Abstract

One hundred and twenty strains were isolated using soil samples collected from vicinities of different sugar mills of Punjab, Pakistan. The strains were primarily identified as yeast on the basis of morphological studies and were screened on the basis of ethanol production. Strain yielding maximum ethanol (1.07%) was subjected to biochemical and molecular identification based on Diazonium Blue B test, urea hydrolysis, ascospore formation and 18S rRNA ITS region sequencing and was identified as a new strain of *Wickerhamomyces anomalus* which was accessioned in Gene Bank (KT883963). It exhibited good tolerance to ethanol (8%) and temperature (37°C) and was able to assimilate several simple and polymeric sugars i.e. glucose, fructose, mannose, maltose, xylose, lactose, sucrose, cellobiose and starch. Fermentation conditions for the production of bioethanol (2.37 %) production were optimized for 72 hours at 25°C with 2.5% inoculum at 5.5 initial pH of medium along with ammonium sulfate as nitrogen source. The addition of growth enhancers did not show significant increase in bioethanol production however use of manganese showed little increase (2.69%).