Screening and identification of an organic solvent-stable protease producer

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Abstract: An organic-solvent-tolerant bacterium strain YP1 producing organic-solvent-stable protease was isolated from crude oil contaminant soil. Strain YP1 was strictly aerobic, motile, gram positive, spore-forming, and rod shaped. The YP1 strain was identified as *Bacillus licheniformis* using culture system BIOLOG analysis (SIM = 0.62, 16 – 24h). The 16S rDNA sequence analysis (GenBank accession number EF105377) suggested that strain YP1 was clustered together with *B. licheniformis* in phylogenetic tree. Based on all the taxonomy, strain YP1 was identified as *B. licheniformis*. YP1 strain could tolerant organic solvents at different levels, especially it can grow well in the presence of water-miscible solvents dimethylformamide (DMF, log*P* = – 1.0) and dimethylsulphoxide (DMSO, log*P* = – 1.35) at a concentration of 10% [V/V]. Strain YP1 can also tolerant middle concentrations of NaCl and extra alkaline conditions (pH12). More than 80% of the biomass remained at pH range 10.5 – 12. However strain YP1 was sensitive to antibiotics such as ampicillin, tetracycline, kanamycin and chloromycetin. The protease production could be enhanced by acetone and repressed by alkanols such as dodecylalcohol and octanol during the fermentation. Compared to trypsin, the YP1 protease had a wider tolerance for organic solvents. YP1 protease tolerated up to at least 11 organic solvents with log*P* ranging from – 1.35 to 5.6 including benzene, toluene, DMSO and DMF etc at 50% (V/V) concentration. Moreover, when solvents such as decane and dodecyl alcohol with log *P* values above 4.0 were added to the crude protease, the enzyme activity levels were 1.08 and 1.21 times higher than the control respectively. Its high tolerance for water-miscible solvents DMF and DMSO makes it an ideal catalyst for kinetic- and equilibrium-controlled synthesis. This organic solvent stable protease could be used as a biocatalyst for enzymatic synthesis in the presence of organic solvents.

Keywords: extremophiles; isolation; identification; solvent-stable protease

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