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PROSPECTING MICROORGANISMS FOR LACTIC ACID PRODUCTION FROM RESIDUAL GLYCERIN FROM BIODIESEL

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1. INTRODUCTION

Lactic acid is an organic acid widely used in the food, chemical, pharmaceutical and cosmetic industries (Yuwaamornpitak and Chookietwatana, 2018; Hong et al., 2009). Lactic acid can be obtained from different raw materials, agri-industrial residues and co-products, which demonstrates the potential that Brazil has to produce this chemical, within the concept of bio refinery. The objective of this work was to select microorganisms capable of producing lactic acid from residual glycerin from biodiesel production.

2. METHODS

A total of 297 bacterial strains preserved in the Collection of Microorganisms and Microalgae Applied to Agroenergy and Biorefineries (CMMAABio) were prospected for their ability to produce lactic acid when cultivated in a medium containing glycerin.

The prospection was carried out in two steps. In the first step, the bacteria were evaluated for their ability to produce lactic acid when grown in a M9 medium containing 2% of pure glycerol (PA) as a carbon source in deep well microplates (28°C, 200 rpm, 72 h). Quantitative analysis of lactic acid was performed by liquid chromatography using an Aminex HPX-87H column, mobile phase of 5 mmol/L H_2SO_4, flow rate of 0.6 mL/min and column temperature of 45°C and refractive index detector. The experiments were performed in triplicate.

After the first step of analysis, the strains capable of producing lactic acid were selected. In the second step, each strain was cultivated in different media containing pure (glycerol PA) and crude glycerin (81.85% purity): the pre-inoculum was prepared in TSB medium (or MRS for *Lactobacillus*). For cultivation, the culture conditions were 28°C (37°C for *Lactobacillus*) at 200 rpm for 72 h. For some strains, the evaluated media were (in 50 mL polypropylene tubes containing 20 mL of medium): A) M9 medium with yeast extract (0.5%) and pure glycerol (2%); B) M9 with tryptone (0.5%) and pure glycerol (2%); C) M9 with tryptone (0.5%) and crude glycerin (to reach 2% glycerol); D) MRS medium with pure glycerol (2%); E) MRS with crude glycerin (2% glycerol). The initial OD_{600nm} was adjusted to 0.3. The cultures were carried out in duplicate. For other strains, each strain was inoculated in Erlenmeyer flasks containing 50 mL of three different culture media: B, C and F) M9 with pure glycerol (2%). The initial OD_{600nm} was adjusted to 0.15. The cultures were carried out in triplicate. Aliquots were collected for chromatographic analysis, which was performed as described for the first step of analysis.

3. RESULTS AND DISCUSSION

In the first step of analysis, all 297 prospected bacterial strains were able to grow in M9 medium containing 2% pure glycerol as the sole source of carbon in miniaturized culture, and the lactic acid producing strains were selected for the second step. In this step, the lactic acid production in at least one of the evaluated media for the 10 strains selected is shown in Table 1.

Table 1. Lactic acid concentration (g/L) detected in the supernatant of bacteria cultures after 72 h of cultivation in different glycerin-based media.

BRM Code	Bacterial strain	Lactic acid concentration (g/L)	Culture médium*
BRM 032754	*Lactobacillus fermentum* 306a	12.50 (± 0.61)	D
BRM 055500	*Lactobacillus plantarum* CTAA179	6.77 (± 0.52)	D
BRM 064893	*Enterobacter hormaechei* MBIA1.18 H3	1.15 (± 0.03)	C
BRM 057993	*Serratia marcescens* Bioenzi B-480A	1.12 (± 0.01)	A
BRM 064894	*Enterobacter hormaechei* MBIA1.26 H11_B	0.62 (± 0.14)	B
BRM 064895	*Enterobacter hormaechei* MBIA1.25 H10_B	0.58 (± 0.10)	C
BRM 051597	*Pantoea dispersa* Bioenzi B-434B	0.47 (± 0.28)	B
BRM 064896	*Enterobacter hormaechei* MBIL1.13 E10_B	0.47 (± 0.04)	B
BRM 064897	*Enterobacter cloacae* MBIA1.11 G8_A	0.34 (± 0.05)	B
BRM 058834	*Serratia marcescens* Bioenzi B-642	0.18 (±0.04)	A

* Medium in which the highest concentration of lactic acid was detected for the respective bacterium.

The highest lactic acid concentration achieved under the conditions tested was 12.5 g/L after 72 h of cultivation. This concentration is higher than many reported in the literature from glycerol, such as the 4.03 g/L obtained by Yuwaamornpitak and Chookietwatana (2018). However, there are also reports such as Hong et al. (2009), describing a very high lactic acid production (85.8 g/L) using a native strain of *Escherichia coli*, isolated from the soil, and glycerol as the sole source of carbon.

4. CONCLUSIONS

A total of 297 bacterial strains were screened for their ability to convert glycerin into lactic acid. The strains *Lactobacillus fermentum* 306a, *Lactobacillus plantarum* CTAA179, *Serratia marcescens* Bioenzi B-480A and *Enterobacter hormaechei* MBIA1.18 H3 were selected to continue the research activities, in which different variables will be investigated in order to increase the production of lactic acid.

5. REFERENCES

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