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Effect of Different Molasses Doses in Bacterial Growth (Commercial Probiotics)

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ABSTRACT

The study investigated the optimal dose of molasses for the cultivation of commercial probiotics containing *Bacillus amyloliquefaciens* and *Bacillus subtilis*. A two-month laboratory experiment was conducted at the Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University, using a Completely Randomized Design (CRD) with four treatments: K (0 ppm), A (5 ppm), B (10 ppm), and C (15 ppm), each with three replications. The study measured optical density (OD) and bacterial growth over 48 hours. The results showed that Treatment C (15 ppm) produced the highest OD value (0.014), indicating substantial bacterial growth. However, it exhibited the slowest growth phase. In contrast, Treatment A (5 ppm) demonstrated the fastest growth phase and achieved the highest bacterial density throughout the study. This suggests that 5 ppm of molasses is ideal for rapid bacterial proliferation. Treatment K (0 ppm) resulted in the lowest OD (0.0042) and led to bacterial death by the end of the experiment, highlighting the necessity of molasses for bacterial survival and growth. In conclusion, a 5 ppm molasses dose (Treatment A) proved to be the most effective for promoting optimal bacterial growth and density, while higher doses resulted in slower growth, and no molasses inhibited bacterial survival.

Keywords: bacterial density, growth pattern, molasses, probiotics

INTRODUCTION

The aquaculture sector is currently one of the main sources of aquatic food for humans that can maintain the stability of world food needs; this causes the demand for cultured organisms to increase every year (Fiorella *et al.*, 2021). The demand for the aquaculture sector has contributed to 52% of world fish consumption, with a demand of more than 150 million tons in 2020 (FAO, 2020). The increasing demand for the aquaculture sector from year to year encourages the intensification process of cultivation. According to Das *et al.* (2017), the intensification process can cause stress for cultivated organisms so that it can reduce the immune system and resistance to pathogen attacks. Decreased immune response and pathogen attacks in the cultivation process can be overcome by using probiotics (Ramos *et al.*, 2017).

Probiotics are microorganisms that can increase host organisms' immune response by improving microbiota composition in the host's intestines (Yilmaz *et al.*, 2022). Probiotics have an important role in the fisheries sector. Namely, they can increase the host's immune response and increase growth and resistance to pathogen attacks (Kaewda *et al.*, 2025). According to Amenyogbe *et al.* (2020), the use of probiotics in cultivated organisms can affect the morphology of the intestines and microorganisms found in the host's digestive organs. Several types of bacteria used as probiotics are *Pediococcus*, *Lactobacillus*, *Bacillus*, *Enterococcus*, *Micrococcus*, *Lactococcus*, *Roseobacter*, and *Pseudomonas* (Kuebutornye *et al.*, 2019).

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According to Hajirezaee *et al*. (2024), giving the probiotic *Lactobacillus rhamnosus* can increase growth, immune response, and resistance to *Streptococcus iniae* bacteria in carp.

In their growth, probiotics require an energy source, one of which comes from carbon (Sasikumar *et al.,* 2024). Carbon sources include macronutrients essential for microorganisms to stay alive (Wicaksono *et al.,* 2017). Carbon sources that can be used include molasses, tapioca flour, bran flour, cornstarch, starch, cassava flour (Sukardi *et al.,* 2018). Molasses used as a source of nutrition to optimize the performance of probiotic bacteria (Popov *et al.,* 2020). Molasses is a thick, dark brown liquid from a by-product of sugar production (Chauhan *et al.,* 2011). Molasses contains amino acids, vitamins, inorganic salts (SO_4^{2-}, Ca^{2+}, Na^{+}), and several color-forming substances such as caramel, melanoidin, and melanin (Zhang *et al.,* 2021). In addition, according to Zhang *et al*. (2019), the glucose content in molasses is higher than other carbon sources. Glucose is a source of energy for microbes and is widely used because it can accelerate the growth of microorganisms due to its metabolic efficiency (Raethong *et al.,* 2025).

The high glucose content in molasses can increase protease activity in *Bacillus subtilis* by up to 63% compared to other carbon sources (Bajaj *et al.,* 2014). Increased protease activity in bacteria can benefit the host by cutting enzymatic reactions in the digestive organs (Zhang *et al.,* 2021). Based on this, this study was conducted to determine the role of molasses with different doses on the growth of commercial probiotic bacteria.

MATERIALS AND METHODS

Experimental set up

This study was conducted at the Faculty of Fisheries and Marine Sciences Laboratory, Jenderal Soedirman University. The experiment employed a Completely Randomized Design (CRD) with four treatments, each replicated three times. The treatments consisted of varying molasses concentrations: 0 ppm (K), 5 ppm (A), 10 ppm (B), and 15 ppm (C), with a constant probiotic dose of 0.004 g per treatment.

Materials and tools

The tools used were hand counters by Joyco, ZX3 Advanced Vortex Mixer, Iwaki test tubes, test tube racks by a local brand, measuring cups by a local brand, cuvettes, biobased spectrophotometers, Bunsen, Airtech laminar air flow, spatulas, oxygen microtube racks, mortars, stationery, and documentation tools. The materials used were molasses, commercial probiotics brand Ariake Kuro, which contains *Bacillus amyloliquefaciens* and *Bacillus subtilis*, 70% alcohol, Tryptic Soy Agar (TSA), sterile distilled water, spirits, latex gloves, cotton, gauze, thread, tissue, paper, aluminum foil, plastic wrap, and plastic.

This study used a 250 ml Erlenmeyer flask. Each treatment received 0.004 g probiotics dissolved in 200 ml water. Prior to experimentation, equipment, and molasses underwent sterilization to prevent contamination. Molasses doses were then added according to treatment specifications. The mixtures were homogenized and incubated for 48 hours. Incubation occurred in a rotary shaking incubator at 37 °C with 150 rpm agitation (Maryanty *et al.,* 2019). Bacterial growth and density were subsequently monitored.

Molasses sterilization

The molasses was boiled and filtered, then autoclaved at 121°C for 15 minutes to ensure sterilization. The sterilized molasses was then dispensed into Erlenmeyer flasks at the

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designated treatment concentrations.

Bacterial density

Multilevel dilutions were performed up to the seventh and eighth levels. The diluted solutions (7 and 8) were analyzed for bacterial density using a spectrophotometer and aquades served as the blank (Dewi, 2018). Optical density measurements were taken at a wavelength of 600 nm (Arfiati *et al.*, 2020). Subsequently, cuvettes containing dilutions 7 and 8 were placed in the cell holder for analysis (Seniati *et al.*, 2019).

Bacterial count

Bacterial growth analysis utilizing the Total Plate Count (TPC) method commenced with multilevel dilutions up to the seventh and eighth levels. Homogeneous solutions from dilutions 7 and 8 were inoculated onto TSA media in duplicate using sterile L-glass and a micropipette. The inoculated media were sealed with plastic wrap and incubated. Sanjaya *et al.* (2023) state that TSA-inoculated bacteria require incubation at 37°C. Observations occurred over 48 hours, with readings taken every 2 hours for the first 24 hours and every 4 hours for the subsequent 24 hours. Following incubation, bacterial density was calculated using the TPC method (Utami *et al.*, 2018).

Statistical analyis

The data were statistically analyzed using Analysis of Variance (ANOVA) with IBM SPSS Statistics 23.

RESULTS AND DISCUSSIONS

Bacterial density

Optical density values with different doses of molasses obtained the highest observation results in treatment C and the lowest in treatment K (Table 1). This is because the concentration of molasses, which increases along with the increase in the absorbance of the solution, will cause the color to become more concentrated, so the value increases along with the increase in the dose of molasses (Neldawati *et al.*, 2013). Carbon sources such as molasses are the main components needed by bacteria to carry out metabolism, so they act as a source of nutrition for bacteria (Raethong *et al.*, 2025). Spectrophotometry can be used to measure bacterial density with the working principle of measuring the turbidity of bacterial suspension using the help of light (Mira *et al.*, 2022).

Bacterial growth

Based on the results of this study, the highest bacterial growth was obtained in treatment A, and the lowest in treatment K. Bacterial growth in this study decreased along with increasing doses of molasses (Figure 1). The decrease in bacterial growth, along with increasing doses can be caused by several factors, one of which is the DO value. Giving too much molasses will cause dissolved oxygen to decrease, which can affect the growth of probiotic bacteria (Sartika *et al.*, 2012). According to Putri *et al.* (2016), giving a low concentration dose of molasses can maximize the performance of probiotic bacteria.

In addition to DO values, molasses contains polyphenols that can damage the structure of cell walls and plasma membranes, causing bacterial death (Chen *et al.*, 2017). Molasses also has a high phenolic compound content of 7.60 mg GAE/g extract, which can inhibit bacterial growth and increase the dose used (Shafiqa-Atiqah *et al.*, 2020). Differences in molasses doses can produce different growth patterns. Differences in bacterial growth patterns at molasses doses indicate that media composition plays an important role in bacterial growth (Supriyanto *et al.*, 2015). The final density of probiotic bacteria obtained in this study ranged from 8.3×10^8 to 69.75×10^8 CFU/mL. Bacterial growth can be influenced

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by several factors, namely the availability of nutrients and environmental factors (Jõers & Tenson, 2016; Français *et al.*, 2019). Bacterial growth occurs in several phases, namely the lag phase, log phase (exponential), stationary phase, and death phase (Bate *et al.*, 2023).

Table 1. Optical Density Observation Value of Bacterial Density

Treatments	Dilutions	
	10^{-7}	10^{-8}
K	0.0045	0.0042
A	0.005	0.0045
B	0.0065	0.0063
C	0.014	0.010

Control: molasses doses 0 ppm; A: molasses doses 5 ppm; B: molasses doses 10 ppm; C: molasses doses 15 ppm.

Based on the results of the study, the lag phase in each treatment has differences, the fastest and slowest lag phases are in treatments A and C, namely 4 hours and 10 hours, respectively. The lag phase is a condition when bacteria adapt to a new environment so that only a few or no bacteria reproduce (Madar *et al.*, 2013). After going through the lag phase, bacteria will enter the log (exponential) phase, in this phase bacterial cells will start to grow exponentially and have a reproductive period at a maximum level (Himeoka & Kaneko, 2017; Ughy *et al.*, 2023).

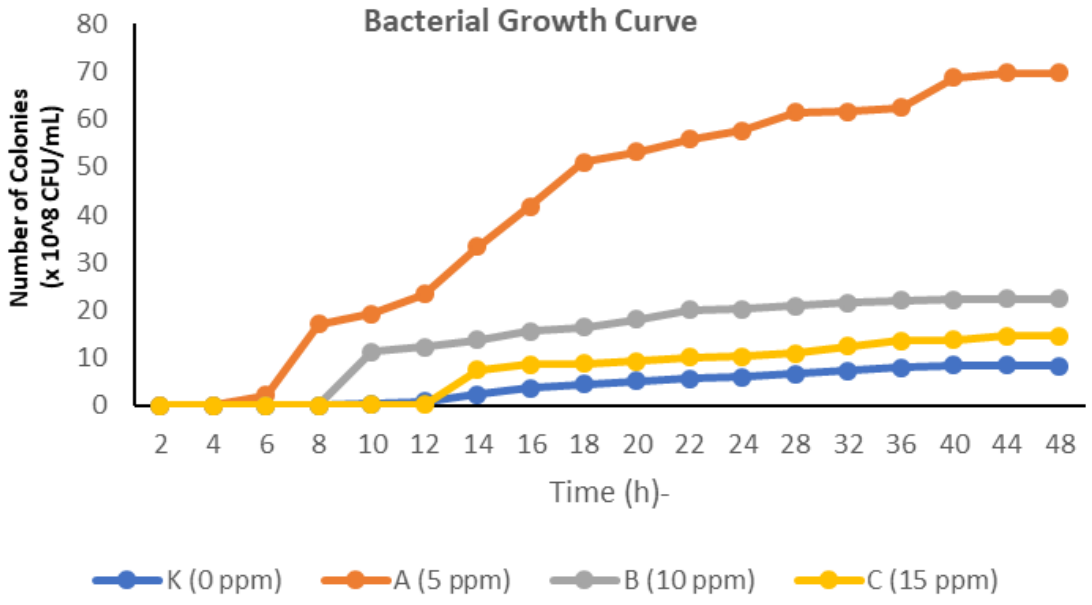

Figure 1. Probiotic Bacteria Growth Curve. Control: molasses doses 0 ppm; A: molasses doses 5 ppm; B: molasses doses 10 ppm; C: molasses doses 15 ppm.

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Observation results obtained the fastest bacterial growth log phase at 6 hours in treatment A and late at 12 hours in treatment C. The log phase occurs because bacteria undergo binary fission to double in size within a certain period (Novianto *et al.*, 2020). Rapid bacterial growth in the exponential phase can stop along with decreasing growth and bacteria entering the stationary phase (Ughy *et al.*, 2023). The fastest stationary phase in this treatment was at 40 hours in treatment K and late at 44 hours in treatment C. The stationary phase can occur due to reduced nutrients and the formation of metabolic compounds that can be toxic to bacteria (Novianto *et al.*, 2020). In addition, the stationary phase can occur when the nutrient content in the media begins to run out, and there is a balance between bacteria that die and divide (Pletnev *et al.*, 2015).

After that, the bacteria will enter the death phase. The death phase of bacteria in this study only occurred in the K treatment at 44 hours. This occurs because of the reduction in available substrates and nutrients so that bacterial cells can no longer grow (Efendi *et al.*, 2017). Bacteria need nutrients, energy sources, and suitable environmental conditions to reproduce (Raethong *et al.*, 2025). The death phase in bacteria can also be caused because bacteria that live in the stationary phase will release toxic compounds in the maintenance media, which cause bacterial death so that bacteria will enter the death phase, which is characterized by a decrease in the number of bacterial cells (Pletnev *et al.*, 2015).

CONCLUSION

The effects of molasses supplementation on bacterial growth and density were evaluated. Treatment with 0 ppm molasses showed the lowest bacterial density and accelerated growth to death. In contrast, Treatment with 15 ppm showed slower growth. In particular, Treatment 5 ppm produced the highest bacterial density.

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AUTHORS' CONTRIBUTIONS

The first author came up with the idea for this study, designed it, analyzed the data, and wrote the report. The second author conducted fieldwork, gathered information, and helped interpret the findings. The third author supervised the research process, helped establish the methodology, and critically examined the work for noteworthy intellectual substance. The fourth author provided resources, oversaw project administration, and managed the acquisition of funds. All writers have read and approved the final draft of the manuscript.

CONFLICT OF INTEREST

The authors state that there are no conflicts of interest related to the publication of this article. This study transparently acknowledges

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all funding sources and collaborative institutions involved and confirms the absence of financial or personal relationships that could influence the reported work.

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