

**GENERATION OF A URACIL AUXOTROPH STRAIN OF THE
PROBIOTIC YEAST SACCHAROMYCES BOULARDII AS A HOST
FOR THE RECOMBINANT PROTEIN PRODUCTION**

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Saccharomyces boulardii is well known as a probiotic yeast and many health-promoting properties has been attributed to it. It is related to, but distinct from, *Saccharomyces cerevisiae* in several taxonomic, metabolic, and genetic properties. *S. boulardii* has been shown to maintain and restore the natural flora in the large and small intestine. In this study, in order to generate auxotrophic mutants, cells were placed under a UV lamp at a distance of 12.5 cm and were irradiated for 22 seconds. Auxotrophic mutants were screened by plating method on 5-FOA selective medium with necessary supplements, uridine and uracil. Confirmation of these isolated strains was performed with grown on a minimal medium (YNB medium with and without uridine and uracil supplements). To confirm the Ura3 gene disruption in uridine auxotroph mutants, a selected mutant was transformed with pYES2 plasmid containing Ura3 gene sequence. The positive transformants were isolated on minimal medium lacking uridine and uracil. Further confirmation was performed by isolation of pYES2 from ura3+ transformants. The probiotic properties of the auxotrophic mutants such as resistance to acid and bile salts in comparison with the commercial probiotic strain were approved.

Keywords: *Saccharomyces boulardii*, probiotic, URA3 auxotroph, mutation

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