Growth optimization of *Bacillus subtilis* 11A isolated from Indonesian native chicken (*Gallus domesticus*) for bacteriocin production

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**Abstract.** This study aimed to obtain the optimal growth of *Bacillus subtilis* 11A isolated from Indonesian native chicken (*Gallus domesticus*). The growth optimization included pH medium conditions (5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5) and different incubation temperatures (31, 33, 35, 37, 39, 41°C). Isolate was incubated for 12 hours using *Luria Bertani* broth. Optical density was observed using spectrophotometer (λ600) at 0 and 12 hours of incubations. Bacteriocin activity against pathogens *Staphylococcus aureus*, *Salmonella typhimurium* and *Escherichia coli* was determined using a well-diffused method. Data was statistically analyzed using a one-way analysis of variance (ANOVA) followed with Duncan's New Multiple Range Test (DMRT) to distinguish the treatment means. The results showed that the optimum pH for bacterial growth was at pH 6.5. Optimum temperature for bacterial growth was at 39°C. The highest bacteriocin activity of *B. subtilis* 11A against indicator pathogens was resulted at stationary phase (1906.14, 2179.79, 2343.07 AU/ml). It can be concluded that *B. subtilis* 11A isolated from Indonesian native chicken grew optimally for bacteriocin production at pH 6.5 and temperature 39°C. The highest bacteriocin production and activity produced by *Bacillus subtilis* 11A was at stationary phase.

**1. Introduction**

Antibiotics in the field of animal health are used as prevention and treatment of diseases, as well as a growth promoter in chickens. The administration of antibiotics in cattle may cause residues in the products. The residues of tetracycline, sulfonamides, fluoroquinolones and enrofloxacin respectively as much as 1,046.3–1,158.9; 20–800; 37.6–164.7; and 18.32 µg/kg in chicken meat in Nigeria, Pakistan, Portugal, and Iran [1–4]. Due to antibiotic residues as the negative effects of antibiotics addition in feed, many researches have been conducted to invent natural antibiotics which may be produced by plants (herbs) and microorganism (probiotics). One of natural antibiotic produced by bacteria was bacteriocin, in exampleis bacteriocin produced by genus *Bacillus*. Bacteriocins produced by *Bacillus* bacteria actively inhibit the growth of pathogenic bacteria [5]. *Bacillus* bacteria play a role in maintaining the balance of intestinal microbiota, enhance the natural defense mechanisms of the
host, so it will positively affect on the performance and the health of chickens [6]. Growth of Bacillus bacteria related to pH medium and temperature of incubation. The production of bacteriocin is also affected by the growth of bacterial cells [7]. So that, optimum pH medium and incubation temperature were needed to optimize the growth and bacteriocin production by Bacillus bacteria isolated from native chickens (Gallus domesticus).

2. Materials and methods

2.1. Materials
This study used microbiology equipments for bacteria isolation and cultivation, B. subtilis 11A isolated from native chicken, three pathogen bacterias (Escherichia coli, Salmonella typhimurium and Staphylococcus aureus), spectrophotometer (Genesys 20™), pH meter (Hanna™), centrifuge model 5810R (Eppendorf®), vorteks mixer model VM-1000, 1.5 mL safelock tube, laminar air flow, autoclave and analytic scale.

2.2. Methods

2.2.1. Growth optimization. This research was conducted by inoculating 2% (v/v)[7] Bacillus subtilis 11A isolate into Luria Bertani broth (LB, Oxoid, UK) on different pH (5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5) and the incubation temperatures (31, 33, 35, 37, 39 dan 41°C) for 12 hours. Then results of the inoculation were measured by spectrophotometer (λ600) at the beginning (0 hour) and at the end of (12th hour) of incubation to determine the growth kinetics of each treatment.

2.2.2. Bacteriocin activity assay. Antimicrobial activity assay of bacteriocins against pathogens every phase of growth was performed by growing the isolate Bacillus subtilis 11A on the medium based on the optimal pH and incubation temperature. Samples of medium were taken every two hours and stored at temperature 4°C for furtherly used in bacteriocins extraction. Extraction of bacteriocin refers to [7], by centrifuging the medium solution at a speed of 10,000×g (4°C) for 15 minutes. Then, a cell-free supernatant was collected for testing against pathogens (Staphylococcus aureus, Salmonella typhimurium and E. coli) as follows: as much as 50 µl of sample is placed in well 5 mm well at Tryptic Soya Agar (TSA, Oxoid, UK) medium and incubated at 37°C for 24 hours. The clear zone was then measured using a caliper and the value of the bacteriocin activity (AU/ml) is determined.

2.2.3. Stastical analysis. The data of bacterial growth were analyzed using a one-way analysis of variance (ANOVA) and continued by Duncan's New Multiple Range Test [9]. Data of bacterial growth and bacteriocin activity were analyzed descriptively.

3. Results and discussion
Incubations of medium at different pH and temperature were conducted to obtain optimum pH and the optimum temperature for bacterial growth. Results showed that the optimum temperature for B. subtilis 11A were 39 and 41°C. Incubation at temperature 39°C had a higher absorbance (0.471) compared to the incubation temperature at 41°C (0.460) and the lowest was at 31°C (P<0.05). The effect of different level temperature on the growth of B. subtilis 11A in this study was summarized in Table 1.

Table 1. The effect of different temperatures on the growth of B. subtilis 11A

| Temperature (°C) | Absorbance (λ600) | p-value |
|-----------------|-------------------|---------|
| 31              | 0.400±            |         |
| 33              | 0.424±            |         |
| 35              | 0.439±            |         |
| 37              | 0.443±            |         |
| 39              | 0.471±            |         |
| 41              | 0.460±            |         |

*a,b,c,d* Means in the same rows with different superscript are significantly different at P<0.05
This study showed that the optimum pH for the growth of *B. subtilis* 11A was at pH 6.5 and the lowest growth was at pH 5, 5.5 and 8.5 (P<0.05). The effect of different pH medium on the growth of *B. subtilis* 11A is presented in Table 2.

**Table 2.** The effect of different medium pH on the growth of *B. subtilis* 11A

| pH  | p-value |
|-----|---------|
| 5   | 0.208±  |
| 5.5 | 0.206±  |
| 6   | 0.420±  |
| 6.5 | 0.453±  |
| 7   | 0.440±  |
| 7.5 | 0.365±  |
| 8   | 0.344±  |
| 8.5 | 0.206±  |

Means in the same rows with different superscripts are significantly different at P<0.05

Growth kinetics of *B. subtilis* 11A at different incubation temperature is presented in Figure 1. Salman [10] and Sumardi [11] reported that the growth of *B. subtilis* RM16 and Bacillus sp. isolated from native chicken had optimum incubation temperature at 40°C, while Nguyen [12] reported that optimum temperature for the growth of Bacillus subtilis CH16 was 42°C.

Growth kinetics of *B. subtilis* 11A at different medium pH is presented in Figure 2. Previous research showed that the optimum pH for the growth of *B. subtilis* was at pH 7 [13] and for *B. licheniformis* at pH 6.5–7.5.

![Figure 1](image1.png)  
**Figure 1.** Growth kinetics of *B. subtilis* 11A at different incubation temperature

![Figure 2](image2.png)  
**Figure 2.** Growth kinetics of *B. subtilis* 11A at different medium pH

This study showed that *B. subtilis* 11A produced bacteriocin at the end of the log phase (10th hour) and its activity reached the peak at the beginning of the stationary phase (12th hour). The bacteriocin activity of *B. subtilis* 11A decreased in line with the decreased in bacterial growth. Antimicrobial activity of bacteriocin produced by *Bacillus subtilis* 11A is presented in Figure 3.

![Figure 3](image3.png)  
**Figure 3.** Antimicrobial activity of bacteriocin produced by *Bacillus subtilis* 11A
Bacteriocin of *B. subtilis* 11A had the highest inhibitory activity to *E. coli* and *S. typhimurium* and the lowest activity to *S. aureus*. The inhibitory activity of bacteriocins against *S. aureus*, *S. typhimurium* and *E. coli* were 1.906.14; 2,179.79; and 2,343.07 AU/ml, respectively. This study showed the inhibitory activity of bacteriocin of *B. subtilis* 11A was more sensitive to Gram negative bacteria than to Gram positive bacteria.

4. Conclusion

This study showed the optimum conditions for the growth of *B. subtilis* 11A isolated from the digestive tract of native chickens were at pH 6.5 and temperature 39°C. The highest bacteriocin activity was produced at the beginning of the stationary phase and was more sensitive to Gram negative pathogenic bacteria (*E. coli* and *S. typhimurium*) than Gram positive pathogenic bacteria (*S. aureus*).

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