**Biosurfactant production by *Piper hispidum* endophytic fungi using cooking oil residue as substrate.**

Segio Duvosin Jr\*, Messe da Silva, Patricia Albuquerque

*Amazonas State University, Laboratory of Chemistry Applied to Technology, 1200 Darcy Vargas Ave., Manaus, Amazonas, Brazil, 69050-020.*

*\*Corresponding author: sjunior@uea.edu.br*

**Highlights**

* *Piper hispidum* endophytic fungi are able to produce tensoactive molecules.
* The fungi were able to use the cooking oil residue as substrate.
* The reduction of superficial tension of the cultivation broths was around 40%.

**1. Introduction**

Fungi found in plant species, called endophytic fungi, present a great potential for the production of new bioactive substances [1]. Among substances of commercial interest are the biosurfactants, amphipathic compounds that present tensoactive properties and are produced by microorganisms [2]. Biosurfactants comprise a wide variety of industrial applications, which include bioremediation, biodegradation, cleaning of oil containers, and have been employed at the food, pharmaceutical, and cosmetic industries. [3] Recently, the use of residues for the production of biosurfactants has gained attention, since it implies in cost reduction [4]. Therefore, this work has evaluated the biosurfactant production on metabolic broths of endophytic fungi isolated from the Amazon species *Piper hispidum* (Piperaceae) using cooking oil residue as substrate.

**2. Methods**

The endophytes were isolated previously and maintained on BDA tubes, being activated on this media at 28oC during 5 to 10 days. It was produced a spore suspension (1,0 x 108 spores/mL), which was inoculated in 125 mL Erlenmeyer flasks containing the liquid media - MgSO4 (0.5 g/L), Na2HPO4 (3.0 g/L), KH2PO4 (1.0 g/L) and yeast extract (1.3 g/L) [5]. After autoclaving the media, it was added 0.5 g/L of filtered cooking oil residue in order to induce the biosurfactant production. The fungi were cultivated in triplicate during 7 days in a shaker at 28oC and 170 rpm. After the experiment, the cultivated media was filtered and the supernatant was used to evaluate the biosurfactant production. In order to access the biosurfactant production it was determined the emulsification index (E24) [6]. The lowering of superficial tension (ST) was verified with the of a tensiometer [7] in order to verify the efficiency of the biosurfactant.

**3. Results and discussion**

The endophytic fungi were able to produce tensoactive molecules using the cooking oil residue, since it was observed a lowering of the superficial tension (ST) of the cultivation broths, and the emulsion formation in the presence of kerosene (Table 1). For the fungi PH I 19F, isolated from the leaf, the ST went from 67.5 to 35.9 Mn/M (46.8% reduction), within 12 days of cultivation in the presence of the residue. The emulsification index (E24) was 30%. For the fungi PH I 12F, also from the *P. hispidum* leaf), the TS reduced from 67.5 to 32.9 mN/m (51.3% reduction) eitihn 8 days, and the E24 was 28%.

**Table 1.** Emulsification index (E24) and superficial tension (ST) of *Piper hispidum* endophytic fungi cultivation broths containing the cooking oil residue.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **PH I 12F** | | | **PH I 19F** | | |
| Cultivation Time  (days) | E24  (%) | ST (mN/m) | Cultivation Time (days) | E24  (%) | ST (mN/m) |
| 0 | 0 | 67.5 | 0 | 0 | 67.5 |
| 4 | 23 | 38.7 | 4 | 24 | 34.3 |
| 8 | 27 | 36.7 | 8 | 28 | 32.9 |
| 12 | 30 | 35.9 | 12 | 22 | 35.2 |

According to same authors, biosurfactants that present low molecular weight have the ability to reduce the superficial tension, and those that have higher molecular weights are more likely to promote stable emulsions, acting as a bioemulsifier [8]. Haba et al [9] affirmed that microorganisms that are considered the best biosurfactant producers are capable to reduce the superficial tension to values below 40 mN/m. Therefore, the *P. hispidum* endophytic fungi may be considered as promising biosurfactant producers.

**4. Conclusions**

It was possible to access *P. hispidum* endophytic fungi that produce biosurfactants which presented promising physical-chemical properties for being used in different industrial areas. The cooking oil residue showed to be a promising substrate for the production of fungi biosurfactant.

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