KLUYVEROMYCES MARXIANUS SELECTION FOR PRODUCING BIOETHANOL USING CHEESE WHEY AS CARBON SOURCE

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Introduction. Whey is a byproduct generated by the production of cheese [1]. Whey is made approximately 4.5% of lactose, 0.6-0.8% of soluble protein, fat 0.03 to 0.01 and 0.5 to 0.8% minerals [2]. Lactose is a disaccharide that is fermentable and is present in high quantities in whey, and this disaccharide can be metabolized primarily by certain yeasts of the *Kluyveromyces* genus, which uses lactose as a carbon and energy source, generating byproducts as bioethanol, biomass, cellular proteins and enzymes [3].
Objective. Select strain of *Kluyveromyces marxianus* yeast to obtain high ethanol yields using cheese whey like substrate.

Methods. Strains were obtained from agave musts and were isolated and identified by PCR-RFLP method from the region ITS-5.8S and were maintained to -80°C with glycerol at 30 %.
1.- Selection of strains: The initial number of strains were 8 and were streak out in solid medium of YPD and two criteria to select the best strain were used:
*The growth on different lactose concentration: Growth tests in Nitrogen Base media (NB) 1% w/v and lactose in two concentrations 2 and 5% w/v.
*Ethanol tolerance: The growth on medium of NB 1%, Lactose 5% and ethanol 5%.
2.- Determination of cell number and optical density: The selected strains were inoculated in YPD medium for 24 hours, cells were washed and then resuspended in 1 mL of sterile distilled water, of which 100 µL were taken and added to YPD medium and incubated for 18 h and 37 °C. After the growth of *K. marxianus* these were centrifuge at 10000 rpm and the pellet obtained washed and resuspended in 1 mL of sterile distilled water. Nine mL of sterile distilled water were added and homogenized to take 100 µL this volume was used to inoculate the whey substrate and then monitored for 48h.
3.- Determination of enzyme activity (β-galactosidase): The methodology above described was used and NB 1% and 2% lactose medium was inoculated and followed for 72h. For each sample the β-galactosidase enzyme activity assay was done and the activity measured by spectrophotometry at 435 nm. The production of ethanol and consume of lactose were measure by HPLC

Results. Strains were streak out, isolated and named as LC11, PA08, PA12, PA15, PA10 PA09, ZA8 and D10. In order to reduce the amount of strains, the colonies chosen where those that grew in ethanol-lactose medium (D10, PA12 and PA15). The results of optical density (absorbance) and cell growth are shown in Fig 1 and 2 respectively.

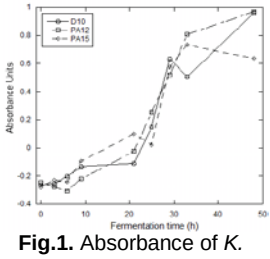

Fig.1. Absorbance of *K. marxianus* (wild) during 48 h of fermentation.

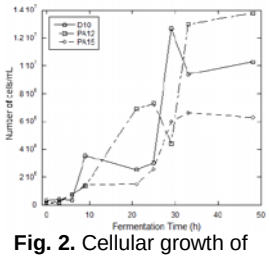

Fig. 2. Cellular growth of *K. marxianus* (wild) during 48 h of fermentation.

The ethanol production found for each strain at 72 hours of fermentation: D10 3.305 g/L, PA12 3.290 g/L and 3.655 g/L. The initial lactose were 76.415 g/L and the final lactose were: D10 21.6435 g/L, PA12 24.476 g/L and PA15 19.051 g/L

Conclusions. The best strain of *K. marxianus* were PA12, PA15 and D10.
The best grow was observed in the strain PA12.
The enzyme activity with this strain is extracellular because show bigger results than intracellular.
The strain which have the best productivity Ethanol-Lactose is PA15 with 0.0637 and this strain will be used to optimize the process.

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(3) Guimarães Pedro, Texeira José, Domingues Lucília. (2010). *Biotechnology Advances*, Vol. 28: 375-384.