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Utilisation of agro alimentary wastes for bio surfactants production by a thermophilic bacterial strain novelty isolated from an Algerian crude oil contaminated soil

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ABSTRACT/RESUME

Abstract: *In this work we have undertaken a study based on the production and characterization of biosurfactants from a thermophilic bacterial strain bacteria isolated from soil contaminated with petroleum hydrocarbons. The results indicate a reduction in the surface tension (32 mN /m) by the strain 1J. The influence of various parameters (carbon source, nitrogen source, pH, salinity) on the emulsifying activity and the surface tension reduction revealed that the olive oil mill effluent (0.5%) and ammonium chloride present the best carbon and nitrogen sources for biosurfactant production by this strain. The maximum of biosurfactant production was obtained at near neutral pH and a salinity of 2%. The biosurfactant produced by our strain improved a resistance to extreme conditions of temperature (4 to 121°C), pH (2-12) and salinity (up to 250 g / l). These interesting characteristics of our local biosurfactant find numerous applications in various food areas.*

I. Introduction

The biosurfactants are surface active molecules synthesized by some microorganisms such as fungus, yeasts and bacteria. Generally, theses biomolecules are classified into low molecular mass (glycolipids, lipopeptides and phospholipids) which efficiently lower surface and interfacial tension, and high molecular weight polymers, which are more effective as emulsion stabilizing agents **[1]**. Industrially advantageous uses of biosurfactants are recently favoured comparatively with chemical surfactants (e.g. textile, leather, metallurgy, oil, petrochemistry, etc). Those are justified by their biodegradability due to the simple chemical structure, compatibility with the environment and low toxicity. These bioproducts are also present in the formulations of the current consumable product such as the detergents, the cosmetics, the agroalimentary (emulsifying, dispersing, anti-adhesive agents) and the medicinal products (antibiotics). Moreover, they are used in the environmental field like the re-mobilization of the grounds contaminated by the insoluble substances (hydrocarbons) **[2].** The aim of the present work is to study the production and characterization of biosurfactants from a thermophilic strain isolated locally. The main factors affecting the production and characterization of biosurfactants such as: the nature and concentration of the carbon and the

nitrogen sources, pH and salinity of medium were studied, the physicochemical stability of biosurfactants against the pH of the medium and the salinity were also studied.

II. Materials and Methods

The biotope used to isolate the aerobic bacterial strain is a sandy soil contaminated by hydrocarbons at Hassi-Messaoud region (south of Algeria). (Article Eddouaouda, 2012).

II.1. Media and culture conditions

To obtain overnight culture, the strain 1J was grown on the Luria-Bertani (LB) liquid medium composed (g.L^{-1} distilled water): 10 peptone, 5 yeast extract, 10 NaCl at pH 7, autoclaved at 121°C for 20 min and inoculated by one colony, and then incubated during 16h at 45 °C to obtain a maximal growth (DO_{620}=1). For biosurfactant production, a mineral salt medium with the following composition (g.L^{-1} distilled water) was utilized: 0.3 KH_2PO_4, 0.4 NH_4Cl, 0.33 $MgCl_2$, 0.05 $CaCl_2$, 0.1 yeast extract, 10 NaCl plus 1 ml of the trace-element solution (g.L^{-1} distilled water): 0,25 H_3BO_4, 0,5 $CuSO_45H_2O$, 0,5 $MnSO_4$ H_2O, 0,06 $NaMoO_4$, 0,7 $ZnSO_4H_2O$. The pH was adjusted to 7 and the medium was sterilized by autoclaving at 121°C for 20 min. Agroalimentary wastes were added as carbon and energy source at desired concentration (g.L^{-1}). This medium was inoculated by overnight culture a raison of 1% (v/v). The incubation was carried out at 45 °C and 150 rpm.

II.2 Optimization of biosurfactant production

A series of experiments were realized in each we change carbon (C) or nitrogen (N) source, pH and the salinity of the medium as follows:

- Four carbon sources were used in the present study in order to select the optimum source for maximum biosurfactant production: olive oil mill effluent (1%, v/v), frying oil (1%, v/v), whey (0,5%, v/v) and orange juice process effluents (0,5%, v/v) **[3]**.
- Various concentrations of the best carbon source were tested (0,5 ; 1 ; 2 ; 5 et 10 %, v/v).
- Four nitrogen sources were used namely: potassium nitrate (KNO_3), ammonium nitrate (NH_4NO_3), ammonium sulfate $(NH_4)_2$ SO_4 and ammonium chloride (NH_4Cl).
- The pH values were adjusted as follows: 3, 5, 7, 9 and 11.
- The sodium chloride (NaCl) concentration was adjusted at: 0, 20, 40, 60 and 80 g.L^{-1}.

Cell-free supernatants, obtained by centrifugation at 4000 rpm during 30 min, used to measure the surface tension reduction and emulsification activity (E24%).

II.3. Biosurfactants recovery

After 24 hours of incubation at optimum conditions, the culture broth was centrifuged at 4000 rpm during 30 min. The supernatant, adjusted to pH 2 employed HCl solutions (3N), was precipitated during one night at 4 °C. The precipitate is recovered by centrifugation with 4000 tr/min during 30 min, and extracted with ethyl acetate this last was then evaporated using a rotavapor **[4]**.

II.4. Biosurfactant Stability

For stability assay, the crude biosurfactant was incubated at different temperatures: 4, 25, 30, 40 and 70 °C for 24h. Sodium chloride was added to the supernatant at different concentrations: 10, 20, 50, 100, 150, 200 and 250 g.L^{-1}. The pH of the supernatant was varied from 2 to 12, using HCl or NaOH stock solution. After a rest period of 24 h, the surface tension and emulsifying index were measured **[5]**.

II.5. Surface tension and emulsification index (E_{24}%)

A manual tensiometer (KRÜSS) was used to measure the supernatants surface tension, using the Wilhelmy standard method. The values reported are the average of three measurements. All measurements were made on cell-free broth obtained by centrifuging the cultures at 4000 rpm for 30 min. A mixture of 4 mL supernatant and 4 mL of motor oil was vertically stirred for 2 min and the height of emulsion layer was measured after 24 h to determine the emulsification index. The equation used to determine the emulsification index (*E*24 %) is as follows:

$$E24 = \frac{E}{E'} \times 100$$

With, *E*: The length of the emulsified layer and *E*': The total length of the mixture.

III. Results and discussion

The quality and quantity of biosurfactant produced by microorganisms dependent highly on the type of the selected strain, available nutrient substrate for cell growth, the nature of the carbon and nitrogen sources and finally the culture conditions (e.g. pH, temperature, agitation , aeration, salinity and oxygen) **[6]**.

III. 1. Optimization of biosurfactant production

III. 1.1. Effect of the carbon source

The influence of the nature of carbon source (olive oil mill effluent, whey, frying oil, orange juice effluent) on biosurfactant production was evaluated by measuring the surface tension of the

culture broth and emulsifying activity. The results obtained are represented on figure 1.

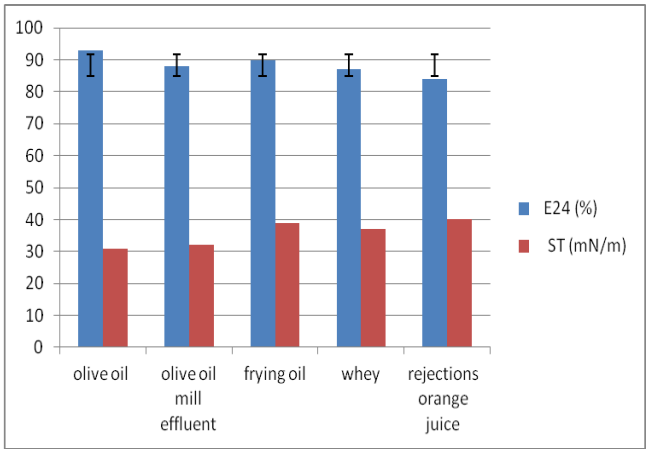

Figure 1. *Influence of the nature of carbon source on biosurfactant production by 1J strain*

According to the results shown in figure 1, oil mill effluent was the best carbon source which allows a good production by the strain 1J witnessed by the reduction of tension surface to 32 mN /m and emulsifying activity of 93% after 24h of incubation. The most active biosurfactant can reduce water surface tension of 72-30 mN / m **[7]**.
A similar results were reported by Mercade and al. **[8]** who showed the use of olive oil mill effluent (OOME) as a new substrate for biosurfactant production (rhamnolipids) by *Pseudomonas* sp. Strains. This strain exhibited a good growth and modified surface tension from 40 to 30 mN/m, interfacial tension from 21 to 5 mN/m and emulsification activity from 20% to 75%.

III. 1.2. Effect of the carbon source concentration

Biosurfactant production can be affected by olive oil mill effluent concentration. As showed in figure 2, the maximum of the production by 1J strain was observed at 0,5% olive oil mill effluent, which leads to an important TS reduction (33 mN/m) and a good emulsifying activity (92%).

Mercadé et al. **[8]** reported that olive oil mill effluent concentration depends on OOME composition, a reduction in surface tension (28 mN/m) was observed using 10% w/v OOME.

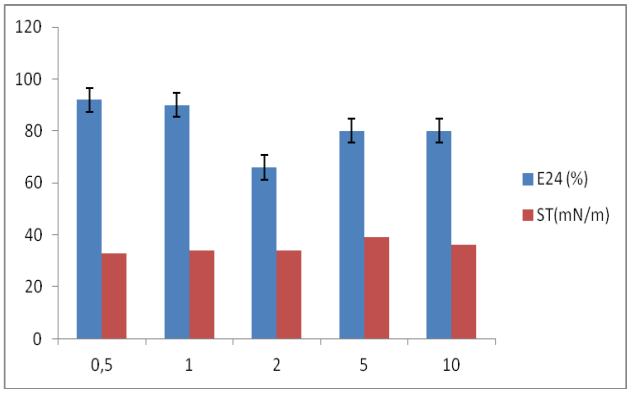

Figure 2. *Effect of the olive oil mill effluent concentration on biosurfactant production*

III. 1.3. Effect of nitrogen source

With olive oil mill effluent as a carbon source, the choice of nitrogen source affects the biosurfactant production (as depicted in figure 3). The strain 1J was able to use nitrogen sources such as ammonia or nitrate. In absence of ammonium chloride the ST was of 33 mN/m. This result confirms that in order to obtain high concentrations of biosurfactants, it is necessary to have limited nitrogen concentrations. Ammonium chloride was the best nitrogen source for growth and biosurfactant synthesis. Similar results were found by Dubey and Juwarkar **[9]**. Forasmuch, the nitrogen limitation increased the production of biosurfactant by *Pseudomonas aeruginosa* BS-2 and *Ustilago maydis* **[10]**. The surface tension is almost constant whatever the nitrogen source added but the emulsifying activity is strongly affected by the nitrogen source present in the medium. The results show that when the olive oil mill effluent, the emulsifying activity recorded in the presence of ammonium chloride (90%) whereas the latter was of 54% with the sulfate of ammonia as nitrogen source. Similar results were reported by Abouseoud and *al* **[5]**.

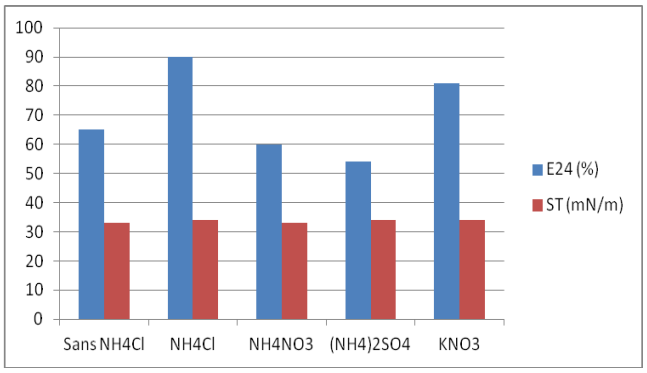

Figure 3*. Effect of the nature of nitrogen source on biosurfactant production by 1J strain*

III. 1.4. pH and salinity effects on biosurfactant production

The environmental factors (pH and salinity) influence the biosurfactant production, as showed in figures 4 and 5, the production was maximal when the medium pH is neutral or near to neutrality, the production decreased in acidic or basic pH domains. On the other hand the relation was inversely proportional, when the salt concentration increased, the production decreased. So the maximal of the production is obtained at 2% NaCl (ST = 30 mN /m). Urai and *al.* **[11]** indicated that A21 and *Haloarcula* sp. D21 were able to produce biosurfactants at 125 $g.L^{-1}$ NaCl and 160 $g.L^{-1}$ $MgCl_2$. The emulsion was stable in the presence of NaCl concentrations of 100 up to 350 $g.L^{-1}$.

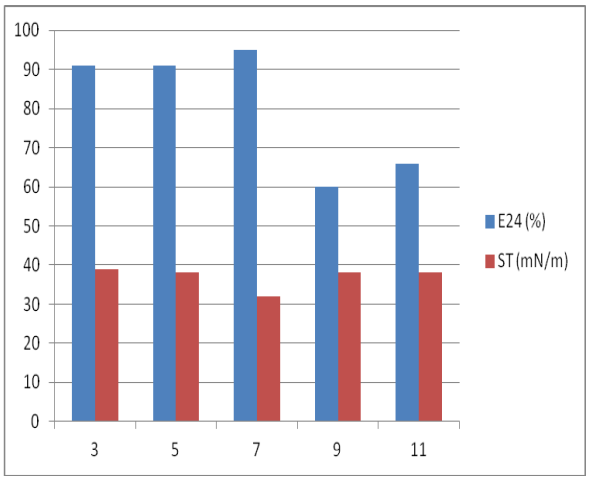

Figure 4*. Effect of pH medium on the 1J biosurfactant production*

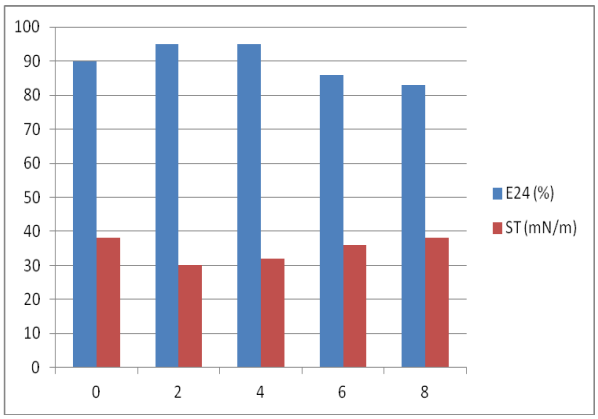

Figure 5. *Effect of salinity medium on the 1J biosurfactant production*

III.2. Biosurfactant stability

The biosurfactants produced by the strain 1J using the olive oil mill effluent as a carbon source show a remarkable stability in salty environments up to 100 $g.L^{-1}$ of salt (figure 6).

A slight increase in the surface tension is observed when the salt concentration is greater than 100 g / l. Abouseoud and al. **[5]** had reported that little changes were observed in the surface active properties of *P. fluorescens* biosurfactant with addition of NaCl up to 2.0 mol /l. Ferhat *and al.***[12]** showed that the surface tension of biosurfactant 1C or 7G remained stable in solution with increasing concentrations of NaCl from 5 g/ l to 100 g /l (average = 31 mN /m). This indicates that biosurfactants produced by isolates 1C and 7G are effective in the presence of monovalent ions (Na+).

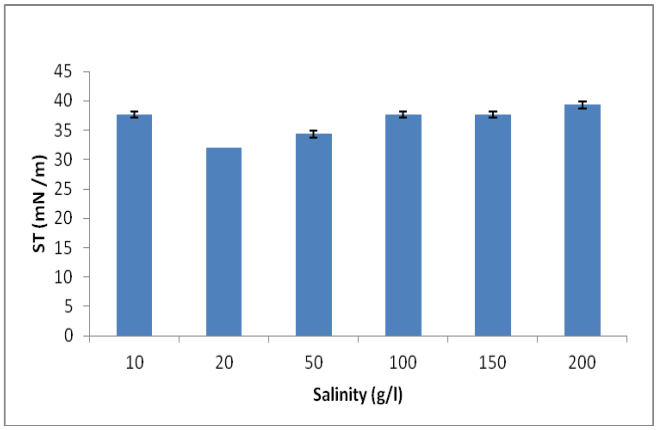

Figure 6*. Salinity effect on the 1J biosurfactant stability*

1J biosurfactant have a very good stability in the acid and basic media in a range of varying pH from 2 to 12 (figure 7). An almost constant value of surface tension in this interval was obtained, which supports its use in industry. The work completed by lee and *al.* **[13]** showed that biosurfactant resulting from *Candida lipolytica* has a good resistance (ST = 33,8 mN/m) in the acid media (pH = 2), however a light increase in the ST (35,1 mN/m) was recorded in the alkaline media (pH = 12).

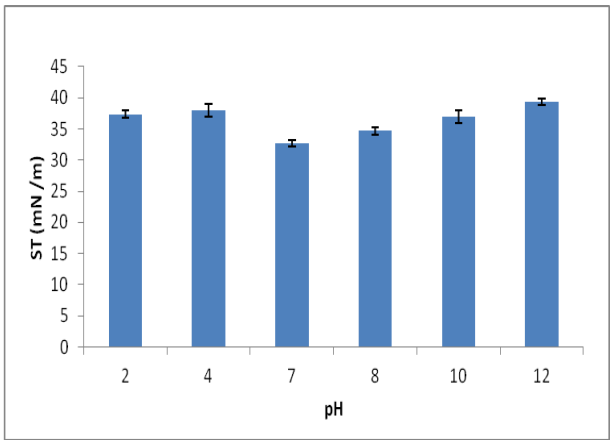

Figure 7*. pH effect on the 1J biosurfactant stability*

The surface tension of biosurfactant 1J was not significantly influenced by temperature (average of 34 mN /m). It presents an excellent stability in the range of temperature from 4 to 70 °C while using the olive oil mill effluent as carbon source. The results obtained by Cameotra and Makkar **[14]** indicate that the exposure of biosurfactant produced by a bacterial strain (indicated by isolate 1165), to extreme temperatures [0, 4,100 and 120 °C] does not affect the thermal stability of biosurfactant. In addition, Deleu and Paquot **[15]** observed that the surface tension preserves its stability after the exposure of biosurfactant produced by the stock *Bacillus subtilis* to a temperature of 100 °C. These results show that 1J biosurfactant produced in our case is considered as excellent stable product, which supports their use in industry and under extreme conditions. Eddouaouda and *al***. [2]** showed that the surface tension was slightly influenced by temperature for 1E biosurfactant. The product was stable during the increase in temperature. The thermal stability of biosurfactant was demonstrated by retaining the same surface tension for a range of temperature from 4 to 55 °C (average of 31 mN /m). At 75 and 100 °C, the surface tension was increased (more than 35 mN /m) indicated the instability of the product at high temperature.

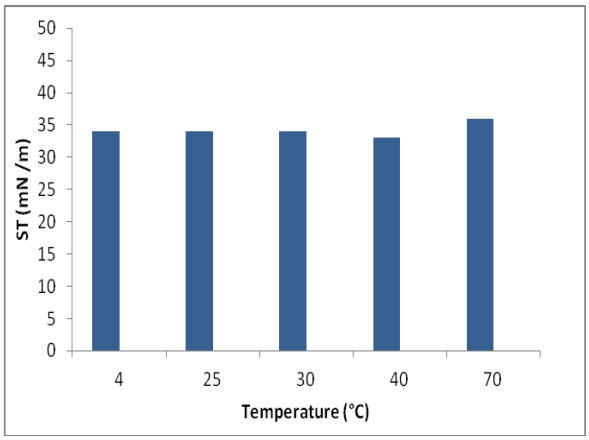

Figure 8*. Temperature effect on the 1J biosurfactant stability*

IV. Conclusion

In conclusion the production of biosurfactants from agro- industrial waste is an economic and environmental interest. The 1J strain is able to produce biosurfactants using the olive oil mill effluent as a carbon source. The production of biosurfactants depend on the culture conditions , a maximum production is obtained with 0.5% of olive oil mill effluent, 0.4% NH_4CL , a neutral pH and salinity of 2%. The biosurfactant produced by this strain has better resistance to extreme pH conditions (2-12) and salinity (up to 250 g / l), thus are used in industrial and environmental field.

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