Microorganism-Based β-Glucan Production and their Potential as Antioxidant

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ABSTRACT
β-glucan can be obtained from various microorganisms, including yeasts, molds and bacteria. Yeasts and molds can produce β-needed from cell walls but the bonding and composition have differences. Meanwhile, bacteria produce β-needed through secondary metabolites. This shows that the β-glucans produced have different characteristics. Saccharomyces cerevisiae is a potential yeast in producing β-glucans because most cell wall structures contain polysaccharides of β-1,3-glucan and β-1,6-glucan types. Aspergillus sp. is a mold that is commonly used in producing fermented food products where most of the cell walls are composed of polysaccharides with β-glucan and α-glucan chains. Meanwhile, Xanthomonas campestris is gram-negative bacteria is known to produce β-(1,2) glucan and Bacillus sp. as gram-positive bacteria showing the activity of the β-glucanase enzyme. Different types of microorganisms will show variations due to different metabolic processes which will produce varying amounts and characteristics of β-glucan affected by the fermentation conditions. β-glucan fermentation of potential and safe microorganisms to be used as additional ingredients. As a food additive, β-glucan can act as an anti-free radical considering its ability to capture free electrons and other biological activities they have. β-glucan extract from microorganisms with different composition will give different antioxidant activity.

INTRODUCTION
β-glucan are derivatives of natural polysaccharides composed of glucose monomers with β-glycoside bonds (1). Food Drug and Administration (FDA) states that β-glucan included the GRAS (Generally Recognized As Safe) category, and have no toxicity or side effects (2). β-glucan can be applied in the food industry, such as thickening, stabilizing, emulsifying and gelation. These properties are used to be adapted to soups, sauces, drinks and other food products (3,4). These various benefits encourage the research and development of β-glucan applications and increase production. The health benefits of β-glucan obtained from microorganisms are having potential biological activity properties such as anti-tumor and immune system (4). Biological activity of β-glucan is influenced by molecular weight and conformation of glucans which will affect the characteristics of glucan so that β-glucan obtained from each source will be different (5). Bacteria, molds and yeasts can be used to produce β-glucan. Bacteria produce β-glucan through secondary metabolites, while β-glucan produced from yeast and mold are present on the cell wall (6). The ties and arrangements of obtained β-glucan from each source will be resulting in different characteristics (3).

One of the yeasts that has the potential to produce β-glucans is Saccharomyces cerevisiae. Most of the cell structure of S. cerevisiae contains most of the protein bound to sugar as a manoprotein, and contains lipids and chitin and polysaccharides type β-1,3-glycans and β- 1,6-glycans which function to strengthen cell structure and as food reserves (7,8). In addition, S. cerevisiae is a yeast that is easily obtained and safe to use. Besides yeast, fungi is one of the potential β-glucan sources and Aspergillus is mold that has been commonly used to produce fermentative food products. Aspergillus cell wall is composed of polysaccharides and proteins, where the polysaccharides contain β-glucan, α-glucan, galactomannan and chitin chains (9). The production of β-glucan also shown by bacteria as secondary metabolites and are produced in the after-stationary phase (10). Xanthomonas campestris is gram-negative bacteria that has been known to produce β-(1,2) glucan, while Bacillus natto is gram positive bacteria that produces β-glucanase enzymes (11,12). Different types of microorganisms will show significant β-glucan characteristics because of their metabolic processes. Metabolism of various microorganisms will produce different amounts and characteristics of β-glucans (4).

β-glucan has been known showing many beneficial biological activities and one of the potentials β-glucan biological activities is antioxidant activity. The antioxidant activities of β-glucan were varies including the anti-lipid peroxidation-reduction ability, scavenging effects on hydroxyl radicals and superoxide anions which dominated with the scavenging activities (13). β-glucans have a few hydroxyl groups that help in bond arrangement with responsive groups of different compounds, prompting changes in their water dissolvability, adaptation, and aggregates capacity (1). It has been proposed that radical scavenging ability was identified with the quantity of hydroxyl groups in polysaccharides that can be shown with the quantity of branches present in the β-D glucans (14,15).

Despite the fact that the mechanisms of β-glucan scavenging activity especially towards the hydroxyl radicals are not yet explained, the different structures of β-glucan may be associated with the antioxidant activity. β-(1,3-1,4)-glucan that extracted from barley and also can be found on mold shown better hydroxyl radical scavenging activity than β-(1,3-1,6)-glucan that extracted from yeast (16). This article will explain the production of β-glucan from various microorganisms such Saccharomyces cerevisiae as yeast, Xanthomonas...
Campestris and Bacillus natto as bacteria and Aspergillus sp. as mold, the potential as antioxidant.

**The Potential of Microorganisms in β-Glucan Production**

β-Glucan production from *Saccharomyces cerevisiae*

*Saccharomyces cerevisiae* is a type of yeast that is known to synthesize β-glucans on its cell wall. The structure of the *S. cerevisiae* cell wall contains proteins that are bound to glucose components as glycoproteins and mannoproteins, and contain mannan, chitin, and polysaccharides of type β-1,3-glucans and β-1,6-glucans which has a function to strengthen cell structure and as a food reserve (17). The structure of the *Saccharomyces cerevisiae* cell wall can be seen in Figure 1.

**β-Glucan Production from Xanthomonas campestris**

Bacteria have the ability to produce various types of polysaccharides such as capsules and extracellular products. Polysaccharides are generally produced by bacteria in response to environmental stresses (24). Polysaccharides derived from bacteria can contain monosaccharide units that are repeated (homo-polysaccharide) or a number of different monosaccharides (hetero-polysaccharide). Most of these polysaccharides have a bacterial origin and are widely used for food and industrial applications because of their rheological and gelling properties (4). Exopolysaccharide bacteria (EPS) xanthan, curdlan, and gellan have been approved by the Food and Drug Administration (FDA) (10). According to Zeković et al. (25), these polysaccharides have β-(1,3)-glucans, β-(1,2)-glucan, and cellulose. β-glucans are resulted by gram negative bacteria namely Xanthomonas campestris (6). Figure 3 shows X. campestris can produce a unique polysaccharide in the form of cyclic β-glucan and contains 16 residues of Glucuronosyl (Glc-p)-15 of which β-binds with C-2 from subsequent residues and one of them α-binds to C-6 from subsequent residues (26). Research of β-(1,2)-glucans production from X. campertis is still lacking, therefore further studies are needed for X. campertis.
**β-Glucan Production of Bacillus sp.**

*Bacillus* sp. is gram-positive bacteria which is interpreted to have a thick peptidoglycan component on the cell wall (27). *B. natto* is generally used to produce microbial fermentation products, that also can be used to produce enzymes in industrial environments (28). *Bacillus* sp. strain has been known to produce the β-glucanase enzyme, where the enzyme can hydrolyze β-glucan. Gummadi and Kumar (29) has obtained β-glucans from the genus *Bacillus subtilis*, although in small amounts. Based on its mechanism of action, β-1,3-glucanase enzyme is classified into 2, that was a β-1,3-exoglucanase enzyme (β-1,3-glucan glucanohydrolase EC 3.2.1.58) and β-1,3-endoglucanase enzyme (β-1,3-glucan glucanohydrolase EC 3.2.1.6 or EC 3.2.1.39) (30). The β-1,3-endoglucanase enzyme works randomly hydrolyzing β-1,3-glucan chain being 2 to 6 glucose units, whereas the β-1,3-exoglucanase enzyme hydrolyzes β-1,3-glucan chain by releasing the monomer glucose from a nonreductive side (31). The released bond during hydrolysis is a β-1,3-glucosidic bond.

**β-Glucan Production from Aspergillus sp.**

Aspergillus sp. is one type of mold that can be used in the β-glucans production. *Aspergillus* sp. is everywhere as a saprophyte. The colonies that easily produce spores. its color becomes yellow, brown, green, or black, and the mycelium which was originally white is no longer visible (32). Aspergillus is commonly used in food fermentation. *Aspergillus wentii*, *Aspergillus oryzae* is an important species in the fermentation of some traditional foods and for producing enzymes. *Aspergillus oryzae* is used in the first stage of making soy sauce and tauco fermentation. *Aspergillus oryzae* is also a mold that has been recognized as GRAS (Generally Recognized as Safe). Figure 3 shows the *Aspergillus* sp. structure of mycelium and conidia.

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**Figure 3. Cell Wall Structure of Aspergillus sp. (33)**

A. *oryzae* commonly do not use for the production of β-glucans, but other species such as *Aspergillus fumigatus* and *Aspergillus niger* are used to produce β-glucan. Garcia-Rubio et al. (34) says the cell wall component of *Aspergillus* sp. is a polysaccharide (at least 90%) and protein. The mycelium and conidia of *Aspergillus* sp. contain a polysaccharide consisting of linear β-(1,3)-glucans (20-35%) branching with β-(1,6)-glucans (4%) chains; linear chain β-(1,3/1,4)-glucans (10%); α-(1,3)-glucans (45-56%); chitin and galactomannan (20-25%) (33).

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**BIOSYNTHESIS OF β-GLUCANS**

Douglas (35,36), say β-1,3-glucans obtained from fungi are synthesized through complex enzymatic reactions. β-Glucan synthetase in the plasma membrane catalyzes the synthesis of β-glucans from UDP-glucose according to the following reactions (37):

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\text{UDP-glucose} + (\beta-(1,3)\text{-glucose})_n \rightarrow \text{UDP} + (\beta-(1,3)\text{-glucose})_{n+1}
\]
Glucose is a substrate that is highly utilized by fungi. Glucose will enter the fungi cells through active transport, then cells will then begin the synthesis of polysaccharides and produce β-glucans (9). β-glucans produced from the bacteria also known as curdlan. Curdlan biosynthesis consists of 3 stages; substrate absorption, metabolism, and polymerization (38). The substrate, which is mostly glucose enters the cytoplasm of cells through active transport, at the stage of substrate metabolism are being catabolism form primary metabolites and precursors for the synthesis of Extracellular polymeric substance (EPS). Glucose glycolysis starts from phosphorylation with hexokinase enzyme in the release of ATP to form Glucose-6-Phosphate, then converts to Glucose-1-Phosphate which occurs in phosphoglucomutase. The key precursor (UDP-glucose) then formed through the catalytic of UDP-glucose phosphorylase from UTP. Glucoolysyl-1-Phosphate from UDP-glucose containing D-glucose which is bound to lipid precursors (isoprenoid-lipid-phosphate) releases UDP which initiates polymerization of β-(1,3)-glycosidic bonds and stripped polymers from UTP by utilizing ATP from the tricarboxylic acid cycle or glycolysis (10,38). The cyclic process continues and synthesizes curdlan. Biosynthesis from curdlan can be seen in figure 4.

**Figure 4.** The biosynthetic pathway for the synthesis of curdlan (10)

**UTILIZATION OF β-GLUCAN AS AN ANTIOXIDANT COMPONENT**

β-Glucans can be an antioxidant component, where β-glucan also has other biological activities, namely as antitumor, anti-cholesterol, and immune system enhancer (20,39). There are many benefits of β-glucan for humans which become an added value to develop innovation using of β-glucan. Non-pathogenic and non-toxic properties of β-glucans are supported by data from the Food and Drug Administration (FDA) which categorizes β-glucan as Biological Defense Modifier (BDM) and Generally Recognized as Safe (GRAS) (40). In this case, β-Glucan as BDM is interpreted as activating the immune system, while categorized as GRAS is interpreted as β-Glucan does not have toxicity or side effects when consuming it (41). The β-Glucan structure can be seen in Figure 5.

β-glucan has been known demonstrating numerous biological advantageous such as antioxidant activities which shown by the scavenging activities. The couple hydroxyl group in β-glucans can make bond game plan with responsive group including the free radicals or superoxides anions (1). It has been suggested that free radical scavenging activities was related to the amount of hydroxyl groups in polysaccharides that can be appeared with the amount of branches present in the β-D glucans (14,15). The various structures of β-glucan might be related with the antioxidant activity, β-(1,3-1,4)-glucan that removed from barley and furthermore can be found on mold demonstrated preferable hydroxyl radical scavenging activity over β-(1,3-1,6)-glucan that extricated from yeast (16).
β-Glucan as an antioxidant is a glucan component that has free electrons from its oxygen, these free electrons can bind free radicals which also have free electrons, so free radicals cannot damage the body's biological cells (43). Without β-Glucan, free radicals will make bonds with the body's biological cells and contaminate these cells to be inactive, this is because the unstable free radicals components that drive free electrons to make bonds with the body's macrophages (44). The contamination of the body's macrophage cells results in inactivation of a cell or cell death.

CONCLUDING REMARKS
Variations in microorganisms will present a variety of characteristics and contents of β-glucans. The cell wall component of S. cerevisiae has β-1,6-glucan 5-10% and more than half the cell wall (50-55%) is composed of β-1,3 glucan. Whereas in Aspergillus sp. contain β-1,3-glucan (20-35%) branched out with β- (1-6) –glucan (4%); linear chain β- (1-3 / 1-4) -glucan (10%). However, the composition of the cell walls of Aspergillus sp. consisting of conidia and mycelium both have β-1,3-glucan component and the cell particle size is much larger compared to S. cerevisiae. So that each cell of Aspergillus sp. contains greater cell biomass compared to S. cerevisiae even though the composition of β-glucan of S. cerevisiae is greater than that of Aspergillus sp. X. campestris and Bacillus sp. produce β-glucan from secondary metabolites, where secondary metabolites are not essential for the growth or reproduction of the organism itself and are only produced in small amounts.

REFERENCES

Figure 5. β-Glucan Structure (42)


