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Detection and Expression of Peroxidase Genes in Trichoderma harzianum KY488466 and Its Response to Crude Oil Degradation

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Abstract : Fungi have potentials for degrading hydrocarbons through the secretion of different enzymes. Crude oil tolerance and degradation by Trichoderma harzianum was investigated in this study with its ability to produce peroxidase enzymes (LiP and MnP). Many fungal strains were isolated from rhizosphere of grasses growing on a crude oil spilled site, and the most frequent strain based on percentage incidence was further characterized using morphological and molecular characteristics. Molecular characterization was done through the amplification of Ribosomal-RNA regions of 18s (1609-1627) and 28s (287-266) using ITS1 and ITS4 combinations and it was identified using NCBI BLAST tool. The selected fungus was also subjected to an in-vitro tolerance test at crude oil concentrations of 5, 10, 15, 20 and 25% while 0% served as control. In addition, lignin peroxidase genes (lig1-6) and manganese peroxidase gene (mnp) were detected and expressed in this strain using RT-PCR technique, its peroxidase producing activities was also studied in aliquots (U/ml). This strain had highest incidence of 80%, it was registered in NCBI as Trichoderma harzianum asemoJ KY488466. The strain KY488466 responded to crude oil concentrations as it increase, the dose inhibition response percentage (DIRP) increased from 41.67 to 95.41 at 5 to 25 % crude oil concentrations. All the peroxidase genes are present in KY488466, and expressed with amplified 900-1000 bp through RT-PCR technique. In this strain, lig2, lig4 and mnp genes were over-expressed, lig 6 was moderately expressed, while none of the genes was under-expressed. The strain also produced 90±0.87 U/ml lignin peroxidase and 120±1.23 U/mil manganese peroxidase enzymes in aliquots. These results imply that KY488466 can tolerate and survive high crude oil concentration and could be exploited for bioremediation of oil-spilled soils, the produced peroxidase enzymes could also be exploited for other biotechnological experiments.

Keywords : crude oil, enzymes, expression, peroxidase genes, tolerance, Trichoderma harzianum
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