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## The Occurrence of Different Species of Yeast Associated With Sweet Sorghum [Sorghum bicolor (L.) Moench], Plants Grown in Brazilian Savanna

I Marriel<sup>1</sup>, M Guimarães<sup>2</sup>, B Camilo<sup>3</sup>, C Oliveira<sup>1</sup>, F Sousa<sup>1</sup>, E Gomes<sup>1</sup>, R Schaffert<sup>1</sup>, R Parrela<sup>1</sup>

<sup>1</sup> Embrapa Maize and Sorghum, Sete Lagoas, Brazil.

<sup>2</sup> Federal University of São João Del Rei, São João Del Rei, Brazil.

<sup>3</sup> University Center of Sete Lagoas – UNIFEMM, Brazil

Biofuel crops are a sustainable alternative to replace fossil fuels and to mitigate effects of carbon dioxide released into the atmosphere. Sweet sorghum, like its close relative, sugarcane, has been selected by Embrapa Maize and Sorghum plant breeding program to accumulate high levels of biomass and of fermentable sugars in the stem. The strategy currently employed to convert sugars to ethanol is based on yeast fermentation. Therefore, the purpose of this research was to evaluate the natural occurrence of yeast in the sweet sorghum juice grown in Brazilian savanna soil. Stalks of different genotypes of sweet sorghum were obtained by cutting off the tips and stripping away the leaves. The juice was extracted by smashing the stalks between two cylinders. After collecting, it was stored at 2-4°C, and used as needed for isolation and characterization studies. The samples were taken from five genotypes cultivated in Brazilian savanna soil during two years, at different planting dates. Isolation of yeast from juice was achieved by the standard dilution method and direct plating on nine culture media with different compositions (FPbr, DPbr, DFPbr, Martin, MEA, YEPD, GYMP, JSSA, CYA). Plates were then incubated in an upright position in the dark at 28° C for 5 days. Subjective observations on differences in size, colour, general appearance and mainly ease of counting colonies indicated the DFPbr medium (dextrose 1,0%, fructose 1,0%, peptone 0,5%, KH<sub>2</sub>PO<sub>4</sub> 1%; MgSO<sub>4</sub>.7H<sub>2</sub>O 5%, bengal rose 0,003%, streptomycin, 0,003%, Chloramfenicol 0,001%, agar 1,5%, pH 5,5) was the best for enumeration and isolation colonies. The various colony types were counted in this medium, and representative colonies of each morphotypes observed were isolated and subcultured on YEPD medium (yeast extract 0.5%, peptone 0.5%, dextrose 4%, agar 2%) for purification; and preserved in GYMP broth (glucose 2%, yeast extract 0.5 %, malt extract 1 %, 0.2 % Na<sub>2</sub>PO<sub>4</sub>) with 20 % glycerol at -86°C; for subsequent identification. Two hundred and twenty yeast strains were isolated from the samples collected in different genotypes and dates. The predominant morphotypes were identified by the rDNA sequencing as species Wickerhamomyces anomalus, Candida intermedia, Candida saopaulonensis e Meyrozyma caribbica. Regardless of genotype and culture media, the number of yeasts ranged from 3.0 x 104 to x 5.5 x 105 CFU mL<sup>-1</sup> juice. Nevertheless, the results showed the existence of a high diversity among the isolates based on genetic, macro and micromorphological variations. Despite of the plant genotypes and sampling dates no pattern was observed. The isolates obtained were deposited in the Collection of Multifunctional Microorganisms of Embrapa Maize and Sorghum. This is probably the first report on the occurrence of diverse population of yeast that colonizing sweet sorghum plants grown in acid soil. The biotechnological potential of these strains for bioethanol production is yet to be accessed.