28 a 31 de julho de 2019
Uberlândia, Minas Gerais, Brasil
XXII Simpósio Nacional de Bioprocessos
XIII Simpósio de Hidrólise Enzimática de Biomassas
XXII SINAFERM
XIII SHEB

SCREENING OF MICROORGANISMS FOR ORGANIC ACIDS PRODUCTION FROM SACCHARINE SORGHUM BROTH

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ABSTRACT

Saccharine sorghum broth is a rich source of fermentable sugars that can be used to produce ethanol and other renewable chemical compounds. Organic acids can be obtained from different raw materials including sugar source such as sorghum broth. These acids have a wide range of applications in various industrial sectors. The objective of this work was to select strains of bacteria and filamentous fungi for production of organic acids using saccharine sorghum broth as carbon source. Six fungal strains were evaluated for citric and kojic acids production. The best strain for citric acid production was able to produce 4.4 g/L and two fungal strains were able to produce between 4 and 5 g/L of kojic acid. When one of the strains was evaluated in medium with almost double TRS concentration, kojic acid production increased more than six times (26.30 ± 6.80 g/L). Among ten strains of Lactobacillus evaluated for lactic acid production, the best one produced 17 g/L. In conclusion, filamentous fungi and Lactobacillus strains capable of producing citric acid (strain GBF 27-13), kojic (GBF 46-13 and PMF 43-11) and lactic acid (CNPC002 AA, 115B AT and CNPC001) in saccharine sorghum broth were selected.

1. INTRODUCTION

Organic acids can be obtained via fermentation processes. Citric, acetic, lactic, gluconic and itaconic acids are already produced on an industrial scale by fermentative processes (CARVALHO et al., 2005). Kojic acid is a topical depigmenting used skin whitening (SATO et al., 2007). Citric acid has a pleasant taste to the human palate and is used in the food industry as acidulant, flavor enhancer, antioxidant, and stabilizer. Lactic acid is a mildly flavored organic acid and is one of the main intermediates/building blocks employed in the food, pharmaceutical and chemical industries (CARVALHO et al., 2005).

Saccharine Sorghum has been shown to be an excellent carbon source of fermentable sugars for ethanol production. However, it can also be applied in the production of chemical compounds of industrial interest, within the biorefinery concept. The objective of this work was to select strains for production of lactic, citric and kojic acids using sorghum broth as carbon source.

2. METHODOLOGY

2.1. Microorganisms, maintenance and growth media

The 6 fungal strains belong to the Collection of Microorganisms and Microalgae applied to Agroenergy and Biorefineries (CMMAABIO), from Embrapa Agroenergia. The 10 strains of *Lactobacillus* sp. were donated by Embrapa Agroindústria Tropical (AT strains) and Embrapa Agroindústria de Alimentos (AA strains) for screening. Strains are preserved in glycerol by freezing or by Castellani method. Fungal strains were grown in Petri dishes containing PDA-Potato-Dextrose-Agar medium (200 g/L potato infusion, 20 g/L glucose and 17 g/L agar) for 7 days at 28^{o}C. Bacterial strains were grown in MRS medium (55 g/L) for 24 h at 48° C and 180 rpm for pre-inoculum production.

2.2. Culture and fermentation conditions for the production of organic acids

The saccharine sorghum broth (raw) donated by Embrapa Milho e Sorgo was sterilized for 20 min at 1 atm and 121^{o}C, and then centrifuged to remove solid sediments.

After growth in PDA medium, three plugs of fungal culture (10 mm diameter) were inoculated in fermentation medium for production of organic acids. Fermentation medium had 95 g/L of total reducing sugars (TRS), derived from diluted sorghum broth, plus 1 g/L of yeast extract and 1 g/L of ammonium sulphate. Fermentation time was 5 days, in 125 mL conical flasks containing 25 mL working volume, under continuous stirring at 180 rpm at 30 °C in triplicate. Posteriorly, the fungus GBF 46/13 was evaluated for production of kojic acid in a medium containing 177 g/L of TRA, 0.7 g/L of yeast extract and 0.7 g/L of ammonium sulphate, and an inoculum of $1x10^5$ spores/mL medium, instead of the plugs.

After the growth of *Lactobacillus* spp. to obtain the pre-inoculum in MRS medium, it was centrifuged and used as inoculum for fermentation medium (OD_{660}=4). Two culture media were tested: with and without nutrients (only diluted saccharine sorghum broth). Concentration of TRS used in both media was 78 g/L. The medium with nutritional supplementation consisted of: tryptone (5 g/L); yeast extract (10 g/L); sodium acetate (1.5 g/L); magnesium sulphate (0.2 g/L); manganese sulphate (0.005 g/L); phosphate monobasic potassium (1.5 g/L) and tween 80 (2 mL/L). Cultivation was performed in 50 mL Falcon tubes containing 25 mL of medium, in duplicate, at 150 rpm and 40 ^{o}C for 5 days.

2.3. Identification and quantification of organic compounds using liquid chromatography

The supernatants of fermentations were analyzed by liquid chromatography (HPLC), equipped with a HPX87H column at 45 °C, and using 5 mM sulfuric acid at a flow rate of 0.6 mL/min as the mobile phase. Identification and quantification were performed using a refractive index detector (RID).

3. RESULTS AND DISCUSSIONS

The production of citric acid by filamentous fungi is shown in Figure 1. The best fungal strain GBF 27-13 produced 4.38 ± 1.52 g/L.

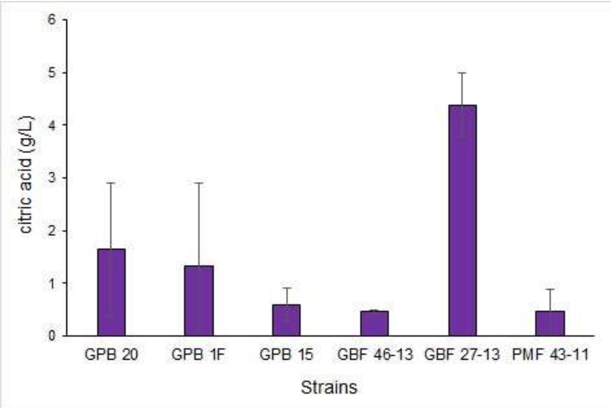

Figure 1. Citric acid production by filamentous fungi cultivated in sorghum broth, after 5 days of fermentation. The columns presented are the means of the triplicates with the standard deviation.

The same fungal strains were also evaluated for production of other acids. The strains GBF 46-13 and PMF 43-11 produced 4.17 ± 1.23 g/L and 5.04 ± 0.14 g/L of kojic acid, respectively (Figure 2) after 5 days of fermentation. When the GBF 46-13 strain was evaluated in medium with almost double ART concentration, the acid production increased more than 6 times (26.30 ± 6.80 g/L).

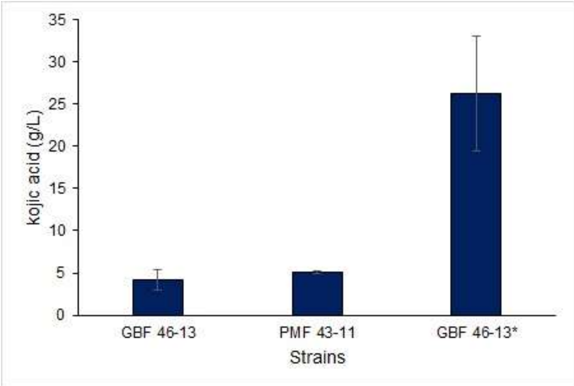

*Figure 2. Production of kojic acid by filamentous fungi in sorghum broth, after 5 days of fermentation. The columns presented are the means of the triplicates and the bars represent the standard deviation of the data. GBF 46-13 * indicates the result obtained in an enriched medium.*

The best production of lactic acid was obtained with the *Lactobacillus* sp. strains CNPC002 AA and 115B AT, which produced, respectively, 9.4 ± 0.49 and 16.9 ± 0.53 g/L, in medium with nutrients supplementation (Figure 3). For the medium without nutrients, the best performance was observed for the strain CNPC001 AA, which produced 11.05 ± 0.04 g/L of lactic acid.

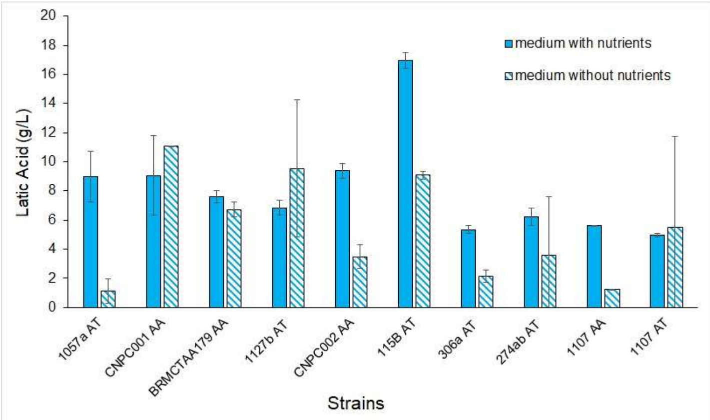

Figure 3. Production of lactic acid by Lactobacillus strains cultivated in sorghum broth after 5 days of cultive.

Organic acids studied in this work are industrially produced by fermentation using *Aspergillus* spp. (kojic and citric) and *Lactobacillus* spp. (lactic) employing sucrose or glucose as carbon source. Saccharine sorghum evaluated in this work can be an alternative source of these sugars. The results found in the screening are still below the industrial indexes (> 100 g / L), but may be reached after process studies (Carvalho, et al., 2005, Shakibaie et al., 2018).

4. CONCLUSION

Filamentous fungi and *Lactobacillus* sp. strains capable of producing citric acid (strain GBF 27-13), kojic (GBF 46-13 and PMF 43-11) and lactic acid (CNPC002 AA, 115B AT and CNPC001) in sorghum broth were selected.

5. ACKNOWLEDGEMENTS

Authors are thankful to FINEP (01.13.0295.00) and Embrapa for the financial support, to CNPq by the scholarship and to centers of Embrapa for donating microbial strains and saccharine sorghum broth.

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