Title: CONSTRUCTION OF A DELETION CASSETTE FOR GENE IME1 STRAINS OF INDUSTRIAL SACCHAROMYCES CEREVISIAE

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Abstract:

The most important aspect of industrial fermentation by *Saccharomyces cerevisiae* is the production of ethanol which is dependent, among other factors, the capacity of the fermentative yeast population and the resistance of these cells to stress, such as pH and heat. The industrial process in Brazil occurs in non-sterile conditions, with an almost inevitable contamination with other yeasts besides, the gain of new cells of S. cerevisiae produces a sequence in the population of yeast where those who are most suitable physiologically tend to dominate making the use of molecular tools to genetically modify yeast, making them more suitable for fermentation, be highly attractive. However, one must acquire a strategy to restrict the propagation of genetically modified yeast in the environment, which would be to make it sterile, i.e., disable it to perform meiosis (sporulate) and therefore avoiding the mating with wild yeasts. This study was performed to construct a deletion cassette of the gene IME1 (a nonessential gene) whose product is the main inducing meiosis activating the early meiotic gene transcription by interacting with Um6p. The construction of the deletion cassette was repeated using PCR techniques, starting from the genomic region corresponding to the regions of the IME1 3'downstream and 5'upstream gene to be deleted. Then it was made by PCR amplification of regions of the IME1 gene, resulting only in regions 5'upstream and 3'downstream, each with a size equivalent to 300pb, and the primers obtained in their end restriction sites for enzymes *Pvu*II , *Bam*HI and *Eco*RI. In this way it was possible to digest the PCR product with *Bam*HI 5'upstream region, and the region 3'downstream with *Eco*RI. From the resistance gene containing the kanR brand responsible gene conferring G418 resistance to yeast to be Knockouted the deletion cassette, previously digested with *Eco*RI and *Bam*HI, it was possible to have a triple bond between the amplicon 5'upstream region, the fragment with kanR brand with size equivalent to 1,6kb, and the amplicon region 3'downstream. With an amplification using the external primers yielded the deletion cassette, 2,2kb in size to being able to use it for Knockout strains of industrial yeast Saccharomyces cerevisiae.

Keywords: Fermentation; *Saccharomyces cerevisiae*; Bioethanol; PCR; Knockout Gene.

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