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Original article

Evaluating comparative β-glucan production aptitude of *Saccharomyces cerevisiae, Aspergillus oryzae, Xanthomonas campestris,* and *Bacillus natto*

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ABSTRACT

β-glucan is a natural polysaccharide derivative composed of a group of glucose monomers with β-glycoside bonds that can be synthesized intra- or extra-cellular by various microorganisms such as yeasts, bacteria, and moulds. The study aimed to discover the potential of various microorganisms such as *Saccharomyces cerevisiae, Aspergillus oryzae, Xanthomonas campestris,* and *Bacillus natto* in producing β-glucan. The experimental method used and the data were analyzed descriptively. The four microorganisms above were cultured under a submerged state in Yeast glucose (YG) broth for 120 h at 30 °C with 200 rpm agitation. During the growth, several parameters were examined including total population by optical density, the pH, and glucose contents of growth media. β-glucan was extracted using acid-alkaline methods from the growth media then the weight was measured. The results showed that S. cerevisiae, A. oryzae X. campestris, and B. natto were prospective for β-glucans production in submerged fermentation up to 120 h. The highest β-glucans yield was shown by B. natto (20.38%) with the β-glucans mass of 1.345 ± 0.08 mg and globular diameter of 600 μm. The highest β-glucan mass was achieved by A. oryzae of 82.5 ± 0.03 mg with the total population in optical density of 0.1246, a final glucose level of 769 ppm, the pH of 6.67, and yield of 13.97% with a globular diameter of 1400 μm.

1. Introduction

β-Glucans can be produced by microorganisms like *Saccharomyces cerevisiae, Pediococcus parvulus 2.6, Aspergillus* spp., *Oenococcus oeni IOEB0205, Xanthomonas campestris, Lactobacillus diolivorans G77, Lasiodiplodia theobromae, Botryosphaeria rhodina, Bacillus natto,* etc., which have commercial values (Abd El Ghany et al., 2016; Pérez-Ramos et al., 2018; Philippini et al., 2019). The biological activities of β-glucans are widely known, including enhancing the body immunity; antitumor, antibacterial and antiviral abilities, and role in wound healing (Özcan and Ertan, 2018; Vetvicka et al., 2019; Chaichian et al., 2020). The molecular weight (21–3100 × 10^3 g/mol) and conformation of β-glucan structure can determine their biological activities (Wang et al., 2017; Philippini et al., 2019). β-glucans have the ability to modify functional attributes of the food product including rheology, viscosity, texture, and sensory properties. They have been successfully used as fat replacers in meat and baked food products (Zechner-Krpan et al., 2010; Kaur et al., 2020). β-glucans can be produced from yeasts or mould cell walls and secondary metabolites of bacteria (Bashir and Choi, 2017; Yoshimi et al., 2017). Yeast and moulds can synthesize β-glucans in the cell wall which serves to strengthen the cell structure and acts as a food reserve. Pengkumsri et al. (2017) reported that as much as 3.72 ± 0.31 g/L β-glucans can be obtained from *Saccharomyces cerevisiae*. Thontowi (2007) also used *S. cerevisiae* and obtained 733.33 ppm β-glucans. On the other hand, the cell wall of moulds consists of

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linear chains of chitin and galactomannan (20–25%) β-(1–3)-need (20–35%) branched out with β-(1–6)-need (4%) chains; linear chain β- (1–3/1–4)-need (10%); α (1–3)-need (45–56%) and protein (Abad et al., 2010). Moulds belonging to the genus *Aspergillus* can be used for β-glucan production (Odabasi et al. 2006). Paulraj et al. (2012) obtained a dry weight of 0.78 g of *A. niger mycelia* and a dry weight of 0.5 g of β-glucan. Another species of *Aspergillus* that can be used for β-glucan production is *A. oryzae*. In addition to moulds and yeasts, bacteria can synthesize β-glucans by producing polysaccharides and other extracellular products. York (1995) reported that *X. campestris* produces polysaccharides in the form of cyclic β-glucans which contained 16 glucuronosyls (Glc p) residues, 15 of which are β-bound to C-2 (B-(1–2) of subsequent residues and one of them α-binds to C-6 (A-(1–6) of the next residue. Gummadi and Kumar (2005) also reported the production of 3 g/L β-glucans from *B. subtilis*. Another gram-positive bacterium of this genus, *B. natto*, which is used to prepare natto, also has the potential to produce β-glucans.

The production of β-glucans from microorganisms is largely determined by the population and is influenced by the condition of the growth media, especially pH and glucose content. It has been reported that *S. cerevisiae* and *A. oryzae* populations can affect the yield because β-glucans are arranged in their cell walls (Lipke and Ovalle, 1998). However, *X. campestris* and *B. natto* synthesize β-glucans through secondary metabolites; therefore, an increase in their population will not affect the yield of β-glucans. *S. cerevisiae* has a particle size of 5–6 μm, and its genome is approximately 12 Mb. *A. oryzae* has a particle size of 37.6 Mb, which is larger than that of other *Aspergillus* species (Machida et al., 2008). *X. campestris* has a particle size of 0.4–1.0 μm in width and 1.2–3.0 μm in length. Valasques Junior et al. (2014) reported that *X. campestris* genome size was 5.3 Mb. Furthermore, *Bacillus* cells are generally rod-shaped, with a length of approximately 4–10 μm and a diameter of 0.25–1.0 μm (Yu et al., 2014). The genome size of *B. natto* is 4.1 Mb (Tan et al., 2016). Differences in microorganisms will, thus, determine the characteristics of β-glucans produced due to differences in their respective sources (Zhu et al., 2016).

The research aimed to determine the prospects of microorganisms such as *S. cerevisiae*, *A. oryzae*. *X. campestris* and *B. natto* in producing β-glucans so as to improve our current knowledge and understanding with context to amount of β-glucans production, their structure and other related technical characteristics from these novel strains for their prospective applications, direct and/or indirect in diverse food systems.

2. Materials and methods

2.1. Microorganisms and growth media

This study used a culture of *S. cerevisiae* obtained from Fermipan® (Lesaffre Yeast Corporation, Wisconsin, USA) and the cultures of *A. oryzae*, *X. campestris,* and *B. natto* that obtained from the Bioprocess Laboratory of the Faculty of Food Science and Nutritions Universiti Malaysia Sabah. The gene accession numbers of *S. cereviseae, A. oryzae, X. campestris,* and *B. subtilis natto* are FNCC 3210, ATCC 10124, ATCC 33913, and ATCC 15245, respectively. The working culture was made by streaking stock culture from slanted agar into potato dextrose agar (PDA) for *A. oryzae* and *S. cerevisiae,* while nutrient agar (NA) used for *X. campestris* and *B. natto*, then incubated for 48 h at 37 °C. Each isolate was grown in a yeast extract glucose (YG) broth containing 15 g/L glucose, 5.2 g/L K_2HPO_4, 3.18 g/L KH_2PO_4, 0.12 g/L $MgSO_4$, 0.5 g/L yeast extract and 0.54 g/L NH_4Cl (Pengkumsri et al., 2017). One loop was placed into the growth media and incubated at 30 °C at an agitation speed of 200 rpm for 120 h. The common temperature (30 °C) was used for culture incubation to avoid the temperature effect on β-glucan yield.

2.2. Growth of microorganisms

The optical density of the culture was measured by collecting 1 ml from the growth media and then diluting it to 10^{-2}. The optical density indicates the number of cell populations measured using wavelength absorbance. The samples were analyzed at an absorbance of 600 nm. The optical density was evaluated at 0, 24, 48, 72, 96, and 120 h according to the Gompertz method (Mytilinaios et al., 2012).

2.3. pH change

The pH of the growth media was evaluated at every 24 h of incubation, starting from 0 h to 24, 48, 72, 96, and 120 h. For this, 2 ml of sample was collected and placed in a beaker to measure the pH using a calibrated pH meter (Eutech pH 2700) (Salari and Salari, 2017).

2.4. Estimation of glucose level

Glucose levels indicate the presence or absence of the growth of microorganisms. Glucose levels were measured at every 24 h of incubation, starting from 0 h to 24, 48, 72, 96, and 120 h. The glucose content was determined by the phenol–sulfuric acid method on a spectrophotometer at a visible light of λ 490 nm (Guo et al., 2019).

2.5. β-glucan extraction

Extraction of β-glucan was conducted according to the method described by Pengkumsri et al. (2017). The incubated cell biomass was collected by centrifugation at a speed of 7500 rpm for 10 min at 4 °C. Then, the cells were autolysed by adding 15% of cell biomass into distilled water at pH 5.0 (adjusted using 1.0 M HCl) and then incubated at 50 °C for 48 h at an agitation speed of 120 rpm. This was followed by an additional incubation step at 80 °C for 15 min in a water bath. Then, the samples were centrifuged at a speed of 7500 rpm at 4 °C for 10 min. Finally, the obtained pellets were dried in an oven at 60 °C.

The autolyzed cells were mixed with 5 ml of 1.0 M NaOH and then incubated at 80 °C for 2 h with a stirrer. Subsequently, the cells were centrifuged at a speed of 6000 *g* for 25 min at 4 °C. The obtained pellet was dissolved in 5 ml of 1.0 M CH_3COOH and re-incubated at 80 °C with a stirrer for 2 h. The pellet was then centrifuged again at a speed of 6000 *g* for 25 min at 4 °C. It was washed three times with sterile water and then freeze-dried. The obtained samples were mashed using mortar until a fine powder was obtained and stored at −20 °C.

2.6. Characterisation of β-glucan microstructure

β-glucans were prepared with Aluminium-Palladium coating and carbon tape affixed to the mounted specimen. Then, it was adjusted to the surface height of the sample and the surface height of the specimen holder. The sample was tightened with the appropriate couplers. Visualization and photography of samples were performed using a JEOL JSM–6360LA electron microscope at an acceleration voltage of 10 kV (Theis et al., 2019).

2.7. Statistical analysis

Descriptive statistical analysis was used to represent the data that collected from each sample. The mean and standard deviation

were calculated for the growth, pH, glucose level, and cell mass, and β-glucan weight, also for the β-glucan yield. The data of growth, pH, and glucose level were represented in a curve to show the changes during observation.

3. Results

3.1. Growth of S. cerevisiae, A. oryzae, X. campestris, and B. natto

As shown in Table 1, *X. campestris* has the largest number of inoculums of 4.9×10^7 cfu/ml, and *A. oryzae* has the smallest inoculum number of 4.3×10^2 cfu/ml. Fig. 1 shows the growth curves for *S. cerevisiae*, *A. oryzae*, *X. campestris,* and *B. natto* plotted using the optical density values. Each microorganism exhibits a growth phase and a significantly different absorbance value. This absorbance value represents the number of microbial cells (Mytilinaios et al., 2012). The higher number of microbial cells obtained more amount of β-glucans produced (Pengkumsri et al., 2017). It can be seen from Fig. 1 that *S. cerevisiae*, *X. campestris,* and *A. oryzae* experienced a logarithmic phase or exponential growth at 96 h, however, *B. natto* experienced this phase at 24 h. Eventually, the stationary phase was experienced by *S. cerevisiae* at 120 h and by *B. natto* at 24 h.

3.2. pH change in the growth media of microorganisms

The changes in the pH level due to the fermentation of each microorganism for a period of 120 h are depicted in Fig. 2. It can be noticed that in the initial hours, the pH of the *S. cerevisiae* medium was decreased, but after 72 h, it was increased. Compared with other microorganisms, *X. campestris* showed the largest decrease in pH; the lowest pH of 3.61 was obtained at 72 h.

Table 1
Total plate count of microorganism inoculum.

Microbial culture	Total plate count (cfu/ml)
S. cerevisiae	1.28×10^7
X. campestris	4.9×10^7
B. natto	7.0×10^6
A. oryzae	4.3×10^2

3.3. Change in glucose level in the growth media of microorganisms

Glucose is a source of nutrients required for the growth of microorganisms. The changes in glucose levels in the medium of each culture for 120 h are shown in Fig. 3. It can be noticed that glucose levels in *S. cerevisiae* growth medium decreased at 72 h, for *X. campestris* at 96 h, for *B. natto* at 24 h while *A. oryzae* showed the greatest decrease in glucose levels compared with other microorganisms.

3.4. β-glucan from S. cerevisiae, A. oryzae, X. campestris and B. natto

Table 2 shows the fermentation results in the form of cell mass and β-glucan mass-produced from *S. cerevisiae*, *A. oryzae*, *X. campestris,* and *B. natto*. It can be stated that *A. oryzae* produced the largest amount of β-glucans and cell biomass, i.e. 82.5 and 590.65 mg, respectively. *X. campestris* produced the smallest amount of biomass and weight of β-glucans, i.e. 0.785 and 4.445 mg, respectively.

3.5. β-Glucan microstructure

The results of scanning electron microscopy regarding the microstructure of β-glucans are depicted in Fig. 5. Based on the appearance of the sample, the extracted β-glucans had an irregular and smaller particle size distribution. At 30× magnification, the average β-glucan particle sizes of *S. cerevisiae*, *A. oryzae*, *X. campestris,* and *B. natto* were 500, 1300, 550, and 400 μm, respectively. However, regarding globular measurements, β-glucans obtained different magnifications of each fermentation media. Globular β-glucans of *S. cerevisiae* had a diameter of 500 μm measured at 250× magnification. Globular β-glucans of *A. oryzae* showed a diameter of 1400 μm at 70× magnification. Those of *X. campestris* had a diameter of 305 μm at 400× magnification. Finally, *B. natto* globular β-glucans exhibited a diameter of 600 μm at 200× magnification.

4. Discussion

4.1. Growth of S. cerevisiae, A. oryzae, X. campestris, and B. natto

S. cerevisiae underwent an adaptation phase until 72 h with an increase and a decrease in the optical density values, which could have been caused due to the difference in the osmotic pressure between the fluids in *S. cerevisiae* with media suspension. Accord-

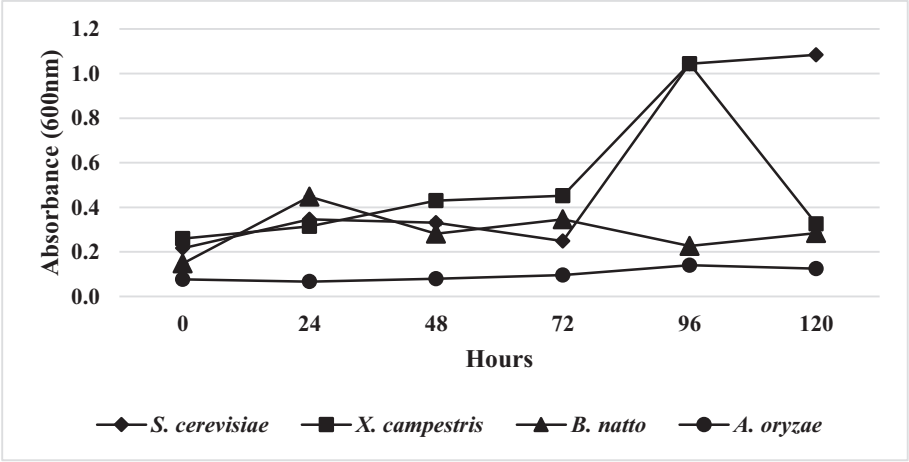


Fig. 1. The growth of microorganisms.

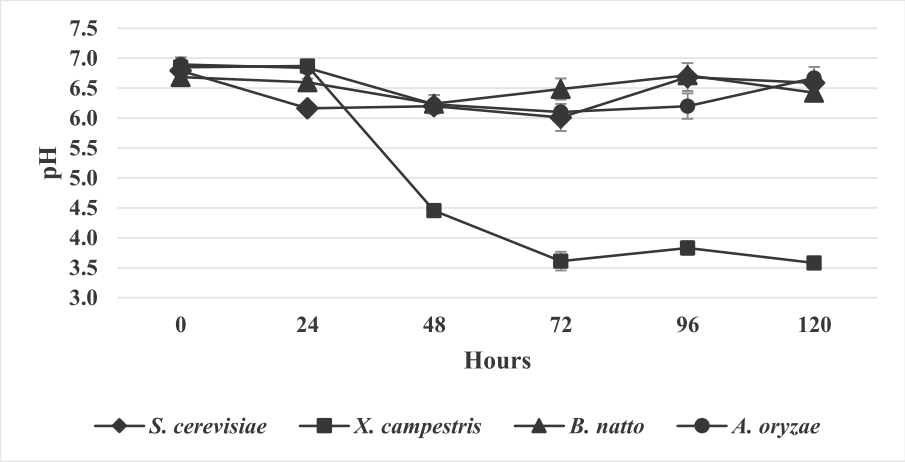


Fig. 2. pH Changes of Growth Media.

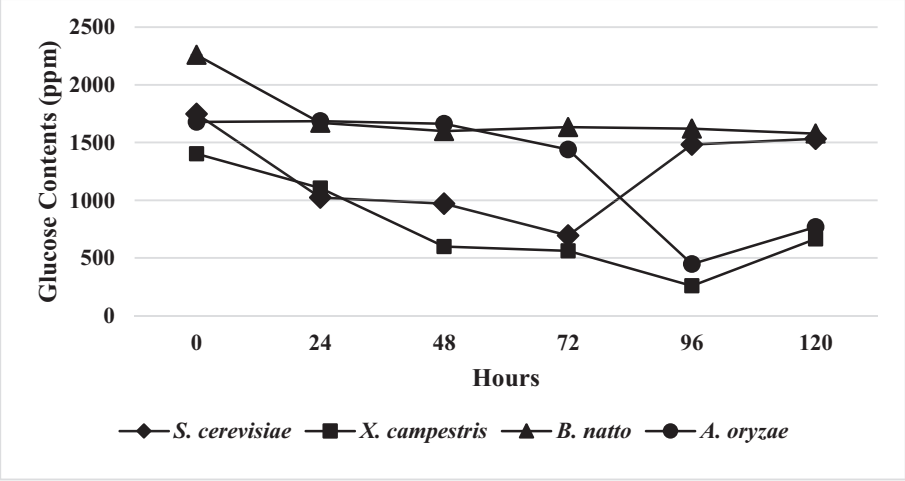


Fig. 3. Glucose contents in the growth media.

Table 2
Microorganisms with their cell mass weight and β-glucan mass weight.

Microbial culture	Cell mass weight (mg)	β-glucan mass weight (mg)
S. cerevisiae	29.445 ± 0.02	3.945 ± 0.05
X. campestris	4.445 ± 0.02	0.785 ± 0.06
B. natto	6.600 ± 0.01	1.345 ± 0.08
A. oryzae	590.650 ± 0.02	82.500 ± 0.03

ing to Falcone and Mazzoni (2016), when yeast cells are placed in a hypertonic solution (rich in solute), plasmolysis occurs, i.e. the liquid inside the cell comes out through the plasma membrane into the outside fluid that has higher solute levels, thereby resulting in the death of the cell. *X. campestris* also undergone an adaptation phase until 72 h, but there was no decrease in the optical density values during this phase. *B. natto* experienced the shortest lag phase, which was till 24 h. Meanwhile, *A. oryzae* mould also experienced the lag phase till 72 h.

It has been reported that the difference in the lag phase time is generally determined by the number of cells inoculated, the appropriate physiological and morphological conditions, and the cultivation media needed (Haruta and Kanno, 2015). Other factors in this context include the size and phase of the initial inoculum, i.e. when the inoculum is collected during the stationary phase, the lag phase will be longer (Rolfe et al., 2012). Bacteria experience a faster lag phase than yeasts and moulds, which can be caused due to their faster multiplication time of approximately 20 min, whereas yeasts take approximately 90 min and moulds can take up to 8 h (Stratford et al., 2014).

S. cerevisiae, *X. campestris* and *A. oryzae* subsequently experienced a logarithmic phase or exponential growth at 96 h, when there was a rapid increase in the number of cells. However, *B. natto* experienced this phase at 24 h, in which the microbial cells divided rapidly and constantly followed the log curve. During the experimental process, a slight decrease in turbidity was observed; it is thought to be due to an increase in glucose concentration and may be also responsible for observing a rapid decrease in the number of bacteria. After 60 h, the increase of glucose contents is shown and the decrease of *X. campestris* that represented by turbidity also occurred. This is happened because of the increase of carbon and nitrogen ratio which followed by secondary metabolites production. High amount of carbon which is not balanced by the amount of nitrogen will resulting the decrease of cell yield and specific growth (Lo et al., 1997). The duration of the log phase is influenced by the previous phase. The longer the lag phase is, the longer the log phase will occur (Rolfe et al., 2012). Other factors that influence the rate of growth are the environmental conditions of the medium such as the temperature, incubation time, substrate

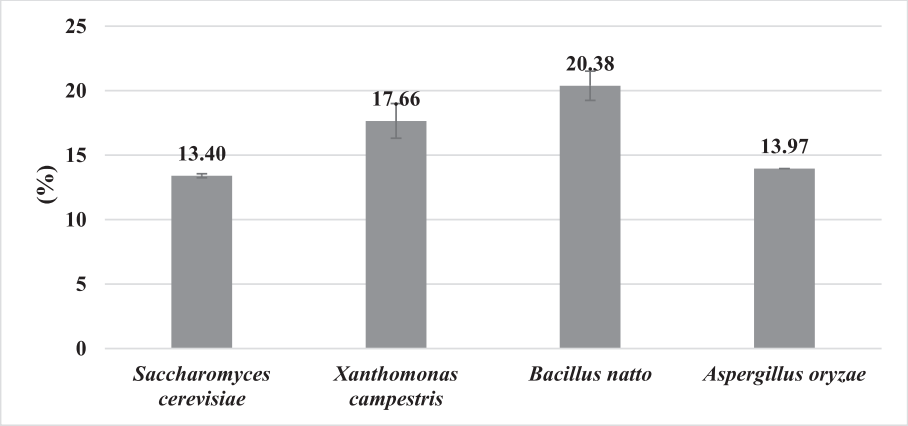
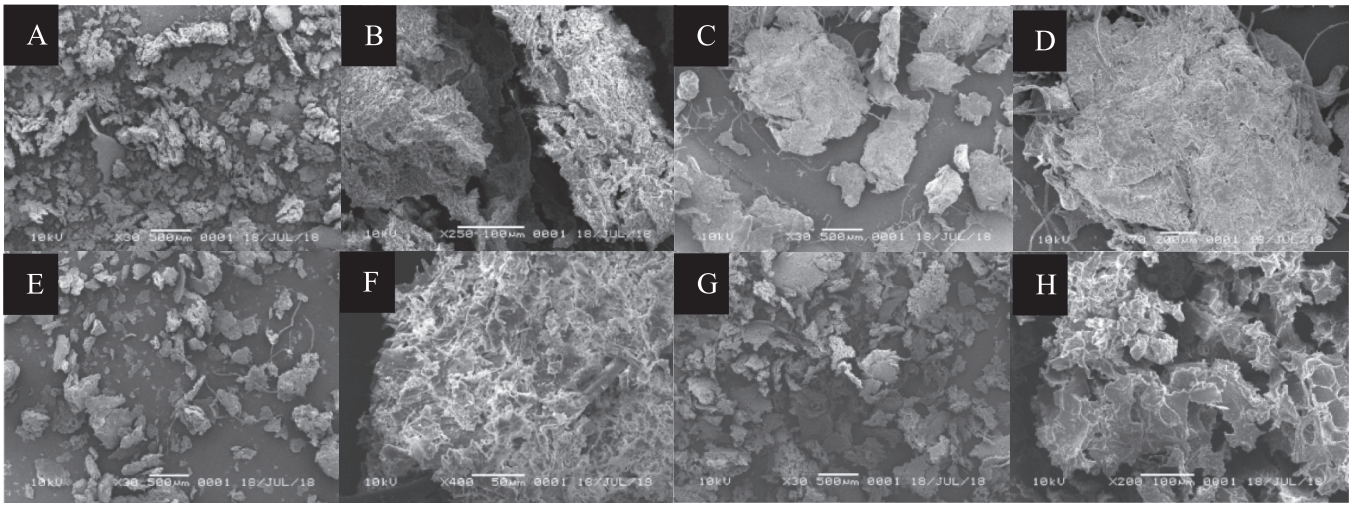


Fig. 4. Yield of β-glucan.



content, and pH (Brooks et al., 2011). In this study, each culture was incubated at 30 °C in a medium with the same substrate concentration. This is the optimal temperature for all cultures. *S. cerevisiae* grows optimally at this temperature, but the new log phase experienced at 96 h can be caused due to the lag phase experienced that was quite long. The *S. cerevisiae* cells produced under the new conditions were suitable for the new environment and could produce acids optimally for growth. At this stage, the logarithmic phase of *X. campestris* also occurs, which is because the optimum temperature for *X. campestris* growth is 26 °C (Ruissen et al., 1993). *B. natto* enters the logarithmic phase at 24 h. According to Dervaux et al. (2014), *Bacillus* has a characteristic feature of a rapid rate of self-division, and a common logarithmic phase is reached at 18–24 h.

After the logarithmic phase, microorganisms enter the stationary and death phases. The stationary phase as experienced by *S. cerevisiae* at 120 h and by *B. natto* at 24 h; this phase occurs at a rate of bacterial growth equal to the rate of death, so that the total number of bacteria remains the same (Allen and Waclaw, 2019). The optical density value for *B. natto* after 24 h was initially decreased and then increased. This could be due to the requirement of a high sufficient temperature by *B. natto*, which is approximately 50 °C. It has been reported that at this temperature, *B. natto* can multiply at the rate of 10^9–10^{10} cfu/g (Nout, 2015).

X. campestris and *A. oryzae* entered the death phase at 120 h, at which there was a decrease in the number of cells because some of the nutrients and energy reserves in the medium were depleted with the accumulation of toxic products (Zengler, 2009). At this stage, the amount of the enzyme produced by *A. oryzae* is reduced due to nutrient deficiencies and the accumulation of toxic compounds (Shafique et al., 2009).

The logarithmic phase is the optimum phase for producing β-glucans in yeasts and moulds such as *S. cerevisiae* and *A. oryzae* (Aimanianda et al., 2009; Yoshimi et al., 2017). This occurs because, in the logarithmic phase, the number of *S. cerevisiae* and *A. oryzae* cells produced is high so that it can produce β-glucans in high amounts as well. More number of *S. cerevisiae* and *A. oryzae* cells produced more amount of β-glucans, which is because β-glucans in *S. cerevisiae* and *A. oryzae* are found in their cell walls (Papaspyridi et al., 2018).

Synthesis of β-glucans in bacteria occurs during the post-stationary phase when nitrogen levels begin to deplete or the fermentation media contains excess carbon sources (Giordano, 2017). Therefore, the production of β-glucans from *X. campestris* and *B. natto* is more optimum at 120 h. Nonetheless, β-glucans could be extracted from the cell wall of *S. cerevisiae* and *A. oryzae* even when the cells are dead or alive (Zhu et al., 2016). Therefore, the extraction was carried out at 120 h to obtain the maximum β-glucan weight.

4.2. pH change in the growth media of microorganisms

The pH has an important role in the process of cell mass propagation of microorganisms that result in increased production of β-

glucans (Zhu et al., 2016). Some studies report that the optimal pH range for β-glucan production by bacteria is 5.5–7.0 (Kalyanasundaram et al., 2012). The decrease in pH in S. *cerevisiae* was caused due to the aerobic state of *S. cerevisiae*, at which glucose is converted into organic acids that render the fermentation media acidic (Abbott et al., 2009). According to Walker and Stewart (2016), the formed acids such as acetic acid, pyruvic acid, and lactic acid can reduce the pH, whereas other acids such as butyric acid and fatty acids have only a slight effect in decreasing the pH of liquids.

After 72 h, the pH of *S. cerevisiae* medium tends to increase as depicted in Fig. 2, which could be due to the decreased oxygen content in the medium and *S. cerevisiae* cells entering anaerobic conditions where the pyruvic acid produced previously are converted into ethanol and carbon dioxide. The ethanol produced is alkaline, which has a pH of 7.33, and thus the pH level of the medium increases (Salari and Salari, 2017).

X. campestris showed the largest decrease in pH compared with other microorganisms; the lowest pH of 3.61 was obtained at 72 h. de Mello Luvielmo et al. (2016) stated that *X. campestris* can use glucose and produce carboxylic acids such as tiglic acid, phenylacetic acid, isovaleric acid, 3-methylthiopropionic acid and *trans*-3-methylthiacrylic acid for up to 72 h. The production of these acids is characterized by a decrease in glucose level in the medium, which continues to decrease until 96 h till the remaining glucose residue reaches 263 ppm.

There was a decrease in pH in the *B. natto* medium until 48 h. Tuan et al. (2015) reported that *B. subtilis* utilizes glucose to produce acetic acid. Besides acetic acid, the metabolites produced from *B. natto* include white mucous substances that result from the formation of poly-γ-glutamic acid during fermentation (Chettri et al., 2016). An increase in pH after 48 h can occur due to prolonged fermentation. This can increase the concentration of ammonia in the medium (Ramos et al., 2000). Organic nitrogen is more easily used by bacteria to enhance their enzyme metabolism. In addition, calcium sources such as NH_4Cl are a driving factor for the formation of enzymes and other nutrients involving the biochemical reaction of bacterial cells (Kwon et al., 2011). The slight increase in pH can be caused by *B. natto* that can produce polyamine, which is a compound whose pH is near neutral (Kim et al., 2012).

The sugar consumed by *A. oryzae* causes the production of amino acids and organic acids (citric acid and gluconic acid, mainly) during fermentation, which affects the acidity of environment and decreases the pH (Lee et al., 2016). *A. oryzae* can tolerate acidic conditions and can thrive at a pH range of 3–7. However, the optimum pH for enzyme formation is 7.0. A lower or higher pH will affect the stability of extracellular enzymes and cause rapid denaturation (Robinson, 2015).

An increase in pH back after 72 h can be caused by the breakdown of sugar in the substrate into simple sugars with the help of the α-amylase enzyme, resulting in the formation of alcohol sugar (Baek et al., 2010). This alcohol sugar increases both the glucose level and pH of the medium (Balia et al., 2018). Yeast extract is a source of organic nitrogen suitable for producing the α-amylase enzyme. The primary metabolites produced are strongly influenced by their growth, which is influenced by the available nutrients (Shah et al., 2014). Meanwhile, secondary metabolism in *A. oryzae* uses acidic compounds to suppress the metabolic pathway, which allows *A. oryzae* to produce secondary metabolites that provide the ability to adapt to various environments (Lee et al., 2016).

4.3. Change in glucose level in the growth media of microorganisms

Glucose consumed by microorganisms is converted into metabolites such as acetic acid, ethanol, formic acid, and CO_2 (Singh et al., 2017). Yeasts and moulds use glucose as a constituent of cell walls consisting of β-glucans (Bashir and Choi, 2017). The metabolism involved in β-glucan formation in bacteria is also highly dependent on the glucose content (Zeković et al., 2005).

Glucose levels in *S. cerevisiae* growth medium decreased at 72 h (Fig. 3). Glucose is being the primary carbon source is absorbed through an active transfer process and is then metabolized to produce energy and synthesize cell-forming materials and metabolites (Saudagar and Singhal, 2004). A decreased in glucose level in the medium indicates the absorption of glucose by *S. cerevisiae* for metabolism and the formation of macromolecules such as β-glucans (Thontowi, 2007). The glucose level at 72 h also reached its lowest point because the greater sugar consumption was marked by a significant decrease in sugar concentration, and the greater cell growth was also marked by the significantly increased optical density values (Mytilinaios et al., 2012). Other nutrients such as nitrogen are used for protein synthesis in cells. The protein formed in this process can be used as an enzyme that plays a role in the formation of β-glucans (Thontowi, 2007).

A. oryzae showed the greatest decrease in glucose levels compared with other microorganisms. This implies that *A. oryzae* is highly dependent on carbon sources for its growth. Beauvais et al. (2014) reported that *A. fumigatus* utilize uridine diphosphate (UDP) glucose as a substrate in producing β-1,3-glucans to build a cell wall.

Yuliana (2012) stated that the higher the rate of bacterial growth, the lower the remaining reducing sugar. The most optimum carbon source for *X. campestris* is sucrose, followed by glucose, pyruvate, and fructose. Carbon dissimulates through glycolysis and forms nucleotide sugars, supports growth, and produces exopolysaccharides in high quantities (Roca et al., 2015). This can be observed from the logarithmic phase of *X. campestris* at 96 h with a low glucose level in the medium. Ruissen et al. (1993) reported that the growth of *X. campestris* reaches a stationary phase after 72 h. After 96 h, the level of glucose in *X. campestris* growth medium showed a slight increase, which could have been caused by other metabolites of *X. campestris* fermentation, such as xanthan. Xanthan is a polysaccharide compound synthesized by *X. campestris* through the Entner–Doudoroff pathway by utilizing glucose. This pathway catalyzes glucose into 2-keto-3-dioxy-6-phosphogluconate acid, which is then converted to phosphoenol pyruvate and pyruvic acid using enzymes produced from *X. campestris* (Lu et al., 2009). This compound is then used to produce xanthan. Therefore, the total sugar content in the medium increases again.

The glucose level in *B. natto* growth decreased at 24 h. The time point 24 h is the hour of the logarithmic phase, so that after this hour, *B. natto* exhibits no growth and the glucose levels remain unchanged. Glucose is synthesized by bacteria to be used as a secondary metabolite in the form of β-glucan (Dhivya et al., 2014).

4.4. β-glucan from S. cerevisiae, A. oryzae, X. campestris and B. natto

The largest amount of β-glucans produced by *A. oryzae* (Table 2) could be due to the presence of β-glucan bonds (1–3) and (1–6), which are obtained by cell walls in both conidia and mycelium (Beauvais et al., 2014). However, *X. campestris* and *B. natto* produced β-glucans only from the secondary metabolites and *S. cerevisiae* from its cell wall (Dhivya et al., 2014). *A. oryzae* converts glucose rapidly and most of the glucose is converted to form mycelium cell walls and their conides. In *Aspergillus*, β-1,3-glucans are synthesized by the membrane-bound plasma glucan synthase complex, which uses uridine diphosphate (UDP) glucose as the donor substrate and extrusion of β-1,3-glucan chain through the membrane to the periplasmic space (Beauvais et al., 2001).

Metabolism of β-glucan formation is in the presence of glucose, which is converted into glucose-6-phosphate wherein the presence of the enzyme phosphoglucomutase is obtained glucose-1-phosphate and decomposed into UDP glucose, which is a component of the yeast cell wall. One of the constituents of the cell wall is β-glucan (Aimanianda et al., 2009). However, in bacteria, UDP glucose and uridine monophosphate (UMP) can be used as precursors in the production of glucans. A decrease in pH can increase the concentration of intra-cellular enzymes such as β-1,3-glucanase, uridine-5′-triphosphate (UTP) glucose-1-phosphate uridylyltransferase, and phosphoglucomutase, which are the enzymes involved in the metabolism and synthesis of β-glucans (Dhivya et al., 2014). The process of the formation of fungal cell walls is influenced by elemental nitrogen obtained from the fermentation media in the form of amino acids and peptides, which can support the metabolism of the growth chains of the cell wall constituents (Yoshimi et al., 2017). Thus, the higher the glucose content, the higher the nutrients available for cell growth and β-glucan end products.

The yield of β-glucan mass and that of the cell mass obtained can be used to determine the percentage or yield of β-glucan produced based on the obtained cell. The highest percentage 20.37% of β-glucans was produced by *B. natto* (Fig. 4). It produces greater cell biomass and converts more glucose than *X. campestris*, although the optical density value of *X. campestris* was greater than that of *B. natto* at 120 h. This could be due to the secondary metabolites produced by *B. natto* in the form of β-1.3 glucans, which are not water-soluble (Lee et al., 2016). However, *X. campestris* produces the largest secondary metabolite in the form of xanthan gum, which is a polysaccharide compared with β-1.2 glucans (Kalogiannis et al., 2003).

In addition to the population, *B. natto* has a higher yield value compared to that of *X. campestris*, which could be because *X. campestris* enters the stationary phase only at 120 h, where at this time point extraction and new *X. campestris* produce secondary metabolites in this phase. However, *B. natto* enters the logarithmic phase at 24 h, and after this time point, the nutrient content in the *B. natto* culture is decreased and utilized by *B. natto* to produce secondary metabolites in the form of β-glucans. Therefore, *B. natto* has more time to produce β-glucans than *X. campestris*. The yield of β-glucans produced by bacteria, in general, has been reported to be approximately 6–7% (Kalyanasundaram et al., 2012). As such there is no data on the production of β-glucans from *B. natto*, but in one of the study it was reported that *B. subtilis* observed the production of 3 g/L β-glucans (Gummadi and Kumar, 2005). The commonly used yeast *S. cerevisiae* can produce a β-glucan yield of about 6–12% (Zechner-Krpan et al., 2010). *A. niger* can produce a yield of 9–11% (Paulraj et al., 2012). In a recent approach, a maximum fungal biomass of 0.76 mg/g with β-glucan at 9.34% (w/w) was obtained during solid-state fermentation of brown rice by *A. oryzae* KCCM 12698 (Ji and Ra, 2021).

4.5. β-Glucan microstructure

Structural differences can occur between samples because each microorganism has a different β-glucan bond (Zhu et al., 2016). *A. oryzae* has a larger cell size due to the shape of the cell consisting of mycelium and spores. Mycelium is a collection of several filaments known as hyphae. Each hyphae is 5–10 μm wide compared with normal bacterial cells (Hogan, 2006). Utama et al. (2019) reported that the size of yeast cells has a width ranging between 1 and 5 μm and a length ranging from 5 to 30 μm or more.

The difference in the size of these particles affects their biological activity. Utama et al. (2020) stated that the smallest microstructure is defined as having the highest solubility to produce a high biological activity. β-glucans produced from *X. campestris* have the smallest microstructure size, followed by those produced by *S. cerevisiae* and *B. natto*, and the lowest biological activity was found in the β-glucans from *A. oryzae*. Results indicate that the β-glucans produced from the microbes in each fermentation medium have different diameters (Fig. 5), whereas commercial globular β-glucans have diameters ranging from 5 to 100 μm (Piotrowska and Masek, 2015).

The four microorganisms despite having different particle sizes and shapes have a common appearance like sponges, i.e. a porous and rough texture and a cell wall structure that is not visible (Fig. 5). Limberger-Bayer et al. (2014) reported that commercial β-glucans have a similar texture and are more round and spongy in shape. Liu et al. (2015) and Singh et al. (2018) demonstrated that the microstructure of β-glucan extracted using an acid-base has characteristics similar to those obtained in this study. The porous particles can be caused due to the drying method used such as spray or freeze-drying. One of the benefits of porous particles is the increase or enrichment of β-glucans with drugs such as nano-drug precipitates or nanocrystals (Upadhyay et al., 2017).

In each globular observation produced similar characteristics between each β-glucan, but when viewed extensively the shape of the particles of each β-glucan have different sizes. The difference in the microstructure size of each β-glucan is due to the differences in bonds between microorganisms (Wang et al., 2017**).**

5. Conclusions

Based on the findings, it can be concluded that the studied microbial strains - S. cerevisiae, A. oryzae, X. campestris, and B. natto have potential for β-glucans production at the industrial level, but studies related to scalabilities must be conducted. The studied strains can be utilized as starter cultures in situ production of β-glucans in fermented foods. The development of such functional foods will improve the gut health of consumers by boosting the growth of probiotic cultures. Therefore, future research should be carried out to explore the production as well as utilization of β-glucans, derived from different novel microbial sources to deve
lop probiotic or functional foods.

CRediT authorship contribution statement

Gemilang Lara Utama: Conceptualization, Data analysis, Funding acquisitions, Writing review, and editing. **Casey Dio:** Data collection, Data analysis and Writing original draft. **Joko Sulistiyo** Resources and Supervising the data collection. **Fook Yee Chye:** Resources and Supervising the data collection. **Elazmanawati Lembong:** Supervising the data collection and Funding acquisition. **Yana Cahyana:** Supervising the data collection, Review and editing. **Deepak Kumar Verma:** Writing, review and editing of the manuscript. **Mamta Thakur** Writing, review and editing of the manuscript. **Smita Singh:** Writing, review and editing of the manuscript. **Ami R. Patel:** Writing, review and editing of the manuscript. All the authors approved the submission of this manuscript.

Author Contribution

Gemilang Lara Utama: Conceptualization, Data analysis, Funding acquisitions, Writing review, and editing. Casey Dio: Data collection, Data analysis and Writing original draft. Joko Sulistiyo Resources and Supervising the data collection. Fook Yee Chye: Resources and Supervising the data collection. Elazmanawati Lembong: Supervising the data collection and Funding acquisition. Yana Cahyana: Supervising the data collection, Review and editing. Deepak Kumar Verma: Writing, review and editing of the manuscript. Mamta Thakur Writing, review and editing of the manuscript. Smita Singh: Writing, review and editing of the

manuscript. Ami R. Patel: Writing, review and editing of the manuscript. All the authors approved the submission of this manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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