Potential of *Bacillus cereus* SN7 as a Single Cell Protein Source

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**Abstract.** Single-cell protein (SCP) is a protein source produced from single-cell organisms, one of which is bacteria. *Bacillus cereus* SN7 is a potential isolate from a group of heterotrophic bacteria that has been isolated from the mouth of the Siak River, Riau Province, Indonesia. This study aims to analyze the potential of *B. cereus* SN7 bacteria using different growth media. The method used is an experimental method using *Bacillus cereus* SN7 bacterial isolate with different protein sources (eggs and skim milk) at different concentrations (8%, 10%, and 12%). All of treatments used the same carbohydrate source, Sago with 3 replications in each treatment. Measurement of bacterial culture growth was carried out every 6 hours, 12 hours, 18 hours and 24 hours based on the total plate count (TPC) method and bacterial cell biomass. The result show that the most optimal growth potential for *Bacillus cereus* SN7 bacteria is sago media added with 12% egg white as a protein source. This data have the same results as growth in commercial culture media (control). The growth of *Bacillus cereus* SN7 isolates in each treatment medium had almost the same growth pattern at the beginning, which experienced an exponential phase at 6 to 12 hours of incubation time. Meanwhile, in milk sago media, the average growth was not very good because the exponential and stationary phases were shorter.

1. **Introduction**

The protein produced by these bacteria is also called single-cell protein (SCP), which has a high protein content reaching 50% - 65%, this indicates where a single protein may be a protein source feed ingredient [1]. Utilization of this protein can be a substitute for protein from conventional sources in agriculture, fisheries, 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 harmful compounds, and have low production costs. However, protein derived from bacteria has not been widely used compared to animals and fungi.

One of the bacteria with the genus *Bacillus* that may be a source of protein production is *Bacillus cereus*, this is evident from several previous studies that stated *B. cereus* to be a probiotic that can inhibit several bacterial pathogens and is also a heterotrophic bacterium that can degrade toxic organic...
matter in the environment, especially aquatic [2]. In addition, B. cereus is a probiotic bacterium that is still rarely applied as a useful product in biotechnology.

Natural sources can be used as an alternative medium for bacterial growth from materials that are easily available and do not require expensive costs. Growth and development of bacteria is influenced by nutritional factors and environmental factors [3]. 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 [4].

One of the media that can be used for the growth of B. cereus bacteria is sago waste. A sago liquid waste which contains quite high carbohydrates and is acidic has the prospect of being used as a medium for bacterial fermentation [5].

Protein serves as a source of nutrients needed by cells as the construction of the cell itself [6]. In bacterial growth, protein is useful in the process of forming bacterial cells. In addition, the low nitrogen levels in sago liquid waste can inhibit bacterial proliferation, so that it is necessary to add protein as a nitrogen source to reduce C/N [7]. Another advantage is that heterotrophic bacteria can increase the survival rate of fish by improving water quality, killing pathogenic bacteria, and improving fish health through digestion. The heterotrophic bacteria used in this study were Bacillus, a probiotic bacterium that usually plays a role in improving the health and survival of some fish given through the digestive tract of fish. The use of Bacillus sp has been proven to increase and maintain the survival rate of aquatic organisms. Probiotic bacteria in aquaculture play a role in maintaining survival rates and boosting the immune system by changing the bacterial community [8].

2. Methodology
This study used an experimental method using B. cereus SN7 bacterial isolate obtained from the Marine Microbiology Laboratory collection, Faculty of Fisheries and Maritime Affairs, University of Riau.

2.1. Preparation of Isolate Culture
A bacterial isolate, B. cereus SN7 was used in six treatments by using different protein sources (egg white and skim milk) at three different concentrations (8%, 10%, and 12%), respectively three replication. Culture media is done by modifying sago liquid waste as the source of carbohydrates. A total of 1.5 ml of sago liquid was added with 0.1 g K2HPO4, 0.15 g KH2PO4, 5 mg Vitamin B12 and 0.9 g NaCl. Then, it was dissolved in distilled water until the volume was 100 ml. The modified culture 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 is then added skim milk or 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 [9]. The addition of protein to the liquid media was carried out at a concentration of 8%, 10%, and 12%.

The bacterial isolate of Bacillus cereus SN7 has suspended in 10 ml of 0.9% NaCl physiological solution aseptically and homogenized using a vortex until the turbidity of the bacterial suspension was equal to standard solution McFarland 0.5. Next, it was put into the modified media which had been added to the protein source. The culture medium containing bacteria was placed on a Water Bath Shaker which was set at 37 °C at 90 rpm for 24 hours.

2.2. Growth of Isolate Culture
Measurement of bacterial culture growth was carried out every 6 hours, 12 hours, 18 hours, and 24 hours using two methods, namely the total plate count (TPC) method. The bacterial sample on the growth medium was diluted firstly at 10⁻² then was taken 0.1 ml using a micropipette and spread into a petri dish containing PCA media. The bacterial samples were incubated for 24 hours (37 °C) then the growing bacterial colonies were counted using a colony counter. The results of the number of colonies obtained are then entered into the bacterial calculation formula as follows.
2.3. Biomass of Isolate Culture
Measurement of bacterial cell 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 was added with 1 ml of the bacteria culture. Microtubes containing bacterial isolates were centrifuged at 3000 rpm for 10 minutes [10]. The supernatant that has been formed was removed 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 to obtain the actual dry weight of the bacteria.

3. Results and Discussion
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 (%) | Observation Time (hour) (x10^8 CFU/ml) | 0   | 6   | 12  | 18  | 24  |
|-----------|----------------------------------------|-----|-----|-----|-----|-----|
| SM        |                                        | 8   | 1,32| 2,22| 2,08| 1,70| 1,20|
|           |                                        | 10  | 1,35| 2,33| 2,17| 1,51| 1,08|
|           |                                        | 12  | 1,30| 2,25| 1,96| 1,38| 1,10|
| EW        |                                        | 8   | 1,64| 2,43| 2,54| 2,70| 2,45|
|           |                                        | 10  | 2,31| 2,43| 2,64| 2,80| 2,82|
|           |                                        | 12  | 1,71| 2,10| 2,46| 2,72| 2,30|
| C (+)     |                                        | 2,10| 2,68| 2,78| 2,86| 2,95|
| C (-)     |                                        | 0,73| 1,12| 1,26| 1,03| 0,57|

Information:
SM : The media with protein source from skim milk
EW : The media with protein source from egg white
C (+) : The Control positive, commercial media (Nutrient Broth)
C (-) : The Control negative, commercial media (NaCl)
Based on the results of TPC measurements, the growth curve of Bacillus cereus SN7 on the modified media, on average the three concentrations experienced almost the same phases. The curve shows the highest growth phase namely exponential phase at 0-6 hours (Figure 1). The next stages were followed by a stationary phase and a death phase after observation along 24 hours. This has similarities with all treatments. Based on the analysis process, media with eggs is better than skim milk contains. This is in accordance with the statement who 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 [11]. This statement declares that eggs have protein content with a better and more complex amino acid composition than skim milk contents.

From all treatments, the exponential growth phase of B. cereus SN7 in modified media with 12% egg white was higher (0.24x10⁸ CFU/ml) than others (Figure 2). The lowest exponential phase was found in modified media with 10% skim milk (0.02x10⁸ CFU/ml). Eggs are a source of high-quality protein and low calories and contain several other important nutrients, such as folic acid, choline, iron, selenium, and vitamin A, B, D, E, and K. Egg whites contain more protein in the form of albumin than egg yolks, while egg yolks contain cholesterol, essential fatty acids (generally in the form of unsaturated fatty acids), and soluble vitamins. in fat (vitamins A, D, E, and K) [12].
Biomass Cell of Bacillus cereus SN7
The results of the measurement of the cell biomass method were averaged based on 3 replications in each treatment, which were then presented in the form of Table 2.

| Media (%) | Observation Time (hour) (gram/ml) |
|-----------|----------------------------------|
|           | 0  | 6  | 12 | 18 | 24 |
| SM        | 8  | 0.076 | 0.095 | 0.118 | 0.037 | 0.112 |
|           | 10 | 0.095 | 0.110 | 0.123 | 0.065 | 0.095 |
|           | 12 | 0.129 | 0.154 | 0.098 | 0.080 | 0.086 |
| EW        | 8  | 0.020 | 0.069 | 0.079 | 0.083 | 0.065 |
|           | 10 | 0.071 | 0.022 | 0.106 | 0.116 | 0.124 |
|           | 12 | 0.068 | 0.096 | 0.141 | 0.130 | 0.094 |
| C (+)     | 0.025 | 0.040 | 0.118 | 0.122 | 0.135 |
| C (-)     | 0.020 | 0.100 | 0.033 | 0.030 | 0.014 |

Information:
SM : The media with protein source from skim milk
EW : The media with protein source from egg white
C (+) : The Control positive, commercial media (Nutrient Broth)
C (-) : The Control negative, commercial media (NaCl)

The medium with the highest biomass value in this isolate had a shorter adaptation phase (lag phase). 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 [13]. Probiotic bacteria, B. cereus SN7 is part of the analyzed heterotrophic bacteria that contain secondary metabolites that have antibacterial properties against several pathogenic bacteria [14, 15]. Furthermore, these heterotrophic bacteria taken from the sea were able to ferment chicken feathers to become more easily digested by pomfret [3].

Based on the research, 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 [16]. It can be increasing 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 SN7 isolates which were incubated for 24 hours.

4. Conclusion
The most optimal growth media for Bacillus cereus SN7 was sago waste media added with 12% egg white as a protein source. The exponential growth phase of Bacillus cereus SN7 was found at 0-6 hours. The use of egg-sago modified media was better than milk-sago modified media for all treatments. The growth and biomass of Bacillus cereus SN7 are not much different in each treatment.

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References

[1] Purwaningtyas Y R 2019 Produksi protein sel tunggal *Gluconacetobacter xylinus* dengan medium limbah cair tempe menggunakan metode air-lift bioreactor *Skripsi Universitas Sanata Dharma Yogyakarta*

[2] Feliatra F, Batubara, U M, Nurulita Y, Lukistyowati I and Setiaji J 2021 The potentials of secondary metabolites from *Bacillus cereus* SN7 and *Vagococcus fluvialis* CT21 against fish pathogenic bacteria *Microbial Pathogenesis* 166 105062

[3] Adelina A, Feliatra F, Siregar Y I, Putra I and Suhanman I 2021 Use of chicken feather meal fermented with *Bacillus subtilis* in diets to increase the digestive enzymes activity and nutrient digestibility of silver pompano *Trachinotus blochii* (Lacepede, 1801) *F1000Research* 10(2) 1-17

[4] Inuhan B, Arreneuz S and Wibowo M A 2016 Optimasi produksi protein sel tunggal (PST) dari bakteri yang terdapat pada gastrointestinal (GI) ikan Nila (*Oreochromis niloticus*) dan ikan Kembung (*Scomber canagorta*) *Journal Kimia Khatulistiwa* 5(1) 24-28

[5] Ahmad S W, Yanti N A and Muhiddin N H 2019 Pemanfaatan limbah cair sagu untuk memproduksi selulosa bakteri *Jurnal Biologi Indonesia* 15(1) 33-39

[6] Nursyirwani N, Feliatra F, Tanjung A and Harjuni F 2020 Isolation of cellulytic bacteria from mangrove sediment in Dumai Marine Station Riau and the antibacterial activity against pathogens *IOP Conference Series: Earth and Environmental Science* 430(1) 012012

[7] Hindersah R, Kalay A M, Jacob A and Talahaturus A 2014 Lifbarkom putup hayat *Jurnal Agrokoteknologi* 6(1) 12-24

[8] Effendi I, Feliatra F, Emrinelson T, Siregar I A and Adelina A 2021 Effect of heterotrophic bacteria on the growth of tilapia (*Oreochromis niloticus*) cultivated in brackish water *IOP Conference Series: Earth and Environmental Science* 744(1) 012016

[9] Wulandari Z, Taufik E and Syarif M 2017 Kajian kualitas produk susu pasteurisasi hasil penerapan rantai pendingin *Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan* 5(3) 94-100

[10] Setyati W A, Martani E and Zainuddin M 2015 Kinetika pertumbuhan dan aktivitas protease isolat 36k dari sedimen ekosistem mangrove Karimunjawa Jepara *Indonesian Journal of Marine Sciences/Ilmu Kelautan* 20(3) 163-169

[11] Rizal B, Hintono A and Nurwantoro N 2012 Pertumbuhan mikroba pada sel telur pasca pasteurisasi *Animal Agriculture Journal* 1(2) 208-218

[12] Khoirunnisa K., Pradani G A P, Alexis J J G, Fahara T and Chrisnanto J O Karakterisasi bakteri kontaminan pada putih dan kuning telur ayam kampung dalam kondisi mentah dan setengah matang (100 °C/4 menit) *Skripsi Institut Teknologi Bandung*

[13] Yeni A M and Sunarti T C 2016 Penggunaan substrat whey tahu untuk produksi biomassa oleh *Pediococcus pentosaceus* E. 1222 *Jurnal Teknologi Industri Pertanian* 26(3) 284-293

[14] Setiaji J, Feliatra F, Teruna H Y, Lukistyowati I, Suhanman I, Muchlisin Z A and Johan T I 2020 Antibacterial activity in secondary metabolite extracts of heterotrophic bacteria against *Vibrio alginolyticus*, *Aeromonas hydrophila*, and *Pseudomonas aeruginosa* *F1000Research* 9(1) 1-11

[15] Feliatra F, Mardalisa M, Setiadi J, Lukistyowaty I and Hutasoit A Y 2020 Potential of secondary metabolite from marine heterotrophic bacteria against pathogenic bacteria in aquaculture *Journal of Physics: Conference Series* 1655(1) 012044

[16] Septiani W D, Slamet A and Herman J 2014 Pengaruh konsentrasi substrat terhadap laju pertumbuhan alga dan bakteri heterotropik pada sistem HRAR *Jurnal Teknik ITS* 3(2) 98-103