DECLARATION

I hereby declare that the thesis entitled "**Studies on cellulase-producing bacteria isolated from a vermicompost-derived consortium and to evaluate their synergism in depolymerization of agricultural residues**" has been prepared by me under the guidance of Prof. Shilpi Ghosh, Department of Biotechnology, University of North Bengal.

No part of thesis has formed the basis for the award of any degree or fellowship previously.

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CERTIFICATE

I certify that Ms. Arijita Basak has prepared the thesis entitled "**Studies on cellulase-producing bacteria isolated from a vermicompost-derived consortium and to evaluate their synergism in depolymerization of agricultural residues**" for the award of Ph.D degree of the University of North Bengal under my guidance. She has carried out the whole work in the Department of Biotechnology, University of North Bengal. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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Studies on cellulase-producing bacteria isolated from a vermicompost- derived consortium and to evaluate their synergism in depolymerization of agricultural residues Thesis submitted to the University of North Bengal For the Award of Doctor of Philosophy in Biotechnology Submitted by Arijita Basak Department of Biotechnology, University of North Bengal Supervisor Prof. Shilpi Ghosh Department of Biotechnology, University of North Bengal, Darjeeling, West Bengal, India-734013 July, 2023

1 Chapter 1 General Introduction and Review of Literature 1.1. Introduction Lignocellulosic biomass (LCB) from plants is the most abundant biomass on earth. It mainly includes various agricultural residues, such as cereal straw, bagasse, forest residues, waste from paper and pulp industry etc. The annual worldwide production of LCB amounts to approximately 181.5 billion tons, of which 8.2 million tons is currently utilized. Traditionally, LCB is utilized for energy requirement and production of paper, textile and construction materials. With advancement in technology, LCB has been realized as a sustainable source of biofuel, bioplastics, enzymes and other value added products (Mujtaba et al. 2023). LCB is primarily comprised of two carbohydrate components, cellulose (40-50 %), hemicellulose (25-30 %), and one non-carbohydrate phenolic polymer, lignin (15-20 %). Cellulose is the major structural polysaccharide of plant cell wall, and is an unbranched homopolymer of 4-O-β-D- glucopyranosyl-D-glucose units. Unlike cellulose, hemicellulose is composed of several heterogenous polymers like xylan, glucuronoxylan, arabinoxylan and xyloglucan, and its main function is to strengthen the cell wall through binding to the cellulose microfibrils. Lignin is a phenylpropanoid polymer composed of sinapyl (S), coniferyl (G) and p-coumaryl (H) alcohol units connected through C-C and ester linkages. It forms an impenetrable network around cellulose which reduces its surface area for hydrolysis by microbial enzymes (Tan et al. 2021, Wu et al. 2020b). Cellulose and hemicellulose are depolymerized to sugars for further conversion by fermentation and biocatalytic processes to value added products, whereas lignin can be extracted for production of aromatics (Weng et al. 2021, Intasian et al. 2021). However, LCB remains unutilized or under-utilized due to its high degree of lignification and therefore, its bioconversion is challenging for development of economic biorefineries (Mora-Sandi et al. 2021). Currently, the LCB refinery requires energy-intensive pretreatment steps for removal of lignin and subsequent hydrolysis with a cocktail of cellulolytic enzymes to liberate fermentable sugars. In recent years, significant progress has been achieved in removal of recalcitrant lignin through physical, chemical and physicochemical pretreatments, however, these pretreatments are energy intensive and produce microbial and enzymatic inhibitors that obstruct the downstream cellulolytic saccharification and fermentation (Sanchez-Munoz et al. 2022). Microbial depolymerization of LCB is a consequence of the synergistic action of hydrolases, lignin-modifying enzymes (LME) and their associated enzymes. The CAZy database classifies carbohydrate active enzymes (CAZymes) into six categories: glycoside hydrolases (GH) that cleave glycoside bonds, glycosyl transferases (GT) that help in the formation of glycoside bonds, carbohydrate esterases (CE) that modify ester bonds, polysaccharide lyases (PL) that cleave glycoside bonds via non-hydrolytic pathways, auxiliary active (AA) enzymes that are oxidoreductases and assist the other enzymes in LCB degradation. Other than these, there are

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PREFACE

Agroresidues are a rich source of lignocellulosic material and can serve as a replenishable feedstock for valorization into value-added products such as green chemicals, second generation biofuels, biosolids, sugars and food supplements. They are composed of lignin (15-35) % which is an aromatic polymer, cellulose (30-50) % which is a linear polymer of glucose linked by β-1,4-D-glycosidic bonds, and hemicellulose (15-40) % which is a soluble heteropolysaccharide. Lignin and cellulose form a complex, hydrolysis-resistant network that holds the hemicellulose. It requires extreme reaction conditions (>300 °C) to degrade these two polymers and cannot be feasibly carried out in biorefineries. Lignocellulose degradation environment harbors microorganisms that produce lignocellulolytic biocatalysts. These enzymes act synergistically to bring about polysaccharide deconstruction and are able to act under milder conditions. Lignocellulolytic consortia have potential for the bioprospecting of novel genes that encode hydrolytic and non-hydrolytic enzymes for the purpose of industrial saccharification. However, they do not offer elucidation of the microbial dynamics and the numerous biochemical pathways involved in their degradation. Co-culturing of microorganisms is a simpler process which helps to explore the enzymatic interactions. Bacterial enzymes are known to tolerate elevated temperatures, a broad range of pH, metal ions, inhibitors, surfectants and reducing agents and are in demand in biorefineries. Despite several advantages, bacteria have largely remained unexplored in the context of co-culturing, due to the underproduction of bacterial enzymes. It is desirable to utilize bacteria in the large scale production of industrially applicable biocatalysts that can replace commercial fungal enzymes in the saccharification of pretreated agroresidues. Current investigation aimed to explore the cellulolytic potential of a thermotolerant, lignocellulolytic consortium prepared from vermicompost using rice straw as substrate and identify the different CAZymes expressed by its metagenome. Culturable bacteria isolated from it were used in the construction of a synthetic consortium whose cellulase production was optimized by both conventional and statistical approaches to explore its saccharification potential on pretreated rice straw relative to the commercial cellulase Celluclast. The cellulase PtCel1 was purified and characterized in terms of pH optima and stability, temperature optima and stability, tolerance to metals, denaturants and inhibitors, and its gene was cloned and sequenced.

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