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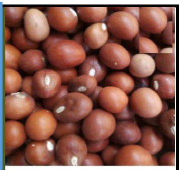

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BAMBARA GROUNDNUT

A Review on Endophytic Fungi: A Natural Source of Industrial Enzymes

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ABSTRACT

Endophytic fungi are microscopic organisms that live inside plant tissue. They can create a vast array of enzymes and metabolites that present a variety of biological activities without causing any visible indications of their existence. Due to their extensive use in many industries and their simplicity in production, stability, and optimization, fungal enzymes have attracted attention. This paper describes potential applications for the enzymes made by endophytic fungus and explores their use in diverse industries. Additionally, endophytic fungi may develop into fresh sources of industrially valuable enzymes like lipases, amylases, and proteases. Endophytes have rarely been used as sources of industrially relevant enzymes due to the diversity of plants that are readily available. In view of that, search for endophytes from plants with the potential of industrial enzymes is of paramount importance.

INTRODUCTION

Bary first used the term "endophyte" in 1866 to refer to any organism that lives inside a living plant tissue [1]. Microbes known as endophytes are those that infiltrate a plant's living interior tissues without immediately manifesting any adverse effects. According to this definition, the host plant and the endophytic microbes have a mutualistic or symbiotic connection [2]. Endophytic fungi have garnered a lot of attention in recent years for two primary reasons. Typically, they exist asymptomatically within the tissues of their host plants. First, mounting data shows that endophytes can be found in any plant, are quite common, and are frequently extremely varied [3]. One source for intelligent screening that meets both requirements is endophytic fungus. They expand within their plant hosts without displaying indications of sickness or growth in this habitat implies an ongoing metabolic interaction between the fungus and the host [4].

There are several reports of fungus endophytes in all types of tissues, including seeds, fruits, roots, leaves, bark, stems, flowers and buds. Additionally, endophytes are quite simple to produce, enabling large-scale production. Globally, study into the useful application of fungal endophytes has risen recently [5]. Since the 1990s, there has been an increase in interest in employing fungi for further biotechnological objectives. Fungi have the potential to contribute to a wide variety of habitats due to their genetic plasticity and capacity to quickly adapt to new dangerous and challenging-to-colonize situations. Their ability to horizontally acquire foreign genes and entire pathways contributes to their overall flexibility. Fungi are perfect for industrial and pharmacological purposes because to these characteristics [6]. Endophytes help protect the host from pathogenic microbes by competing for habitat and food sources [7].

Enzymes are biocatalysts that facilitate biochemical processes in living organisms. Enzymes can also be isolated from cells and utilized to catalyze a range of vital industrial processes [8]. Most of the industrial enzymes in current use are hydrolytic and are used to degrade a variety of natural products [9]. Microbial enzymes are attracting attention as they are widely used in medicines and many industries because of their stability, simplicity of production as well as optimization. A variety of enzymes and secondary metabolites are derived from endophytic fungi inside plant tissues, which exhibit diverse biological actions [10].

Several studies have shown that the extracellular enzymes including amylase, pectinase, cellulase, laccase and protease are produced by endophytes as strategies for resistance to infection and host food acquisition [7,11,12]. Among microorganisms, endophytic fungi and their enzymatic cascades are currently becoming an important part of the toolkit supporting process chemical/synthetic processes to produce industrially interesting molecules [13]. Endophytes produce extracellular biocatalyst as mechanisms of resistance to disease-causing organisms. The most frequently synthesized extracellular enzymes are amylases, cellulases, lipases, pectinases and proteases [14, 15].

Most of the industrial request for enzymes comes from the microbial source. Microbes are desired in industries for enzyme synthesis due to their high viability, short lifespan, and ease of genetic manipulation [16]. The review explains promising uses of biocatalyst synthesised by endophytic fungi and discusses it application of the endophytic fungal enzymes in various industries.

Endophytic fungi-host plant interaction

Plant endophytic fungi are understood to be fungi that colonize between and/or intracellularly in healthy host plant tissues during entirely or portion of their lifecycle and usually do not cause evident disease symptoms, endophytes are vital constituents of plant micro-ecosystems [17]. Although fungi can inhabit the intracellular or intercellular spaces of plants, systematic and widespread colonization most probable occurs in roots and not aboveground leaves and stems [18].

Considering the functional diversity, lifestyle, biology, and modes of transmission pursued by endophytes, they can be systemic/true endophytic fungi [shuttling throughout life] or transient/non-systemic endophytic fungi. It is classified as a fungus [which adapts to a temporary lifestyle]. Symbiosis and parasitism are the two most common indices at various stages of the life cycle [19, 20]. Plant-associated endophytes reduce damage course by the pathogen, presumably through the accumulation of secondary metabolites [21].

Plants have developed unique adaptations to relieve the abiotic and biotic stresses in nature, but they also depend on microbial associates for survival and defense against microbial attackers [22]. Abiotic stress in the host plant can be managed by the induction of a stress response in the invader and subsequent production of stress-relieving metabolites by these endophytes [23]. Symbiotic endophytes can benefit a wide variety of plants and suppress pathogens [24]. Endophytic microbes improve plant immune response against chewing insects by encouraging endogenous defense reactions mediated by the jasmonate pathway [25].

Endophytes live without seeming symptoms within plants but may provide competitive benefits such as resistance to stresses [abiotic and biotic] [26]. Endophytes link plant, rhizosphere and soil interactions to facilitate nutrient dissolution and transport those nutrients further to plant roots, forming the soil-plant-microbe continuum. In addition, plant roots internalize microorganisms and oxidatively deprive them of nutrients in the nodule feeding cycle [27].

Industrial enzymes from fungal entophytes

Chemicals are used in various industries around the world, and the request for industrial biocatalyst is growing, mainly because of the necessity for new, supportable industrial processes that has no negative effects on human health. In addition, enzymes are also used in waste management, used in wastewater management and detoxification, bioindicators of pollution, renewable energy sources, biosensors, and help maintain a clean environment [28]. Microbial hydrolases are preferred for use in various industries due to their availability, high stability, cost-effectiveness, and environmental friendliness [29-31].

Endophytes are considered to be an excellent source of secondary metabolites, bioactive natural products, and industrially important enzymes [32]. Endophytes are perhaps one of the most remarkable microbes for screening the manufacture of industrial bio-compounds. These microbes are abundant in plants and colonize their tissues without causing noticeable symptoms in the host [33, 34].

Enzymes extracted from endophytes have been used commercially in food processing, detergent, textile, pharmaceutical, and medical therapy production, as well as in the field of molecular biology [35]. In addition, several studies have shown that endophytes are involved in the synthesis of industrially important enzymes; including amylase, cellulase, lipase and laccase [36, 37]. Different enzymes have been extracted from various strains and utilized as detergents to soften and launder clothes, preserve the fabric's quality, color and other properties. These various fungal enzymes are amylases, cellulases, lipases and proteases, they are used as detergents to increase their effectiveness [38].

The study was aimed to identify several endophytes associated with plant stems, leaves and roots in Southeastern Algeria. Eleven endophytic fungi were examined for their ability to produce extracellular enzymes such as amylases, cellulases, laccases, lipases, proteases, ligninases and proteases on solid media. The results showed that cellulase activity was detected in 27.27% of the fungi tested for enzymatic activity, 27.27% for amylase, and 18.18% for ligninase [39]. In another study, a number endophytes representing 22 different species were recovered from the roots, leaves and flowers of *Cymbidium alloifolium*. The results revealed that 93% of endophytes produced phosphatase, 80% produced ylase, 70% produced amylase, 63.33% produced protease, 30% produced pectinase, 23.33% produced lipase and 10% produced laccase [40]. Table 1 below shows some endophytic fungi that have potential for enzymatic production.

Industrial enzyme applications

Enzymes with cellulolytic and hemicellulolytic activities have been comprehensively studied as tools for profitable production of second-generation ethanol. Hemicellulose includes collateral enzymes, a group of enzymes that can increase the yield of reducing sugars during enzymatic hydrolysis of lignocellulosic substrates [33].

Cellulases are commonly used in the paper industry [49]. Cellulose is the most common polysaccharide in nature, as it is the main component of plant biomass. Cellulose is connected in plants with hemicellulose, lignin, and numerous extractives [alcohols, alkanes, fats, phenols, proteins, terpenes and waxes] that form the complex, rigid cell wall structure of the plant [50]. Enzymatic hydrolysis of cellulose is an important industrial activity, but it is often a difficult and time-consuming process. Cellulases are often used in the paper industry [49]. In recent years, cellulases have acknowledged specific attention because they are used to saccharify cellulose from lignocellulosic resources, releasing glucose that can be converted to cellulosic ethanol via microbial fermentation. Cellulosic ethanol is recognized as the best alternate biofuel to fossil fuels, and its combustion is not only non-renewable but also has environmental impacts [51].

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Table 1. Endophytic fungi with the potential of enzyme production.

Endophytic fungi	Source of isolate	Enzyme	Reference
Aspergillus sp.	Salt marsh plant [Sueada maritime]	L-asparaginase	[41]
Beauveria bassiana,	Onion leaves	Xylanase and endoglucanase	[42]
Colletotrichum sp., *Fusarium solani*, *Acrophomina phaseolina*, *Nigrospora* and *sphaerica*	*Catharanthus roseus*	*Colletotrichum* sp. and *Fusarium solani*_strains were also amylase positive, but only *Fusarium solani*_was able to produce the protease	[43]
Aspergillus niger	Orchid	*Aspergillus niger* produced the highest pectinase activity	[44]
Penicillium oxalicum r4	*Taxus cuspidate*	Higher cellulase activity was observed	[45]
Monotospora sp.	*Cynodon dactylon*	Laccase production by an endophytic fungus was observed	[46]
A. Niger	*Ziziphus* spina	*A. Niger* produced amylase	[47]
Pestalotiopsis sp. and *Aspergillus* sp.	*Euterpe oleracea* Mart. [Açaizeiro]	A lipase activity was observed that exhibited the highest lipolytic activity in solid media	[48]

It is worth noting that various microbes in nature, particularly fungi and bacteria, can synthesize enzymes capable of biomass degradation. Cellulolytic microorganisms can evolve as individual degrading organisms or as part of a 'chain reaction' in microbial communities in some ecosystems. The cellulolytic enzymes produced by such microorganisms are a class of glycoside hydrolases and at times contain lignin-modifying biocatalysts [52]. Recently, endophytes were shown to be potential producers of lignocellulolytic enzymes e.g cellulases and hemicellulases [33, 53, 54].

Xylan is the second most abundant natural polysaccharide among lignocellulosic materials, its hydrolysis is achieved via the synergistic actiivity of endoxylanases that hydrolyze backbone internal glycosidic bonds randomly [51]. Endophytes have been testified to produce xylanases. However, it has been stated that very few microorganisms live in plants e.g the p-1,cendoxylanase-producing ericoid mycorrhizal fungus hymenoscyphus ericae produces xylanases even when xylan appeared to be one of the main constituents of the plant cell wall [55, 56].

Robl *et al.* [2013] studied hemicellulase producers using a fluid rich in pentose obtained from hydrothermal pretreatment of sugarcane bagasse. Amongst the many microbes tested, the endophyte strain, *A. Niger* DR02 appeared as a potential producer because of its high xylanase secretion rate [33]. Proteases are now recognized for their various physiological and commercial uses, and also serve a variety of other roles. They have wide applications in biological processes, metabolic regulations, and digestion of dietary proteins so that amino acids can be absorbed [57].

Interest in microbial proteases is increasing because plant and animal proteases cannot meet current global requirements [58]. Proteases are used across multiple industries including the leather industry, pharmaceutical industry, detergents as well as food industry [59-61]. Proteases of microbial origin are preferred over enzymes of plant and animal sources, because they have almost all the desired properties for biotechnology applications [62]. Microbial proteases are preferred to plant and animal proteases because of their higher biochemical diversity and ease of gene manipulation. Microbial proteases of microbial source account for roughly 40% of all enzyme sales worldwide [63]. Advantages include reduced production costs, potential for large-scale production in industrial fermenters, wide range of physical and chemical properties, potential for genetic manipulation, lack of seasonal influences, rapid culture development and reduction of stress. Microbial enzymes enable the development of new enzymatic systems not available from plants or animals, achieving important advances in the food industry [62]. Based on study by Zaferanloo *et al.,* three endophytic fungi including; *Alternaria alternata*, *Phoma herbarum,* and an uncategorized fungus were isolated from *Eremophilia longifolia* [a native australian plant] and investigated for protease synthesize. The endophytic properties of this fungus have shown that it is a potential source of enzymes specifically applicable to the dairy industry [64].

There is currently a growing need for innovative and more effective sources of industrial enzymes. Among several industrial enzymes, fungal pectinases dominate the global enzyme market because of their broad sort of applications in the bioindustry [44]. Pectinase is a biocatalyst which breaks down pectin. Pectin is one of the components present in the cell walls of plants, in the middle layer it arises as the first part of the wall during cytokinesis after cell division. Thus, pectinases contribute to cell wall breakdown. This increases juice recovery [higher yield], reduces juice viscosity [wateriness], and reduces juice turbidity due to floating cell wall debris [65].

Pectinases play a significant role in the food industry. This biocatalyst help extract juice and clarify wine, concentration and fermentation of tea, cocoa and coffee and extraction of vegetable oil [66]. Of these enzymes of industrial significance, pectinase are particularly important due to their widespread practice in main industries such as the fiber, food, beverage, pulp and paper, and biofuel industries [5]. Pectinases of microbial origin account for 25% of the world's food and industrial enzyme volume, and the market is growing from time to time [16].

Laccase act on a wide-ranging of substrates that can be used for industrial purposes. The simple requirements for laccase catalysis [presence of substrate and oxygen], and its obvious stability and lack of inhibition make this enzyme appropriate and attractive for industrial applications. In addition, laccases can oxidize various organic and inorganic substrates such as monophenols, diphenols, polyphenols, aminophenols, methoxyphenols and metal complexes. This is the main reason why laccase is attractive for dozens of biotechnological applications [67]. Laccase producing fungal endophytes fungi from different aquatic plants were isolated from Hulimavu Lake, bengaluru. Differential production rates for the enzyme laccase were observed through various endophytic fungi; rates of production also varied between fungi isolated from different parts like leaf, node, root and stem of the same plant species too. Phylogenetic study of an isolates with uppermost laccase production was carried out and the species was found to be *Cladosporium tenuissimum* [68].

Fungal lipases [triacylglyceroacyl hydrolases] are important industrial enzymes with multiple uses. Lipases are considered a group of potential industrial enzymes that can catalyze the hydrolysis of triglycerides [soluble fats], liberating monoglycerides. Diglycerides, glycerol and free fatty acids across the oil-water interface [69]. Lipases are valuable enzymes as they accept a wide range of substrates, they are stable and active in organic solvents, and are available from several microbes. Lipase-catalyzed reactions play an imperative role in organic synthesis to meet the rapidly growing demand for strategically pure compounds such as agrochemicals, pharmaceuticals and natural products [70].

CONCLUSION

Endophytic fungi are a class of fungi that has received a lot of attention recently, but are still unexplained. New approaches and more comprehensive studies using known techniques of fungal culture are essential to optimize enzyme production. Subsequently, studies on the physicochemical properties and characterization of enzymes are urgently needed to assess the true potential of endophytes as sources of industrial enzymes.

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