



# A Review on Mycorrhizae and Related Endophytic Fungi as Potential Sources of Enzymes for the Bio-Economy

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## Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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## ABSTRACT

Ericoid mycorrhizal (ERM) fungi and related fungal root endophytes do form symbiotic associations with roots of ericaceous plants. These groups of fungi can have profound impact on community of plants in soil environment. Studies conducted on *Hymenoscyphus ericae* revealed that *H. ericae* can produce extracellular enzymes such as phosphatases, proteases, cellulases and pectinases, which support the utilization of nitrogen, phosphorus and permitting access to other valuable nutrient embedded within the soil and decaying plant tissues. Most studies conducted on extracellular enzymes from these fungi majorly focused on the use of plating method to determine activity. Currently, there is little information on extracellular enzymes from ERM for the bio-economy, but there are proofs that some ericoid, ectomycorrhizal and dark septate endophytic fungi have the potential to produce a good number of hydrolytic enzymes *in vitro*. Therefore, this review seeks to employ available information on these fungi and their ability to produce enzymes when growing in liquid medium where their production can be optimized for commercial purposes.

**Keywords:** Mycorrhizae; endophytic fungi; hydrolytic enzymes; bio-economy.

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## 1. INTRODUCTION

Ericaceous plants are able to associate symbiotically with soil fungi to form a distinctive type of mycorrhiza, termed ericoid mycorrhiza. This association was initially investigated among members of the family Ericaceae [1], but a morphologically similar mycorrhizal association was described also in the family Epacridaceae [2, 3]. The phylogenetic analyses indicated that Ericaceae, Epacridaceae and Empetraceae are traditionally considered as separate families of the order Ericales but most common ericoid mycorrhizal (ERM) fungi are found in the genera that include; *Erica*, *Calluna*, *Rhododendron*, *Empetrum* and *Vaccinium* [4].

Plants contribute substantially to ecosystem development and soil formation [5]. They add to available nutrients and carbon, their rhizospheres harbor a great diversity of microorganisms [6], including mycorrhizal fungi. Dickie *et al.* [7] proposed that the distribution pattern of mycorrhizal fungi in newly developing ecosystems cannot be predicted with exactness but are believed to be driven by two main factors. Firstly, the edaphic conditions and plant community composition. Secondly, the ecosystem disturbances. According to Petrini [8] most plants in natural ecosystems form symbiotic associations with mycorrhizal fungi and fungi endophytes. These fungi symbionts can have huge effects on the plant fitness, evolution and ecology [9] and thereby determining the plant communities in a given area [10]. Mycorrhizal fungi generally grow and colonize plant roots and rhizosphere, while endophytic fungi on the other hand reside within plant tissues and sometimes grow within roots, stems and/or leaves emerging to sporulate host-tissue senescence [11]. Plants in the Ericaceae have a unique mycorrhizal association, where fungi colonize their fine roots (hair roots) and produce hyphal complexes in epidermal cells [12]. This association plays important roles in plant nutrition and ecological adaptation to unsuitable environments [12].

Researchers [12-16] have shown that most plants in natural ecosystems form a symbiosis with mycorrhizal fungi and fungal endophytes (Table 1). Fungal symbionts can have a dramatic effect on plant fitness, evolution, and ecology [17]. They regulate nutrient and carbon cycles, and influence soil structure [18] thereby determining the plant communities in a given area [10]. Mycorrhizal fungi grow and colonise plant roots and the rhizosphere, while endophytic

fungi reside within plant tissues, sometimes growing within roots, stems, and leaves and sporulate on host-tissue at senescence [11]. Fungi found growing around and within roots are termed root endophytic fungi (Fig. 1). Molecular and biotechnological techniques have been extensively applied to the study of mycorrhizae and root endophytic fungi, and the knowledge gained has substantially modified the view of researchers on the biology, evolution, and biodiversity of mycorrhizal fungi [18]. Therefore, this study focuses on enzymes from ericoid mycorrhizal and associated root endophytic fungi, their characterisation and production for the bio-economy and related studies.

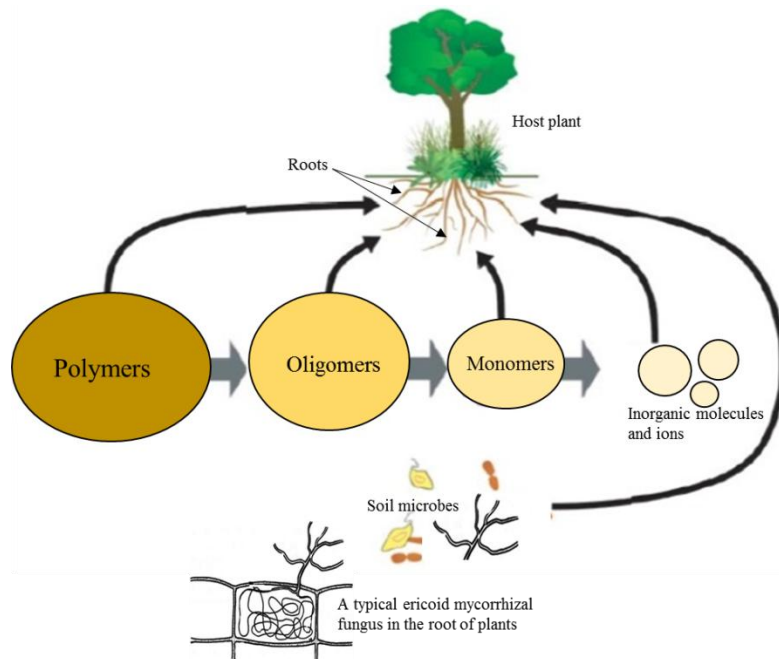
The morphology of ericoid mycorrhizal fungi is conserved in many plant species. The epidermal cells of the fine ericaceous hair roots harbour more or less dense coils of fungal mycelium [20] which appear to remain enclosed within single root cells. As in all endo-symbioses, the intracellular fungal symbiont is separated from the plant cytoplasm by a plant-derived membrane, which invaginates to follow fungal growth. Ericoid mycorrhizal plants are mostly found as understorey vegetation in boreal and Mediterranean forests. Ericaceous shrubs can become dominant in many natural and semi-natural heathland communities. This happens especially in an environment where a slow decomposition of the plant litter is found, resulting in acidic soils rich in recalcitrant organic matter but low in available mineral nutrients such as nitrogen and phosphorus [21]. The survival of ericaceous plants under such nutrient-stressed conditions is thought to depend on the formation of mycorrhizal symbiosis, and the evolution of the association is noted to be mediated by the selective advantages conferred by fungal infection [22].

### 1.1 Ericoid Mycorrhizal (ERM) Fungi

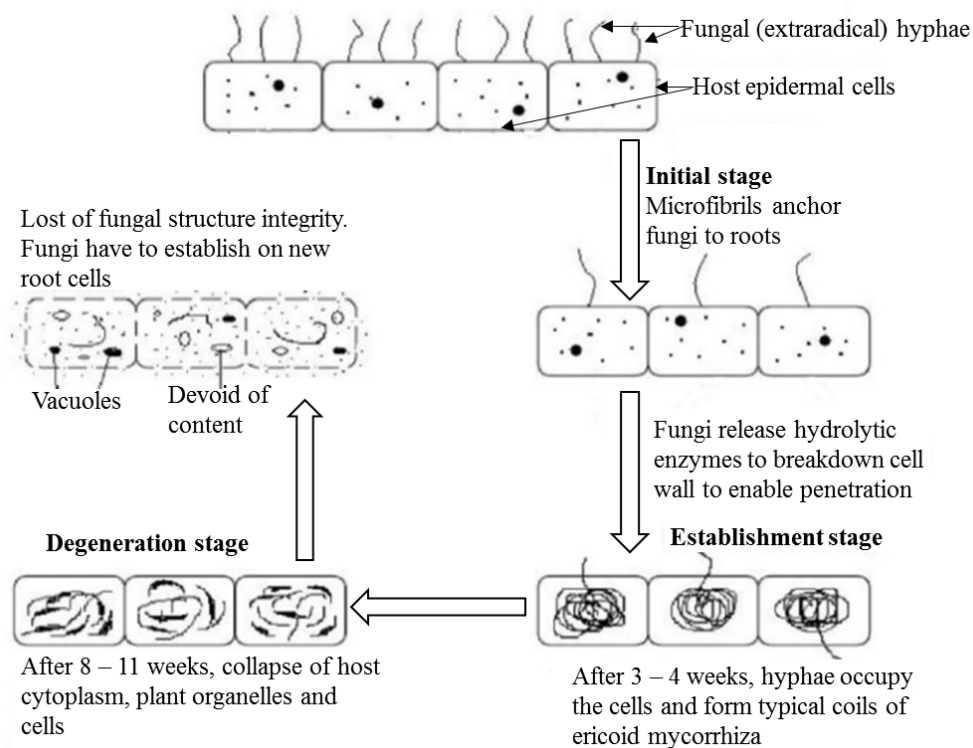
The relationship that exists between ericoid mycorrhizal fungi and ericaceous plants is described as obligate with the formation of several distinctive mycorrhizal categories and endophyte relations [11]. Plants belonging to Ericaceae have a distinct mycorrhizal association, where fungi colonise fine roots (hair roots) and produce hyphal complexes in epidermal cells (Fig. 2 and Table 1) [11]. Culturable ERM fungi that have been isolated are mainly from the phylum of Ascomycota [11] with *Scytalidium vaccinii* as the first isolated and identified ericoid mycorrhizal fungus [23].

**Table 1. Plant-mycorrhizal/root endophytic fungal relationships**

	<b>Ericoid mycorrhizal fungus</b>	<b>Ectomycorrhizal fungus</b>	<b>Root endophytic fungus</b>	<b>Reference(s)</b>
Hyphae	Hyphae cover root tips and colonise the rhizodermis forming hyphal coils.	Hyphae include root tips creating 'Hartig' net and do not penetrate cells of cortex in root tips. Hyphal 'Hartig' net surrounds cortical cells.	Hyphae in root tips penetrate cells of the cortex	[11]
Host plant	Ericaceous plants (common genera are <i>Erica</i> , <i>Calluna</i> , <i>Pieris</i> , <i>Empetrum</i> , <i>Vaccinium</i> , and <i>Rhododendron</i> ).	Commonly found on trees and many smaller perennial plants (Pinaceae, e.g. cedars, firs, hemlocks, larches, pines, and spruces. Fagaceae, e.g. oak and beech Betulaceae, e.g. birch).	All plant roots with mycorrhizal fungi.	[38, 41]
Soil condition	Mostly found in acidic soil.	Mostly found in the soil with moderate pH.	All soil types.	[39]
Benefit	Enhanced nutrient uptake particularly from organic sources.	Enhance nutrient absorption and protects plants against nematodes and soil pathogens.	Secrets bioactive metabolites such as alkaloids, flavonoids, and phenolics that inhibit soil pathogens.	[42, 43]



**Fig. 1. Proposed scheme of plant nutrient sources in which soil microbes and root-derived enzymes contribute to depolymerisation and mineralisation of organic matter. Plants acquire inorganic nutrients directly from natural nutrients via mycorrhizal symbionts and also take up organic compounds and microbes [19]**



**Fig. 2. Series of events that occur during the formation of the ericoid mycorrhizal association in the Ericaceae hair roots. The stages include initiation, establishment, and degeneration, lasting up to 11 weeks (Modified from Bizabani [25])**

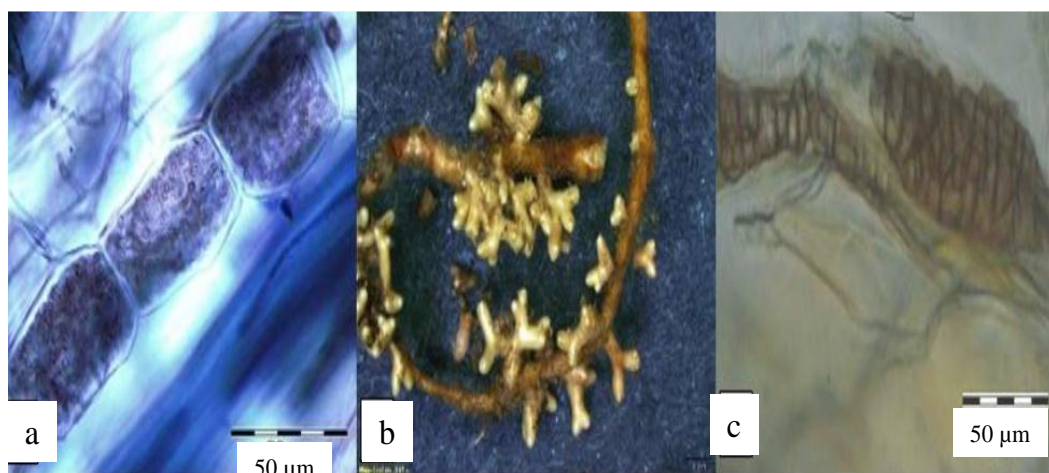
Ericaceous plants are naturally found on well-drained and acidic soils in temperate climatic zones; as well as at high elevations in the mountainous areas of the tropic, where they can become a dominant plant community [24]. The survival of ericaceous plants under nutrient-stressed conditions is thought to depend on the formation of the mycorrhizal symbiosis (Table 1), and the evolution of the association is noted to be mediated by the selective and competitive advantages conferred by fungal colonisation [22].

The ability of ERM to degrade complex substrates (e.g., starch and cellulose) have been studied and performed over the years by some authors [21]. Their results indicated that the enzymatic degradation of organic polymers in the soil and the transfer of some of the resulting products to the root is a significant benefit to the growth and development of ericaceous plants (Table 1) [11]. Subsequently, host plants can access nutrients from the unavailable organic sources. The production and activity of enzymes from mycorrhizal fungi influence the development of the host plant (Fig. 2) and confers on their host the ability to compete successfully with other plant species. For example, *Calluna vulgaris* is the most common ericaceous species in the oceanic North West of Europe, where it was found to form nearly a pure plant community [26]. The competitive ability allows *C. vulgaris* to compete over *Nardus stricta* as demonstrated using pot cultures under different growth conditions [27]. There is now a concerted effort

geared towards the study of ERM fungi because of their ability to grow on polluted sites contaminated by heavy metals [24] which could suggest the possibility of using them for bioremediation purposes.

## 1.2 Ectomycorrhizal (ECM) Fungi Hyphae Surrounding the Plant Cells Within the Root Cortex

ECM is dominant group of the soil microbial community in temperate and boreal forests. Tropical rainforests harbour the highest number of trees, and different ecological studies have attributed this to the presence of ECM fungi [28]. They form a fungal mantle (sheath) surrounding the plant's roots and an intercellular network of hyphae (Fig. 3 and Table 1) forming a 'Hartig' net around cortical root cells [11]. They mainly belong to members of Basidiomycota, Ascomycota and some Zygomycota [29]. *Suillus* species possess different traits that make them suited for co-invasion with *Pinus* invasions these include a large number of fruit bodies, spores resistant to environmental factors, dispersal mode, and rapid rate of colonising roots of pines [30]. Another trait suggested in host invasion by mycorrhizal fungi is the ability of these fungi to break down substrate with the help of specific enzymes involved in the degradation of woody substrates [31]. ECM can as well facilitate the acquisition of some vital mineral nutrients in the establishment of plants on sites contaminated by toxic metals [32].



**Fig. 3. Typical ericoid, ectomycorrhizal and root endophytic fungal structures observed under the light microscope. (a) Ericoid coils within the individual cells, (b) ectomycorrhizal mantle surrounding roots, (c) dark septate root endophyte within individual cells (Photo credits: Dr Christine Bizabani and Dr Veronique Chartier-Fitzgerald)**

### 1.3 Endophytic Fungi

The word endophytes refer to fungi belonging mainly to the Ascomycota, which usually colonise healthy plant tissue causing no immediate or overt adverse effects [33]. It should be noted that many endophytes may cause diseases under stressed conditions. An endophytic fungus can inhabit all available tissues including leaves, roots, stems, twigs, barks, fruits, flowers and seeds within the host system. Root endophytic fungi are those mainly found in the roots of plants (Fig. 1). Fungal endophytes exhibit complex web interactions with host plant and have been extensively studied as rich sources of new bioactive natural products [34]. They can produce some vital enzymes that include cellulases, amylases, laccases, and pectinases [35]. Starch-degrading endophytic fungi such as *Gibberella pulicaris* and *Acremonium* sp. have been reported [36].

Dark septate endophytic (DSE) fungi can form symbiotic associations with Ericaceae (e.g. *Phialocephala fortinii*) and produce extracellular enzymes [37]. DSE are conidial or sterile ascomycetous fungi that colonise living plant roots without causing apparent tissue disorganisation [38]. DSE colonisation has been recorded in about 600 plant species which represent about 320 genera and 114 families [38]. They are widely distributed in soil and most common in environments with intense abiotic stress [39]. Some DSE (*Cadophora*, *Leptodontidium*, *Phialophora*, and *Phialocephala*) isolated from roots of poplar trees on metal-polluted sites [40], produced auxin while some accelerated plant growth by the release of volatile organic compounds (VOCs) [40]. The ability of metal-resistant DSE strains producing both soluble and volatile compounds can discern the possibility of using them for phytoremediation and enzyme production.

### 1.4 Other Mycorrhizal Fungi

Arbuscular, ectendomycorrhizal (arbutoid and monotropoid) and orchid mycorrhizal fungi are among other mycorrhizal fungi that exist in the rhizosphere. Arbuscular mycorrhizal (Vesicular-Arbuscular Mycorrhizas, VAM or AM) associations are ubiquitous endomycorrhizal fungi, which associate with more than 74% of all terrestrial plants [9]. They belong to the phylum Glomeromycota and can produce arbuscules, hyphae, and vesicles within root cortex cells [44,45].

Ectendomycorrhizas consist of arbutoid and monotropoid fungi that are characterised by a hyphal sheath, Hartig net and intracellular hyphal colonisation (i.e. they show the characters of both ectotrophic as well as endotrophic mycorrhizal fungi) [46,47]. Arbutoid mycorrhizal fungi can be found in the Ericaceae subfamily Arbutioideae, while monotropoid fungi occur with the Ericaceae subfamily Monotropoideae and Orchidaceae. In orchid mycorrhizal associations, coils of hyphae (pelotons) penetrate the root cortex, root tubers in the plant family Orchidaceae [48].

Recently, cavendishoid and sheathed ERM associations have been discovered with roots of plants [11]. Cavendishoid mycorrhizal (CVM) is characterised by the unclamped hyphal sheath, mantles with intercellular fungal tissue that resembles a Hartig net and hyphal colonisation in cortical cells [46]. Sheathed ERM, on the other hand, has clamped hypha and does not possess any structure that resembles a Hartig net that is present in CVM associations [16].

Saprotrophic potential of ericoid fungi and their ability to degrade complex and recalcitrant polymeric substrates has been demonstrated over the years by several authors [21,49,50,51]. Based on their results, it is widely accepted that the major benefit is the enzymatic degradation of organic polymers in the soil, and the transfer of some of the resulting products to the root [4]. Subsequently, host plants can access the unavailable organics such as nitrogen and phosphorus [52]. Also, it has been demonstrated that ericoid mycorrhizal fungi confer to their host the ability to compete successfully with other plant species. For example, *Calluna vulgaris* is the most common ericaceous species in the oceanic North West of Europe, where it can form almost pure plant communities [53]. The competitive ability of mycorrhizal fungi e.g., *C. vulgaris* to compete over *Nardus stricta* was demonstrated in pot cultures under different nutrient conditions [27], and suggested to depend on allelopathy rather than competition for nutrients [54]. There is a renewed interest in the study of ericoid mycorrhizal fungi because of the ability of ericoid mycorrhizal plants to grow on polluted sites contaminated by heavy metals, and their potential applications in bioremediation.

Endophytic fungi are those fungi that are capable of colonizing plant tissue without causing immediate, overt negative effects [34]. Within the host system, the fungi inhabit all available tissue

including stem, twig, barks, root, fruit, flower and seeds. They improve the resistance of the host plant to adversity by secreting bioactive metabolites such as alkaloids, flavonoids, phenolic acids and steroids. These fungi are also able to produce some vital enzymes that include; cellulases, amylases, laccases and pectinases [35].

## 2. MICROBIAL ENZYMES AND FUNGI

The potential of using microorganisms as biotechnological sources of industrially relevant enzymes has stimulated renewed interest in the exploration of extracellular enzymatic activity in several microorganisms [55]. Production of extracellular enzymes in microorganisms is significantly mediated by a number of factors which include: temperature, pH, aeration and medium constituents [56]. The relationship between these variables has a remarkable effect on the ultimate production of the enzymes. There have been reports on various fermentation parameters that influence amylase production by different fungi [57] which include the traditional “one-variable-at-a-time approach” for medium optimization. Fungi produce a variety of extracellular enzymes, and some DSE fungi have been tested for their enzymatic capabilities [39].

Microbial enzymes are known to play crucial roles in biocatalysis. This encourages their use for various industrial applications. Their use as biotechnological sources of industrially relevant enzymes has stimulated renewed interest in the exploration of extracellular enzymatic activity in several microorganisms [55] such as mycorrhizal fungi. Over 500 essential industrial products have been produced using enzymes [58]. The production of extracellular enzymes in microorganisms is significantly mediated by some factors which include temperature, pH, aeration and medium constituents. The relationship between these variables has a remarkable effect on the final production of the enzymes. DSE such as *Periconia* and *Microdochium* fungi have been tested for their enzymatic capabilities. These fungi tested positive for amylases, cellulases (Table 2), polyphenol oxidases and gelatinases [39].

ERM are among culturable fungi [11,25,59]. *Scytalidium vaccinii* (formerly, *Rhizoscyphus ericae*) was the first isolate from ericaceous plants [60,61]. Species such as *Oidiodendron*, especially *O. maius* have also been found widely in association with different Ericaceae

populations [24]. Other fungi that form ERM associations with Ericaceae include species of *Capronia* [62], DSE such as *Phialocephala fortinii* [37] and some basidiomycota (*Clavaria*). Moreover, molecular techniques have contributed greatly to gain insights on fungal diversity as well as the molecular ecology of mycorrhizal fungi. These techniques have been applied extensively to the study of ericoid fungi, and the knowledge gained has modified substantively the view of researchers on ericoid mycorrhizal and endophytic fungi symbiosis.

### 2.1 Amylases

Amylases are enzymes which hydrolyse starch molecules to produce various products that are composed of glucose units [63]. These enzymes are of high significance to modern biotechnology with applications ranging from food to bioethanol production [64,65]. Amylases are one of the most common biomolecules which account for about 25% of the world's enzyme production [66]. Amylases are capable of hydrolysing  $\alpha$ -1,4-glucosidic bonds of amylose, amylopectin, glycogen and their degradation products [66]. They hydrolyse bonds between adjacent glucose units to produce products typical for the particular enzyme involved. *Tricholoma matsutake* has been reported to produce high  $\alpha$ -amylase activity when the production medium was supplemented with rice powder [67]. Hur *et al.* [68] indicated that the amylase activity of *T. matsutake* was higher with starch originated from barley grain than from other sources. Microbial amylases have almost replaced chemical hydrolysis in the starch processing industry. Today, amylases have impacted the world enzyme market positively [66, 69]. Several amylase preparations are now available for various industrial applications.

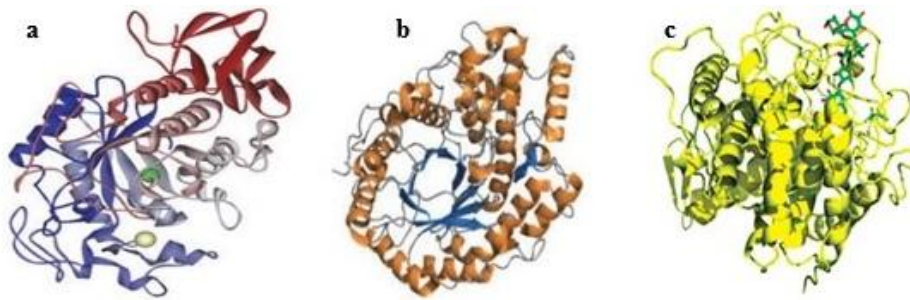
Amylases are broadly classified into  $\alpha$ ,  $\beta$ , and  $\gamma$  subtypes (Fig. 4) viz.  $\alpha$ -amylase (also called 1,4- $\alpha$ -D-glucan glucanohydrolase, glycogenase, EC 3.2.1.1) cleaves at random locations on the starch chain, to yield maltotriose and maltose, and glucose from amylose and amylopectin [70].  $\beta$ -amylase (also called 1,4- $\alpha$ -D-glucan maltohydrolase, glycogenase, saccharogen amylase, EC 3.2.1.2) catalyses the hydrolysis of  $\alpha$ -1,4 glycosidic bond from the non-reducing end to yield two glucose units (maltose) [65]. Amyloglucosidase (AMG), (also called  $\gamma$ -amylase, glucoamylase, glucon 1,4- $\alpha$ -glucosidase, exo-1,4- $\alpha$ -glucosidase, EC 3.2.1.3) cleaves  $\alpha$ -1,6 glycosidic linkages, as well as the

**Table 2. Some selected mycorrhizal and endophytic fungal enzymes, their sources, relationship with the host plants and methods of detection**

Fungus	Enzyme	Source	Relation-ship	Method for detection	Reference(s)
<i>Hymenoscyphus ericae</i>	Cellulase	Ericaceous Plant	ERM	p-nitrophenol based substrate	[82]
<i>Acremonium</i> sp.	Amylase	Ericaceous plant	RE	Plating	[90]
<i>Phialophora finlandia</i>	Cellulase	<i>Pinus strobus</i>	DSE	Plating	[91]
<i>Periconia macrospinosa</i>	Amylase, Cellulase	Tallgrass	DSE	Plating	[90]
<i>Suillus variegatus</i>	Cellulase	Ericaceous plant	ECM	Plating	[92]
<i>Mortierella hyaline</i>	Cellulase, Xylanase	<i>Osbeckia stellata</i>	RE	Plating, DNSA	[93]
<i>Paecilomyces variabilis</i>	Amylase, Xylanase	<i>Osbeckia chinensis</i>	RE	Plating, DNSA	[93]
<i>Hymenoscyphus ericae</i>	Endoxylanase	Ericaceous plant	ERM	DNSA	[82]
<i>Lyophyllum shimeji</i>	Glucoamylase	Barley	ECM	Somogyi–Nelson	[67]
<i>Cylindrocephalum</i> sp.	Amylase	<i>Alpinia calcarata</i>	RE	Plating, DNSA	[34]
<i>Tricholoma matsutake</i>	Amylase	<i>Pinus densiflora</i>	ECM	Somogyi-Nelson	[68]
<i>Leohumicola</i> sp.	Endoglucanase	Ericaceous plant	ERM	DNSA	[94]
<i>Leohumicola incrustata</i>	Amylo-glucosidase	Ericaceous plant	ERM	DNSA	[95]

[RE = root-endophyte, DNSA = Dinitrosalicylic acid]





**Fig. 4. Computer simulated three-dimensional amylose structure: (a) alpha-amylase, (b) beta-amylase [70] and (c) amyloglucosidase [72]**

last  $\alpha$ -1,4 glycosidic linkages at the nonreducing ends of amylose and amylopectin to produce glucose [71].

## 2.2 Cellulases

There are some studies aimed at obtaining novel microorganisms that are capable of producing cellulases with higher specific activities and greater stability. ERM and related fungal species have been demonstrated to produce or secrete extracellular hydrolytic enzymes in agar media with appropriate nutrient composition [50]. Cellulase has a broad range of industrial applications such as in the textile, laundry, pulp and paper industries, in fruit juice extraction, and animal feed additives, as well as in biofuel production [73]. Cellulases can be used for extraction and clarification of fruit and to increase the yield of juices [74].

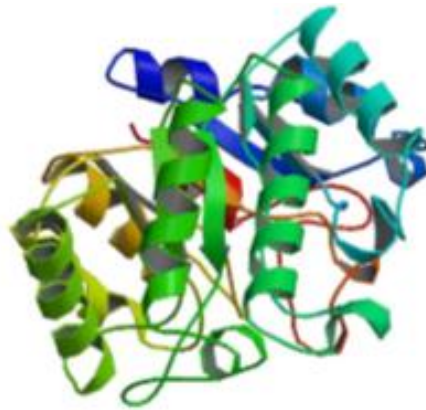
Cellulase may cleave either glucose dimers from the end of the cellulose polymer (exoglucanase) or randomly fragment the polymer into smaller molecules by internal digestion (endoglucanase). These two types of actions usually take place simultaneously, but the amount of each enzyme expressed and rate of activity differs between microbial species [75]. Hydrolysis of cellulose is carried out by three components of cellulose: endo-(1,4)- $\beta$ -glucanase (EC 3.2.1.4), exo-(1,4)- $\beta$ -glucanase (EC 3.2.1.91) and  $\beta$ -glucosidases or cellobiase (EC 3.2.1.21) [76]. The endoglucanase cleaves internal  $\beta$ -1,4-glucan linkages in cellulose randomly [77], which opens up the cellulose molecules structure for cellobiohydrolase to hydrolyse the bonds at the nonreducing end of the crystalline cellulosic chain to produce cellobiose. The cellobiases then split the disaccharide units, converting them into glucose [78] (Gautam *et al.*, 2011). A 61

glycoside hydrolase (GH61) family, structurally similar to 33 carbohydrate-binding module (CBM33) proteins, has been discovered [79]. It promotes the efficiency of cellulases by acting on the surfaces of the insoluble substrate and introduces chain breaks in the polysaccharide chains without the need of first "extracting" these chains from their crystalline matrix [79].

## 2.3 Xylanase

Xylanases are enzymes that degrade  $\beta$ -1,4-xylan, a linear polysaccharide found as hemicellulose in plant cell walls [80]. Microbial enzymes have been involved in the hydrolysis of xylan, which is a significant step towards the degradation of most lignocellulosic material [81]. A  $\beta$ -1,4-endoxylanase has been purified and characterised from *Hymenoscyphus ericae* (Table 2), and the enzyme was reported to have an isoelectric point of 4.85 to 5.20 and a molecular weight of 58.4 kDa [82]. The hydrolysis of lignocellulosic materials usually requires the synergistic action of different enzymes because of the structural heterogeneity of its components [83]. It has been reported that supplementation of xylanase with acetyl xylan esterase enhances the solubilisation of hemicellulose to a certain level and increases the subsequent hydrolysis of cellulose [84].

The structure of an endoxylanase (Fig. 5) has been described using a thermophilic fungus, *Thermomyces lanuginosus* [85]. The endo-1,4- $\beta$ -xylanase (EC 3.2.1.8) initiates the degradation of xylan into xylose and xylooligosaccharides of varying sizes [86,87]. Xylanases can be used for the bioconversion of lignocellulosic materials to produce higher value products, e.g. biofuel [88]. Xylanases have also been noted to be generated concurrently with production of cellulases [89].



**Fig. 5. A structural front view of endo-xylanase from *Thermoascus aurantiacus* [85]**

### 3. MYCORRHIZAL FUNGAL ENZYME

Microbial enzymes can be secreted into the fermentation broth by the producer (e.g., ERM, DSE and ECM fungi), enhancing fast downstream processing of the biocatalyst as compared to those obtained from other sources (e.g., plants and animals) and encouraging an increase in production. The shift from chemical processes to biological processing, attained by using fungal enzymes rather than chemical processes in industries has significantly eliminated most negative impacts associated with the use of chemicals on the environment [96]. Enzyme activity has been investigated in some ERM, DSE and ECM fungi (Table 2) using plating methods [34,97,98,99]. The highest cellulase activity of 0.74 units/ml was recorded for *Coriolus versicolor* when production medium was supplemented with 1% (w/v) peptone at a temperature of 28°C [100]. Burke and Cairney [82] produced, purified and characterised a  $\beta$ -1,4-endoxylanase from the ericoid mycorrhizal fungus (*H. ericae*). This enzyme had an isoelectric point of 4.85-5.20 and a molecular weight of 58.4 kDa [82]. The pH optimum for activity was 4.5 and was stable between pH 3.5-4.0 [82].

#### 3.1 Media for Culture Growth

Pure isolate can be made by the process where a single colony is taken from its natural habitat and then grown without contaminants. In short, either a spore or hypha is extracted from the substrate. Some fungi have specific requirements for growth, or the capacity to tolerate inhibitors of potential competitors. This factor is used in formulating selective media. One of such media may enable a range of fungi to

grow, but the target fungus will take a particular colour or form enabling it to be subcultured to pure culture [94, 100]. Ericoid mycorrhizal and endophytic fungi grow better in media such as malt extract agar (MEA) (Table 3) and Modified Melin Norkrans (Table 4) agar for both agar and broth cultures.

**Table 3. Composition of malt extract agar (MEA) medium**

<b>Malt extract</b>	30.0g
<b>Soy peptone</b>	5.0g
<b>Agar</b>	15.0g
<b>Water</b>	1000ml

**Table 4. Composition of Modified Melin Norkrans (MMN) medium**

<b>Malt extract</b>	3.0g
<b>Glucose</b>	10.0g
<b>(NH<sub>4</sub>)<sub>2</sub>C<sub>4</sub>H<sub>4</sub>O<sub>6</sub></b>	0.25g
<b>KH<sub>2</sub>PO<sub>4</sub></b>	0.5g
<b>MgSO<sub>4</sub>·7H<sub>2</sub>O</b>	0.15g
<b>CaCl<sub>2</sub></b>	0.05g
<b>FeCl<sub>3</sub> (1% solution)</b>	1.2ml
<b>NaCl</b>	0.025g
<b>Thiamine-HCl</b>	100µg
<b>Agar</b>	1.5%
<b>Water</b>	1000ml

Source: Marx [101]

### 4. CONCLUSION

The bio-economy is the knowledge-based production and application of renewable resources to make products, processes and services available for various economic sectors. This review on ericoid mycorrhizae and endophytic fungi has revealed the potential of

using these organisms for commercial enzyme production. It is cleared that if appropriate media formulations are available, coupled with the right conditions of growth, some of these fungi can be used in the production of desired enzymes using chemically defined media.

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## COMPETING INTERESTS

Author has declared that no competing interests exist.

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