World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-4, November 2021
ISSN: 2749-3644

CELLULASE FROM *TRICHODERMA LONGIBRACHIATUM* FUNGUS: A REVIEW

Noor T. Hamdan [1] and Hameed M. Jasim [2]
1 Biology Department, College of Science, Mustansiriyah University, Baghdad- Iraq
2 College of Biotechnology,University of Nahrain, Baghdad - Iraq
Corresponding author e-mail: noor.t.hamdan@uomustansiriyah.edu.iq

Article history:

Received: August 30th 2021
Accepted: September 26th 2021
Published: November 17th 2021

Abstract:

Biocatalysis involves the utilization of natural catalysts, i.e. enzymes, to perform chemical transformations of organic compounds. Living, whole-cells producing enzyme, or isolated enzymes, are used in biocatalysis. The key advantage of biocatalysis is the specificity of the biocatalyst, which potentially results in high yields of a particular product. New biocatalytic processes are based on the availability of interesting, useful new enzymes, usually obtained by screening for microbes that carry out the desired target reaction. Exploration of extreme environments can provide unique microbial culture collections that can be used in screening for suitable enzymes to perform a desired biocatalytic reaction. These enzymes may then be used as biocatalysts in industrially relevant bioprocesses**.** Cellulases are industrially important enzymes with a market share of 500 million dollars that is expected to rise to 1.5 billion dollars by 2018. Cellulases play a crucial role in generating sugar feedstock for lignocellulosic based biorefinery platform. In addition, their demand in textile, paper, feed and food industries is rising steadily. However, these industrial applications require thermostable, catalytically highly efficient cellulases for making the processes commercially viable Cellulase has been produced from different organism, mainly fungi, bacteria, and protozoans, but the fungus Trichoderma longibrachiatum was recorded as one of the most important commercial cellulase producers and has been widely used in a variety of industries. Trichoderma longibrachiatum is a soil fungus which often found on dead wood, other fungi, building material and sometimes animals. It is found all over the world but mainly in warmer climates. This review assesses the following topics: Genus Trichoderma , Morphology of Trichoderma spp, Molecular Identification of Trichoderma spp. using ITS regions ,Trichoderma longibrachiatum, Cellulases, Mechanism of cellulose hydrolysis by microorganisms, Cellulolytic Domains, Cultural conditions for the cellulase production, Submerged fermentation (SmF), Solid State fermentation (SSF), General aspects of solid state fermentations, Microorganisms used in solid state fermentations, Advantages of SSF, Lignocellulosic Residues/Wastes as Solid Substrate, Physical mutagenesis using ultraviolet light(UV), Optimizations affecting cellulase production by SSF, Inoculum size, Fermentation medium, Initial pH, Temperature, Incubation time, Moisture content of the substrate, Biotechnological application of cellulose, Food industry, Animal feed industry, Pulp and paper industry Pulp and Paper, Agriculture industry, Biofuel industry, Pharmaceutical industries, waste management, Textile.

Keywords: Cellulase, Trichoderma longibrachiatum, Submerged fermentation, Solid state fermentation, Optimization, Biotechnological applications.

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-4, November 2021
ISSN: 2749-3644

INTRODUCTION

1.1.1.Genus Trichoderma

Scientific Classification of Trichoderma was described by Samuels and Hebbar, (2015)
Kingdom: Fungi
Division: Ascomycota
Subdivision: Pezizomycotina
Class: Sordariomycetes
Order: Hypocreales
Family:Hypocreaceae
Genus: Trichoderma

Trichoderma sp. are widely distributed all over the world and occur in nearly all soils and other natural habitats, especially in those containing organic and inorganic mattar (Montoya et al., 2016).
They are ubiquitous colonizers of cellulosic materials and can thus often be found wherever decaying plant material is available as well as in the rhizosphere of plants, where they can induce systemic resistance against pathogens (Atanasova et al., 2013).

They are filamentous fungi commonly found in the soil community that are facultative saprophytes. Thereafter, numerous new species of Trichoderma were discovered, and the genus already comprised more than 100 phylogenetically defined species (Druzhinina et al., 2006).

The genus Trichoderma was established in 1794 including four species (Samuels, 1996). In recent years, the number of Trichoderma species increases dramatically. Bissett et al., (2015) presented a list of 254 names of species and two names of varieties in Trichoderma with name or names against which they are to be protected. More recently, in a large-scale survey of Trichoderma from rotten wood and soil in China, Qin and Zhuang (2016, 2017) published 27 new species based on the collections producing ascomata on woody substrates. Until now, 287 Trichoderma species have been described**.**

Species of *Trichoderma* have been isolated from a wide diversity of sources, including immersed marine habitats (Paz *et al*., 2010), water- damaged building materials (Polizzi *et al*., 2011), animal feed (Caballero *et al*., 2007), and other indoor niches (Beezhold *et al*., 2008), also there are occur on basidiomes of polypores, jelly fungi, and other basidiomycete hosts that occur in forests and other natural habitats (Jaklitsch, 2011).

The evident success of *Trichoderma* species in the environment is evidently due to the development of a number of mechanisms that enhance their ability to survive and proliferate. These mechanisms include an aggressive ability to attack or inhibit other fungi and the production of hydrolytic and other enzymes that degrade complex carbohydrates. These mechanisms have been exploited for human benefit (Lantz *et al*., 2010).

Individual species were reported to exhibit some restriction in their geographic distribution and were also formed to show preference to certain soil temperature and moisture content. *T.viride* and *T. polysporum* for example, were reported to be restricted to areas where low temperature prevail and *T. harzianum* were mostly found in warm climatic regions whereas, *T, hamatum* and *T. Koningii* occur widely under diverse climatic conditions. *T.hamatum* and *T. pseudokoningii* were reported to be adapted to conditions of excessive soil moisture (Samuels, 1996).

1.1.1.1. Morphology of *Trichoderma* spp.

Colonies of *Trichoderma* spp. are growing slowly or rapidly depending on the species, aerial mycelium usually limited, floccose to arachnoid, reverse colourless to dull yellowish. Some isolates with a distinctive aromatic odour resembling coconut. Conidiation variably effuse, loosely tufted, or forming compact pustules, white at first, eventually green (rarely brown). Chlamydospores present in most isolates, frequently abundant. Conidiophores usually relatively narrow and flexuous, with primary branches arising at regular intervals, usually paired or in whorls of three, usually short and not extensively rebranched. Phialides mostly in verticils of 2 or 3, in some strains up to 5-verticillate, lageniform to subulate. They are attached to the conidiophores at right angles. Conidia green (rarely brownish), smooth walled to distinctly verrucose, subglobose to obovoid or ellipsoid colourless to pale yellowish or greenish, smooth-and sometimes thick-walled (to 4 µm) (Samuels and Hebbar, 2015).

1.1.1.2. Molecular Identification of *Trichoderma* spp. using ITS regions

Number of molecular studies have been employed to characterise *Trichoderma* species. Molecular techniques differentiate between isolates by differences in their DNA and RNA. Techniques focusing on DNA have an advantage over morphological because species identification based on morphology is difficult at best because of the paucity and high similarity of useful morphological characters (Lidia *et al*., 2011), and increasing numbers of morphologically cryptic species that can be distinguish only through their DNA characters are being described (Atanasova *et al*., 2010).However, recently many molecular methods and identification tools were developed, which are based on DNA sequence analysis. Therefore, it is now possible to identify every *Trichoderma* isolate to its species (Lidia *et al*., 2016). There are several molecular methods to characterize fungi species. Sequence analysis of the ITS (internal transcribed spacers) region is the most famous method among molecular characterization methods. In

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-4, November 2021
ISSN: 2749-3644

eukaryotic cells, there are two internal transcribed spacers flanking the 5.8S gene. The two spacers, together with the 5.8S gene, are normally referred to as the ITS region (Schoch *et al*., 2012). The rRNA genes are universally conserved, while the ITS region are highly variable (Kannangara *et al*., 2017). The ITS region is one the fastest evolving region and they may vary among species within a genus. Thus, the sequences of these regions can be used for identification of closely related species (White *et al*., 1990). Sequence analysis of the ITS region have been used successfully to generate specific primers capable of differentiating closely related fungal species (Chakraborty *et al*., 2010). It has typically been most useful for molecular systematic study at species level, and even within species (Lee and Hseu, 2002).

The ITS regions of ribosomal DNA(rDNA) were the first studied gene (Kindermann *et al*., 1998).The ITS spacer, approximately 600 to 1000 bp, is amplified by universal primers (ITS1 / ITS4), specific to fungi (ITS1f / ITS4) or specific to Basidiomycota (ITS1f / ITS4b)(White *et al*., 1990; Hamdan and Jasim, 2021). Nuclear rDNAs, and particularly the ITS regions, are a good target for phylogenetic analysis in fungi (Bruns *et al*., 1991). Phylogenetic analysis of whole genus of *Trichoderma* was achieved sequence analysis of the ITS region of rDNA (Lee and Hseu, 2002). The non-coding internal transcribed spacer (ITS), 18S and 28S region of genomic DNA are well explored for taxonomic identification of fungi. The rDNAs genes of fungi, similar to eukaryotes is composed of 18S, 5.8S, 28S rRNA transcribed by RNA polymerase I as a 35S to 40S precursor, with both internal and external transcribed spacers (ITS and ETS). The large part (LSU) contains 28S, 5.8S & 5S rRNAs whereas smaller subunit (SSU) contains the 18S rRNA (SSU rRNA). The 18S small subunit rRNA gene (SSU) is also commonly used in phylogenetics whereas 28S large subunit rRNA gene (LSU) either alone or in combination with ITS is capable of discriminating species (Singh and Gupta, 2017).

The 18S and 28S markers are recommended for taxonomic studies at family and generic level where as internal transcribed spacers (ITS) is useful at the species level resolution (Bridges *et al*., 2005). So far the use of ITS in phylogenetic studies of species in *Trichoderma* has been used by several workers eg the molecular systematics of the Hypocreales was reassessed (Lieckfeldt *et al*., 1998; Lieckfeldt and Seifert, 2000) and similarly, ITS markers have been used for *Trichoderma* sect. Longibrahiatum (Kuhls *et al*. 1997 ; Samuels *et al*., 1998). Kindermann *et al.* (1998) analyzed the phylogeny of species in *T*. sect *Pachybasium* while Dodd *et al*., (2000) also used ITS based markers for studying the relationship of species of *Hypocrea* / *Trichoderma*. Lieckfeldt *et al*., (2001) also studied the taxonomoic position of *H. aureoviridis* and *T*. *aureoviride* using DNA and morphological features.

1.1.2.Trichoderma longibrachiatum

Trichoderma longibrachiatum is a soil fungus which often found on dead wood, other fungi, building material and sometimes animals. It is found all over the world but mainly in warmer climates (Druzhinina *et al*., 2012). Many species from this clade have been adopted in various industries because of their ability to secrete large amounts of protein and metabolites (Samuels *et al* ., 2012).

It was found a fast-growing fungus and it typically produces off- white colonies that change to greyish green with age (De Hoog, 2000). This species is able to grow over a wide range of temperature; however the optimal temperature for growth is ≥ 35 °C (Samuels *et al* ., 2012) .*T. longibrachiatum* is a clonal species that reproduces through 1- celled, smooth-walled conidia (Samuels and Hebbar, 2015).

T. longibrachiatum uses cellulases to digest cellulose from decaying plant biomass, and chitinases to digest the chitinous walls of other fungi also, It is able to digest proteins with the aid of aspartic proteases, serine proteases, and metalloproteases(Xie *et al*., 2014; Hamdan and Jasim, 2021). *T. longibrachiatum* produces many secondary metabolites including: peptaibols, polyketides, pyrones, terpenes and diketopiperazin e-like compounds (Hermosa *et al*., 2014).

Its enzymatic capacity could potentially be useful in bioremediation, for use in remediation of polycyclic aromatic hydrocarbons (PAHs) and heavy metals (Rosales *et al*., 2011).Other industrial uses include using the various cellulases for staining fabrics in the textile industry, increasing digestibility of poultry feed, potentially in the generation of biofuels, promoting plant growth, and biocontrol agent for its parasitic and lethal effects on the cysts of the nematode *Heterodera avenae* and against *Meloidogyne incognita* (Maurer*et al*., 2012; Zhang *et al.,* 2017; Srinivasa *et al*., 2017).

T. longibrachiatum is not thought to pose risk to human health, although it has been isolated as an indoor contaminant with high allergenic potential (Druzhinina *et al* ., 2012). This species has also been implicated in the colonization of immunocompromised people (Xie *et al*., 2014) and has been found in the blood cultures derived from a neutropenic patient with lymphoma, bone marrow transplant patients, and patients with severe chronic kidney disease (Howard, 2003).

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-4, November 2021
ISSN: 2749-3644

1.1.3. Cellulases

Cellulases are glycoside hydrolases (GHs) that decompose cellulose, a hydrophilic, water-insoluble polymer composed of repeated units of D-glucose interlinked by β-1,4- glycosidic bonds into shorter chain polysaccharides such as cellodextrin, cellobiose, and glucose. (Moraïs *et al.*, 2012; Garvey *et al.*, 2013). Cellulases are distinctly categorized into three groups endoglucanases, exoglucanases or cellobiohydrolases, and β-glucosidases or cellobiases) as per their structure and function, but work collaboratively to enforce the hydrolysis of the complex cellulose microfibrils of the plant cell wall. The endo- and exoglucanases functionally perform the same task the hydrolysis of glycosidic bonds but they differ structurally in terms of the site (loop) for cellulose binding (Juturu and Wu, 2014). For instance, endoglucanases (E.C.3.2.1.4) are characterized by short loops, defining open active site clefts that can bind to any accessible site (especially the amorphous sites) along cellulose chains to yield long-chain oligomers (Wilson, 2015) as shown in figure (1-1).

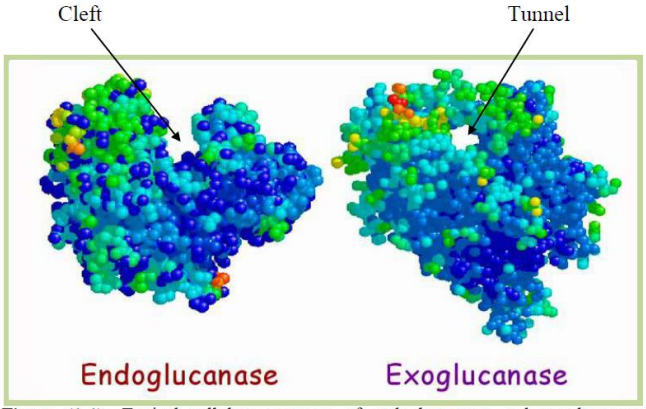


Figure (1-1): Typical cellulase structure of endoglucanases and exoglucanases (Bayer *et al.*, 2006).

For exoglucanases, they have long loops and affinity for the crystalline sites along cellulose chains and yield primarily cellodextrin (Segato *et al.*, 2014). Most often, the loops form a tunnel around the catalytic residues; therefore, substrates usually are directed from the end of the tunnel to encounter the active site of the enzyme (Juturu and Wu, 2014). On the other hand, β-glucosidases possess a rigid structure with active site residing in a large cavity, called the active site pocket, which favors the entry of disaccharides (Nam *et al.*, 2010); even though β- glucosidases are also capable of hydrolyzing soluble cellodextrins (Zhang and Lynd., 2004).

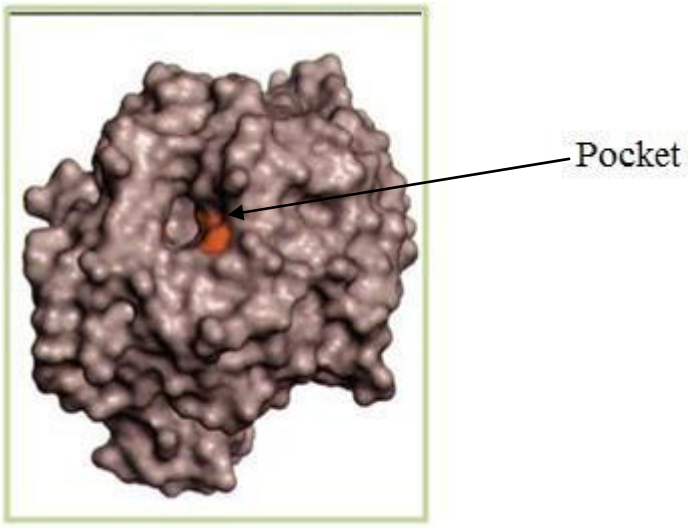
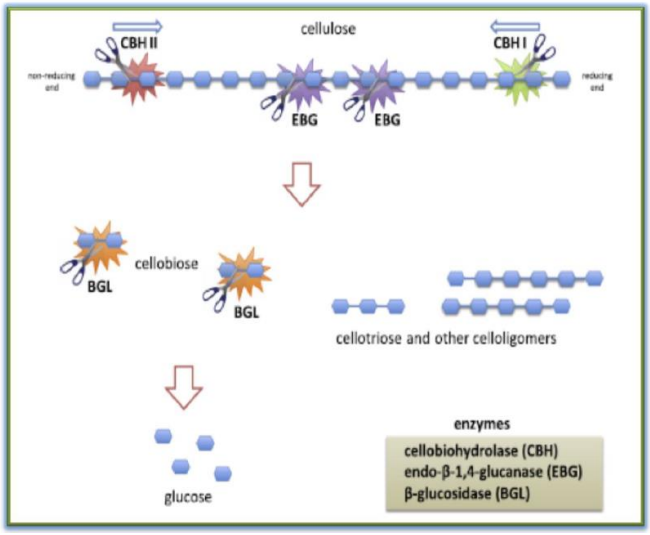


Figure (1-2): The structure of glycoside hydrolases,pocket-shaped groove active site (Henrissat *et al.*, 1995).

1.1.4. Mechanism of cellulose hydrolysis by microorganisms

The hydrolysis of cellulose into glucose requires the synergetic action of at least three enzymes, endoglucanases (EG; endo-1,4-β-D- glucanase, EC 3.2.1.4), preferably, attack amorphous regions and randomly cleave the accessible intramolecular β-1,4-glucosidic bonds of cellulose chains randomly in a nonprocessive manner with formation of new chain ends. Furthermore, exoenzymes (exoglucanases or CBH 1,4- b-D-glucan-cellobiohydrolase, EC3.2.1.91) cleave cellulose chains in a processive way at the reducing or nonreducing ends to release cellobiose, as shown in Figure (1-3). Lastly,βglucosidase(BG;cellobiase,Dβglucosideglucanohydrolase, EC 3.2.1.21) further hydrolyses cellobiose to glucose and also releases glucose from the non reducing ends of soluble cellooligosaccharides (Jørgensen *et al.*, 2007). There is a high degree of synergy between cellobiohydrolases(exoglucanases) and endoglucanases, which is required for the efficient hydrolysis of cellulose (Gupta *et al.*, 2009).The products of endoglucanases and cellobiohydrolases, which are cellodextrans and cellobiose, respectively, are inhibitory to the enzyme's activity. Thus, efficient cellulose hydrolysis requires the presence of β- glucosidases which cleaves the final glycosidic bonds producing glucose (end product).

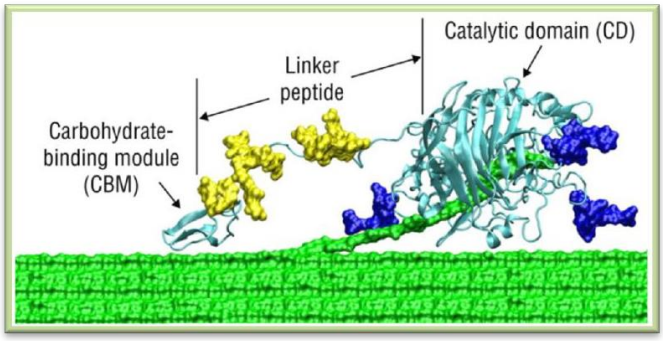
World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-4, November 2021
ISSN: 2749-3644



Figure(1-3):The enzymatic breakdown of cellulose. (Vale *et al*., 2014).

1.1.5.Cellulolytic Domains

Most fungal cellulases are organized in to two structurally independent domains; a larger catalytic core domain (CD), and a smaller cellulose-binding module (CBM). These two domains are usually interconnected via a short flexible linker (Glycosylated linker peptide) (Bhat and Hazlewood, 2003). The catalytic core domain is the part of the cellulase where the hydrolysis of the cellulose chain takes place. This domain is the largest part of the enzyme. It varies greatly in size between different cellulases (Bhat and Hazlewood, 2003).



Figure(1-4):Schematic diagram of cellulase modules (Beckham *et al*., 2011).

The cellulose-binding module is a small wedge-shaped domain consisting of approximately 35 amino acids. The function of the CBM is to bind on the surface of cellulose, and serve as an "anchor" for the enzyme, keeping it strongly adsorbed to the cellulose surface. The two domains in the enzyme are interconnected via a flexible linker. The linker is usually very rich in threonines, serines, and prolines, and it is heavily glycosylated. The role of the linker is probably to keep the two domains apart, and to restrict their movements with respect to one and another, and the glycosylation of the linker probably makes it less flexible, and probably decreases its sensitivity to proteolytic enzymes (Wilson and Irwin, 1999).

1.1.6.Cultural conditions for the cellulase production

Two main fermentation types that are generally used for the production of commercial enzymes are submerged fermentation (SmF) and solid state fermentation (SSF). There are two major differences between submerged and solid state conditions: (i) CMCase yield or productivity is higher in SSF than in Smf (ii) CMCase location under SSF conditions is mostly extracellular, while it is bounded to the mycelium under SmF conditions. Maximum CMCase activity expressed intracellularly is also 18 times more in SSF than in Smf, while the extracellular activity is 2-5 times higher in SSF than SmF.

1.1.6.1. Submerged fermentation (SmF)

Submerged Fermentation is a type of fermentation which is used for most of the industrial enzyme production because of its ease of process and separation and good control of environmental factors such as temperature, aeration, agitation and pH, but this technique is not only expensive but also of energy intensive (Reddy *et al* ., 2015). It involves the production of enzymes by microorganism in a liquid nutrient media which utilizes the liquid substance such as molasses and broth (Farinas, 2015). The biomolecules are secreted into fermentation broth and after the process it's easy to purify it. This process is highly suitable for the microbes that require high moisture conditions e.g. bacteria and fungus. The yield of product in this fermentation differs for each substrate so it is important to select the correct substrate and conditions for fermentation should be optimized (Limayem and Ricke, 2012). Cellulase production is carried out in aerobic aseptic culture. The growth medium contains salts, nutrients, surfactant and inducers which are required for the fungus to survive and grow in medium. The important metal ions which are required for cellulase production are iron, cobalt, copper, magnesium, calcium, potassium, manganese and ammonium. The progress in cellulase production was averaged doubling every two years due to the strain improvement and optimization of fermentation process (Jabasingh and Nachiyar, 2012).Major advantages of submerged cultivations are the well- established technological basis for scaling the processes to industrial production capacity. This scalability is mainly due to control of pH, temperature, oxygen and nutrient availability (Hansen *et al*., 2015).

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-4, November 2021
ISSN: 2749-3644

1.1.6.2. Solid State fermentation (SSF)

Solid-state fermentation (SSF) is a process whereby an insoluble substrate is fermented with sufficient moisture but in the absence of free- flowing water. However, the substrate must possess enough moisture to support growth and metabolism of the microorganism (Agrawal, 2015). Solid state fermentations have been reported to have high enzyme productivities as compared to submerged fermentations (Prévot *et al*., 2013). The exact reasons for higher titres in SSF as compared to submerged fermentations are currently not well known. There are however suggestions that higher biomass and lower protein breakdown contribute to better production in SSF. SSF holds a tremendous potential for the production of enzymes especially where the crude fermented products maybe used directly as an enzyme source. SSF processes have lower energy requirements and produce less wastewater (Singhania *et al*., 2010).

1.1.6.2.1. General aspects of solid state fermentations

Several important aspects that need to be considered in SSF include selection of a suitable microorganism and substrate, optimisation of process parameters and isolation and purification of the product (Hansen *et al*., 2015). Selection of the substrate is important in SSF. The solid material is non-soluble and acts as a physical support and a source of nutrients (Abdullah *et al.,* 2016). The material could be naturally occurring e.g. agricultural crops and agro-industrial residues Agro- industrial products that have been used in SSF include sugar canebagasse, rape seed cake, wheat straw, rice, soya hull, saw dust, barley, grains, etc (Soccol *et al*., 2017; Hamdan and Jasim, 2018A; Hamdan and Jasim, 2018B).

1.1.6.2.2. Microorganisms used in solid state fermentations

A large number of organisms including mainly fungi , yeast and some bacteria, have been used in SSF. The selection of the microorganisms growing on solid substrates depends on the requirements of water activity, capacity to adhere and penetrate the substrate and ability to assimilate mixtures of different polysaccharides in the often complex substrates used (Agrawal, 2015). Theoretically, based on the water activity of SSF systems fungi and yeasts are best suited for SSF (Yazid *et al*., 2017). Filamentous fungi are best adapted for SSF because of their physiological and enzymological and biochemical properties. Besides that, the production of cellulase is hypothesized to be greatly affected by the fungal morphology as reviewed by (Singhania *et al* ., 2010), i.e their hyphae can grow on particle surfaces and penetrate into the inter particle spaces and thereby colonizing solid substrates.These features also give them a major advantage over bacteria for their colonization of the substrate and the utilization of the available nutrients.

Fungal genera-*Trichoderma, Humicola, Penicillium, Aspergillus, Thermomonospora fusca, H. insolens;* Bacterial genera -*Bacilli, Pseudomonas, Cellulomonas; and among Actinomycetes-Streptomyces, Actinomucor* are commonly used for cellulase production (Mussatto and Teixeira, 2010; Imran *et al*., 2016; Hamdan and Jasim, 2018A; Hamdan and Jasim, 2018B). However, bacteria and yeasts have been used in traditional SSF processes. Bacteria have been used for enzyme production, composting, ensiling and some food process (e.g. sausages, Japanesenatto, fermented soybean paste, Chinese vinegar)(Martins *et al*., 2011). Yeasts have mainly been used for ethanol production and protein enrichment of agricultural residues (López-Pérez and Viniegra-González, 2016).

1.1.6.2.3. Advantages of SSF

SSF offers numerous advantages lower cost and reduced need for asceptic techniques compared to submerged fermentations (SmF) (De la Cruz Quiroz *et al*., 2015).Advantages of using SSF lie in higher fermentation productivities, higher end-concentration of the products, high product stability, cultivation of microorganisms specialised for water-insoluble substrates or mixed cultivation of various fungi and lower demand for sterility due to lower water activity used (Singhania *et al*., 2010). Other advantages include the simplicity of the fermentation media, no requirement for complex machinery, equipment and control systems (Kheng and Omar, 2005).

1.1.6.2.4. Lignocellulosic Residues/Wastes as Solid Substrate

The selection of a substrate for cellulase production in a SSF process depends upon several factors, mainly related with cost and availability of the substrate and thus may involve screening of several agro-industrial residues. In a SSF process, the solid substrate not only supplies the nutrients to the microorganism(s) growing on it but also serves as anchorage for the cells. The substrate that provides all the needed nutrients to the microorganism growing on it should be considered as the ideal substrate (Farinas, 2015).

Some of the substrates that have been used include sugar cane bagasse, cassava bagasse, rice bran, wheat bran, maize bran, wheat straw, rice straw, rice husk, soy hull, grapevine trimming dust, saw dust,

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-4, November 2021
ISSN: 2749-3644

corncobs, coir pith, banana waste, etc. Wheat bran however, holds the key and has most commonly been used in production of cellulase and these wastes are mainly composed of cellulose (35%–50%), hemicellulose (25%–30%), and lignin (25%–30%) (Behera and Ray, 2016; Soccol *et al*., 2017)

1.1.7. Physical mutagenesis using ultraviolet light(UV)

Physical mutagenesis has been applied to improve cellulolytic activity of the strain for its industrial application. The creation of different mutant strains with several-fold increase in the amount of secreted cellulolytic enzymes compared to the wild-type strain has been achieved by both academic and industrial research programs (Shahbazi *et al*., 2014).

When deoxyribonucleic acid (DNA) is exposed to UV light (254nm), the most frequent DNA damage, or lesions, results at dimers of any two adjacent pyrimidine bases (T, thymine; C cytosine) causing T-T, C-T, and C-C dimers, but T-T dimers are the most common cyclobutane pyrimidine dimers. Another type of DNA damage is the 6-4 pyrimidine- pyrimidone photoproducts. These occur at a lesser frequency than cyclobutane pyrimidine dimers but are less mutagenic because they are more efficiently repaired than cyclobutane pyrimidine dimmers.

Many fungal strains have been subjected to extensive mutagenesis studies due to their ability to secrete large amounts of cellulose- degrading enzymes, for example *T. reesei* QM6a has been improved by using different mutagenesis techniques including UV-light at the US Army Natick Laboratory, resulting in the mutant QM 9414 with higher filter paper activity (Mandels *et al*., 1971). Other studies in different laboratories have also made significant contributions to strain improvements using mutagenesis techniques, leading to development of the mutant strains of *T. reesei* M7, NG14 (Montenecourt and Eveleigh., 1977) and RUT-C30 (Montenecourt and Eveleigh., 1979). *T. reesei* RUT-C30 is one of the best known mutants, producing 4-5 times more cellulase than the wild-type strain (QM 6a)(Peterson and Nevalainen, 2012). A study by Kovács and *et al*., (2008) has shown that wild-type *Trichoderma atroviride* (F-1505) produces the most cellulase among 150 wild-type *Trichoderma*. Cellulase and xylanase activities in *Penicillium verruculosum* mutants were improved about 3-fold using four cycles of UV mutagenesis. The enzyme production was further improved by 2- to 3- fold in a two- stage fermentation process using wheat bran, yeast extract medium and microcrystalline cellulose as the inducer (Solov'eva *et al*., 2005).

1.1.8. Optimizations affecting cellulase production by SSF

1.1.8.1. Inoculum size

The inoculum size reported being one of the important parameters that should be considered in the SSF processes. Concentration of inoculum may influence the yield of enzymes of fungal strains (Krishna, 2005). Colonization of fungi on lignocellulosic substrate might take a relatively longer time if a low dosage of inoculum is used. This might correspondingly raise the risk of contamination where other fast growing fungi might colonize the substrate in a faster rate compared to that of the intended microbial species. Higher inoculum size might accelerate the fungal growth rate but at the same time increase the rate of nutrient depletion. Upon nutrients depletion, the growth of the fungi is affected and this might not be helpful in improving the yield of cellulase (Kumaran *et al* ., 1997). The important fact in the inoculum size is that there should be a balance between the amount of spores and amount of available oxygen and nutrients i.e. solid substrate and moistening agent to maintain their metabolic activities resulting in enzyme production. The optimum inoculum size in order to produce enzymes may be different for SmF and SSF using the same substrate (Patil and Dayanand, 2006).

1.1.8.2. Fermentation medium

Fermentation medium used in SSF influences the types and titres of enzymes produced using fungi. fermentation medium for cellulase production consists of carbon source, nitrogen source, phosphorus source, trace element solution and other minerals. The sources and optimal concentration of carbon is an important factor for the production of carboxymethy1 cellulase enzyme. Different types of carbon sources (paddy straw, wheat straw, sugarcane bagasse, jute stick, carboxymethylcellulose, corn cobs ground nut shells, cotton, ball milled barley straw, delignified bail milled oat spelt xylan, sulfite pulp, printed papers and mixed waste paper) have been reported for the production of cellulase enzyme (Behera and Ray, 2016). Since any cellulose biotechnological process is likely to base on crude enzymes, it is important to increase their activities in the culture supernatants by selecting the best carbon and nitrogen sources and optimizing their concentrations (Gomes *et al*., 2000). While nitrogen sources are the secondary energy sources for the organisms which plays an important role in the growth of the organism and enzyme production. A wide range of nitrogenous compounds either organic or inorganic can affect the productivity of cellulase. Organic nitrogen sources responded in positive cellulase activity more than inorganic ones in general. The effects of nitrogen sources on cellulase production were variable with

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-4, November 2021
ISSN: 2749-3644

respect to the fungi and compounds tested (Kachlishvili *et al.*, 2006). The enzyme production was affected significantly under different concentrations of nitrogen sources (Panagiotou *et al.*, 2003). Besides nitrogen sources, phosphorus sources and other minerals were also supplemented to the fermentation medium for cellulase production. Phosphorus is an essential requirement for fungal growth and metabolism. It is an important constituent of phospholipids involved in the formation of cell membranes. Besides its role in linkage between the nucleotides forming the nucleic acid strands, it is involved in the formation of numerous intermediates, enzymes and coenzymes essential in carbohydrate metabolism, other oxidative reactions and intracellular processes. Different phosphate sources such as potassium dihydrogen phosphate, tetra-sodium pyrophosphate, sodium β glycerophosphate and dipotassium hydrogen phosphate have beenevaluated for their effect on cellulases production (Kumar *et al.*, 2008).

1.1.8.3. Initial pH

The initial pH of the SSF medium may be adjusted via the addition of weak acids or bases, buffers or moistening agents containing mineral salts. However, pH may shift throughout the process with respect to the metabolic activities of the fungi . It has been reported that during SSF, the initial pH dropped after 4 days of fermentation and increased at the end of SSF on day 8. This alteration may be due to the consumption of the substrate such as ammonium salt consumption may decrease pH, on the contrary hydrolysis of urea in the medium may result in alkali pHs (Khan*et al.*, 2007; Chen, 2013). Other obvious reason of pH reduction will be the release of organic acids such as citric, acetic or lactic secreted by the microorganisms and vice versa the assimilation of the organic acids present in the prepared media will increase the pH. The trend in the pH variation is mostly dependent on the type of microorganism (Ray *et al.*, 2008).

1.1.8.4. Temperature

One of the most important environmental factors affecting the development of biological processes is temperature which may lead to protein denaturation, enzymatic inhibition, promotion, or inhibition on the production of a particular metabolite, cells death (Rodriguez-Leon *et al.*, 2008). It is well known that both fungal growth and metabolite production is sensitive to this factor in the solid-state fermentation processes (Smits *et al.*, 1998). Therefore, in order to support the growth of fungi and yield the targeted enzyme from the fermentation medium, the temperature of incubation should be optimized. However, the optimum temperature required for the growth and the product formation could be different from each other. Nevertheless, the optimum temperature and should be compatible with the product formation temperature should be in the range of 20 to 55 °C, where a mesophile fungus is involved in the bioprocess (Bhargav *et al.*, 2008).

1.1.8.5. Incubation time

When fungi are cultivated on lignocellulosic substrate under suitable condition, fungi starts to grow by extending their hyphal tips and the fungal mycelium spreads throughout the substrate forming a network of mycelia (Montoya *et al.*, 2012). The first sign of fungal growth was reported on day 2 of SSF in most of the studies involving cellulase production , after 7 to 11 days, the fungus cultivated might have completely colonized the substrate, depending on the amount of substrate used (Elisashvili *et al.*, 2009). During colonization phase of fungal growth, extracellular enzymes are produced to degrade the lignocellulosic substrate into smaller soluble molecules that could be utilized as nutrients for growth. Due to the hydrolytic action of the enzymes produced, depolymerisation of lignocellulosic components occurs (Hong *et al.*, 2011; Montoya *et al.*, 2012). It is important to optimize the incubation period especially in the enzyme production processes, since less time may lead to synthesis of the enzyme with low enzymatic activity and contrary to this; excessive incubation time increases the risk of contamination. Besides, the produced enzyme will be denatured with the generation of metabolic heat or inhibited due to the accumulation of inhibitory secondary metabolites or proteases secreted or due to the shift of pH of the medium at the later stages of the fungal growth (Pal and Khanum, 2010).

1.1.8.6. Moisture content of the substrate

The optimum moisture content required for the fungal growth or enzyme production may show difference depending on the type of the substrate. Moisture content below the optimum level may lead to low nutrient diffusion, microbial growth, enzyme stability, substrate swelling and sporulation (Kumar *et al* ., 2011), whereas above this level the problems will be particle agglomeration, gas transfer limitation and competition with bacteria. In addition, the risk of contamination is greater if higher moisture content was applied in the SSF as the condition encouraged the growth of unfavorable microorganisms (Deswal *et al.*, 2011).

On the other hand, optimum moisture content should be set, where the essentials of the SSF such as fungal growth, metabolite production and physicochemical properties of the substrate meet (Krishna, 2005). The moisture content of the solid

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-4, November 2021
ISSN: 2749-3644

substrate may show variation throughout the SSF process due to the evaporation or consumption of water for the metabolic activities. Therefore, one needs to add water, humidifiers or water-saturated air flow as the fermentation proceeds (Perez-Guerra *et al*., 2003).

Water content of solid substrate between 55% and 70% is found optimum for the growth of most of the cellulase producing organisms, i.e. *Trichoderma* (Sun *et al*., 2010), *Aspergillus* spp. (Lee, 2007), *Penicillium* (Pericin *et al*., 2008). The initial moisture level of 70% was found optimum for cellulase production by *Trichoderma* sp. on apple pomace under SSF(Sun *et al*., 2010).

1.1.9. Biotechnological application of cellulase

1.2. 9.1. Food industry

Cellulases play a key role in food industry (Kuhad *et al*., 2011). Macerating enzyme which mainly consists of cellulases with pectinases and hemicellulases are used to macerate fruit pulps to maximum possible liquefaction and results in more nutritive juice yield with better stability and reduction of processing time. Macerating enzymes are used to improve the cloud stability and texture of nectars and purees and decreasing their viscosity rapidly. For the first time an enzyme named Olivex was prepared by mixing pectinase with cellulase and hemicellulase from *Aspergillus aculeatus* to improve the extraction of olive oil (Grassin and Fauqemberge, 1996). Cellulases are also used for food colouring agent production. The main group of colouring substances in nature which are being responsible for many plant colours from red to yellow are Carotenoid (Gupta *et al*., 2013).

1.2.9.2. Animal feed industry

Important role of cellulase in animal feed industry, which is an important sector of agribusiness comprising of ruminants, poultry, pigs, pet foods and fish farming. The dietary fibre in animal food consists of non starch polysaccharides such as arabinoxylans, cellulose, many other plant components including dextrins, inulin, lignin, waxes, chitins, pectins, β-glucan, and oligosaccharides, which can act as antinutritional factor for several animals. Cellulase improves the nutritional value of feed by degrading certain cereal components or by eliminating these anti- nutritional factors present in raw materials by providing supplementary digestive enzymes such as proteases, amylases, and glucanases (Bhat, 2000).

1.2.9.3. Pulp and paper industry

Cellulases are use in paper industries for most biomechanical pulping, reduced energy requirement, reduced chlorine requirement, improved fiber brightness, strength properties, and cleanliness, improved drainage in paper mills, production of biodegradable cardboard, paper towels, an sanitary paper (Behera *et al*., 2017).

1.2.9.4. Agriculture industry

For enhancing growth of crops and controlling plant diseases, various enzyme preparations consisting of different combinations of cellulases, hemicellulases, and pectinases have potential applications in agriculture. Enhanced seed germination, rapid plant growth and flowering and increased crop yields are known to be facilitated by many cellulolytic fungi such as *Trichoderma* sp., *Geocladium* sp., *Chaetomium*sp. and *Penicillium* sp. (Chet *et al*., 1998).

1.2.9.5. Biofuel industry

A potential application of cellulases is the conversion of cellulosic material to glucose and other fermentable sugars, which in turn can be, used as microbial substrate for the production of single cell protein or fermentation products like ethanol. Production of ethanol from renewable resources via fermentation represents an important process for production of alternative fuels (Sukumaran *et al*., 2005).

1.2.9.6. Pharmaceutical industries

Since cellulose fibres are not easily digested, people are using adigestive enzyme product, like digestin, that contains cellulose enzymes which is important for healthy cells. Fungal hemicellulase and cellulase enzyme system helps in rapid hydrolysis of cellulose, hemicellulose and beta-glucan polymers in food (Gupta *et al*., 2013).

1.2.9.7. Waste management

The wastes generated from forests, agricultural fields, and agro industries contain a large amount of unutilised or underutilized cellulose, causing environmental pollution. Nowadays, by the use of enzyme like cellulase, amylase, lipase, protease etc, these so-called wastes are judiciously utilised to produce valuable products such as enzymes, sugars, biofuels, chemicals, cheap energy sources for fermentation, improved animal feeds, and human nutrients (Kuhad *et al*., 2010)

1.2.9.8. Textile (Bio-polishing technique)

Cellulase enzyme was used for biopolishing of jute fiber. This biopolishing technique have considered an environmental friendly way of improving the property of fabrics, their desirable appearance and soft handle reduces brittleness and stiffness and promotes its affinity to readily accept dyes. Jute is a ligno-cellulosic fibre that is partially fibre and partially wood. India is the world's largest jute growing country with its cultivation localized in the states of West Bengal, Orissa, Bihar, Assam, Meghalaya, Tripura and Andhra Pradesh (Saravanan *et al*., 2013). Jute is an important natural fiber which has a great potential to produce multipurpose products in daily routine life. Unprocessed

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-4, November 2021
ISSN: 2749-3644

raw fiber is being utilized as an input source to textile sector for products with high mechanical properties. Jute is one of the longest and most commonly used natural fibers for various technical applications. It is obtained from the inner bark of the plant's stem as shown in figure (1-5). It belongs to the genus *Corchorus* in the basswood family, Tiliaceae. Jute is being known as Golden Fiber due to its golden and silky shine. Jute fibers consist of lignin (12-14%), cellulose (58–63%), hemicellulose (21- 24%), nitrogenous matter (0.8-1.5%), traces of pigments, inorganic matter (0.6-1.2%) and fats and waxes (0.4-0.8 %). Thease have many applications in garment linings, household items, furniture upholstery, automotive carpeting, automobile sound absorption materials, carpet underlays, building materials for insulation and roofing felt, and low-end blankets (liew *et al*., 2017).

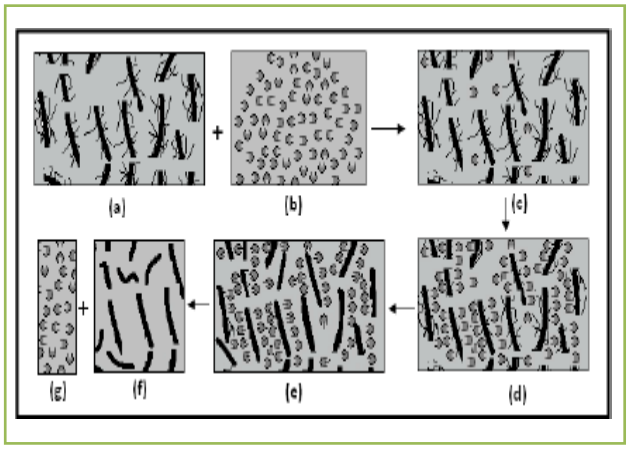
-a- -b-

Figure(1-5): a: jute plant, b: jute fiber (Akshay *et al*., 2017)

Jute has some major shortcomings like harsh prickly feel and poor drapeabiity.Therefore, it is necessary to minimize some of the shortcomings of jute for improved feel by chemical processing through an eco-friendly route. Cellualase enzyme has, therefore, attracted the attention of many scientists. Cellualase enzymes have already been recognized to remove cellulosic impurities, individual and loose jute fibre ends that protrude from jute fabric surfaces to provide an enhanced appearance and handle of the jute fabric (Chattopadhyay and Sharms, 2000).

Success of jute biofinishing is influenced by pH, temperature, substrate concentration, enzyme concentration time and mechanical action. Endoglucanases are key enzymes in biofinishing. Studies conducted to evaluate the best component of cellulase for high performance in biofinishing was favorable towards endoglucanase. Commercial cellulases for biofinishing originate from *Trichoderma reesei* and *Humicola insolens* (Jabasingh and Nachiyar, 2011).

Representation of the possible mechanism of enzymatic finishing of jute fiber is given by a schematic representation in figure(1-6).



Figure(1-6): Possible mechanism of the cellulase bio-finishing of Jute fiber (Jabasingh and Nachiyar, 2012).

This Figure represents the following: (a) Jute fibers with protruding micro fibrils; (b) Addition of cellulase to act upon microfibrils; (c) Cellulase action on microfibrils; (d) Establishment of lock and key mechanism by endoglucanase component with protruding micro fibrils; (e) Action of endoglucanase on protruding fibers and hydrolysis of protruding micro fibrils; (f) Completely de-microfibrilled and biofinished Jute fiber and (g) cellulase after the biofinishing action. (Jabasingh and Nachiyar, 2012).

CONCLUSION

Fungi have evolved to be the most powerful and prevalent biomass degrading organisms in nature, exhibiting a diverse range of lifestyles for the turnover of lignocellulosic material on Earth. Given their significant activity and ability to be readily produced at high titers on the industrial scale, fungal cellulase cocktails are an excellent starting point for industrial biorefinery applications.

Hence, recent development in cellulases from *Trichoderma longibrachiatum* fungus has led to speculation and anticipation of their enormous commercial potential in biotechnology and will undoubtedly play a key role in future biotechnological research in the 21st century.

ACKNOWLEDGMENTS

The authors would like to thank Mustansiriyah University (www. uomustansiriyah.edu.iq) Baghdad Iraq and University of Nahrain Baghdad Iraq for its support in the present work.

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-4, November 2021
ISSN: 2749-3644

REFERENCES

1. Abdullah, JJ.; Greetham, D.; Pensupa, N.; Tucker, GA. and Du, C. (2016). Optimizing Cellulase Production from Municipal Solid Waste (MSW) using Solid State Fermentation (SSF). J Fundam Renewable Energy Appl, 6, 206-212. https://doi.org/10.4172/2090-4541.1000206**.**
2. Agrawal, Sh. (2015). Solid state fermentation for cellulase production, Biotec.Res.J. Vol 1(1):108-112**.**
3. Akshay, M.; Mohan, K.; Adarsh, N.; Arvind, B. A. and Balaji, S. C. (2017).Fabrication and testing of hybrid composites using jute and mango endocarp. International Research Journal of Engineering and Technology, 4 (11): 1760-1764
4. Atanasova, L.; Jaklitsch, WM.; Komoń-Zelazowska, M.; Kubicek, CP. and Druzhinina, IS. (2010). Clonal species *Trichoderma parareesei* sp. nov. likely resembles the ancestor of the cellulase producer *Hypocrea jecorina/T. reesei*. Appl. Environ. Microbiol ,76: 7259-7267.
5. Atanasova, L.; LeCromS.; Gruber, S. and Coulpier, F. (2013). Comparative transcriptomics reveals different strategies of *Trichoderma* mycoparasitism. BMC Genomics 14:121.
6. Bayer, E.; Shoham, Y.and Lamed, R. (2006) Cellulose-decomposing Bacteria and Their Enzyme Systems. The Prokaryotes 2:578-617
7. Beckham ,G. ; Bomble,Y. ; Bayer,E.; Himmel,M. and Crowley,M. (2011). Applications of computational science for understanding enzymatic deconstruction of cellulose. Current Opinions inBiotechnology. 22(2): 231–238 .
8. Beezhold, D. H.; Green. B. J.; Blacbere, F. M.; Schmechel, D .;Weissman, D. N.; Veickoff; D.; Hogan, M. B. and Wilson, N. W. (2008). Prevalence of allergic sensitization to indoor fungi in West Virginia. Allergy Asthma Proc. 29:29-34.
9. Behera, S.S.and Ray, R.C.(2016). Solid state fermentation for production of microbial cellulases: Recent advances and improvement strategies. Int. J. Biol. Macromol., 86, 656–669.
10. Bhargav, S.; Panda, B.P.; Ali, M. and Javed, S. (2008). Solid-state Fermentation: An Overview. Chem. Biochem. Eng. Q. 22(1), 49-70.
11. Bhat, M. K. and Hazlewood, G. P. (2003). Enzymology and other characteristics of cellulases and xylanases. In: Enzymes in Farm Animal Nutrition pp, 11-60 (Bedford M. R. and Partridge, G. G. eds). CAB international, Cambradge.
12. Bhat, M. K. (2000). Cellulases and related enzymes in biotechnology. Biotechnology advances, 18(5), 355-383.
13. Bissett, J.; Gams, W.; Jaklitsch, WM. and Samuels, GJ. (2015). Accepted *Trichoderma* names in the year 2015. IMA Fungus, 6: 263–295. https://doi.org/10.5598/imafungus.2015.06.02.02.
14. Bridges, P.D.; Spooner, BM. and Roberts, PJ. (2005).The impact of molecular data in fungal systematic. Adv Bot Res.;42:34–67.
15. Bruns, T.D.; White, T.J. and Taylor, JW. (1991). Fungal molecular systematics. Ann. Rev. Ecol. Syst. 22:525–564.
16. Chakraborty, B.N.; Chakraborty, U.; Saha, A.; Dey, P.L. and Sunar, K. (2010). Molecular characterization of *Trichoderma viride* and *Trichoderma harzianum* isolated from soils of North Bengal based on rDNA markers and analysis of Their PCR-RAPD profiles. Global J Biotech and Biochem. 5: 55-61.
17. Chattopadhyay, D. P and Sharmsa, J. K (2000). Improvement in jute fabric handle through biofinishing. indian Journal of Fibre and Textile Research 25: 121-129.
18. Chen, H.(2013). Modern Solid State Fermentation Theory and Practice: Springer Netherlands,p 424.
19. Chet, I.; Benhamou,N.; Haran,S.; in: G.F. Harman, C.P. Kubicek. (1998). *Trichoderma* and *Gliocladium*-Enzymes, Biological Control and Commercial Applications, Taylor and Francis, London, pp. 327–342
20. De Hoog, G.S. (2000). Atlas of clinical fungi (2.ed.). Utrecht: Centraalbureau voor Schimmelcultures [u.a.] ISBN 9070351439.
21. De la Cruz Quiroz, R.; Roussos, S.; Hernández, D.; Rodríguez, R.; Castillo, F. and Aguilar, C.N. (2015). Challenges and opportunities of the bio-pesticides production by solid-state fermentation: Filamentous fungi as a model. Crit. Rev. Biotechnol. 35, 326–333.
22. Deswal ,D.; Khasa, YP. and Kuhad, RC. (2011). Optimization of cellulase production by a brown rot fungus *Fomitopsis* sp RCK2010 under solid state fermentation. Bioresource Technology; 102:6065.

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-4, November 2021
ISSN: 2749-3644

23. Dodd, SL.; Crowhurst, R.N.; Rodrigo, AC.; Samuels, GJ.; Hill, RA. and Stewart, A. (2000). Examination of *Trichoderma* phylogenies derived from ribosomal DNA sequence data. Mycol Res.; 104:23–34.
24. Dong, X. W.; Pang, X. H.; Huang, F.; Liu, W.; Chen, X. L.; Zhou, B. C.; Zhang, Y. Z. and Song, X. Y. (2014)."Comparative Genomics Provide Insights into Evolution of *Trichoderma* Nutrition Style". Genome Biology and Evolution. 6 (2): 379–390.
25. Druzhinina, I.S.; Komoń-Zelazowska,M.; Ismaiel,A.; Jaklitsch,W.; Mullaw, T.; Samuels,G.J.; and Kubicek,C.P. (2012) ."Molecular phylogeny and species delimitation in the section *Longibrachiatum* of *Trichoderma*". Fungal Genetics and Biology. 49 (5): 358–368.
26. Druzhinina, IS, Schmoll, M.; Seiboth, B. and Kubicek, CP.(2006). Global carbon utilization profiles of wild -type, mutant, and transformant strains of *Hypocrea jecorina*. Appl Environ Microbiol; 72: 2126-2133.
27. Elisashvili, V.; Kachlishvili, E.; Tsiklauri, N.; Metreveli, E.; Khardziani, T. and Agathos, SN. (2009). Lignocellulose-degrading enzyme production by white-rot Basidiomycetes Isolated from the forests of Georgia. World Journal of Microbiology and Biotechnology; 25:331.
28. Farinas, C. S. (2015). Developments in solid-state fermentation for the production of biomass degrading enzymes for the bioenergy sector. Renewable and Sustainable Energy Reviews, 52, 179-188
29. Garvey, M.; Klose, H. and Fischer, R. (2013). Cellulases for biomass degradation: comparing recombinant cellulase expression platforms. Trends Biotech- nol 31:581–593. doi:10.1016/j.tibtech.2013.06.006
30. Gomes, I.; Gomes, J.; Gomes, D.J.; and Steiner, W. (2000). Simultaneous production of high activities of thermostable endoglucanase and β-glucosidase by the wild thermophilic fungus *Thermoascus aurantiacus*. Appl. Microbiol. Biotechnol., 53, 461-468.
31. Grassin,C. and Fauquembergue, P. (1996) in: T. Godfrey, S. West (Eds.), Industrial Enzymology, second ed., Macmillan, UK, 226–4.
32. Gupta, R.; Mehta, G.; Deswal, D.; Sharma, S.; Jain, K. K.; Kuhad, R. C. and Singh, A. (2013). Cellulases and their biotechnological applications. In Biotechnology for Environmental Management and Resource Recovery (pp. 89-106). Springer India.
33. Gupta, R.; Sharma, K. K. and Kuhad, R. C. (2009). Separate hydrolysis and fermentation (SHF) of Prosopis juliflora, a woody substrate, for the production of cellulosic ethanol by *Saccharomyces cerevisiae* and *Pichia stipitis*-NCIM 3498. Bioresource Technology, 100(3), 1214-1220.
34. Hamdan, N. T., & Jasim, H. M. (2018A). Purification and characterization of cellulase enzyme from *Trichoderma longibrachiatum* isolated in Iraqi soil. J Biotechnol Biochem, 4(1), 32-41.
35. Hamdan, N. T., & Jasim, H. M. (2018B). Purification and characterization of cellulase enzyme from *Trichoderma longibrachiatum* isolated in Iraqi soil. J Biotechnol Biochem, 4(1), 32-41.
36. Hamdan, N. T., & Jasim, H. M. (2021). TRICHODERMA LONGIBRACHIATUM: FIRST RECORD IN IRAQ. Web of Scientist: International Scientific Research Journal, 2(10), 86-103.
37. Hansen, G.; Lübeck, M.; Frisvad, J.; Lübeck, P. and Andersen, B. (2015). Production of cellulolytic enzymes from ascomycetes: Comparison of solid state and submerged fermentation. Process Biochemistry, 50(9), 1327-1341.
38. Henrissat, B.; Callebaut, I.;Fabrega, S.; Lehn, P.; Mornon, J. and Davies, G. (1995). Conserved catalytic machinery and the predictionof a common fold for several families of glycosyl hydrolases. Proceedings of National Academy of Science. (92): 7090-7094.
39. Hermosa, R.; Cardoza, R. E.; Rubio, M. B.; Gutiérrez, S, and Monte, E. (2014). Secondary metabolism and antimicrobial metabolites of the internal transcribed spacer region 1 of the rDNA clade. Fungal Genet Biol., 24:298–309.
40. Hong, LS.; Ibrahim, D. and Omar, I.C. (2011). Lignocellulolytic materials as a raw material for the production of fermentable sugars via solid state fermentation. Asian Journal of Scientific Research, 4:53.
41. Howard, ed. by Dexter H. (2003). Pathogenic fungi in humans and animals (2nd ed.). CRC

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-4, November 2021
ISSN: 2749-3644

Press. New York, NY: Dekker.

42. Imran, M.; Anwar, Z.; Irshad, M.; Asad, M. J.; Ashfaq, H. and Imran, M. (2016). Cellulase Production from Species of Fungi and Bacteria from Agricultural Wastes and Its Utilization in Industry: A Review. Advances in Enzyme Research, 4(4), 44–55. https://doi.org/10.4236/aer.2016.42005
43. Jabasingh, S. A. And Nachiyar, C. V. (2012). Process Optimization for the Biopolishing of Jute Fibers with Cellulases from *Aspergillus Nidulans* AJ SU04, International Journal of Bioscience, Biochemistry and Bioinformatics, 2(1).
44. Jabasingh, S.A. and Nachiyar, CV. (2011). "Utilization of pretreated bagasse for the sustainable bioproduction of cellulase by *Aspergillus nidulans* MTCC344 using response surface methodology", Industrial Crops and Products, 34, 1564–71
45. Jaklitsch, W. M. (2011). "European species of *Hypocrea* part II: species with hyaline ascospores". Fungal Diversity. 48 (1): 1–250. doi:10.1007/s13225-011-0088-y.
46. Jørgensen, H.; Kristensen, J. B. and Felby, C. (2007). Enzymatic conversion of lignocellulose into fermentable sugars: challenges and opportunities. Biofuels, Bioproducts and Biorefining, 1(2), 119-134.
47. Juturu, V. and Wu, JC. (2014). Microbial cellulases: engineering, production and applications. Renew Sustain Energy Rev 33:188–203. doi:10.1016/j. rser.2014.01.077.
48. Kachlishvili, E.; Penninckx, M.J.; Tsiklauri, N. and Elisashvili, V. (2006). Effect of nitrogen source on lignocellulolytic enzyme production by white-rot basidiomycetes under solid- state cultivation. World J Microbiol Biotechnol, 22, 391–397
49. Kannangara, S.; Dharmarathna, R. M. G. C. S. and Jayarathna, D. L. (2017). Isolation , Identification and Characterization of *Trichoderma* Species as a Potential Biocontrol Agent against *Ceratocystis paradoxa,* 12(1), 51–62.
50. Khan, MMH.; Ali ,S.; Fakhru'L-Razi, A. and Alam, MZ. (2007). Use of fungi for the bioconversion of rice straw into cellulase enzyme. Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes , 42:381.
51. Kheng, P.P, and Omar, IC. (2005). Xylanase production by a local fungal isolate, *Aspergillus niger* USM AI 1 via solid state fermentation using palm kernel cake (PKC) as substrate. Songklanakarin J. Sci. Technol. 27(2):325-336.
52. Kindermann, J.; El-Ayouti ,Y.; Samuels, G.J. and Kubicek, C.P. (1998). Phylogeny of the genus *Trichoderma* based on sequence analysis
53. Kov acs, K.; Megyeri, L.; Szakacsa, G.; Kubicekc, CP.; Galbeb, M. and Zacchi, G. (2008). *Trichoderma atroviride* mutants with enhanced production of cellulase and β-glucosidase on pretreated willow. Enzyme and Microbial Technology, 43: 48–55.
54. Krishna, C. (2005). Solid-state Fermentation Systems-An Overview. Crit. Rev. Biotechnol., 25, 1-30.
55. Kuhad, R. C.; Gupta, R. and Singh, A. (2011). Microbial cellulases and their industrial applications. Enzyme research, Volume2011(2011). Article ID 280696, 10 pages .http://dx.doi.org/10.4061/2011/280696
56. Kuhad, R. C.; Gupta, R.; Khasa, Y. P. and Singh, A. (2010). Bioethanol production from lantanacamara (red sage): pretreatment, saccharification and fermentation. Bioresource technology, 101(21), 8348-8354.
57. Kuhls, K.; Lieckfeldt, E.; Samuels, GJ.; Borner, T.; Meyer, W. and Kubicek, CP. (1997). Revision of *Trichoderma* sect. Longibrachiatum including related teleomorphs based on analysis of ribosomal DNA internal transcribed spacer sequences. Mycologia. 89:442–60.
58. Kumar, R.; Singh, S. and Singh, O.V. (2008). Bioconversion of lignocellulosic biomass: biochemical and molecular perspectives. Journal of industrial microbiology and biotechnology, 35(5), pp. 377–391.
59. Kumar, S.; Sharma, H. and Sarkar, B. (2011). Effect of substrate and fermentation conditions on pectinase and cellulase production by *Aspergillus niger* NCIM 548 in submerged (SmF) and solid state fermentation (SSF). Food Science and Biotechnology; 20:1289
60. Kumaran, S.; Sastry, CA.and Vikineswary, S. L. (1997). Cellulase and xylanase activities during growth of *Pleurotus* sajorcaju on sago hampas. World Journal of Microbiology and Biotechnology, 13:43.
61. Lantz, S. E.; Goedegebuur, F.; Hommes, R.;

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-4, November 2021
ISSN: 2749-3644

Kaper. T.; Kelemen, B. R.; Mitchinson, C.; wallace, t.; Stahlberg, j.and Larenas, E. A. (2010). *Hypocrea jecorina CEL6A* protein engineering. Biotechnol. Biofuels, 3:20. Doi: 10.1186/ 1754-S834-3-20.

62. Lee, CF. and Hseu, TH. (2002). Genetic relatedness of *Trichoderma* sect. *Pachybasium* species based on molecular approaches. Can J Microbiol 48: 831-840.
63. Lee, N. S. (2007). The production of fungal mannanase, cellulase and xylanase using palm kernel meal as a substrate. Walailak J. Sci. Technol. 4: 62-82.
64. Lidia, B.; Popiel, D. and Che, J. (2011). Species diversity of *Trichoderma* in Poland, Journal of applied genetics, 52(2) 233–243. https://doi.org/10.1007/s13353-011-0039-z.
65. Lidia, B.; Strakowska, J. and Che, J. (2016). *Trichoderma* species occurring on wood with decay symptoms in mountain forests in Central Europe : genetic and enzymatic characterization, Journal of applied genetics, 57(3): 397–407. https://doi.org/10.1007/s13353-015-0326-1
66. Lieckfeldt , E.; Samuels, GJ.; Borner, T. Gams ,W. (1998). *Trichoderma koningii*: neotypifi cation and Hypocrea teleomorph. Can J Bot., 76:1507–22.
67. Lieckfeldt, E. and Seifert, KA. (2000). An evaluation of the use of ITS sequences in the taxonomy of the Hypocreales. Stud Mycol, 45:35–44.
68. Lieckfeldt, E.; Kullnig, CM.; Kubicek, CP.; Samuels, GJ.and Borner, T. (2001). *Trichoderma aureoviride* : phylogenetic position and characterization. Mycol Res,105:313–22.
69. Liew, F. K.; Hamdan, S.; Rahman, R. and Rusop, M. (2017). Thermomechanical Properties of Jute / Bamboo Cellulose Composite and Its Hybrid Composites: The Effects of Treatment and Fiber Loading, Advances in Materials Science and Engineering, 2017, (1-10).
70. Limayem, A. and Ricke, SC. (2012). "Lignocellulosic biomass for bioethanol production: current perspectives, potential issues and future prospects", Progress in Energy and Combustion Science, 38, 449–67.
71. López-Pérez, M. and Viniegra-González, G. (2016). Production of protein and metabolites by yeast grown in solid state fermentation: Present status and perspectives. J. Chem. Technol. Biotechnol., 91, 1224– 1231.
72. Mandels, M.; Weber, J. and Parizek, R. (1971).Enhanced cellulase production by a mutant of *Trichoderma viride*. Appl Microbiol;21:152- 154.
73. Martins, S.; Mussatto, S.I.; Martínez-Avila, G.; Montañez-Saenz, J.; Aguilar, C.N. and Teixeira, J.A. (2011). Bioactive phenolic compounds: Production and extraction by solid-state fermentation. A review. Biotechnol. Adv., 29, 365–373.
74. Maurer, S.A.; Brady, N.W.; Fajardo, N.P. and Radke, C.J. (2012)"Surface kinetics for cooperative fungal cellulase digestion of cellulose from quartz crystal microgravimetry". Journal of Colloid and Interface Science. 394: 498–508.
75. Montenecourt, B.S. and Eveleigh, D.E. (1977). Preparation of mutants of *Trichoderma reesei* with enhanced cellulase production. Appl Environ Microbiol,34:777-782.
76. Montenecourt, BS. and Eveleigh, DE. (1979). Selective screening methods for the isolation of high yielding cellulase mutants of *Trichoderma reesei*. Adv. Chem. Ser.;181:289-301.
77. Montoya, Q. V.; Meirelles, L. A.; Chaverri, P. and Rodrigues, A. (2016)."Unraveling *Trichoderma* species in the attine ant environment: description of three new taxa," Antonie van Leeuwenhoek, Int. J. Gen. Mol. Microbiol., vol. 109, no. 5, pp. 633–651.
78. Montoya, S.; Eduardo, O.C. and Levin, L. (2012). Growth, fruiting and lignocellulolytic enzyme production by the edible mushroom Grifola frondosa (maitake). World Journal of Microbiology and Biotechnology, 28:1533.
79. Moraïs, S.; Barak, Y.and Lamed, R . (2012). Paradigmatic status of an endo- and exoglucanase and its effect on crystalline cellulose degradation. Bio- technol Biofuels 5:78. doi:10.1186/1754-6834-5-78
80. Mussatto, S.I. and Teixeira, J.A. (2010). Lignocellulose as raw material in fermentation processes. Current research technology and education topics in applied microbiology and microbial biotechnology, A. Mendez-Vilas (Ed.) 897–907.
81. Nam, KH.; Sung, MW. and Hwang, KY. (2010).

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-4, November 2021
ISSN: 2749-3644

Structural insights into the substrate rec ognition properties of β-glucosidase. Biochem Biophys Res Commun 391:1131–1135. doi:10.1016/j.bbrc.2009.12.038.

82. Pal, A.and Khanum, F. (2010). Production and Extraction Optimization of Xylanase from *Aspergillus niger* DFR-5 through Solid- state Fermentation. Bioresour. Technol., 101, 7563-7569.
83. Panagiotou, G.; Kekos, D.; Macris, B.J. and Christakopoulos, P. (2003). Production of cellulolytic and xylanolytic enzymes by *Fusarium oxysporum* grown on corn stover in solid state fermentation. Ind Crop Prod, 18, 37–45.
84. Patil, S. and Dayanand, A. (2006). Optimization of Process for the Production of Fungal Pectinases from Deseeded Sunflower Head in Submerged and Solid-state Conditions. Bioresource Technol. 97, 2340- 2344.
85. Paz, Z.; Komori-Zelazowska, M., pruzhinina, I. S.; Aveskamp, M, M.; Schnaiderman, A.; Aluma, Y.; Canneli, s.; Ilan, M. and Yarden, O. (2010). Diversity and potential anti-fungal properties of fungi associated with a Mediterranean sponge. Fungal Diversity, 42:17-26.
86. Pérez-Guerra, N.; Torrado-Agrasar, A.; López-Macias, C. and Pastrana, L. (2003). Main Characteristics and Applications of Solid Substrate Fermentation. Electron. J. Environ. Agric. Food Chem., 2, 343- 350.
87. Pericin, D.; Madarev-Popovic, S.; Radulevi-Popvic, L. and Skrinjar, M. (2008). Evaluate of pumpkin oil cake as substrate for cellulose production by *Penicillium roquefortii* in solid state fermentation. Roman. Biotecnol. Lett. 13 : 3815-3820.
88. Peterson, R. and Nevalainen, H. (2012). *Trichoderma reesei* RUT- C30–thirty years of strain improvement. Microbiology 158, 58–68. doi: 10.1099/mic.0. 054031-0
89. Polizzi, V.; Adams, A.; picco, A. M.; Adriaens, E.; Lenoir, j.; Van peteghem C.; De Saeger, s. and De Kimpe, N.(2011). Influence of environmental conditions on production of volatiles by *Trichoderma atroviride* in relation with the sick-building syndrome. Build. Environ. 46:945-954.
90. Prévot, V.; Lopez, M.; Copinet, E. and Duchiron, F. (2013). Comparative performance of commercial and laboratory enzymatic complexes from submerged or solid-state fermentation in lignocellulosic biomass hydrolysis. Bioresource technology, 129, 690-693.
91. Qin, WT. and Zhuang, WY. (2016). Four new species of *Trichoderma* with hyaline ascospores from central China. Mycological Progress 15: 811–825. https://doi.org/10.1007/s11557-016-1211-y.
92. Qin, WT. and Zhuang, WY. (2017). Seven new species of *Trichoderma* (Hypocreales) in the Harzi anum and Strictipile clades. Phytotaxa 305: 121–139. https://doi.org/10.11646/phyto-taxa.305.3.1.
93. Ray, R.C.; Shetty, K. and Ward, O.P. (2008). Solid-state fermentation and value-added utilization of horticultural processing wastes. In: Microbial Biotechnology in Horticulture, Volume 3 (Eds., Ramesh C. Ray and O.P. Ward), Science Publishers, New Hampshire, pp.231- 272
94. Reddy, G. P. K.; Narasimha, G.; Kumar, K. D. and Ramanjaneyulu, G. (2015). Original Research Article Cellulase production by *Aspergillus niger* on different natural lignocellulosic substrates, 4(4), 835–845.
95. Rodriguez-Leon, J.A.; Soccol, C.R.; Pandey, A.; and Roddguez, D.E. (2008). Factors Affecting Solid-state Fermentation. In Current Developments in Solid-state Fermentation; Pandey, A. Larroche, C. Soccol C.R. Eds.; Springer: Delhi, pp. 230-252.
96. Rosales, E.; Pérez-Paz, A.; Vázquez, X.; Pazos, M. and Sanromán, M. A. (2011)."Isolation of novel benzo[a]anthracene-degrading microorganisms and continuous bioremediation in an expanded-bed bioreactor". Bioprocess and Biosystems Engineering. 35(5): 851–855.
97. Samuels, G. J.; Ismaiel, A; Mulaw, T. B.; Szakacs, G.; Druzhinina, I.S.; Kubicek, C. P. and Jaklitsch, W. M. (2012). "The *Longibrachiatum* Clade of *Trichoderma*: a revision with new species". Fungal Diversity. 55(1): 77–108.
98. Samuels, G.; Petrini, O.; Kuhls, K.; Lieckfeldt, E. and Kubicek, CP.(1998). The *Hypocrea schweinitzii* complex and *Trichoderma* sect. *Longibrachiatum.* Stud Mycol.;41:1–54.
99. Samuels, G.J. and Hebbar, P.K. (2015). *Trichoderma* Identification and Agricultural Applications. The American Phytopathological Society, 1st Edn., St. Paul, Minnesto, USA, pp196.

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-4, November 2021
ISSN: 2749-3644

100. Samuels, G.J. (1996). *Trichoderma*: a review of biology and Systematic of the genus. Mycol. Res., 100: 923–35.

101. Saravanan, D.; Lakshmi, S. N. S.; Raja, K. S. and Vasanthi, N. S. (2013). Biopolishing of cotton fabric with fungal cellulase and its effect on the morphology of cotton fibres. Indian Journal of Fibre and Textile Research 38: 156–160.

102. Schoch, C. L.; Seifert K. A.; Huhndorf S.; Robert, V.; Spouge, J. L.; Levesque, C. A. and Chen, W. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi, Proceedings of the National Academy of Sciences of the United State of America. 109 (16): 6241-6246.

103. Segato, F.; Damásio, ARL. and de Lucas, RC. (2014) Genome analyses highlight the different biological roles of cellulases. Microbiol Mol Biol Rev 78:588–613. doi:10.1128/MMBR.00019-14

104. Shahbazi, S.; Ispareh, K.; Karimi, M.; Askari, H. and Ebrahimi, M. A. (2014). Gamma and UV radiation induced mutagenesis in *Trichoderma reesei* to enhance cellulases enzyme activity. Int'l J. of Fam. and Alli Sci, 3(5), 543-554.

105. Singh, B. P.and Gupta, V. K.(2017). Molecular Markers in Mycology. In Molecular Markers and Their Use in Taxonomic Characterization of *Trichoderma* spp(First edn), p37-52, Springer .Switzerland.

106. Singhania, R.R.; Sukumaran, RK.; Patel, AK.; Larroche, C. and Pandey, A. (2010). Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. Enzyme and Microbial Technology, 46:541.

107. Smits, J.P.; Rinzema, A.; Tramper, J.; van Sonsbeek, H.M.; Hage, J.C.; Kaynak, A. and Knol, W. (1998).The Influence of Temperature on Kinetics in Solid-state Fermentation. Enzyme and Microbial Technology, 22, 50-57.

108. Soccol, C. R.; Costa, E. S. F. da, Letti, L. A. J.; Karp, S. G.; Woiciechowski, A. L. and Vandenberghe, L. P. de S. (2017). Recent developments and innovations in solid state fermentation. Biotechnology Research and Innovation,1(1): 52-71 https://doi.org/10.1016/j.biori.2017.01.002.

109. Solov'eva, IV.; Okunev, ON.; Vel'kov, VV.; Koshelev, AV.; Bubnova TV.; Kondrat'eva, EG.; Skomarovskii, AA. and Sinitsyn, AP. (2005). The selection and properties of *Penicillium verruculosum* mutants with enhanced production of cellulases and xylanases. Mikrobiologiia, 74:172-178.

110. Srinivasa, N.; Kamil, D.; Singh, C.; Singode, A. and Gupta, D. (2017). Molecular and Biochemical Characterization of Potential Isolates of *Trichoderma* Species Effective against Soil-Borne Pathogens, 6(6), 3132–3149.

111. Sukumaran, R. K.; Singhania, R. R. and Pandey, A. (2005). Microbial cellulases-production, applications and challenges. J. Sci. Ind. Res. 64 (2005) 832–844.

112. Sun, H.; Ge, X.; Hao, Z. and Peng, M. (2010). Cellulase production by *Trichoderma* sp. on apple pomace under solid state fermentation. Afr. J. Biotechnol. 9: 163- 166.

113. *Trichoderma*. Biotechnology and Biology of *Trichoderma*, 125-137.

114. Vale, L. H. F. Do.; Filho, E. X. F.; Miller, R. N. G.; Carlos, A. O. and Sousa, M. V. De. (2014). Cellulase Systems in *Trichoderma*: An Overview. In Biotechnology and Biology of *Trichoderma* (pp. 229-244). https://doi.org/10.1016/B978-0-444-59576-8.00016-3.

115. White, T. J.; Bruns, T.; Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocoles: A Guide to Methods and Applications. (Innis, M. A. Gelfand, D. H. Sninsky, J. J. White, T. J. Eds.). Academic Press, Inc, California. Pp 315-322.

116. Wilson, D. B. (2015). Processive cellulases. In Direct Microbial Conversion of Biomass to Advanced Biofuels, Elsevier B.V, (pp. 83-89).

117. Wilson, D. B. and Irwin, D. C. (1999). Genetics and Properties of Cellulases. Advances in Biochem. Eng. Biotech.,65:2-18.

118. Xie, B. B.; Qin, Q. L.; Shi, M.; Chen, L. L.; Shu, Y. L.; Luo, Y.; Wang, X. W.; Rong, J. C.; Gong, Z. T.; Li, D.; Sun, C. Y.; Liu, G. M.(2014). Comparative Genomics Provide Insights into Evolution of *Trichoderma* Nutrition Style". Genome Biology and Evolution. 6 (2): 379–390.

119. Yazid, N. A.; Barrena, R.; Komilis, D. and

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-4, November 2021
ISSN: 2749-3644

Sánchez, A. (2017). Solid- state fermentation as a novel paradigm for organic waste valorization: A review. Sustainability (Switzerland), 9(2),1–28. https://doi.org/10.3390/su9020224

120. Zhang, S.; Gan Y.; Ji, W.; Xu, B.;Hou, B. and Liu, J. (2017) "Mechanisms and Characterization of *Trichoderma longibrachiatum* T6 in Suppressing Nematodes (*Heterodera avenae*) in Wheat," .Front. Plant Sci., vol. 8, pp. 1–20.

121. Zhang, Y-HP. and Lynd , LR. (2004) .Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems. Biotechnol Bioeng 88:797–824. doi:10.1002/bit.20282.