CHAPTER II

REVIEW OF THE LITERATURES

2.1 Chemical structure of wood component

Wood consists of elongated cells, most of which are oriented in the longitudinal direction of the stem and are connected to each other through openings, referred to as pits. These cells, varying in shape according to their function, provide the necessary mechanical strength to the tree and also permit liquid transport and provide storage of reserve food supplies. The tracheids in softwoods and fibrils form cells in hardwoods are generally called wood fibers (Fujita and Harada, 1991). These cell wall wood fibers can be divided into three main layers, i.e. the primary wall, secondary wall, and warty wall (Figure 2.1).

Cellulose, hemicellulose, and lignin are the major constituents of wood material. Cellulose is the main component, accounting for approximately 40-50% of the dry substance in most wood species and located mainly in the secondary cell wall. Like cellulose, most hemicelluloses function as supporting material in the cell walls. Hemicelluloses appear in close association with cellulose, especially in lignified tissues. However, no covalent bonds have been found between cellulose and hemicelluloses, although marked mutual adhesion is provided by hydrogen bonding and van der Waals interactions. The location of different hemicelluloses in the fiber wall is dependent on the wood species and growth season, and the amount of hemicelluloses is usually 25-40% of the wood dry weight. Lignin is located in primary and secondary cell walls and in the middle lamellae. It is closely associated with wood carbohydrates and covalently linked to hemicelluloses. The covalent cross-linking, lignification acts as glue to cement cellulose microfibrils and produce rigid woody tissue able to withstand the compressive force of gravity.

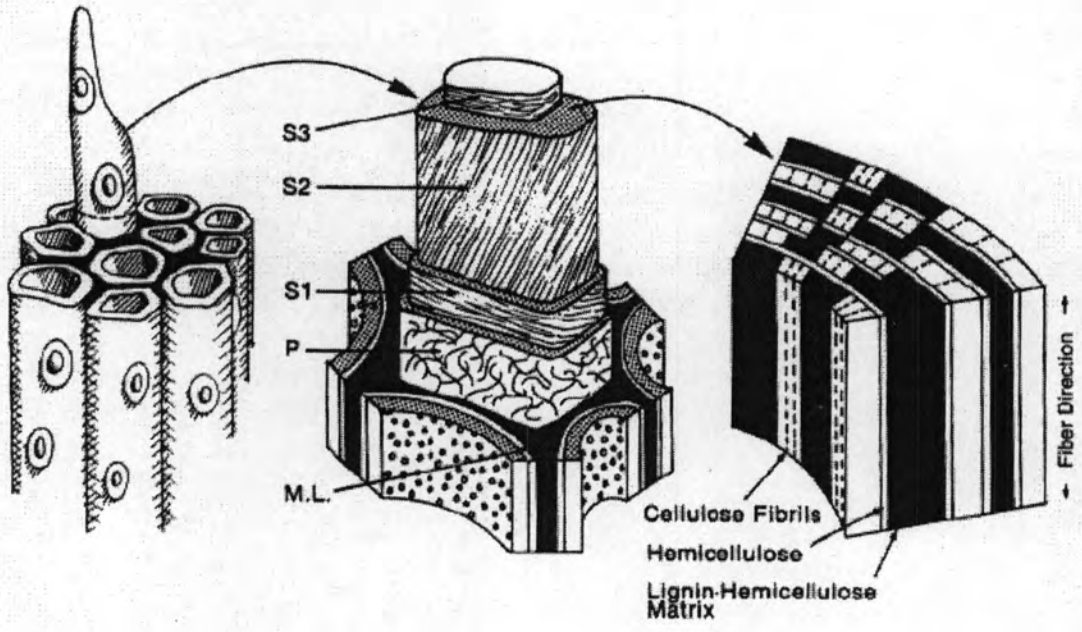


Figure 2.1 Schematic illustration of the molecular architecture of wood tissue, showing the relationship of contiguous cells *(left),* cut away view of the cell wall layers *(center),* and one depiction of the relationship of the lignin, hemicelluloses, and cellulose in the secondary wall (*right*). The diameter of each cell is approximately 25 μm. S1-S3; secondary cell wall layers, P; primary wall, and M. L.; middle lamella (Kirk and Cullen, 1998)

2.2 Fungal degradation of wood

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. The microbes that degrade wood include bacteria and fungi. Microbial degradation is either parasitic or saprophytic. Saprophytic degradation can be specific to certain cell wall components or can involve degradation of all cell wall components (Post, 1990).

Fungi are heterotrophic eukaryotes and one of their major roles in the ecosystem is bioconversion, including wood degradation. The saprophytic wood degrading fungi are either unicellular or filamentous during the vegetative part of their life cycle. Filamentous fungi are predominant wood degraders and their filamentous morphology helps them to creep over and grow into the wood structures. In general wood-degrading fungi secrete the enzymes that degrade complex cell wall polymers and consume the resultant smaller polymers, sugars and other products. Based on the extent of degradation of wood components, the fungal species are referred to as wood rot fungi, white rot fungi, brown rot fungi, soft rot fungi, etc. There are many fungal strains that are used industrially for various applications. *Trichoderma reesei* is one such extensively used forerunner. Many enzymes from *Trichoderma reesei* are used for industrial applications such as pulp and paper, textile, food processing, laundry etc. The white rot fungus *Ceriporiopsis subvermispora*, though not well studied, is an effective delignifier and is used for biopulping (Blanchette, 1991; and Wall *et al.*, 1993). In recent times, the white rote fungus *Phanerochaete chrysosporium*, with its unique ability to degrade lignin and many organic pollutants, has emerged as a potential candidate for future application in hazardous waste remediation and other industrial applications such as paper and textiles.

2.3 Types of wood decay

Wood decay fungi are categorized based on 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). The first is soft rot, in which enzymatic decay of cellulose and hemicelluloses is accompanied by little or no lignin degradation. This is characteristic of species in many ascomycete and anamorphic genera and is restricted to surface layers in wood where characteristic soft-rot cavities are observed. The most

widely studied soft-rot fungi are as follows: *Trichoderma* spp., *Fusarium* spp. (Mackenzie *et al.*, 1997), *Humicola* spp. (Davies *et al.*, 2000), *Penicillium* spp., and *Aspergillus* spp. (Gielkens *et al.*, 1999). The second is white rot, where rapid and extensive decay of all wood components due to enzymatic degradation is observed, with characteristic wood bleaching due to lignin removal. White-rot decay is found only among a number of basidiomycetes and a few higher ascomycetes (Pointing *et al.*, 2003). The most studied white-rot fungi are *Phanerochaete chrysosporium* (Broda, 1996), *Trametes versicolor* (Archibald, 1992) and *Agaricus bisporus* (Armesilla *et al.*, 1994). Finally, in brown-rot, very rapid cellulose and hemicelluloses decay is attributed to nonenzymatic oxidation with relatively little or no associated lignin degradation. The brown rot fungi are not colonizers of very wet or waterlogged wood and so are not found in aquatic environments. The most studied brown-rot fungi are *Gloeophyllum trabeum* (Varela *et al.*, 2003 and Roni *et al.*, 2005), *Coniophora puteana* (Schmidhalter and Canevascini 1992), and *Postia placenta* (Clausen and Ferge, 1995). In terrestrial environments white rot and brown rot are the most important decay types in terms of cellulose and hemicelluloses turnover. Lignin degradation is achieved appreciably only by white-rot fungi, and so their role is key in lignocelluloses turnover since lignin is also the most recalcitrant component of wood.

2.4 The family Xylariaceae

The family Xylariaceae is one of the commonly encountered groups of the Ascomycota. The ascomycetes produce ascospores as a means of sexual reproduction. Members of the Ascomycota also reproduce asexually by mean of *conidia*. The sexual phase is known as the *teleomorph* and the asexual phase is referred to as the *anamorph*. Three main subclasses constitute the Ascomycota, these are Plectomycetes, Discomycetes, and Pyrenomycetes. The family Xylariaceae is in subclass pyrenomycetes; their fruiting bodies (*ascocarps*) are flask-like shaped structures and, they bear a pore like opening at the top (*ostiole*), called the *perithicia* (Whalley, 1996). The Xylariaceous fungi generally grow on wood and these fungi are known to produce a type of decay of the wood tissues known as a soft rot. They are saprotrophs on decaying plant material such as logs and stumps, dead twigs and branches of tree, 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). Some are weakly parasitic and some members of the family

are responsible for causing various types of disease. For example, *Hypoxylon mammatum* (canker of aspen), *Hypoxylon mediterraneum* and *Hypoxylon atropunctatum* (canker of oak), *Hypoxylon serpens* (root rot of tea, coffee, and rubber), and *Rosellinia necatrix* (white root rot of fruits) (Whalley, 1996).

The members of the family xylariaceae are distributed throughout the temperate and tropical regions of the world. To date 37 genera and hundreds of species are accepted and identified worldwide (Laessoe and Lodge, 1994). The members are mainly distributed in the genera; *Anthostomella*, *Biscogniauxia*, *Camillea*, *Daldinia*, *Hypoxylon*, *Kretzschmaria*, *Kretzschmarialla*, *Podosordania*, *Poronia*, *Rosellinia*, *Thamnomyces*, and *Xylaria*. The xylariaceae are regconised by the presence of dark-coloured nonseptate *ascospores* enclosed in smooth perithecia, clustered on or embedded on *stromata*. The genera of this family are sometimes distinguished by their stromata. For example, in *Hypoxylon* the stromata appear as a crustilike growth under the bark, in *Daldinia* their stromata are large black hemispherical structures with internal dark-coloured distinctive zone lines, and the stromata of *Xylaria* appear like human fingers.

As the Xylariaceae can reproduce either sexually (teleomorphically) producing ascospores or asexually (anamorphically) producing externally unicellular asexual reproductive spores (condidia), the erratic fruiting behaviour and the ephemeral nature of many species make identification fraught with difficulty. Classical taxonomy makes use of teleomorphic structures such as colour, size, and shape of stromata, ascospore shape and size, and spore surface ornamentation to classify these fungi. Taking this into account, there is need for an efficient and consistent system of classification. More stable and natural characters are therefore needed to clarify the inter-generic relationships. There have been many attempts to identify the xylariaceae, these include; making use of the usual mycological data, anamorphic and/or teleomorphic state together with biochemical data such as cultural characters (Whalley, 1996), secondary metabolite production (Whalley and Edwards, 1995), or isozyme patterns (Rodrigues *et al.*, 1993 and Brunner and Petrini, 1992).

2.5 The genus *Daldinia*

The genus *Daldinia* was named by the mycologists Cesati and De Notaris to honour their friend, the Swiss catholic monk Agosto Daldini (1817- 1895) (Crivelli *et al.,* 1981). It is a member of the family Xylariaceae, and comprises approximately 20 taxa of wood-inhabiting pyrenomycetes with perithecia embedded in large stromata, that are internally concentrically zoned (Ju *et al.,* 1997). The type species of the genus is *D. concentrica* (Bolt.: Fr.) and was originally described by Bolton (1789) as the common fungus growing on ash (*Fraxinus excelsior*) in England (Figure 2.2). Stromata of *D. concentrica* have been called "cramp balls" in Great Britain because of its assumed positive relieving effect on leg cramps (Ainsworth, 1976). They have also been called "King Alfred's cakes" because of their supposed resemblance to some pastries that this Saxon king reputedly burnt while falling asleep during a baking session (Ju *et al.,* 1997).

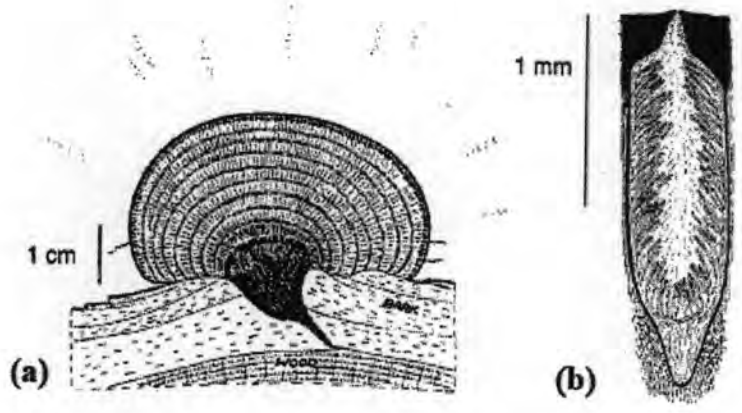


Figure 2.2 Section through perithecial stroma of *Daldinia concentrica.* (a) Discharged spores are seen in the air. (b) A single perithecium (Ingold, 1959)

Species of *Daldinia,* along with a large number of species belonging to the Xylariaceae, are suggested to be endophytes, i.e. organisms that live inside the plant tissue for at least part of their life cycles without causing any disease symptom in the host (Whalley, 1996). Several endophytic fungi are suggested to be present as latent propagules, such as yeast spores or hyphal fragments, in the sap stream of the living host. The propagules remain passive when the sapwood is functional in water conduction, but develop into mycelia and start to decay dysfunctional sapwood when this begins to dry out. This phenomenon is a possible explanation of the rapid development of extensive columns of decay caused by a single fungal genet, in the sapwood and trunks of

angiosperm trees with no sign of exterior wounds (Boddy, 1985). The initial phase of endophytic interactions is largely unknown, and there are only a few examples studied. In the xylariaceous endophytes *Hypoxylon mediterraneum, Hypoxylon fuscum* and *Hypoxylon fragiforme,* contact with tissue of the most common host has been shown to trigger germination or increase the germination rate of ascospores (Chapela *et al.,* 1991; and Vannini *et al.,* 1996). In the latter species, ascospores germinate rapidly on the specific host, *Fagus sylvatica,* by a mechanism mediated by monolignol glucosides in the host acting as specific recognition messengers (Chapela *et al.,* 1990, 1993).

Daldinia spp. cause a wood rotting of host, i.e. both cellulose and lignin are degraded (Cartwright and Findlay, 1958; and Rogers, 1979). *D. concentrica* is an effective wood-decayer, in two months; it can cause a 62.9 % weight loss in sapwood blocks of birch (Nilsson *et al.,* 1989). Wood decayed by *D. concentrica* characteristically has a gross appearance of concentric dark speckled rings, resulting from dark coloured mycelium in the vessels of spring wood, and is sometimes termed Calico wood (Panisset 1929; and Cartwright and Findlay, 1958). In later stages of decomposition, the wood exhibits a patchy appearance as a result of some areas of wood being more decayed than others (Boddy *et al.,* 1985).

Teleomorphs (the sexual stage) of *Daldinia* species are believed to be exclusively angiosperm associates, and probably all produce conidia prior to, or on very young, stromata. However, the *Daldinia* anamorphs (the asexual stage), found in the genus *Nodulisporium* Preuss., are also free-living in nature. *Daldinia* spp. are found to occur in a diverse range of host plants in which they fail to produce a teleomorph (Petrini and Petrini, 1985; and Petrini *et al.,* 1995) indicating a broader host-range than reflected by collections of stromata. However, several difficulties are encountered when performing studies of the host-specificity and distribution of *Daldinia* species. First, species of *Daldinia* are morphologically similar and the interpretations of taxa are somewhat confused. Thus, the name *D. concentrica* has been used for almost any entity within the genus, including taxa growing on both burned and non-burned wood of *Alnus, Betula, Corylus* and *Fraxinus*. Moreover, apart from the difficulties of isolating endophytic fungi from wood (Chapela and Boddy, 1988; and Chapela, 1989), anamorphs of *Daldinia* species can be difficult to identify to species level because the cultural characteristics are few and have been described for only a limited number of species (Petrini and Petrini, 1985; Petrini and Muller, 1986; Petrini *et al.*, 1995; and Ju *et al.*, 1997). The problem

with limitations of cultural characteristics has been solved for different genera of the Xylariaceae, by careful investigation and description of a combination of cultural and e.g. biochemical or molecular characteristics of single ascospore isolates, obtained from investigated teleomorphs (Gowan and Vilgalys, 1991; Brunner and Petrini, 1992; Rodrigues *et al.*, 1993; and Whalley and Edwards, 1995).

The mechanisms behind the formation of the *Daldinia* teleomorph is unknown, but it has been suggested for xylariaceous fungi in general to be linked to a narrow range of host species, as well as the stage of decomposition and water-potential of the wood (Whalley 1985, 1996). Perithecial development of *Daldinia* has not been studied in detail, but Ingold (1954) described the morphology of the ascogenous system of *D. concentrica.* He found that the special feature of this extensive system of straight, unbranched, non-septate ascogenous hyphae is the elongation of the growing tip between successive stages of ascus formation. In the base of each perithecium, a coiled archicarp can be observed, from which the ascogenous hyphae most probably arise. The young perithecium is criss crossed with ascogenous hyphae from which asci grow into the mucilage-filled perithecial cavity (Ingold, 1954).

2.6 Biodegradation of cellulose

Cellulose, in association with hemicellulose, lignin, and other polysaccharides, is a major structural component of plant cell walls and is the most abundant renewable material in the biosphere. It is estimated that plants synthesize about 4×10^9 tones of cellulose annually (Tomme, 1995). Cellulose consists of long chains of β-1,4-linked glucose units, which in turn form higher-order fibril structures. It is present to a large extent in plant materials in a crystalline, water-insoluble form cannot be utilized directly by most which organisms (Clarke, 1997). However, in nature, the cellulolytic bacteria and filamentous fungi produce families of enzymes that synergistically hydrolyze crystalline cellulose to smaller oligosaccharides and finally to glucose which supports their own growth and that of other microorganisms (Ghosh and Ghosh, 1992).

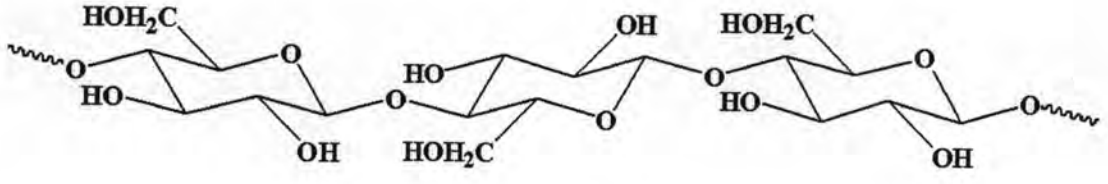
Since cellulose is the most abundant form of polymeric organic carbon on earth, cellulose biodegradation by cellulolytic organisms plays a very important role in carbon recycling ecosystems. Cellulases have attracted wide attention due to the possibility of utilizing these enzymes for converting the huge quantities of renewable cellulose biomass

to fermentable sugars. A major research goal during recent decades has been the economical production of cellulases for converting this biomass into a safe, alternative energy source (Eveleigh and Monetecourt, 1979, Eveleigh *et al.*, 1983, Grohmann and Himmel, 1991, and Ingram *et al.*, 1997).

Research on cellulose biodegradation began over fifty years ago (Reese *et al.*, 1950, and Reese, 1956), and present knowledge has been obtained through the concerted efforts of taxonomists, microbial physiologists, biochemists, geneticists, and molecular biologists. However, due to its complex physical organization and its interactions with other plant cell wall components, the hydrolysis of cellulose is a highly complex multi-enzymatic process.

2.7 Cellulose structure

Cellulose is an unbranched, insoluble, homopolymer composed of up to 14,000 anhydro-D-glucose units, linked together by β-1,4-D-glucoside bonds (Figure 2.3). The cellulose chains aggregated together via intra- and inter-molecular hydrogen bonding to form rigid fibrils with a highly ordered structure. The degree of order found within and between fibrils varies from regions where the glucan chains are firmly held in parallel, the crystalline regions, to region in which there is a lesser degree of order, the so called amorphous region (Young and Rowell, 1986). The higher the ratio of crystalline to amorphous regions, the more resistant the cellulose is to enzymatic attack.



Cellulose
(β-1,4 linkages)

Figure 2.3 A schematic structure of a cellulose chain

Most of the cellulose is produced as a component of plant cell walls, but other organisms such as bacteria, marine invertebrates, fungi, slime molds, and amoebae can

also synthesize cellulose. In the plant cell walls, cellulosed fibrils are usually embedded in amorphous matrices consisting of hemicellulose and lignin. There are covalent cross-linking bonds between hemicellulose and lignin chains involving ester or ether linkages (Atalla, 1993). This arrangement makes plant cell walls resistant to attack by many microorganisms under natural circumstances. However, cellulolytic organisms can produce an array of polysaccharide hydrolases and lignin degradation enzymes which enable them to completely mineralize lignocellulose materials.

2.8 Enzymes required for the hydrolysis of cellulose

An efficient cellulolytic enzyme system requires endo-, and exo-type enzymes and β-glucosidases. Microbial cellulases have been classified into more than 60 different families depending on their structure and substrate specificity, but also according to the mode of action: some microbial cellulases display both endo-, and exo-type attack features (Henrissat and Davies, 1997). The complementary activities of endo- and exo-type enzymes lead to synergy, an enhancement of activity, which is more than the added activities of individual enzymes. Exo-exo synergy is also observed, and may indicate a low, inherent endoglucanase activity of the exoglucanases (Tomme *et al.*, 1995; Shen *et al.*, 1995). **Endoglucanases** (EGs, E.C. 3.2.1.4) belong to endo-type enzymes that hydrolyse cellulose microfibrils preferentially in the amorphic parts of the fibril (Figure 2.2). The catalytic region of the enzyme is groove-shaped that enables the attachment of the enzyme and the hydrolysis in the middle part of the cellulose fiber (Divne *et al.*, 1994). Endoglucanase activity in other white rot fungi is a common feature, and it probably exists in all wood-degrading fungi including brown-rot fungi (Highley, 1988). **Cellobiohydrolases** (CBHs, E.C. 3.2.1.91) are exo-type enzymes that attack cellulose fibres from both reducing and non-reducing ends. In *Trichoderma reesei*, CBH I attacks reducing ends, and CBH II the non-reducing ends of the fibre (Teeri, 1997). No exo-type activity has been observed in brown-rot fungi, so they cannot degrade pure crystalline cellulose. Exceptions to this generalisation can be found in a few brown rot fungi belonging to the family *Coniophoraceae* (Nilsson and Ginns, 1979). In *Coniophora puteana* two CBHs have been isolated (Schmidhalter and Canevascini 1993). The product of CBH action, cellobiose is hydrolysed by **β-glucosidases** (BGs, E.C. 3.2.1.21) to two glucose units. Cellobiose and glucose can be taken up and assimilated by the hyphae. The cellobiose taken up is probably hydrolysed to glucose by cell wall bound or intracellular β-glucosidases.

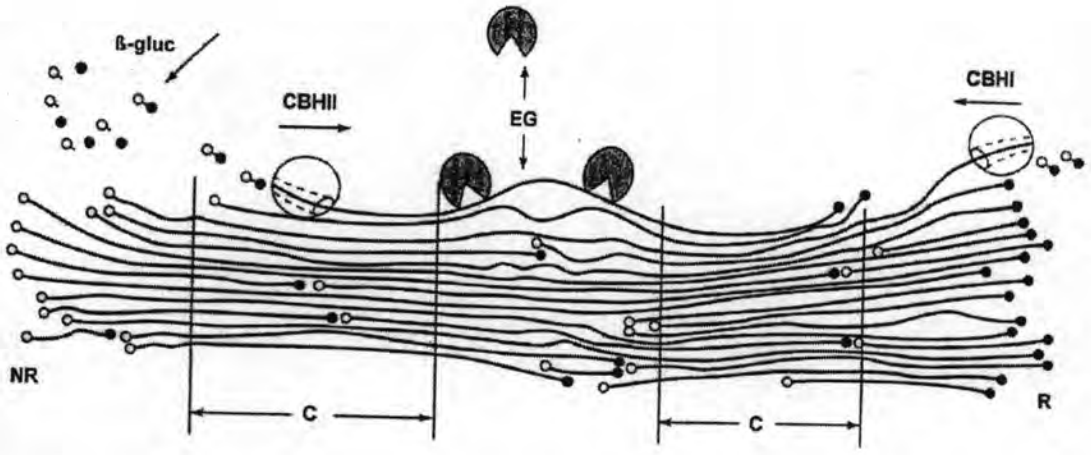


Figure 2.4 A schematic view of the cellulose structure and action of the Endoglucanase (EG), Cellobiohydrolase (CBH), and β-glucosidases (β-gluc) in *Trichoderma reesei*. C; defines the highly ordered crystalline region, R; the reducing ends (filled circles), and NR the nonreducing ends (open circles). Modified from Teeri (1997)

2.9 β-Glucosidases: Mechanism and applications

β-Glucosidases are often referred to as cellobiases (Kovar *et al.*, 1987). They hydrolyze the β-1,4-glycosidic bond of cellobiose, and they play a role in cellulose degradation since they are involved in the final step that forms glucose (Kuriyama *et al.*, 1995). β-Glucosidases are found in many prokaryotes and eukaryotes. They have a variety of functions depending on their location and substrate specificity. In humans, acid β-glucosidase is responsible for catabolism of glucocerebroside (Mikhaylova *et al.*, 1996). Some β-glucosidases in plants are utilized in the chemical defense against pathogens and herbivores. Toxic compounds are released from cyanogenic glucosides, or arbutin when they are hydrolyzed. Others are utilized in the regulation of phytohormones such as cytokinin, gibberellin, and auxin by causing the release of the active forms from the inactive hormone-glucoside conjugates (Leah *et al.*, 1995). In many fungi and bacteria, β-glucosidases are utilized in the conversion of cellobiose to glucose. Many of these enzymes are also capable of utilizing other sugars as acceptors so that

transglycosylation occurs rather than hydrolysis. This additional activity has been noted in mammals (Vanderjagt *et al.*, 1994), plants (Kuriyama *et al.*, 1995), fungal (Christakopoulos *et al.*, 1994), and bacterial (Watt *et al.*, 1998) β-glucosidases. There has been some interest in using transglycosylation for the production of oligosaccharides and glycosides. This could provide a more economical method of production since it occurs in one step and the sugar product can be altered depending on the initial substrate and acceptor (Yzaki *et al.*, 1997). Additionally, β-glucosidases can be used in ethanol production by including an ethanol producing organism in the saccharification process (Hoh *et al.*, 1992). Studies of β-glucosidases that function in cellulose breakdown are important because the degradation of cellulose to glucose may provide a solution to resource problems involving fuel, feedstock, and food materials that may occur in the future (Skory *et al.*, 1996).

At present there is no well-defined method for the classification of these enzymes. However, on the basis of substrate specificity, β-glucosidases have been grouped into three classes, namely, (1) aryl-β-glucosidases, (2) true cellobiases, and (3) broad substrate specificity enzymes, which are active on a wide variety of substrates. In another approach, a classification scheme based on sequence and folding similarities was proposed for glycosidases by Henrissat and co-workers (Henrissat, 1991; and Henrissat and Bairoch, 1996), and this is the currently accepted method for classification of these enzymes. In this method, β-glucosidases along with other carbohydrases have been assigned to various families under the glycosylhydrolase category and out of 88 such families defined (last update July 15, 2002), β-glucosidases have been placed in family 1 and family 3. Both these families comprise of retaining enzymes that hydrolyze their substrates with net retention of anomeric configuration that occurs via a double-displacement mechanism (Withers and Street, 1989, and Withers, 2001). Family 1 of glycosylhydrolases includes β-glucosidases from archaebacteria, plants, and mammals. The crystal structures have been solved for a few family 1 β-glucosidases, which are also designated as members of the 4/7 super family with a common eightfold β/α barrel motif, consisting of similar amino acid sequences at the active site (Jenkins *et al.*, 1995). Family 3 comprises of β-glucosidases of some bacteria, molds, and yeasts.

Mechanism of catalysis of β-glucosidases and other glycosidases can be divided into two classes, inverting and retaining glycosidases by inversion or retention of the anomeric configuration of a released monosaccharide (Figure 2.5). The mechanisms of both enzymes differ in that inverting glycosidases catalyze via a direct displacement of aglycone (leaving group) by water (nucleophile), whereas retaining glycosidases catalyze through a glycosyl-enzyme intermediate as a double displacement mechanism. In spite of the difference, both enzymes employ the same principle of catalytic mechanism, general acid-base catalysis. They have a pair of conserved carboxylic amino acids, which may be aspartate (Asp) or glutamate (Glu). In inverting glycosidases, one residue acts as a general acid and the other as a general base. In retaining glycosidases, one residue functions both as a general acid and a general base, while another residue functions both as a nucleophile and a leaving group. The cause of distinct mechanisms in both enzymes is the distance between the two carboxylic acid groups. In retaining glycosidases, they are close enough to form a glycosyl-enzyme intermediate. However, in inverting glycosidases, the carboxylates have greater distance separation allowing the insertion of a water molecule for direct attack (McCarter and Withers, 1994).

Application of β-glucosidases can be used to synthesize both oligosaccharides and glycosides via two approaches (Figure 2.6). The first is "reverse hydrolysis" (or equilibrium controlled synthesis) that shifts the equilibrium of the reaction from hydrolysis to synthesis by using conditions such as high concentration, high temperature, trapping or removal of product from the reaction. The second approach is "transglycosylation" (or kinetically controlled synthesis) where the equilibrium of the reaction is preserved, but synthesis can be achieved by using an efficient glycosyl-donor as substrate and excess of glycosyl-acceptor (nucleophile) which competes within the hydrolysis (Nilsson, 1988; and Ichikawa *et al.*, 1992). Both approaches have been used for synthesis of oligosaccharides and glycosides using microbial β-glucosidases from organisms such as *Aspergillus niger* (Yan and Liau, 1998), *Fusarium oxysporum* (Makropoulou *et al.*, 1994) *Trichoderma pseudokoningii* (Dong *et al.*, 1996). Interest in the synthesis of β-glucosides has arisen owing to a variety of functions exhibited by these molecules because of their potential detergent, food and pharmaceutical applications (Vandamme and Soetaert, 1995).

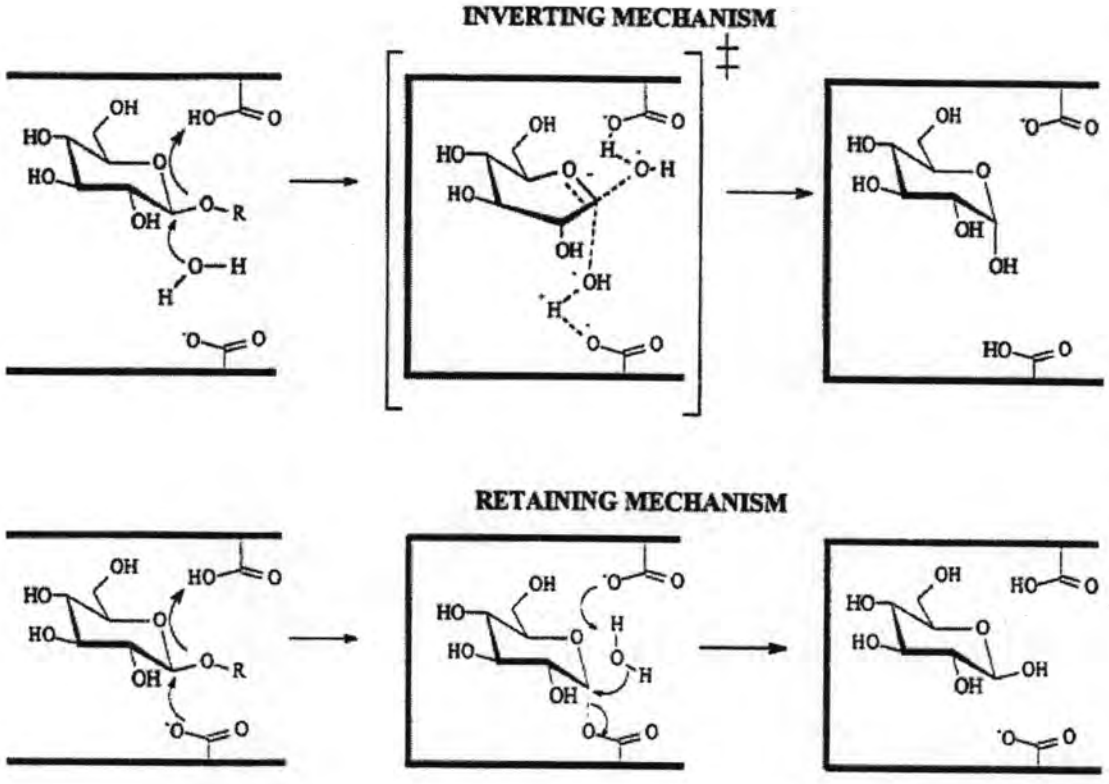


Figure 2.5 Mechanisms of retaining and inverting glycosidases

The transferase activity of β-glucosidases may be used in the synthesis of a variety of compounds such as oligosaccharides and glycoconjugates. The role of these sugar-linked molecules is becoming understood in the biological and pharmaceutical sciences, necessitating their availability on a large scale. In this regard, considerable progress has been made in the use of the enzymatic route of biosynthesis of these compounds. Chemical methods of synthesis are often slow and nonspecific, and this is the major barrier to their widespread application. Biotransformations are now well established as a means for the manufacture of pharmaceuticals, fine chemicals, and food ingredients, owing to the high selectivity of enzymes and the use of mild reaction conditions (Bhatia *et al.*, 2002). Alkyl-glucosides are a new generation of biodegradable, nonionic surfactants with good emulsifying and antimicrobial activities. These have been used as drug carriers and as solubilizing agents for biological membranes, particularly hexyl-, heptyl-, and octyl-glucosides (Kiwada *et al.*, 1985; and Shinoyama *et al.*, 1991). Butyl-glucoside is also valuable because it serves as a precursor in the synthesis of gemini

surfactants and other pharmaceutical compounds. The former are useful as liquid crystal generators (Castro *et al.*, 1997). Esterification of butyl-glucoside in the presence of phenyl butyric acid in a coupled β-glucosidase/ *Candida* sp. lipase reaction resulted in synthesis of an aromatic *n*-alkyl glucoside ester that was effective in the treatment of fever, rheumatism, headache, and other ailments (Otto *et al.*, 1998). The role of methyl-glucoside as a precursor for the synthesis of methyl-laminario oligosaccharides was also established. These have utility in the therapy of AIDS (Dainipon-Ink-Chem, PN 1996). Apart from primary alcohols, secondary, tertiary, monoterpene, and aryl alcohols or even diols may serve as acceptors of the glucosyl group in β-glucosidase-catalyzed biotransformations. For instance, the glucosides of organosilicon alcohols, synthesized by free and immobilized *Pyrococcus furiosus* enzymes, have potential applications as agrochemicals and drugs (Fischer *et al.*, 1996). Enzymatic synthesis of glucosides of monoterpenyl alcohols is also gaining importance in the food industry (Gunata *et al.*, 1994). The market is growing because of increased public concern for the safety of food ingredients. Thus, these may be commercialized as "natural food flavors". The synthesis of some natural compounds, like aryl-glucosides possessing repellant and antifeedant properties, was achieved with thermostable β-glycosidases from *Sulfolobus solfataricus* (Tricone and Pagnotta, 1995).

The application of β-glucosidases in a well-defined manner requires large-scale production of the enzymes and a detailed knowledge of their reaction mechanisms. Currently, research in several laboratories is underway to understand the molecular basis of their wide substrate specificity and assembly into multimodular entities. The identification of amino acid residues occurring at the enzyme active site is of considerable importance for revealing the structure-function relationship as well as designing mutant enzymes with improved characteristics.