CHAPTER I

INTRODUCTION

Microbial degradation of wood is an important biological process helping nature to complete the carbon cycle by channeling the carbon stored as carbohydrates back to carbon and hydrogen (Kirk and Cowling, 1984; and Post *et al.*, 1990). The cell walls of woody tissues of higher plants have a complex structure that is physically robust and chemically stable. The structure and chemical composition of wood have a significant influence on its degradation by microorganisms and the resulting patterns of decay. The type of cell, chemical composition, and cell wall morphology may all govern the effects of enzymes on the woody substrate (Eriksson *et al.*, 1990). In general, wood-decaying fungi secrete the enzymes that degrade complex cell wall polymers and consume the resultant smaller polymers, sugars, and other products. Those products of this decay are then absorbed as food and energy. Wood is made up largely of cellulose (40-50%), hemicelluloses (25-40%), and lignin (20-35%) in various proportions (Rayner and Boddy, 1988). Wood decay fungi are categorized based on the components utilized and characteristics of the decayed wood. These categories include the soft-rot fungi, the brown-rot fungi, and the white-rot fungi (Schwarze *et al.*, 2004).

Cellulose is the most abundant and renewable carbon source. Great interest has been focused on the hydrolysis of cellulose for the production of glucose, which can be used for fuel, food, and chemical production. Cellulosic material can be hydrolyzed using acid or enzyme. Acid hydrolysis of cellulose requires a high temperature and more by products are formed compared with enzymatic hydrolysis. Intensive efforts have been made to develop more efficient cellulolytic enzymes and to increase the productivity of the enzyme-producing microorganisms (Lunjungdahl and Eriksson, 1985; and Beguin and Aubert, 1993). A complete set of cellulolytic enzymes is minimally composed of the following enzymes: endoglucanase (1,4-β-D-glucan glucanohydrolase, EC 3.2.1.4), cellobiohydrolase (1,4-β-D-glucan cellobiohydrolase, EC 3.2.1.91), and β-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21). The enzyme action is generally initiated by random acting endoglucanases at amorphous regions within cellulose chain to produce cello-oligosaccharides. The cellobiohydrolases then act sequentially by removing cellobiose units from non-reducing as well as reducing ends of the cellulose chains.

Finally, β-glucosidase completes the hydrolysis by degrading cellobiose to glucose (Eveleigh, 1987).

Current research on β-glucosidases has significant scientific, medical and economic implications. β-Glucosidases catalyze the hydrolysis of β-glucosidic linkages, such as those in alkyl- or aryl- β-glucosides as well as diglucosides and oligosaccharides. They represent an important group of enzymes because of their potential use in various biotechnological processes, including biomass degradation (Coughlan, 1985), production of fuel ethanol from cellulosic agricultural residues (Bothast and Saha, 1997), release of aromatic compounds in the flavour industry (Gueguen *et al.*, 1996) and synthesis of useful β-glucosides (Makropoulou *et al.*, 1998). The role of the β-glucosidase in the saccharification of cellulose is to degrade cellobiose, an inhibitor of the depolymerizing enzyme, and cellulooligosaccharides to glucose. However, β-glucosidase is frequently a rate-limiting factor during enzymatic hydrolysis of cellulose and is very sensitive to glucose inhibition, which limits its activity (Howell and Stuck, 1975).

The Xylariaceae are wood-decaying fungi found in terrestrial habitats, are particularly diverse in tropical regions and are able to degrade lignocellulose. Many of the Xylariaceae are saprotrophs on decaying plant material such as logs and stumps, dead twigs and branches of trees, dead leaves and stems of herbaceous plants (Whalley, 1996). It is also clear that many species can degrade lignin (Sutherland and Crawford, 1981) and that others exhibit impressive production of cellulolytic enzymes (Wei *et al.*, 1992). *Daldinia* (*Xylariaceae*) is a genus of wood-inhabiting pyrenomycetes with perithecia embedded in large stromata that are internally concentrically zoned and produce enzymes that digest the cell wall components for nutrition and energy (Johannesson *et al.*, 2000).

The objective of this work is to undertake the purification and to determine the detailed biochemical characteristics of an extracellular β-glucosidase and synthesis of alkyl-glucosides using β-glucosidase from the wood-decaying fungus *Daldinia eschscholzii*. It is noted that the activities of *D. eschscholzii* have not as yet been reported.