

15th World Congress onBIOTECHNOLOGY AND BIOTECH INDUSTRIES MEET
&2nd International Conference on

ENZYMOMOLOGY AND MOLECULAR BIOLOGY

March 20-21, 2017 Rome, Italy

Identification and characterization of a novel thermo stable and oxidant stable protease from Chumathang hot spring using functional metagenomics**Shafaq Rasool, Vishnu Kumar Gupta, Tishu Devi and V Verma**
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Metagenomics is the genomic analysis of microorganisms by direct extraction and cloning of DNA from an assemblage of microorganism. The common strategies for metagenomics analysis include functional and sequence based approaches. Functional metagenomics is a promising strategy for the exploration of the bio-catalytic potential of micro biomes in order to uncover novel enzymes for industrial processes. However, such methods suffer from low hit rates of positive clones and hence, the discovery of novel enzymatic activities from metagenomes is highly challenging. In the present study, functional metagenomics as a promising approach was applied for exploring the potential of hot springs for various industrial enzymes. Hot spring metagenomics offers the possibility of exploiting the potential of unique niches in order to unravel the functional aspects of the hidden micro biomes. Hot spring metagenomic library of Chumathang-a hot spring of Ladakh region was constructed in *E. coli* using pUC 18 as cloning vector. Functional screening of approximately 10,000 clones was done for protease activity on protease substrate plates. Screening of the metagenomic library led to the identification of one clone with potent protease activity. The clone was designated as pCHpro1. The protease positive clone (pCHpro1) derived from the Chumathang sediment metagenomic library showed 41% identity with subtilase family (sediment metagenome) and 35% structural similarity with crystal structure of Pro-Tk SP from *Thermococcus kodakaraensis*. MEROPS peptidase database analysis showed that it belonged to peptidase S8-S53 superfamily. The enzyme was purified to a final specific activity of 84.51 IUmg⁻¹ proteins with a yield of 15.4%. The purified enzyme had a molecular mass of about ~38 kDa as revealed by SDS-PAGE. The present study indicates that metagenomics without doubt offers the possibility of exploring novel genes/ ORF's which can be characterized and applied in various industrial processes.

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