Maestría en Ingeniería en Diseño de Bioprocesos

Title

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Fructanase Activity of Yeasts Isolated From Fermenting Musts of Mezcal

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1. Introduction

The Mezcal is an alcoholic beverage produced from musts Agave spp. The industrial production of this drink is a process not standardized, because there is little information about the microbial community present in these musts. Sugar concentrations musts are characterized by high concentrations of fructose (90 g/L) (1). The high fructose content in these musts, resulted of fructan hydrolysis, enzymatically. The yeasts that inhabit these musts have demonstrated the ability to degrade *Agave* fructans spp. Showing fructanase activities between 0.02 and 0.27 U/mL on the substrate sucrose, inulin, levan and FAT (2). Also *S. cerevisiae* isolated from tequila must show high fructanase activity (31.1 U/mL) using as substrate ATF (3). These high activity demonstrated by these yeasts could be of adaptive capacity on fructose-rich substrate from which it was isolated.

2. Aim

The aim of this study is to determine the fructanase activity in five yeasts isolated from mezcal of Tamaulipas and compare their activities with the level of expression of fructanase genes. Also know whether the polymorphisms present in fructanase genes could affect the transcription and enzyme activities.

3. Method.

In this study four strains of *S. cerevisiae* and *K. marxianus* were included. The final strategy consist of three parts. Part I (Fig. 1) consisted in a preliminary screening yeasts, culturing in inductor medium containing 20 gL^{-1} of inulin, levan or sucrose. The fructanase activity was determined using inulin, levan sucrose as substrates. For determination of protein curves BSA (0.5 mg/mL) and curves DNS (5g/L) were constructed. The results allowed us to select the LCGB-3Y8 and LCGB-IY9, which were used to determine the fructanase activity by enzyme kinetics monitored for ten days, using sucrose and inulin substrates. The part II (fig. 2), consisted in primer desing from sequence analysis of SCD gene *suc2* and construction of libraries, clones sequenced were used for determination of polymorphis present in the gene. The part III, consisted in the evaluation of expression of *Suc2* gene in a time selected from enzimatic kinetic, expression relative is evaluated by qPCR, method comparative ΔΔct.

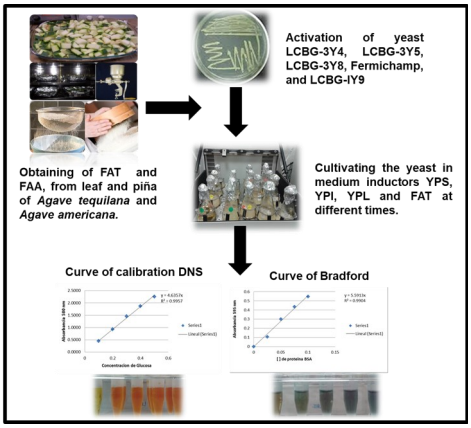

Fig. 1. Represent the strategy experimental for determination of Fructanase activity of yeast isolated from musts of Agave.

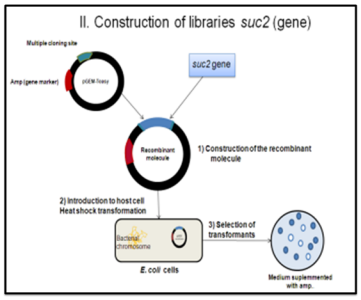

Fig. 2. The construction of libraries was realized cloning the gene Suc2 in the vector pGEM. Transformation was realized by heat shock, and screening of transformants was by selection of white colonies. The clones were sequenced and analysis of polymorphism was conducted.

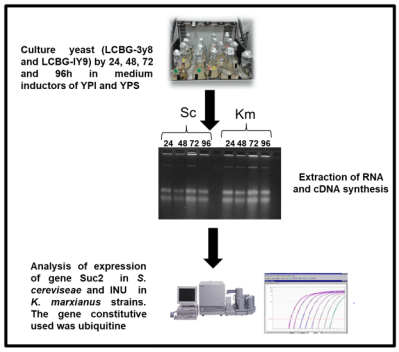

Fig. 3. The analysis of expresion relativa of genes Suc2 and Inu1, was realized in conditions that showed high activity fructanase. The time selected was 24, 48, 72 and 96h in medium inductoresYPI and YPS. The strain selected was LCBG-3Y8 and LCBG-IY9.

4. Results

The activities fructanase determinated in yeast analized showed that the induction medium used for the induction of enzyme it is important (fig. 4). In a medium induction YPS was observed that strain glucophilics (LCBG-3Y4 and LCBG-3Y5) and *K. marxianus* only showed fructanase activity between 0.278 ± 0.072 and 24.017 ± 0.639 U/mL. For this part activities fructanase obtained in induction medium YPI, around 0.501 ± 0.125 and 10.674 ± 1.351 U/mL, the yeast fructophilics (LCBG-3Y8 and Fermichamp) showed higher activities. For this part, in induction medium YPL, the fructanase activity was about 0.467 ± 0. 0.0378 and 0.86 ± 0.22 U/mL. The yeast K. marxianus and LCBG-3Y8 in enzyme kinetics monitored for ten days, using sucrose and inuline substrates, indicated that is possible incremented values of fructanase activities incremented culture time and used medium inductors YPI (fig. 5)

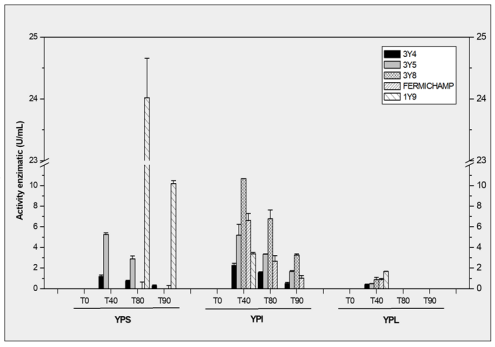

Fig. 4. The fructanase activity, was determined using three inductors medium (YPS, YPI and YPL). The reaction enzymatic was realized with three times of incubation (substrate and enzyme) 40, 80 and 90 min. The experiment was realized for triplicate and se(yEr±) is showed.

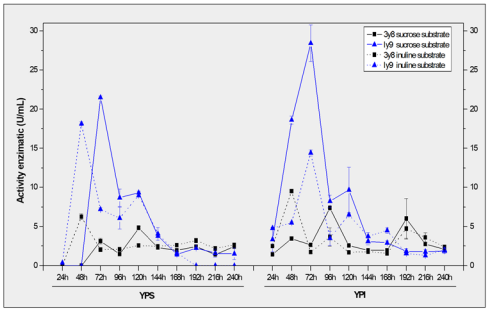

Fig. 5. Fructanase activity of strain LCGB-3Y8 and LCGB-1Y9, cultivating in inductors medium YPS and YPI and monitored for ten days. The experimentes was realized for duplicated and se(yEr±) is showed.

The analysis of secuences corresponding to gene *Suc2* from SCD, allow the desing the primers to amplify 186 pb corresponding to domain B-fructosidases (fig. 6A). For this part the desing of primers of *K. marxianus* comprented a region of 526pb (fig. 6B). The tm of primers was of 66ºC.

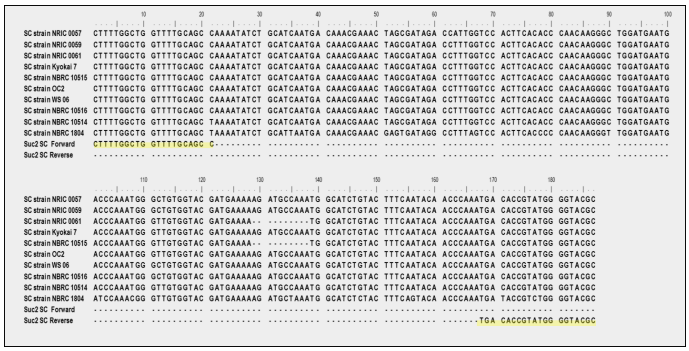

Fig. 6. Alignment of genes Suc 2 of S. cereviseae strains (A) and aligament of gene INU from K. marxianus (B). The sequences incorporated was obtained from SCD and NCBI data base, respectively.. The software used were BlastN, BlastP, CCD, Mega V. 4.0 and Bioedit V. 5.0

5. conclusion

The medium inductor YPI showed increase the fructanase activities of yeast. The strains LCBG-3y8 and LCBG-IY9 showed high fructanase activity on substrate inuline and sucrose, respectively. The enzyme kinetics monitored for ten days showed that time of cultivate for a high fructanase activity of *S. cerevisiae* is 48h and *K. marxianus* is 72h in medium inductor YPI, reaching a 5% increase over fructanase activity in comparison with the others times of cultivation and inductors medium (YPS and YPL).

6. References

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