The Effectiveness of the Growth of a Consortium of \textit{Bacillus cereus} Bacteria with Different Protein Sources

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Abstract. One of the benefits of probiotic bacteria is to become a source of protein that can be used as a product in the field of biotechnology with high use value. Proteins derived from bacteria are less widely used compared to animals and fungi. The aim of this study is to analyze the composition of the media and the optimal time for the growth of a consortium of \textit{Bacillus cereus} bacteria. The experimental method used is a consortium of \textit{B. cereus} bacteria isolates (5 Strains) six treatments in each \textit{B. cereus} consortium, namely the addition of a different protein source (eggs and skim milk, the same carbohydrate source, namely Sago) and 3 different concentrations in each protein source (8%, 10%, and 12%) so that the treatment obtained was 6 treatments with 3 replications in each treatment. Measurement of bacterial culture growth was carried out every 6 hours for 24 hours using two methods, namely the TPC method and bacterial cell biomass. Optimal growth was found in sago media which was added in different concentrations, namely 12% due to growth in this medium which was close to the same results as growth in positive control. Growth on biomass measurements showed similar results to the growth pattern similar to TPC. While in milk sago media, the growth is less because the exponential and stationary phases are shorter.

1. Introduction.
A good fish feed should contain balanced energy and protein to optimize fish growth. One of the obstacles in the fish cultivation business is the high price of commercial feed due to the increase in the price of feed raw materials. Until now, the main raw material for protein feed is fish meal, the supply of which still relies on imported products with relatively expensive prices, as a result, the price of feed produced will be expensive.

Protein from bacteria has not been widely used compared to protein from animals and plants. Protein sourced from bacteria should have more potential to be utilized because the growth of bacteria is very fast, does not require large media or space and the regeneration process is very fast [1]. The protein produced by bacteria is also called single cell protein (SCP), which has a high protein content reaching 50% - 65%, this shows where single cell protein has the potential as a protein source feed material for cultured fish [2]. Utilization of this protein can be a substitute for protein from conventional sources in agriculture, fishery, and animal husbandry. Bacteria that can be a source of protein must have criteria
that are not pathogenic (probiotics), have good nutritional value, can be used as food or feed, do not contain toxic compounds, and have low production costs [3].

One of the bacteria with the genus Bacillus that has the potential as a source of protein production, namely *Bacillus cereus*. *B. cereus* becomes a probiotic that can inhibit several pathogenic bacteria and is also a heterotrophic bacteria that can degrade toxic organic matter in the environment, especially waters [4]. In addition, *B. cereus* is a probiotic bacteria that has not been widely applied as a useful product in biotechnology.

One of the problems in the development of bacterial culture is the living media of bacteria. Several alternative media for bacterial growth are made from materials that are easily available and do not require expensive costs. The growth and development of bacteria is influenced by nutritional factors and environmental factors [5]. Single cell protein-producing microbes generally grow in waste that has carbon and nitrogen elements which are the main components of carbohydrates and proteins as growth media [6].

One of the media that can be used for the growth of *B. cereus* bacteria is sago waste. Wulandari et al, 2017 [7] which states that sago liquid waste which contains quite high carbohydrates and is acidic has the prospect of being used as a medium for bacterial fermentation. Sources of protein media that have the potential and are easily obtained to be used as media are eggs and skim milk. Addition of protein as a nitrogen source to meet the needs of the C/N ratio. Therefore, media from *B. cereus* must contain good protein to be utilized optimally for the growth of this bacterium.

2. Methodology

The method used in this study is an experimental method using a consortium of bacterial isolates (a combination of 6 isolates of *B. cereus* with different strains). This method used 6 treatments for each isolate of *B. cereus*, namely the addition of different protein sources (egg white with concentrations of 8%, 10% and 12% and skim milk with concentrations of 8%, 10% and 12%) with 3 replications.

The media used in the culture of *B. cereus* is a growth medium by modifying liquid waste into a growth medium for *B. cereus*. The sago liquid waste that has been obtained is mixed with other micronutrients such as Vitamin B12, buffer solutions such as K2HPO4 and KH2PO4 solutions, and also mineral salts such as NaCl dissolved in aquadest. A total of 1.5 ml of sago liquid waste was added with 0.1 g K2HPO4, 0.15 g KH2PO4, 5 mg Vitamin B12 and 0.9 g NaCl, then dissolved in distilled water until the volume was 100 ml. Modified waste media was then sterilized using an autoclave at a temperature of 121°C, a pressure of 1 atm.

Modified liquid waste media that has been sterilized was then added skim milk and egg white as a protein source that has been previously pasteurized at a temperature of 63 – 66 °C for 30 minutes according to SNI 01-3951-1995 [6]. The addition of protein to the sago liquid waste media was carried out at a concentration of 8%, 10%, and 12%.

The consortium bacteria were suspended in 10 ml of 0.9% NaCl physiological solution aseptically and homogenized using a vortex until the turbidity of the bacterial suspension was equalized with a standard solution of 0.5 McFarland. 10 ml of *B. cereus* bacterial suspension was then put into the growth medium of sago liquid waste which had been added to the protein source treatment. The culture medium containing bacteria was placed on a Water Bath Shaker which was set at 37 °C at 90 rpm for 24 hours.

The measurement of bacterial culture growth was carried out every 6 hours (0 hours, 6 hours, 12 hours, 18 hours and 24 hours using two methods, namely the TPC method and measuring bacterial cell biomass. The technique used in the TPC method in this study was the spread dish technique). (spread plate). The sample of bacteria in the growth media is diluted first to a dilution of 10-5, then 0.1 ml is
taken using a micropipette and put into a petri dish containing PCA media. The bacterial sample on the media is then spread and flattened using a Drgalski rod aseptically near Bunsen, after that the bacterial samples were incubated for 24 hours in the incubator then bacterial colonies growing on PCA media were counted using a colony counter. The results of the number of colonies obtained were then entered into the bacterial calculation formula as follows:

$$CFU = \frac{1}{Volume \times \sum \frac{1}{Dilution Factor} \times \sum colony}$$

Measurement of bacterial biomass was carried out by determining the dry weight of bacterial cells. The microtube which became the container for bacterial cells was first weighed with an analytical balance, recorded, and 1 ml of the bacteria taken was added to the culture medium. Microtubes containing bacterial isolates were centrifuged at 3000 rpm for 10 minutes [8]. The supernatant that has been formed is removed using a dropper that has been sterilized with alcohol until only the bacterial cell precipitate remains. The microtube containing bacterial cells was then placed in an oven at 100 °C for 15 minutes. After being in the oven, the microtubes were cooled in a desiccator and then their dry weight was weighed. The dry weight that has been obtained is reduced by the weight of the previous microtube until the actual dry weight of the bacteria is obtained.

3. Results and Discussion

Growth of the Number of Bacterial Colonies of *B. Cereus*.

The measurement results of the TPC method were averaged based on 3 replications for each treatment, which were then presented in the form of Table 1.

| Media (%) | 0 (x10^6 CFU’s/ml) | 6 | 12 | 18 | 24 |
|-----------|---------------------|----|----|----|----|
| SS        | 8                   | 2.30 | 2.59 | 2.45 | 1.43 | 1.26 |
|           | 10                  | 2.57 | 2.87 | 2.72 | 2.31 | 1.90 |
|           | 12                  | 2.69 | 2.95 | 2.61 | 2.36 | 1.80 |
| ST        | 8                   | 1.77 | 2.65 | 2.75 | 2.95 | 2.43 |
|           | 10                  | 2.29 | 2.46 | 2.77 | 2.80 | 2.40 |
|           | 12                  | 2.57 | 2.70 | 2.88 | 2.94 | 2.90 |
| K (+)     | 1.85                | 2.41 | 2.33 | 2.35 | 2.21 |
| K (-)     | 0.87                | 1.06 | 1.21 | 1.18 | 0.76 |

Description: SS: Sago waste media + skim milk; ST: Sago waste media + egg white; K: Control.

Based on the results of TPC measurements, growth in sago and egg media, the average of the three concentrations experienced almost the same phases. The results obtained indicate that at the 6th and 12th hours is an exponential phase where bacterial growth increases but with different cell numbers. There are even some that are not too significant. Furthermore, at the 18th and 24th hours, the number of bacterial cells began to decrease, indicating that a stationary phase had begun (Fig 1). The exponential phase is the phase of cell division that occurs rapidly to a maximum extent. The length of the exponential phase varies depending on the type of bacteria and the composition of the medium. In general, the exponential phase occurs in the range of 6-12 hours. In contrast to the previous phase, stationary is an
advanced phase in the bacterial growth curve. In this phase the cells run out of nutrients so that some bacterial cells die and release toxins into the environment which results in a decrease in the number of cells [9].

Figure 1. Growth of B. cereus consortium bacteria with Egg (12%) Sago media.

Overall, the growth of bacteria in egg sago media was better than that of milk sago, where the three concentrations within the incubation time of 12 to 24 hours had experienced a death phase (Fig 2). Sago is a liquid waste containing an organic composition that can be utilized by heterotrophic microbes. Sago waste contains cellulose and starch which can be utilized optimally as a carbon source. Ahmad et al, 2016 [6] reported that sago waste contains 65.7% starch, the remainder in the form of crude fiber, crude protein, fat and ash. The addition of eggs in the bacterial growth medium can also meet the complexity of the media. Rizal et al, 2012 [10] said that "eggs contain a fairly high protein, namely 12%, and the amino acid composition contained in it is quite comparative compared to meat and milk" which means that eggs have protein content with a better and more complex amino acid composition compared to milk.

Figure 2. Growth ratio of Milk (8%) + sago and Egg (8%) + sago. Bacterial Cell Biomass Growth B. Cereus

The results of the measurement of the cell biomass method were averaged based on 3 replications
for each treatment, which were then presented in the form of Table 2.

### Table 2. Measurement results of bacterial cell biomass *B. cereus* Consortium

| Media (%) | Measurement time (Hour to-
| (gram/ml) | 0 | 6 | 12 | 18 | 24 |
|-----------|------------------|---|---|---|---|---|
| SS 8      | 0,072            | 0,113 | 0,121 | 0,098 | 0,080 |
| 10        | 0,091            | 0,133 | 0,135 | 0,056 | 0,055 |
| 12        | 0,100            | 0,115 | 0,137 | 0,027 | 0,080 |
| ST 8      | 0,044            | 0,088 | 0,125 | 0,111 | 0,064 |
| 10        | 0,069            | 0,099 | 0,129 | 0,136 | 0,108 |
| 12        | 0,057            | 0,131 | 0,138 | 0,107 | 0,084 |
| K (+)     | 0,097            | 0,117 | 0,131 | 0,128 | 0,123 |
| K (-)     | 0,015            | 0,029 | 0,101 | 0,024 | 0,009 |

Description: SS: Sago waste media + skim milk; ST: Sago waste media + egg white; K: Control.

Milk and sago media gave biomass that resembled the bacterial growth curve, which occurred in the lag phase at 0-6 and 6-12 growth, followed by a death phase at 12-18 and 18-24 hours respectively. (Fig 3). Based on the results obtained, it can be seen that there is a correlation between the increase in the number of cells and the production of biomass which is expressed in dry weight of cells.

According to Marugan et al, 2021 [11], biomass is the mass of living organisms that expresses the average mass per unit area or total mass in a community. The addition of bacterial biomass greatly affects the properties and composition of the media used. This is in accordance with the statement of [12], who stated that the bacterial growth medium must contain nutrients that meet the needs of the bacteria and must have similarities with the production medium so as to minimize the adaptation time of the starter culture, reduce the lag phase and optimize the time of the stationary phase.

![Figure 3. Biomass development of *B. cereus* bacteria with sago milk media](image-url)
Figure 4. Biomass development of *B. cereus* bacteria using egg + sago media.

The use of media Sago eggs have the same mass development as sago milk, during which there is a lag phase at incubation of 0-6 hours and 6-12 hours, and then followed by a stationary phase of 12-18 hours and a death phase at 18-24 hours. (Fig 4). Based on research conducted by [13], sago liquid waste media with sufficient nutrients in certain levels can produce microalgae and heterotrophic bacteria cultures that are able to convert organic matter into new cells from the waste itself so that increase the growth of biomass from microbial culture, this shows that sago liquid waste can be a source of organic matter as a nutrient for *B. cereus* isolates as evidenced by the increase in biomass of *B. cereus* isolates consortium which is incubated for 24 hours. the time container is achieved faster [14].

4. Conclusions
Egg and Sago growth medium is better than milk and Sago. From all treatments, 12% egg + sago treatment was the most effective medium, because it had more growth and higher biomass. The growth pattern of the bacterial consortium with the media Egg + milk and milk + sago had the same growth phase, namely the lag phase formed at 0-6 hours and 6-12 hours, followed by a stationary phase 12-18 hours and a death phase at 18-24 hours. 24 hours. The biomass of the *B. cereus* consortium has the same pattern of growth, where the maintenance phase should only be carried out at a maximum growth of 12 hours.

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