Screening and evaluation of lipase from extremophilic bacteria: A study on kinetic and thermodynamic characteristics

THESIS

SUBMITTED TO

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

LUCKNOW

BABASAHEB
BHIMRAO
AMBEDKAR
UNIVERSITY

LUCKNOW

प्रज्ञा शील करुणा

ESTABLISHED 1996

FOR THE DEGREE OF

Doctor of Philosophy

IN

ENVIRONMENTAL SCIENCE

Submitted by

Kanchan Sonkar

ENROLMENT NO.: 1176/15

Under the Supervision of

Prof. Naveen Kumar Arora

DEPARTMENT OF ENVIRONMENTAL SCIENCE
SCHOOL FOR ENVIRONMENTAL SCIENCES
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A Central University, NAAC Accredited 'A' Grade)
VIDYA VIHAR, RAEBARELI ROAD
LUCKNOW-226 025

2021

SUMMARY

Lipases from microbial source have valuable industrial applications in enhancing cheese flavour, organic synthesis, oil structural modification, and biodegradation of plasticizers and phthalic acid esters (PAEs). Due to their multifunctionality and various properties, lipases have attracted great interest in biodegradation of plasticizers and PAEs and many research efforts have been made to find lipases suitable for different phthalic esters degradation.

The production of lipase from bacteria may depend upon the nutrient availability, physical and environmental conditions. However, the production of microbial lipase in extreme environmental conditions could be a new approach and may be an area of interest as the production of bioactive molecules may get modified and enhanced.

The present study was an attempt to explore the bacterial lipase from two different extremophlic strains isolated from two diverse climatic zone. *Pseudomonas punonensis* isolated from cold-environment soil was psychrotolerant whereas, *Bacillus gibsonii* isolated from saline water halotolerant in nature. Both the isolated strains produced lipase, which was further optimized in different environmental and physico-chemical conditions of pH, temperature, metal ions, inhibitors and surfactants. Further, its kinetic and thermal properties were also studied for environmental application. Thermal activation and inactivation of enzyme helps in the implementation of its environmental aspects. The result of thermal inactivation of *B. gibsonii* was further confirmed by UV-CD spectra. The change in secondary structure of protein was found as reversible after high temperature treatment. Whereas, *P. punonensis* CD spectra of enzyme was found as irreversible after temperature treatment.

Biodegradation of DEHP (di (2- ethylhexyl) phthalate) was examined by isolated bacterial

strains. DEHP utilization and degradation were estimated by mass spectrophotometer. The residual concentration of DEHP and intermediated products formed during biodegradation were detected by GC-MS/MS. However, several studies on phthalate degradation were conducted by different types of bacteria but we have chosen DEHP due to their complexity and worldwide use as plasticizer.

The findings of the study are summarized as below-

- Two extremophilic bacterial strains were isolated from two different climatic zones of India, one from agricultural soil of dry temperate region of Leh, Ladakh, India and another from saline water from Dwraka Gujrat, India.
- The microscopic morphological characters were used for the identification of bacterial strains and it was concluded that one strain belong to genus *Pseudomonas* other to *Bacillus*.
- Based on 16 S rRNA gene sequencing and comparison with NCBI GenBank database, strains were identified as psychrotolerant *P. punonensis* (accession no. MH 393314) and halotolerant *B. gibsonii* (accession no. MK835660).
- The present work revealed that the psychrotolerant *P. punonensis* has potential to grow at low temperature (15°C) with growth optima at 25°C and alkaline pH 7.0. The halotolerant *B. gibsonii* showed optimum growth between 30-35°C and alkaline pH 7.0-8.0.
- Further, it was observed that mesophilic *B. gibsonii* was able to tolerate NaCl concentrations (upto 1200 mM) and showed optimum growth at 600 mM salt concentration. Hence, *B. gibsonii* was found to be halotolerant in nature.
- Biochemical characteristics of both extremophilic strains revealed that both could efficiently produce extracellular lipase enzymes.

- Screening of lipase enzyme was done by using tributyrin agar method and production was enhanced by adding olive oil.
- Lipase was purified by ammonium sulphate purification method with purification fold 1.40 in *P. punonensis* and 1.36 purification fold in *B. gibsonii* than crude enzyme.
- Optimization of lipase activity was observed under different physico-chemical environmental conditions such as pH, temperature, metal ions, enzyme inhibitors, surfactants and organic solvents.
- Optimum pH and temperature were found as 7.0 and 30°C for *P. punonensis* lipase, whereas, it was found 9.0 at 60°C for *B. gibsonii* lipase.
- Among different metal ions Ca^{+2} and Al^{+3} was found as enhancers for both extremophilic lipases activity whereas, Mn^{+2}, Ba^{+2}, Co^{+2} inhibited the activity of enzyme.
- Among different inhibitors, PMSF, DTT and β-2 mercaptoethanol, PMSF enhanced the activity of lipase isolated from both bacterial strains.
- Among different surfactants and organic solvents such as Tween 20, Tween 80, CTAB, EDTA, SDS, acetone, 2-butanol, ethanol, methanol and 2-propanol, lipase activity was enhanced by EDTA by 172 % in *P. punonensis* whereas, SDS slightly enhanced lipase activity for *B. gibsonii*. It was found that *P. punonensis* lipase was enhanced by acetone whereas, ethanol and 2- propanol enhanced the *B. gibsonii* lipase activity.
- Kinetic study was examined by using p-NPP as substrate at different concentrations ranging from 0.1-1.0 mM at different temperature range (10, 30, 60°C). Kinetic constant such as Michaelis-Menten constant (Km) and Vmax were calculated by Lineweaver-Burk plot.

- Kinetic study of both types of lipase revealed that maximum velocity and Michaelis-Menten constant (Km) was found at 30°C as 86.22 U/mL and 0.163 mM for *P. punonensis* lipase. Whereas, *B. gibsonii* lipase showed its maximum velocity158.72 U/mL at 0.53 mM substrate concentration at 60°C.
- The catalytic efficiency and turn over number of lipases revealed that enzyme activity substrate enzyme binding complex was found high at 30°C in *P. punonensis*. However, it was estimated high at 60°C for *B. gibsonii* lipase.
- The activation energy was calculated as 27.74 kJ/mol for *P.punonensis* and 45.28 kJ/mol for *B. gibsonii* lipase from Arrhenius plot.
- Thermal stability of enzyme was estimated by calculating residual activity at different temperature *P. punonensis* and *B. gibsonii* lipase. It was found that *P. punonensis* lipase was found maximum stable at 30°C whereas, at 60°C, *B. gibsonii* lipase achieved its maximum stability.
- Thermal inactivation was also studied by calculating inactivation energy, deactivation constant, Desidual, Gibbs free energy, enthalpy, entropy and half-life of enzyme. The result of thermal inactivation of enzyme suggested that 56.89 kJ/mol deactivation energy was needed by *P. punonensis*. However, 66.98 kJ/mol energy is needed to inactivate *B. gibsonii* lipase.
- The effect of temperature on protein secondary structure were determine by far UV-CD spectra. The change in α-helix of protein was observed by increasing temperature above optimum. The change in secondary structure were found irreversible for *P. punonensis*, whereas, it was found reversible after 60 min in *B. gibsonii*.

- Both the isolated extremophilic strains were used to degrade phallic acid esters. DEHP was chosen to study the potential use of isolated strains in environment pollution management.