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THE EXPIRATION TIME OF STARTER COMPOSED OF INDIGENOUS BACTERIAL AND FUNGI CONSORTIUM ISOLATED FROM FERMENTATION FEED OF SELLULOSIC MATERIALS IN ROOM AND COLD TEMPERATURE

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Abstract

The aim of this study determined the storability of the starter which was composed of bacteria and fungi consortium isolated from fermentation feed of cellulosic waste at room and cold temperatures. Cellulosic waste were composed of water hyacinth (Eichhornia crassipes) and corn cobs (Zea mays) which were undergoing a fermentation process was isolated these bacteria and fungi. Furthermore, the bacteria and fungi were purified and given a symbol. Each isolate was tested for cellulolytic activity on CMC media. Isolates with large cellulolytic activity were selected as starter candidates. These candidates were tested for antagonism / synergism work each other. The isolates that were consorted to be starter were isolates with the largest cellulolytic activity and work synergistically. The starter expiration time was determined by the calculate the total number of microorganisms (TPC = total plate count) periodically. Furthermore, the calculation results was matched with the SNI standard for the number of microorganisms for starter (10^8 cfu / ml). The results of this research was obtained that the total microorganism content of each formula until storage for 65 days at room temperature at 10^{-13} level dilutions was 147,21 cfu/ml and at cold temperatures 181,37 cfu/ml. The conclusion that can be stated was that the starter is still active at room temperature storage and cold for 65 days. The expiration time of the starter at cold temperatures is longer than the room temperature.

Keywords: ***storability (expiration time), bacteria, fungi, indigenous, cellulosic.***

Introduction

Several studies on the using of water hyacinth, which is a wild water weed, that has damaging effects to various high-value economic materials through fermentation process have been widely carried out. Water hyacinth has been used as feed for rabbits (Phioneer, et al., 2016). Duck eggs which are one of the elements of the feed are water hyacinth, have high protein content (Sarian, 2016). Water hyacinth is used by red tilapia as feed (Okoye et al., 2002). According to Fitrihidajati *et al*., (2013), the fermented feed made from water hyacinth has high protein content and undigested fiber. The applications of the feed can trigger the increasing of goat biomass (Fitrihidajati *et al*., 2014). Suparno *et al*., (2015), has applied this fermented feed and produced goat meat that was high in protein and low in the fat. According to Ratnasari, *et al*., (2017) feed containing water hyacinth can trigger an increase in the number of children in female goats. Fermented water hyacinth feed can also improve the quality of male goat spermatozoa (Ratnasari, et al., 2018). The economic value of fermented feed made from water hyacinth can increase with the addition of corn cobs, which is agricultural waste that has not been widely used.

The protein content of corn cobs was 5.6%, still higher when compared to protein content of rice straw (4.9%). Therefore it is suitable for animal feed. So far, corn cobs are only used as a fuel (wood substitute), as a wood mushroom growing medium (Sarian, 2016). Somaoang uses it as animal feed (Sarian, 2016). Through the fermentation process, the corn cobs as a favorite food for pigs, goats and poultry (Sarian, 2016). The crushed corn cobs can also be used to replace sliced cassava as a feed for buffaloes (Wanapat, et al., 2012). Rostika and Safitri (2012) used crushed corn cobs and fermented with various molds for fish feed. Lardy and Anderson (2009) confirmed that corn cobs can be used as an alternative for ruminant feed.

The main problem in utilizing water hyacinth and corn cobs for fermentation feed is it take a long fermentation time. The problem solving for this case was using indigenous starter, that was done by isolation of indigenous microorganisms in traditional culture. The fermentation process always involves bacteria and fungi (Sha, et al., 2017). Likewise, the fermentation process of water hyacinth and corn cobs also involves bacteria and

fungi that are suitable as consortia of indigenous microorganisms. Therefore, it is very important to produce and the applicate of indigenous microorganism consortium as a starter, in order to the fermentation process can be accelerated with the best results. There are several requirements that must be fulfilled by starter products, including the total population of microorganisms in each unit (ml or g), at least 10^6 cfu / g (Palavecino Prpich, et al., 2015), the water content may not be more than 4.05% (Shokri, et al., 2015) and starters must be able to survive at least 3 months at room temperature. Therefore, it is very important to monitor the number of microorganisms in the starter periodically so that they are known for their expiration time. Monitoring the number of microorganisms can be done by TPC (Total Plate Count) method to determine the total number of bacteria and fungi contained in it (Biyani, et al., 2018). In this study have been made the microorganisms starter and will be monitored the expiration time of the starter.

Research Methods

The starter was developed from indigenous microorganisms isolated from fermented feed made from a mixture of water hyacinth and corn cobs. The feed was made by some stages (1) collecting of water hyacinth and corn cobs (2) cutting the two raw materials (3) steaming ingredients to be soft and easy to be fermented (4) drying of materials so that the water content is not more than 15% (5) fermenting the materials under microaerophilic conditions. After 5 days, the feed is ready to be used as a source of indigenous microorganisms.

The next stage, isolated of indigenous microorganisms from the feed was done by streak technique on general medium). Microorganisms that grow in the general medium then purified to produce a pure culture. In each pure isolate, species were identified using Microbact Identification Kits (Microbact TMGNB12A and 12B) and the Bergey's Manual of Determinative Bacteriology. The isolates were then tested for cellulolytic activity and selected for species whose cellulolytic activity was large. Cellulolytic activity test was carried out using a medium with a single source of energy and carbon CMC. Isolates with large cellulolytic activity will show clear zones around the colony after being colored with Congo Red. The isolates that have large cellulolytic activity were then tested for antagonism and synergism work each other.

Antagonism / synergism test was done by growing two isolates in one disk in opposite positions and observing the growth of both colony. If the isolates show the growth block zone it mean that the isolates antagonist each others. Conversely, if there is no visible growth inhibition of other colonies, it mean that the two isolates work synergistically. Based on these results a consortium of indigenous microorganisms was made by combined the isolates which had a large cellulolytic activity and worked synergistically to produce a starter. Starters were stored at room and cold temperatures. At a certain time, TPC is carried out to determine the total number of microorganisms in the starter.

The Total Plate Count was carried out by pouring plate technique. Dilution series of culture was done. From the last dilution was poured into a petri dish and into it was added a medium. Then, the mixture was homogenized and incubated. Colonies were calculated after 24 hours of incubation periode.

Research Results and Discussion

The results were obtained related to expiration time of starter that storage in the room and cold temperature for 65 days are shown in Table 1 and Table 2 below.

Table 1. Results of the starter TPC that storage at room temperature

The long of storage	Total cfu (*colony forming unit*)/ml x 10^{13} in experiment unit				Average
1	189	190	175	187	185.3
2	201	187	177	185	187.5
5	186	187	173	190	184,0
12	174	185	189	189	184.3
19	188	187	176	187	184.5
29	185	185	173	184	181.8
39	184	182	170	185	180.3

51	180	178	165	180	175.8
65	173	170	160	172	168.8

Table 2. Results of the starter TPC that storage at cold temperature

The long of storage	Total cfu (*colony forming unit*)/ml x 10^{13} in experiment unit				Average
1	158	149	143	161	152.8
2	148	155	143	160	151.5
5	157	158	138	146	149.8
12	143	162	159	145	152.3
19	160	142	148	155	151.3
29	162	140	145	150	149.3
39	152	135	140	150	144.3
51	148	130	136	145	139.8
65	145	122	130	138	133.8

Based on Table 1 above it can be stated that until the 65th day of storage the starter that was developed was still above the SNI threshold value even though it was stored at room temperature. This is because the medium was used in the research until the 65th day can still supply the needs of microorganisms in the starter, even though the starter remains in a state of active metabolism. The medium used is nutrient broth that contain carbon sources, energy sources, macro and micro elements sources that needed for the growth of microorganisms in the starter. The starter that stored at room temperature can be used directly for various purposes such as fermentation in the manufacture of feed or complete degradation of compost / fertilizer, as well as biogas production from cellulosic materials. Alternatively, the starter was storage in cold temperatures (in the refrigerator) too. The results are presented in Table 2 below.

Based on Table 2 above, as in starter that storage at room temperature, up to 65 days of starter storage age is still feasible to use because the number of microorganisms in it is still above the threshold value SNI standard. In fact, it was seen that the starter stored in cold temperatures after TPC was carried out, the number of microorganisms contained in it was more compared to the number of microorganism that storage at room temperature. Cold temperature was better in maintaining the starter's expiration time was compared to storage at room temperature. This can be explained that cold temperatures cause a decrease in metabolism of microorganisms. In cold temperatures microorganisms only maintain their basal metabolism, so they will consume less food, respiratory gases and other necessities of life. Therefore the medium composition in the starter can be maintained for longer time, so the starter expiration time that is stored in cold temperatures was longer too.

Conclusion

The starter was developed in this research remain feasible until 65 days of storage both at room and cold temperature storage. Cold temperatures provide better condition for starters compared the condition storage at room temperature. The starter that was storaged in the cold temperature shown longer expiration time.

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