
The preliminary study of cell membrane stability of *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm strains related to the production of fruiting body

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Abstract One of the most widely cultivated edible mushroom in Indonesia is *Pleurotus ostreatus* (Jacq. Ex Fr.) P. Kumm or commonly called “white oyster mushroom”. The growth and fruit body formation of *P. ostreatus* is influenced by environmental factors, especially temperature. High temperature tended to cause disadvantages because it can reduce the rate of mushroom growth. Heat stress affects cell membrane function. The increased permeability and leakage of electrolyte out of the cell has been used as a measure of cell membrane stability (CMS) and as a screen test for stress tolerance. CMS can be done by measuring cell’s relative injury (RI). Based on result, the lowest damage was found in InaCC F10 strain (31,21% RI), while the highest damage was found in InaCC F209 strain (52,98%). Result stated that different types of *P. ostreatus* strains significantly affected the stability of the fungal cell membrane. Further test stated that InaCC F10 [RI=31,21(a)] and InaCC F216 [RI=32,11(a)] strains were the same effect and the lowest damage levels, while InaCC F196 [RI=47,18(c)] and InaCC F209 [RI=52,98(c)] strains were the same effect, but the highest damage levels. The biological efficiency (BE) was observed to determine the productivity of *P. ostreatus* strains. Result stated that different types of *P. ostreatus* strains significantly affected the productivity of mushroom. Further test stated that InaCC F10 was the highest productivity [BE=21.62% (c)], while the lowest productivity was found in InaCC F196 strain [BE=11.10%(a)] and InaCC F131 strain [BE=12,46%(a)].

Keywords: Biological efficiency, electrolyte leakage, heat stress, mushroom, relative injury

Introduction

Mushrooms have been widely known and various benefits for human life, one of them is the benefit in food source. One of the most widely cultivated edible mushrooms is *Pleurotus ostreatus* (Jacq. Ex Fr.) P. Kumm or commonly called “white oyster mushroom”. The growth and fruit body formation of *P.*

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ostreatus is influenced by environmental factors. One of the main factors that influence the growth and development of the mushroom is temperature. An environment that suitable for mushroom growth will determine the quality of its growth. (Sanchez, 2010 and Aliffahrana, 2012). High temperatures tend to cause disadvantages because it can reduce the rate of mushroom growth. This condition is a problem for regions that have high temperatures.

Temperature that is not optimal for the growth of *P. ostreatus* will reduce the level of mushroom production, therefore the selection of superior mushroom against high temperatures is important in mushroom production. According to Bajji *et al.* (2002) and El Basyoni *et al.* (2017), heat stress can occur suddenly and cause a decrease in crop yields in plants. Temperature is one of the factors that can cause stress in some organisms that cause disruption of the function of cell membrane stability. Cell membrane is one of the initial targets of environmental stress. Heat stress can interfere with the permeable selective of cell membrane, therefore cells cannot maintain their composition. Disruption of cell membrane permeability can cause electrolyte leakage which causes release of electrolyte from cell. The ability of cells to maintain membrane integrity and stability against stress conditions is a major component of organism's tolerance to environmental conditions. Increased permeability and leakage of ions out of cells were used as criteria to measure cell membrane stability which was also used as a screening method for heat stress tolerance.

Cell membrane stability or commonly called CMS is a trait that is used to study the response to environmental conditions such as heat stress (El Basyoni *et al.*, 2017). Nowadays, cell membrane stability considered as a character that related to heat stress. Research on cell membrane stability has been carried out in many plants, but not much in mushrooms. The similarity of cell structure between plant and mushroom's cell which are both composed of phospholipid bilayer is the reason for adopting the techniques for cell membrane stability test in mushroom. According to Agarie *et al.* (1995), many studies have found that cell membranes have an important role in dealing with environmental stress, especially in heat stress. According to El Basyoni *et al.* (2017), the stability of cell membranes can be used to select and produce superior genotypes that tolerate to heat stress. The cell with stronger character of cell membrane stability has a higher tolerance in handling environmental stress. Several studies were carried out previously with *in vitro* technique, to obtain superior genotypes that are tolerant to heat stress. The character of cell membrane stability is one of the traits that can be inherited from generation to generation and closely related to productivity. According to Beltrano and Ronco (2008), the character of cell membrane stability can be determined by measuring the conductivity of released electrolyte due to exposure at high temperatures.

Measurement of cell membrane stability can be done by measuring cell conductivity by testing using a heat stress. Cell conductivity that has been measured then calculated into relative injury (RI) value or damage to cells using the following equation:

$$\text{Heat_RI (\%)} = \left\{ 1 - \frac{1 - (T_1/T_2)}{1 - (C_1/C_2)} \right\} \times 100$$

The letters T and C respectively refer to the conductance values for heat and control tests, while subscripts 1 and 2 respectively refer to the initial and final conductance measurements. The higher RI value (%) indicates that the more electrolytes that come out means that cell damage is getting worse, on the contrary, the low RI value (%) shows that the electrolyte comes out a little and the cell becomes more stable (El Basyoni *et al.*, 2017).

In this study mushroom cultivation was carried out in areas with high temperatures, namely Sukra, Indramayu. According to Karsid *et al.* (2015), Indramayu district has a high daily temperature, the temperature can reach 34°C. Gaitan and Salmones (2008) argued that *P. ostreatus* cultivation would be relatively optimal at lower temperatures, it should be 16-25°C. Higher temperatures tend to cause disadvantages because they can reduce the productivity of mushrooms. This condition can be a problem for locations that have high temperatures. The high temperature will be a problem in the cultivation of *P. ostreatus*, because according to Zhararae *et al.* (2010) temperatures above 30°C are considered to be too high and thus damage the growth of mycelia. The damage will be more significant as the temperature increases.

Based on the previous explanation, CMS test needs to be selected superior strains for cultivation that are able to tolerate high temperature. The objectives of research were to determine the effect of strains on the cell membrane stability of *P. ostreatus* in heat stress conditions, to know which strains were the best cell membrane stability, to determine the effect of *P. ostreatus* strains on the production of fruiting bodies at high temperatures and to know which strains were the best in the production of fruiting bodies at high temperatures.

Materials and methods

Research material and experimental design

This study was conducted in two parts, the first was the determination of cell membrane stability of various *P. ostreatus* strains and the second was the cultivation of *P. ostreatus* strains. The objectives were studied the various strains of *P. ostreatus* obtained from the Indonesian Culture Collection (InaCC),

Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia (www.inacc.biologi.lipi.go.id). These strains are InaCC F10, InaCC F131, InaCC F196, InaCC F209, and InaCC F216. The five isolates were *P. ostreatus* strains originating from different regions, InaCC F10 strain is a strain originating from LIPI Cibinong, Indonesia; InaCC F131 and InaCC F 209 strains originating from Applied Plant Research (APR), The Netherland; InaCC F196 strain originating from Alam Mushroom Ciwidey – Bandung, Indonesia/Canada and InaCC F216 originating from Bandung, Indonesia.

The measurement of CMS was conducted in the Food Microbiology Laboratory, Indonesian Institute of Science, Cibinong and the cultivation of mushroom was conducted in Sukra, Indramayu regency, Indonesia.

The first stage experiment was designed using Completely Randomized Design (CRD), with treatments consisted of five strains and repeated three times. The second stage experiment was done using Randomized Complete Block Design (RCBD) with treatments consisted of five strains and it was divided into five group for each treatment.

Pure culture of P. ostreatus

The strains of *P. ostreatus* are maintained under paraffin oil. They were cultured on Potato Dextrose Agar (PDA) medium (Schlegel, 1993) and incubated at 25°C for 7 days for growing.

Preparing mycelium for heat treatment

The strains were cultivated in Potato Dextrose Broth (PDB) (Bai and Abraham, 2001) to obtain fungal mycelia. One plug of *P. ostreatus* mycelia (0.5 cm of colony diameter) was transferred to 300 ml of Erlenmeyer flask containing 100 ml of PDB medium and incubated using a rotary shaker incubator at 25°C and 80rpm for 4 days.

Heat treatment

The mycelia from PDB in each strain of *P. ostreatus* were harvested and transferred into test tubes, and then the mycelia were rinsed 3 times with sterilize distilled water. After that, mycelia were transferred to test tube added with 75 ml of sterilize distilled water. Heat treatment was done by soaking the test tube at 50°C for 1 hour in waterbath and control treatment (without heatshock) was left at 25°C (El Basyoni *et al.*, 2017).

Measurement of electrolyte leakage

After treatments, the test tubes were stored for 24 hours at 10°C in a dark to let the electrolyte diffuse out of the cells. Then, samples were warmed at 25°C. The conductivity was measured and the initial conductance value from heat treatment (T_1) and control (C_1) were obtained. Then, the test tubes were autoclaved at 121°C for 15 minutes to kill the cell and released the entire electrolyte from the cell. The test tubes were cooled to 25°C. Cell conductivity was measured again and the final conductance value from the heat treatment (T_2) and control (C_2) were obtained. The damage of cell was expressed in the percentage of relative injury (RI%) using the following equation (El Basyoni *et al.*, 2017):

$$\text{Heat_RI (\%)} = \left\{ 1 - \frac{1 - (T_1/T_2)}{1 - (C_1/C_2)} \right\} \times 100$$

Descriptions:

T_1 : initial conductance of heat treatment

T_2 : final conductance of heat treatment

C_1 : initial conductance of control

C_2 : final conductance of control

Spawn preparation

Spawn used in the *P. ostreatus* cultivation was containing 99% milled corn and 0,5-1% CaCO_3 as a substrate. It contained about 60% of water. Then, the substrate was put into a spawn bottle and sterilized at 121°C for 30 min. After that, the bottle was inoculated with an agar disk 6 mm in colony diameter of *P. ostreatus* and incubated at 25-30°C in the dark for 15 days.

Substrate Preparation for P. ostreatus cultivation

P. ostreatus was cultivated in sawdust with the addition of rice bran (15%), CaCO_3 (2%), CaSO_4 (2%) and corn flour (2%). The tap water was added to obtain moisture content of around 70% The polypropylene bags (17 x 35 cm) were filled with 1 kg of substrate, the substrate, called “baglog” then was sterilized using steamer at 90°C for 3 hours.

Cultivation and harvesting of P. ostreatus strains

After sterilization, the baglog was cooled down for about 16 hours. The spawn was inoculated in baglog and incubated for about 40 days until the mycelia grown in full the baglog. Substrate in the baglog covered with mycelia

was then transferred into incubation the room cotton cap of baglog was opened to initiate the formation of fruiting bodies. The fruit bodies were harvested and biological efficiency was calculated using the following equation (Apetorgbor *et al.* (2015):

$$\text{Biological efficiency} = \frac{\text{Total fresh fruiting body weight}}{\text{Total media weight}} \times 100\%$$

Statistical analysis

Data were statistically analyzed using analysis of variance (ANOVA) at $P = 5\%$. The difference between means was evaluated using Duncan Multiple Range.

Results

Cell membrane stability

Cell membrane stability against heat stress was measured by the amount of electrolyte release from cells due to cell membrane damage expressed in relative injury (RI) percentage. Relative cell membrane injury (RI) ranged from 31.21% to 52.98%. The lowest damage (the lowest RI) was found in InaCC F10 strain (31.21% RI), while the highest damage was found in InaCC F209 strain (52.98%). The results of cell membrane stability after CMS test in *P. ostreatus* strains of InaCC F10, InaCC F131, InaCC F196, InaCC F209, and InaCC F216 are completely shown in Table 1.

Table 1. The value of relative injury (%) in several strains of *P. ostreatus* after cell membrane stability test

Strains	Repetition			RI mean
	1	2	3	
F10	29,3940	28,8232	35,4140	31,21 (a)
F131	35,7777	44,5564	40,6335	40,32 (b)
F196	44,9386	48,9399	47,6475	47,18 (c)
F209	51,5305	58,8683	48,5345	52,98 (c)
F216	30,4518	32,5702	33,2941	32,11 (a)

Based on the results, it was found that the effect of different *P. ostreatus* strain on cell membrane stability was stated to be significant. It meant that the different types of *P. ostreatus* strains were significantly affected the stability of the fungal cell membrane. The character of cell membrane stability from each

strain or genotype in the same species showed different results (Table 1.) It can be seen that InaCC F10 and InaCC F216 strains were the same effect and lowest damage level, while InaCC F196 and InaCC F209 strains also showed the same effect, but in the highest damage level.

In addition to the main parameters observed in the first phase of the study, the diameter of hyphae was carried out using phase-contrast microscope and scanning electron microscope (SEM). The hyphae diameter was measured in all strains (InaCC F10, InaCC F131, InaCC F196, InaCC F209, and InaCC F216) with a mean value of each strains were 2.42 μm , 2.59 μm , 2.64 μm , 2.51 μm and 3.08 μm . Based on the results, the size of diameter of hyphae did not always in positive correlation with cell resistance. There were strains which, despite having small diameter values, actually was better cell membrane stability than other strains and *vice versa*.

Biological efficiency

After measuring the cell membrane stability of five strains in the first stage, the second stage of the research was the cultivation of strains in Sukra, a location that was high temperature. Before measuring the BE, mushrooms were harvested.

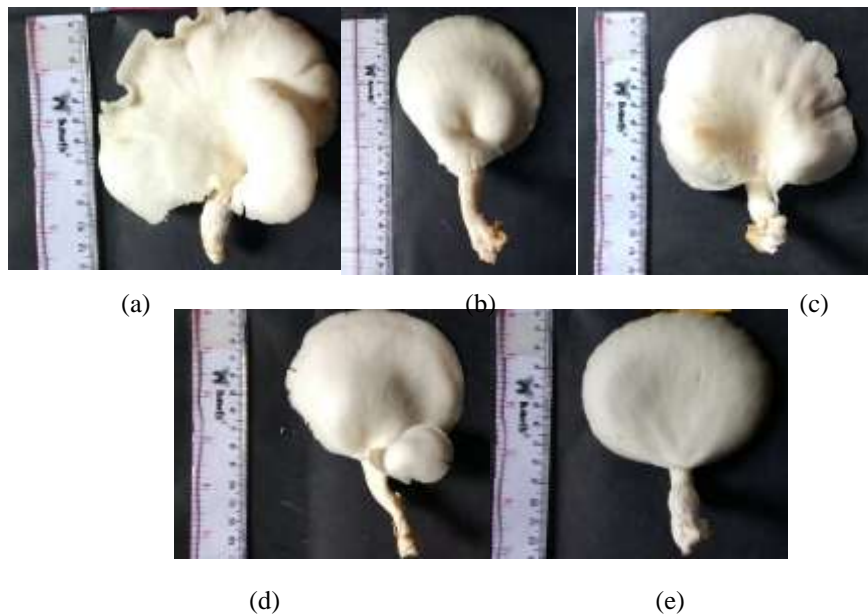


Figure 1. The fruiting bodies of *P. ostreatus* strains (a) InaCC F10, (b) InaCC F131, (c) InaCC F196, (d) InaCC F209, and (e) InaCC F216

The biological efficiency (BE) was observed to determine the productivity of *P. ostreatus* strains. The complete data of BE results are shown in Table 2.

Table 2. The value of biological efficiency (%) in several strains of *P. ostreatus*

Strains	Group				
	1	2	3	4	5
F10	18,00	24,00	24,10	18,00	24,00
F131	13,10	12,00	13,00	13,00	11,20
F196	11,00	10,30	12,00	10,20	12,00
F209	12,00	17,30	12,50	12,00	17,30
F216	13,90	13,80	22,00	13,90	20,30

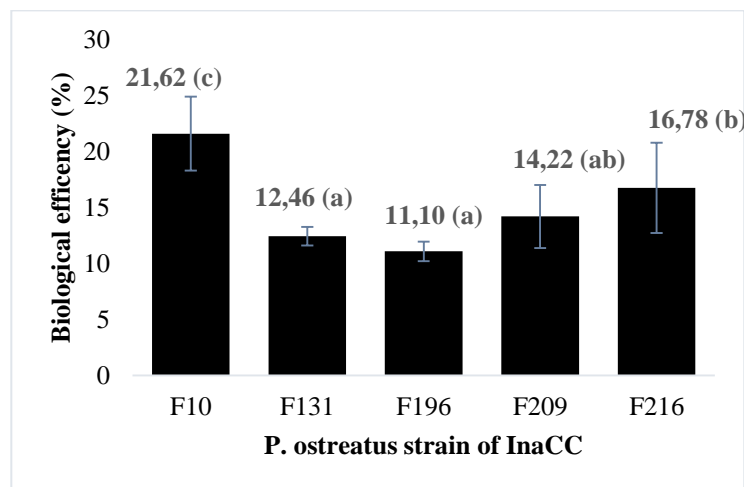


Figure 2. Diagram of biological efficiency (BE) means from several strains of *P. ostreatus*

The results showed that InaCC F10 strain had the highest BE mean (21.62%), while the lowest BE mean was found in InaCC F196 strain (11.10%). (Table 2). The results stated that the effect of different *P. ostreatus* strain on biological efficiency was significantly differed. InaCC F10 strain gave the highest value of biological efficiency compared to other strains (Figure 2).

Discussion

In this study, we observed the stability of membrane cells of various strains of *P. ostreatus* which evaluated from cell membrane damage caused by heat stress. The procedure is performed to determine cell membrane damage,

which is based on measuring electrolyte leakage from fungal mycelium cells. According to Bajji *et al.* (2002), cell membrane stability can be seen from the degree of cell membrane damage. The degree of cell membrane damage caused by environmental stress that can be estimated by measuring electrolyte leakage from the cell. Agarie *et al.* (1995) stated that changes in electrical impedence and electrolyte leakage can be a technique for detecting the level of cell membrane damage due to stress. These leaks are related to the ability of the membrane to retain cell fluid. The potential and permeability of the membrane could be changed because the damage due to environmental stress. Ali *et al.* (2009) added that the stability of cell membranes refers to the ability of cells or tissues to maintain the electrolyte inside them in stress condition, so that the cell does not suffer damage.

Results found that the differences in *P. ostreatus* strains affected the stability of the cell membrane. The character of cell membrane stability from each strain or genotype in same species can show different results. Based on previous research by Handayani *et al.* (2013) stated that the stability of cell membranes in 20 potato varieties showed that there were significant differences between varieties in same potato species. According to Arvin and Donelly (2008) and Handayani *et al.* (2013) stated that varieties or genotypes that lower cell membrane damage are related more tolerant against heat stress compared to varieties that higher cell membrane damage.

The results indicated that InaCC F10 and InaCC F216 were the strains with the lowest level of damage, while InaCC F196 and InaCC F209 strains were the species that the highest levels of membrane cell damage characterized by relative injury values. Mushroom strains were low percentage of damage in high temperature stress that better cell membrane stability and can protect cellular functions inside them. This possibility can occur because of the influence of the origin of the strain, according to Indonesian Culture Collection (2018). InaCC F10 and InaCC F216 are strains originated from Indonesia, while other strains were introduced from the Netherlands and a mixture of Indonesia/Canada which is an area relatively lower temperatures. The origin of InaCC F10 and InaCC F216 strains originating from Indonesia allowed the mushroom strain to be able to withstand the heat stress compared to other strains originating from the Netherlands and Canada. Indonesia is a tropical country that has a higher temperature than the Netherlands and Canada. Indonesian indigenous strains have better adapted to high temperature. Referring to the opinion of El Basyoni *et al.* (2017) stated that the genotype factors of each strain greatly affected the ability of an individual to heat stress.

Damage of the membrane resulted from exposure to heat. When a tissue is exposed to high temperature, electrical conductivity will increase due to an

electrolyte leakage from the cell due to damaged cell membranes and increased permeability. This condition causes the cell membrane unable to maintain electrolytes inside cells (Kumar *et al.*, 2012). Cell membranes are composed of the main components in the form of phospholipids and proteins which benefits to protect and support cell function. High temperature causes the two components to be disrupted, and further the thickness of the lipid membrane decreases and also the protein will be denatured. Damage to these components causes the cell membrane to lose its ability to maintain its function and the permeability will increase that causes the electrolytes in the cell released out of the cell (Wahid *et al.*, 2007 and Handayani *et al.*, 2013). Based on measurement of diameter of hyphae, the diameter of hyphae was not correlated with cell membrane stability. Genetic factors are thought to play a major role in the cell membranes stability. El Basyoni *et al.* (2017) stated that the ability to stresses will be different in each genotype.

Biological efficiency values obtained from research results are relatively low according to Fernandes *et al.* (2015). The value of biological efficiency (BE) is a parameter used to evaluate the productivity of mushroom cultivation. Mushroom production is good if the BE value reaches 70% or more. This condition can be caused by high temperature of cultivation location.

InaCC F10 strains that had the best cell membrane stability which showed the highest biological efficiency. This is because the strain is more resistant to high temperature compared to other strains. According to Handayani *et al.* (2013), organisms that higher cell membrane stability will be more tolerant and able to adapt to extreme environmental conditions than organisms with low cell membrane stability. This is strengthened by the statement of El-Basyoni *et al.* (2017) regarding the relation of CMS to crop yields on wheat, that the stability of cell membranes against heat stress of several wheat plant varieties influences the final crop's yield. Plant varieties that better character of membrane cell stability can provide better yields compared to less stable varieties. The yield will be directly proportional to the character of the cell membrane stability. It can be concluded the strain of InaCC F10 and InaCC F216 showed the best cell membrane stability, InaCC F10 was the highest biological (BE) value compared to other strains.

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