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The Functional Aspects and Potential of Fungi Based β-Glucan

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ABSTRACT: β-glucan is a natural polysaccharide derivatives composed of glucose monomers with β-glycoside bonds that can be synthesized by various microorganisms including fungi. So far, there have been many studies revealed that the main source of β-glucan production is cereal, yeast, and fungi. However, the study was more dominated by cereal and yeast as producer of β-glucan, therefore in this study will be explored the potential production of filamentous fungi based β-glucan. *Rhizopus sp.* and *Aspergillus sp.* are members of filamentous fungi genus that can be used as producer of β-glucans because they have cell walls which composed of polysaccharide components, especially glucans. β-glucans categorized as Generally Recognized As Safe (GRAS), FDA has recommended a daily consumption of 3 g β-glucan to achieve such health benefits. β-glucan can be consumed directly as food supplement or incorporated in a wheat, biscuit, bread, and beverage such as daily fruit drink. Supplementation of β-glucans from fungi has high potential as functional bioactive as antioxidant, and that could be as an alternative to overcome may diseases including cholesterol.

Keywords: *Aspergillus*, *Rhizopus*, β-glucans, functional aspects

Abbreviations: GRAS, Generally Recognized As Safe;SCFAs, Short-Chain Fatty Acids; UDP, uridine diphosphate; FDA, Food and Drugs Administrations, GPI, Glycosylphosphatidylinisotol, AGS, α-1,3-glucan synthase; BGS, β-1,3-glucan synthase; CHS, chitin synthase; ECM, extra cellular matrix; CAZy, Carbohydrate Active enzyme' BDM, Biological Defense Modifier; LDL, Low Density Lipoprotein; GI, Gastrointestinal; HMG, hydroxymethylglutaril.

I. INTRODUCTION

β-Glucans are natural polysaccharide derivatives composed of glucose monomers with β-glycoside bonds [48]. β-glucans are active substances that categorized as Generally Recognized As Safe (GRAS) and according to the Food and Drug Administration (FDA), β-glucans are safe for human consumption and have no toxicity or side effects[34].

β-glucans can be applied in the medicine, pharmaceutical, cosmetic, chemical industries along with food production. In food production, β-glucans also has various suitable functional properties such as for emulsification, thickening, gelation, and stabilizing [5]. These properties can be adjusted and used for soups, sauces, drinks, and other food products [29, 23]. In addition, β-glucans have potential biological activity properties. FDA has recommended a daily consumption of 3 g β-glucan to obtain the health benefits of β-glucan [35]. β-glucanscan play a role as antioxidants by releasing an electron contained in the oxygen component of its structure to bind to the free radical components which have unpaired electrons so that they can be stable if they bind to the electron from the β-glucans [54].

Besides, β-glucans are *neutraceutical* which can reduce cholesterol level [88]. β-glucans are classified as having high-molecular weight and high-viscosity food fibres, so they have a positive effect on lipid metabolism [65, 72]. The fermentation of β-glucans in the colon will form Short-Chain Fatty Acids (SCFAs) which can inhibit cholesterol-forming enzymes in the human body [6, 49]. These benefits encourage the research and development of β-glucans applications and increase production.

In previous studies, many of the potential sources reported as producer of β-glucan such as cereal (e.g. oats [98],[78] barley [19, 79, 33], and wheat [28, 68]), microorganisms especially yeast (e.g. *Saccharomyces cerevisiae*) [66, 82, 93], and several fungi [50,76]. Fungi have cell walls which the main components are polysaccharides consisting of glucans, chitin, and mannan [63]. Glucans are the most important and abundant component of polysaccharides from the cell walls of most fungi [63, 70]. Formation of β-1,3-glucans is needed to form cell walls and normal fungal development [21, 100].

Many studies haven't clearly explained the content of β-glucan in filamentous fungi. In this study will be explained how potential filamentous fungi mainly from the *Aspergillus sp* and *Rhizopus sp* genus which are viewed based on its cell components and some of its ability to produce β-glucan-degradation enzymes. In addition, will be discussed about various functional aspects of the filamentous fungi based β-glucan, especially related its activities as antioxidants and anti-cholesterol. This study was carried out because in the

recent years there have been many fungi based natural products that were attracting researcher's attention to be developed as antioxidant and anti-cholesterol [74, 89].

II. MATERIALS AND METHODS

The paper was written with non-research methodology based on scientific information obtained from various literatures such as textbooks and published articles from various sources that divided into two stages.

The first stage is the stage which related to the data collected, consisting of: 1) Problem identification with the keywords Fungi, β-glucan, and functional aspects; 2) Determination of literature sources based on eligibility criteria and inclusion or exclusion criteria. The references obtained are then sorted by several criteria that must be fulfilled (inclusion criteria) and the exclusion criteria; 3) Collecting and sorting (screening) data based on the suitability with the topics discussed. The number of literature sources that have been identified and passed the feasibility and also sorting test were calculated based on Fig. 1.

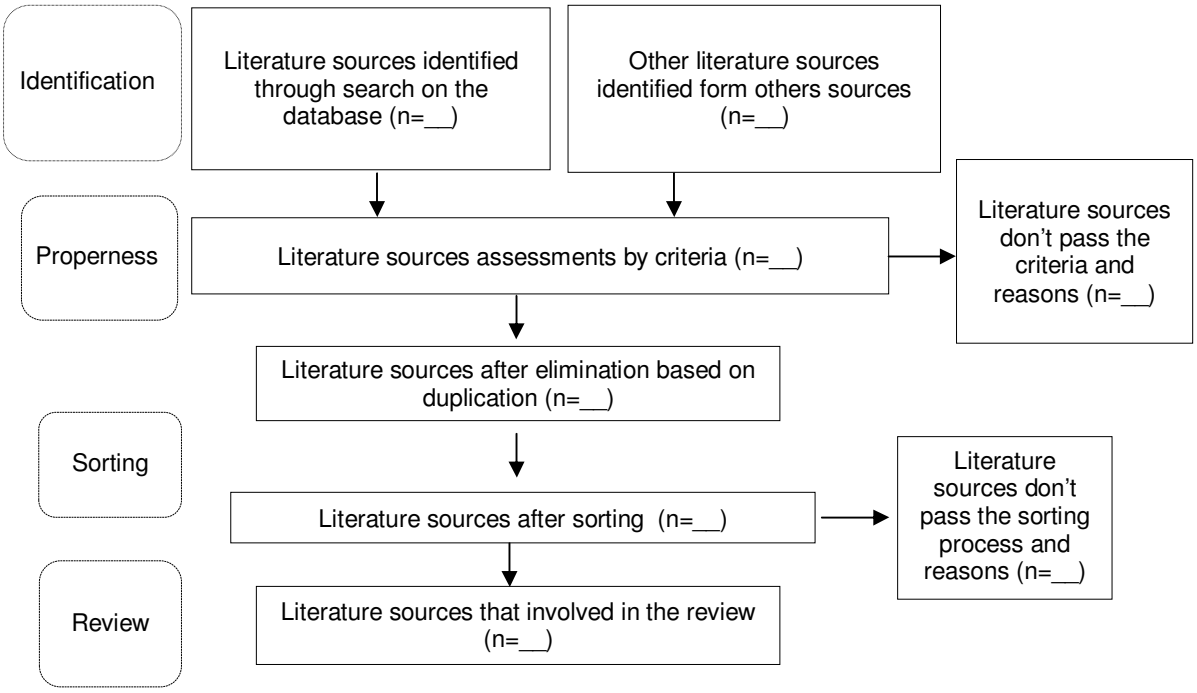

Fig. 1. Determination System of The Number of Literature Sources on Review [36]
Information: n = The number of literature sources.

The second stages is data processing that have passed the sorting process, consisting of: 1). Data analysis and interpretetion. Data analysis and interpretation conducted on the literature sources that have been sorted are as follows: Potential of *Aspergillus sp* and *Rhizopssp*in producing β-glucan and functional aspects of β-glucan as antioxidant and anticholesterol; 2) Confirmation. Confirmation conducted by comparing the data in a literature with other similar literature or other supporting literature. Unconfirmed data will be re-interpreted or compared to other data or re-sorted to determine its relevance to the topic, while the confirmed data will be elaborated and compiled into a descriptive review. The descriptive review is based on interpretation of similarities and differences in the objectives, methods, and results of research from the various literature sources found. Then, the conclusion can be withdrawn based on the results of the review.

III. β-GLUCAN FROM VARIOUS SOURCES

β-glucans are polysaccharides composed of D-glucose monomers connected by β-glyosidic bonds. β-glucans formed by D-glucose monomer bonds are generally in position (1,3) and have branches in position (1,4) and (1,6) [30]. β-glucans in nature usually appear as large lumped white compound, not crystallize, have no sweet taste, insoluble in water under neutral conditions, can be separated in alkali solution, when mixed with water will form a colloidal solution, and can become gel at 54℃ [27].

Many kinds of β-glucans are existing in nature and are commonly found in algae, seaweed, mushrooms, and cereal grains, especially in the bran of barley, wheat, and oats [98, 101]. Aside from cereals, the production of β-glucan nowadays mostly comes from microorganisms, namely bacteria, yeast, and fungi include filamentous fungi. These microorganisms generally store β-glucans in their cell walls and as a result of their metabolism [12, 48].

Structurally, β-glucan from cereal grains consists of long linear chains of glucose having β-(1,3) and β-(1,4)-linkages but these linkage are not arranged in a random and repeating fashion [11, 92]. However, β-glucan from baker's yeast has a different type of linkage; it consists of β-(1,3) as well as (1,6) linkages [40]. In fungi, between 65% and 90% it consists of β-1,3-glucan, but

other glucans, such as β-1,6-, mixed β-1,3- and β-1, 4-, α-1, 3-, and α-1,4-linked glucans, have been found in various fungi [42, 60].

β-Glucans from different sources and with different structure and molecular weigh has different biological activity [32].In addition, the physicochemical properties of the different β-glucans depending on characteristics of their primary structure, including degree of branching, linkage type and conformation (e.g., single helix, triple helix, and random coil structures) [94, 97].

To determine the molecular-structural characteristics of extracted β-glucans, the proper extraction method is very necessary to optimize the β-glucan yield and purity and to retain the integrity of β-glucan molecules [22]. The most proper extraction method depends on the structure and the sources of β-glucans. The most common extraction method of β-glucan is hot water extraction. There are other extraction methods include solvent extraction [19], acidic extraction [80], alkali extraction [56], microwave-assisted extraction [77], ultrasound-assisted extraction [33], and enzymatic extraction [3].

Indigenousenzymes can influence recovery and characteristic of β-glukan that was extracted. The main indigenous enzymes that responsible in hydrolyze β-glucan compound in cereal is endo-β-$(1\rightarrow 3)$ $(1\rightarrow 4)$-glucanase [47], while in fungi is 1,3-β-D-glucan synthases [13], and endo-1,4-β-D-glucanases [71]. Some several enzymes, such as endo-xilanase, arabino furanosidase, xyloacetylesterase, and feruloyl esterase also play a role in extrication of β-glucan from various sources [3].

Table 1: Production of β-Glucan from Various Sources.

Group	Source	Production process	Production time	Amount of β-glucan	Reference
Cereals	Hull-less barley	Remove starch and protein, accelerated solvent extraction and precipitate with anhydrous ethanol	9 min	16.39%[a]	[33]
	Barley	Extraction with water, centrifugation, precipitation with ethanol and homogenization	30 min	5.54%[a]	[95]
	Barley	Water extraction, enzymatic removal of starch and protein and subsequent precipitation with ammonium sulfate saturation	2 h	5.93%[a]	[51]
	Barley	Hot water Extraction, high speed stirring and centrifuge	90 min	5.4%[a]	[4]
	Oat	Enzymes extraction	6 h	5.14%[a]	[3]
	Barley grains and malt	Acidic extraction with perchloric acid	2 to 3h[f]	4.3 to 6.0%[b]	[2]
Cereals	Barley	Acidic extraction with citric acid	10 to 12h[f]	4.65%[b]	[4]
	Barley bran	Alkaline extraction with NaOH	20 to 24h[f]	5.6 to 11.9%[b]	[20]
	Wheat bran		24h[f]	2.6%[ab]	[28]
	Wheat bran		96 to 120h[f]	2.15 to 2.51%[b]	[68]
	Barley		10 to 12h[f]	3.94%[b]	[4]
	Oat bran	Alkaline extraction with Na_2CO_3	10 to 12h[f]	8.57%[b]	[17]
	Barley	Water extraction	168h[f]	2.5 to 5.4%[b]	**[79]**
	Barley	Enzymatic extraction	14 to 16h[f]	[ab]5.22%[b]	[4]
Yeast	Yeast	Alkaline extraction, DEAE-cellulose and ConA chromatography	ND	4%[a]	[66]
	Brewer's yeast	Alkaline extraction	1h	51%[a]	[93]
	S. cerevisiae strains HII31	NaOH/ HCl extraction	54 to 72h	41.69[c]	[82]
	S. cerevisiae strains TISTR5003			36.31[c]	[82]
	S. cerevisiae strains TISTR5024			38.48[c]	[82]
	Candida spp.	Extraction with 0.25 M KOH and 1.2 M KCl. Assayed using a the Glucatell (now Fungitell) assay	ND	414-3765 pg/ml[d]	[76]
	C. albicans			1141-1992 pg/ml[d]	[76]
	C. dubliniensis			549-3258 pg/ml[d]	[76]
	C. tropicalis			1093-2283 pg/ml[d]	[76]
	B. capitatus			1859 pg/ml[d]	[76]

	Saccharomyces cerevisiae			1468-2336 pg/ml[d]	[76]
	Rhodotorula rubra			1174-1815 pg/ml[d]	[76]
	Cryptococcus neoformans			153-265 pg/ml[d]	[76]
Fungi	*Bipolaris spicifera*	Extraction with 0.25 M KOH and 1.2 M KCl. Assayed using a the Glucatell (now Fungitell) assay	ND	2747-4389 pg/ml[d]	[76]
	Penicillium marneffei			1685-2297 pg/ml[d]	[76]
	Aspergillus spp.			1311-2480 pg/ml[d]	[76]
	A. flavus			1311-2191 pg/ml[d]	[76]
	A. fumigatus			2191-2480 pg/ml[d]	[76]
	A. niger			1334-1843 pg/ml[d]	[76]
	A. terreus			1660-2476 pg/ml[d]	[76]
	Paecilomyces sp.			1199-2539 pg/ml[d]	[76]
	Fusarium spp.			952-1860 pg/ml[d]	[76]
	Basidiobolus ranarum			1418 pg/ml[d]	[76]
	Phialophora verrucosa			1062-1281 pg/ml[d]	[76]
	Rhizomucor pusillus			191-412 pg/ml[d]	[76]
	Rhizopus arrhizus			89-209 pg/ml[d]	[76]
	Mucor sp.			111-131 pg/ml[d]	[76]
	Agaricus bisporus	Ultrasonic-assisted extraction, precipitation with ethanol, centrifugation	62 min	6.02%[a]	[96]
	A. chevalieri	Assayed using the Limulus Amebocyte lysate (LAL) after extraction pf 0.5 ml of 0.6 M NaOH by shaking	ND	4.61 pg/Spore volume[e]	[50]
	A. flavus			0.48 pg/Spore volume[e]	[50]
	C. cladosporioides			4.19 pg/Spore volume[e]	[50]
	C. herbarum			240.54 pg/Spore volume[e]	[50]
	E. nigrum			21.02 pg/Spore volume[e]	[50]
	P. brevicompactum			13.81 pg/Spore volume[e]	[50]
	A. chevalieri	Assayed using the Enzyme Immunoassay (EIA) after extraction pf 0.5 ml of 0.6 M NaOH by shaking	ND	5.08 pg/Spore volume[e, f]	[50]
	A. flavus			1.64 pg/Spore volume[e, f]	[50]
	C. cladosporioides			120.55 pg/Spore volume[e, f]	[50]
	C. herbarum[a]			1.72 pg/Spore volume[e, f]	[50]
	E. nigrum			32.72 pg/Spore volume[e, f]	[50]
	P. brevicompactum			228.39 pg/Spore volume[e, f]	[50]
	A. versicolor	Assayed using the Limulus Amebocyte lysate (LAL) assay after extraction of 1mg in 1mL of 0.5N NaOH modified LAL assay	ND	22.0 pg glucan spore μm^{-2} (×1,000)[g]	[38]
	Cladosporium cladosporiodies			59.8 pg glucan spore μm^{-2} (×1,000)[g]	[38]
	A. versicolor	Assayed using the Limulus Amebocyte lysate (LAL) assay after extraction of 1mg in 1mL of 0.5N NaOH modified LAL assay	ND	22.0 pg glucan spore μm^{-2} (×1,000)[g]	[38]
	Cladosporium cladosporiodies			59.8 pg glucan spore μm^{-2} (×1,000)[g]	[38]
Bacteria	*Agrobacterium* sp. ATCC31750	Two-step culture, centrifugation, washing with distilled water	120h	6.6%[a]	[55]
	Agrobacterium spA1.5	Extraction with 1N NaOH and 5N HCl	ND	2,3-7,5%[h]	[62]
	Agrobacterium spB4.4			2,49-7,21%[h]	[62]

*ND=not defined
[a]Production yield (%).
[b]Production yield (%), Yield inclusive of fiber, protein, and other grain components in addition to β-G (major). Time calculated (≅) right from the extraction process till obtaining the dried β-glucan.
[c]β-glucan (%, w/w).
[d]BG Range (Beta glucan concentrations detected in broth media culture supernatants of clinical mould and yeast isolates)
[e]($pg/\mu m^3 x10^{-3}$). [f]=The value for this fungi (1-3)-β-D-glucan concentration was below the lower detection limit of the EIA assay (250 ng/ml). These values were replaced with LOD divided by the square root of two.
[g]Glucan contents per spore.
[h]Levels of β-glucans equivalent to glucose in β-1,3-glucans (crude).

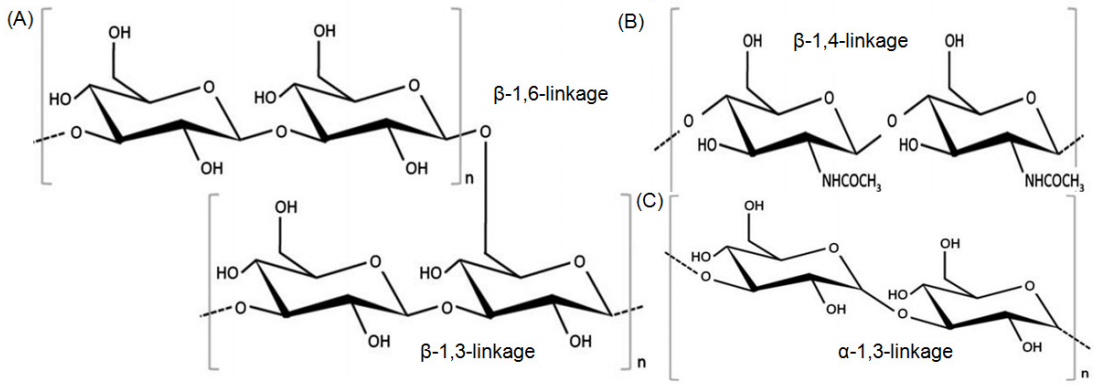

Fig. 2. Chemical structure of polysaccharides associated with fungi cell wall, (A) β-1,6-branched β-1,3-glucan (B) Chitin, (C) linear chains α-1,3-glucan [99].

IV. FUNGI BASED β-GLUCAN

Fungi are found all over the world. They play an important role in ecosystems to decompose plant biomass. Fungi's cell wall not only play a role in keeping cell morphology, but also help to protect the cells from various kinds of extracellular environmental stress. In their life cycles, fungi are exposed by changes in osmotic pressure, temperature, pH, and other environmental factors. Fungi's cell wall is the key of defense lines to keep it still survive of these environmental changes that give the pressure on fungi [99].

Fungi has complex structure cell wall. It mainly composed of polysaccharides include glucans [99]. The production of glucans is mostly done in the cell walls of fungi because of their high composition reaching 50%-60% of the dry weight of the cell walls [37, 57]. Glucan are composed of repeating glucose residues that are assembled into chains through a variety of chemical linkages. In general, between 65% and 90% of the cell wall glucan is found to be β-1,3-glucan [18], [42].

The β-1,3-glucan serves as the main structural constituent to which other cell wall components are covalently attached. As a result, the synthesis of β-1,3-glucan is required for proper cell wall formation and the normal development of fungi [21].

Other types of glucans, such as a branched β-1,3-/1,6-glucan (Fig. 2A), which is crosslinked to chitin (Fig. 2B), galactomannan and linear chains α-1,3-glucan (Fig 2C), have been found in various fungi cell walls [41, 64]. The glucan matrix and chitin are cross-linked together to form a chitin/glucan matrix. GPI-anchored glycoproteins and non-glycoproteins are covalently attached to the cell wall (Fig. 3) [39].

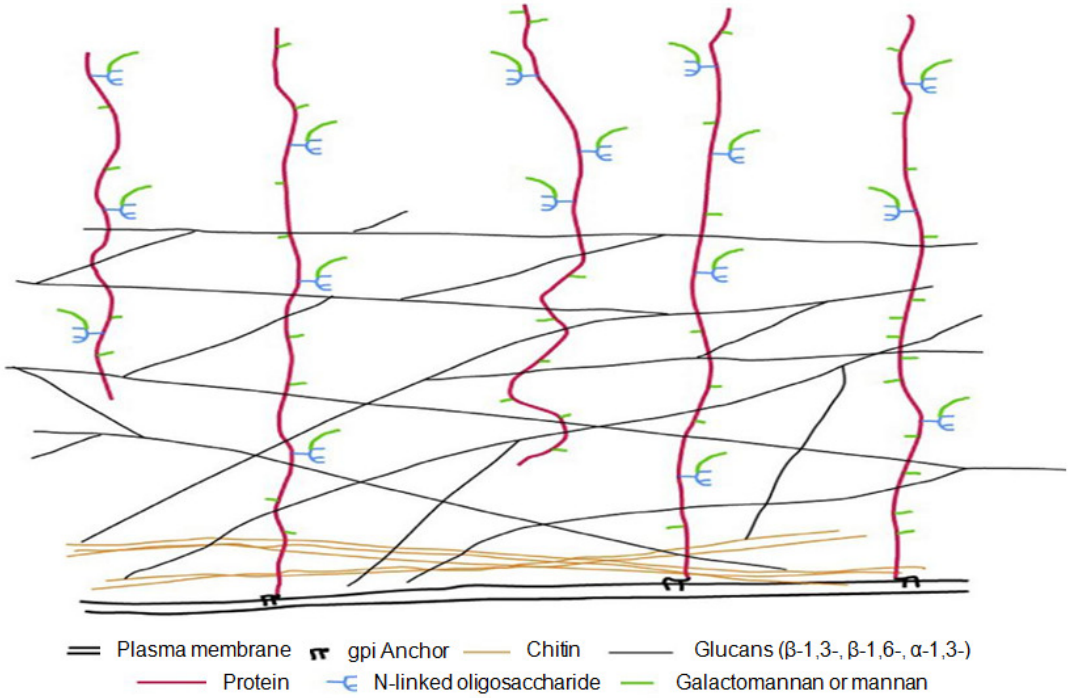

Fig. 3. Basic fungi cell wall structure is composed of glucans, glycoproteins, and chitin [39, 21].

Filamentous Fungi

There are a lot of fermented foods that are made using these filamentous fungi. *Aspergillus* genus include the kojimold *Aspergillus oryzae*, *A. niger*, and other species used in fermentation industries.

Aspergillus species such as *A. oryzae*, *A. sojae*, and *A. luchuensis* generally known as koji-molds which play a role in producting traditional Japanese fermented products such as *saké* (rice wine), *shoyu* (soy sauce), *miso* (soybean paste), *shochu* (distilled beverage) [46] and *miso* (*tauco*) [84]. Besides, *A. niger* also play a role in traditional cassava based fermented food such as *gaplek* or *gatot* in Indonesia [10], and *gari*in Nigeria [75].

Aspergillus genus's long history of utilization in the food industries has got them recognition from the FDA as GRAS organisms [1], and also recognized as safe microorganisms from the World Health Organization (WHO) [34]. Besides, *Rhizopus* genus also has the widely potential that very play a role in the human life especially fermented foods. *Rhizopus* is consists of 10 species, including plant spoilage species (like *Rhizopus artocarti* or *Rhizopus arrhizus*) or food related species, like *Rhizopus oligosporus* and *Rhizopus oryzae*. *R. oryzae*and *R. oligosporus* categorized as GRAS by FDA and used as traditional food fermentation agent, e.g. tempeh in Indonesia and koji in China and Japan [100].

The outstanding safety and productivity displayed by *Asspergillus and Rhizopus* makes it an ideal host organism for the production not only of fermented foods, but also of various enzymes and chemicals with pharmacological effects that could be applied to medical treatments in the future include production of β-glucan.

The production of β-Glucan from Aspergillus

Aspergillus is a potential filamentous fungiin the production of β-glucans. *Aspergillus fumigatus* is a species of *Aspergillus sp*. which is reported to contain β-glucans. In *A. fumigatus*, β-1,3-glucan is synthesized by a plasma membrane-bound glucan synthase complex, which uses UDP (uridine diphosphate)-glucose as a donor-substrate and extrudes β-1,3-glucan chains through the membrane into the periplasmic space [14, 15].

The *Aspergillus fumigatus* cell wall is almost exclusively composed of polysaccharides (Fig. 5). The mycelium and conidia cell walls in *A. fumigatus* contain polysaccharides consisting of linear chains β-(1-3)-glucan (20-35%), branched with chains of β-(1-6)-glucan; linear chains β-(1-3/1-4)-glucan; α-(1-3)-glucan (35-46%); also chitin (7-15%) and galactomannan (20-25%) [41]. Based on research, the level of β-glucan supernatant in *A. fumigatus*s 2191-2480 pg/ml [76].

The cell wall central core is mainly amorphous α-1,3-glucan and composed of β-1,6-branched β-1,3-glucan crosslinked to chitin is present in the cell wall outer layer. Polysaccharides, such as galactomannan and galactosaminogalactan, and proteins, such as surface proteins, GPI-anchored are also present in cell wall. Abbreviations: α-1,3-glucan synthase (AGS); β-1,3-glucan synthase (BGS); chitin synthase (CHS); and extra cellular matrix (ECM) [99].

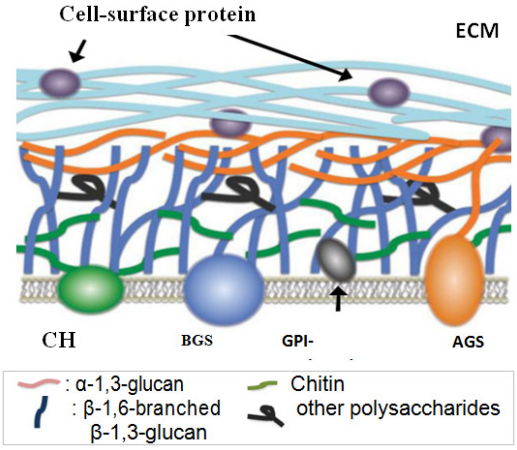

Fig. 4. Schematic illustration of cell wall architecture in *Aspergillus* species [99].

Besides *A. fumigatus*, *Aspergullis niger* also has the potential to produce β-glucan. The cell walls of *A. niger* are composed of glucan components with a percentage reaching 37% and other components such as galactomannan and chitin. α-glucans (mainly α-(1,3)-glucan and α-(1,4)-glucan in low concentration), β-glucans (β-(1,3)-glucan with branches of β-(1,6)-glucan) [27, 83].

The average level of β-glucan supernatant from *A. niger* is 1559 mg/L of *A. niger* biomass [76]. From a mycelia of 0.78 grams (dry weight) *A. niger* gains 0.5 g (dry weight) β-glucan with a percentage (yield) of 64.1% (w/v) [81].

The production of β-Glucan from Rhizopus

Rhizopus oryzae has cell walls composed of chitin, chitosan, phosphomannan, and glucans [86]. The cell walls of *zygomycetes,* including *R. oryzae*, are composed of chitin and chitosan, and contain small amounts of β-1,3-glucan [13]. β-1,3-glucan has also been suggested to be included in the cell wall morphology regulation of *zygomycete Mucor rouxii* [9].

The β-glucans in the cells of *R. oryzae* are bound to the cell walls and the separation can be done by acid-base neutralization method or with the help of the Carbohydrate Active enzyme (CAZy). These enzymes can also be produced by *R. oryzae* to degrade the β-glucan bonds from the long polysaccharide bonds [13]. The *R oryzae* genome is able to encode three putative 1,3-β-D-glucan synthases (GT48 family)[13], and endo-1,4-β-D-glucanases [71].

The β-1,3-glucanase enzyme can be divided into exo-β-1,3-glucanase and endo-β-1,3-glucanase. Four of the seven GH5 proteins of *R. oryzae* are functionally annotated as a candidate β-glucosidase related to exo-1,3-β-glucanases, while five of the six models of GH3 proteins are functionally annotated as a candidate β-glucosidase or exo-1,3-β-glucosidase[13].

R. oryzae is also able to encode two family GH72 proteins, a family typically containing GPI-anchored β-1,3-glucanosyltransferases. This enzyme is known to play a central role in the cross-bond of β-1,3-glucan cells with other cell walls of β-glucans [44].

Based on its action mechanism, the β-1,3-glucanase enzyme is classified into two types, namely the β-1,3-exoglucnase enzyme (β-1,3-glucanglucanohydrolase EC 3.2.1.58) and the β-1,3-endoglucanase enzyme (β-1,3-glucan glucanohydrolase EC 3.2.1.6 or EC 3.2.1.39). The β-1,3-endoglucanaseenzyme works by randomly cutting the β-1,3-glucan bond into two to six glucose units, while the β-1,3-exoglucanase enzyme cuts the β-1,3-glucan bond by releasing glucose monomers from the non-reductive side [83]. The bond released during hydrolysis is the β-1,3-glucosidic bond. The majority of these enzymes are endogluacase [8]. This enzyme can be used as a biocontrol agent and characterize β-glucans [83]. Based on research, the average level of β-glucan supernatant from *Rhizopus arrhizus*is 149 pg/ml [76].

V. FUNCTIONAL ASPECTS OF FUNGI BASED β-GLUCAN AS ANTIOXIDANT AND ANTI-CHOLESTEROL

In the application, a lot of references expressed β-glucan has functional characteristic as Biological Defense Modifier (BDM) that can activate body's immune system [52], reduce onset of colorectal cancer [31], reduction in glycemic index [25], prevention of insulin resistance [45], flattening of the postprandial blood glucose levels and insulin rises [43], prevention of coronary heart disease [53], prevention of hepatic damage by reducing taxol-induced hepatic damage [58]. β-glucans can also be used as an wound healing, protection against radiation, antiaging, hematopoiesis-stimulating, antitumor, and anti-inflammatory properties [69], [85].

In addition, β-glucans are also useful as antioxidant [26] and anti-cholesterol [59]. β-glucans have the ability to release an electron present in the oxen component of their structure to bind to free radicals that have unpaired electrons so that they can be stable by binding to electrons from the β-glucans. These free electrons can ward off free radicals which also have free electrons, so free radicals cannot damage the body's biological cells [54]. In the absence of β-glucans, free radicals will make bonds with biological cells of the body and contaminate these cells to be inactive [61]. The antioxidant activity of β-glucans can simultaneously act as anti-cholesterol [7]. Antioxidants can inhibit and prevent damage to LDL-cholesterol due to oxidation, which can ultimately reduce blood cholesterol levels [15].

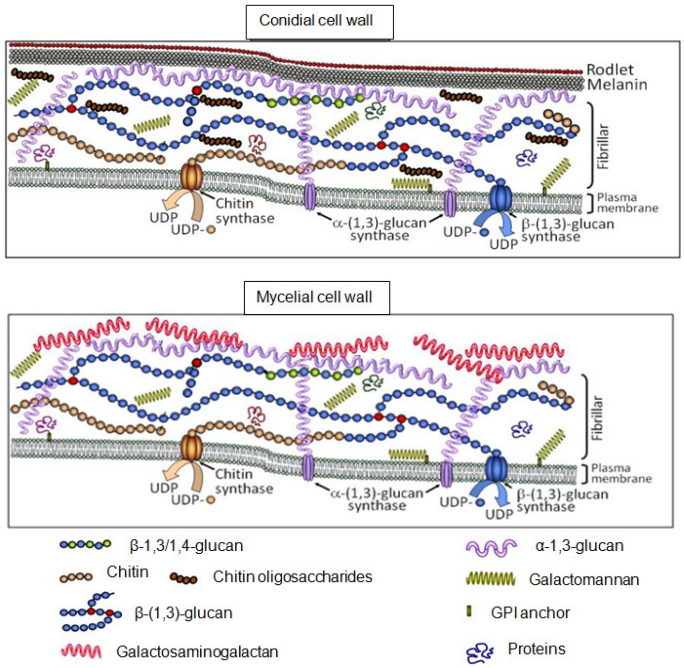

Fig. 5. Schematic representation of the *A. fumigatus* conidial and mycelial cell walls [64].

Low Density Lipoprotein (LDL) is the main cholesterol-carrying agent in the blood. Free radicals (reactive oxygen) can oxidize LDL-cholesterol. If this oxidized LDL-cholesterol builds up in the walls of blood vessels, the cholesterol level in the blood will automatically increase. In this case, the body needs antioxidants to prevent the oxidation of LDL-cholesterol so that there is no buildup of LDL-cholesterol in the walls of blood vessels [87, 90].

Another mechanism of β-glucans in reducing cholesterol levels is through the formation of a gel layer in the gastrointestinal (GI) tract especially in the small intestines due to β-glucans being able to dissolve water at low concentrations and have high viscosity [72]. The formation of the gel layer is able to increase intestinal viscosity and increase the excretion of bile acids so that inhibition occurs in the re-absorption of bile acids [65], [78]. Inhibited re-absorption of bile acid can increase the formation of bile acids from cholesterol as well as reduce the circulation of LDL-cholesterol [24].

In addition, cholesterol-reduction occurs as a secondary reaction of fermentation of β-glucans by intestinal bacteria [67, 91]. The fermentation of β-glucans produces Shot-Chain Fatty Acids (SCFAs) especially

acetate (C1), propionate (C2), and butyrate (C3). SCFAs is significantly able to inhibit cholesterol synthesis by hampering the action of the HMG-Coenzyme A reductase enzyme [6, 49]. The inhibiton of the action of that enzyme will inhibit the conversion of acetyl-CoA into mevalonate, which will ultimately inhibit cholesterol formation. It is due to the formation of cholesterol in the liver will pass through the phases of the formation of a compound that starts from the formation of mevalonate, mevalonate conversion into isopren unit, polymerization of 9 of 5 isopren carbon units forming 30 carbon molecules which is skualen, and the fourth skualen cyclications forms 4 steroid rings and forms cholesterol [73].

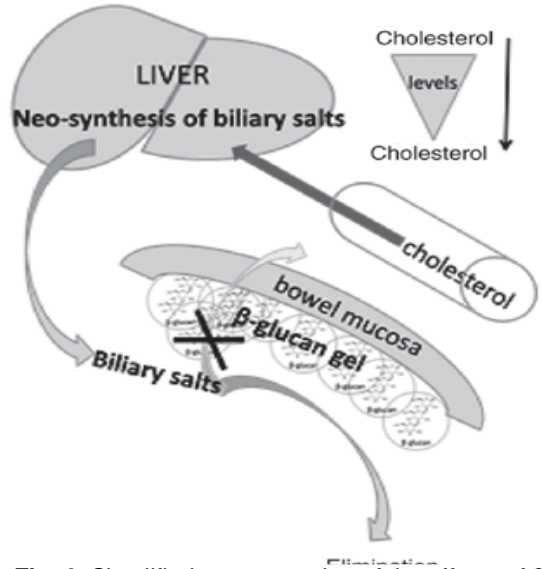

Fig. 6. Simplified representation of the effects of β-glucan on cholesterol balance [88].

Notes: β-glucan as fibers are able to form a gel on the bowel mucosa surface of the intestines, thus inhibiting the reabsorption of billiary salts and stimulating the neo-synthesis of billiary salts in the liver. Increased billiary salts have an impact on the utilization of cholesterol circulation, thus reducing cholesterol levels in the blood.

VI. CONCLUSION

The production of β-glucan has been carried out mostly from cereals, and currently has been developed a lot of yeast *S. cerevisiae* based β-glucans, because *S. cerevisiae*'s cell wall components is dominated by β-glucan. In the future, it is predicted that the demand or the necessity for β-glucan will increase because its has many roles in human life.The increasing demand or necessity for β-glucan must necessarily be accompanied by an increase in β-glucan production which is currently still dependent on cereals and yeast or macrofungi. Thus, it is certainly necessary to update the study for the development of alternative sources of β-glucan production.

We assume that filamentous fungi of the *Rhizopussp.* and *Aspergillus sp.* genus can be used as alternative sources of β-glucans production based on microorganisms. The production of filamentous fungi based β-glucan is very possible to be developed, it is because fungi include microorganism that are easy to grow and are found in many regions of the world. Moreover, *Rhizopus sp.* dan *Aspergillus sp.* also known as fungi which have been categorized as GRAS, so it has also been widely involved in various sectors of chemical, food, and medical industries to support human life.

If viewed from its cell wall components, *Rhizopussp.* and *Aspergillus sp.* also composed of a number of β-glucan with β-(1,3-1,6)-D-glucan bonds which are also commonly found in *S cerevisiae,* so the physicochemical characteristics of β-glucan from these filamentous fungi are thought to be the same as *S. cerevisiae* based β-glucan, including its functional aspects as antioxidant and anti-cholesterol.

The development of filamentous fungi based β-glucan extraction is also possible with modification of its extraction media, namely by utilizing various food industries waste. It is because the waste is still possible to contain the compounds which needed for the growth of filamentous fungi. So, the development of filamentous fungi based β-gulcan production will be more environmentally friendly, low-cost and can also support three sustainable development programs (SDGs) in the environmental, health, and food sectors.

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