Development of an enzyme assay and preliminary kinetic studies for the enzyme(s) from Candida tropicalis RETL-Cr1 involved in phenol degradation

Abstract

In this study, RETL-Cr1 was studied for its growth and degradation properties in M3 and modified Ramsay media and in the latter with varying saline concentrations. There was no significant effect of medium salinity towards the growth and degradation of phenol in RETL-Cr1. From the growth profile, μ was calculated to be 0.288 h^{-1} while t_d was 2.971 h. The significantly higher intracellular enzyme activity of the crude extract was assayed against varying pHs, ethylenediaminetetraacetic acid (EDTA) concentrations, temperatures and Nicotinamide adenine Dinucleotide (NADH) concentrations, in that order with the resulting optimized conditions of pH 6.5, 1.8 mM EDTA, 37°C and 0.4 mM NADH. Enzyme stability was assayed against varying pH and temperature where it was most stable at pH 6.5 and between temperatures ranging from 25 and 30°C. The crude phenol degrading enzyme was further subjected to kinetic studies at the optimized conditions to determine its affinity towards phenol at varying concentrations. From the Lineweaver-Burk plot it was found that the crude enzyme has a high V_{max} value of 4.963 μM phenol degraded per minute and a low K_m value of 2.115 μM suggesting high affinity towards the substrate. Confirmation of phenol degradation intermediate was determined upon the presence of catechol via thin layer chromatography.