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SIDEROPHORE PRODUCTION ACTIVITY OF RHIZOSPHERIC & LACTIC ACID BACTERIA FOR PAPAYA GROWTH PROMOTION

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Siderophore Production Activity of Rhizospheric& Lactic Acid Bacteria for Papaya Growth Promotion

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Abstract

Siderophore produced by microbial cells helps the plant to acquire iron from the surrounding. Iron uptake able to promote plant growth at the same time, acts antagonistically against pathogen by limiting iron availability. Preliminary study on Lactic Acid Bacteria (LAB) showed its potential against papaya dieback disease which was caused by causative pathogen (*Erwinia mallotivora*). The ability of LAB to produce siderophore was investigated in order to enhance their properties as biocontrol and plant growth promoter. The microbes present in rhizosphere of healthy papaya plant were screened for LAB that capable of producing siderophore using Chrome Azurol S (CAS) agar. CAS agar effectively differentiates bacteria capable of producing siderophore with non producer with simple colour changes to form orangy colony zone. Out of twelve rhizospheric and endophytic bacteria grown in selective LAB media, three isolates were tested positive for siderophore production. The characteristics of the positive isolates were confirmed by biochemical test for LAB and their species were identified using 16S rRNA sequencing.

Keywords :Siderophore, CAS agar , Rhizospheric bacteria.

Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel & Molekul Sebagai memenuhi keperluan untuk ijazah (Biologi Sel & Molekul)

Aktiviti Pengeluaran Siderophore dari Rhizospheric & Asid Laktik Bakteria untuk Promosi Pertumbuhan Betik

Oleh

Nursuhailla bt AbdMajid

Supervisor : Dr Amalia bt Mohd Hashim

Faculti : Fakulti Bioteknologi & Sains Biomolekul

Abstrak

Siderophore dihasilkan oleh sel mikrob membantu tumbuhan untuk memperoleh besi dari sekitarnya. Pengambilan zat besi dapat menggalakkan pertumbuhan pokok, pada masa yang sama, bertindak secara antagonis terhadap pathogen dengan mengehadkan ketersediaan besi. Kajian awal mengenai Bakteria Asid Laktik (LAB) menunjukkan potensi melawan penyakit betik dieback yang disebabkan oleh patogen (*Erwiniamallotivora*). Keupayaan LAB untuk menghasilkan siderophore disiasat dengan lebih teliti bagi meningkatkan ciri - ciri mereka sebagai kawalan biologi dan factor pertumbuhan pokok. Mikrob yang hidup di dalam tanah pokok betik yang sihat telah disaring untuk Bakteria Asid Laktik yang mampu menghasilkan siderophore dengan menggunakan Chrome Azurol S (CAS) agar. CAS agar berkesan membezakan bacteria yang mampu menghasilkan siderophore dengan perubahan warna yang mudah untuk dilihat seperti membentuk oren zon disekitar kultur. Daripada dua belas bakteria endofitik dan bacteria tanah yang di tumbuh di media LAB , tiga kultur telah diuji positif untuk pengeluaran siderophore. Ciri-ciri kultur yang positif telah disahkan oleh ujian biokimia sebagai LAB dan spesies mereka telah dikenal pasti menggunakan 16S rRNA penjujukan.

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Approval

This thesis was submitted to the Department of Cell & Molecular Biology , Faculty of Biotechnology & Biomolecular Sciences accepted as fulfillment of the requirement for the degree of (Cell & Molecular Biology). The member of the Supervisor Committee was as follows :

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Declaration

Declaration by under graduate student

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LIST OF SYMBOLS AND ABBREVIATIONS

LAB	Lactic Acid Bacteria
GM17	Glucose M17
H_2O_2	Hydrogen peroxide
rRNA	ribosomal Ribonucleic acid
MWS	Membrane wash solution
MBS	Membrane binding solution
SDS	Sodium dodecyl sulfate
$MgCl_2$	Magnesium chloride
MRS	de Man, Ragosa and Sharpe
LB	Luria Bertani
dNTP	Deoxyribonucleic acid Triphosphate
16S	16 sequencing
TAE	Tris base, acetic acid and EDTA
Amp	Amphicillin
ATP	Adenosine triphosphate
CAS	Chrome Azurol S
PCI	Phenol Chloroform Isoamyl alcohol
$FeCl_2.6H_2O$	Iron(II) chloride hydrate
HCl	Hydrochloric
HDTMA	Hexadecyltrimethylammonium bromide
KH_2PO_4	Potassium phosphate
MM9	Minimal Media 9
NH_4Cl	Ammonium chloride
NaCl	Sodium Chloride
NaOH	Sodium hydroxide
PIPES	Piperazinediethanesulfonic acid
NaOAc	Sodium acetate
EtBr	Ethidium bromide

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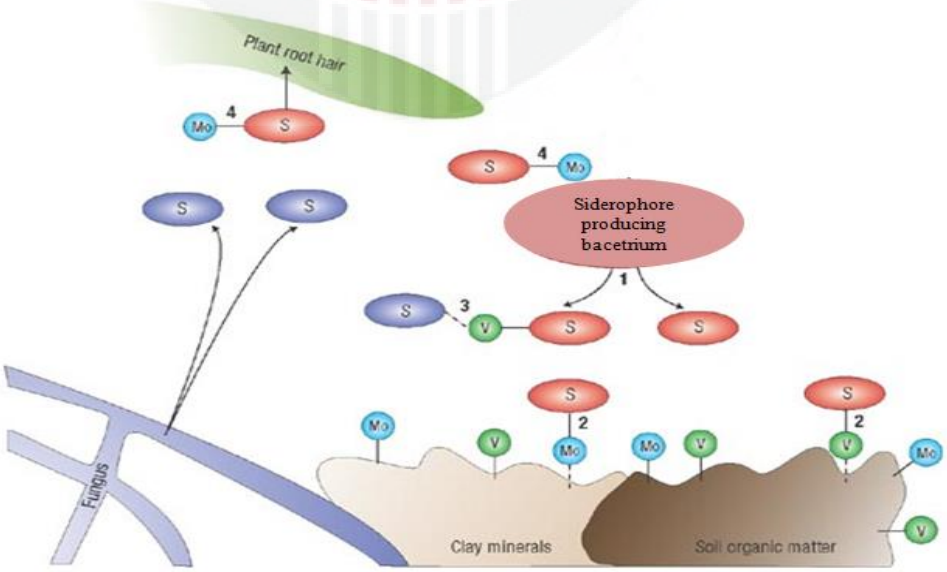
CHAPTER 1

INTRODUCTION

1.1 Background

Generally, iron uptake is essential for development in any living cell. Iron is crucial element in ATP generation, oxygen transport, cell cycle and proliferation. Plant growth is substantially depends on iron absorption for metabolic production of chlorophyll and the depletion will cause chlorosis yellowish leaf like symptom. Although iron is present abundantly in earth crust and soil, plant do not specialize in capturing iron molecule naturally. Thus, the plant utilizes the relationship with microorganism to supply soluble iron from the surrounding. Under metal limiting environment, most of bacteria produce low molecular weight of metal scavenging compound known as siderophore. Siderophore is known as chelating agent, transporter, or carrier of iron for microbe . Iron form a water soluble complexes with the help of siderophore which posses a specific ligand for Fe^{3+}. Siderophore production is part of biocontrol mechanism to fight deleterious pathogen from infecting the plant. By overpopulating certain microbial group, pathogen have limited access to iron, which subsequently hinder their growth and survival. Nutrient competition is an effective and natural process to control disease especially causes by bacterial pathogen.



Based on figure above, siderophore producing bacterium; secrete metal scavenging siderophore (S) under limiting condition. These compounds interact with free metal such as vanadium (V) and molybdenum (Mo) on surface of clay or soil. Siderophore also release by other organism such as fungus will compete for iron uptake and once it is capture, the iron will be returned to the host or be absorbed by plant root. Ironis an important metal for nitrogenase enzyme building.

1.2 Problem Statement

Major disease attacking papaya such as papaya dieback disease, papaya ringspot and powdery mildew cause stunted growth and lethality which subsequently cause economic impact. Thus, isolation of Lactic acid bacteria is necessary since this group of bacterial are not well explored and studied as biological control agent in papaya.

1.3 Objective

The objectives of this study are :

- To investigate siderophore producing capability of LAB isolate such as siderophore using CAS agar.
- To determine the characteristics of LAB isolates by biochemical test.
- To extract bacterial genomic DNA using conventional PCI method.
- To identify the species of isolated LAB using 16S rRNA sequencing.

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