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Regulation of synthesis for non-cellulolytic fungal β-(1,3)- and β-(1,6)- glucanases

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[1]Jayus

[1] University of Jember, Indonesia

ABSTRACT

The non-cellulolytic β-(1,3)- and β-(1,6)- glucanases capable of degrading the naturally occurring β-(1,3)- and β-(1,6)- glucans have been reported to be produced by many filamentous fungi, although unlike the β-1,3-glucanases, the production of multiple β-1,6-glucanases appears to be quite rare in fungi. To support the effort to realize the industrial potential of these enzymes, cloning of the gene/s encoding them and their expression in host cells in sufficiently large quantities is required. However, clarification is needed on how the synthesis and the formation of their multiple forms are regulated differentially. This review considers this group of enzymes and in particular their multiple isoform and regulation of synthesis. This information and discussion will assist us to get insight their functions in the fungi that synthesize the enzyme.

Key words; regulation of synthesis; fungal β-(1,3)- and β-(1,6)-glucanases; fungal glucans

1. The non-cellulolytic β-glucans – substrates for the β-glucanases

β-Glucans other than cellulose β-(1,4)-glucan (not discussed here) are being the most abundant widely found in nature, which include in plants, some invertebrates and microorganism, mostly fungi [1]. Their polymers are consisted of glucose residues joined by glycosidic linkage in a β- configuration [1,2]. The non-cellulolytic β-glucans may be classified according to the predominant linkage types and their chain of configuration [3,4]. Some are constructed in a simple structure, consisting of only one linkage type in an unbranched chain of the β-glucosidic linkage as were found in curdlan and paramylon [β-(1,3)-glucans] and pustulan [a β-(1,6)-glucan]. Meanwhile, the more complex β-glucans structure consist of a diversity of linkage types in either linear or branched chains as were found in laminarin scleroglucan, schizophylan, and epiglucan (all β-(1,3-1,6)-glucans), and barley glucan (a β-(1,3-1,4)-glucan) [1,2,3,4].

Even though many fungi have been reported to synthesize β-glucans, the mechanisms for their biosynthesis, and the relationship between the chemical structure and the properties of β-glucans are not clearly understood [1,4]. These β-glucans may be synthesized either extracellularly or cytoplasmically [1,4]. Their occurrence and major function usually as the major components for the structure of fungal cell walls providing rigidity and strength, often work together in combination with other polymers [5,6,7].

Cytoplasmic and exocellular β-glucans are believed to have a survival function act as carbon storage materials. When the conditions of carbon limitation occur, the β-glucans might be reutilized by the producing fungus [2,8]. More over, exocellular β-glucans from pathogenic fungi *Phanerochaete chrysosporium* appear to have ability to induce wilting symptoms in plants [9]. Glucan sheaths from *Botrytis cinerea* was also described to be involve in mycoparasitism.

This glucan is able to protec the fungus from antagonistic host responses, and also aiding the initial points of infection through the entrapment of the hydrolytic enzymes within it [10].

2. Functions of fungal β-glucanases

The functions of exocellular fungal β-glucanases probably diverse. Even though some evidence are available, for some microorganism found, they still consider as still largely unknown [2]. The structure function relationships between the secreted exocellular β-glucanases and those related with the cell wall secretion from the organism is also not clear. To corroborate this, recognition of their peptide signal sequence for the protein secretion from its sequence data is needed [12].

Other possible function of β-glucanases is locating the secretion of enzyme from fungi, act in some fungal structures like compound fungal sporophores, as observed in *Agaricus bisporus* where the exo-β-(1,3)-glucanase gene sequenced from its basidiocarps possessed a signal sequence responsible for the enzyme secretion[13].

The fungal β-glucanase may function in cell wall glucan metabolism and morphogenesis of omicroorganism. It has been reported that a similar exocellular enzyme exo-β-(1,3)-glucanase (Exg) from *C. albicans* and EXG1p from *Saccharomyces cerevisiae* may have functions in cell morphogenesis and cell wall glucan metabolism, since both were exhibiting transferase and hydrolase activity [11].

Fungal β-(1,3)-glucanases might play as an important roles in mobilization of β-glucans when the producing fungus is in the culture conditions where the energy or carbon source become exhausted [2]. Some other glucanases might also involve in morphogenetic-morpholytic processes during fungal cell wall development and differentiation [7]. Moreover, they also have been reported to have an important nutritional role in saprophytic and mycoparasitic fungi, where they act primarily as biochemical offensive weapons to degrade the parasitized fungal cell wall [1,2,11,12].

Other β-(1,6)-glucanase from plant pathogenic *Neotyphodium* sp. might also serve the ability to degrade host plant wall callose material during parasitic attack, although the role of this exocellular β-(1,6)-glucanase, which can be expressed in the host of the *Neotyphodium* sp. [13] is remains unclear since the plants lack of its β-(1,6) glucan substrate. It may function to remove other possible competing plant pathogenic fungi.

Some β-glucanase might assist the cell wall extension and growth representing a trivial equilibrium between the degradation of the existing or remaining cell wall as well as new cell wall synthesis of the producing fungus [7]. It seems to be true that β-glucanases could play a crucial role in this process, since β-glucans are the major components of fungal cell walls. These enzymes will partially hydrolyze confined zones, allowing insertion of new cell wall material without damaging its overall cell integrity [7], as it was the case in *Sclerotium rolfsii* [14]. There will be so many factors triggering cell autolysis in fungi [15]. As has been reported in *Botrytis* spp. [16], *Penicillium oxalicum* [17] and *Aspergillus nidulans* [18], β-glucanases will play a crucial role in the cell autolysis process in fungi, working together with other hydrolases. Those enzymes activity often increase when the producing fungus was in conditions of carbon limitation and this condition usually occur immediately prior to the cell wall autolysis.

Other probable role for the exocellular β-glucanases is in the degradation of their own exocellular β-glucans, which usually work when the producing fungus is grown under a limited carbon availability [2]. For example, *Phanaerochaete chrysosporium* produces an exocellular β-glucan on a glucose rich medium, but when the glucose is exhausted from the medium, the

fungus was utilize the glucan in the medium. At this stage, β-glucanase production were increase, allowing hydrolysis of this exocellular β-glucan and releasing glucose as the substrate for fungal growth [19].
In other fungus, which include *Sclerotium glucanicum* [20, 21], *B. cinerea* [16,22] and *A. persicinum* [23], were also found that the β-glucanases produced by these fungi, possessed a similar functions in cell wall degradation, working in combination with other glucan degrading enzymes such as β-glucosidases from the same fungus. Both enzymes might working simultaneously, involved in assisting the β-glucanases in this cell wall degradation task [23].

Since not all β-glucanase producing fungi secrete exocellular β-glucans, so that the role of β-glucanase might not always involve in the digestion of exocellular β-glucan. For instance, some members of the genus *Trichoderma* were playing the role in mycoparasitism, work antagonistically against other fungi as well as some plant-pathogenic species [24-27]. Substantial effort has been made by [24-27] to elucidate the role of β-glucanases in this mycoparasitism using some fungal strains producing β-(1,3)- and β-(1,6)-glucanases. These enzymes have been reported effective in hydrolyzing other fungal cell walls. However, not much what we know regarding this role in mycoparasitic organisms, since the current molecular biology studies of these enzymes' role is limited. Only a few fungi have been reported to increase the levels of mRNA specific for β-(1,3)-glucanases during their mycoparasitic activity [28,29].

3. Regulatory control mechanism of synthesis for fungal β-glucanases

It has been concluded by [23] in the earliest studies, that both β-(1,3)-glucanases and β-(1,6)-glucanases produce by fungi were constitutive enzymes. As in the case of the endo-β-(1,3)-glucanase from *A. fumigatus* [30], it was reported indeed these enzymes were produced constitutively, but others were reported to be regulated under a control mechanisms such as induction and/or carbon catabolite repression [2], although mainly the researcher examined only crude enzymic activities.
Interpretation of these experimental data is difficult when many fungi secrete multiple exocellular β-glucanases, where its individual enzymes was found synthesized under different regulatory control mechanisms of synthesis, as has been seen in *A. persicinum* [8]. Therefore, mRNA analysis and transcription studies on the individual enzyme genes responsible for the synthesis is required, and the conclusions must be retrieved carefully.
In one case, the exocellular β-(1,6)-glucanase from *Neotyphodium* sp. [31] was clearly induced by their own β-glucan substrates, proved by its transcript analysis of mRNA. Meanwhile in other case of fungal β-glucanase, using also transcript analysis of its specific mRNA synthesis of *Coniothyrium minitans*, it was shown that their synthesis of its β-(1,3)-glucanase can be regulated by the presence of glucose [28].

3.1 Catabolite repression of fungal β-glucanases by carbon and nitrogen

When a readily utilizable substrate such as glucose is present in the medium, the production of exocellular β-glucanase may be repressed by this such glucose, regulated under catabolite repression [2, 21], no matter where this glucose available from, either supplied as the result of hydrolysis product of available substrates or provided naturally. However, the precise mechanism of this catabolite repression in many fungi is still not well understood, because mainly it was measured only from its enzymatic activities.

Derepression were often observed when the repressing carbon source is utilized, and then the repressed β-glucanase were again redetected [1,2]. This finding may support the possible survival functions of β-glucanase in fungi, where this might be needed for storage reserve materials degradation and/or the mobilization of cell wall glucan [2,21,32,33]. Furthermore, beside it could lead to catabolite repression, the presence of glucose in the medium could also deactivate the existing β-glucanases, as described in the Basidiomycete QM806 [34]**.** Meanwhile in *S. glucanicum*, the presence of readily utilizable glucose in the medium could inhibit the product formation of β-(1,3)-glucanase [35]**.**

Carbon catabolite repression occur in *A. nidulans* and *A. niger*, were reported to be mediated by the CREA repressor protein. The inhibition of the β-glucanases transcription occur by the binding of this repressor protein CREA to a specific short sequence along the promoter regions of the related genes [36]. This mechanism might also occur in *A. nidulans* and *A. niger* [37-39], and from *T. reesei* [40,41], *T. harzianum* [42], *Metarhizium anisopliae* [43], *Sclerotina sclerotiorum* [44], *Gibberella fujikuroi* [45**]** and *B. cinerea* [45]. From the cloning and sequencing studies performed, it was confirmed that the gene encoding the production of β-glucanases from those fungi were also exhibit a similar repressor protein CREA, *cre*A. [37-45]. However, the role of this repressor protein CREA in catabolite repression with fungal β-glucanases is still not yet clear, since other putative carbon catabolite binding elements have been found in sequences upstream of the genes encoding fungal β-(1,3)- and β-(1,6)-glucanases in some fungi which include *T. atroviride* [46], *T. harzianum* [47], *A. saitoi* [48], and *Acremonium* OXF C13 [16].
In *T. atroviride*, it was found two such elements upstream of the *gluc78* gene [46], while in *T. harzianum*, there is one upstream of the *lam1.3* gene [47], and from *A. saitoi* it was found four upstream of *exgS* gene [48]. Moreover, *Acremonium* OXF C13 represented three elements altogether, two upstream of the *BGN3.2* and one upstream of the *BGN6.2* gene [49].
A CREA-like protein was also believed to play some role in the regulation of transcription of the βglucanase genes in *Neotyphodium* sp., even though the present of this elements was not discussed. [31].
However, it seems that not all *creA* consensus sequences bind the regulatory protein, even though *creA* involvement in the regulation of several fungal genes is now confirmed in some β-glucanases in fungi [36], including the endochitinase (*ech*42) in *T. harzianum* [50]. Thus, the importance of these putative binding sequences in the regulation of fungal β-(1,3)- and (1,6)-glucanase genes has yet to be confirmed agreeably, since *in vitro* binding is not always representation of any functional significance *in vivo* [45].

As has been mentioned earlier that under the conditions of nitrogen starvation, the *gluc78* gene encoding the exo-β-(1,3)-glucanase was expressed in *T. atroviride* [51]. However, how general this regulatory feature is among fungi is need to be confirmed, since the gene encoding the β-(1,6)-glucanase which has multiple GATA boxes was found in *V. fungicola* [38]. This indicate that enzyme synthesis in yeast might be sensitive to nitrogen repression, the situation which is parallel with some other β-glucanase synthesis in yeasts [52].

3. 2 The fungal β-glucanases induction

Many fungi have been described to produce both exocellular β-(1,3)- and β-(1,6)-glucanase activities by induction mechanism [2]. When grown in laminarin or scleroglucan or

fungal cell wall preparations containing mixtures of complex glucans as sole carbon sources, they produce higher yield of β-1,3-glucanase. Meanwhile, highest β-(1,6)-glucanase production are often obtained from fungi by growing them with pustulan (a β-(1,6)-glucan) [2]. However, it cannot be simply generalized in this way since in some fungi, higher β-(1,3)-glucanase yields were produced with compounds against which they have no enzymic activity, such as chitin or pustulan [8, 53, 54,55]. These polymeric substrates are unlikely to be the true inducers for synthesis of these enzymes, because of their size. The possible presence of β-glucan recognition proteins on the cell surface of β-glucanase synthesizing fungi has not been approved, although these such glucan are known as the receptors in modulation of the host immune system in animal cells [56, 57]. It is also possible that the β-glucan may be contaminated with other smaller saccharides, since the β-glucan used as the substrates are all obtained from natural sources. These smaller oligosaccharides might be acting as actual inducer molecule/s, although the possibility of the involvement of smaller oligosaccharides as the inducer was considered unlikely in *A. persicinum*, since none screened supported high β-(1,3)-glucanase activities in this organism [8]. Transcript analysis of β-(1,3)-glucanase synthesis in mycoparasitic fungi such as *T. harzianum*, *V. fungicola* and *S. elegans*, reveals that its induction is generated by the presence of their host fungal cell wall [38,58, 59]. The concentration of β-(1,3)-glucan, laminarin present in the medium were correlated well with the fungal β-(1,3)-glucanase synthesis in both *Chaetomium indicum* [60] and *T. harzianum* [61]. In other case, synthesis of the *T. atroviride* exo-β-1,3-glucanase was induced by nitrogen starvation Donzelli and Harman [57]

Thus, approach for optimizing the manufacture on a large scale of exocellular β-(1,3)-glucanases may largely depended on a cautious choice of the glucan used as the carbon source for their production. As an example, *T. harzianum* TC [61] and *Trichoderma asperellum* [62] produced higher activities when cultured with purified fungal cell walls rather than with single glucans. Meanwhile, in *Trichoderma harzianum* IMI 206040, the opposite occurred [51]. It was also noted that these fungi produced different numbers of glucanase isoforms when grown with different substrates. This maybe the explanation why those fungi produced different yield. Only two isoforms were detected when *T. harzianum* TC was grown with laminarin as carbon source, while three were existing with fungal cell walls [61]. Furthermore, seven isoforms were detected in *T. asperellum* when grown with laminarin but only three with cell walls [62]. More isoforms (four) were produced by *S. elegans* cultured with laminarin than with fungal cell walls (only two) even though lesser β-(1,3)-glucanase activities were observed with laminarin [59]. In this case, it is appeared that the carbon source used does not affect in the same way to the activities of individual β-(1,3)-glucanase isoforms produced.

The explanation why *Acremonium* IMI 38068 produced higher β-(1,3)-glucanase activity when cultured in scleroglucan containg media than in pustulan, it was because of the presence of an additional β-(1,3)-glucanase produced when scleroglucan is present [53]. The expression of the gene encoding the β-(1,3)-glucanase in *T. atroviride* was also reported to be enhanced by scleroglucan [46], but the case might differ to *Acremonium* IMI 383068, since this fungus produced an identical molecular weight and N-terminal amino acid sequence protein, but inactive β-(1,3)-glucanase, to that produced in scleroglucan cultures. It is appeared that scleroglucan generally increases β-(1,3)- glucanase activity in fungi, but this finding yet need to be proved by the evidence.

4. Conclusion

Fungal β-(1,3)- and β-(1,6)-glucanases are mostly constitutive enzymes, their synthesis seems to be under induction and/or carbon catabolite repression as their regulatory control

mechanisms. Many fungi produce multiple exocellular β-glucanases. However, each enzyme can be controled under independent regulatory mechanisms. Some β-glucanases are clearly inducible in the presence of their substrates, as corroborated by their transcript analysis of mRNA, although other transcript analysis in other fungi suggests that their synthesis might arise when glucose is present.

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