ภาคผนวก 1

Enterobactin Released from *Enterobacter asburiae* Strain RS83 Involved with Induced Disease Resistance and Plant Growth Promotion

Jetiyanon, K.[1*], Plianbangchang, P.[2], Kloepper, J. W.[3]

[1]*Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Muang, Phitsanulok 65000, Thailand*
[2]*Faculty of Phamaceutical Sciences, Naresuan University, Muang, Phitsanulok 65000, Thailand*
[3]*College of Agriculture, Auburn University, Alabama 36849, USA*

Abstract

The objective of this study was to investigate plant disease resistance and growth promoting potential of a siderophore released from strain RS83. Random insertion mutagenesis by electroporation was carried out using EZ-Tn5™ <KAN-2> Tnp Transposome™ Kit. All mutants were cultured on CAS medium for screening deficiency of siderophore production. Inverse PCR was performed to amplify DNA sequences flanking transposon Tn5 insertions of mutant strain. The amplified products were sequenced and compared to BLAST program. The HPLC method was used to identify the type and quantity of siderophore production released from wild type strain compared with the mutant. Induced systemic resistance (ISR) activity and plant growth enhancement elicited by wild type and mutant strains were evaluated in greenhouse experiments. Results showed that the glycerol-3-phosphate dehydrogenase (NAD (P)+) gene was indirectly responsible for reduced siderophore production by the mutant strain. Enterobactin was the type of siderophore released from both wild type and mutant. The mutant strain produced about 80% less enterobactin than the wild type. Greenhouse experiments also showed that the mutant strain lost the ability of plant growth promotion compared to the wild type strain. ISR activity against southern blight disease on solanaceous plants is currently under investigation.

Keywords: enterobactin, *Enterobacter asburiae* strain RS83, induced disease resistance, plant growth promotion

*Corresponding author.
Tel.: 0-5596-2722; Fax: 0-5596-2704
E-mail: kanchaleej@nu.ac.th

ภาคผนวก 2

Auburn University
Department of Entomology and Plant Pathology

Joseph W. Kloepper
Professor
209 Life Sciences Bldg.
Auburn University, AL 36849-5409

Telephone: (334) 844-1950
FAX: (334) 844-5067
E-Mail: kloepjw@ auburn.edu

April 26, 2011

Assoc Prof Kanchalee Jetiyanon, PhD
Department of Agricultural Sciences
Faculty of Agriculture, Natural Resources, and Environmental Sciences
Naresuan University
Phitsanulok THAILAND 65000

Dear Dr. Jetiyanon,

This letter is to invite you to come to my laboratory at Auburn University. We would like for you to arrive in Auburn on September 15, 2011 and continue your visit through December 15, 2011.

As we have communicated you will conduct research and participate in training related to molecular and physiological aspects of rhizobacteria, including isolation and sequencing of DNA, identification of bacteria by 16s DNA techniques, studying volatile organic compounds produced by bacteria, use of fluorescent *in situ* hybridization (FISH) microscopy for visualizing introduced rhizobacteria, and use of next-generation sequencing. Your financial support will be provided by your university.

We very much look forward to your visit.

Sincerely,

Joseph W. Kloepper
Professor, Auburn University

ภาคผนวก 3

Lipopolysaccharide of *Enterobacter asburiae* Strain RS83: A Bacterial Determinant for Induction of Early Defensive Enzymes in *Lactuca sativa* Against Soft Rot Disease

Jetiyanon, K.[1*], Plianbangchang, P.[2]

[1]*Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Muang, Phitsanulok 65000, Thailand*
[2]*Faculty of Pharmacy, Rangsit University, Muang, Pathumthani 12000, Thailand*

Abstract

Lipopolysaccharide (LPS) is one of bacterial determinants involving with induced systemic resistance (ISR) for plant disease suppression. Various plant defense-related responses were associated with LPS from phytopathogens. Unfortunately, little has been investigated on LPS from beneficial rhizobacteria. *Enterobacter asburiae* strain RS83 previously demonstrated its ability for ISR against several diseases under greenhouse and field trials. The objectives of this study were (1) to examine the ability of LPS extracted from strain RS83-mediated ISR in *Lactuca sativa* against bacterial soft rot disease and (2) to evaluate the enhanced early defensive-related enzymes induced by LPS of strain RS83 implicated for disease restraint. LPS from strain RS83 was firstly recovered in a hot $MgCl_2$-Triton X-100 solution, solubilized with EDTA Triton X-100, and finally precipitated with $MgCl_2$. The LPS was further tested for its ability of ISR in lettuce against *Pectobacterium catovorum* subsp. *catovorum* (Pcc) under *in vitro* bioassay system. A colorimetric assay for superoxide dismutase (SOD) and peroxidase (PO) activities was performed with spectrophotometer. The results showed that lettuce stimulated by LPS of strain RS83 had significantly ($P \leq 0.05$) fewer disease incidences than non-induced pathogen control. Both SOD and PO activities in plants induced with LPS of strain RS83 were significantly enhanced before challenge. Such activities were also significantly accelerated at higher levels during 24-72 hours after challenge compared to the control. This suggested that LPS of strain RS83 played a role for induction of early defensive-related enzymes in lettuce against soft rot disease caused by Pcc.

Keywords: lipopolysaccharide, induced systemic resistance, soft rot disease, *Enterobacter asburiae* strain RS83

*Corresponding author.
Tel.: 0-5596-2722; Fax: 0-5596-2704
E-mail: kanchaleej@nu.ac.th, jetiyanon@gmail.com

ภาคผนวก 4

Biological Control 67 (2013) 301–307

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Lipopolysaccharide of *Enterobacter asburiae* strain RS83: A bacterial determinant for induction of early defensive enzymes in *Lactuca sativa* against soft rot disease

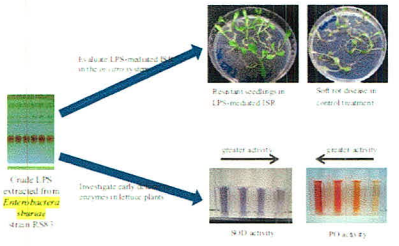
Kanchalee Jetiyanon [a,*], Pinyupa Plianbangchang [b]

[a] Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Muang, Phitsanulok 65000, Thailand
[b] Faculty of Pharmacy, Rangsit University, Muang, Pathumthani 12000, Thailand

HIGHLIGHTS

- LPS was extracted from *Enterobacter asburiae* strain RS83.
- Evaluate the capacity of LPS extracted from strain RS83 mediated ISR in lettuce.
- Lettuce treated with LPS of strain RS83 had less disease incidence than the control.
- LPS of strain RS83 plays a role in the induction of SOD and PO enzymes in lettuce.
- This bioassay method is advantageous for investigations of LPS as an elicitor of ISR.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 August 2013
Accepted 20 September 2013
Available online 29 September 2013

Keywords:
Lipopolysaccharide
Induced systemic resistance
Soft rot disease
Enterobacter asburiae strain RS83

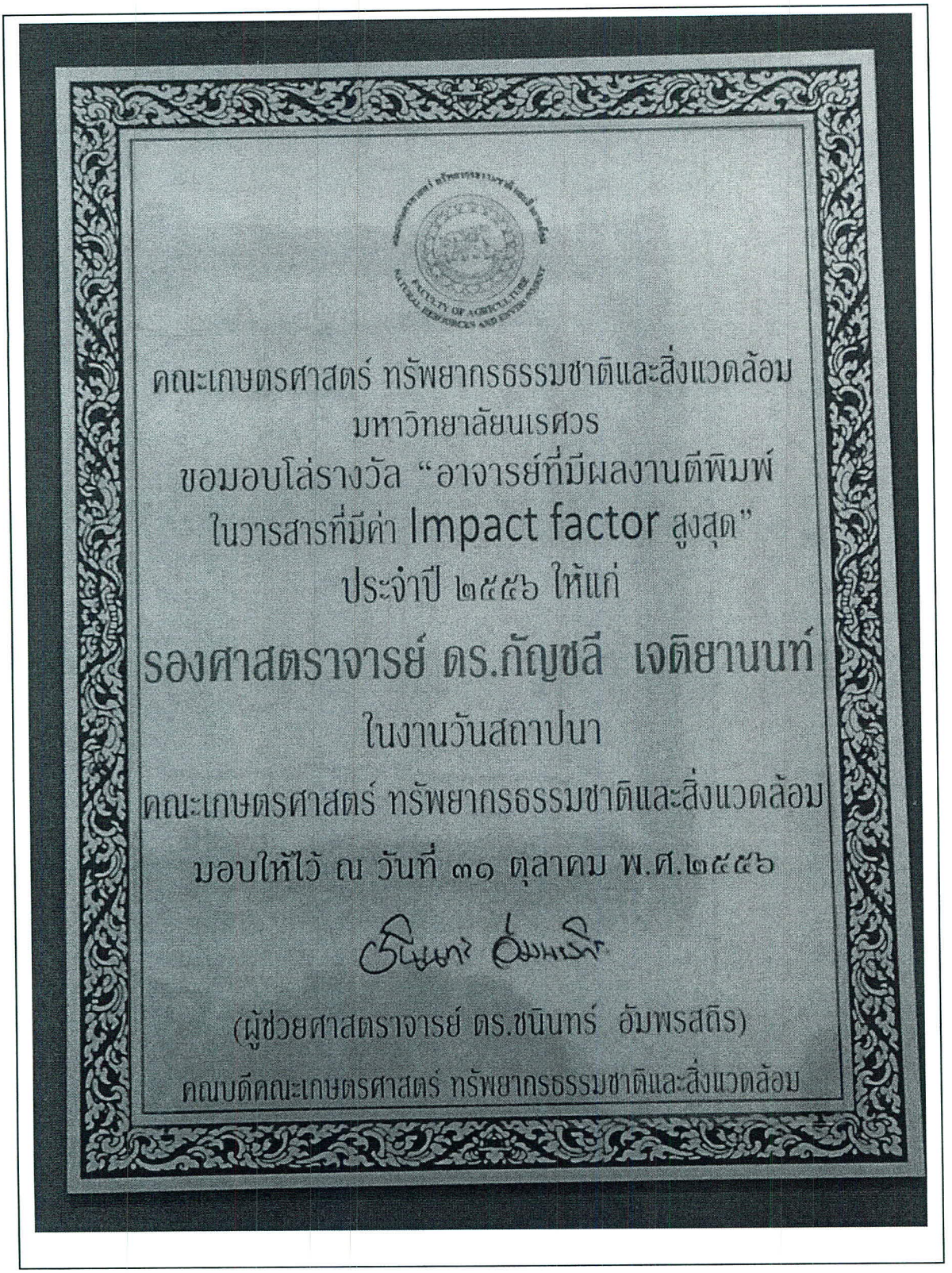
ABSTRACT

Lipopolysaccharides (LPS) from plant pathogenic bacteria have been reported to be determinants of induced systemic resistance (ISR) for plant disease suppression. The role of bacterial LPS in ISR elicited by beneficial rhizobacteria has not been extensively reported. The capacity of *Enterobacter asburiae* strain RS83 to induce ISR against several diseases was previously demonstrated under greenhouse and field trials. The objectives of this study were (1) to examine the capacity of LPS extracted from strain RS83-mediated ISR in *Lactuca sativa* against bacterial soft rot disease and (2) to evaluate the enhanced early defensive-related enzymes induced by LPS of strain RS83 implicated for disease restraint. LPS from strain RS83 was first recovered in a hot $MgCl_2$–Triton X-100 solution, solubilized with EDTA Triton X-100, and finally precipitated with $MgCl_2$. The LPS was further tested for elicitation of ISR in lettuce against *Pectobacterium catovorum* subsp. *catovorum* (Pcc) under *in vitro* bioassay system. A colorimetric assay for superoxide dismutase (SOD) and peroxidase (PO) activities was performed. The results showed that lettuce treated with LPS of strain RS83 had less ($P \leq 0.05$) disease incidence about 90% than the non-induced pathogen control. Before challenge with the pathogen, significant increase of SOD activity in plants induced with LPS of strain RS83 were 92% greater than the non-induced pathogen control. Only little increase of greater PO activity occurred in treatment induced with LPS of strain RS83 compared to the control. Both SOD and PO activities were significantly accelerated from 24 h to 72 h after challenge compared to the control. These results suggest that LPS of strain RS83 plays a role in the induction of early defensive-related enzymes in lettuce against soft rot disease caused by Pcc.

* Corresponding author. Fax: +66 5596 2704.
E-mail addresses: kanchaleej@nu.ac.th, jetiyanon@gmail.com (K. Jetiyanon), pplianbangchang@gmail.com (P. Plianbangchang).

1049-9644/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.biocontrol.2013.09.014

ภาคผนวก 5


คณะเกษตรศาสตร์ ทรัพยากรธรรมชาติและสิ่งแวดล้อม
มหาวิทยาลัยนเรศวร
ขอมอบโล่รางวัล "อาจารย์ที่มีผลงานตีพิมพ์
ในวารสารที่มีค่า Impact factor สูงสุด"
ประจำปี ๒๕๕๖ ให้แก่
รองศาสตราจารย์ ดร.กัญชลี เจติยานนท์
ในงานวันสถาปนา
คณะเกษตรศาสตร์ ทรัพยากรธรรมชาติและสิ่งแวดล้อม
มอบให้ไว้ ณ วันที่ ๓๑ ตุลาคม พ.ศ.๒๕๕๖
(ผู้ช่วยศาสตราจารย์ ดร.ชนินทร์ อัมพรสถิร)
คณบดีคณะเกษตรศาสตร์ ทรัพยากรธรรมชาติและสิ่งแวดล้อม

ภาคผนวก 6

Abstract ของ Manuscript ที่จะส่งไปลงตีพิมพ์ในวารสารต่างประเทศ

Bacterial Determinants of *Enterobacter asburiae* strain RS83 for Plant Growth Enhancement

Jetiyanon, K.

Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Muang, Phitsanulok 65000

Abstract

Enterobacter asburiae strain RS83 was isolated from rhizosphere of cassava in Phitsanulok province having the capability to promote growth and yield of various vegetable crops. The objective of this study was to investigate possible bacterial determinants of strain RS83 involving with plant growth enhancement. Different methods such as particular media, biochemical methods and high performance liquid chromatography were used to analyze specific bacterial determinants relevant to plant growth promotion. Results demonstrated that several bacterial determinants of strain RS83 implicating for plant growth improvement were detected. These included indole-3 acetic acid (IAA), siderophore (enterobactin type) production, gluconic acid relating to phosphate solubilization, and the presence of pellicles in nitrogen free medium correlating with nitrogen fixation. In conclusion, the strain RS83 seemed to provide combinations of several determinants mentioned above for plant growth enhancement. Detailed of each bacterial determinant will be discussed.

Keywords: *Enterobacter asburiae* strain RS83, bacterial determinants, plant growth enhancement