

**OPTIMIZATION OF BACTERIOCIN PRODUCTION BY LACTIC
ACID BACTERIA ISOLATED FROM AMPEL BAMBOO SHOOTS
(*Bambusa vulgaris*) PICKLE UNDER DIFFERENT
FERMENTATION CONDITIONS USING
SUPPLEMENTED WHEY MEDIUM**

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PADA KONDISI FERMENTASI YANG BERBEDA MENGGUNAKAN
MEDIA WHEY YANG DISUPLEMENTASI***

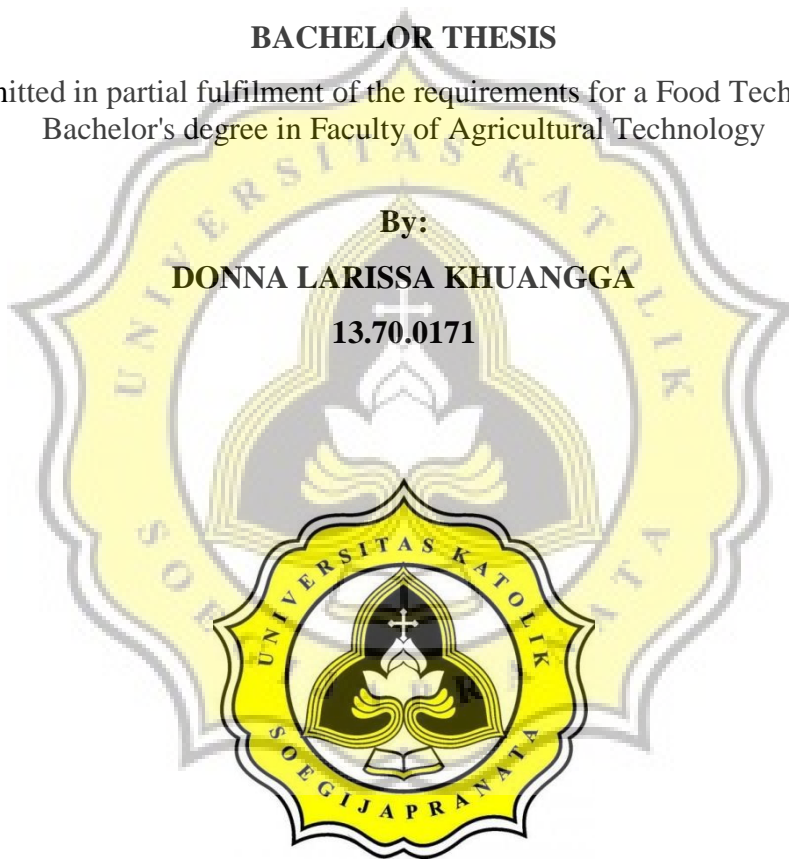
BACHELOR THESIS

Submitted in partial fulfilment of the requirements for a Food Technology
Bachelor's degree in Faculty of Agricultural Technology

By:

DONNA LARISSA KHUANGGA

13.70.0171



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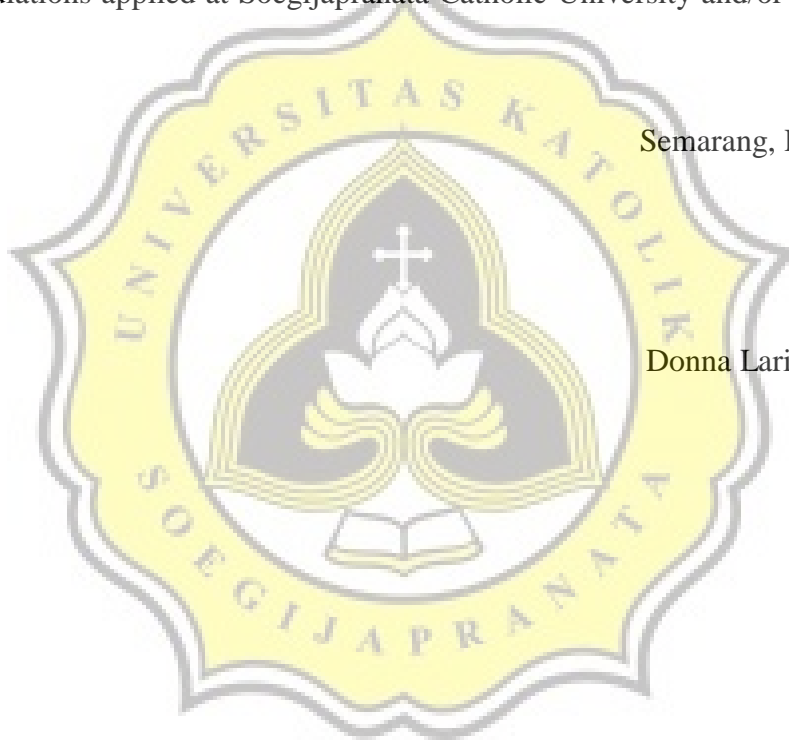
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STATEMENT OF THESIS AUTHENTICITY

I hereby declare that the thesis entitled **“OPTIMIZATION OF BACTERIOCIN PRODUCTION BY LACTIC ACID BACTERIA ISOLATED FROM AMPEL BAMBOO SHOOTS (*Bambusa vulgaris*) PICKLE UNDER DIFFERENT FERMENTATION CONDITIONS USING SUPPLEMENTED WHEY MEDIUM”** contains no work that ever proposed to acquire a bachelorship title in a University, and along to my knowledge, there is no work ever written or published by others, except the ones used as references in this thesis and mentioned in the list of references.

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Semarang, February 2017



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**This thesis has been approved and defended in front of the examination
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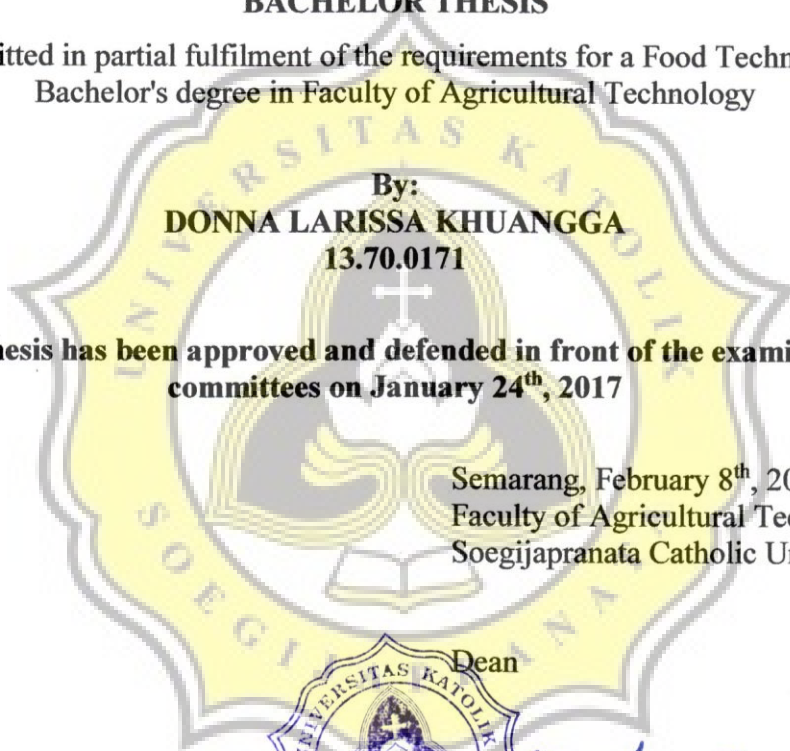

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SUMMARY

Ampel Bamboo Shoots is one of the foodstuffs derived from native bamboo species (*Bambusa vulgaris*) in Indonesia. Since bamboo shoots have a relatively short shelf-life, they are often consumed as pickle. Pickle is known as the product of fermentation carried out by natural lactic acid bacteria (LAB). LAB have the capability to produce bacteriocins which have been found to have antagonistic activity against pathogenic bacteria. Bacteriocin production is usually performed in growth complex medium, however its high cost limits its suitability for large-scale production. Therefore, by-product from food industry such as whey has been used as an alternative medium for bacteriocin production. However, supplementation on whey medium is still needed due to its lack of some essential nutrients for growth and bacteriocin production. The aim of this study was to investigate the effect of initial fermentation conditions and supplementation on whey medium towards bacteriocin production of LAB isolated from Ampel Bamboo Shoots Pickle, also to find out the isolate with optimal bacteriocin inhibitory activity to inhibit *Escherichia coli* (FNCC 0091), *Listeria monocytogenes* (FNCC 0156), and *Staphylococcus aureus* (FNCC 0047). Fermentation was carried out under the following conditions: 2.5% of salt concentration at 15°C for 5 days (code A) and 5% of salt concentration at 30°C for 4 days (code B). LAB isolates were identified and selected based on results of cell morphology, motility test, physiological test, probiotic potentials test, and antimicrobial activity test. A total of 32 LAB isolates selected were analyzed for their bacteriocin inhibitory activity using agar well diffusion method against pathogenic bacteria. Bacteriocin inhibitory activity was observed by measuring the clear zone formed around the wells and calculating the Activity Unit (AU in mm² ml⁻¹). The results showed that *Lactobacillus* was the predominant LAB in Ampel Bamboo Shoots Pickle and considered as probiotics due to their resistance to acid and bile salts, as well as their ability to produce antimicrobial substances. Fermentation of Ampel Bamboo Shoots in condition B yielded less bacteriocin-producing LAB strains compared to fermentation in condition A. Among 32 LAB isolates tested, 3 isolates which were grown in MRS-B without supplementation could produce bacteriocin. Supplementation of carbon and nitrogen on whey medium can stimulate the bacteriocin production of LAB. However, only 6 LAB isolates which were grown in supplemented whey medium showed bacteriocin inhibitory activity against pathogenic bacteria. Inhibitory activity levels of bacteriocin ranged from 24.90-1048.67 mm² ml⁻¹. The optimal bacteriocin inhibitory activity was found in bacteriocin A8 (produced by *Lb. pentosus*) which was grown in whey medium supplemented with 1% of glucose.

RINGKASAN

Rebung Ampel merupakan salah satu bahan makanan yang berasal dari spesies bambu (*Bambusa vulgaris*) asli Indonesia. Namun, rebung memiliki umur simpan yang relatif singkat sehingga rebung sering dikonsumsi sebagai acar. Acar merupakan produk hasil fermentasi oleh bakteri asam laktat (BAL) alami. BAL memiliki kemampuan untuk menghasilkan bakteriosin yang ditemukan memiliki aktivitas antagonis terhadap bakteri patogen. Produksi bakteriosin biasanya dilakukan di media pertumbuhan kompleks, namun biayanya yang mahal menyebabkan media pertumbuhan kompleks tidak cocok digunakan untuk produksi bakteriosin dalam skala besar. Oleh karena itu, produk sampingan dari industri makanan seperti whey digunakan sebagai media alternatif untuk produksi bakteriosin. Namun, suplementasi perlu dilakukan pada whey karena kurangnya beberapa nutrisi yang penting untuk pertumbuhan dan produksi bakteriosin. Tujuan dilakukannya penelitian ini adalah untuk mengetahui pengaruh kondisi fermentasi dan suplementasi pada media whey terhadap produksi bakteriosin oleh BAL yang diisolasi dari acar rebung Ampel, serta untuk menemukan isolat dengan aktivitas penghambatan bakteriosin yang optimal untuk menghambat *Escherichia coli* (FNCC 0091), *Listeria monocytogenes* (FNCC 0156), dan *Staphylococcus aureus* (FNCC 0047). Fermentasi dilakukan pada kondisi berikut: kadar garam 2,5% pada suhu 15°C selama 5 hari (kode A) dan kadar garam 5% pada suhu 30°C selama 4 hari (kode B). Isolat BAL diidentifikasi dan dipilih berdasarkan hasil dari uji morfologi sel, uji motilitas, uji fisiologis, uji kemampuan probiotik, dan uji aktivitas antimikroba. Sebanyak 32 isolat BAL terpilih dianalisa aktivitas penghambatan bakteriosinnya dengan metode difusi sumuran agar terhadap bakteri patogen. Aktivitas penghambatan bakteriosin diamati dengan mengukur zona bening yang terbentuk di sekitar sumuran dan menghitung Activity Unit (AU dalam $\text{mm}^2 \text{ml}^{-1}$). Hasil penelitian menunjukkan bahwa *Lactobacillus* merupakan BAL yang dominan pada acar rebung Ampel dan merupakan bakteri probiotik karena ketahanannya terhadap asam dan garam empedu, serta kemampuannya dalam memproduksi senyawa antimikroba. Fermentasi rebung Ampel pada kondisi B menghasilkan strain BAL penghasil bakteriosin lebih sedikit dibandingkan fermentasi pada kondisi A. Dari 32 isolat yang diuji, sebanyak 3 isolat BAL yang ditumbuhkan pada MRS-B tanpa suplementasi dapat memproduksi bakteriosin. Suplementasi karbon dan nitrogen pada media whey dapat mendorong produksi bakteriosin oleh BAL. Namun, hanya 6 isolat BAL yang ditumbuhkan pada media whey tersuplementasi yang menunjukkan aktivitas penghambatan terhadap bakteri patogen. Tingkat aktivitas penghambatan bakteriosin berkisar antara 24.90-1048.67 $\text{mm}^2 \text{ml}^{-1}$. Aktivitas penghambatan bakteriosin yang paling baik ditemukan pada bakteriosin A8 (diproduksi oleh *Lb. pentosus*) yang ditumbuhkan pada media whey dengan suplementasi 1% glukosa.

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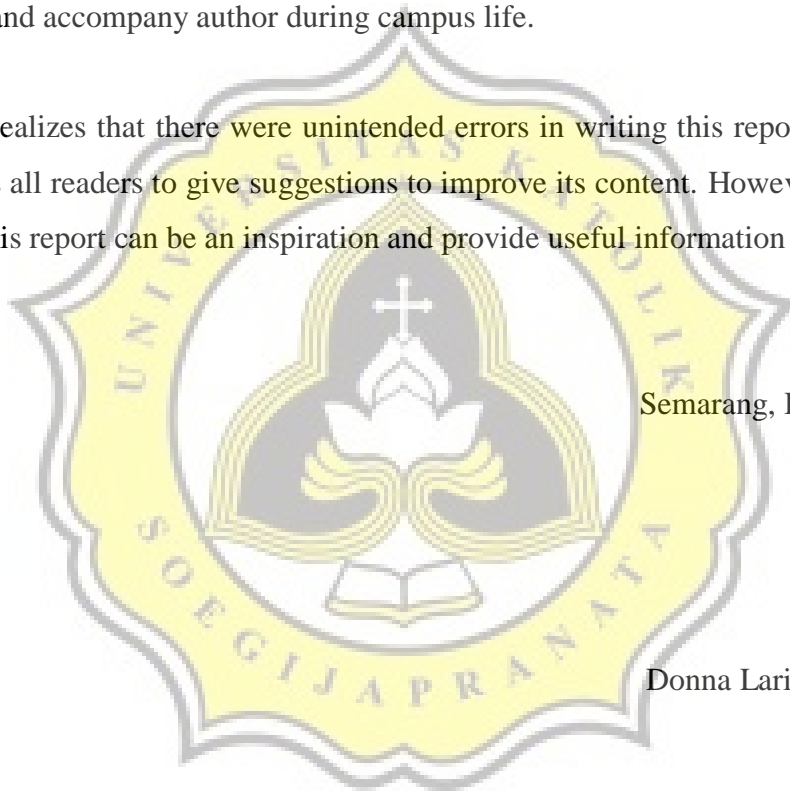
Praise the Lord because of His grace and blessing, the author would have the opportunity to complete the thesis entitled: “OPTIMIZATION OF BACTERIOCIN PRODUCTION BY LACTIC ACID BACTERIA ISOLATED FROM AMPEL BAMBOO SHOOTS (*Bambusa vulgaris*) PICKLE UNDER DIFFERENT FERMENTATION CONDITIONS USING SUPPLEMENTED WHEY MEDIUM”. This thesis was written to fulfill the requirement to acquire Bachelor Degree of Food Technology in Soegijapranata Catholic University, Semarang, Indonesia. This thesis is a part of the second year of Penelitian Unggulan Perguruan Tinggi (PUPT) 2016/2017 entitled “Efek Probiotik dan Mikrostatik dari Bakteri Asam Laktat yang Berperan dalam Fermentasi Acar Rebung”.

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The author realizes that there were unintended errors in writing this report. The author really allows all readers to give suggestions to improve its content. However, the author hopes that this report can be an inspiration and provide useful information for others.



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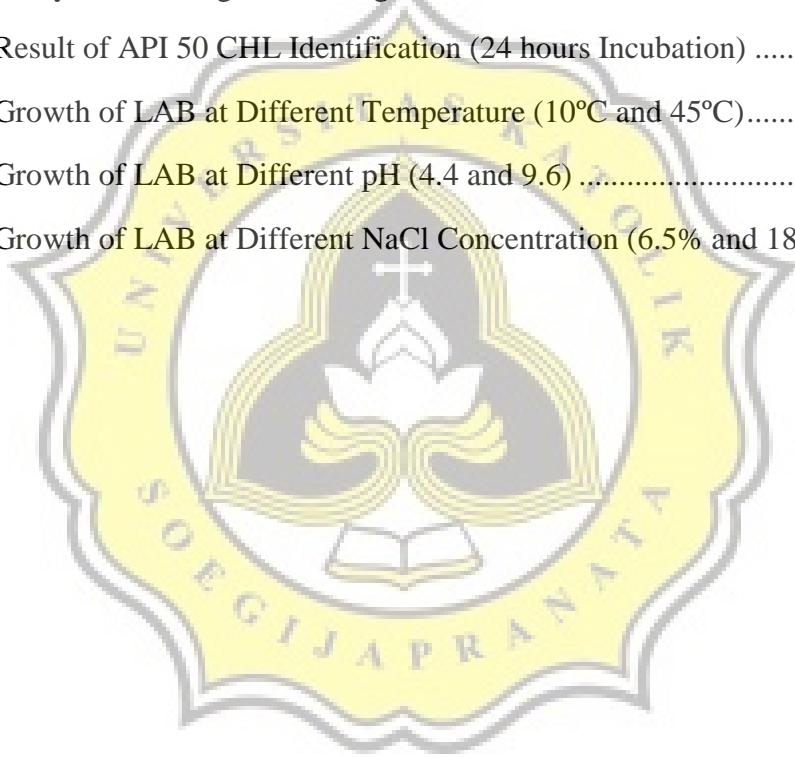
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