UNIVERSITI PUTRA MALAYSIA

EXPLORING THE POTENTIAL OF LACTIC ACID BACTERIA FROM RHIZOSPHERE OF PAPAYA FOR HYDROGEN CYANIDE PRODUCTION

NOOR SUHAILLA AZZUANA MARZIHA

FBSB 2015 156

EXPLORING THE POTENTIAL OF LACTIC ACID BACTERIA FROM RHIZOSPHERE OF PAPAYA FOR HYDROGEN CYANIDE PRODUCTION

By

NOOR SUHAILLA AZZUANA BINTI MARZIHA

Thesis Submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Bachelor of Science (Hons.) Cell and Molecular Biology

JUNE 2015

Abstract of thesis presented to the Department of Cell and Molecular Biology in fulfillment of the requirement for the degree (Bachelor of Science (Hons.) Cell and Molecular Biology)

EXPLRORING THE POTENTIAL OF LACTIC ACID BACTERIA FROM RHIZOSPHERE OF PAPAYA FOR HYDROGEN CYANIDE PRODUCTION

By

NOOR SUHAILLA AZZUANA BINTI MARZIHA

June 2015

Chairman : Amalia Mohd Hashim, PhD

Faculty : Biotechnology and Biomolecular Sciences

Hydrogen cyanide (HCN) produced by plant growth promoting bacteria plays an important role in enhancing plant growth. This chemical compound helps in suppressing plant pathogens and act as biological control agent against plant diseases. HCN is also known as an antimicrobial compound involved in biological control of many root diseases. In Malaysia, the spread of Papaya Dieback Disease (PDD) has caused great losses to papaya production. Unfortunately, the effective remedies to combat this disease are lacking. This study was carried out to investigate the potential

of LAB as biological control agent in producing hydrogen cyanide (HCN) as a mechanism to inhibit the causing pathogen of PDD, *Erwinia mallotivora*. Five endophytes and twenty rhizospheric microbes from papaya plant were tested for their HCN-producing ability and it was found that the produced HCN levels were successfully detectable. These findings revealed one of the alternatives that can be used in agriculture to control plant diseases. The use of these promising isolates in bio-fertilizer is anticipated to restrain the pathogen and enhance plant growth.

Keywords: Lactic acid bacteria, HCN production, plant growth promoting bacteria, plant diseases, biological control agent

Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel dan Molekul

Sebagai memenuhi keperluan untuk ijazah (Ijazah Sarjana Muda (Kepujian) Biologi Sel dan Molekul)

MENEROKA POTENSI BAKTERIA ASID LAKTIK DARI RIZOSFERA POKOK BETIK UNTUK PENGHASILAN HYDROGEN SIANIDA

Oleh

NOOR SUHAILLA AZZUANA BINTI MARZIHA

Jun 2015

Pengerusi : Amalia Mohd Hashim, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Hidrogen sianida (HCN) yang dihasilkan oleh bakteria penggalak pertumbuhan tumbuhan memainkan peranan penting dalam memantapkan tumbesaran pokok. Sebatian kimia ini membantu membenteras patogen-patogen tumbuhan dan bertindak sebagai agen kawalan biologi terhadap penyakit tumbuhan. HCN juga dikenali sebagai sebatian antimikrob yang terlibat dalam kawalan biologi untuk kebanyakan penyakit akar. Di Malaysia, penyebaran Penyakit Mati Rosot Betik (PDD) telah menyebabkan kerugian yang besar terhadap pengeluaran betik. Malangnya, penawar yang berkesan

untuk memerangi penyakit ini tidak mencukupi. Kajian ini telah dijalankan untuk menyiasat potensi bakteria asid laktik sebagai agen kawalan biologi dalam menghasilkan HCN sebagai mekanisma untuk menghalang patogen penyebab PDD, *Erwinia mallotivora*. Lima sampel endofitik dan dua puluh sampel mikrob rizosfera daripada pokok betik telah diuji keupayaan mereka untuk menghasilkan HCN dan penghasilan HCN berjaya dikesan. Penemuan ini mendedahkan salah satu alternatif yang boleh digunakan dalam bidang pertanian untuk mengawal penyakit tumbuhan. Penggunaan bakteria yang menjanjikan ini di dalam baja-bio dijangka dapat menghalang patogen dan meningkatkan pertumbuhan tumbuhan.

Kata kunci: bakteria laktik asid, pengeluaran HCN, bakteria penggalak pertumbuhan, penyakit tumbuhan, agen kawalan biologi

ACKNOWLEDGEMENT

Special dedications:

I want to give my big thanks to these persons for guiding me and for giving me tons of helps while preparing and finishing this thesis.

Me, Myself and I

Academic advisor - Dr. Adam Leow Thean Chor

Dedicated, awesome supervisor - Dr. Amalia Mohd Hashim

Family – Mum, Dad, Sisters: Cik Fieza, Hazlieyn and adik Aleesyh

Lab seniors – Kak Nana, Kak Anis, Kak Mirah and Kak Balkis

Friends – Maya, Nana and the whole classmates

Beloved - you know who you are ^_^

My special thanks also go to all lecturers who teach me to become a better person from first semester up to this very end. Thank you for the knowledge, thank you for the courage, thank you for everything that I couldn't express in words. I just want to say thank you.

One word to describe this: Alhamdulillah

APPROVAL

This thesis was submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences and has been accepted as fulfillment of the requirement for the degree of Bachelor of Science (Hons.) Cell and Molecular Biology. The member of the Supervisory Committee was as follows:

Dr. Amalia Mohd Hashim, PhD
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia

.....

(Associate Professor Dr. Janna Ong Abdullah)
(Janna Ong Abdullah, PhD)
Head of Department
Cell and Molecular Biology,
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia

Date: .....

DECLARATION

Declaration by undergraduate student:

I hereby confirm:

- that this thesis is my original work;
- the quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions.
- intellectual property from the thesis and copy right of thesis are fully-owned by the Department of Cell and Molecular Biology.
- written permission must be obtained from supervisor before thesis is published (in the form of written, printed, or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials.
- there is no plagiarism or data falsification/fabrication in the thesis.
- the thesis has undergone plagiarism detection software (TURNITIN).

Signature: ……... Date……...

Name and Matric No.: Noor Suhailla Azzuana binti Marziha (163832)

Declaration by Supervisor

This is to confirm that:

- the research conducted and the writing of this thesis was under supervision.

Signature: .....

(Dr. Amalia binti Mohd Hashim)

Date: .....

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

dH_2O	Distilled water
HCN	Hydrogen cyanide
H_2O	Water molecule
H_2O_2	Hydrogen peroxide
IAA	indole-3-acetic acid
LAB	Lactic acid bacteria
MRS	de Man, Rogosa and Sharpe
NH_3	Ammonia
O_2	Oxygen gas
PGPB	Plant growth promoting bacteria
ml	Milliliter
µl	Microliter
^0C	degree Celsius
h	Hour
ha	Hectare
%	Percent

CHAPTER 1: INTRODUCTION

The research entitled 'Exploring the Potential of Lactic Acid Bacteria from the Rhizosphere of Papaya for Hydrogen Cyanide Production' is a study of HCN-producing property by lactic acid bacteria isolated from different region of papaya trees. Twenty five isolates consists of five endophytes from papaya and another twenty are from the rhizosphere of papaya. These isolates are tested for their capability to produce HCN, a chemical compound which act as biological control agent in suppressing plant pathogens. There are two sets of isolates used in this study. The first set contains five isolated endophytes designated as PPS seed 1, PPK SCT 1 and PPK SCT 4S, A28 and A29. On the other hands, the second set contains twenty isolates from papaya rhizosphere. The morphology of all isolates is studied to confirm their belonging to the lactic acid bacteria (LAB) group. This study is carried out at Plants and Molecular Biology Laboratory, Universiti Putra Malaysia (UPM).

The production of hydrogen cyanide (HCN) by rhizospheric bacteria has been reported as an important antifungal trait to control fungi which infect the plant root as well as suppressing plant pathogen by the mean of antimicrobial property. Previous research by Heydari et al., (2008) reported that *Pseudomonas* sp. could produce HCN. However, not many researches have been conducted on LAB in order to investigate their HCN-production property, especially on how they deal with pathogen that attack papaya trees.

This study hypothesizes that the microbes isolated from the rhizosphere of papaya which likely to be LAB may have the ability to produce HCN. The aim of this study is to examine capability of hydrogen cyanide (HCN) production by beneficial microorganisms isolated from papaya fruits and its rhizosphere which make them beneficial to be applied as biological control agent. To achieve that, two tests which are LAB biochemical tests and qualitative assay of HCN production were performed. The LAB biochemical tests included Gram staining and catalase test. The HCN-production was carried out on isolates which positives to LAB biochemical test.

REFERENCES/BIBLIOGRAPHY:

Aravind G., Debjit Bhowmik, Duraivel S., Harish G. (2013). Traditional and Medicinal Uses of Carica papaya. Journal of Medicinal Plants Studies. 1: 7-15

Bergey, David H., John G. Holt, Noel R. Krieg, Peter H.A. Sneath. (1994). Bergey's Manual of Determinative Bacteriology (9th Ed.) Lippincott Williams & Wilkins. ISBN 0-683-00603-7.

Berg G. (2009). Plant–microbe Interactions Promoting Plant Growth and Health: Perspectives for Controlled Use of Microorganisms in Agriculture. Journal of Applications of Microbiology and Biotechnology. 84: 11-18.

Beveridge T.J (2001). Uses of the Gram Stain in Microbiology. Biotech Histochemistry 76 (3): 111–8.

Clarke H. and Cowan S.T (1952). Biochemical Methods for Bacteriology. Journal of General Microbiology. 6**:** 187–197.

Compant S., Clément C., Sessitsch A. (2010). Plant Growth-Promoting Bacteria in the Rhizo and Endosphere of Plants: Their Role, Colonization, Mechanisms Involved and Prospects for Utilization. Soil Biology and Biochemistry. 42: 669-678.

De Man, Rogosa J.C., Elisabeth Sharpe M. (1960). A Medium for the Cultivation of Lactobacilli. Journal of Application Biology 23: 130-135

Dey R., Pal K.K, Bhatt D.M and Chauhan S.M. (2004). Growth Promotion and Yield Enhancement of Peanut (*Arachis hypogaea* L.) by Application of Plant Growth Promoting Rhizobacteria. Microbiology Res. 159: 371-394.

Finegold S.M, Martin W.J, Scott E.G. Reagents and Tests, in Bailey & Scott's Diagnostic Microbiology, 5th ed., CV Mosby Co., St. Louis, MO, p. 482, 1978.

Gaby S.K. and Singh V.N. (1991). Vitamin C; Vitamin Intake and Health: A Scientific Review, Gaby S.K., Bendich A., Singh V. and Machlin, L. (eds.). Marcel Dedder, N.Y

Hammes W.P, Vogel R.F (1995). The Genus Lactobacillus. In Wood B.J.B, Holzapfel W.H (eds), The genera of Lactic Acid Bacteria. Blackie Academic & Professional, London, 19–54.

Herman M.A.B., Nault B.A. and Smart C.D. (2008). Effect of Plant Growth Promoting Rhizobacteria on Bell Pepper Production and Green Peach Aphid Infestations in New York. Crop Protect. 27: 996-1002.

Heydari S., Moghadam P.R, Arab S.M (2008). Hydrogen Cyanide Production Ability by Pseudomonas Fluorescence Bacteria and Their Inhibition Potential on Weed. In Proceedings. Competition for Resources in a Changing World: New Drive for Rural Development.

Kim M., Moore P., Zee F., Fitch M.M.M., Steiger D., Manshardt R., Paull R., Drew R.A, Sekioka T., Ming R. (2002). Genetic Diversity of *Carica papaya* as revealed by AFLP markers. Genome 45(3): 503–512

Kokalis-Burelle N., Kloepper J.W and Reddy M.S (2006). Plant Growth-Promoting Rhizobacteria as Transplant Amendments and Their Effects in Indigenous Rhizosphere Microorganisms. Application of Soil Ecosystem. 31: 91-100.

Leroy F., Falony G., Vuyst L. (2008). Latest Developments in Probiotics. In: Toldra F., editor of Meat Biotechnology. Brussels, Belgium: Springer. 217-229.

MacFaddin J.F (2000). Biochemical Tests for Identification of Medical Bacteria, (3rd eds.) Lippincott Williams & Wilkins, Philadelphia, PA.

Mahon C.R, Lehman D.C, and Manuselis G. (2011). Textbook of Diagnostic Microbiology, (4th eds.) Saunders W. B Co., Philadelphia, PA.

O'Meara R.A.Q (1931). A Simple Delicate and Rapid Method of Detecting the Formation of Acetyl-methylcarbinol by Bacteria Fermenting Carbohydrate. Journal of Pathology Bacteria. 34: 401–406.

Perrig D., Boiero M., Masciarelli O., Penna C., Ruiz O., Cassán F., et al., (2007). Plant-Growth Promoting Compounds Produced by Two Agronomically Important Strains of *Azospirillum brasilense*, and Implications for Inoculant Formulation. Journal of application of Microbiology Biotechnology. 75: 1143-1150.

Reddy G., Altaf Md., Naveena B.J, Venkateshwar M., Vijay Kumar E. (2008). Biotechnology Advances 26: 22-34.

Salminen S., Wright A., Ouwehand A.C (eds.) (2004). Lactic Acid Bacteria: Microbiological and Functional Aspects, (3rd eds.) Marcel Dekker, New York.

Schippers B., Bakker A., Bakker P., van Peer R., (1990). Beneficial and Deleterious Effects of HCN-producing Pseudomonads on Rhizosphere Interactions. Plant and Soil, 129 (1): 75-83.

Tannock G. (ed.) (2005). Probiotics and Prebiotics: Scientific Aspects, (1st eds). Caister Academic, Wymondham.

Van den Berg D.J.C, Smith A., Pot B., Ledeboer A.M., Kerstens K., Verbakel J.M.A., and Verrips C.T. (1993). Isolation, Screening and Identification of Lactic Acid Bacteria from Traditional Food Fermentation Processes and Culture Collections. Journal of Food Biotechnology. 7: 189-205.

Voisard C., Keel C., Haas D. and Defago G. (1989). Cyanide Production by *Pseudomonas fluorescens* Helps Suppressing Black Root Rot of Tobacco under Gnotobiotic Conditions. EMBOJ. 8: 351-358.

Wade W.E., Smiley K.L, and Boruff C.S (1946). An Improved Method for Differentiating Acid-forming from Non-acid-forming Bacteria. Journal of Bacteriology. 61: 787-788.

Wani P.A, Khan M.S, Zaidi A. (2007). Co-inoculation of Nitrogen-fixing and Phosphate Solubilizing Bacteria to Promote Growth, Yield and Nutrient Uptake in Chickpea. 55: 315-323.