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Production of Organic Solvent Tolerant Hydrolytic Enzymes (Amylase and Protease) by Bacteria Isolated from Soil of a Dairy Farm

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Abstract : Organic solvent tolerant amylases and proteases of microbial origin are in great demand for their application in transglycosylation of water-insoluble flavanoids and in peptide synthesizing reaction in organic media. Most of the amylases and proteases are unstable in presence of organic solvent. In the present work two different bacterial strains M-11 and VP-07 were isolated from the soil sample of a dairy farm in Delhi, India, for the efficient production of extracellular amylase and protease through their screening on starch agar (SA) and skimmed milk agar (SMA) plates, respectively. Both the strains (M-11 and VP-07) were identified based on morphological, biochemical and 16S rRNA gene sequencing methods. After analysis through Ez-Taxon software, the strains M-11 and VP-07 were found to have maximum pairwise similarity of 98.63% and 100% with Bacillus subtilis subsp. inaquosorum BGSC 3A28 and Bacillus anthracis ATCC 14578 and were therefore identified as Bacillus sp. UKS1 and Bacillus sp. UKS2, respectively. Time course study of enzyme activity and bacterial growth has shown that both strains exhibited typical sigmoid growth behavior and maximum production of amylase (180 U/ml) and protease (78 U/ml) by these strains (UKS1 and UKS2) was commenced during stationary phase of growth at 24 and 20 h, respectively. Thereafter, both amylase and protease were tested for their tolerance towards organic solvents and were found to be active as well stable in p-xylene (130% and 115%), chloroform (110% and 112%), isooctane (119% and 107%), benzene (121% and 104%), n-hexane (116% and 103%) and toluene (112% and 101%, respectively). Owing to such properties, these enzymes can be exploited for their potential application in industries for organic synthesis.

Keywords : amylase, enzyme activity, industrial applications, organic solvent tolerant, protease
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