THE EFFECT OF SUCROSE AND NITROGEN SUPPLEMENTATION TO BACTERIOCIN PRODUCTION OF LACTIC ACID BACTERIA ISOLATED FROM AMPEL BAMBOO SHOOT (*Bambusa vulgaris*) PICKLE UNDER DIFFERENT FERMENTATION CONDITIONS

BACHELOR THESIS

Submitted in partial fulfillment of the requirements for a Food Technology Bachelor’s degree in Faculty of Agricultural Technology

By:

AGATHA PUTRI ALGUSTIE

13.70.0126

DEPARTMENT OF FOOD TECHNOLOGY
FACULTY OF AGRICULTURAL TECHNOLOGY
SOEGIJAPRANATA CATHOLIC UNIVERSITY
SEMARANG

2017
THE EFFECT OF SUCROSE AND NITROGEN SUPPLEMENTATION TO BACTERICIN PRODUCTION OF LACTIC ACID BACTERIA ISOLATED FROM AMPEL BAMBOO SHOOT (Bambusa vulgaris) PICKLE UNDER DIFFERENT FERMENTATION CONDITIONS

PENGARUH SUPLEMENTASI SUKROSA DAN NITROGEN TERHADAP PRODUKSI BAKTERIOSIN BAKTERI ASAM LAKTAT YANG DIISOLASI DARI ACAR REBUNG AMPEL (Bambusa vulgaris) DALAM KONDISI FERMENTASI YANG BERBEDA

By:
AGATHA PUTRI ALGUSTIE
13.70.0126

This thesis has been approved and defended in front of the examination committees on 24th January 2017
Semarang, 9th February 2017
Faculty of Agricultural Technology
Soegijapranata Catholic University

Supervisor,
Dra. Laksmi Hartayanie, MP.

Dean,
Dr. V. Kristina Ananingsih, ST., MSc.

Co-Supervisor,
Dr. Ir. Lindayani, MP., PhD.
STATEMENT OF THESIS AUTHENTICITY

I hereby declare that this thesis entitled “THE EFFECT OF SUCROSE AND NITROGEN SUPPLEMENTATION TO BACTERIOCIN PRODUCTION OF LACTIC ACID BACTERIA ISOLATED FROM AMPEL BAMBOO SHOOT (Bambusa vulgaris) PICKLE UNDER DIFFERENT FERMENTATION CONDITIONS” 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.

If it is proven in the future that partially or whole thesis is the result of plagiarism, therefore I will be willing to be revoked with all the consequences in accordance with the law and regulation applied at Soegijapranata Catholic University and/or valid law and regulations.

Semarang, February 2017

Agatha Putri Algustie
13.70.0126
SUMMARY

Ampel bamboo shoot was originate from bamboo plant (*Bambusa vulgaris*) which abundant in Indonesia. It was an edible product that perishable in nature and had a relatively short shelf life. Fermentation was one way to preserve bamboo shoots. In bamboo shoot fermentation, lactic acid bacteria (LAB) were the major natural strain that took role in the process, which could give typical taste and extending the shelf life. It was because of its ability to produce antimicrobial compounds, such as organic acid, hydrogen peroxyde and bacteriocins. To obtain optimal production of bacteriocins, appropriate environment and nutrient in culture media were required. The presence of carbon and nitrogen source could induce bacteriocin production from LAB strain, but studies on its applications for LAB from Ampel bamboo shoot pickle was still limited. Therefore, this study aimed to evaluate the effect of initial fermentation conditions and the supplementation of sucrose and nitrogen in culture medium towards bacteriocin production of LAB isolated from Ampel bamboo shoot pickle under different fermentation conditions, also to find the optimum bacteriocin produced by the isolates. Fermentation of bamboo shoots were done in 2.5% of salt concentration at 15ºC for 5 days (code A) and in 5.0% of salt concentration at 30ºC for 4 days (code B). After that, 35 isolates which isolated from Ampel bamboo shoot pickle were screened and analyzed. The analysis included identification of LAB based on cell morphology, motility test, physiological test and the probiotic potential analysis that consist of acid tolerance test (pH 3 and 7) and bile salts tolerance (0.3% and 0.5%). As much as 32 LAB isolates were selected then tested for antimicrobial activity against pathogenic bacteria *E. coli* FNCC 0091, *L. monocytogenes* FNCC 0156 and *S. aureus* FNCC 0047. The results of the antimicrobial activity analysis showed all isolates had antimicrobial activity against pathogenic bacteria. Bacteriocin activity analysis were done to isolates that had been grown in MRS-B media supplemented with sucrose as carbon source and yeast extract and peptone as nitrogen source. The test was done using agar-well diffusion method by measuring the clear zone and calculate the Activity Unit (AU in units of mm$^2$ mL$^{-1}$). Thirteen isolates from fermentation B and 10 isolates from fermentation A were shown to have bacteriocin inhibitory activity ranging between 13.10-3274.99 mm$^2$ mL$^{-1}$. The largest inhibition was found on isolate B16 against *S. aureus* FNCC 0047 with supplementation of 2% sucrose, while bacteriocin B9 had the optimum inhibitory against pathogens *E. coli* FNCC 0091, *L. monocytogenes* FNCC 0156 and *S. aureus* FNCC 0047 when supplemented with 2% sucrose and 0.8% yeast extract. In this study, it was found that supplementation of sucrose and nitrogen on culture medium could induce bacteriocin production of LAB, which the results depended on each LAB isolate.
RINGKASAN

Rebungan Ampel berasal dari tanaman bambu (Bambusa vulgaris) yang melimpah di Indonesia. Rebungan merupakan bahan makanan yang mudah rusak dan memiliki umur simpan yang relatif singkat. Fermentasi merupakan salah satu cara pengolahan rebungan. Dalam fermentasi rebungan, bakteri asam laktat (BAL) merupakan strain alami yang memiliki peran dalam proses fermentasi dalam pembentukan cita rasa serta memperpanjang umur simpan. Hal ini disebabkan oleh kemampuannya untuk menghasilkan senyawa antimikroba, seperti asam organic, hidrogen peroksida dan bakteriosin. Untuk mendapatkan produksi bakteriosin yang optimal maka diperlukan kondisi lingkungan dan nutrisi media kultur yang sesuai. Penambahan sumber karbon dan nitrogen dapat menginduksi produksi bakteriosin BAL, namun studi mengenai aplikasinya pada BAL dari asinan rebungan masih terbatas. Oleh karena itu, penelitian ini bertujuan untuk mengevaluasi pengaruh kondisi fermentasi awal dan suplementasi sukrosa dan nitrogen dalam media terhadap produksi bakteriosin BAL yang ditidurakan dari acar rebungan Ampel, serta untuk mengetahui bakteriosin yang paling optimal yang diproduksi dari isolat BAL. Fermentasi rebungan dilakukan pada kadar garam 2.5% dengan suhu 15ºC selama 5 hari (kode A) dan kadar garam 5.0% dengan suhu 30ºC selama 4 hari (kode B). Selanjutnya dilakukan analisa dan seleksi terhadap 35 isolat hasil isolasi asinan rebungan Ampel. Pengujian meliputi identifikasi BAL berdasarkan morfologi, uji motilitias, identifikasi secara fisiologis dan uji potensi probiotik yang meliputi uji ketahanan asam (pH 3 dan 7) serta ketahanan garam empedu (0.3% dan 0.5%). Sebanyak 32 isolat BAL yang terpilih selanjutnya diuji aktivitas antimikroba terhadap bakteri patogen E. coli FNCC 0091, L. monocytogenes FNCC 0156 dan S. aureus FNCC 0047. Hasil dari uji antimikroba menunjukkan semua isolat memiliki aktivitas antimikroba terhadap bakteri patogen. Uji aktivitas bakteriosin dilakukan terhadap isolat yang telah ditumbuhan dalam media MRS-B yang disuplementasi sukrosa sebagai sumber karbon serta ekstrak yeast dan pepton sebagai sumber nitrogen. Uji tersebut menggunakan metode difusi sumuran agar yang diamati dengan mengukur zona bening serta menghitung Activity Unit (AU dalam satuan mm\(^2\) mL\(^{-1}\)). Tiga belas isolat dari fermentasi B dan 10 isolat dari fermentasi A menunjukkan aktivitas penghambatan bakteriosin yang berkisar antara 13.10-3274.99 mm\(^2\) mL\(^{-1}\). Aktivitas penghambatan terbesar terdapat pada isolat B16 terhadap indikator patogen S. aureus FNCC 0047 setelah disuplementasi dengan 2% sukrosa, sedangkan bakteriosin B9 memiliki penghambatan yang paling optimal terhadap patogen E. coli FNCC 0091, L. monocytogenes FNCC 0156 and S. aureus FNCC 0047 ketika disuplementasi dengan 2% sukrosa dan 0.8% ekstrak yeast. Dalam penelitian ini dapat diketahui bahwa suplementasi sukrosa dan nitrogen dalam media kultur mampu menginduksi produksi bakteriosin BAL yang hasilnya tergantung pada masing-masing isolat BAL.
ACKNOWLEDGEMENT

Praise to God, only because of His grace and bless, the author would have the opportunity to join the research team and complete this thesis. This thesis which entitled “THE EFFECT OF SUCROSE AND NITROGEN SUPPLEMENTATION TO BACTERIOCIN PRODUCTION OF LACTIC ACID BACTERIA ISOLATED FROM AMPEL BAMBOO SHOOT (Bambusa vulgaris) PICKLE UNDER DIFFERENT FERMENTATION CONDITIONS,” has been written to fulfill the requirements for a Food Technology Bachelor’s degree in Faculty of Agricultural Technology. This thesis is financially supported by Penelitian Unggulan Perguruan Tinggi (PUPT) 2016 and being part of 2nd year PUPT research entitled “Efek Probiotik dan Mikrostatik dari Bakteri Asam Laktat yang Berperan dalam Fermentasi Acar Rebung”.

The accomplishment in this research and the report making was cherished by the support and guidance given by amazing people around the author. There are my special thanks for:
1. Dr. Victoria Kristina Ananingsih, ST., MSc., Dean of Faculty of Agricultural Technology, Soegijapranata Catholic University, for giving the chance to finish this final research.
2. Dra. Laksmi Hartayanie, M.P. as my supervisor and Dr. Ir. Lindayani, MP as my co-supervisor for their encouraging guidance and advices for doing the research and making this report better.
3. Katharina Ardanareswari, S.TP, M.Sc., as thesis coordinator for arranging the administration and schedule during this process.
4. All lecturers, laboratory assistants and administration staff for the help and for giving the joyful of learning in Food Technology Department
5. My parents (Agus Sri Haryanto and Albertine Sendang N.), my brother, Alexandre Donny Algustie and sisters, Anna Karina Algustie and Anastasia N. Algustie who always support and bring my name in their prayer.
6. Donna Larissa Khuangga as my partner who cherish, support and help me during the process.
7. Amelia Juwana, Agata Apriliana, Simon Armando and Ita Mariana as PUPT team partners for the help, advices and experience to work as a research team.

8. Lukas Bryandito Wicaksono, for always be there for me, believe in me and support me through good and hard times.

9. Chikita Eljo, Rosa Xena, Christian P, all my friends in PAVALI members, Student Organization Team, GLORY 6 Team, NUDC Kopertis VI Team, 2nd ASEC Team, ASEACCU Team, AU Internship Team, Student Senate Team, Assistant Laboratory Team, Djarum Beasiswa Plus and all student in Food Technology for giving me wonderful experiences and had been part of my self-development during campus life.

10. All people who have directly and indirectly helped me during the research work until finishing the report.

The author realized that this report is still far from perfect and there are still many shortages due to the limitation of the author. However, the author hopes that this report could provide useful information for the readers. The author really allows all readers to give suggestions to future studies. The author was very grateful for such a valuable and unforgettable experience from this research process.

Semarang, February 2017

Author,

Agatha Putri Algustie
LIST OF CONTENTS

SUMMARY ............................................................................................................. i
RINGKASAN........................................................................................................ ii
ACKNOWLEDGEMENT....................................................................................... iii
LIST OF CONTENTS ............................................................................................. v
LIST OF TABLES ................................................................................................ . vii
LIST OF FIGURES .............................................................................................. viii
LIST OF APPENDICES .......................................................................................... x

1. INTRODUCTION ...................................................................................... 1
   1.1. Background of research ............................................................................... 1
   1.2. Literature review .......................................................................................... 2
       1.2.1. Lactic Acid Bacteria (LAB) .................................................................. 2
       1.2.2. Bamboo Shoots ............................................................................... 4
       1.2.3. Important Role of Lactic Acid Bacteria in Bamboo Shoots Fermentation 4
       1.2.4. Bacteriocins ...................................................................................... 5
       1.2.5. Sucrose as Medium for Bacteriocin Production ................................ 6
   1.3. Objectives ................................................................................................... 7

2. MATERIALS AND METHODS ................................................................ 8
   2.1. Materials ..................................................................................................... 8
   2.2. Methods ...................................................................................................... 9
       2.2.1. Experimental Design ........................................................................... 9
       2.2.2. Fermentation of Ampel Bamboo Shoots .......................................... 10
       2.2.3. Screening, Isolation, and Identification of Lactic Acid Bacteria ..........10
       2.2.4. Screening Probiotic Potentials of Lactic Acid Bacteria ......................12
       2.2.5. Determination of Antimicrobial Activity .......................................... 13
       2.2.6. Determination of Bacteriocin Activity .............................................. 13
       2.2.7. LAB Species Identification ............................................................... 15

3. RESULTS ................................................................................................ .16
   3.1. Fermentation of Ampel bamboo shoots ..................................................... 16
   3.2. Isolation of bacteria from Ampel bamboo shoot ....................................... 16
   3.3. Screening of Lactic Acid Bacteria (LAB) from Ampel bamboo shoot .......17
       3.3.1. Cell Morphology and Motility Test ................................................. 17
       3.3.2. Physiological Test .......................................................................... 20
   3.4. Screening Probiotic Potentials of LAB ..................................................... 22
   3.5. Determination of Antimicrobial Activity of LAB .................................... 25
   3.6. Determination of Bacteriocin Inhibitory Activity ..................................... 27
   3.7. Identification of LAB Species ................................................................... 43

4. DISCUSSIONS ......................................................................................... 44
   4.1. Fermentation of Ampel Bamboo Shoots ............................................... 44
   4.2. Isolation of bacteria from Ampel bamboo shoot ....................................... 45
4.3. Screening of Lactic Acid Bacteria (LAB) from Ampel bamboo shoot........45
  4.3.1. Cell Morphology and Motility Test .............................................45
  4.3.2. Physiological Test .....................................................................47
4.4. Screening Probiotic Potentials of LAB ..........................................48
4.5. Determination of Antimicrobial Activity of LAB ...........................49
4.6. Bacteriocin Inhibitory Activity and Identification of LAB species ....50

5. CONCLUSIONS AND SUGGESTIONS ..............................................57
  5.1. Conclusions ................................................................................57
  5.2. Suggestions ................................................................................57

6. REFERENCES ....................................................................................58

7. APPENDICES ....................................................................................64
LIST OF TABLES

Table 1. Result of Cell Morphology and Motility Test ............................................17
Table 2. Result of Physiological Test of LAB Isolates.............................................21
Table 3. Result of Probiotic Potentials Test to LAB Isolates....................................22
Table 4. Result of Antimicrobial Activity Assay .....................................................25
Table 5. Bacteriocin Inhibitory Activity (mm$^2$mL$^{-1}$) on Supplemented MRS-B Medium Against Pathogenic Bacteria........................................28
Table 6. Result of API Software Identification (24 hours Incubation)......................43
Table 7. Growth of LAB at Different Temperature (10°C and 45°C) ......................66
Table 8. Growth of LAB at Different pH (4.4 and 9.6) ............................................67
Table 9. Growth of LAB at Different NaCl Concentration (6.5% and 18%)..........68
LIST OF FIGURES

Figure 1. Ampel Bamboo Shoots (Bambusa vulgaris) .......................................................... 8

Figure 2. Flowchart of Research Design ............................................................................. 9

Figure 3. Five days of fermentation in 2.5% of salt concentration at 15°C (a); Four days of fermentation in 5% of salt concentration at 30°C (b). .................................................................................. 16

Figure 4. Single colony of LAB (arrow) from purification in MRS agar medium supplemented with CaCO₃ ................................................................. 17

Figure 5. Gram-positive bacteria (isolate B16) under 10 x 100 magnification (a); Gram-negative bacteria (isolate B17) under 10 x 100 magnification (b) ............................................................................. 19

Figure 6. Non-spore forming bacteria (isolate A11) under 10 x 100 magnification ................................................................................................................................. 19

Figure 7. Motility Test on Non motile bacteria (isolate B1 and B13) ..................................... 20

Figure 8. Motility Test on Isolate B7 and B18 (No Visible Growth) ..................................... 20

Figure 9. Resistance of Isolate B13 to pH 3 at 0 hour (a-i); 1.5 hours (a-ii); 3 hours (a-iii); and pH 7 at 0 hour (b-i); 1.5 hours (b-ii); 3 hours (b-iii) ..................................................................................................... 24

Figure 10. Resistance of Isolate B13 to 0.3% bile salt at 0 hour (a-i); 2 hours (a-ii); 4 hours (a-iii); and 0.5% bile salt at 0 hour (b-i); 2 hours (b-ii); 4 hours (b-iii) ............................................................................. 25

Figure 11. Antimicrobial Activity of Isolate B16 Against E. coli FNCC 0091 (B16a); L. monocytogenes FNCC 0156 (B16b); and S. aureus FNCC 0047 (B16c) .................................................................................. 27

Figure 12. Effect of Medium Compositions on Bacteriocin Inhibitory Activity (mm²/mL¹) of LAB Isolates Against E. coli FNCC 0091, L. monocytogenes FNCC 0156 and S. aureus FNCC 0047 .................................................................................. 33

Figure 13. Effect of Medium Compositions on Bacteriocin A14 Inhibitory Activity. ................................................................................................................................. 37
Figure 14. Effect of Medium Compositions on Bacteriocin A17 Inhibitory Activity. .................................................................38

Figure 15. Effect of Medium Compositions on Bacteriocin A22 Inhibitory Activity. .................................................................39

Figure 16. Effect of Medium Compositions on Bacteriocin B9 Inhibitory Activity. .................................................................40

Figure 17. Effect of Medium Compositions on Bacteriocin B14 Inhibitory Activity. .................................................................41

Figure 18. Effect of Medium Compositions on Bacteriocin B16 Inhibitory Activity. .................................................................42

Figure 19. Identification of Isolate A17 using API 50 CH .................64
LIST OF APPENDICES

Page

Appendix 1. Species Identification of LAB using API 50 CHL ............................. 64
Appendix 2. Medium Used for Growth and Bacteriocin Inhibitory Activity .......... 65
Appendix 3. Compositions of Standard Solution McFarland 2, 3 and 5 ............... 66
Appendix 4. Result of Growth Capability Test on LAB Isolates at Different Temperature, pH, and NaCl Concentration .............................................. 66