Biopolymers from Fungi and Their Applications

S. G. Tupe, Sunil K. Deshmukh, R. B. Zambare, A. A. Tripathi, and Mukund V. Deshpande

Abstract The cell wall polysaccharides of fungi *viz.* β-glucan, chitin, chitosan, and mannan and different fungal exopolysaccharides find wide-ranging applications in various industries. The fungal exopolysaccharides (EPSs) such as pullulan (*Aureobasidium*), scleroglucan (*Sclerotium*), and botryosphaeran (*Botryosphaeria*) are recognized as high value bio-macromolecules for pharmaceuticals, medicine, foods and other industries. The fungal waste is generated in mushroom industry, wineries (*Saccharomyces* and non-*Saccharomyces* yeasts), enzyme (*Aspergillus, Trichoderma*), and antibiotic (*Penicillium*) industries, to name a few. Whereas, the genera like *Absidia, Benjaminiella, Gongronella, Rhizopu, Saccharomyces* and others have been studied extensively for chitin and/or chitosan, glucan, mannan production. Usually the fungal strains, solid or submerged fermentation, nutritional parameters decide the quality of the biopolymers produced.

Keywords Biopolymers · Cell wall · Chitin/chitosan · Commercial potential · Exopolysaccharides · Glucans hydrophobins · Mannans

1 Introduction

Every year up to 180 m tons of different polymers are generated for their use in different fields (Castillo et al. 2015). The plant as well as petroleum -based polymers though widely used have the disadvantage of limited resources and the negative impact on the environment of latter is a concern too. In this regard, use of microbial sources can be a viable option (Donot et al. 2012). Fungal biopolymers have attracted researchers for their variety of applications owing to their biological and biophysical characteristics. In general, biopolymers being complex chains of exo-polysaccharides of microbial origin and have capacity to form viscous solutions or gels in aqueous system compatibility with different salts for a varied range of

S. G. Tupe · S. K. Deshmukh · R. B. Zambare · A. A. Tripathi · M. V. Deshpande (✉)
Research and Development Division, Greenvention Biotech Pvt. Ltd., Pune, Maharashtra, India
e-mail: greenventionbiotech@gmail.com

S. K. Deshmukh et al. (eds.), *Fungal Biopolymers and Biocomposites*,
https://doi.org/10.1007/978-981-19-1000-5_1

pH and temperature, high solubility water, and compatibility along with other polysaccharides. In addition to the exopolysaccharides, in case of fungi the polymers of cell wall like chitin, chitosan, glucan and mannans have also significant applications in industry, agriculture and in healthcare too (Ghormade et al. 2017). The fungal biopolymers have not been sufficiently explored for their commercial potential. Furthermore, the increasing interest in biopolymers production is because of availability of excess of glycerol generated in biodiesel production which could be employed as a substrate for fungal growth for biopolymer production (Araújo et al. 2016).

The following section will give the overview of fungal biopolymers.

1.1 The Fungal Cell Wall Polymers

The fungal cell wall is a protective cover with immense plasticity and is indeed essential for the cellular integrity and viability. It facilitates interactions with the exterior environment through adhesins as well as signal transduction complexes. The cell wall of fungi is specifically designed in different layers with main innermost structural component, chitin, β-1,4- linked *N*-acetylglucosamine polymer and its deacetylated form chitosan (mainly glucosamine), while matrix components like glucans and mannans and glycosylated proteins (Table 1). Glucan and chitin are synthesized at the plasma membrane (PM). Usually, the nascent enzyme complexes are transported to the PM through secretory vesicles and eventually activated when insertion into the PM. While endoplasmic reticulum and Golgi are sites for the synthesis of mannans and other glycoconjugates. Then they possibly conjugate to proteins in cell wall, and subsequently brought to the cell wall by secretory vesicles. Nucleoside diphosphate-sugars are substrates for all synthases. Indeed, enzymes

Table 1 Common fungal cell wall components

Division	Structural/fibrous polymers	Matrix/gel-like polymers
Chytridiomycota (*Allomyces*)	Chitin (> 50%), Glucan (>16%)	Glucan
Zygomycota (*Absidia, Benjaminiella, Mucor, Rhizopus*)	Chitin (10%), Chitosan (30%)	Polyglucuronic acid, α-1,2/ α-1,6-mannosyl and β-1,5/β-1,6-Glucuro nomannoproteins, Polyphosphate
Ascomycota (*Aspergillus,* yeasts)	Chitin (1–40%), β-1,3 and β-1,6, Glucan (40–60%)	α-1,2/ α-1,6- Mannosyl and β-1,5/β-1,6-galacto-furanosyl residues in Galactomannoproteins, α-1,3-Glucan
Basidiomycota, (mushroom)	Chitin (5–30%) β -(1–3), β-(1–6) Glucan (40–60%)	Xylomannoproteins, α-1,3-Glucan

Modified from Gooday (1990), Gow et al. (2017), Khale and Deshpande (1992)

involved in the synthesis of nucleoside sugars are found to be necessary for the construction of the cell wall as well as for rate-limiting too (Gow et al. 2017).

1.1.1 Chitin and Chitosan

Naturally abundant chitin is a polysaccharide, it was first isolated from the cell wall of mushrooms by Braconnot, where it was designated as fungine. During 1823, fungine was re-designated as chitin by Odier. It is a polymer of β 1,4-linked *N*-acetyl glucosamine (GlcNAc) abundantly present in crustaceans, insects, fungi, including a few algae as a main structural component (Deshpande 1986; Ghormade et al. 2017).

Chitin exists in three forms in nature: alpha (α), beta (β) and gamma (γ). The first one has antiparallel chains of β-1,4-linked GlcNAc. Most of the natural chitin is in α-form. The β-chitin has parallel alignment of chains while γ-chitin, which is exists in insects, which has two chains those are parallel in one direction and the third chain is antiparallel. The β-chitin is not common in living entities and reported in spines of diatoms and squid pens to name a few. In nature, the exocellular β-chitin spines of *Thalassiosira fluviatilis*, a centric diatom, are the only in pure form of chitin *i.e.* 100% degree of acetylation. Otherwise in rest of the organisms, including fungi, chitin is a polymer of both GlcN and GlcNAc with variable percentages. In general, chitosan is >70% in deacetylated form. Chemically chitin is a linear polymer of β-1,4-linked *N*-acetylglucosamine (GlcNAc) units, whereas its deacetylated form, chitosan contains glucosamine (GlcN) residues (Ghormade et al. 2017).

The content of chitin in the fungal wall differs (2–61%) depending on the vegetative morphological phase (unicellular yeast or filamentous) of the fungus (Khale and Deshpande 1992). In chitin synthesis, the enzymes from carbon as well as nitrogen metabolism are brought together by an enzyme glucosamine 6 phosphate synthase (EC 2.6.1.16) to synthesize uridine diphosphate-*N*-acetyl glucosamine (UDP-GlcNAc) from glucose and glutamine. The chitin synthase (CS, EC 2.4.1.16) is an important enzyme for further synthesis of chitin from UDP-GlcNAc.

Briefly, glucosamine 6 phosphate is synthesized with the help of glucosamine 6 phosphate synthase (EC 2.6.1.16) which is further acetylated to form *N*-acetylglucosamine 6 phosphate by an enzyme glucosamine 6 phosphate *N*-acetyl transferase (EC 2.3.1.4). UDP-N-GlcNAc was further synthesized in two steps with the involvement of 2 enzymes, namely phosphoacetyl glucosmine mutase (EC 5.4.2.3) and UDP *N*-acetylglucosamine phosphorylase (EC 2.7.7.23). The final step of chitin synthesis involves sequential transfer of GlcNAc from UDP-GlcNAc to non-reducing end of growing polymer, which is catalysed by CS. The zymogenic CS, transported to the plasma membrane through a vesicle (chitosome), is activated by proteolysis (Bartnicki-Garcia 2006; Deshpande et al. 1997; Phadatare et al. 1989). Multiplicity of CSs is a common occurrence. Chitnis et al. (2002) reported eight discrete CS genes in *B. poitrasii* as confirmed based on DNA sequence as well as Southern analysis. Earlier six CSs were reported in *Ustilago maydis*, while three are present in *S. cerevisiae* and a dimorphic *Sporothirx*

schenckii. Ten CSs were reported in a zygomycetous fungus *Phycomyces blakesleeanus* (from Chitnis et al. 2002). The role of chitin metabolism in the morphogenesis was explicitly studied using *B. poitrasii* protoplast regeneration in presence of different cell wall polymer synthesis inhibitors (Chaudhary et al. 2013; Chitnis and Deshpande 2002).

The chitosan is not directly synthesized in nature. Chitin deacetylase (CDA; E.C. 3.5.1.41) drives the hydrolysis of acetamido groups of GlcNAc in chitin, thus promoting the conversion to chitosan (Ghormade et al. 2010).

For the hyphal tip elongation the old cell wall chitin is hydrolysed to supply GlcNAc to the growing tip. There are two different pathways to hydrolyze chitin (Chavan and Deshpande 2013). In the first one, a chitinase complex consisting of: endo chitinases (EC 3.2.1.14), *N*-acetyl hexosaminidase (EC 3.2.1.52) and exo-chitinase (EC 3.2.1.201). The second pathway is comprised of two enzymes namely, chitin deacetylase (CDA, EC 3.5.1.41) hydrolyses acetamide group in GlcN by removing acetic acid. Chitosanase (EC 3.2.1.132) perform endo hydrolysis of β-1,4 linkage between GlcN residue in partially acetylated chitosan while exo chitosanase (GlcNase, EC 3.2.1.165) successively removes GlcN residues from non-reducing end of chitosan oligosaccharides (Chavan and Deshpande 2013).

1.1.2 Glucans

Glucan is one of the most abundant structural polysaccharides which represents 40–60% of the cell walls in ascomycetous fungi. Most of the glucans are β- 1,3 and β-1,6 polymers of glucose. In some fungi glucans have α-1,3 and -1,4 linkages too. They are either linear or branched, and also either microfibrillar or amorphous (Ruiz-Herrera and Ortiz-Castellanos 2019). The α-1,3-glucans are copious in the fungal cell walls. While in glycogen, α-1,4 and α-1,6 bonds are present between glycosyl units. The structure of β-glucans is highly complex, linear as well as branched mainly β-1,3 and β-1,6. The glucans and chitin are major structural components of the cell wall of fungi. The most studied β 1,3- glucans come from *Saccharomyces cerevisiae*. Another polysaccharide in *S. cerevisiae* is made up of glucosyl units having β- 1,6 linkages, is called pustulan. While in lichens, lichenan, a polysaccharide having β -1,3 as well as β- 1,4 linkages is mainly present.

Most of the secreted polysaccharides have β-1,6 branches of different lengths and characteristics: botryosphaeran extracted from *Botryosphaeria* sp., grifolan from *Grifola frondosa*, schizophylan from *Schizophylum commune* and scleroglucan from *Sclerotium* sp. The basidiocarp of *Calocybe indica* contains calocyban, chain of glucosyl units linked by β 1,3-bonds with β 1,4- linked branches (For more details refer Ruiz-Herrera and Ortiz-Castellanos 2019). The plasma membrane bound β-glucan synthase (EC 2.4.1.34) is involved in the synthesis of β-1,3-glucan (Garcia-Rubio et al. 2020).

The synthesis of α-1,3 glucan was very well studied in *Schizosaccharomyces pombe*.

The multiplicity of α -1,3- glucan synthases (EC 2.4.1.183) was reported in *S. pombe* (three members), four in *Rhizoctonia solani* and up to seven in *Aspergillus niger* (Ruiz-Herrera and Ortiz-Castellanos 2019).

Many fungi produce extracellularly β-(1,3)- and β-(1,6)-glucans -hydrolysing enzymes, β-(1,3)- and β-(1,6)-glucanases (Kéry et al. 1991). These enzymes are O-glycoside hydrolases (EC 3.2.1-58 and EC 3.2.1.75, respectively), catalyzing the hydrolysis of glycosidic bonds (Martin et al. 2007). *Trichoderma harzianum* produces at least ten different forms of endo-acting β-1,3- glucanases. While *Acremonium persicinum* produced three β-(1,3)-glucanases (two exo- and 1 endo-acting) which were attributed to the expression of three vried genes. Though multiple β-1,6-glucanases were rarely reported, one of the exception is *T. harzianum*, which produces three different β-(1,6)-glucanases coded by three different genes (Martin et al. 2007). It was reported further by Martin et al. (2007) that post-synthesis glycosylation may generate multiple isoforms. For instance, extracellular β-glucanases (Exo1a and Exo1b) in *S. cerevisiae* were reported. All these enzymes have applied value in the food industries, wine industries (to lower viscosity) and also in treating feed grains and serve as a tool for the structural analysis of β-glucans.

1.1.3 Mannans

Mannans represent about 35–40% of fungal cell wall as matrix components. As compared to chitin and β-glucans, mannans are less rigid. Mannans have low permeability as well as porosity, which contribute to the resistance of the fungus against antifungal drugs and other host defence mechanisms. In fungal cell wall short *O*-linked and *N*-linked mannans along with glycoproteins as well as long mannan chains are present. The *O*-linked mannans contain mannose residues linked to the hydroxyl group of either threonine or serine residues, while in *N*-linked mannans, highly branched, are connected to amide group of aspargine residues. The phospholipomannan (PLM) is mainly comprised of long linear chain of β-1,2 mannose unit and inositol covalently linked through a phosphate diester bond to a lipid moiety. In filamentous fungi, galactomannan consists of α-1,2/ α-1,6-mannosyl and β-1,5/β-1,6- galactofuranosyl residues, is observed at the cell wall surface.

Synthesis of *O*- and *N*- linked mannans is similar in both unicellular yeasts and filamentous fungi. However, a difference among unicellular yeasts and filamentous fungi is the structural organization of the long mannan chains in the cell walls. For instance, in yeasts highly branched *N*-linked mannans (100 mannosyl residues) are bound per molecule of protein. Interestingly, mannans without covalent bonding with polysaccharide core of the cell wall cover the surface of the cell wall. On the other hand in*Aspergillus fumigatus*, the long mannans (average, 50 mannose residues/chain) bind to glucan and chitin and thus become an integral part of the cell wall (Henry et al. 2016).

Mannosylation of proteins occurs in endoplasmic reticulum and then in Golgi. Some protein mannosyltransferases are important in the first step of *O*-linked

mannan biosynthesis, addition of mannose residue to a threonine or serine residue. Additional mannose residues are added by the α-1,2 mannosyltransferases (EC 2.4.1.131) which result in a short α-1,2 mannose chains and addition of an α-1,3 mannose by α-1,3 mannosyltransferases (EC 2.4.1.258). While an attachment of α-1,6 mannose residue to core structure is done by the action of α-1,6 mannosyltransferase (EC 2.4.1.232). The enzyme mannosylphosphate transferase (EC 2.4.1.B72) is involved in attachment of phosphomannan group to the branches. For the synthesis of phospholipomannan, mannose inositolphosphoceramide mannosyltransferase (EC 2.4.1.370) is needed (Garcia-Rubio et al. 2020).

Hydrolysis of mannan is catalysed mainly by endo-acting β-mannanases (EC 3.2.1.78) and axo-acting β-mannosidase (EC 3.2.1.25) (Moreira and Filho 2008).

1.1.4 Proteins

Fungi are considered as a promising source for novel proteins with exceptional features. These include: lectins, enzymes and enzyme inhibitors and hydrophobins. These have applications in several medical and biotechnological processes (Erjavec et al. 2012). Number of proteinases have role in fungal morphogenesis (Deshpande 1992; Phadatare et al. 1989).

Heavily glycosylated proteins are usually anchored on cell membrane and cell wall. These proteins play a significant role in morphogenesis, adhesion, and pathogenicity. Mannoproteins are made up of several classes. For instance, glycosyl phosphatidyl inositol (GPI)-modified proteins (almost 88%) are responsible for cell wall synthesis, organisation, remodelling, and virulence too (Ibe and Munro 2021; Pitarch et al. 2008).

1.1.5 Hydrophobins

Hydrophobins are amphipathic proteins play significant role in fungal growth as well as differentiation. The classification of hydrophobins in two classes (I and II) is based on distribution of the cysteines and the clustering of both hydrophobic and hydrophilic residues which affect the solubility and morphology. The aggregates of class I hydrophobins are highly insoluble even in sodium dodecyl sulfate (2% SDS at 100 °C) and can only be dissociated with high concentration of formic acid or trifluoracetic acid. The Class II hydrophobins will be readily dissolved with ethanol (60%) or SDS (2%). Under the microscope, class I hydrophobins show rodlet structures outside the fungal cell wall. Hydrophobins are well characterized with eight conserved cysteine residues (Wösten 2001). The characteristic spacing between cysteine residues distinguishes hydrophobins of Class I and Class II (Wösten and Wessels 1997). According to Kershaw et al. (1998) in the class II hydrophobins the eight residues of cysteine form four disulphide linkages resulting in the formation of four loop-like structures. The characteristic order of cysteine

residues is useful to distinguish the fungal hydrophobins from other cysteine rich proteins.

1.2 Fungal Exopolysaccharides

Number of exopolysaccharides with different structural complexities are synthesized by microorganisms. Some of them remain attached to the cell wall or found in the extracellular medium. The exopolysaccharides are high molecular weight, long-chain polysaccharides consist of branched and repeating units of sugars linked either by β- 1, 4 or β-1, 3linkage or α-1,2 or α- 1,6 linkage. They have distinct physiological roles which include: communication, defence against predation, detoxification of chemicals, to name a few (Osemwegie et al. 2020, Tang and Zhong 2002).

A bacterium, *Leuconostoc mesenteroides* was the first organism identified as exopolysaccharide (EPS) producer (Mahapatra and Banerjee 2013). While different fungi such as *Agaricus blazi*, *Cordyceps* sp., *Ganoderma lucidum*, Grifola *frondosa* and *Lentinus edodes* were reported initially to produce different exopolysaccharides in submerged fermentation. Presently number of fungi are being reported to produce EPS in the fermentation under defined nutritional and fermentation parameters. Mahapatra and Banerjee (2013) have given exhaustive list of EPS producing fungi from all the classes. Only few fungi have been studied to understand the biosynthesis pathway for EPS. Donot et al. (2012) reviewed microbial EPS for their synthesis, secretion, genetics and extraction. The main fungal EPS are: pullulan (*Aureobasidium pullulans*), scleroglucan (*Sclerotium* sp.), schizophyllan (*Schizophyllum commune*), galactan (*Sporobolomyces* sp) and glucan (*Cryptococcus* sp., *Ganoderma lucidum*, *Rhodotorula* sp., *Tremella aurantia, T. fusiformes* and *T. mesenterica*). The synthesis of EPS is not very well understood. Osemwegie et al. (2020) have reviewed current status of EPS from fungi and bacteria with respect to biosynthesis, production and structural studies. According to the literature, the biosynthesis of homopolysaccharides is theoretically differs from that of heteropolysaccharides synthesis. In case of bacteria, heteropolysaccharides are synthesized intracellularly followed by transport into the extracellular environment. On the other hand homopolysaccharides are produced extracellularly. The enzymes such as hexokinases (involved in phosphorylation), enzymes involved in formation of sugar nucleotides (UDP-glucose pyrophosphorylase); glycosyl transferases and finally enzymes required for acylation, acetylation, sulphation and methylation are involved in EPS synthesis, in general. Then EPSs are exported to the exterior surface with the help of specific enzymes or transporters (flippase, permease and ABC transporters) (Osemwegie et al. 2020). The identifiable characteristics of any particular exopolysaccharide are consistent with monomeric units, the kind of linkage between the monomers, and the sequence of linkage.

1.2.1 Pullulan

Bauer (1938) observed the viscous culture broth when a fungal isolate, the then *Pullularia* was grown with excess glucose. In 1958, Bernier suggested the viscosity is due to the exopolysaccharide produced in the medium (from Kelkar 1991). Eventually it was named as pullulan. It is a polymer composed of malto-triose units with linkages as Glu-α-(1–4)- Glu-α-(1–4)-Glu-α-(1–6) (Fig.1a). Many other types of linkages also found in pullulan as a succession of maltotetraose units (-(1–4)-Glu-_-(1–4)-Glu-_-(1–6)-Glu-_-(1–6)) (Kelkar 1991). *Aureobasidium pullulans* (Black yeast) produces high concentrations of pullulan (Jiang 2010; Wu et al. 2009). In fact, *A. pullulans* produces three extracellular glucan components. Major is pullulan (α-1–4 and α-1–6) while acidic β-linked glucan containing β-1,3 and β-1,6 linkages and an acidic heteropolysaccharide containing galactose, glucose, mannose and hexuronic acid are quantitatively less. Pullulan synthesis is not possible by all the strains of *A. pullulans* (Mishra et al. 2018).

The mechanism of pullulan synthesis is not completely understood LeDuy et al. (2014). Simon et al. (1998) suggested that the pullulan and others too are synthesized within the cell in *A. pullulans*, and then are secreted out. Three major enzymes are reported to play crucial role in the biosynthesis of pullulan: α-phosphoglucose mutase, (UDPG, uridine 5′-diphosphate glucose EC 5.4.2.2), which facilitates the interconversion of glucose 1 phosphate and glucose 6 phosphate, while pyrophosphorylase (EC 2.7.7.9) involved in the synthesis of UDP-glucose from glucose 1 phosphate and glucosyltransferase (EC 2.4.1.B64), which catalyzes glycosidic bond formation using sugar donors.

The lipid phosphate is involved in pullulan synthesis from UDPGlc. The lipid phosphate receives two successive glucosyl moieties from UDPGlc to form an

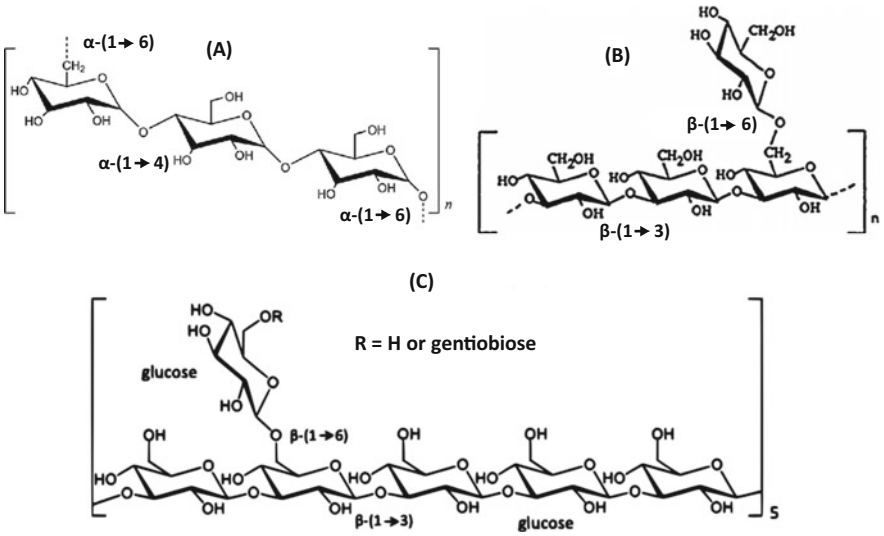

Fig. 1 Structures of Pullulan (**a**), Scleroglucan (**b**) and Botryosphaeran (**c**)

isomaltose molecule (Lipid-P-Glc-α-1-6-Glc), finally receiving a glucosyl unit from UDPG to form an isopanosyl intermediate (Lipid-P-Glc-α-1-6-Glc-α-1-4 Glc). These isopanosyl moieties are further proceed for polymerization to form the pullulan.

The enzymes, namely pullulanase, isopullulanase, neopullulanase, and glucoamylase hydrolyze pullulan. Pullulanase (Pullulan 6-glucanhydrolase, EC 3.2.1.41) can hydrolyse pullulan into maltotriose. It has linkage specificity to α-(1–6) linkage only. Isopullulanase (Pullulan 4-glucanohydrolase, EC 3.2.1.57) hydrolyses pullulan into isopanose (maltosyl- α-1,4-glucose) as the sole product. From pullulan the enzyme produces a mixture of isopanose, isomaltose, and glucose as end products. Neopullulanse (Pullulan 4-glucanohydrolase, EC 3.2.1.135) attacks the α-(1–4) glycosidic linkages on the non-reducing end of the α-(1–6) linkage in pullulan at random and produces panose (D-glucosyl-α-1,6-maltose). Glucoamylase (1,4-α-glucan glucohydrolase, EC 3.2.1.3) hydrolyses pullulan from the non-reducing point producing glucose as a sole product of hydrolysis (Kelkar and Deshpande 1993).

1.2.2 Scleroglucan

A fungus from mycelia sterilia group (fungi which do not produce any sexual or asexual spores), *Sclerotium* sp. produces a β- 1,3 and -1,6 glucan known as Scleroglucan, having diverse branching frequencies, side chain length as well as molecular weight relaying on the strain and /or growth conditions (Fig. 1b). Due to its water-solubility, viscosity and stability against a wide range of temperature, pH and salinity, it has lot of applications as food additives, for enhanced oil recovery, and in cosmotic and pharmaceutical products (Castillo et al. 2015).

The biosyntheses of scleroglucan and oxalic acid are closely linked (Schmid et al. 2011). The uptake of glucose into the cell will be by facilitated diffusion as well as phosphorylation by hexokinase (EC 2.7.1.1) to glucose-6-phosphate (G6P), which is then converted in to G1P by phosphoglucomutase (EC 5.4.2.2) which eventually starts scleroglucan synthesis (Schmid et al. 2011). UTP-glucose-1-phosphate uridylyltransferase synthesizes UDP-glucose from glucose-1- phosphate and UTP. A (1 → 3)-β-glucan synthase (EC 2.4.1.34) polymerizes the backbone chain. The last few steps to the definite branching at every third glucose unit are still unclear.

1.2.3 Botryosphaeran

Botryosphaeria rhodina causes a stem canker in *Eucalyptus* tree. It produces β-glucan type EPS in submerged fermentation on glucose. Structurally it is β-glucan with (1 → 3) (1 → 6)-linkages (Dekker and Barbosa 2019). According to Barbosa et al. (2003) botryosphaeran is a (1 → 3)-β-D-glucan with almost 22% side branching at C-6 (Fig.1C). Furthermore, partial acid hydrolysis studies indicated that on the side branches a single (1 → 6)-β-linked glucosyl, and

(1 → 6)-β-linked gentiobiosyl residues are present. When grown on varied carbohydrates, *B. rhodina* produced botryosphaerans with a same1,3-linked backbone chain but with different levels of branching on C-6 with glucose as well as gentiobiose. Dekker and Barbosa (2019) reported that when *B. rhodina* was grown on glucose or sucrose a branch point occurred on every fifth glucose residue along the backbone chain for the botryosphaeran, while grown on fructose one in every third glucose residues branch point occurred. The membrane-modifying agents such as Tween 80 and soybean oil enhanced production of botryosphaeran without changing its chemical structure. The botrypsphaeran has the status as GRAS (Generally Regarded As Safe). The biopolymer exhibits strong antimutagenic/anticlastogenic activity as chemoprotective agent against many cancer chemotherapy drugs. Similar to other β-glucans, botryosphaeran is also synthesized through complex enzymatic pathway from UDP-glucose (Utama et al. 2020).

Botryosphaeran, is hydrolysed by β-1,3-glucanases mainly produced by *B. rhodina* and *Trichoderma harzianum* (Giese et al. 2006). The hydrolysis products mainly were glucose as well as gentiobiose with some trisaccharide, however, no laminaribiose or tetrasaccharide when hydrolysed by *T. harzianum* glucanase. On the other hand, *B. rhodina* β-1,3-glucanases generated predominantly glucose during botryosphaeran hydrolysis.

1.3 Future Perspectives

The fungal cell wall polymers especially chitosan, glucan and mannan have a lot of applications especially in healthcare industry. A number of fungi produce exopolyssacharides which have been extensively studied using advanced analytical techniques. However, the predictable uniformity in the structural features and functional characteristics is lacking. The structural features are dependent on the composition and combination of monosaccharides used to grow the fungal organism. It is also relevant that in addition to cultivation conditions, the downstream processing to ensure a high recovery yield, with respect to molecular weight, degree of polymerization, purity is also important. The continuous efforts are necessary to understand structure-function relationship and eventually application potential and cost-effective production of fungal biopolymers.

References

Araújo TR, De Oliveira PCL, Cardoso VL, Filho UC, Vieira PA (2016) Biopolymer production using fungus *Mucor racemosus* Fresenius and glycerol as substrate. Polímeros 26:144–151

Barbosa AM, Steluti RM, Dekker RFH, Cardoso MS, Corradi da Silva ML (2003) Structural characterization of botryosphaeran: a (1→3;1→6)-β-d-glucan produced by the ascomyceteous fungus, *Botryosphaeria* sp. Carbohydr Res 338:1691–1698

Bartnicki-Garcia S (2006) Chitosomes: past, present and future. FEMS Yeast Res 6:957–965

Bauer R (1938) Physiology of Dematium Pullulans de Bary. Zentralbl Bacteriol Parasitenkd Infektionskr Hyg Abt2 98:133–167

Castillo NA, Valdez AL, Farina JI (2015) Microbial production of scleroglucan and downstream processing. Front Microbiol 6:1106. https://doi.org/10.3389/fmicb.2015.01106

Chaudhary PM, Tupe SG, Deshpande MV (2013) Chitin synthase inhibitors as antifungal agents. Mini-Rev Med Chem 13:222–236

Chavan SB, Deshpande MV (2013) Chitinolytic enzymes: an appraisal as a product of commercial potential. Biotechnol Progress 29:833–846. https://doi.org/10.1002/btpr.1732

Chitnis M, Deshpande MV (2002) Isolation and regeneration of protoplasts from the yeast and mycelial form of a dimorphic fungus *Benjaminiella poitrasii*: role of chitin metabolism for morphogenesis during regeneration. Microbiol Res 157:29–37

Chitnis M, Munro CA, Brown AJP, Gooday GW, Gow NAR, Deshpande MV (2002) The zygomycetous fungus, *Benjaminiella poitrasii* contains a large family of differentially regulated chitin synthase genes. Fungal Genetics Biol 36:215–223

Dekker RFH, Barbosa A (2019) Botryosphaeran – A fungal exopolysaccharide of the (1→3)(1→6)-β-D-glucan kind: structure and biological functions. In: Extracellular Sugar-Based Biopolymers Matrices, Biologically Inspired Systems, vol 12. Springer Nature, Cham, pp 433–484. https://doi.org/10.1007/978-3-030-12919-4_11

Deshpande MV (1986) Enzymatic degradation of chitin and its biological applications. J Sci Ind Res 45:273–281

Deshpande MV (1992) Proteinases in fungal morphogenesis. World J Microbiol Biotechnol 8:242–250

Deshpande MV, O'Donnell R, Gooday GW (1997) Regulation of chitin synthase activity in the dimorphic fungus *Benjaminiella poitrasii* by external osmotic pressure. FEMS Microbiol Lett 152:327–332

Donot F, Fontana A, Baccou JC, Schorr-Galindo S (2012) Microbial exopolysaccharides: Main examples of synthesis, excretion, genetics, and extraction. Carbohydr Polym 87:951–962

Erjavec J, Kos J, Ravnikar M, Dreo T, Sabotič J (2012) Proteins of higher fungi – from forest to application. Trends Biotechnol 30:259–273

Garcia-Rubio R, de Oliveira HC, Rivera J, Trevijano-Contador N (2020) The fungal cell wall: *Candida, Cryptococcus,* and *aspergillus* species. Front Microbiol 10:2993. https://doi.org/10.3389/fmicb.2019.02993

Ghormade V, Kulkarni S, Doiphode N, Rajamohanan PR, Deshpande MV (2010) Chitin deacetylase: A comprehensive account on its role in nature and its biotechnological applications. In: Mendez-Vilas A (ed) Technology and education topics in applied microbiology and microbial biotechnology. Current Research, Formatex Research Center, Badajoz, pp 1054–1066

Ghormade V, Pathan EK, Deshpande MV (2017) Can fungi compete with marine sources for chitosan production? Int J Biol Macromolec 104(B):1415–1421. https://doi.org/10.1016/j.ijbiomac.2017.01.112

Giese EC, Covizzi LG, Dekker RFH, Monteiro NK, Da Silva CM d L, Barbosa AM (2006) Enzymatic hydrolysis of botryosphaeran and laminarin by β-1,3-glucanases produced by *Botryosphaeria rhodina* and *Trichoderma harzianum* Rifai. Process Biochem 41:1265–1271. https://doi.org/10.1016/j.procbio.2005.12.023

Gooday GW (1990) Inhibition of chitin metabolism. In: Kuhn PJ, Trinci APJ, Jung MJ, Goosey MW, Copping LG (eds) Biochemistry of cell walls and membranes in fungi. Springer, Berlin, pp 60–79

Gow NAR, Latge J-P, Munro CA (2017) The fungal cell wall: structure, biosynthesis, and function. Microbiol Spectrum 5(3) FUNK-0035-2016. https://doi.org/10.1128/microbiolspec

Henry C, Fontaine T, Heddergott C, Robinet P, Aimanianda V, Beau R, Beauvais A, Mouyna I, Prevost M-C, Fekkar A, Zhao Y, Perlin D, Latge J-P (2016) Biosynthesis of cell wall mannan in the conidium and the mycelium of *aspergillus fumigatus*. Cell Microbiol 18:1881–1891

Ibe C, Munro CA (2021) Fungal cell wall: an underexploited target for antifungal therapies. PLoS Pathog 17:e1009470. https://doi.org/10.1371/journal.ppat.1009470

Jiang L (2010) Optimization of fermentation conditions for pullulan production by *Aureobasidium* pullulan using response surface methodology. Carbohydr Polym 79:414–417
Kelkar HS (1991) Studies on pullulan hydrolysing activity from *Sclerotium rolfsii*. A PhD thesis submitted to Pune University, Pune
Kelkar HS, Deshpande MV (1993) Purification and characterization of a pullulan-hydrolysing glucoamylase from *Sclerotium rolfsii*. Stärke/Starch 45:361–368
Kershaw MJ, Wakley G, Talbot NJ (1998) Complementation of the *Mpg1* mutant phenotype in *Magnaporthe grisea* reveals functional relationships between fungal hydrophobins. EMBO J 17:3838–3849
Kéry V, Kogan G, Zajacová K, Slámová K, Masler L, Alföldi J (1991) Hydrolysis of yeast cell-wall glucan by extracellular (1→3)-β-glucanases from *aspergillus Niger*. Enz Microb Technol 13: 87–90
Khale A, Deshpande MV (1992) Dimorphism in Benjaminiella *poitrasii*: cell wall chemistry of parent and two stable yeast mutants. Anton van Leeuwenhoek 62:299–307
LeDuy A, Choplin L, Zajic J, Luong J (2014) Pullulan: properties, synthesis, and applications. In: Encyclopedia of polymer science and technology. https://doi.org/10.1002/0471440264.pst620
Mahapatra S, Banerjee D (2013) Fungal exopolysaccharide: production, composition and applications. Microbiol Insights 6:1–16. https://doi.org/10.4137/MBI.S10957
Martin K, McDougall BM, Mcllroy S, Chen JJ, Seviour RJ (2007) Biochemistry and molecular biology of exocellular fungal β-(1,3)- and β-(1,6)-glucanases. FEMS Microbiol Rev 31:168–192
Mishra B, Zamare D, Manikanta A (2018) Selection and utilization of agro-industrial waste for biosynthesis and hyper-production of pullulan: A review. In: Varjani S, Parameswaran B, Kumar S, Khare S (eds) Biosynthetic technology and environmental challenges. Springer Nature, Singapore, pp 89–103. https://doi.org/10.1007/978-981-10-7434-9_6
Moreira LR, Filho EX (2008) An overview of mannan structure and mannan-degrading enzyme systems. Appl Microbiol Biotechnol 79:165–178
Osemwegie OO, Adetunji CO, Ayeni EA, Adeiobi OI, Arise RO, Nwonuma CO, Oghenekaro AO (2020) Exopolysaccharides from bacteria and fungi: current status and perspectives in Africa. Heliyon 6:e04205. https://doi.org/10.1016/j.heliyon.2020.e04205
Phadatare S, Srinivasan MC, Deshpande MV (1989) Evidence for the involvement of serine protease in the conidial discharge of *Conidiobolus coronatus*. Arch Microbiol 153:47–49
Pitarch A, Nombela C, Gil C (2008) Collection of proteins secreted from yeast protoplasts in active cell wall regeneration. Meth Mol Biol 425:241–263
Ruiz-Herrera J, Ortiz-Castellanos L (2019) Cell wall glucans of fungi. A review. Cell Surface 5: 100022. https://doi.org/10.1016/j.tcsw.2019.100022
Schmid J, Meyer V, Volker S (2011) Scleroglucan: biosynthesis, production and application of a versatile hydrocolloid. Appl Microbiol Biotechnol 91:937–947. https://doi.org/10.1007/s00253-011-3438-5
Simon L, Bouchet B, Bremond K, Gallant DJ, Bouchonneau M (1998) Studies on pullulan extracellular production and glycogen intracellular content in *Aureobasidium pullulan*. Can J Microbiol 44:1193–1199
Tang YJ, Zhong JJ (2002) Exopolysaccharide biosynthesis and related enzyme activities of the medicinal fungus, *Ganoderma lucidum*, grown on lactose in a bioreactor. Biotechnol Lett 24: 1023–1026
Utama GL, Dio C, Lembong E, Cahyana Y, Balia RL (2020) Microorganism-based β-glucan production and their potential as antioxidant. Sys Rev Pharm 11:868–873
Wösten H, Wessels J (1997) Hydrophobins, from molecular structure to multiple functions in fungal development. Mycoscience 38:363–374
Wösten HAB (2001) Hydrophobins: multipurpose proteins. Ann Rev Microbiol 55:625–646
Wu S, Jin Z, Tong Q, Chen H (2009) Sweet potato: a novel substrate for pullulan production by *Aureobasidium pullulans*. Carbohydr Polym 76:645–649