



ISOLATION AND CHARACTERIZATION OF YEAST STRAINS FOR BIOETHANOL PRODUCTION

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The industrial scale sugar fermentation process for bioethanol production applies yeast strains. The final ethanol yield depends on the viability of yeast under stressful conditions such as temperature, osmotic pressure by sugars and ethanol concentration which emerge inside a fermenter. Therefore this particular study was done to characterize isolated yeast strains by means of their thermo, osmo and ethanol tolerance. Rotten and fresh fruits (Apples, grapes, oranges) from market, garden soil and compost samples were inoculated into Yeast Extract Peptone Dextrose (YEPD) broth to isolate yeast strains. Discrete yeast colonies were obtained by streaking loopful from each flask on YEPD agar plates supplemented with tetracycline 30 mg/ml. Yeast pure cultures were maintained at 4 °C in YEPD agar slants. Light microscopic observation of typical morphology was helpful in confirming yeast strains. Throughout the study, loopful from 24 hour-old culture in YEPD agar slant was used as initial inoculum. Ten yeast strains were individually inoculated into YPD broth (5 ml) tubes with different ethanol concentrations (2%, 10%, 20%, 30%, 40%, 50%, and 60%) and sugar concentrations (2%, 5%, 10%, 20%, 30%, 40%, and 50%) separately and the tubes were incubated at 37 °C for 24 hours without shaking. To determine the temperature tolerance, they were inoculated into YPD broth (5 ml) tubes and incubated at different temperatures (10°C, 25°C, 37°C, 44 °C and 50 °C) for 24 hours without shaking. The growth of strains was measured as an indication of absorbance at 595 nm by UV-Visible spectrophotometer after 24 hour incubation. The stress tolerance of each strain was studied on Yeast Extract Peptone (YEP) agar modified with different sugar and ethanol levels to determine the combined effect of sugar (substrate) and ethanol (product) on viability of yeast strains. The maximum growth temperature tolerated by all the tested stains was 37°C. Sugar tolerance varied from 2% to 5% for majority and ethanol tolerance varied from 30% to 40%. However, when these conditions were combined for growth, the tolerable ranges decreased and best growth of 5 strains were observed on 200 g/L glucose + 80 ml/L ethanol at 30 °C for 72 hrs. Y1 and Y5 yeast strains tolerated most of the combined stress conditions applied.

Keywords: *Bioethanol, Sugar fermentation, Stress tolerance, Yeast*