





# **ABSTRACT BOOK**

## Conference on Plant-Microbe Symbiosis and Nitrogen Fixation (6<sup>th</sup> APMNF)



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### WELCOME MASSAGE

It is undeniable that the agricultural sector is the most crucial player for feeding the world. Utilization of microbes to replace chemicals used in food production, be it fertilizers, hormones or herbicides and pesticides, has been under constant development and improvement to achieve sustainable natural resource and environment conservation.



Scientists working on Plant-microbe interaction research, especially those in Asia-Pacific, have formed a group for collaborative endeavors. Particularly in Asia, initial steps of such collaboration took place in 2010, Miyazaki, Japan. Now, the 6<sup>th</sup> Asia-Pacific Conference on Plant-Microbe Symbiosis and Nitrogen Fixation takes place from 7-9 January 2024 in Chieng Mai, Thailand. The organizing committee would like to invite scientists and researchers to participate in this conference in order to discuss, share and exchange ideas, experiences and knowledge, which will lead to strengthening of Asia-Pacific's agricultural technology.

The conference theme covers a wide spectrum of topics, ranging from genome research to its applications to the community and society. The conference also offers an opportunity for oral and poster presentations to young scientists whose results are outstanding.

Welcome to Chiang Mai, Thailand



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The 6<sup>th</sup> Asian-Pacific Conference on Plant-Microbe Symbiosis and Nitrogen Fixation

#### Schedule "6<sup>th</sup> APMNF" 7-9 January, 2024

Date	Time	Activities	
Sunday 7-1-24	8.00 - 16.45	Excursion Route 1: Cultural Track: Old beautiful Northern style temples in Chiang Mai City, Doi Suthep Temple, Doi Kam Temple, Royal Flora Ratchapruek Park Route 2: Natural Track: Nong Hoi Agricultural Research Station, Royal Project, Queen Sirikit Botanical Garden	<ul> <li>Appoint place: Kantary Hills Hotel Lobby (Near Reception front desk, please look for the Excursion sign)</li> <li>Appoint Time: Registration for excursion 7.30-8.00 am</li> </ul>
	16.00-18.45	<b>Registration</b> / Drop ppt presentation file / Poster Decoration/Preparation	Room: Doi Luang-Doi Nang
	19.00-21.00	<ul> <li>Opening Ceremony</li> <li>Welcome speech by Prof. Dr. Neung Teaumroong and Prof. Dr. Nantakorn Boonkerd</li> <li>Buffet Dinner</li> </ul>	Room: Doi Suthep 1-2
Monday 8-1-24	9.00-9.45	Plenary session 1 Prof. Dr. Kiwamu Minamisawa Tohoku University, Japan Cool earth via nitrogen-fixing bacteria: Methanotrophs and bradyrhizobia	Room: Doi Luang-Doi Nang
	9.45-10.30	Plenary session 2 Dr. Eric Giraud PHIM, France Alternatives symbioses between <i>Bradyrhizobium</i> strains and legumes: identification of new Type 3 effectors triggering nodulation independently of Nod factors	
	10.30-11.00	Plenary session 3 Prof. Dr. Neung Teaumroong SUT, Thailand Progress in bradyrhizobial effector protein from secretion system and symbiotic role in non-leguminous plants	
	11.00-11.15	Coffee Break	
	11.15-11.35	Chairman: Prof. Shusei Sato Co-Chair: Asst. Prof. Dr. Kamonchanok Umnajkitikorn Session: Bioresource and Genomics	
		Tohoku University, Japan	



Date	Time	Activities	
		Updated information on legume experimental resources in NBRP Lotus/Glycine	
	11.35-11.50	<b>speaker 2</b> Deciphering how faba bean rhizobia adapt to acidic soils via comparative genomics	
		Dr. Jian JIAO	
	11.50-12.05	<b>speaker 3</b> The Methylomonas Pangenome Reveals A Novel Endophytic Bioresource	
		Mr. Sahassawat Chailungka	
	12.05-13.30	Lunch	Room: Lunch Room (Nimman)
	13.30-13.50	Chairman: Prof. Chi-Te Liu Co-Chair: Dr. Takashi Goto	Room: Doi Luang
		Session: Plant – Microbe Interaction	
		Invited speaker 1 Prof. Dr. Chi-Te Liu National Taiwan University, Taiwan	
		From Lab to Farm: application of photosynthetic bacterial inoculant for sustainable agriculture	
	13.50-14.10	Invited speaker 2 Dr. Takashi Goto NIBB, Japan	
		Auxin Methylation Recruited into Leguminous Nodule Symbiosis	
	14.10-14.25	<b>speaker 3</b> The New Putative Type III Effector SkP48 in <i>Bradyrhizobium</i> sp. DOA9 is Involved in Legume Nodulation	
		Dr. Pongdet Piromyou	
	14.25-14.40	<b>speaker 4</b> An evolutionarily shared formin protein INFO-mediated different symbiotic intracellular infections in legume and non-legume plants	
		Dr. Liang Pengbo	
	14.40-14.55	<b>speaker 5</b> Molecular Basis of Mung Bean Symbiotic Incompatibility: A Pull-Down Assay Identifies Plant Proteins Targeted by NopP2 from <i>Bradyrhizobium</i> sp. ORS3257	



Date	Time	Activities	
		Dr. Pongpan Songwattana	
	14.55-15.15	Coffee Break	
	15.15-15.30	Flash poster presentation (Odd number) (1 min/person)	
	15.30-15.50	Chairman: Dr. Didier Lesueur Co-Chair: Prof. Dr. Graham Ohara	
		Session: Application of N <sub>2</sub> – fixation & N – cycle Invited speaker 1 Dr. Didier Lesueur CIAT, Hanoi, Vietnam	
		Why scaling up cowpea inoculated with effective native rhizobia should be good for soil health and yields in Yen Bai Province of Northern Vietnam?	
	15.50-16.10	Invited speaker 2 Prof. Dr. Graham Ohara Murdoch University, Australia	
		Consequences of inoculation - evolution of diverse rhizobia symbionts of agricultural legumes	
	16.10-16.25	<b>speaker 3</b> Effect of nitrogen fixation enhancing gene on soybean growth and yield	
		Dr. Akihiro Suzuki	
	16.25-16.40	<b>speaker 4</b> Screening and evaluation of efficient nitrogen fixing rhizobia specific to cultivars of <i>Vigna radiata</i> recommended for different agro climatic regions of India	
		Dr. Nisha Tak	
	16.40-16.55	<b>speaker 5</b> The denitrification pathway of <i>Bradyrhizobium</i> <i>ottawaense</i> and its potential application in N2O mitigation	
		Dr. Atsuo Suzuki	
	17.00-18.00	Poster presentation (odd No.)	
Tuesday 9-1-24	9.00-9.20	Chairman: Prof. Dr. Toshiki Uchiumi Co-Chair: Dr. Kamonluck Teamtisong	Room: Doi Luang-Doi Nang



Date	Time	Activities	
		Session: Application of $N_2$ – fixation & N – cycle (continue)	
		Invited speaker 1 Prof. Dr. Toshiki Uchiumi Kagoshima University, Japan	
		Class 1 plant hemoglobin functions in two different symbiotic systems of <i>Lotus japonicus</i>	
	9.20-9.35	<b>speaker 2</b> Transcriptome Analysis of Two RpoN Sigma Factors in <i>Bradyrhizobium</i> sp. Strain DOA9: Insights into Nitrogen Fixation Regulation under Free-Living Conditions	
		Dr. Jenjira Wongdee	
	9.35-9.50	<b>speaker 3</b> Mechanism of endophytic bradyrhizobial cell viability, differentiation, and nitrogen fixation in rice, chili, and tomato	
		Dr. Teerana Greetatorn	
	9.50-10.15	Coffee Break	
	10.15-10.35	Chairman: Prof. Dr. Arinthip Thamchaipenet Co-Chair: Dr. Katsuharu Saito	
		Session: Non – legume and Microbial Symbiosis	
		Invited speaker 1 Prof. Arinthip Thamchaipenet KU, Thailand	
		Beneficial endophytic streptomycetes allivate salinity tolerance in rice	
	10.35-10.55	Invited speaker 2 Prof. Dr. Katsuharu Saito Shinshu University, Japan	
		Enhancing asymbiotic growth in arbuscular mycorrhizal fungi using mixed fatty acids	
	10.55-11.10	<b>speaker 3</b> Arbuscular mycorrhizal <i>Acaulospora morrowiae</i> suppresses growth and nutrient uptakes of host plants regardless of soil P availabilities	
		Dr. Lompong – Klinnawee	



Date	Time	Activities	
	11.10-11.25	<b>speaker 4</b> Matching of cannabis endophytic bacterial microbiomes for plant growth stimulation and metabolite promotions	
		Dr. Panlada Tittabutr	
	11.25-13.00	Lunch	Room: Lunch Room (Nimman)
	13.00-13.20	Chairman: Prof. Dr. Shin Okazaki Co-Chair: Prof. Dr. Chang-Fu Tian	Room: Doi Luang-Doi Nang
		Session: Legume and Rhizobial symbiosis	
		Invited speaker 1 Prof. Dr. Shin Okazaki TUAT, Japan	
		Symbiotic roles of rhizobial type III effectors in soybean nodulation	
	13.20-13.40	Invited speaker 2 Prof. Dr. Chang-Fu Tian China Agricultural University, China	
		Optimization mechanisms for symbiotic function of broad-host-range <i>Sinorhizobium fredii</i>	
	13.40-13.55	<b>speaker 3</b> Wild mungbean resistance to the nematode Meloidogyne enterolobii involves the induction of phenylpropanoid metabolism and lignification	
		Dr. Sook-Kuan Lee	
	13.55-14.10	<b>speaker 4</b> Performance in nurseries and analysis of native microbial symbionts of Australian Acacia hybrid seedlings in Northern Vietnam (Phu Tho Province)	
		Ms. Anh Phuong Ngo	
	14.10-14.25	Flash poster presentation (even No.) (1 min/person)	
	14.25-14.45	Coffee Break	Committee meeting
			Room: Small Party Room
	14.45-15.45	Poster presentation (even No.)	Room: Doi Luang-Doi Nang



Date	Time	Activities	
	15.45-16.00	Chairman: Dr. Toungporn Uttarotai Co-Chair: Dr. Pongdet Piromyou	
		Session: Young Scientist (Oral presentation)	
		<b>speaker 1</b> Phylogenetic diversity of rhizobia nodulating <i>Crotalaria</i> pallida intercropped with coffee trees in Gia Lai province, Vietnam	
		Mr. Long Nguyen Van	
	16.00-16.15	<b>speaker 2</b> Unveiling the Tripartite Synergistic Enhancement of Plant-Arbuscular Mycorrhizal Fungus Symbiosis by endophytic <i>Bacillus velezensis</i> S141 in <i>Lotus japonicus</i>	
		Mr. Sutee Kiddee	
1	16.15-16.30	<b>speaker 3</b> Enhancement of Rhizobial Inoculant for Peanut through Co-inoculation with Phosphate-Solubilizing Bacteria	
		Dr. Jean Louise Cocson Damo	
	16.30-16.45	<b>speaker 4</b> Basidiomycetes Fungi isolated from rice in northern Thailand: Advantage or Disadvantage?	
Ms. Sahar Absalan	Ms. Sahar Absalan		
	16.45-17.00	<b>speaker 5</b> Isolation and characterization of cowpea rhizobia in Myanmar	
		Dr. Aung Zaw Htwe	
	17.00-17.15	<b>speaker 6</b> Role of type IV secretion system (T4SS) in <i>Bradyrhizobium</i> -legumes symbiosis	
		Ms. Praneet Wangthaisong	
	17.15-17.30	<b>speaker 7</b> Identification of a genomic region related to host specificity in nodule symbiosis of <i>Lotus burttii</i>	
		Mr. Yuhei Chiba	



Date	Time	Activities	
	17.30-17.45	<ul> <li>speaker 8</li> <li>Phyllospheric Bacteria Isolated from Broccoli (<i>Brassica oleracea</i> var. <i>italica</i>) as Biocontrol Agent against <i>Ralstonia solanacearum</i> and as Plant Growth Promoting Bacteria</li> <li>Mr. Ariya Putra</li> </ul>	
	17.45-18.30	<b>Special lecture by Prof. Shusei Sato</b> Overview & Inspiration on Plant-microbe interaction Research: The future and the possible collaboration	
	18.30-18.50	Closing Remark - Summarize the overall research from the 6 <sup>th</sup> APMNF by Prof. Dr. Kiwamu Minamisawa - Summarize of the meeting and closing the conference by Prof. Dr. Neung Teaumroong	
	19.30-23.00	Banquet & Awards Ceremony	Room: Room: Doi Suthep 1-2
		(Dressing with Traditional Thai Northern Style)	



#### Poster Presentation

Poster No.	Торіс	Name	Theme
1	Identification of cannabis strains using Inter-Simple Sequence repeats (ISSRs) molecular marker	Ms.Souphaphone Keomany	1. Bioresource and Genomics
2	Impacts of Humic Acid Applications on Soil Bacterial Abundance and Community under Sweet Corn Rhizospheric soil	Ms.Mona Ayu Santi	
3	The National BioResource Project (NBRP) for Lotus and Glycine in Japan: QTL analysis for seed storability in soybean under different temperature	Dr. Pakanan Jiamtae	
4	Effects of rice straw and its derived biochar incorporation on soil enzyme activity in a flooded rice field	Ms. Saowalak Somboon	2. Plant – Microbe Interaction
5	The role of SUMO protease from Bradyrhizobium sp. DOA9 on Arachis hypogaea L. symbiosis	Mr. Beedou Aphaiso	
6	Lettuce Promoting Activity of Siderophore Producing Streptomyces sp. Rh1-5-22	Ms. Benyapa Kitwetch	
7	Unveiling Drought-Mitigating Potential of Streptomyces thermocarboxydus S3 in Pepper (Capsicum annuum L.) Seedlings: Mechanistic Insights from Genome Mining	Dr. Pharada Rangseekaew	
8	Unlocking the Power of Bacillus velezensis S141: Unraveling Synergistic Growth Promotion and Biocontrol in Legumes	Dr. Nantakorn Boonkerd	



9	Protein-protein interaction assay between	Ms. Natcha Purksametanan	
	Vigna radiata proteins and effector		
	protein, NopP2, from Bradyrhizobium		
	elkanii USDA61		
10	The molecular interaction between Lotus	<mark>Ms. Cui Ying</mark>	
	japonicus and Bradyrhizobium mediated		
	by type 3 effector protein, NopM		
11	Application of Recombinant Human scFv	Mr. Chayanan Singtothong	3. Application of Nitrogen –
	Antibodies for the Enumeration of		fixation & N – cycle
	Individual Viable Cells in Mixed-Culture		
	Bradyrhizobial Inoculants		
12	Amino-vinyl-glycine (AVG) and Indole-3-	Dr. Kamolchanok	
	butyric acid (IBA) in Seed Coating	Umnajkitikorn	
	enhance Plant growth and nitrogen		
	fixation of soybean cv. CM60 under stress		
	condition		
13	Al-designing based on likelihood of	Prof. Qi Cheng	
	evolutionarily structural origin between		
	multi-subunit nitrogenase-like enzymes		
	and single-polypeptide LPOR-like		
	Catalysis		
14	Transcriptomic study on the growth-	Dr. Pakpoom Boonchuen	4. Non-legume and
	promoting impact of Bacillus velezancia		microbial symbiosis
	S141 on Connabia activa		
	STATION Cannabis Saliva		
15	Plant root and only tic bacteria for	Dr. Shun Hashimoto	
10	mitigation of groophouse gas from		
	iniugation of greenhouse gas from		
	agricultural fields		



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16	Production of Arbuscular Mycorrhizal Fungal spores in Zea mays using Light Emitting Diode (LED) condition	Dr. Kamonluck Teamtisong	
17	Improve the Production of Edible Cassava (Manihot esculenta Crantz) cultivar Phiroon 6 using Biofertilizers for Sustainable Agriculture	Ms. Niramon Lakkason	
18	A genome DNA sequencing analysis of a root bacteria from water floating legume, Neptunia natans, indicated it as Stenotrophomonas pavidii.	Dr.Shigeyuki Tajima	5. Legume and Rhizobial symbiosis
19	Black pepper (Piper nigrum) – Arachis pintoi intercropping system in the Central Highlands in Vietnam: Impact on the soil fertility and the diversity of native rhizobia	Mr. Duy Quang Nguyen	
20	Unveiling a novel paradigm in Bradyrhizobium strains triggering NF- independent nodulation in soybean	Ms. Safirah Tasa Nerves Ratu	
21	Nod-factor independent symbiotic phenomena and symbiotic inhibition factor of Bradyrhizobium and Aeschynomene genus	Mr. Shogo Fukunaga	
22	Function of cysteinyl-tRNA synthetase of Mesorhizobium loti in root nodule symbiosis	Dr. Mitsutaka Fukudome	
23	Modulating Plant Defense Pathways: <i>Bradyrhizobium</i> sp. DOA9 Priming Induces Resistance to <i>Cercospora canescens</i>	<mark>Mr. Apisit Songsaeng</mark>	



24	Assessing the Antagonistic Potential of Rhizobium sp. Isolates Against Sclerotium rolfsii, Causative Agent of Stem and Root Rot in Yard- Long Beans	Dr. Pilunthana Thapanapongworakul
25	Screening and Efficiency Assessment of Rhizobium Strains for Growth Enhancement of Yard- Long Beans	Ms. Tawanchai Khuendee
26	Screening of Rhizobium Strains Isolated from Yard-Long Bean for Their Plant Growth-Promoting Ability	Dr. Yupa Chromkaew
27	Selection and evaluation of Bradyrhizobium inoculum for peanut, Arachis hypogea production in the Lao People's Democratic Republic	Ms. Tarnee Phimphong
28	Enhancing Siamese Rosewood (Dalbergia cochinchinensis) Reforestation Success: The Impact of Rhizobial Inoculation on Nodulation and Growth	Dr. Janpen Prakamhang



## **Plenary session**



#### Cool earth via nitrogen-fixing bacteria: Methanotrophs and bradyrhizobia

#### KIWAMU MINAMISAWA<sup>1</sup>

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Abstract

Chemical nitrogen fixation by the Haber–Bosch method permitted industrial-scale fertilizer production that supported global population growth, but simultaneously released serious reactive nitrogen into the environment (2, 6). My talk highlights the potentials for bacterial nitrogen fixation and mitigation of greenhouse gas (GHG) emissions from paddy rice and soybean fields. Type II methane (CH<sub>4</sub>)-oxidizing bacteria (methanotrophs) are endophytes in paddy rice roots and fix N<sub>2</sub> using CH<sub>4</sub> (a GHG) as an energy source, mitigating the emission of CH<sub>4</sub> and reducing nitrogen fertilizer usage (3, 9). Nitrous oxide (N<sub>2</sub>O), a GHG, is mainly emitted from agricultural use of nitrogen fertilizer and symbiotic nitrogen fixation (7). Some bradyrhizobia (*B. diazoefficiens* and *B. ottawaense*) have a denitrifying enzyme system that includes a N2O reductase and are able to mitigate N<sub>2</sub>O emission from the rhizosphere of leguminous plants (1, 5, 8). The application of T3SS-dependent interactions between host plants and endosymbionts might enhance such GHG mitigation technologies (4,10). In conclusion, the microorganisms with symbiotic nitrogen fixation shows potentials for GHG mitigation in paddy rice and soybean fields while simultaneously supporting sustainable agriculture (2, 6).

#### References

- 1) Wasai-Hara et al. 2023. Sci Rep 13. 2023 18862.
- 2) Minamisawa K. 2022. Biosci Biotechnol Biochem. 87:7-12.
- 3) Hara et al. 2022. mBio. 13: e0125522.
- 4) Arashida et al. 2022. ISME J. 16:112-121.
- 5) Wasai-Hara et al. 2020. Microbes Environ. 35: ME19102.
- 6) Yoneyama et al. 2019. Plants (Basel). 8: E408.
- 7) Sánchez C and Minamisawa K. 2019. Front Microbiol. 10:1943.
- 8) Hara et al. 2019. Front. Microbiol. 10: 407.
- 9) Shinoda et al. 2019. Soil Biol. Biochem. 132: 40-46.
- 10) Sugawara et al. 2018. Nature Communications 9: 3139. December 7-9, 2024, Chiang Mai, Thailand.



## Alternatives symbioses between Bradyrhizobium strains and legumes: identification of new Type 3 effectors triggering nodulation independently of Nod factors

<u>Giraud Eric</u><sup>1</sup>, Camuel Alicia<sup>1</sup>, Carcagno Melanie<sup>2</sup>, Haq Fazal<sup>3</sup>, Teulet Albin<sup>1</sup>, Pacquit Valerie<sup>2</sup>, Gully Djamel<sup>1</sup>, Okazaki Shin<sup>4</sup>, Luo Haiwei<sup>5</sup>, Mergaert Peter<sup>3</sup>, Deslandes Laurent<sup>2</sup>

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Abstract

The establishment of the rhizobium/legume symbioses is generally based on a Nod factor signal synthetized by the rhizobia that controls both the nodulation and the infection processes. However, some Bradyrhizobia have the original ability to nodulate certain legume species, such as *Aeschynomene* spp. or *Glycine max*, thanks to two alternative NFs-independent symbiotic processes that differs by the requirement or not of the type III secretion system (T3SS) [1,2]. ErnA is the first type 3 effector (T3E) shown to trigger nodulation in *Aeschynomene indica* [3]. To better understand these alternative symbioses, a large collection of *Bradyrhizobium* strains was tested on *A. indica*. We focused on strains whose genome was sequenced to establish correlations between their genomic content and their symbiotic properties.

Through a combination of comparative genomic analyses and mutagenesis of some candidate genes emerging from these analyses, we identified divers T3Es triggering nodulation in *A. indica* [4]. During this talk, I will present some characteristics of these T3Es that we propose to call ET-Nod for Effectors triggering Nodulation. Together, our results highlight an unsuspected distribution and diversity of T3Es within the *Bradyrhizobium* genus that may contribute to their symbiotic efficiency by participating in triggering legume nodulation.

1. Okazaki S et al. Proc. Natl. Acad. Sci. USA. 2013; 110:17131-17136.

2. Okazaki S, et al. ISME J. 2016; 10:64-74.

3. Teulet A et al. Proc. Natl. Acad. Sci. USA. 2019; 116:21758-21768.

4. Camuel et al. ISME J. 2023; 17:1416-1429.

Keywords : Type III effectors; Bradyrhizobium; Nod factor Independent Symbiosis; Aeschynomene



#### Progress in bradyrhizobial effector protein from secretion system and symbiotic role in nonleguminous plants

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#### Abstract

Research has predominantly involved selecting specific microbial strains to enhance plant growth, such as selecting rhizobium biofertilizers, or strains with properties to combat plant diseases. Our research focus on the molecular mechanisms of interaction between legume plants and *Bradyrhizobium* sp. SUTN9-2 and *B. elkanii* USDA61 effector proteins. It has been discovered that proteins, specifically NopM and NopP2, entering plant cells, play a significant role in mutualistic interactions. We have successfully identified potential interactions of both proteins with various types of plant proteins. Additionally, within the T3SS gene system of *Bradyrhizobium* sp. strains isolated directly from the root nodules of peanuts, three strains have shown implications for enhancing mutualistic relationships in mung bean (*Vigna radiata*) and *V. mungo*.

For the endophytic bradyrhizobium, we investigate the mechanisms of bacterial entry into plant cells, bacterial cell morphology changes, and nitrogen fixation of *Bradyrhizobium* endophytes in rice tissues and other economically important plants such as chili and tomatoes. The results revealed that the *Bradyrhizobium* sp. SUTN9-2 is capable of residing within chili and tomato tissues. Moreover, its residency in different plant tissues influences cell properties and survival, motivating researchers to study deeper into the mechanisms controlling cell properties for further exploration.

In case of plant immunity system, we focus on studying the efficacy and resistance mechanism against brown spot disease in *V. radiata* CN72. Our findings revealed that the presence of cultivated *Bradyrhizobium* sp. DOA9 notably decreased the lesion area caused by the *Cercospora* sp. fungus. Remarkably, when applying the DOA9 strain lacking the T3SS (Type III Secretion System), the lesion area was even lower compared to non-inoculated plants. These results demonstrate the potential of DOA9 in effectively suppressing the severity of *Cercospora* sp. fungus in CN72. Additionally, DOA9 T3SS may also play a pivotal role in triggering plant resistance in CN72.



## **Oral presentation**

## **Theme: Bioresource and Genomics**



#### Updated information on legume experimental resources in NBRP Lotus/Glycine

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#### Abstract

The National BioResource Project (NBRP), which was launched by the Japanese government in 2002, has entered into 5th phase from April 2022, and the legume bio-resources program, NBRP *Lotus* and *Glycine*, renewed its web database, "LegumeBase", as a kickoff of the new phase. In the phase 5 of NBRP *Lotus* and *Glycine*, we are continuing our efforts on providing the material resources, such as seeds of experimental strains, wild accessions and recombinant inbred lines (RILs) of *L. japonicus* and *G. soja*. Also we are going to improve our material resources by propagating the native retrotransposon (*LORE1*) insertion tag lines of *L. japonicus* and establishing the collection of the pairs of wild accession of *L. japonicus* and its natural symbionts. In addition, we are providing the information resources, such as updated reference genome sequence of *L. japonicus* experimental strain "Gifu" based on the sequences produced by PacBio sequencer, and genome resequencing based genotype information of RILs and wild accessions of *L. japonicus* and *G. soja*, and the analysis of mechanism of environmental adaptation in *L. japonicus* as an example of NBRP resource application.

Keywords : Lotus japonicus; Glycine soja; genome information; wild accessions; GWAS



#### Deciphering how faba bean rhizobia adapt to acidic soils via comparative genomics

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#### Abstract

The rhizobium-legume symbiotic system is the most efficient biological nitrogen fixation system in nature, but the artificially inoculated high-efficiency nitrogen-fixing rhizobial strains in agricultural production usually exhibit suboptimal inoculation performance due to their inability to acclimate to local soil conditions. Previous research has shown that soil pH plays a pivotal role in shaping the adaptive diversity of rhizobia populations and their biogeographic distribution at the genus and species levels. In this study, we sequenced the genomes of 120 faba bean rhizobia strains using high-throughput genome sequencing. Through comparative genomics analysis, we identified a lineage-specific acid fitness island (AFI) that equips Rhizobium anhuiense with the ability to thrive in acidic soil. The AFI comprises 19 genes, including those encoding transmembrane transport proteins enzymes involved in the amino acid deamination, ion channel and antiporter proteins, and two-component system etc. Further genetic investigations revealed that the deletion of AFI in the representative strain Ran651241 resulted in the loss of the deamination capability to produce alkalic NH<sub>3</sub>. Gene gain/loss analysis and genome collinearity analysis demonstrated that various genes within the AFI were acquired through multiple lateral gene transfer events following the emergence of the *Rhizobium* genus. Subsequently, these genes were integrated into a functional island through genome recombination. Some lineages subsequently lost the AFI, possibly due to the diminished selection pressure upon migrating to alkaline environments. In summary, our findings unveil the genetic mechanism behind the differentiation of faba bean rhizobia and their adaptation to varying soil pH levels. These insights provide valuable genetic resources and practical insights for engineering highly stress-resistant and broadly adaptable rhizobia strains through synthetic biology.

Keywords : faba bean; rhizobia; comparative genomics; pH adaptation; acid fitness island



#### The Methylomonas Pangenome Reveals A Novel Endophytic Bioresource

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#### Abstract

Aerobic methanotrophs are bacteria that utilize methane as their sole carbon and energy source under oxic conditions, contributing to methane mitigation. They typically inhabit submerged areas where methane and oxygen are available, such as peatlands and rice paddy fields. Moreover, aerobic methanotrophs have been found in the endosphere of various plants. However, methanotroph adaptation to plant-microbe interaction has not been extensively reported. This study aims to isolate aerobic methanotrophs from the root endosphere in the heavy metal contamination area to investigate the capacity of endosphere colonization, plantmicrobe nutrient exchange, and heavy metal resistance via whole genome information. Methylomonas sp. TEB was isolated from the plant root endosphere in a cadmium-contaminated area. The 16S rRNA gene sequence was phylogenetically related to Methylomonas denitrificans FJG1 with a similarity of 99.46%. Though the percentage similarity of bacterial marker genes exceeded the 97.0% cut-off value, the genomic comparisons, including digital DNA-DNA hybridization (dDDH), average nucleotide identity (ANI), and average amino acid identity (AAI) between strain TEB and FJG1 showed values below cut-off points. Additionally, the Methylomonas pangenome visualized the different genome patterns between strain TEB and M. denitrificans FJG1. Core genes of aromatic pangenome hydrocarbon degradation, abilities showed to exopolysaccharide production, and defense mechanisms including divalent metal cation efflux pump and multidrug efflux pump, suggesting that the genus Methylomonas could be used as the bioremediation agent under heavymetal contamination environments. The accessory genes of strain TEB elucidated the specific adaptation for endospheric lifestyle as mutualism such as nitrogen fixation, phosphate solubilization, and siderophore production facilitating the

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available nutrients for plants, while the plant root provided a special niche for methanotrophs. Finally, this is the first report of the pangenome of *Methylomonas* that demonstrated the methanotrophs could be a micro-bioresource for submerged plant symbiosis with their capability of methane sink.

Keywords : Methanotroph; Methane; Pangenome; Bioremediation; Endophyte



## **Oral presentation**

## **Theme: Plant – Microbe Interaction**



#### From Lab to Farm: application of photosynthetic bacterial inoculant for sustainable agriculture

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Abstract

Photosynthetic bacteria possess versatile metabolic abilities and are widely applied in environmental bioremediation, bioenergy production and agriculture. They include improvement of nutrient acquisition, production of phytohormones, induction of immune system responses, interaction with resident microbial community. We isolated a photosynthetic bacterium designated as *Rhodopseudomonas palustris* strain PS3 from paddy soil. This bacterium can utilize different kinds of carbohydrate sources, possesses versatile extracellular hydrolytic enzymes, can fix nitrogen and produce Indole-3-acetic acid (IAA) under free-living condition. *R. palustris* PS3 was applied as a microbial inoculant in fields, and we found that it could not only exert beneficial effects on plant growth, but also enhance the use efficiency of applied fertilizer nutrients. This talk mainly focuses on the influences of this microbial inoculant on soil health, plant growth, and microbial communities. We also elucidated the underlying plant growth-promoting mechanisms of PS3 from the viewpoint of both microbes and plants.

**Keywords** : PGPR (plant-growth promoting rhizobacteria); plant-associated microbe; microbial inoculant; soil microbial community; soil fertility



#### Auxin Methylation Recruited into Leguminous Nodule Symbiosis

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Abstract

Leguminous plants attract symbiotic bacteria, called rhizobia, and create nodules on their roots. Nodule development consists of bacterial infection of root epidermis and subsequent primordium formation in the root cortex. The cortical development occurs just below the site of rhizobial infection of the epidermis, suggesting a spatiotemporal coordination between epidermis and cortex during nodulation. However, little is known about the molecular mechanism. A Lotus japonicus mutant "daphne" has uncoupled symbiotic events in epidermis and cortex, in that it promotes excessive bacterial infection in epidermis but does not produce nodule primordia in cortex. Therefore, *daphne* should be useful for exploring unknown signals that coordinate these events across tissues. Here, we conducted time-course RNA-seq using daphne after rhizobial infection. We noticed that IAA carboxyl methyltransferase 1 (IAMT1), which encodes the enzyme that converts auxin (IAA) into its methyl ester (MeIAA), showed differential expression dynamics in wild-type and daphne roots. IAMT1 serves shoot development of Arabidopsis, but the function of IAMT1 in roots has not been reported. Interestingly, phylogenetic tree suggested a gene duplication of IAMT1 in the legume lineage, and one of two paralogs (named IAMT1a) was expressed in L. japonicus roots after rhizobial infection. The IAMT1 gene duplication may have contributed to the evolutionary acquisition of auxin methylation in roots and its involvement in nodule symbiosis. IAMT1a was mainly expressed in root epidermis, but its function was required in root cortex, where it promotes nodule development. The finding implies that IAMT1a is involved in coordinated epidermal and cortical regulation. LC/MS-MS succeeded MeIAA detection in roots infected with rhizobia, and exogenous MeIAA induced the development in rhizobium susceptible zone. These findings illuminate our understanding of auxin methylation and intertissue communication acquired during evolution of nodule symbiosis.

Keywords : Nodule Symbiosis; Auxin



## The New Putative Type III Effector SkP48 in Bradyrhizobium sp. DOA9 is Involved in Legume Nodulation

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#### Abstract

*Bradyrhizobium* sp. DOA9 can nodulate a wide spectrum of legumes; however, unlike other bradyrhizobia, DOA9 carries a symbiotic plasmid harboring type III secretion system (T3SS) and several effector (T3E) genes, one of which encodes a new putative type III effector—SkP48. Here, we demonstrated the pivotal roles of SkP48 from *Bradyrhizobium* sp. DOA9 in inhibiting nodulation of various *Vigna* species and *Crotalaria juncea* and suppressing nodulation efficiency of *Arachis hypogea*. By contrast, the nodulation efficiency of a SkP48 mutant did not differ significantly with the DOA9 wild-type strain on *Macroptilium atropurpureum* and *Stylosanthes hamata*. An evolutionary analysis revealed that the SkP48 effector which contains a shikimate kinase and a SUMO protease (C48 cysteine peptidase) domain is distinct from the others effectors previously identified in others bradyrhizobia and pathogenic bacteria. Our findings suggest that the new putative T3E SkP48 is a key factor suppressing nodulation and nodule organogenesis in several legumes by activation of effector-triggered immunity through salicylic acid biosynthesis induction, which is deleterious to rhizobial infection. In addition, nodulation may be modulated by the function of defensins involved in jasmonic acid signalling in *V. radiata* SUT1.

Keywords : T3SS; SkP48; symbiosis; Bradyrhizobium sp. DOA9; Vigna radiata



#### An evolutionarily shared formin protein INFO-mediated different symbiotic intracellular infections in legume and non-legume plants

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#### Abstract

Symbiotic infections own specific cellular inventions to entrap the symbionts to allow intracellular infection. This whole process involves stepwise and distinct actin cytoskeleton dynamics. Here, we identified a novel actin nucleator gene *INFO*, which is required for cytoskeleton remodeling at the onset of rhizobia and Arbuscular Mycorrhizal Fungi (AMF) intracellular entry in *Medicago truncatula*. Rhizobia infections on *info* mutant roots are mostly blocked at the stage of IT initiation. INFO protein itself localizes to the plasma membrane but undergoes a pattern transition from a homogenous distribution in the absence of rhizobia to punctate confinement in SYMREM1-positive nanodomains upon rhizobia inoculation. FLIM-FRET analysis and Co-IP assay support their physical interaction *in vivo*. Additionally, the INFO cytosolic domain FH1FH2 could bind G-actin with high affinity (Kd=825.23 nM). This cytoskeleton remodeling is mediated by protein condensation between INFO and SYMREM1.

Given that INFO is evolutionary not restricted to the nodulating clade but well confined in AM-forming species, a specific correlation between INFO promoter activation and AMF infection is further confirmed. Here the protein localizes to the cell periphery of cells hosting freshly branched arbuscules. In line with this, AMF colonization is compromised in *info* mutants.

Taken together, INFO, as an evolutionarily shared formin protein, could mediate different symbiotic intracellular infections in legume and non-legume plants as a key component in the common symbiotic signaling pathway.

**Keywords** : Rhizobia infection; Arbuscular Mycorrhizal Fungi infection; Cytoskeleton dynamics; Evolution of symbioses; Common Symbiotic Signaling Pathway



#### Molecular Basis of Mung Bean Symbiotic Incompatibility: A Pull-Down Assay Identifies Plant Proteins Targeted by NopP2 from Bradyrhizobium sp. ORS3257

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#### Abstract

Bradyrhizobium sp. ORS3257 is one of the most efficient strains for symbiosis with Vigna unguiculata and V. mungo. However, it renders the symbiotic interaction with V. radiata (mung bean) incompatible. Mutation of the nodulation outer protein P2 (NopP2) effector revealed that NopP2 is the cause of this incompatibility. However, the specific gene for gene-interaction involved in this process remains unknown. This study used an *in vitro* pull-down assay to detect physical interactions between NopP2 and proteins from mungbean roots. Subsequent protein analysis by MS/MS and individual pull-down assays confirmed that NopP2 interacts with plant enolase, glyceraldehyde-3-phosphate dehydrogenase (G3PDH), and monodehydroascorbate reductase (MAHAR). Transcriptomic analysis of NopP2 effects revealed that it stimulates the expression of genes in the group of resistance proteins, including TAO1-like (TIR-NBS-LRR), TMV resistance protein N-like (TMV3192) (TIR-NBS-LRR), and a putative disease resistance protein 1 of the RPP13 family (CC-NBS-LRR). Additionally, ORS3257 induced the expression of peroxidase, pathogenesis related-5 (PR5), and the transcription factor MYB13-like, which are important in secondary metabolites synthesis. All these genes are involved in plant defense mechanisms, and are likely to be factors inhibiting the nodule formation process for ORS3257. These results suggest that NopP2 of ORS3257 can completely block the symbiotic interaction by interacting with many different partners and inducing a multifaceted plant defense response, leading to incompatible symbiosis in V. radiata. However, the specific binding site between these proteins remains unknown, and its elucidation could be an interesting avenue for future research.

Keywords : Effector protein; Type III secretion system; Nodulation; Protein-protein interaction; Bradyrhizobium



## **Oral presentation**

## Theme: Application of Nitrogen fixation and N-cycle

December 7-9, 2024, Chiang Mai, Thailand.



## Why scaling up cowpea inoculated with effective native rhizobia should be good for soil health and yields in Yen Bai Province of Northern Vietnam?

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#### Abstract

Legume crops have long played a major role in sustainable cropping systems in Southeast Asia, representing up to 44% of the total cropped area in Myanmar for example. However, in Vietnam, due to increasing population pressure and demand for agricultural land, the current agricultural system is dominated by intensive practices that include conventional tillage, mono-cropping and overuse of mineral fertilizers.

Farmers in Northern Vietnam (Yen Bai province) rely on intensive monocropping fields of maize and cassava whilst facing serious issues of soil erosion and loss of natural soil biodiversity. Cowpea (*Vigna unguiculata* L.) is highly valued by farmers as it is locally consumed and easily sold at local markets at a good price. To reduce the damages caused by mono-cropping cassava, the intercropping cassava-cowpea system was tested by farmers and the impacts on soil health were assessed. Our results showed intercropping significantly enhanced macrofauna richness and evenness, increased diversity and abundance of soil microfauna as well as the abundance and richness of the total bacterial communities. Nodulation of cowpeas intercropped with cassava or maize in 2 districts showed that the natural nodulation of the legume plants remained limited regardless of the soil characteristics, seasons and landscape. Native strains were isolated, screened for their symbiotic effectiveness and successfully tested under field conditions to compensate for the total absence of commercial rhizobial inoculants for cowpeas on the market. The high expansion rate of intercropping with cowpeas shows the high adoption level of these agroecological practices by local farmers and justifies our current efforts to scale it up. Future projects will focus on further testing and legume intercropping promotion and dissemination to increase soil health and farmer incomes in mountainous regions of Vietnam.

Keywords : cowpea; intercropping with legumes; soil health; rhizobia; Field inoculation



#### Consequences of inoculation - evolution of diverse rhizobia symbionts of agricultural legumes

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Abstract

Rhizobia are phylogenetically diverse soil proteobacteria capable of infecting legumes to form a nitrogenfixing symbiosis. In Australian agriculture, this symbiosis, associated with pasture and pulse legumes, contributes nitrogen with a value of \$3.5 billion annually. Rhizobia compatible with agricultural legumes are not naturally present in Australian soils, so exotic highly effective strains are used as inoculants to maximise benefits of legumes for Australian farmers. After more than a century of legume cultivation genetically diverse communities of rhizobia have developed in Australian soils. A key mechanism for emergence of diverse rhizobia is horizontal transfer of symbiosis genes, encoded on mobile genetic elements, from inoculant strains to soil bacteria. For rhizobia in the genus Mesorhizobium, symbiosis genes are chromosomally encoded on genomic islands called symbiosis Integrative and Conjugative Elements (ICEs). Symbiosis ICEs can excise from the inoculant chromosome, transfer to a recipient cell by conjugation, and integrate into the chromosome at specific conserved sequences, resulting in a new symbiotic strain. The ICE recipients seem to be non-symbiotic Mesorhizobium spp. that are natural members of the soil microbiome. Crucially, while symbiosis ICE recipients can nodulate legumes, they may not fix nitrogen efficiently, even though the symbiosis ICE was acquired from a highly effective inoculant strain. This suggests interactions between the core and symbiosis ICE accessory genomes in rhizobia can significantly affect nitrogen fixation. Where large populations of sub optimally effective novel strains develop, this could pose a significant constraint to maximising symbiotic nitrogen fixation in agricultural systems.

Keywords : rhizobia; inoculants; symbiosis; mobile genetic elements; agriculture



#### Effect of nitrogen fixation enhancing gene on soybean growth and yield

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#### Abstract

We have already reported that the SEN1, which is essential for nitrogen fixation on *Lotus japonicus* polymorphism could be used to promote plant growth through enhanced nitrogen fixation. In this study, we examined whether the same strategy could be applied to soybean. We found that the soybean variety Enrei is different from others by comparing the sequence of soybean *SEN1*. We defined the standard sequence that most of the cultivars retained as Peking type SEN1, and the mutated sequence found in Enrei as Enrei type SEN1. Using near-isogenic lines (NILs) prepared by crossing Enrei with Fukuyutaka, which has Peking type SEN1, we investigated whether polymorphisms in the *SEN1* gene affect nitrogen fixation activity and plant growth. The results showed that Enrei type SEN1 can achieve an increase in soybean yield depending on the growing conditions. These results indicate that Enrei type SEN1 is a gene that enhance nitrogen fixation and that the introduction of Enrei type SEN1 may reduce nitrogen fertilizer application and increase yield.

Keywords : nitrogen fixation enhancing gene; soybean; Lotus japonicus; symbiosis



## Screening and evaluation of efficient nitrogen fixing rhizobia specific to cultivars of Vigna radiata recommended for different agro climatic regions of India

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Abstract

Vigna radiata (L.) R. Wilczek (Green gram) commonly known as mung bean, is one of the principally cultivated Zaid pulse crop and several varieties have been recommended for cultivation in different agro climatic regions of India. Aim of the present study was to screen four cultivars of V. radiata {RMG 975 (Keshwanand Mung-1), IPM 99-125 (Meha), PDM 139 (Samrat) and MH-1142} for their symbiotic nitrogen fixation efficacy (number of nodules and improved plant biomass) under controlled glass house conditions. Variety RMG 975 was developed for Rajasthan, PDM 139 for Uttar Pradesh, MH-1142 for North Western Plain Zone (NWPZ) and North Eastern Plain Zone (NEPZ); and Meha for NEPZ. Well characterized strains of Ensifer aridi (TW1, TW10) and novel strains of Bradyrhizobium (CPS12, TF4, TV10 and TVS2) isolated from root nodules of native legumes [species of Tephrosia (T. wallichii, T. villosa, T. falciformis) and Chamaecrista pumila] found in alkaline soils of Rajasthan and acidic soils of Shillong, Meghalaya were cross inoculated on four cultivars. Strains TW1 and TW10 harboring novel E. aridi type of nodulation genes effectively nodulated all cultivars except Meha, they even outperformed the N+ controls, in terms of improved plant biomass. These results suggest that Ensifer strains are best candidates as bio-inoculum for cultivars adopted for broad agro climatic regions except NEPZ. Similarly we observed that novel Bradyrhizobium (belonging to Mega Clade-I) strains TF4, TV10 harboring novel nod genes divergent from B. yuanmingense were more efficient on all four tested cultivars in comparison to strains CPS12 and TVS2 (belonging to Mega Clade-II) with nod genes divergent to B. elkanii. Multiple reports on dominance of B. yuanmingense strains as root nodule symbionts in different agro climatic zones of India suggests that while designing rhizobia based bioformulation preference to be given to B. yuanmingense strains in addition to E. aridi.

Keywords : Vigna radiata; Cultivars; Bradyrhizobium; Ensifer; Nodulation genes



## The denitrification pathway of Bradyrhizobium ottawaense and its potential application in N2O mitigation.

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#### Abstract

Nitrous oxide (N<sub>2</sub>O) poses a substantial threat due to its global warming potential (GWP), which is 298 times higher than that of carbon dioxide (CO<sub>2</sub>) over a 100-year period. Rhizobia, capable of biological nitrogen fixation (BNF), are widely utilized as biological inputs (inoculants) and can perform the denitrification process that, in its entirety, has the capacity to convert N<sub>2</sub>O into atmospheric nitrogen (N<sub>2</sub>) by utilizing the *nosZ* gene, encoding the nitrous oxide reductase (N2OR) enzyme. Our aims are (i) to explore soybean bradyrhizobia with higher N2OR activity and (ii) to reveal the relation between nitrate reductase and N2OR activities. From field-grown sorghum roots we isolated *B. ottawaense* with higher N2OR activity (1). Thus, we explored soybean bradyrhizobia with higher N2OR activity in Japan by PCR analysis of *B. ottawaense nosZ* gene. As a results, we obtained 104 isolates of B. ottawaense. From 82 measured strains, 70 strains (85%) showed higher N2OR activities (1000-2000 nmol/h/OD<sub>660</sub>) (2). In addition, we will present the results of gene comparison and functions of denitrification such as nitrate reductase (3) among soybean bradyrhizobia including *B. ottawaense* with higher N2OR activity.

#### Keywords: -

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Keywords : Bradyrhizobium ottawaense; Nitrous oxide; nosZ; Denitrification; N2O mitigation


#### Class 1 plant hemoglobin functions in two different symbiotic systems of Lotus japonicus

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Abstract

Hemoglobin is widely spread in plant kingdom. Leghemoglobin, which is the first plant hemoglobin identified by Dr. Kubo in 1939, is exclusively expressed in the root nodules of legumes and control oxygen partial pressure in the nodules for nitrogenase activity of rhizobia. Other plant hemoglobins (phytoglobins, Glbs) of land plants are classified into three classes, class 1, class 2 and class 3, based on their amino acid sequences. We focused on the function of a class 1 Glb of *Lotus japonicus*, LjGlb1-1, in two different symbiotic systems, root nodule symbiosis and mycorrhizal symbiosis.

The gene *LjGlb1-1* is the only gene whose expression is induced by nitric oxide (NO) among the eight *Glb* genes in *L. japonicus*, and the recombinant LjGlb1-1 protein exhibits NO dioxygenase activity. A null mutant line of *LjGlb1-1*, Kd1, exhibited higher NO level in the roots, lower number of nodules and lower nitrogenase activity compared with those of the wild type of Gifu. Two over-expression lines of *LjGlb1-1*, Ox1 and Ox2, exhibited lower NO level in the roots and higher nitrogenase activity, whereas no significant difference was detected in the number of nodules compared with that of Gifu. The nodules formed on Ox1 and Ox2, were tolerant to flooding and delayed in senescence.

In the mycorrhizae of *L. japonicus* with *Rhizophagus irregularis*, the expression of *LjGlb1-1* was observed at the infection site of *R. irregularis* where NO was detectable. Both of the tubulin gene of *R. irregularis* and *LjPT4* of *L. japonicus*, a phosphate transporter specific to mycorrhizal symbiosis, exhibited higher expression in mycorrhizae of Ox1. The line Ox1 exhibited better growth than the wild type, whereas the null mutant Kd1 exhibited poor growth. These results confirm that NO modulation by LjGlb1-1 is involved in mycorrhizal symbiosis in *L. japonicus*, as well as in root nodule symbiosis.

Keywords : root nodule; mycorrhiza; hemoglobin; nitric oxide; Lotus japonicus



### Transcriptome Analysis of Two RpoN Sigma Factors in Bradyrhizobium sp. Strain DOA9: Insights into Nitrogen Fixation Regulation under Free-Living Conditions

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Abstract

The alternative sigma factor, RpoN, is a crucial regulator governing diverse cellular processes in bacterial cells. This research investigates the unique regulatory model of rpoN genes in Bradyrhizobium sp. strain DOA9, which possesses two copies of *rpoN*—one on the chromosome (*rpoNc*) and another on a plasmid (*rpoNp*). DOA9RpoNc plays a crucial role in regulating nitrogen fixation under free-living conditions, while both RpoN are essential for nitrogen fixation during symbiosis with Aeschynomene americana. Analysis of free-living rpoN mutants revealed that individual inactivation of chromosomal and plasmid-encoded rpoN impacts colony morphology, swimming motility, carbon and nitrogen source utilization, extracellular production, biofilm formation and survival to stress conditions. The study determined free-living growth in Bnm broth without added nitrogen source and oxygen for a 7-day cultivation period. Metabolomic analysis investigated the metabolic responses of different DOA9 strains under free-living conditions, and integrative analysis with transcriptome data was performed. In total, 20 differential metabolites from the integrated transcriptomic and metabolomic analysis were identified, mainly involved in ABC transporter systems, nitrogen fixation, urea metabolism, nitrate metabolism, ribosomal synthesis, electron exchange, hydrogenase activity, responsive systems, cell division, quorum sensing, and cellular surface polysaccharide, controlled by regulatory RpoNc. Additionally, 10 differential metabolites were identified for regulatory RpoNp, including metabolites of active transport systems, cellular surface polysaccharides, responsive systems, and carbon utilization. Data analysis revealed that the regulation of nitrogen-fixing DOA9 under free-living conditions primarily requires RpoN on the chromosome, with additional regulation from RpoN on the plasmid. Furthermore, RpoN-regulated genes were verified by prediction of RpoN-binding sites, identification of five classical types of protein domains, and conserved domains of ATPases associated with various cellular activities (AAA+). Finally, a model of the regulation of the two RpoN in the freeliving life of Bradyrhizobium sp. strain DOA9 under low oxygen and no added nitrogen conditions was proposed.

Keywords : RpoN; Sigma factor; Bradyrhizobium; Nitrogen fixation; free-living state



### Mechanism of endophytic bradyrhizobial cell viability, differentiation, and nitrogen fixation in rice, chili, and tomato

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Abstract

Bradyrhizobium sp. strain SUTN9-2 underwent cell enlargement, increased its DNA content, and efficiently performed nitrogen fixation in response to rice extract due to the correlation of plant cationic antimicrobial peptides (CAMPs) and Bradyrhizobium BacA-like transporter (BclA), similar to bacteroid in legume nodules. In addition to rice, the present study revealed that SUTN9-2 could also establish a functional endophytic interaction with chili (Capsicum annuum L.) and tomato (Lycopersicon lycopersicum L.). Similarly, chili extract triggers SUTN9-2 cell differentiation, a process involving cell elongation, polyploidy, and increased membrane permeability, resulting in a loss of bacterial cell viability. In contrast, these effects on SUTN9-2 cells were retarded in response to tomato extract. Moreover, a combination of transcriptomic and cytological analyses was performed to investigate similarities in the physiology and mechanisms among endophytic SUTN9-2 cells in response to these three plant extracts. The upregulated differentially expressed genes (DEGs) in response to three plant extracts are related to the CAMPs resistance pathway (degP, only in rice extract), nitrogen metabolism (glnB, fixK, and ntrX), plant defense response (filC), and secretion system (types I, II, III, and IV). In contrast, the down-regulated DEGs encode genes involving cell cycle (gcrA, ctrA, ccrM, and ftsZ) and the CAMPs resistance pathway (AcrAB-TolC system, chili extract, especially). These results implied distinct CAMPs production in rice, chili, and tomato plants and responses by protease-chaperone DegP and AcrAB-TolC multidrug efflux pumps, possibly mediating efflux of CAMPs or by proteolytic degradation, affecting SUTN9-2 cell viability, differentiation, and nitrogen fixation in response to rice, chili, and tomato extracts.

Keywords : endophytic bradyrhizobia; cell viability; cell differentiation; nitrogen fixation; plant extracts



The 6<sup>th</sup> Asian-Pacific Conference on Plant-Microbe Symbiosis and Nitrogen Fixation

## **Oral presentation**

## Theme: Non – legume and Microbial Symbiosis



#### Beneficial endophytic streptomycetes allivate salinity tolerance in rice

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Abstract

Endophytic actinomycetes isolated from crops and medicinal plants in Thailand are rich sources of new taxa and bioactive compounds. Amongst them, beneficial *Streptomyces* spp. carrying plant growth promoting (PGP) traits including phytohormones, siderophores, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, chitinases, phosphate solubilization, and anti-microbial activities have been proven to promote growth, enhance stress tolerance, and protect phytopathogens *in planta*. ACC deaminase-producing streptomycetes improve abiotic tolerance of plants by reducing stress ethylene *via* conversion of ACC, a precursor of ethylene, to alpha ketoglutarate and ammonia under stress conditions. ACC deaminase (*acdS*) deficient and overexpressed mutants were then constructed to demonstrate the effect of this important trait towards salinity tolerance in rice. Further analysis of gene expression and transcriptome under stress conditions have revealed the molecular interaction of such beneficial endophytes to rice. Extensive understanding of the mutual association will sustainably enable application of these microbes as environmentally friendly added value bio-agents for green agriculture under climate change.

Keywords : Endophyte; Streptomyces; ACC-deaminase; Abiotic stress; Rice



#### Enhancing asymbiotic growth in arbuscular mycorrhizal fungi using mixed fatty acids

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Abstract

Most land plants form symbiotic associations with arbuscular mycorrhizal (AM) fungi. Plants acquire soil minerals through AM hyphal networks in the soil. In return, AM fungi obtain carbon compounds from the host plants, depending entirely on the hosts for their growth. Previous studies have shown that AM fungal genomes do not code any fatty acid synthases and that AM fungi utilize lipids as a carbon source, which is provided by the host plants. Recently, we showed that myristic acid (C14:0) increases the biomass of AM fungi under asymbiotic conditions, enabling AM fungi to be cultured without a host. During the incubation period, hyphal growth was extensive, and the formation of next-generation spores was observed. However, the size of the next generation spores was smaller than that of the spores formed in co-culture with plants. We observed that fatty acids were absorbed from the apical portion of the branched hyphae. Myristate was not only used as a carbon skeleton for biological components but also as an energy source to produce ATP. The major fatty acid supplied by plants is thought to be palmitic acid (16:0). To investigate the effects of fatty acid mixture, AM fungi were cultured in a medium supplemented with a mixture of palmitate and myristate. AM fungal biomass was increased in the mixture of the fatty acids compared to that in myristic acid alone. Additionally, a mixture of myristate, palmitate, and palmitoleate (C16:1) further enhanced both biomass and spore production. Our findings suggest that fatty acid mixtures containing myristate play a crucial role in the asymbiotic growth of AM fungi. This insight is key to advancing the pure culture of AM fungi.

Keywords : arbuscular mycorrhizal fungi; fatty acid; pure culture; Rhizophagus irregularis; spore



#### Arbuscular mycorrhizal Acaulospora morrowiae suppresses growth and nutrient uptakes of host plants regardless of soil P availabilities

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#### Abstract

Arbuscular mycorrhizal fungal (AMF) symbiosis plays a crucial role in enhancing plant growth, performance, and tolerance to biotic and abiotic stresses. However, mycorrhizal growth response (MGR) in AMF host plants depends on the interactions between AMF identity, plant genotype, and soil property and environmental condition. Previously, we found that the Acaulospora mycorrhizal fungus, isolated from lowland organic rice paddy, significantly suppressed the growth of Nipponbare lowland *japonica* rice grown in the sterile compost and sand mixture and fertilized with the 0.5x Hoagland solution without phosphorus (P). In this study, we elucidated the effect of Acaulospora morrowiae inoculation on MGR in various host plants under different soil P conditions. The result showed that inoculation of A. morrowiae inhibited the growth of Dawk Kha (DK) upland indica rice grown in the sterile compost and sand mixture. Subsequently, MGRs of the DK rice and maize were verified in the sterile nutrient deficient growing substrate composed of sand, perlite, and peat moss in a ratio of 8:1:1. However, the growth of host plants was inhibited by A. morrowiae. The fungus reduced the nutrient concentrations and contents in the host plants. Additionally, we perform the standardized *in vivo* bioassay by growing maize seedlings in the sand and perlite in the ratio of 9:1 containing 20 ppm of P in form of insoluble  $CaH_2PO_4$  and fertilized with the 0.5x Hoagland solution lacking P. The results showed that A. morrowiae inhibited the growth of maize seedlings. Based on our investigations, A. morrowiae suppressed the growth of host plants through reduction of nutrient concentrations and contents in the plants. The negative MGRs in rice and maize under various planting substrates reveal the parasitic association between A. morrowiae and the mycorrhizal host plants.

Keywords : Arbuscular mycorrhizal fungi; Acaulospora morrowiae; Rice; Maize; Mycorrhizal growth response



### Matching of cannabis endophytic bacterial microbiomes for plant growth stimulation and metabolite promotions

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#### Abstract

Normally, plant endophytic microbiomes inhabit plant tissues internally, and play important roles in plant development and growth. The beneficial endophytic bacterial microbiomes of *Cannabis sativa* were isolated from different plant tissues growing in the soil and planting material. Based on the 16s rRNA sequence, the *Gammaproteobacteria* are the main cannabis endophytic bacterial microbiome, as being strongly associated with plant growth promotion. The beneficial isolates were used as single inoculations and in mixed cultures to improve cannabis growth. Significant growth promotion was founf in both inoculations at the early seedling and harvesting stages. The secondary metabolitetetrahydrocannabinol (THC) production was also increased, particularly in consortia of eight identifiedisolates inoculation. The DGGE fingerprint confirmed the capability of these isolates for cannabis plant recolonization. Hence, the compatibility between endophytic bacteria inoculants and their host plants is crucial for ensuring the efficacy of the mixed culture inoculum. The application of mixed inoculum has enabled the development of synthetic microbial communities (SynComs), allowing more matching of the endophytic bacterial microbiome in *C. sativa* and its product is suitable for further medical usage as organic product.

Keywords : endophyte; cannabis; microbiome; DGGE; plant growth promotion



The 6th Asian-Pacific Conference on Plant-Microbe Symbiosis and Nitrogen Fixation

## **Oral presentation**

# Theme: Legume and Rhizobial symbiosis



#### Symbiotic roles of rhizobial type III effectors in soybean nodulation

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#### Abstract

Rhizobia induce the formation of nitrogen-fixing nodules on the roots of legumes. Nodules are formed when the roots initiate a molecular dialogue with compatible rhizobia in the soil. Key components for the establishment of symbiosis are rhizobiumderived lipochitooligosaccharides (Nod factors, NFs) and their leguminous receptors (NFRs) that initiate nodule development and bacterial entry. Previously, we demonstrated that symbiosis in the soybean rhizobium *Bradyrhizobium elkanii* is promoted by the type III secretion system (T3SS), which delivers virulence factors via pathogenic bacteria. Intriguingly, *B. elkanii* but not with its T3SS mutant was able to form nitrogen-fixing nodules on soybean *nfr* mutant, suggesting that T3SS activates host nodulation signaling by bypassing NF recognition. On some soybeans carrying *Rj4* allele, however, T3SS of *B. elkanii* caused restriction of nodulation. Recent progress in the effector functional analysis will be presented.

Keywords : nodulation; effector; soybean



#### Optimization mechanisms for symbiotic function of broad-host-range Sinorhizobium fredii

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Abstract

The rhizobium-legume symbiotic nitrogen fixation system is an essential component of green agriculture, and its nodulation and nitrogen fixation (Nod-Fix) efficiency should be considered during the iteration process of both legume cultivars and rhizobial inoculants. *Sinorhizobium fredii* can nodulate on more than 110 genera of legumes, it is therefore of important scientific and practical value to investigate optimization mechanisms of its Nod-Fix efficiency. We have systematically revealed that uptake pathways of P, Zn, K and Fe, type three secretion system and its effector protein, uptake and secretion systems of quorum sensing molecules, intracellular community of transposable elements are involved in optimizing Nod-Fix efficiency of *S. fredii*. These optimization modules are regulated by a xenogeneic silencer MucR in a global way. These findings can support further elite rhizobial inoculant development using synthetic biology procedures.

Keywords : Rhizobium; Soybean; Sinorhizobium fredii; MucR; T3SS



### Wild mungbean resistance to the nematode Meloidogyne enterolobii involves the induction of phenylpropanoid metabolism and lignification

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#### Abstract

Root-knot nematodes (Meloidogyne spp.) are plant parasites that attack crops and cause annual economic losses amounting to several billion US dollars worldwide. One of the most aggressive species is M. enterolobii, a growing threat to agriculture due to its broad host range and ability to overcome many existing resistance genes. Mungbean, a nutritionally and economically valuable crop, is particularly vulnerable because it is susceptible to many pests, including nematodes. However, research focusing on mungbean accessions with resistance to M. enterolobii is scarce and the corresponding defense mechanisms are poorly understood. We therefore screened wild mungbean resources and identified an accession with resistance to M. enterolobii. Transcriptome analysis revealed 2730 differentially expressed genes (DEGs) in this resistant accession (CPI106939) compared to 1777 in the susceptible accession (Crystal) 7 days after nematode inoculation. The genes upregulated in CPI106939 were similar to those downregulated in Crystal, with functions related to plant-pathogen interactions, plant hormone signaling, oxidative stress, and plant immunity. Plant defense-related genes (WRKY, PAL, MAPK, POD and PR) were also significantly induced in CPI106939. Metabolome analysis showed that six secondary metabolites, related to phenylpropanoid metabolism and lignification, were significantly enriched in CPI106939. The induced immune response and secondary metabolites may underpin the enhanced resistance to *M. enterolobii*, providing insight into the resistance mechanisms in accession CPI106939 as well as candidate genes controlling the interaction between mungbean and its nematode parasite. Our study therefore provides foundations for the breeding of new varieties with intrinsic *M. enterolobii* resistance.

**Keywords** : Plant–nematode interactions; Transcriptomics; Meloidogyne enterolobii; Wild mungbean; Secondary metabolites



## Performance in nurseries and analysis of native microbial symbionts of Australian Acacia hybrid seedlings in Northern Vietnam (Phu Tho Province)

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#### Abstract

Australian Acacia hybrids (*Acacia mangium* x *Acacia auriculiformis*) have been crucial in Vietnam's afforestation and economic development since 1990. As leguminous trees, their sustainable and productive growth in poor soils relies crucially on effective microsymbionts in the soil. Despite considerable attention given to the selection of high-yielding Acacia hybrid clones, research on their symbionts (including both root-nodule rhizobia and Arbuscular Mycorrhizal Fungi) has been widely neglected. This oversight has resulted in the suboptimal performance of Acacia hybrid plantations in Vietnam.

Phu Tho Province, a key province for production of Acacia hybrid clones, provides seedlings to both Northern and Central Vietnam. Seedlings produced either by tissue culture or cutting methods were included in the investigation of thirteen private nurseries in the province. Plant performance, root mycorrhization, nodulation and rhizobia diversity were measured. Interviews conducted with nursery owners revealed a lack of traceability of the clone origins and intensive management practices (ie. high rate of mineral fertilizers). Consequently, AMF colonization was very low (3% to 16%) and nodulation was poor (from 14 to 49 nodules per plant). Despite a low nodulation, a high genetic diversity among rhizobia was discovered. Surprisingly, clustering based on restriction fragments length polymorphism (RFLP) of the intergenic spacer region (IGS) did not align with the seedling type (tissue culture or cuttings), the nursery or the soil characteristics. Predominant genera of rhizobium discovered from the nodules included *Mesorhizobium Presenter 7:9:2024*. Chiang Mai, Thailand dyrhizobium (Bradyrhizobium sp., Bradyrhizobium elkanii,

*Bradyrhizobium guangdongense)* and *Rhizobium (Rhizobium tropici)*. Our study paves the way for further benefits on soil health and plantation productivity of Acacia hybrid in Vietnam by unveiling the potential of effective native rhizobia strain utilization to reduce chemical inputs.

Keywords : Australian Acacia hybrids; Vietnam; rhizobia genetic diversity; Root mycorrhization; Nurseries



The 6th Asian-Pacific Conference on Plant-Microbe Symbiosis and Nitrogen Fixation

## **Oral presentation**

## **Young Scientist**

December 7-9, 2024, Chiang Mai, Thailand.



#### Phylogenetic diversity of rhizobia nodulating Crotalaria pallida intercropped with coffee trees in Gia Lai province, Vietnam.

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#### Abstract

Robusta coffee is the largest cash crop in Vietnam and intensive farming practices significantly increased coffee production in recent decades. However, applications of fertilizers and pesticides significantly reduced the soil biodiversity and increased the incidence of soil soil-borne pests and diseases, mainly nematodes and *Fusarium*. *Crotalaria pallida* is a legume that can excrete nematicide compounds to control the populations of *Pratylenchus* and *Meloidogyne* in soils. However, the efficacy of the practice to reduce nematode incidence remains unknown. As this nematicide effect is produced by the complex legume-rhizobial interactions, it is important to have *C. pallida* nodulated with effective strains of rhizobia. At this stage, however, there is very limited knowledge about the genetic diversity of native rhizobia nodulating *C. pallida* in Vietnam.

The objective of our study is to investigate the phylogenetic diversity of rhizobia nodulating *C. pallida* intercropped with coffee trees from five different farms in the Gia Lai province. The genetic diversity of native rhizobia strains was identified by Polymerase Chain Reaction/Restriction Fragment Length Polymorphism of the Inter Genic Spacer (IGS) between rDNA 16S and 23S obtained with Msp I and Hae III restriction enzymes. The phylogenetic positions of strains were determined by using 16S rRNA sequencing. Our results identified 11 different IGS Groups within a total of 88 strains isolated from 125 nodules (25 nodules/farm). Strains for these different IGS groups were sequenced and the results showed that *Bradyrhizobium* strains (*B. arachidis, B. liaoningense, B. elkanii,* and *Bradyrhizobium sp.*) and *Rhizobium* strains (*R. multihospitium* and *R. tropici*) were able to nodulate *C. pallida*. The screening of potential elite inoculum strains from the 88 isolates will be performed in field trials with *C. pallida* intercropped in young-replanted coffee plantations in the Central Highlands to determine their efficacy for controlling nematode populations.

**Keywords** : Nematode infestation on coffee plantation; Crotalaria pallida; Phylogenetic diversity of rhizobia; Nematicide compounds; Legume-rhizobia interactions



#### Unveiling the Tripartite Synergistic Enhancement of Plant-Arbuscular Mycorrhizal Fungus Symbiosis by endophytic Bacillus velezensis S141 in Lotus japonicus

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#### Abstract

The crucial roles played by arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) in enhancing plant nutrient uptake and soil quality are widely recognized across various plant species. This study explored the effects and potential of *Bacillus velezensis* S141 as a plant growth-promoting rhizobacterium on promoting a symbiotic relationship of AMF, *Rhizophagus irregularis* with *Lotus japonicus*. *B. velzensis* S141 inoculation positively influenced fungal growth and development. *B. velzensis* S141 promoted fungal abundance, such as AM root colonization and spore number in addition to the plant nutrient uptake under the tripartite interaction. However, the indole-3-acetic acid producing capability of *B. velzensis* S141 was not the key mechanism for promoting this symbiotic interaction. Surprisingly, *B. velzensis* S141 induced not only marker gene expression related to the early phases of AMF colonization and nutrient uptake in the host plant; in addition, the AMF genes responsible for cell cycle regulation were also upregulated. These findings highlight the potential of *B. velzensis* S141 as a helper bacterium to support plant–AMF symbiosis and for further application in sustainable agriculture practices.

Keywords : Bacillus velezensis S141; Lotus japonicus; Rhizophagus irregularis; symbiosis; tripartite interaction



#### Enhancement of Rhizobial Inoculant for Peanut through Co-inoculation with Phosphate-Solubilizing Bacteria

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#### Abstract

Enhancement of legume N<sub>2</sub> fixation by co-inoculation of rhizobia with beneficial bacteria is a way to improve N availability in sustainable agriculture production systems. Specifically, phosphate-solubilizing bacteria (PSB) can assist in the P nutrition of legumes thereby in nodule formation and N2 fixation. Hence, this study aims to enhance a BIOTECH-UPLB developed rhizobial inoculant called NitroPlus<sup>TM</sup> through co-inoculation with PSB. Characterization of PGP traits and molecular identification of PSB were performed. Quantitative estimation of the phosphate solubilizing ability of the isolates was done using tricalcium phosphate as insoluble P source. Then, the viability of the PSB with the rhizobial inoculant on peanut seeds and roots were monitored. Lastly, the effectiveness of the rhizobial inoculant with PSB on peanut 'Biyaya' was evaluated in a screenhouse condition at zero, half, and full rates of P fertilizer using unsterile soil. PSB isolates; Bacillus sp. (345 and 349a), Serratia sp. (PS9), and Enterobacter sp. (LG7), possess other PGP attributes. The amount of Psolubilization by the isolates varied from 46 ppm to 217 ppm. In seed coating, NitroPlus<sup>TM</sup> (NP) with PS9 had the highest number of viable colony forming unit (cfu) per seed followed by LG7 and 349a. Furthermore, these isolates were able to colonize the peanut roots and survive during several weeks while the population of rhizobia was maintained. Significant interaction was observed between P fertilization and inoculation on plant height and nodulation of peanut 'Biyaya.' Irrespective of the P levels, NP+PS9 (?= 0.04) improved the total number of nodules than NP alone. NP+LG7 can augment the N and P uptake of peanut than NP alone. Lastly, NP+PS9 (?= 0.03) gave significant increase in postharvest soil P content than NP alone under full P fertilization. This study demonstrated the potential of PSB to enhance the performance of rhizobial inoculant for peanut under screenhouse condition.

Keywords : rhizobia; inoculant; phosphate-solubilizing bacteria; peanut; co-inoculation



#### Basidiomycetes Fungi isolated from rice in northern Thailand: Advantage or Disadvantage?

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#### Abstract

Rice (*Oryza sativa* L.) is one of the staple foods in Asia as the largest consuming region. In particular, rice is the main economic crop in Thailand. Various fungi including endophytes and saprobes are associated with rice plants that play significant roles. On the one hand, they can provide benefits in growth and health. On the other hand, they can cause disease in their host by changing their lifestyle. Endophytic fungi can colonize internal plant tissues without causing apparent harm to the host. Therefore the study of endophytic and saprophytic fungi on rice seems to be essential for disease management purposes, yield increasing as well as improving our knowledge on fungal diversity. In this study, we present three species belonging to *Pseudolagarobasidium*, *Pyrrhoderma*, *Schizophyllum* as endophytes, and one species of *Akenomyces* as a saprophytic fungus isolated from rice, collected from Chiang Rai province in Thailand. The isolates were identified based on morphology and phylogenetic analyses, including the ITS and LSU loci.

Keywords : Rice; Taxonomy; Endophytes; Saprobes

#### Isolation and characterization of cowpea rhizobia in Myanmar

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#### Abstract

This study was conducted to isolate cowpea rhizobia contributing to Myanmar's agriculture. Soil samples were collected from the fields in Sagaing, Mandalay, Nay Pyi Taw, and Magway regions in tropical savanna climate zone, and Bago and Yangon regions in tropical monsoon climate zone. These fields have a cultivation history of cowpea. These soils suspensions were inoculated into white cowpea. A total of 51 isolates were obtained from cowpea nodules. Based on 16S rRNA sequence analysis, a total of 47 Bradyrhizobium lineages, 2 Rhizobium dioscoreae, and 2 Paraburkholderia lineages were identified. Inoculation test of isolated R. dioscoreae and Paraburkholderia strains indicated that these strains had no ability to nodulate cowpea. Bradyrhizobium sp. (B. frederickii/B. agreste/B. glycinis), B. yuanmingense, Bradyrhizobium sp. (B. liaoningense/B. diversitatis), and "B. zhanjiangense" were dominant in tropical savanna climate zone of Sagaing, Mandalay, Nay Pyi Taw and Magway region, respectively. Bradyrhizobium sp. (B. elkanii/B. pachyrhizi/B. tropiciagri/B. ripae) was dominant in tropical monsoon climate zone of Bago and Yagon. Phylogenetic tree constructed by the sequence of internal transcribed spacer (ITS) region between 16S and 23S rRNA genes well depicted intraspecies variation in isolated Bradyrhizobium lineages. For example, almost all isolates of Bradyrhizobium sp. (B. elkanii/B. pachyrhizi/B. tropiciagri/B. ripae) in Bago and Yangon formed cluster with type strain of B. elkanii. Among the tested isolates, B. elkanii YGN-C1 and YGN-C4 produced the significantly highest nodule number and nitrogen fixation. Co-inoculation test of these two selected strains with non-nodulating R. dioscoreae YGN-C7, P. heleia BGO-C5, and P. *phymatum* BGO-C8 was conducted because non-nodulating bacteria isolated from nodules were reported to show the plant growth promoting ability. The results showed that the co-inoculation of B. elkanii YGN-C1 with P. phymatum BGO-C8 increased the nitrogen fixation significantly. These effective strains will be useful for the development of microbial inoculants for cowpea in Myanmar.

#### Keywords : Bradyrhizobium; 16S rRNA; 16S-23S rRNA (ITS); nodulation; nitrogen fixation



#### Role of type IV secretion system (T4SS) in Bradyrhizobium-legumes symbiosis

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Abstract

A broad host range Bradyrhizobium sp. SUTN9-2 was selected to study the role of the type IV secretion system (T4SS) during symbiosis interaction with legumes. SUTN9-2 carries two copies of gene encoding T4SS belongs to *tra/trb*cluster. A unique gene arrangement of *copG*, traG, and virD2 restricted in the Bradyrhizobium genus. Phylogenetic analysis of traG revealed evidence of horizontal gene transfer of the T4SS gene between Bradyrhizobium and Mesorhizobium. Different copies of the T4SS genes are found in different clades within the Bradyrhizobium genus. The T4SS copy 1 (T4SS<sub>1</sub>) is similar to B.diazoefficiens USDA110 and B. yuanmingense. To investigate the role of  $T4SS_1$  under symbiosis condition,  $T4SS_1$ deletion and their complementation  $(T4SS_{1compl})$  were constructed and tested with representation legumes in Dalbergioids, Millettiods, and Genistoids. The ?T4SS1 reduced nodule number and nitrogenase activity while, T4SS<sub>1compl</sub> resembling levels observed in the wild type. Further investigations focused on the roles of copG, traG, and virD2 genes within the T4SS<sub>1</sub> fragment. The  $?traG_1$  and  $?virD2_1$  reduced infection efficacy but gradually recovered their ability of invasion. In contrast,  $?copG_1$  showed completely abolished nodule organogenesis and nitrogenase activity. While,  $2copG_2$  slightly increased the number of nodules but decreased nitrogenase activity compared to the wild type. Moreover, the expression of nodulation genes was not detected in the lack of  $copG_1$  but high expression levels of T4SS<sub>1</sub> structural genes. These findings suggest that  $copG_1$  in T4SS<sub>1</sub> may function as a global regulator during the symbiotic interaction between Bradyrhizobium SUTN9-2 and legumes.

Keywords : Symbiosis; Type IV secretion system (T4SS); Bradyrhzobium sp. SUTN9-2



#### Identification of a genomic region related to host specificity in nodule symbiosis of Lotus burttii

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#### Abstract

The interaction of various molecular factors between legumes and rhizobia plays a crucial role in the establishment of root nodule symbiosis. On the other hand, interactions induced by an incompatible partner can disrupt nodule symbiosis, thereby contributing to host specificity. However, the molecular mechanisms underlying host specificity remain unclear. To understand the mechanisms in legumes, we isolated the *Rhizobium* sp. Chiba-1, which is capable of forming nodules on *Lotus burttii* B-303, but not on *L. japonicus* Gifu B-129. To clarify the genetic characteristics of these nodule phenotypes, we investigated nodule formation in  $F_1$  and  $F_2$  plants (*L. burttii* B-303 × *L. japonicus* Gifu B-129) after inoculation with *Rhizobium* sp. Chiba-1. All  $F_1$  plants formed approximately five mature nodules, and  $F_2$  plants exhibited 239 : 75 segregation as nodulation plants to non-nodulation plants (3:1, ?<sup>2</sup>?0.21) 3 weeks after inoculation. Furthermore, we observed the nodulation phenotype for *Rhizobium* sp. Chiba-1 in 60 RILs crossed between *L. burttii* B-303 and *L. japonicus* Gifu B-129, and QTL analysis using high-resolution SNP markers (Shah *et al., DNA Res.,* 2016) indicated a significant LOD peak at the SG0028 on chromosome 1. Additionally, QTL-seq was performed based on two classified bulks: "more than 7 nodules in plants" and "0 nodules in plants" of the  $F_2$  plants inoculated with *Rhizobium* sp. Chiba-1, and identified a locus within the region of chromosome 1 in *L. burttii* is the locus related to the broad host range induced by *Rhizobium* sp. Chiba-1.

Keywords : Symbiotic nodulation; Host specificity; Host range; Lotus species; Rhizobium



#### Phyllospheric Bacteria Isolated from Broccoli (Brassica oleracea var. italica) as Biocontrol Agent against Ralstonia solanacearum and as Plant Growth Promoting Bacteria

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#### Abstract

Phyllospheric bacteria, a group thriving on the above-ground parts of plants, intricately shape the plant microenvironment. Within this, plant growth promoting bacteria (PGPB) play a crucial role, enhancing plant's health through various mechanisms, such as stimulating growth by providing adequate nutrients and protecting the plants against phytopathogens. *Ralstonia solanacearum* is one of the most destructive phytopathogens, with a large group of strains, a wide host range, and known for causing bacterial wilt. The concern over the use of chemical fertilizers and pesticides has prompted the exploration of PGPB as an excellent alternative to boost plant productivity as well as protection from pathogens. This study aimed to investigate the characteristics of bacterial isolates from the phyllosphere of broccoli, to screen the isolates against *R. solanacearum*, to assess its PGPB abilities, and to identify the best isolate with the 16S rRNA barcoding method. The isolation of broccoli phyllosphere bacteria yielded 10 isolates, in which FB5 isolate had the best antagonist activity against *R. solanacearum*, based on the inhibition zone 16.37 mm formed in the disc diffusion test. The nitrogen fixation qualitative assay of FB5 showed negative results, while the assay of phosphate solubilization and IAA production gave positive results. Based on 16S rRNA molecular identification, FB5 was *Bacillus velezensis* strain FI383, with a percentage homology identity of 91.43%.

Keywords : Crucifers; Bacillus velezensis; Antagonistic activity; PGPB; 16S rRNA barcoding



## **Poster presentation**

## **Theme: Bioresource and Genomics**



#### Identification of cannabis strains using Inter-Simple Sequence repeats (ISSRs) molecular marker

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#### Abstract

Cannabis, a member of the Cannabaceae family renowned for its diverse medicinal and industrial applications, a taxonomic challenge due to distinct categorizations of its varieties: marijuana, distinguished by THC content of 0.2% or higher, and hemp, characterized by a THC content below 0.2%. Nonetheless, delineating these cannabis types has remained a difficult task. This study aimed to identify various cannabis strains through application of Inter-Simple Sequence Repeats (ISSRs). Twelve DNA samples from cannabis strains underwent analysis utilizing eleven ISSR primers. The results revealed 230 discernible DNA bands across eleven ISSR primers, averaging 20.19 bands per primer. The amplified products spanned a range of 150 to 3000 base pairs, comprising 8.26% monomorphic (19 bands) and 91.74% polymorphic (211 bands) traits. To elucidate genetic relationships among the twelve cannabis strains, a dendrogram was constructed using UPGMA method within NTSYS-pc software. Upon grouping using the Dendrogram technique, it was observed that the strains could be segregated into two clusters. Cluster 1 comprised ten cannabis strains: Foithong (FT), Hangkrarok (HR), Charlotte's Angel (CA), Baox, Blue Venom (BV), Charlotte's Web (CW), Cannafule, West Slop (WS), Golden Tiger (GT), and Orange Valley (OV). Meanwhile, Cluster 2 consisted of two Thai cannabis (hemp) strains: RPF1 and RPF2. Within Cluster 1, it was evident that the Thai cannabis (marijuana) strains FT and HR (clade 1) distinctly separated from foreign cannabis strains (clade 2). These results demonstrate that the application of these ISSR primers effectively distinguishes between native Thai and foreign cannabis strains. Furthermore, it appears that this technique is capable of distinctly differentiating between hemp and marijuana. These findings not only advance the precision in identifying diverse cannabis varieties but also contribute significantly to the refinement of marker-assisted selection methodologies. The research outcomes yield invaluable insights for future breeding programs directed towards the development of novel cannabis cultivars.

Keywords : Cannabis; marijuana; hemp; ISSR marker



#### Impacts of Humic Acid Applications on Soil Bacterial Abundance and Community under Sweet Corn Rhizospheric soil

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Abstract

Soil bacterial communities regulate several ecosystem functions and ecological processes that are crucial to maintaining plant productivity. Due to the naturally low fertility, lack of organic matter, and high phosphorus fixation of the soil in northeastern Thailand, agricultural intensification to enhance plant productivity consistently relies on the use of chemical fertilizers. Considering these issues, organic fertilizers like humic acid (HA) promote a great approach to improving soil health and productivity. Hence, humic acid recently used as a supplemental material incorporated with chemical fertilizer to optimize soil fertility and increase available phosphorus. In this study, we investigated the impact of humic acid on bacterial abundance and community under sweet corn (Zea mays L.) cultivation. The soil samples were collected from rhizosphere of corn after harvesting consisted of 4 treatments including control, chemical fertilizer (CF), humic acid 1% (HA 1%), and humic acid 2% (HA 2%). The q-PCR (quantitative polymerase chain reaction) of 16S ribosomal RNA gene (16S rRNA gene) based on gene copy number was used to analyze bacterial abundance, and the metagenomic information of the 16S rRNA gene sequencing was used to identify the potential PSB (phosphate-solubilizing bacteria) genus. This investigation revealed that the highest gene copy number obtained in CF with significantly different to control and HA 1%, but CF was not significant to HA 2%. Moreover, the observed genera, which included prominent genus denoted as Bacillus, Sphingomonas, Solirubrobacter, Nocardioides, Gaiella, and Vicinamibacteraceae, and the potential PSB genus were Bacillus, Pseudomonas, Rhizobium, and Enterobacter. Additionally, the application of humic acid significantly impacted the available phosphorus, with the highest content observed in HA 1%, followed by HA 2%, CF, and the lowest was control. We concluded that humic acid application promoted bacterial abundance and available phosphorus content among control and chemical fertilizer application.

Keywords : bacterial communities; phosphate-solubilizing bacteria; humic acid; sweet corn; rhizosphere diversity



## The National BioResource Project (NBRP) for Lotus and Glycine in Japan: QTL analysis for seed storability in soybean under different temperature

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#### Abstract

The aim of National BioResource Project (NBRP) in Japan is the collection, conservation and distribution of biological materials that form the basis of life science research. It consists of 30 biological resources, including animals, plants, microorganisms, DNAs and an information center. A new systematic collaboration was established with University of Miyazaki and Tohoku University in 2017 for the bioresource of *Lotus japonicus* and *Glycine max*. The fundamental information on the collected *Lotus* and *Glycine* resources has been rebuilt and expanded to users via the web database, LegumeBase (https://legumebase.nbrp.jp/).

Soybean (*Glycine max*) is the most important crop since the seed contains large amounts of protein and lipid which is widely used for human food and as a feed crop. Moreover, the research society has developed a large amount of basic research and valuable bioresources for soybeans. However, soybean was categorized in the poor storability group. The understanding of soybean seed storability should be studied for basic information in soybean breeding program. For QTL analysis, the recombinant inbred lines (RILs) crossed between *Glycine max* cv. Misuzudaizu and *Glycine gracilis* cv. Moshidou Gong 503 were cultivated in 2010 and 2019. Each seed was used to determine the seed viability and the seed vigor after storage under two storage temperatures (25°C and 35°C) for 6 months. Five QTLs were detected in the two traits, seed viability and seed vigor at all detected QTLs. Most QTLs were found near loci controlling seed viability, maturity, germination, seed hardness, and seed surface micromorphology, suggesting that seed storability is related to these traits. In addition, two new QTLs were found to be associated with seed storability.

Keywords : NBRP; quantitative trait locus (QTL); seed viability; seed vigor; soybean seed storability



## **Poster presentation**

## **Theme: Plant – Microbe Interaction**



## Effects of rice straw and its derived biochar incorporation on soil enzyme activity in a flooded rice field

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Abstract

Methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) emissions from rice paddy soils significantly contribute to global warming. Biochar has the potential to promote carbon sequestration and reduce N<sub>2</sub>O emissions. The activity of extracellular enzymes plays a crucial role in the cycling of carbon and nitrogen. However, limited research has explored how biochar affects enzyme activities and their involvement in  $CH_4$  and  $N_2O$ emissions in paddy soil. A field experiment was conducted to analyze ?-glycosidase and protease activities throughout the rice growth stages (the seedling, tillering, panicle initiation, and harvest stage) using chemical fertilizer (CF), rice straw combined with CF (RS+CF), and rice straw-derived biochar combined with CF (BC+CF). Compared to CF, ?-glycosidase activity increased with BC+CF at the seedling to the tillering stage and with RS+CF at the tillering to harvest stage. However, ?-glycosidase activity in BC+CF is lower than in RS+CF during the panicle initiation and harvest stage. The protease activity in BC+CF did not differ significantly from that in CF at all growth stages. In contrast, RS+CF increased protease activity at both the tillering and harvest stages compared to CF alone. Pearson correlation analysis indicated that a positive correlation was observed between ?-glycosidase activity and CH<sub>4</sub> emission in both BC+CF and CF treatments, while no correlation was identified in the RS+CF treatment. Additionally, the results showed that there was no correlation between protease activity and N<sub>2</sub>O emission in all treatments. Our findings indicated that incorporating rice straw-derived biochar into paddy soil reduced ?-glycosidase activity compared to directly adding straw at the latter stage. This has implications for CH<sub>4</sub> emission mitigation in biochar-treated soil. On the contrary, protease activity did not increase in biochar-treated soil. Furthermore, protease activity does not contribute to controlling N<sub>2</sub>O emissions in paddy soil.

Keywords : biochar; enzyme activity; CH4; N2O; paddy soil



**P05** 

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#### Abstract

The establishment of the rhizobium-legume symbiosis is a fascinating process involving intricate communication between plants and bacteria. A key participant in Nod-dependent nodulation is the SUMO protease, identified as a type III effector (T3E). To explore the genetic relationships between different Bradyrhizobial strains, phylogenetic tree and proteins domains of four SUMO proteases (p0490, p0871, p0903, and Skp48) from DOA9 were examined. The investigation unveiled diversity compared to other strains, with the SUMO protease (C48 cysteine peptidase) and catalytic core amino acids Histidine (H), Aspartic (D), and Cysteine (C) exhibiting similarities to XopD of Xanthomonas campestris. To deepen our understanding of these SUMO proteases functions, clean deletion mutant strains ?p0490, ?p0871, ?p0903, and ?Skp48 were generated and inoculated into A. hypogaea. Results indicated that strains ?p0903 and ?Skp48 exhibited an increase in nodules, whereas ?p0490 and ?p0871 did not. Additional investigation into the SUMO-p0903 protease domains and catalytic core amino acids during symbiosis with A. hypogaea revealed that the complementation of the ULP lack domain in the ?p0903 strain (?p0903+p0903-ULP-lack) and point mutations in amino acids D728A of the ?p0903 strains (?p0903+p0903-D728A) resulted in a increasing in nodulation on the host plant. In A. hypogaea inoculated with ?p0903, and ?p0903+p0903-ULP-lack, both mutant strains exhibited downregulation in plant defense genes of salicylic acid pathway and jasmonic acid pathway. Conversely, the symbiotic signaling pathway showed downregulation in SYMRK and CYCLOPS and upregulation in CNGC and SYMREM genes. This suggests that SUMO-p0903 protease has a negative impact on nodulation in A. hypogaea, while the N-terminal part (repeat domains) and catalytic amino acid core D728A in the ULP domain showed incomplete inhibition of nodulation. Consequently, SUMO-p0903 appears to play a suppressive role in the symbiotic interaction between Bradyrhizobium sp. DOA9 and its A. hypogaea host.

Keywords : Bradyrhizobium sp. DOA9; Arachis hypogaea; Type III effector: SUMO protease; Symbiosis



#### Lettuce Promoting Activity of Siderophore Producing Streptomyces sp. Rh1-5-22

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#### Abstract

Iron availability in soil is generally low due to its limited solubility. Presently, microorganisms are employed as alternatives to chemical interventions through their direct introduction into the soil. Microorganisms play a pivotal role in producing chemical compounds known as siderophores, serving as iron-chelating molecules. In this study, Streptomyces sp. Rh1-5-22 which showed high siderophore production was selected to test its its potential to enhance the growth of lettuce. Strain Rh1-5-22 demonstrated the capability to produce both hydroxamate-type  $(438.33\pm9.46 \text{ ?mol}.\text{L}^{-1})$  and catecholate-type  $(110.53\pm8.47 \text{ ?mol}.\text{L}^{-1})$  siderophores. Additionally, the introduction of 10 mM FeCl<sub>3</sub> into the culture medium resulted in the inhibition of siderophore production. For lettuce growth promotion, treatments were prepared as follows: the first treatment served as the control (sterile distilled water), the second treatment involved the application of Rh1-5-22 supernatant (siderophores), and the last treatment utilized Rh1-5-22 supernatant cultured in King B broth supplemented with 10 mM FeCl<sub>3</sub> (non-siderophore). Lettuce cultivation was conducted in perlite within a growth chamber for one month. Both supernatant treatments exhibited greater fresh weight and dry weight compared to the control. The siderophore treatment displayed the highest fresh weight (0.1511±0.0063 g) and shoot length (4.83±0.40 cm). Meanwhile, the non-siderophore treatment showed the highest dry weight (0.0129±0.0012 g), root length (5.82±0.55 cm), and chlorophyll content (0.2975±0.0883 mg.g<sup>-1</sup>). These outcomes imply that Streptomyces sp. Rh1-5-22 holds the capacity to enhance lettuce growth possibly by siderophore production to provide iron for plants.

**Keywords** : Siderophores; Plant growth promoting actinobacteria; Streptomyces; Plant-microbe interactions; Sustainable agriculture



## Unveiling Drought-Mitigating Potential of Streptomyces thermocarboxydus S3 in Pepper (Capsicum annuum L.) Seedlings: Mechanistic Insights from Genome Mining

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Abstract

The decline in global agricultural productivity is caused by climateinduced drought events. Drought stress exerts deleterious effects on crop yield, thereby posing a formidable threat to global food security. Consequently, there is an urgent need for strategies to ensure the sustainability of agricultural and food production. The utilization of plant growth-promoting actinobacteria emerges as a promising approach Drought-tolerant address these challenges. to **Streptomyces** thermocarboxydus S3 was previously isolated from arbuscular mycorrhizal spores. This strain demonstrates notable activities in indole-3-acetic acid and siderophore productions, as well as tri-calcium phosphate solubilization. S. thermocarboxydus S3 has previously demonstrated efficacy in mitigating drought effects in rice. However, the precise mechanisms through which this particular Streptomyces species mitigates such stress remain unclear. Therefore, this research aims to 1) evaluate the potential of S. thermocarboxydus S3 to mitigate drought stress in pepper (Capsicum annuum L.) seedlings and 2) elucidate the mechanisms employed by S. thermocarboxydus S3 in drought mitigation through a genome mining approach. The findings indicate that pepper seedlings inoculated with S. thermocarboxydus S3 exhibit superior growth compared to non-inoculated control under drought conditions, as evidenced by an increased in shoot and root length, dry weight, and total chlorophyll and carotenoid content. December 7:9, 2024, Chiang Mai, Thailand. Significantly, S. thermocarboxydus S3 alleviates drought stress by reducing hydrogen peroxide accumulation in pepper seedlings. Genome mining of *S. thermocarboxydus* S3 revealed numerous predicted genes associated with plant growth-promoting properties and the mitigation of osmotic and oxidative stresses. This corroborates its potential in enhancing plant growth and alleviating drought stress. Biosafety testing provides evidence affirming the environmental safety of this strain. In summary, this research shows the efficacy of *S. thermocarboxydus* S3 in alleviating drought stress in pepper seedlings and unveils its molecular-level mechanisms, offering a sustainable solution to drought stress challenges in crop plants.

**Keywords** : Drought stress mitigation; Genomic analysis; Plant-growth promoting actinobacteria; Streptomyces; Climate resilience agriculture



#### Unlocking the Power of Bacillus velezensis S141: Unraveling Synergistic Growth Promotion and Biocontrol in Legumes

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#### Abstract

*Bacillus velezensis*S141 is one of effective Plant Growth Promoting Rhizobacteria (PCPR) which was isolated from soybean rhizosphere soil in Thailand. The whole-genome sequence of S141 was published in 2017 to revealed valuable insights into its capabilities. The co-inoculation of either S141 cells or free-cell culture with soybean nodulating bradyrhizobia (*B. diazoefficiens*strains USDA110 and THA6) significantly boosts soybean growth and yield in field trials. While, the disruption of genes related to IAA and cytokinin (CK) biosynthesis in S141 leads to reduce the number of large nodule. This finding indicates that, IAA and (CK) plays a crucial role on the symbiosis interaction. Moreover, S141 also exhibits strong antifungal activity against *Cercospora canescens* PAK1 (causing agent of leaf spot disease in munbean) through multiple enzymatic hydrolases and secondary metabolites from secreted compounds. Interestingly, the cell-free secretions from a single culture of S141 and dual culture of S141+PAK1 showed inhibition of fungal growth. The biocontrol efficiency of S141 against *Cercospora*leaf spot on mungbean was also evaluated, with a control efficiency of 83% after 2 days of infection. Transcriptome analysis further confirms its production of potent antifungal enzymes like protease, ?-glucanase, and N-acyl glucosaminase, alongside genes for secondary metabolite synthesis. These remarkable findings position S141 as a promising biocontrol agent against *C. canescens* and a superior co-inoculant for efficient legume production, offering a sustainable alternative to chemical fungicides.

Keywords : Bacillus velezensis; Plant growth promotion; Biocontrol; Cercospora leaf spot; Mung bean



#### Protein-protein interaction assay between Vigna radiata proteins and effector protein, NopP2, from Bradyrhizobium elkanii USDA61

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#### Abstract

Rhizobia is one of an important bacteria that have a beneficial effect on the growth of legumes. However, the interaction of legume symbiosis is highly specific. Rhizobia employ diverse strategies to enhance symbiosis within their hosts, including the targeted delivery of effector proteins using the type three secretion system (T3SS). Earlier research shown that *Bradyrhizobium elkanii* USDA61 Type 3 effector, T3E-*innB* as a novel rhizobium-specific effector that negatively impacts symbiotic relationships with *Vigna radiata* (mung bean). Moreover, our investigation provides compelling evidence that the *T3E-nopP2* as a pivotal factor crucial for early infection establishment and the formation of nodules. However, the intricate interplay between T3Es and plant proteins remains elusive. In this study, pull down assay revealed the interaction between *nop*P2 and some interesting proteins from mung bean. Three interesting proteins, Adenosylhomocysteinase, Pathogenesis-related protein 10, and Glutaredoxin, were identified by LC-MS/MS and SDS-MS/MS and will be further investigated to clarify molecular mechanisms of interaction.

**Keywords** : Type three secretion system (T3SS); Vigna radiata (mung bean); Type 3 effector proteins (T3Es); Bradyrhizobial T3SS; T3E-nopP2



## The molecular interaction between Lotus japonicus and Bradyrhizobium mediated by type 3 effector protein, NopM

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#### Abstract

Microsymbionts *Bradyrhizobium elkanii* USDA61 possesses a functional type III secretion system (T3SS) that can secrete effector proteins into host cells to modulate host-cellular functions. One such effector protein, USDA61 NopM, triggers brownish nodules on *Lotus* spp. by inducing an early senescence-like response. However, the intricate molecular mechanisms governing the interaction between *Lotus* spp. and *B. elkanii* mediated by NopM remain unclear. This study was focused on NopM's functional domains, unveiling its role in the symbiosis between *Lotus* spp. and *B. elkanii*. We identified two key domains within NopM: The Leucine-rich repeat (LRR) domain which provides a general structural framework for protein-protein interactions, likely facilitating NopM's binding to specific *Lotus* spp. proteins. The Novel E3 ligase (NEL) domain, which functions as an E3 ubiquitin ligase, potentially makes host proteins for degradation, ultimately leading to the observed senescence-like response. To elucidate the roles of these domains, LRR or NEL deletion mutants were constructed and inoculated on *Lotus* spp. The nodule numbers and fresh weight of plants inoculated with these mutants were significantly changed when compared to the wild-type. These findings suggest that both the LRR and NEL domains are essential for NopM's functionality in inducing the early senescence-like response in *Lotus* spp. Moreover, the protein-protein interactions between NopM and plant interacting partner will be performed *in vitro* pull-down assays using *Lotus* root proteins. Subsequently, we plan to utilize LORE1

mutant lines to validate the functions of discovered interacting proteins. Therefore, this study could provide a valuable foundation for comprehending the intricate molecular mechanisms underlying the symbiosis between *Lotus* spp. and *Bradyrhizobium*. This knowledge can potentially pave the way for developing strategies to enhance nitrogen fixation in legumes, leading to increased agricultural productivity and sustainability.

Keywords : Bradyrhizobium elkanii; Lotus japonicus; type III secretion system; effector protein NopM


# **Poster presentation**

# Theme: Application of Nitrogen – fixation and N – cycle



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Abstract

The application of nitrogen biofertilizer *Bradyrhizobium* is necessary for legume production. The mixed-culture inoculant of bradyrhizobia is a promising strategy to successful nodulation in the field. Ensuring the production of a mixed-culture inoculant with high amounts of viable cells and less or no contamination is imperative. This study focuses using the recombinant human scFv antibodies as a quality control technique for mixed-culture inoculant production of peanut bradyrhizobia. The mixed-culture inoculant of *Bradyrhizobium* sp. SUTN9-2 and *Bradyrhizobium* sp. DASA03028 was used as a model. The specific scFv antibody yiN92-1e10 and yi028-F11 which against to strain SUTN9-2 and strain DASA03028, respectively were selected for this research. There was no cross-binding reactivity between these two scFv antibodies and among other tested bradyrhizobia. These recombinant scFv antibodies were successfully applied with the fluorescent antibody (FA) technique to assess viable cell counts of individual bradyrhizobial strains in liquid and peat-based mixed-culture inoculants, providing a concise and effective quality control method.

Keywords : Recombinant scFv; phage display; Bradyrhizobium; legume inoculant; Quality control

### Amino-vinyl-glycine (AVG) and Indole-3-butyric acid (IBA) in Seed Coating enhance Plant growth and nitrogen fixation of soybean cv. CM60 under stress condition

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Abstract

Soybean have been used as a source of food, animal feed, and oil in Thailand. The import soybean was more than 2.7 million tons per year. Soybean production in Thailand is still limited due to the poor knowledge in production technology, high yield and resistance cultivars, and water deficit condition. At reproductive phrase, drought condition can cause 74% yield reduction. Under stress conditions, plant growth regulators (PGRs) play a main role in severity of stress and stress This study aims to investigate the potential of PGRs on tolerance. soybean's physiological traits, growth, nodulation, and nitrogen fixation efficiency. Bradyrhizobium diazoefficiens strain USDA110 with Gustagged was mixed with 0.6% Xanthan gum with or without PGRs: 5 nM ASA, 50 nM AVG, 5 nM IBA were thoroughly coated onto soybean cv. CM60 seeds. The laboratory experiment in sterilized modified Leonard jar showed that 50 nM AVG or 5 nM IBA promote infection tread and nodule formation as well as shoot length in the first 7 daysafter-inoculation. Under greenhouse conditions, soybean seeds inoculated with B. diazoefficiens strain USDA110 and PGR as described above were sown in 2-L pots filled with sandy-roam soil with low organic matter, supplemented with the nitrogen-free fertilizer. Onemonth old soybean plants (anthesis stage) were divided into 2 groups: well water and water stress (withheld water for 7 days until soil projecture 2 content Tisanaround 10-15% relative soil water content). The

photosynthetic rate of USDA110 + 5 nM IBA plants were significantly higher than other treatments both under stress and well-water condition. Nitrogenase activity (ARA activity) was also significantly higher in both conditions. This study suggested the possibility of using IBA as an additive to the soybean seed coating material as a viable method for soybean nitrogen fixation and growth promoting factor.

Keywords : Soybean; water deficit; auxin; ethylene; stress tolerance



### AI-designing based on likelihood of evolutionarily structural origin between multi-subunit nitrogenase-like enzymes and single-polypeptide LPOR-like catalysts

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### Abstract

The nitrogen cycle, along with the carbon cycle, is one of the key processes for sustaining life on Earth, in which biological nitrogen fixation catalysed by the multi-subunit nitrogenase remains essential. Over the past three decades, the remarkable and considerable progress has been achieved in our understanding of the structure of nitrogenase, and their structures display a notable similarity to an enzyme in the biosynthesis of the photosynthetic pigment chlorophyll, namely dark-operative protochlorophyllide oxidoreductase (DPOR?subsequently COR). However, the reduction of nitrogen by nitrogenase is a very energy-demanding reaction, powered by over sixteen ATPs per N<sub>2</sub> fixed. Intriguingly, there is an alternative enzyme, light-dependent protochlorophyllide oxidoreductase (LPOR), that catalyses the same reaction but using light rather than chemical energy in the form of ATP. Here, by directly comparing the structural similarities between N2ase/DPOR/COR subunits (NifHDK/ChlLNB/BchXYZ) with LPOR, evolutionary clues for how a porphyrin reduction catalyst could evolve from the less efficient version, ATP-driven DPOR, to a highly efficient light-driven LPOR can be obtained. Such insights could provide valuable guidance in designing a light-utilising version of nitrogenase (LUN) that shares similarities with LPOR or a related SDR [shortchain dehydrogenase reductase] family protein. These breakthroughs have significantly expanded the possibilities for engineering both nitrogen reduction and porphyrin reduction processes, leading to the development of more effective forms of biological nitrogen fixation and photosynthesis. Furthermore, this methodology may be extended to the biosynthesis of a wide range of catalysts. The key structural milestones achieved in N2ase, DPOR, and LPOR researches have laid the groundwork for future perspectives, particularly in proposing a direction for a potential nitrogen-fixing scenario using a light-utilizing nitrogenase (LUN) and photosynthesis with bacteriochlorophyll using light-driven chlide reductase (LCOR).

Keywords : nitrogenase; chlorophyll biosynthesis; protochlorophyllide; chlorophyllide; light-driven



### Enhancing Siamese Rosewood (Dalbergia cochinchinensis) Reforestation Success: The Impact of Rhizobial Inoculation on Nodulation and Growth

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Abstract

Siamese Rosewood (*Dalbergia cochinchinensis*) is a leguminous tree of economic significance, primarily cultivated for reforestation. However, the growth of Siamese Rosewood is hampered by nutrient deficiencies. Legumes, including Siamese Rosewood, can form nodules in collaboration with rhizobia, which play a crucial role in fixing nitrogen for the host plant. Thus, the focus of this study was to investigate the impact of rhizobial inoculation on the nodulation and growth of Siamese Rosewood seedlings. Out of the 30 Rhizobium isolates tested, only 12 demonstrated successful nodulation with Siamese Rosewood seedlings. The isolate B1 exhibited the highest average nodule number (3.60 nodules/plant). However, isolate I5 proved to be the most effective in promoting plant growth during pot experiments, displaying the highest plant dry weight (66.65 mg/plant). In addition, isolate I5 induced the highest levels of Chlorophyll a, Chlorophyll b, and total Chlorophyll content (0.57, 0.36, and 0.98 mg/g leaf fresh weight, respectively). These findings lead to the conclusion that Rhizobium holds promise as a biofertilizer for

Siamese Rosewood reforestation efforts.

Keywords : Siamese Rosewood; Reforestation; Nodulation; Rhizobial Inoculation; Biofertilizer



The 6<sup>th</sup> Asian-Pacific Conference on Plant-Microbe Symbiosis and Nitrogen Fixation

# **Poster presentation**

# Theme: Non – legume and Microbial Symbiosis



### Transcriptomic study on the growth-promoting impact of Bacillus velezensis S141 on Cannabis sativa

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### Abstract

Nowadays, *Cannabis sativa* has been increasingly regarded to possess positive benefits in the contexts of alternative choices for medical users for instance as a pain-relieving administration in cancer patient. This study was therefore of interest in implementing a well-studied *Bacillus velezensis* S141 as a supplementing probiotic proficient in increasing the growth performance of the cannabis. Furthermore, transcriptomic analysis was carried out to discern an insight into the biological processes entailed in how *B. velezensis* S141 can encourage the augmented development of the plant and what pathways the bacteria can provoke in the cannabis. The annotated genes involved in each pathways categorized by KEGG, such as phenylpropanoids biosynthesis, photosynthesis, carbon metabolism, plant hormone signal transduction, and plant-pathogen interaction, were putatively found to play pivotal roles in the stimulation of plant growth, whereby the transcriptional levels of these genes were analyzed by qRT-PCR and conformed to the transcriptomic data. Hence, our finding can unveil a comprehensive information detailing the behind biological mechanisms participating in the growth performance of *Bacillus velezensis* S141 on *Cannabis sativa*.

Keywords : Bacillus velezensis S141; Cannabis sativa; Transcriptomic study



### Plant root endophytic bacteria for mitigation of greenhouse gas from agricultural fields

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Abstract

Methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) are greenhouse gases with a global warming potential 25 and 265, respectively. CH<sub>4</sub> and N<sub>2</sub>O emission from agriculture account for 11% (CH<sub>4</sub>) and 59% (N<sub>2</sub>O) of anthropogenic emissions. To reduce these greenhouse gas emissions, we focus on methanotrophs, which convert CH<sub>4</sub> to CO<sub>2</sub> and *Bradyrhizobium* harboring N<sub>2</sub>O-reducing activity.

Rice paddy field is a major source of  $CH_4$ , which is produced by methanogen decomposition of organic matter. The  $CH_4$  is released to the atmosphere through the soil and the rice plant tissue. Methanotrophs, oxidize the  $CH_4$  as a carbon source, live in the rice root tissue containing  $CH_4$  and atmospheric oxygen, such as aerenchyma. In addition, some methanotrophs fix nitrogen using the energy from methane oxidization. We aim to mitigate  $CH_4$  emission and nitrogen fertilizer in rice field by utilizing methanotrophs. Previous study showed that the methanotroph abundance was higher in the rice roots cultivated in paddy fields without N fertilizer rather than conventional fertilizer applications. Therefore, rice might be regulating the endophytic interactions with methanotrophs depending on their nitrogen nutrient. By 16S rRNA amplicon analysis, we compared the abundance of methanotrophs between *Oryza sativa* L. cv. Nipponbare and its *NLP4* (master regulator of nitrate response and assimilation) mutant cultivated in with/without N fertilizing fields. As we hypothesized, the abundance of methanotrophs in *Osnlp4* root was higher than that of the Nipponbare root.

 $N_2O$  is emitted from agricultural fields after N fertilization and soybean rhizosphere. To mitigate  $N_2O$  emission, we focused on *Bradyrhizobium ottawaense* harboring higher  $N_2O$ -reducing activity. Generally, microbial  $N_2O$  reduction occurs under anaerobic conditions. However, we found that *Bradyrhizobium ottawaense* SG09 showed low levels of  $N_2O$ -reducing activity even under an aerobic condition. Thus, we compared the transcription levels of *nosZ* gene encoding  $N_2O$  reductase enzyme under aerobic and anaerobic conditions by *nosZ* qPCR.

Keywords : Methane; Nitrous oxide; Methanotroph; Nitrous oxide reducing Bradyrhizobium; NosZ



### Production of Arbuscular Mycorrhizal Fungal spores in Zea mays using Light Emitting Diode (LED) condition

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Abstract

The production of arbuscular mycorrhizal (AM) fungal spores often faces the problems with light intensity, temperature fluctuations, nutrient availability constraints and time consuming. This study aimed to apply the LED (Light Emitting Diode) technology to increase the spore productivity. *Rhizophagus irregularis* was inoculated in maize (*Zea mays*) by comparing the LED light with greenhouse conditions. A significant improvement in AMF colonization at 45 DAI and spore production was approximately 1.5 times at 45 and 90 DAI under LED lighting compared to greenhouse conditions. The LED light treatments tended to enhance maize growth by increasing nitrogen (N) in shoot and phosphorus (P) in root tissues. Under LED light treatments, genes related to photosynthesis were significantly upregulated compared to the green house (GH) condition. Additionally, LED light condition influenced the expression of marker genes associated with the AMF-related cell cycle, indicating an upregulation during symbiosis. This method may be used for accelerate the large-scale spore production under substrate-based method.

Keywords LED light, maize, Rhizophagus irregularis, symbiosis, gene expression

Keywords : LED light; maize; Rhizophagus irregularis; symbiosis; gene expression



### Improve the Production of Edible Cassava (Manihot esculenta Crantz) cultivar Phiroon 6 using Biofertilizers for Sustainable Agriculture

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### Abstract

Cassava (Manihot esculenta Crantz) is a crucial staple crop for an economic stability. Among of cultivars for tapioca starch production, cultivar Phiroon 6 is an edible cassava used directly for cooking as food, snack, or desert. In this study, the beneficial biofertilizers including arbuscular mycorrhiza fungus (AMF), rhizobium, and some PGPR that act as Mycorrhiza Helping Bacteria (MHB) were used to improve cassava production. The intercropping system of cassava and peanut (Arachis hypogea L.) cultivar Kalasin 2 was firstly examined. Rhizophagus irregularis (AMF) was assured to colonized cassava Phiroon 6 after 45 days after planting (DAP), while Bradyrhizobium sp. SUTN9-2 was selected as Nbiofertilizer for peanut under organic farming system. Bradyrhizobium could promote the growth of peanut with yielding of pod fresh weight at 3,622.70 kg/ha while pod fresh weight 1,594.70 kg/ha was shown when using organic fertilizer at 90 DAP. However, no differences in plant growth promotion for cassava in all treatments. Then, the legume plant residues were incorporated into the soil as green manure and the cassava yield will be later collected at 8-10 months after planting. Meanwhile, the other hypothesis was focused on using MHB to promote AMF colonization on cassava root and may later promote cassava growth. The coinoculation of AMF with SUTN9-2 or Bacillus velezensis strain S141 as MHB, resulted in increasing frequency of mycorrhiza in the root system (%F) to 14 % and 2 %, respectively in pot experiment. Arbuscule abundance in the root system (%A) was increased to 1.49 % and 1.87 % in the cassava co-inoculated with SUTN9-2 and S141 at 45 DAP.

Here's feasibility study to gain the knowledge and understanding in viewpoint of biofertilizers interaction to cassava and the potential using as the combination biofertilizer in future cassava crop production under organic system.

Keywords : Cassava; Biofertilizers; Bradyrhizobium sp.; Mycorrhiza Helping Bacteria; Bacillus velezensis



The 6th Asian-Pacific Conference on Plant-Microbe Symbiosis and Nitrogen Fixation

# **Poster presentation**

# Theme: Legume and Rhizobial symbiosis



### SHIGEYUKI TAJIMA<sup>1</sup>

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Abstract

A genome DNA sequencing analysis of a root bacteria from water floating legume, *Neptunia natans* indicated it as *Stenotrophomonas pavidii* 

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Root samples of water mimosa (*Neptunia natans* (L.f.) Druce) were randomly collected from three areas in Mae Rim District and Mae Wang District, Chiang Mai Province, Thailand. The samples were processed to isolate bacteria by washing the plant roots under running water for 5 min. to remove soil and loosely attached microorganisms. Afterward, the roots were rinsed three times with sterilized distilled water. The washed roots were then shaken in sterilized distilled water for 10 min. to separate microorganisms that may adhere to the root surface. Subsequently, the solution was spread on nutrient agar (NA) and incubated at 28 C for 3 days.

The collected isolates were grown on nitrogen-free broth and the most positive culture was subjected genome sequencing analysis. By hybrid assembling using short-read and long-read sequencing data, one circle chromosome DNA having 4,750,146bp was revealed and the GC content was 66.9%. The data concluded the isolate is *Stenotrophomonas pavanii*. The *Stenotrophomonas sp.* was reported to detected in various soils and environments as antibiotic or PET degrading bacteria. *Stenotrophomonas pavanii* is reported from sugar cane stem in organic farming in Brazil?Ramos et.al, 2011?. In this presentation, we report about collecting of the isolates, broth culture and genome sequencing analysis.

**Keywords** : Neptunia natans; Stenotrophomonas pavanii; genome sequencing analysis; organic farming; nitrogen fixation



### Black pepper (Piper nigrum) – Arachis pintoi intercropping system in the Central Highlands in Vietnam: Impact on the soil fertility and the diversity of native rhizobia

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### Abstract

Vietnam is leading the growth of black pepper worldwide, accounting for more than one-third of the global production. However, this is achieved by the intensive use of chemical fertilizers and pesticides, causing pollution to the environment and reduction of soil health. Overall, it favors the development of soil-borne pathogens, such as *Fusarium*, *Phytophthora*, and *Pythium/Phytopythium*. *Arachis pintoi* is a legume that can contribute to biological nitrogen fixation (BNF), due to its symbiosis with rhizobia. It is widely planted as a cover crop to reduce soil erosion, suppress weeds, control several pests and soil-borne diseases. For instance, in Vietnam, *A. pintoi* has been intercropped with black pepper to enhance soil health and sustain the yield. However, its impacts on reducing weeds, pests and soil-borne pathogens remain largely unknown. Meanwhile, limited information is available about the rhizobia nodulating *A. pintoi* in Vietnam.

In this study, we investigated the impact of *A. pintoi* intercropped in black pepper plantations in Gia Lai and Dak Lak provinces in the Central Highlands in Vietnam. Soil samples were analyzed, and the results showed that plantations intercropped with *A. pintoi* significantly improved the total nitrogen. Besides, nodules of *A. pintoi* from these provinces were collected and screened for their rhizobial strains. The intergenic spacer (IGS 16-23S) region of the rhizobial DNA was amplified by PCR, and restriction was digested with two enzymes (MspI and HaeIII) to group rhizobia based on their restriction profiles. During the initial phase of our project, 38 strains have been isolated from 108 nodules and grouped into 13 different groups. The objective is to identify elite strains associated with *A. pintoi* and to formulate effective rhizobial inoculants to allow effective BNF by this legume, hence, reducing the need for fertilizer inputs, and assisting in soil health restoration of black pepper plantations.

Keywords : Arachis pintoi; Black pepper; Rhizobia; Soil health; Vietnam



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Abstract

We previously reported that *Bradyrhizobium elkanii* USDA61 can hijack soybean nodulation signaling via its type three secretion system (T3SS), which commonly functions in pathogenic bacteria to deliver effector proteins (T3Es), in the absence of Nod factors (NFs). Moreover, we identified the rhizobial effector Bel2-5 as the key T3E responsible for triggering soybean NF-independent nodulation. However, the presence of Bel2-5 causes strong nodulation restriction in soybean carrying the *Rj4* allele by activating plant immune responses.

In our current study, we isolated a novel *bradyrhizobium* strain capable of inducing nodules in non-nodulating soybeans, including Clark-*rj*1, En1282 (*nfr1*), and supernodulation (*nod*+++) comes from nitrate-tolerant symbiotic (*nts*) mutant. Intriguingly, inoculation tests in diverse legume collections revealed that this strain exhibits similar phenotypes to USDA61 across tested plant species, except for the soybean *Rj4* allele. In contrast to USDA61, which demonstrates a robust symbiotic incompatibility, this isolated strain can induce effective N<sub>2</sub>-fixing nodules in *Rj4* soybean, thus promote plant growth.

A phylogenetic tree based on the internal transcribed spacer (ITS) region of isolated strain, classifies it within the *B*. *elkanii* group. The presence of the T3SS was confirmed in the isolated strain through PCR and sequencing, using universal primers targeting the canonical *rhcN* gene. In parallel, partial sequence of Bel2-5 in isolated strain was also confirmed by PCR amplification. The infection processes of isolated strain in En1282 and *Rj4* soybeans are currently being investigated.

Together, these pre-screening results open the possibilities of: (1) a novel rhizobial T3E(s) triggers NF-independent nodulation in soybean; (2) the recognition of variations in Bel2-5 effector that may affect symbiosis differently; and (3) another TE3(s) may play as complementary and synergetic roles with Bel2-5 in triggering NF-independent nodulation. This finding is expected to improve our understanding on T3SS as an alternative symbiotic system to optimize the symbiotic capacity between legume-rhizobium.

Keywords : symbiosis; nodulation; rhizobia; type III secretion system; effector



### Nod-factor independent symbiotic phenomena and symbiotic inhibition factor of Bradyrhizobium and Aeschynomene genus

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Abstract

The establishment of rhizobial symbiosis with many legumes depends on Nod-factor (NF). *Aeschynomene* genus Cross-Inoculation group 3 (CI-group3) has a NF-independent symbiosis, and rhizobial Type 3 Secretion System (T3SS) and effectors (T3SEs) has an essential role. In fact, recent studies showed that some *Bradyrhizobium* genus possesses T3SS effectors that induce nodule formation (ET-Nod). However, there remain unknown T3SS-independent symbiotic pathways, such as photosynthetic *Bradyrhizobium* genus and little is known about T3SEs that cause negative effects on nodulation.

In this study, we conducted inoculation tests on some CI-group 3 using *Bradyrhizobium* sp. SUTN9-2, *Bradyrhizobium elkanii* USDA 61 and USDA 94. SUTN9-2 couldn't nodulate *Aeschynomene indica* group, but occasionally confirmed small nodules in *A. evenia* var. *evenia*. About *B. elkanii* USDA61/USDA94, both strains showed similar phenotypes in *A. indica* group. However, USDA 61 showed better phenotypes than USDA 94 in *A. evenia*. All ?T3SS mutants couldn't nodulate in CI-group3. From these points, these *Bradyrhizobium* strains try to nodulate in CI-group3 by T3SS-dependent manner. In addition, the existence of inhibition factors is suggested.

To unravel the inhibition factor, additionally conducted Transposon Tn5 mutagenesis of SUTN9-2 and inoculation tests with *A. indica* Thai ecotype. In this experiment, big white nodules were rarely observed. Isolation of SUTN9-2 mutants and identification of insertion sites have progressed. Our results suggested the presence of symbiotic inhibitors and multiple checkpoints in *Bradyrhizobium-Aeschynomene* CI-group3 symbiosis.

Keywords : Bradyrhizobium; Aeschynomene; Symbiotic phenotype; T3SS; ET-Nod



### Function of cysteinyl-tRNA synthetase of Mesorhizobium loti in root nodule symbiosis

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Abstract

Reactive molecular species such as ROS and RNS are required for root nodule symbiosis between legumes and rhizobia, while excessive accumulation of these reactive molecules inhibits the symbiosis. However, there are few studies on reactive sulfur species (RSS) in plants and its involvement in symbiosis is unknown. We have recently discovered that RSS is produced in infection threads and infected cells of the nodule and that treatment of the rhizosphere of *Lotus japonicus* with RSS-donors promotes nodulation. Based on these backgrounds, RSS is considered to be a molecule involved in root nodule symbiosis.

In the present study, we focused on cysteinyl-tRNA synthetase (CARS) and investigated its role in the root nodule symbiosis. We examined the effect of mutations in the *Cars* gene of *Mesorhizobium loti* on the symbiosis with *L. japonicus*. The *cars* mutants had lower nitrogenase activity, smaller host plant growth, and fewer RSS production compared to the WT. We also examined the effect of mutation of the *Cars* gene on metabolism in nodules. We measured the amount of 22 amino acids by LC-MS/MS analysis and found that sulfur-containing amino acids such as cysteine and methionine were all lower in the nodules of the *cars* mutants. Therefore, focusing on the involvement of CARS in sulfur metabolism, 68 sulfur compounds were analyzed by sulfur index analysis. Twenty-eight sulfur compounds were detected in the nodules. In the nodules of the *cars* mutants, 21 compounds were lower, and 3 compounds were higher compared to the WT. The sulfur compounds detected included six RSS, and four were less abundant in the *cars* mutants. Furthermore, the expression of sulfur metabolism genes in *L. japonicus* was altered in infected nodules of *cars* mutants.

These results indicate that CARS in *M. loti* functions in the production of RSS during symbiosis and contributes to successful symbiosis establishment.

**Keywords** : Reactive sulfur species (RSS); Sulfur metabolism; Cysteinyl-tRNA synthetase (CARS); Root nodule; Mesorhizobium loti



### Modulating Plant Defense Pathways: *Bradyrhizobium* sp. DOA9 Priming Induces Resistance to *Cercospora canescens*

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### Abstract

Cercospora leaf spot (CLS), caused by the fungus Cercospora canescens, is a major disease of Mung bean (Vigna radiata) that leads to significant economic losses in production areas. To reduce fungicide application, this study investigated the potential of biofertilizers to enhance plant resistance. The mung bean roots were inoculated with Bradyrhizobium sp. DOA9 before infection with C. canescens. The results showed that short priming and double priming with *Bradyrhizobium* sp. DOA9 effectively reduced the size of lesions caused by C. canescens. This suggests that priming enhances CLS resistance by stimulating plant immunity. To understand the mechanisms behind this, gene expression related to plant defense in leaves with and without CLS infection was evaluated. The heatmap gene expression analysis revealed a wide response in mung bean to DOA9 root inoculation and with CLS infection. At 1 day postinfection (dpi), up-regulation of Ptil, PR-1, and PR-2 were prominent under specific priming conditions. Ptil induction occurred under all DOA9 priming at 2 dpi, while its expression and that of the HR gene decreased in DOA9 priming with C. canescens. Additionally, suppression of the PR-5 gene was observed in certain treatments at 1 and 2 dpi, while PR-9 expression increased with DOA9 priming before C. canescens infection. These findings demonstrated that DOA9 priming might prevent fungal infection by modulating the function of transcriptional regulators responsible for PR-gene expression, influencing cell wall lignification and cross-linking deformation. These mechanisms contribute to enhance plant defense, preventing fungal infection and reducing damage from disease lesions.

Keywords : Biopriming; Bradyrhizobium; Cercospora canescens; Vigna radiata; Cercospora leaf spot



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### Abstract

To explore the potential of the root-nodulating bacterium Rhizobium sp., isolated from the leguminous plant Vigna unguiculata subsp. sesquipedalis (L.) Verde, as a biocontrol agent against certain air-borne and soilborne plant pathogenic fungi, a series of dual agar assays were conducted on PDA medium. The study focused on 19 isolates, specifically YL01 and YL03-YL20. During the primary screening, it was found that only 5 isolates exhibited complete inhibition against all fungal pathogenic assays. Subsequently, the 5 isolates were further assessed via dual agar assays. Among them, YL20 and YL17 showed the greatest antagonistic activity against S. roflsii, with inhibition of 57.31% and 54.97%, respectively. Additional isolates demonstrated varying degrees of mycelial growth suppression: YL6 suppressed Fusarium sp. by 32.29%, YL15 suppressed Colletotrichum sp., Chi-1 by 36.67%, and YL20 by 34.00%. Notably, YL7 exhibited inhibitory activity against three fungal species, namely Alternaria sp., C. gloeosporioides Ali-1, and *Phytophthora* sp., with inhibition rates of 35.48%, 27.78%, and 4.85%, respectively. Furthermore, YL6, YL7, and YL20 underwent agar well diffusion assays using supernatant, pellet, and cell culture. These isolates demonstrated considerable variations in their ability to inhibit the growth of S. rolfsii, Alternaria sp., Fusarium sp., Colletotrichum sp. Chi-1, and C. gloeosporioides Ali-1, except for R. solani and Phytophthora sp. Moreover, in controlled greenhouse settings, the inoculation of S. rolfsii and YL20 into yard-long bean seedlings was precisely detected 7 days after the inoculation. The tested isolate showed a marginal reduction in the disease severity index by 34.67%, disease incidence by 66.67% and the occurrence of disease reduction by 33.33%, compared to the controls that were not inoculated or infected. These results suggest that YL20 plays a crucial role in preventing the invasion of root pathogens and could serve as a potential biocontrol agent.

Keywords : Rhizobium sp.; Sclerotium rolfsii; antagonistic activity; biocontrol agent; leguminous plant



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#### Abstract

The research aimed to assess the efficiency of *Rhizobium* strains in enhancing the growth of Yard-Long Beans and their capacity to solubilize phosphorus and potassium from inorganic insoluble substances. The findings highlighted specific proficiencies among the isolates. Phosphorus Solubilization: Isolate YL1 demonstrated outstanding proficiency in phosphorus solubilization, registering a value of 103.20 ppm. Potassium Solubilization: Isolate YL3 exhibited the highest proficiency in potassium solubilization, recording a value of 22.55 ppm. Indole-3-Acetic Acid (IAA) Production: Isolate YL14 displayed the greatest production level of Indole-3-acetic acid (IAA) at 52.20 mg/L. Acid Resistance: The Rhizobium strains exhibited resistance to acidity at pH levels of 4.5, 4.7, 5.5, and 6.5. These findings collectively suggest the potential efficacy of Rhizobium strains in enhancing the growth of Yard-Long Beans. Their ability to solubilize essential nutrients and produce plant growth-promoting substances such as IAA indicates their promise for integration into sustainable agricultural systems. This research underscores the significance of these *Rhizobium* strains as potential contributors to sustainable agricultural practices, offering avenues for further exploration and application in agricultural settings to enhance crop productivity while maintaining environmental sustainability.

Keywords : Rhizobium; Yard-Long Beans; PGPR



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Abstract

The study aimed to assess the screening process and the potential of yard-long bean rhizobia for promoting plant growth. The experiment was conducted at Faculty of Agriculture, Chiang Mai University. From the screening process, there were a total of 19 isolates of rhizobium examined. The research involved an analysis of the structure and chemical compounds produced by rhizobium. Both acidic and basic compounds were produced as secondary metabolites. All 19 strains of rhizobium showed the ability to form nodules. Additionally, they demonstrated a greater impact on the growth and nutritional absorption of yard-long beans compared to the control group. The beans treated with these additives exhibited a dry weight of 3.8310 grams and a fresh weight of 0.3977 grams per plant. Moreover, they displayed higher levels of total phosphorus (51.54 grams per plant) and total potassium (186.10 grams per plant). The nitrogen content in each plant was determined to be 1.0844 grams, with 16 plants being analyzed. Fourteen separate samples were taken, each containing a total of 86.19 grams of calcium and 25.23 grams of magnesium per plant. Consequently, certain isolates possess a significant capacity for the cultivation of yard-long beans.

Keywords : rhizobia; yard-long bean; PGPR



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#### Abstract

The interaction between leguminous plants and *Bradyrhizobium* is limited, known as host specificity. Therefore, the selection of an appropriate Bradyrhizobia for use as biofertilizer inoculum for legumes is necessary. The Arachis hypogea L. is the most popular legume produced in the Lao People's Democratic Republic (PDR). Therefore, this research aimed to obtain the appropriate Bradyrhizobia that provides high efficiency in A. hypogea production in the Lao PDR. The 14 isolates were obtained from root nodules of A. hypogea L. trapped with Lao PDR soil samples. Three were the top isolates PMVTL-01, SMVTL-02, and BLXBL-03 showing high efficiency for peanut growth promotion. Strains PMVTL-01 and SMVTL-02 were closely related to the Bradyrhizobium geno sp. SA-3 Rp7b and B. zhanjiangense, respectively, whilst strain BLXBL-03 was closely related to Bradyrhizobium sp. CCBAU51745 and B. manausense BR3351. The competitiveness of these strains with Bradyrhizobium sp. SUTN9-2::GFP was analyzed, and only Bradyrhizobium sp. SMVTL-02 performed a number of occupied nodules higher than SUTN9-2::GFP. In addition, the competitiveness of the selected strain Bradyrhizobium sp. SMVTL-02 in a soil sample from the Lao PDR in the pot level was employed by tagging the SMVTL-02 with the DsRed gene. The results demonstrated that the DsRed expressing tagged strain showed higher nodule occupancy than indigenous strains. Moreover, the results of the acetylene reduction assay (ARA), nodule number, nodule dry weight, and total plant dry weight from the pot experiment that inoculated with the SMVTL-02::DsRed were presented as having high potential to promote peanut growth as compared to non-inoculation. Thus, Bradyrhizobium sp. SMVTL-02 could be considered a potential biofertilizer inoculum for A. hypogea production in the Lao PDR.

Keywords : Arachis hypogea L.; Biofertilizer; Bradyrhizobium sp.; Isolation; Nitrogen fixation