on the biosurfactant production (i.e. on the reduction of the culture medium surface tension), while OMW showed a positive and significant effect ($p < 0.1$). Several studies reported the suitability of yeast extract for the production of biosurfactants (Dzięgielewska and Adamczak, 2013; Maass et al., 2015; Manivasagan et al., 2014; Mousavi et al., 2015; Solaiman et al., 2007). Therefore, given the high negative impact of CSL on biosurfactant production, yeast extract was the nitrogen source selected for further optimization of the biosurfactant production process.

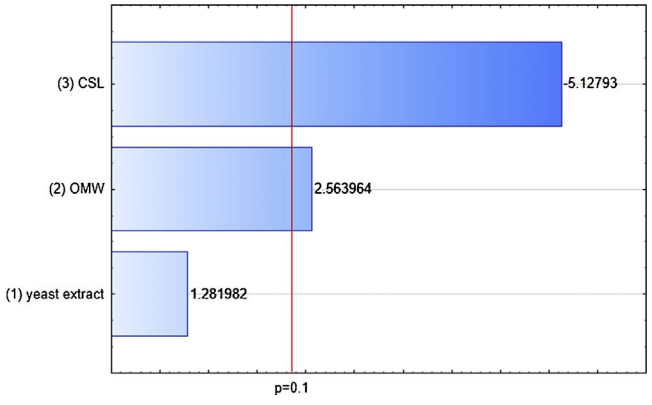

Fig. 1. Pareto diagram of the fractional factorial design used to select the variables (nitrogen and carbon sources) that affect the production of biosurfactant by *Aureobasidium thailandense* LB01.

The results obtained with the central composite design (CCRD) carried out to optimize the biosurfactant production process are summarized in Table 3. As it can be seen, surface tension reductions ranging from 20 mN/m to 30 mN/m were obtained after 48 h of fermentation. The regression models obtained to predict the surface tension reduction after 24 h and 48 h of fermentation are given in Eqs. (1) and (2), respectively:

$$Z = 20.18 + 4.41x - 1.87x^2 + 3.3y - 0.94y^2 + 0.5xy \quad (1)$$

$$Z = 17.9 + 3.95x - 0.87x^2 + 5.85y - 1.97y^2 + 0.21xy \quad (2)$$

where Z is the surface tension reduction, (mN/m); Y is the OMW concentration, % (w/w) and X is the yeast extract concentration, (g/L).

The models are adequate to fit the experimental data according to the ANOVA and F-test. For Eq. (1), the calculated $F_{5,5}$ value ($F_{calculated} = 13.47$) was higher than the listed value ($F_{5,5} = 5.05$) at a confidence level of 95%, meaning that the model is statistically significant. For Eq. (2), the calculated $F_{5,5}$ value ($F_{calculated} = 15.45$) was also higher than the listed one at 95% of confidence interval. The correlation coefficients (R^2) were higher than 0.90, thus demonstrating a good similarity between the experimental results and the values predicted by the fitted equations. The response surface graphs are shown in Fig. 2a and b.

The regression models were validated by repeating the fermentation (in triplicate) at the maximum response (i.e. higher surface tension reduction), which corresponds to 2 g/L of yeast extract and 1.5% (w/w) of OMW. The optimal values were mathematically obtained at the equation critical point and were the same for both fermentation times (24 and 48 h). The experimental values did not differ from those predicted by the fitted models (Eqs. (1) and (2)) (Table 5).

In the optimized culture medium, the concentration of yeast extract was reduced by 33% when compared with the synthetic medium (Table 1). It was found that the use of 2 g/L of yeast extract in combination with 1.5% (w/w) of OMW was the most favorable condition for the production of biosurfactants by *A. thailandense* LB01.

Similar surface tension reductions (between 14 and 30 mN/m) than the obtained in the present work were reported for other biosurfactant-producing yeasts (*Candida bombicola*, *Pseudozymaaphidis*, *Pseudozyma antarctica*) and bacteria (*Bacillus subtilis*, *Brevibacterium luteolum*, *Geobacillusstearo thermophilus*, *Paenibacillus alvei*), in some cases using agro-industrial wastes as substrates (Delbeke et al., 2015; Dzięgielewska et al., 2013; Jara et al., 2013; Maass et al., 2015; Najafi et al., 2011; Solaiman et al., 2007; Vilela et al., 2014).

In the current study, high surface tension reductions were obtained with *A. thailandense* LB01 even at 24 h of fermentation, using lower amounts of yeast extract as compared to those reported in other works (5–10 g/L) (Dzięgielewska and Adamczak, 2013; Maass et al., 2015; Mousavi et al., 2015; Solaiman et al., 2007). The results herein gathered clearly demonstrate that *A. thailandense* LB01 produces an effective biosurfactant using a residue generated during olive oil production as an inducer. The use of this residue can reduce the production costs of this biosurfactant and, at the same time, is interesting to promote other applications for OMW and to reduce the environmental impact caused by its disposal (Dermeche et al., 2013; Gudiña et al., 2016a,b; Mousavi et al., 2015).

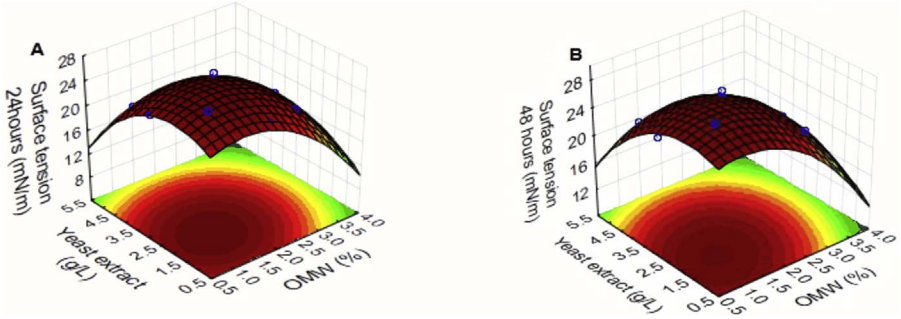

Fig. 2. Response surfaces representing the effect of OMW and yeast extract on the surface tension of the culture medium (which is the indicative of biosurfactant production) at 24 h **(A)** and 48 h **(B)**.

Table 5
Validation of the experimental design at the optimum point. Results correspond to the average of three independent experiments ± standard deviation.

Time(h)	Surface tension reduction (mN/m)	
	Observed	Predicted
24	27 ± 2.5^{aA}	27^{aA}
48	28 ± 2.6^{aA}	28^{aA}

Values with lowercase letters in the same line and the same capital letters equal in the same column do not differ by Tukey test ($p < 0.05$).

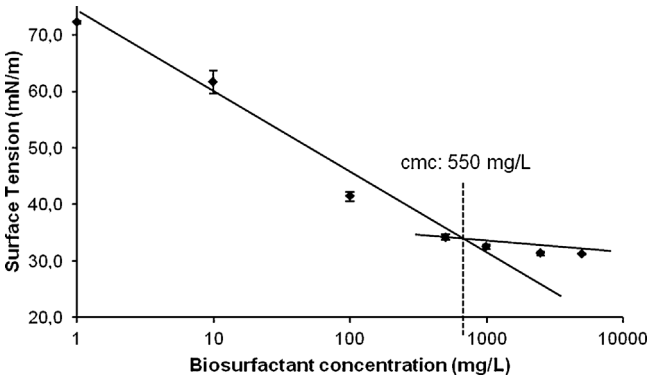

Fig. 3. Surface tension values (mN/m) *versus* logarithm of biosurfactant concentration (mg/L) obtained with the biosurfactant produced by *Aureobasidium thailandense* LB01 dissolved in distilled water. Results represent the average of three independent measurements and error bars represent standard deviations of the mean values.

3.2. Critical micelle concentration (CMC)

The CMC determined for the biosurfactant produced by *A. thailandense* LB01 was 550 mg/L, and it reduced the surface tension of water from 72.2 to 31.2 mN/m (Fig. 3). A surface active molecule is considered a good surfactant when it reduces the surface tension of water to values close to 35 mN/m (Gudiña et al., 2010). Thus, the biosurfactant herein produced can be considered an effective surface active compound.

Other authors reported CMC values for biosurfactants produced by different microorganisms between 64 mg/L and 2500 mg/L (Bhardwaj et al., 2015; Gudiña et al., 2010; Marin et al., 2015; Oliveira et al., 2013). Synthetic surfactants such as sodium dodecyl sulfate (SDS) and tetra decyl trimethyl ammonium bromide (TTAB) exhibit CMC values between 1000 mg/L and 2000 mg/L. Overall, the CMC value obtained for the biosurfactant produced by *A. thailandense* LB01 is in good agreement with the general values reported in the literature for other biosurfactants, and better than those reported for synthetic surfactants.

The biosurfactant production yield achieved with *A. thailandense* LB01 was 139 ± 16 mg/L after 48 h of fermentation. As far as we know, biosurfactant production by *A. thailandense* strains has never been reported before. However, an *Aureobasidium pullulans* strain which

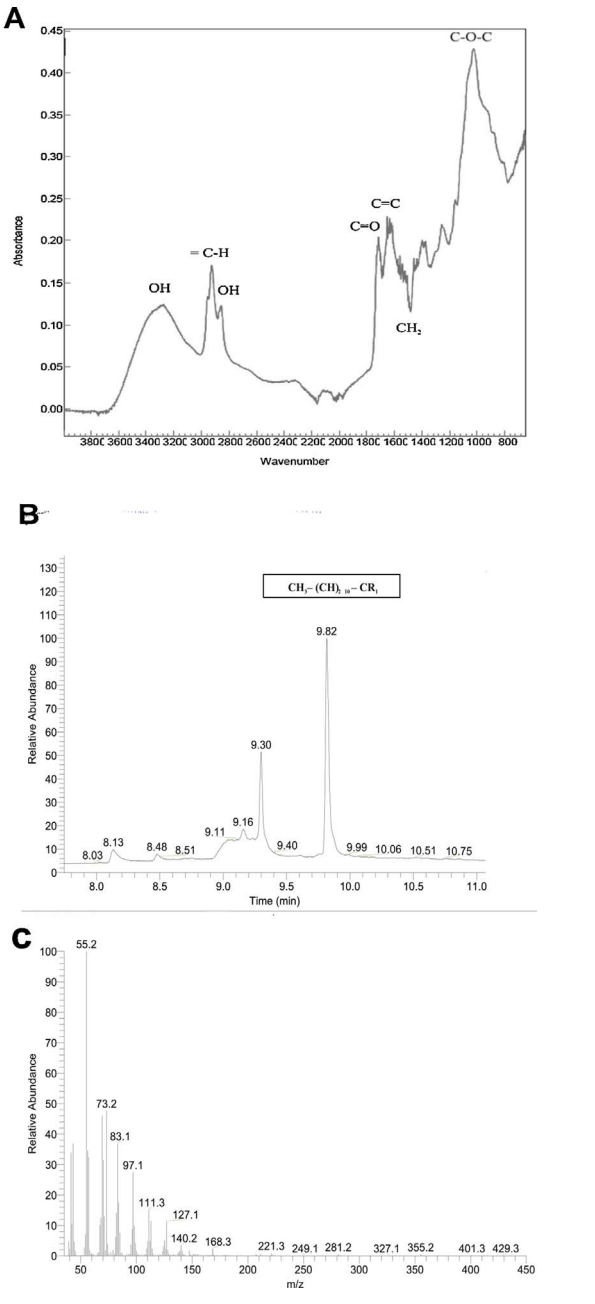

Fig. 4. Fourier Transformed Infrared Spectroscopy (FTIR) spectrum **(A)** mass spectrum **(B)** and chromatogram **(C)** obtained for the biosurfactant produced by *Aureobasidium thailandese* LB01.

Table 6
Diameter of the clear zones formed in the crude oil layer due to the addition of the biosurfactant, SDS-C1 (10 mg/mL) and SDS-C2 (42 mg/mL), at time 0 and after 24 h. Results correspond to the average of three independent experiments ± standard deviation.

Sample	Diameter of the clear zone (cm)	
	0 h	24 h
Biosurfactant	4.2 ± 0.3	4.2 ± 0.3
SDS-C1	3.6 ± 0.4	0.0
SDS-C2	3.5 ± 0.4	2.9 ± 0.0
Distilled water	0.0	0.0

produced a glycolipid biosurfactant (glycerol-liamocin) was reported by Kim et al. (2015). In this case, the biosurfactant reduced the surface tension of water up to 31.5mN/m. However, the production yield (37 mg/L) was considerably lower when compared with that achieved with *A. thailandense* LB01 in the present work.

3.3. Chemical characterization

The FTIR spectrum of the biosurfactant produced by *A. thailandense* LB01 is shown in Fig. 4.A. From the spectrum, it can be observed the carbohydrate −OH group at 3285 cm^1; the =C−H corresponding to fatty acid peaks at 2953 cm^{-1}; and the characteristic ester group peak at 2852 cm^{-1}. At 1712 cm^{-1} and 1633 cm^{-1}it is possible to confirm the presence of the unsaturated ester groups (C=O−C=C), and the peak at 1407 cm^{-1} that corresponds to the $-CH_2$ group.

The mass spectrum and chromatogram showed that the molecular structure of the biosurfactant is similar to a C_{12} molecule at the peak at 9.82 min. This structure corresponds to CH_3-$(CH_2)_{10}$-, which is similar to a lauric acid ester. The peak at 9.30 min corresponds to an impurity (Fig. 4B and C). According to these results, the biosurfactant comprises aliphatic hydrocarbons combined with a lipid moiety, resulting from the presence of unsaturated fatty acid groups. Thus, the molecule holds in its composition both a hydrophobic and a hydrophilic part, which is characteristic of surface active compounds.

3.4. Oil displacement studies

The oil dispersion ability of the biosurfactant produced by *A. thailandense* LB01 was evaluated using crude oil. The diameter of the clear zones obtained with the synthetic surfactant SDS and the biosurfactant are presented in Table 6. Fig. 5 shows the clear zones formed due to the action of both surface active compounds.

The results obtained demonstrate that the biosurfactant produced by *A. thailandense* LB01 holds a higher oil spreading efficiency than SDS, dispersing almost entirely the oil. Furthermore, contrary to the biosurfactant, the lowest concentration of SDS tested (SDS-C1) did not maintain its dispersing activity after 24 h. Using the higher SDS concentration (42 mg/mL, SDS-C2), an improvement of the surface activity was found but still less pronounced than the biosurfactant (Table 6). These results were similar to those reported for the biosurfactants produced by *Candida lipolytica* UCP0988 and *Geobacillus stearothermophilus* UCP 0986 (Jara et al., 2013; Santos et al., 2013),indicating a good surfactant activity against hydrocarbons and the potential application of this biosurfactant in bioremediation processes.

4. Conclusions

In this work, biosurfactant production by an *A. thailandense* strain was reported for the first time. Different agro-industrial by-products were evaluated as substrates for biosurfactant production, and the best results were obtained with a culture medium containing glucose, yeast extract, and OMW. The biosurfactant reduced the surface tension of water up to 31.2 mN/m, and its molecular structure was preliminarily determined as similar to a lauric acid ester. The ability of this biosurfactant of dispersing crude oil was also demonstrated, highlighting its potential applicability in bioremediation processes.

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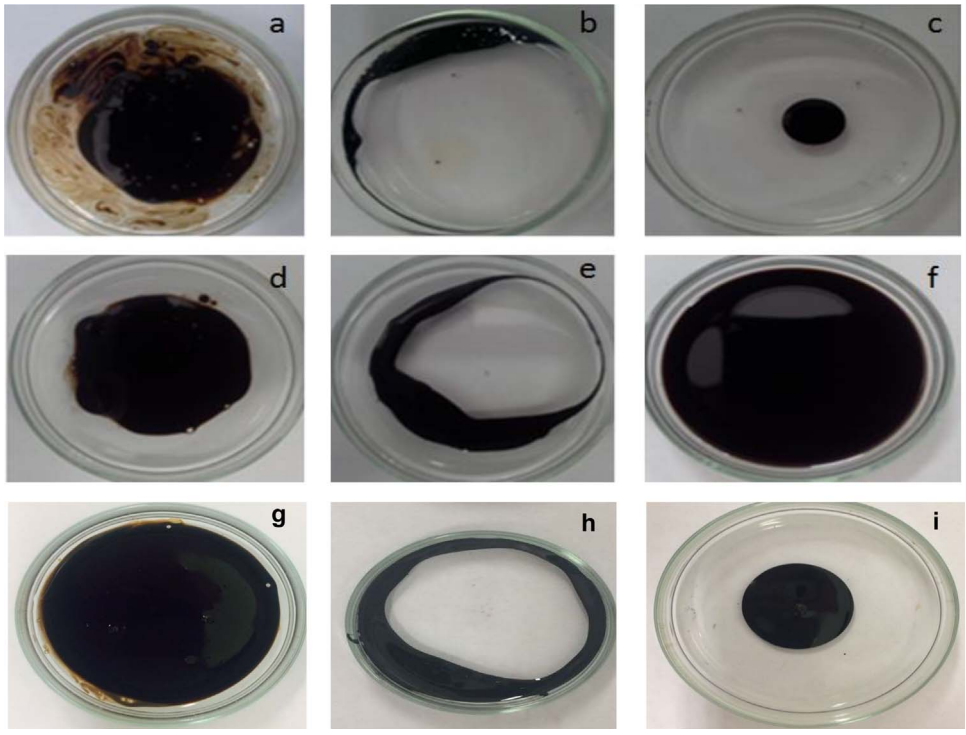

Fig. 5. Results of the oil dispersion assays performed using the biosurfactant produced by *Aureobasidium thailandense* LB01 and SDS. (a), (d) and (g): distilled water; (b), (e) and (h): clear zone formed immediately after the addition of the biosurfactant produced by *Aureobasidium thailandense* LB01 (10 mg/mL), SDS-C1 (10 mg/mL) and SDS-C2 (42 mg/mL), respectively; (c), (f) and (i): clear zones formed 24 h after the addition of the biosurfactant produced by *Aureobasidium thailandense* LB01 (10 mg/mL), SDS-C1 (10 mg/mL) and SDS-C2 (42 mg/mL), respectively.

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