Growth of probiotics for poultry in glucose yeast pepton media, production of biomass, viability and stability after encapsulation with spray drying

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Abstract. This study aimed to determine specific growth rate and biomass cells production of probiotics bacteria isolated from Indonesian native chicken gastroitestinal tract. Three bactrias manely Lactobacillus murinus Ar-3, Streptococcus thermophilus Kp-2 and Pediococcus acidilactici Kd-6 were grown in glucose yeast peptone (GYP) medium. A mixture of probiotic bacteria base on rasio of specific growth rate were employed to the experiment for biomass production study. Fermentation was conducted in 2 litres bioreactor, and encapsulation was carried out by centrifugation. Coating process was done by skim milk/maltodextrin medium and dried with spray drying. The results showed that L. murinus Ar-3 had a generation time of 3.25 ± 0.006 hours, S. thermophilus Kp-2 3.68 ± 0.1 hours and P. acidilactici Kd-6 3.74 ± 0.04 hours, thus ratio of generation time was 1 : 1.18 : 1.16. The growth of mixture of probiotic showed the same pattern. Its cells were harvested at maximum cell yield 9.33±0.12 log 10 cfu/ml was achieved. The fermentation effiency was 51 %, the viability of mixed probiotic culture were 10.12 log 10 cfu/ml before spray and 9.12 log 10 cfu/g after spray drying or 90.11%. The stability of encapsulated probiotics in room temperature during 21 days strorage was 90.89%.

1. Introduction
Probiotics bacteria which is ideal for poultry should be isolated from the gastrointestinal tract of poultry, because in its application probiotics bacteria will easily adapt to its environment [1]. Supplementation of multispecies probiotics bacteria in chickens is more effective than monospecies probiotic bacteria. [2]. Bacterial growth undergo to an adaptation phase or lag phase, log phase, stationary and death phases Log phase is usually used to determine the rate of bacterial growth by calculating its specific growth rate and generation time. In general, for application purposes in poultry, the probiotics chosen are those that have a short generation time [3, 4] and mixed into feed in an encapsulated form. The encapsulation of probiotics is expected to have the high viability and stability. This finding will be useful for determining the strain culture ratio in the manufacture of mixed probiotics that have high stability during storage.
2. Material and methods

2.1. Bacteria strain and medium
Probiotic bacteria of *Lactobacillus murinus* Ar-3, *Streptococcus thermophilus* Kp-2 and *Pediococcus acidilactici* Kd-6 isolated from gastrointestinal tract of Indonesian native chicken [5] were used throughout the study. Medium used throughout the study were glucose yeast peptone (GYP) broth for the starter culture and sugar cane molasses medium for production of probiotic biomass.

2.2. Methods
Pure cultures of probiotic strains in skim-glycerol milk medium were rejuvenated and grown in 25 ml of sterile GYP medium at 37°C for 24–48 hours or until they reached pH 3.5–4.5. Growth of probiotics during the incubation was measured by the absorbance of its turbidity at a wavelength of 600 nm using a spectrophotometer. Absorbances were plotted versus incubation time and calculated their generation time.

Production of cell biomass was carried out in a 2 liters capacity of bioreactor. A total of 100 ml mixed probiotics starter culture was prepared by mixing each strain bacteria starter culture based on the ratio of their generation time. The mixed strater culture was then added to 2 liters of molasses hydrolyzate fermentation medium (10% v/v sugar content). Incubation was carried out at 37°C for 24–48 hours. The pH of the medium was stabilized at pH 6.0 with a 0.4M NaOH solution. Probiotic biomass was harvested after 24-hour incubation time (OD<sub>600</sub> showed the number of cells was 10<sup>9</sup>–10<sup>10</sup> bacterial cells/ml) by centrifugation at 3000xg for 20 minutes. Then it was encapsulated with 20% skim milk/maltodextrin solution, and dried with spray drying at 110°C inlet and 78°C outlet temperatures. The encapsulated probiotic before and after drying were tested for the viability and stability for 21 days at room temperature using the plate count method.

3. Results and discussion
The probiotic growths of lactic acid bacteria were observed every hour for 24 hours incubation time at 37°C and indicated their growth curve as shown in Figure 1.

![Figure 1](image_url)  
**Figure 1.** Growths of probiotic bacteria in GYP medium. A: bacterial growth was based on actual values. B: based on estimated values since the line was not on the actual values.

As shown in the Figure 1 the growth of probiotic bacteria were gradually increased up to 16 hours of incubation time and began level off after that. However, growth of exponential (log) phases were occurred from 1<sup>st</sup>–9<sup>th</sup> hours of incubation time, where from those can be calculated the specific growth rate (μ) of each probiotic strain. The generation time was then calculated according to the formula given by Maier and by Painter, \( t = \frac{\mu}{\ln 2} \) where \( t \) is generation time [6, 7] and found for *L. murinus* Ar-3 *S. thermophilus* Kp-2 and *P. acidilactici* Kd-6 were 3.25±0.006 hours, 3.74±0.04 hours and 3.68±0.1 hours respectively. Therefore, according to their generation times the fastest growth was *L. murinus* Ar-3, followed by *S. thermophilus* Kp-2 and the slowest among the three strains of probiotic bacteria was *P.
acidilactici Kd -6, and the generation time ratio can be obtained Ar-3: Kp-2: Kd-6 was 1: 1.16: 1.18. This ratio will be used as the basis for preparation of mixed probiotics starter culture for biomass production. As showed in Figure 1-B, the growth of mixed probiotics in molasses medium has similar pattern as in the GYP medium. The production of probiotic cell biomass and their microencapsulated product were given in Table 1.

**Table 1.** Production of probiotic biomass in molasses medium and their encapsulated product

| Replication | Initial probiotic cell number (log CFU/ml starter culture) | Number of bacteria after fermentation (log CFU/ml molasses medium) | Encapsulated biomass before spray drying (g) | Encapsulated biomass after spray drying (g) |
|-------------|-----------------------------------------------------------|------------------------------------------------------------------|---------------------------------------------|--------------------------------------------|
| 1           | 8.31                                                      | 9.31                                                             | 610.25                                      | 60.39                                      |
| 2           | 8.12                                                      | 9.35                                                             | 612.31                                      | 66.82                                      |
| 3           | 8.02                                                      | 9.32                                                             | 610.05                                      | 60.57                                      |
| Average     | 8.15 ± 0.03                                               | 9.33 ± 0.12                                                      | 610.87 ± 1.25                               | 62.59 ± 3.66                               |

From the data as showed in Tabel 1, it can be calculated the efficiency of fermentation regarding ti biomass production was approximately 51%, while the production of encapsulated biomass was only about 3.13 % w/v. These result has not been said to be high. The lower efficiency of fermentation was probably due to the fermentation conditions that are not suitable for the production of probiotic biomass, while the low production of encapsulated biomass tend to be caused by a lack of conformity to the encapsulation method. However, the viability of microencapsulated probiotic biomass was still high as well as the viability during storage at room temperature (Table 2).

**Table 2.** Viability and stability of encapsulated mixed probiotic bacteria

| Viability (stability) | Log CFU/g | %     |
|-----------------------|-----------|-------|
| Before spray drying   | 9.36 ± 0.033 | 100   |
| After spray drying    | 7.90 ± 0.008 | 84.4  |
| Zero time of storage  | 7.90 ± 0.008 | 100   |
| Day 7 of storage      | 7.71 ± 0.06  | 97.59 |
| Day 14 of storage     | 7.38 ± 0.34  | 93.42 |
| Day 21 of storage     | 7.18 ± 0.11  | 90.89 |

The main cause of cell death is the high temperature received by cells during the encapsulation process by spray drying. *Lactobacillus* and *Pediococcus* are mesophilic bacteria. These bacteria do not have a stable protein at high temperatures. If cells are exposed to high temperatures due to imperfect encapsulation, the protein will be damaged so that the cell dies. The survivability as showed in the Table 2 indicated that mixed lactic acid probiotics were able to survive at high temperatures during the spray drying process. The viability of mixed probiotics in this study was higher compare to that found by Triana et al.,[8] which only gained 76% viability in their experiments with *Lactobacillus* sp Mar 8 encapsulated with 10% skim milk [8]. If the decrease in viability during storage at room temperature is considered linear (regression analysis of the data shows $Y = 100.65 - 0.45X$), then the viability of microencapsulated probiotic biomass is only 50% when storage lasts 111 days.

4. Conclusion

The results of the study concluded that the growth of *Lactobacillus murinus* Ar-3 was the fastest, followed by *Streptococcus thermophilus* Kp-2 and *Pediococcus acidilactici* Kd-6 which were the slowest in GYP as indicated by their generation time 3.25±0.006 hours, 3.74±0.04 hours and 3.68±0.1 hours (1:1.16:1.18) respectively, so the growth of mixed probiotics in molasses medium showed the same pattern. Fermentation using molasses medium (10% sugar content) at 37°C for 24 hours yielded
maximum cell biomass of $9.33 \pm 0.12 \log_{10} \text{cfu/ml}$, so that the efficiency regarding to biomass cell production was 51%. Spray drying of microencapsulated mixed probiotic biomass decreased the viability from $10.12 \log_{10} \text{cfu/ml}$ before spray drying to $9.12 \log_{10} \text{cfu/g}$ after spray drying, so that the viability after drying was 90.11%. Storage of microencapsulated mixed probiotics at room temperature reduced its viability linearly. Its stability was only 90% after being stored for 21 days at room temperature. The viability is only 50% when stored for 111 days calculated by regression analysis.

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