

STUDY OF PROTEASE ACTIVITY OF BACTERIA ISOLATED FROM SOLID WASTE

**A
DISSERTATION
SUBMITTED TO THE CENTRAL DEPARTMENT OF MICROBIOLOGY
TRIBHUVAN UNIVERSITY**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS THE AWARD OF
THE DEGREE OF MASTER OF SCIENCE IN MICROBIOLOGY
(ENVIRONMENT AND PUBLIC HEALTH)**

**BY
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KIRTIPUR, KATHMANDU, NEPAL
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ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and appreciation to my supervisor **Mr. Binod Lekhak**, Lecturer, Central Department of Microbiology for his valuable suggestions, comments, continuous guidance and support throughout this work.

I am equally indebted to **Dr. Dwij Raj Bhatta**, Associate Professor and Head, Central Department of Microbiology for providing me the laboratory facilities to carry out this work.

My special regards goes to **Mr. Dev Raj Joshi**, Lecturer, Central Department of Microbiology for his guidance, constant inspiration and valuable suggestions during the whole study period. I would like to acknowledge **Mrs. Reshma Tuladhar**, Lecturer, Central Department of Microbiology and **Mr. Janardan Pandey**, Central Department of Biotechnology for their valuable suggestions and guidance during this work. I am specially thankful to **Mr. Ramesh Khadka**, **Mr. Madhukar Thapa**, **Mr. Navaraj Karki**, **Mr. Raiman Shakya** and all the laboratory staffs of Central Department of Microbiology for their kind co-operation for accomplishment of this dissertation work.

I am indebted to Ratnashree, Mrs. Niru Rana, my parents and family members, my husband and in-laws for their continuous support, inspiration, encouragement and co-operation during this study period.

I would like to acknowledge my friends Nanu Maiya Khadka, Bijaya Laxmi Maharjan, Ram Prasad Awal, Esha Shrestha, Saurav Aryal, Manita Aryal, Shiv Nandan Sah and Geeta Pandey for their supportive contribution and suggestions during the study period.

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ABSTRACT

The study was carried out at Central Department of Microbiology, Tribhuvan University, Kirtipur from September 2008 to May 2009. Random sampling was adopted to collect altogether 20 samples of municipal solid waste from different sites within Kahtmandu Valley. The temperature of the solid waste was recorded at site using mercury thermometer while pH was recorded after transporting the samples to the laboratory. The indigenous proteolytic bacteria were isolated by spread plate technique on mineral base agar supplemented with 1% gelatin. Out of 113 isolates obtained, only 22 degraded gelatin incorporated in agar media and the rest were chemolithotrophs. On the basis of degree of hydrolysis, 3 potent gelatinase producers (laboratory code: K2.2i, R3.3i and R5.2iv) with diameter of zone of hydrolysis more than 20 mm were selected and identified on the basis of their morphological, cultural and biochemical characteristics. The isolates K2.2i and R5.2iv were *Micrococcus* spp. while R3.3i remained unidentified. Enzyme was extracted from these most potent isolates by fermentation. The crude enzyme extracts were assayed for secondary screening by cup plate assay and the isolates K2.2i and R3.3i exhibited similar gelatinase activity while R5.2iv was slightly less active. The enzymes from K2.2i and R3.3i were purified by acetone precipitation. The kinetics (temperature and pH) was studied and enzyme from K2.2i was found optimally active at 4⁰C and pH 8 while that from R3.3i was optimally active at 37⁰C and pH 9. The enzymes were further purified by ammonium sulphate fractionation. The 30%, 60% and 90% fractions of enzyme from K2.2i and only 30% and 60% fractions of enzyme from R3.3i revealed gelatinase activity during assay at 37⁰C and pH 7. The activities of 60% fractions of both the enzymes were the highest while studied at different temperatures and pH. The obtained data was analyzed for analysis of variance (ANOVA) and paired samples t-test using statistical software (SPSS version 11.5). From these analyses, significant effect of temperature (P=0.045 and P=0.014 for enzymes from R3.3i and K2.2i respectively) and pH (P=0.032 and P=0.078 for enzymes from R3.3i and K2.2i respectively) was observed on the enzyme activity. Also, ammonium sulphate fractionation can be significantly applied for purification of the enzymes. From the study of the temperature and pH condition of the piled up solid waste and the activity profile of the obtained enzymes, it can be concluded that both of these enzymes are suitable for their application in solid waste management; enzyme from K2.2i in the cold seasons while that from R3.3i at in warmer seasons. The study could be extended to field trial.

Key words: Solid waste, protease activity, proteolytic bacteria

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LIST OF ABBREVIATIONS

AIDS	Acquired Immuno Deficiency Syndrome
ANOVA	Analysis of Variance
CBS	Central Bureau of Statistics
ICIMOD	International Centre for Integrated Mountain Development
IUCN	International Union for Conservation of Nature
KMC	Kathmandu Metropolitan City
MOPE	Ministry of Population and Environment
WHO	World Health Organization

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