BORANG ERGS – P3(R)

Code Project :

KEMENTERIAN PENDIDIKAN MALAYSIA

FINAL REPORT
EXPLORATORY RESEARCH GRANT SCHEME (ERGS)

Laporan Akhir Skim Geran Penyelidikan Eksploratori (ERGS) IPT
Pindaan 1/2015

A

RESEARCH TITLE: The role of 1-Aminocyclopropane-1-Carboxylate Deaminase (ACCD) in plant-microbe interaction and lycopene content of tomato

PHASE & YEAR: 1/2012

START DATE: 1 August 2012
END DATE: 31 July 2014
EXTENSION PERIOD (DATE): RMC LEVEL: 1 August 2014 - 31 January 2015
KPM LEVEL: 1 February 2015 – 30 April 2015

PERPUST

PROJECT MEMBERS: 1) Prof Mohd Nazalan b. Mohd Najimudin.
(including GRA) 2) Sajid Khan (GRA)

PROJECT ACHIEVEMENT *(Prestasi Projek)*

B

ACHIEVEMENT PERCENTAGE				
Project progress according to milestones achieved up to this period	Evaluation and identification of suitable diazotrophic isolates	Plant phytohormone study	Morphological study	Plant Phytohormones
Percentage	**100%**	**100%**	**100%**	**100%**

RESEARCH OUTPUT		
Number of articles/ manuscripts/ books *(Please attach the First Page of Publication)*	**Refereed Journal**	***Non-Refereed Publication***
Conference Proceeding *(Please attach the First Page of Publication)*	**International**	***National***
	Salwani, S., Amir Hamzah Ghazali and Nazalan N. (2013). Presence of 1-Aminocyclopropane-1-C arboxylate (ACC) Deaminase Enzyme in Phytohormone-Producing *Burkholderia sp.* USMB20. International Congress of the Malaysian Society for Microbiology 2013 (ICMSM 2013). 12-15 December 2013. Langkawi Malaysia. p. 155-158.	Sajid Khan and Amir Hamzah Ahmad Ghazali (2014). Influence of diazotrophs and minimal N supply on plant morphological traits, yield and lycopene content of tomato (*Lycopersicon esculentum*) The 13th Symposium Malaysian Society of Applied Biology. 8-10 June 2014. Cherating Pahang.

	Nazalan Najimudin, Amir Hamzah Ghazali, Salwani Shaffie, Nurohaida Abd. Aziz, Ahmad faisa; Mohamed and Yamin Abdul Rahman (2014). *Burkholderia* as a model to unravel the mechanisms of plant microbe interaction. 3rd. Asian Conference on Plant-Microbe Symbiosis. Chengdu China. 28 October – 2 November 2014. Salwani Shaffie, Amir Hamzah Ghazali and Nazalan Najimudin (2014). Inoculation of phytohormone producing *Burkholderia sp.* USMB20 promoted effective root nodules of *Mucuna bracteata.* The 9th Regional IMT-GT UNINET Conference. Gurney Hotel Penang, Malaysia. 3-5 November 2014.	
Intellectual Property *(Including Paten,Copyright, Industrial Design,layout Design of Integrated Circuit & Trademarks)*		

HUMAN CAPITAL DEVELOPMENT

Human Capital	**Number**				**Others** (please specify)
	On-going		**Graduated**		
Citizen	Malaysian	Non Malaysian	Malaysian	Non Malaysian	
No. PHD STUDENT	01	01			
Student Fullname: IC / Passport No: Student ID:					
No. MASTER STUDENT					
Student Fullname: IC / Passport No: Student ID:					

No. UNDERGRADUATE STUDENT	03		03		
Student Fullname: IC / Passport No: Student ID:					
Total	04	01	03		

EXPENDITURE *(Perbelanjaan)*

C **Budget Approved** *(Peruntukan diluluskan)*
Amount Spent *(Jumlah Perbelanjaan)*

Balance *(Baki)*
Percentage of Amount Spent
(Peratusan Belanja)

ADDITIONAL RESEARCH ACTIVITIES THAT CONTRIBUTE TOWARDS DEVELOPING SOFT AND HARD SKILLS
(Aktiviti Penyelidikan Sampingan yang menyumbang kepada pembangunan kemahiran insaniah)

D

International

Activity	Date (Month, Year)	Organizer
(e.g : Course/ Seminar/ Symposium/ Conference/ Workshop/ Site Visit)	International Congress of the Malaysian Society for Microbiology 2013 (ICMSM 2013). 12-15 December 2013. Langkawi Malaysia.	Malaysian Society for Microbiology
	3rd. Asian Conference on Plant-Microbe Symbiosis. Chengdu China. 28 October – 2 November 2014.	Chinese Academy of Agricultural Sciences.
	The 9th Regional IMT-GT UNINET Conference. Gurney Hotel Penang, Malaysia. 3-5 November 2014.	IMT-GT UNINET

National

Activity	Date (Month, Year)	Organizer
(e.g : Course/ Seminar/ Symposium/ Conference/ Workshop/ Site Visit)	13th Symposium of the Malaysian Society of Applied Biology 2014	Malaysian Society of Applied Biology

E **PROBLEMS / CONSTRAINTS IF ANY** *(Masalah/ Kekangan sekiranya ada)*

F **RECOMMENDATION** (*Cadangan Penambahbaikan*)

G **RESEARCH ABSTRACT – Not More Than 200 Words** (*Abstrak Penyelidikan – Tidak Melebihi 200 patah perkataan*)

Plants require an optimal level of ethylene (phytohormones) for normal growth and function, any increase in ethylene may cause deleterious effects. However, certain plant growth promoting rhizobacteria (PGPR) were able to produce 1-aminocyclopropane-1-carboxylate deaminase enzyme (ACC deaminase), a cleaving enzyme that optimized the deleterious level of ethylene and promoted normal plant growth. In the present study, our main focus will be on the cumulative effects of ACC deaminase of locally isolated *Burkholderia* sp. USM B20 and *E. coli* USM L2, *Azospirillum brasilense* (Sp7) and *Herbaspirillum seropedicae* (Z 78) on ethylene levels and plant growth promotion of host plants. Earlier results have shown the ability of *A. brasilense* Sp7 and locally isolated *Burkholderia* sp. USMB 20 to produce ACC deaminase and optimize the endogenous ethylene levels and enhanced nodule formation of *Mucuna bracteata*. Our results also showed highly significant ($p \leq 0.01$) interaction of *A. brasilense* Sp7, *H. seropedicae* Z 78 and inoculation frequency in promoting growth and lycopene content of tomato. The effects were influenced by ACC deaminase activities in controlling the synthesis of ethylene and indole acetic acid. Similar effects on plant growth promotion were also observed on rice seedlings inoculated with *H. seropedicae* Z 78.

H

International Congress of the Malaysian Society for Microbiology 2013 (ICMSM2013)
12-15 December 2013 Langkawi Lagoon Resort, Malaysia

PRESENCE OF 1-AMINOCYCLOPROPANE-1-CARBOXYLATE (ACC) DEAMINASE ENZYME IN PHYTOHORMONE-PRODUCING *Burkholderia* sp. USMB20

Salwani S.*, Amir Hamzah G. and Nazalan N.

School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia.

*Corresponding author email: salwaniusm@yahoo.com

ABSTRACT

The genus *Burkholderia* that belongs to the beta-proteobacteria class has been isolated from many legume nodules. Nevertheless, inoculation of these species has shown to form effective nodules (determinate and indeterminate) with legumes from family Mimosoideae and Papilionoid. Nodulation is a very complex process involving a variety of genes that control *NOD* factors (bacterial signaling molecules), which are essential for the establishment, maintenance and regulation of this process. Ethylene is an established potent plant hormone that is also known for its negative role in nodulation. Locally isolated *Burkholderia* sp. strain USMB20 was reinoculated and observed to promote nodulation as well as plant growth for leguminous plant, *Mucuna bracteata*. USMB20 has ACC deaminase enzyme that will lower down the endogenous ethylene levels in roots for enhancing nodule development. This enzyme converts ACC, the precursor of the plant hormone ethylene, to alpha-ketobutyrate and ammonium. USMB20 grown in medium containing 3mM ACC produced 11.099 uM/H of alpha-ketobutyrate comparable to *Azospirillum brasilense* that produced 8.935 uM/H and not detected in *Rhizobium leguminosarum* within 24H of incubation. In addition, USMB20 was able to produce phytohormone, Indole-3-Acetic Acid, IAA (0.031 mg/ml) within 24H incubation. IAA is important for plant root cell elongation and will enhance symbiosis process of USMB20. Plant growth-promoting characteristics shown by USMB20 caused effective nodulation with *M. bracteata* and stimulated plant growth.

INTRODUCTION

In nodulation, ethylene generally promotes root hair elongation, germination process, flowering and plant defense system (Ma *et al.*, 2003). In legumes, ethylene appears to play negative roles as it suppresses the nodulation process. The elongation of infection thread was inhibited in *Pisum sativum* when exogenous ethylene was treated. In contrast, inhibition of ethylene synthesis and responses has increased nodules formation in some legumes tested, *Medicago sativa, Medicago truncatula, Lotus japonicas* and *Macroptilium atropurpureum* (Nukui *et al.*, 2000; Oldroyd *et al.*, 2001). Many rhizobacteria encode ACC deaminase, which catalyses degradation of the ethylene precursor 1-aminocyclopropane- 1-carboxylic acid (ACC), tuning down the plants defense response (Glick, 2005). The gene that encoded this enzyme is *acds* and was found in many bacteria including *Burkholderia* sp. (Ma et al., 2003). Consistent with this function, rhizobial ACC deaminase gene deletion mutants induce fewer nodules compared to the wild-type strain (Ma et al., 2003). Furthermore, increased expression of ACC deaminase will give more infection opportunity for rhizobia to infect root and lead a successful nodule development for leguminous plants.
Ethylene along with IAA alters root tip cells and initiates cell elongation of lateral roots. Thus, it provides more infection area for bacterial infection prior nodulation process. Root nodules have been shown to contain more IAA than non nodulated roots and this phytohormone could be important for maintaining a functional of root nodule (Lambrecht *et al.*, 2000). There