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SELECTION OF FUNGAL STRAINS FOR THE BIOCONVERSION OF BIODIESEL CRUDE GLYCEROL INTO CITRIC ACID

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1. INTRODUCTION

Citric acid (CA) is one of the most valuable organic acids in the market as it can be used in diverse biotechnological applications, such as in the food industry, as flavoring agent and as food preservative, besides cosmetics, pharmaceuticals, and cleaning sectors. This acid can be produced by a biotechnological route and the most common producing microorganisms are filamentous fungi and yeasts (Amato, Becci and Beolchini, 2020). Considering that the Brazilian biodiesel production in 2021 was 6.76 million m^3, and that 10 kg of crude glycerol are generated for each 100 kg of biodiesel produced, a large amount of that byproduct is available in the market. Then, the objective of this work was to screen filamentous fungi capable of producing citric acid from biodiesel crude glycerol (BCG) on submerged fermentation.

2. METHODS

A total of 40 filamentous fungi strains preserved in the Collection of Microorganisms and Microalgae Applied to Agroenergy and Biorefineries (CMMAABio) were prospected for their ability to produce CA when cultivated in a medium containing BCG (82% purity), the crude glycerol, a byproduct of biodiesel production. Firstly, the strains were cultivated in a medium with BCG as sole carbon source (in g/L: glycerol (60); yeast extract (1.1); KH_2PO4 (1.0); $MgSO_4$ (0.5); $(NH4)_2SO_4$ (0.5)). Flasks were inoculated with two mycelial plugs and incubated in a rotary shaker at 30°C and 120 rpm. Aliquots were collected for chromatographic analysis after 5, 10, and 15 days of cultivation. The experiments were performed in triplicate. Quantitative analysis of CA was performed by liquid chromatography (Aminex HPX-87H column at 45°C; 5 mmo/L H_2SO_4 mobile phase; 0.6 mL/min flow rate; refractive index detector).

After the screening, two selected strains were cultivated in 5 different media to define the best source of nitrogen and the effect of micronutrients on CA production (Table 1). Flasks were inoculated with $1x10^6$ conidia/mL and incubated in a rotary shaker at 30°C and 180 rpm for 15 days. Quantitative analysis of CA was performed as described previously. The means were compared, two by two, by t-test (alpha/2=0.025).

Taxonomic identification of fungal strains was carried out evaluating the sequences of the ITS1-5.8-ITS2 region of the rDNA and the β-tubulin and calmodulin genes.

2. RESULTS AND DISCUSSION

Among 40 fungal strains evaluated, only four were able to produce citric acid from BCG. The strains *Aspergillus niger* and *Penicillium pulvillorum* produced, respectively, 3.0 and 7.5 g/L of CA; while the

strain *Penicillium daleae* and an unidentified strain produced low levels of CA, 0.6 and 0.8 g/L, respectively. *A. niger* and *P. pulvillorum* were selected for the next step and were cultivated in 5 different culture media (Table 1). For *P. pulvillorum*, the highest CA concentration (8.93 g/L) was achieved when culture medium containing $NaNO_3$ as a nitrogen source (medium E) was used. The addition of Fe_2SO_4 and $MnSO_4$ had no statistically significant effect on CA production. For *A. niger*, the highest CA concentrations were achieved in the media A and B, respectively, when $(NH_4)_2SO_4$ was used as a nitrogen source. The addition of Fe_2SO_4 and $MnSO_4$ had no statistically significant effect on CA production.

Table 1. Citric acid concentration (g/L) detected in the supernatant of filamentous fungi cultures after growth in BCG-based media (15 days).

Culture medium (in g/L)	***A. niger* BRM 052099**	***P. pulvillorum BRM 052091***
	Citric acid [1] (g/L)	**Citric acid [2] (g/L)**
A) Glycerol (60); KH_2PO_4 (1.0); $MgSO_4$ (0.5); $(NH_4)_2SO_4$ (1.0)	20.55±1.54^{A}	1.47±0.28 $^{A'}$
B) Medium A + Fe_2SO_4 (0.2)	22.36±3.36 ^{B}	1.61±0.16$^{B'}$
C) Medium A + $MnSO_4$ (0.5)	16.80±3.93^{C}	1.69±0.22 $^{C'}$
D) Glycerol (60); KH_2PO_4 (1.0); $MgSO_4$ (0.5); urea (1.0)	4.93±1.71^{D}	4.06±0.20 $^{D'}$
E) Glycerol (60); KH_2PO_4 (1.0); $MgSO_4$ (0.5); $NaNO_3$ (1.0)	13.15±3.97^{E}	8.93±0.41 $^{E'}$

1- A=B; A=C; A>D; A>E 2- A'=B'; A'=C'; A'<D'; A'<E'

Although the CA concentration obtained by the elite strain evaluated (*A. niger*), was increased about 7 times (medium A and B) in comparison with the first screening experiments, the value achieved (~20 g/L of CA) is still lower than the concentration obtained by a genetic engineered yeast *Yarrovia lypolitica* (124.2 g/L) also using crude glycerin as carbon source (Rymowicz et al., 2010).

4. CONCLUSIONS

Forty fungal strains were screened for citric acid production from glycerin. The strain*A. niger* BRM 050299 was selected to continue the studies for the development of the bioconversion process.

5. REFERENCES

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