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Solvent-tolerant DNase-producing gram-negative bacteria: Isolation characterisation and optimisation

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Solvent-tolerant microbes are an upcoming group of microorganisms capable of surviving and functioning in environments rich in organic solvents. Their enzymes are highly stable in the presence of organic solvents and are designed to mediate cellular and metabolic processes in an environment rich in solvents. Among these, DNases (Deoxyribonucleases) have gained significant attention for their diverse applications in molecular biology, diagnostics, and therapeutics due to their ability to degrade DNA. They are used in genomic DNA removal, biofilm disruption, and in the treatment of diseases such as cystic fibrosis and autoimmune disorders. The discovery of solvent-tolerant DNases with high thermal and pH stability enhances their potential for industrial applications, particularly in non-aqueous or extreme environments. This study focuses on isolating and characterising novel organic and aliphatic solvent tolerant bacteria producing DNase from the unique ecosystem of the Arabian Sea coast in India. These strains were screened for DNase activity using DNase plates and DPA test. Molecular identification of the strains was done by MALDI-TOF mass spectroscopy and 16s rRNA sequencing. The ammonium sulfate precipitation method was used to purify and characterise the enzyme. Using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the purified enzyme with a molecular weight of 31kDa displayed maximum activity at 70°C and pH 9, sustaining good viability and activity in an environment of organic solvents such as diethyl ether, petroleum ether and methanol. Optimisation studies involving different carbon/nitrogen sources, pH, temperature, metal ions, and salt concentrations further enhanced enzyme production and stability. These findings underscore the significance of marine extremophiles as sources of industrially relevant enzymes. To the best of our knowledge, this is the first report of DNase activity in aliphatic solvent-rich environments, with promising implications for biotechnological and pharmaceutical applications.

Keywords: Marine extremophiles, Non-aqueous enzymology, Extracellular nucleases, Organic solvent stability, Solvent resistant enzymology