

Natural beneficial microorganisms for suppression of Fusarium wilt of banana and its promoting for organic production



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The outline of this presentation

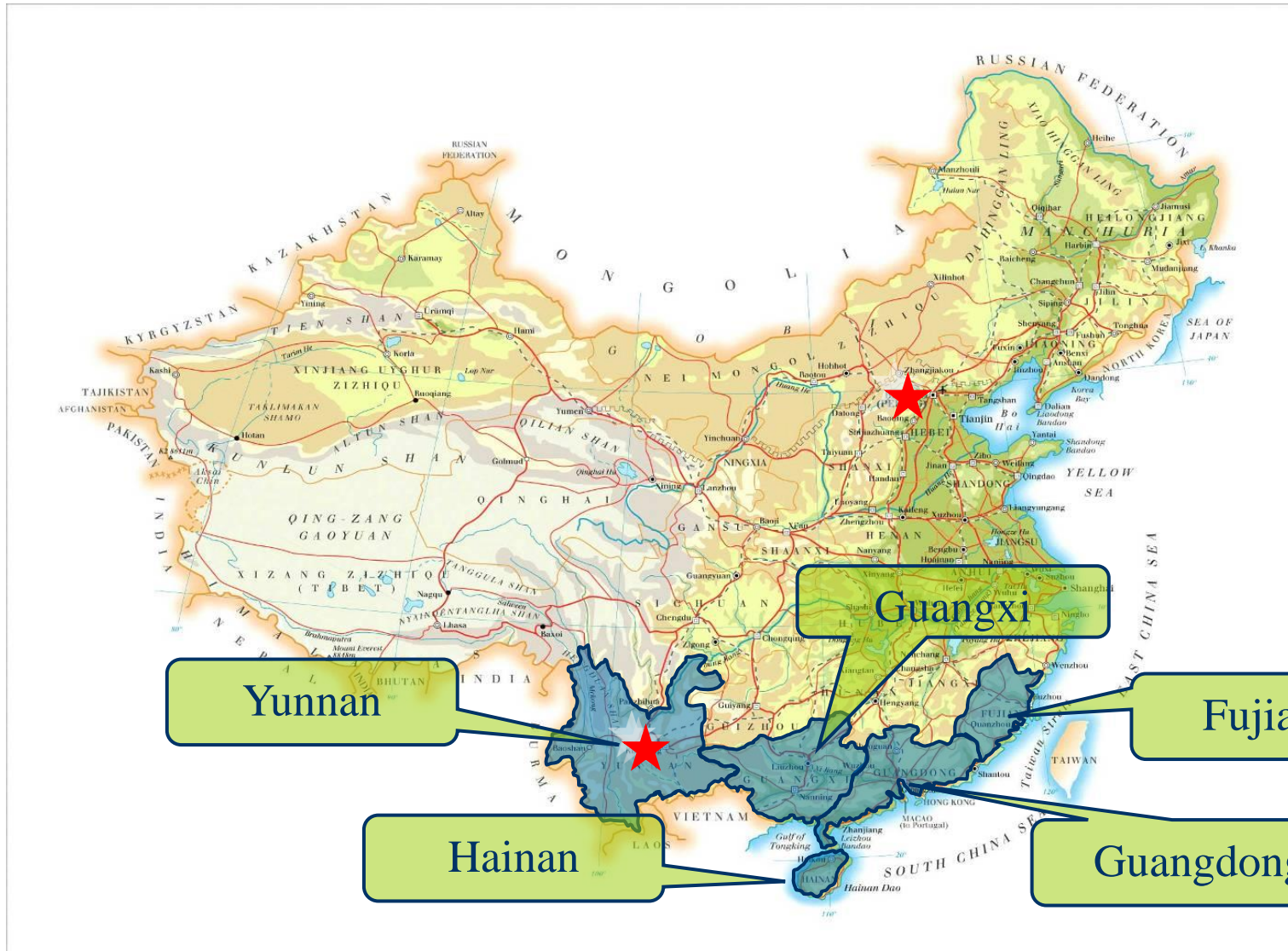
- **Current status of Fusarium Wilt of Banana (FWB)**
- **Isolation and Screening of Antagonistic Endophytic Bacteria against FWB**
- **Molecular Identification of Antagonistic Strains**
- **Biological Characteristics of Antagonistic Strains**
- **Future perspective for banana sustainable production**



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Banana production in China



Main banana production regions in mainland China

Yunnan is the biggest banana producing province



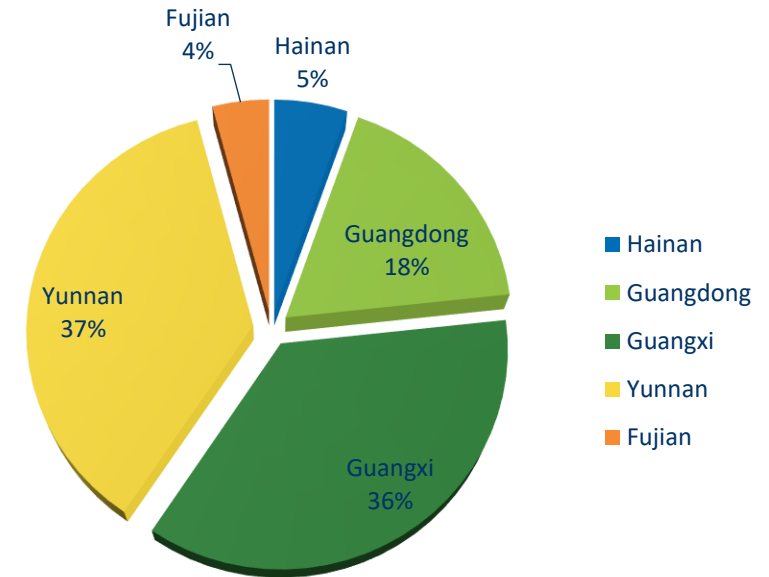
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Banana production in China

China is the second largest banana-producing country in the world, and has a long history of banana cultivation.

Banana producing areas in mainland China are mainly in Guangdong, Guangxi, Hainan, Yunnan and Fujian.



In the past years, China's banana area has been shrinking. In 2017, the planting area and output dropped to 393,000 hectares and 12.5 million tons respectively. Affecting by banana wilt and unfavorable weather, the planting area was further reduced in 2018. Now it is back to be stable.

Banana plantations significantly improve local farmer's livelihood



Miao, Hani, and Dai ethnic groups in Yunnan

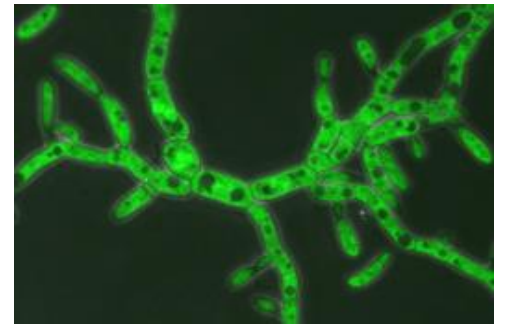
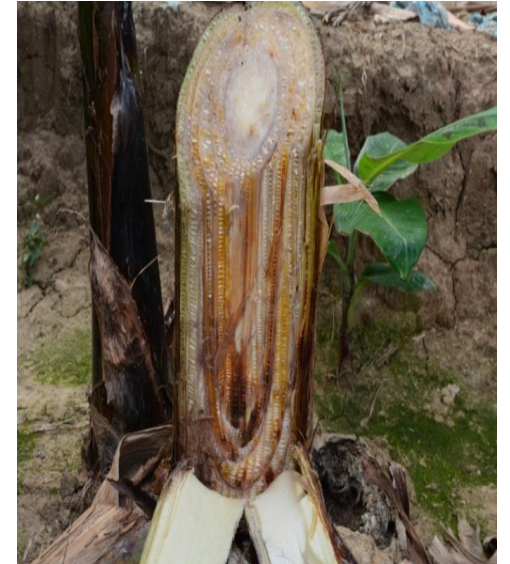
Zheng et al. (2018) FAO Proceedings of the International Symposium on Agroecology in China



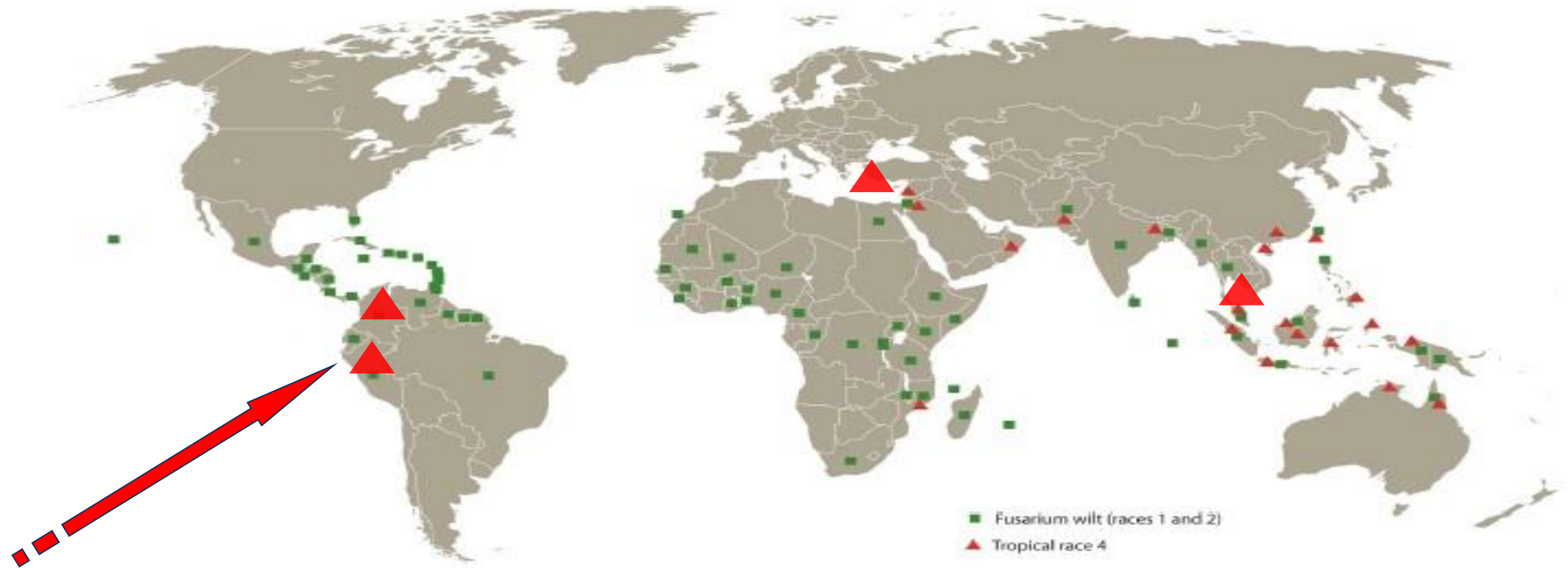
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TR4 is one of the most crucial factors which affect China banana industry and the stability of agricultural ecosystem



The distribution of Fusarium wilt of banana in globe



TR4 in Colombia, Thailand and Turkey (2019)

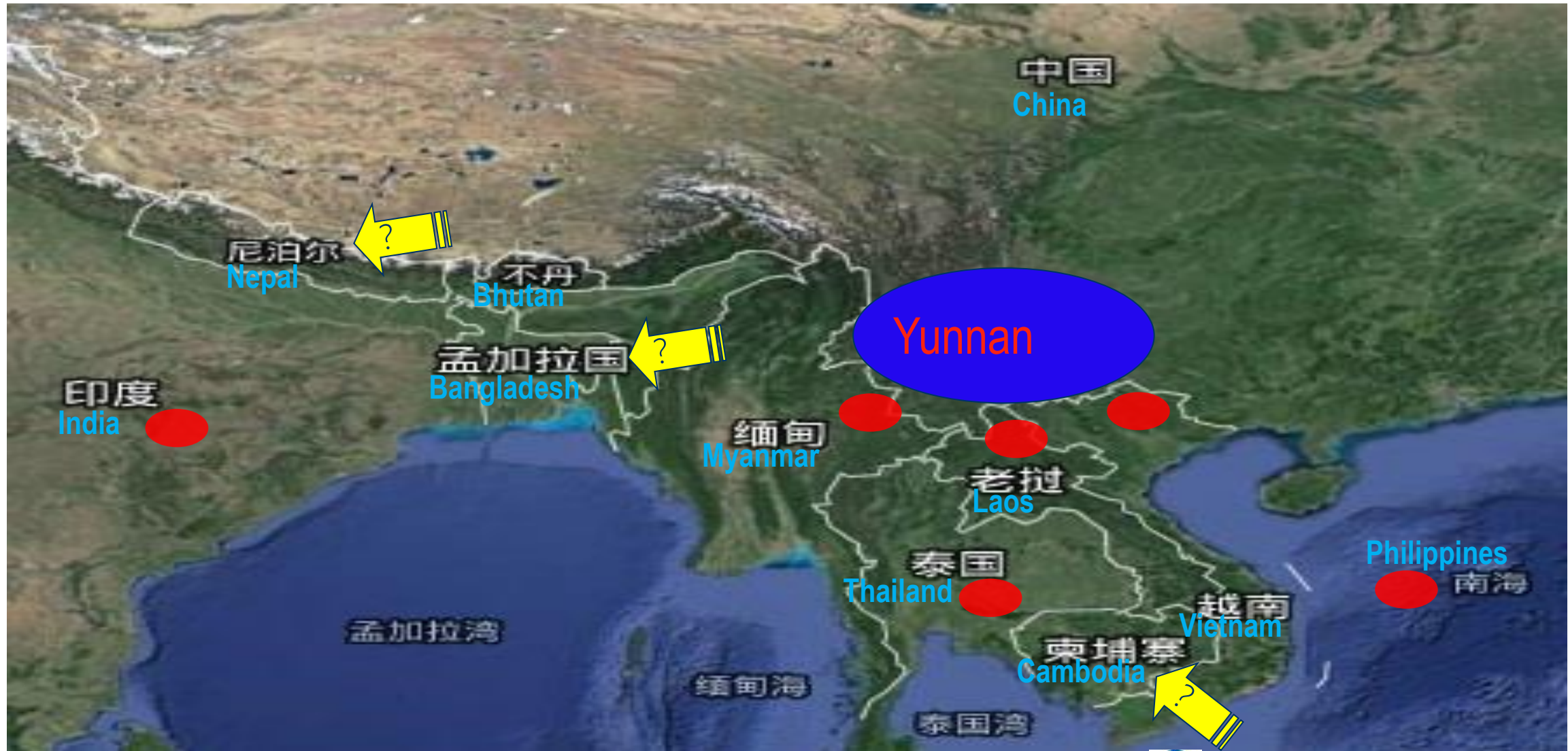
TR4 in Peru (2021)



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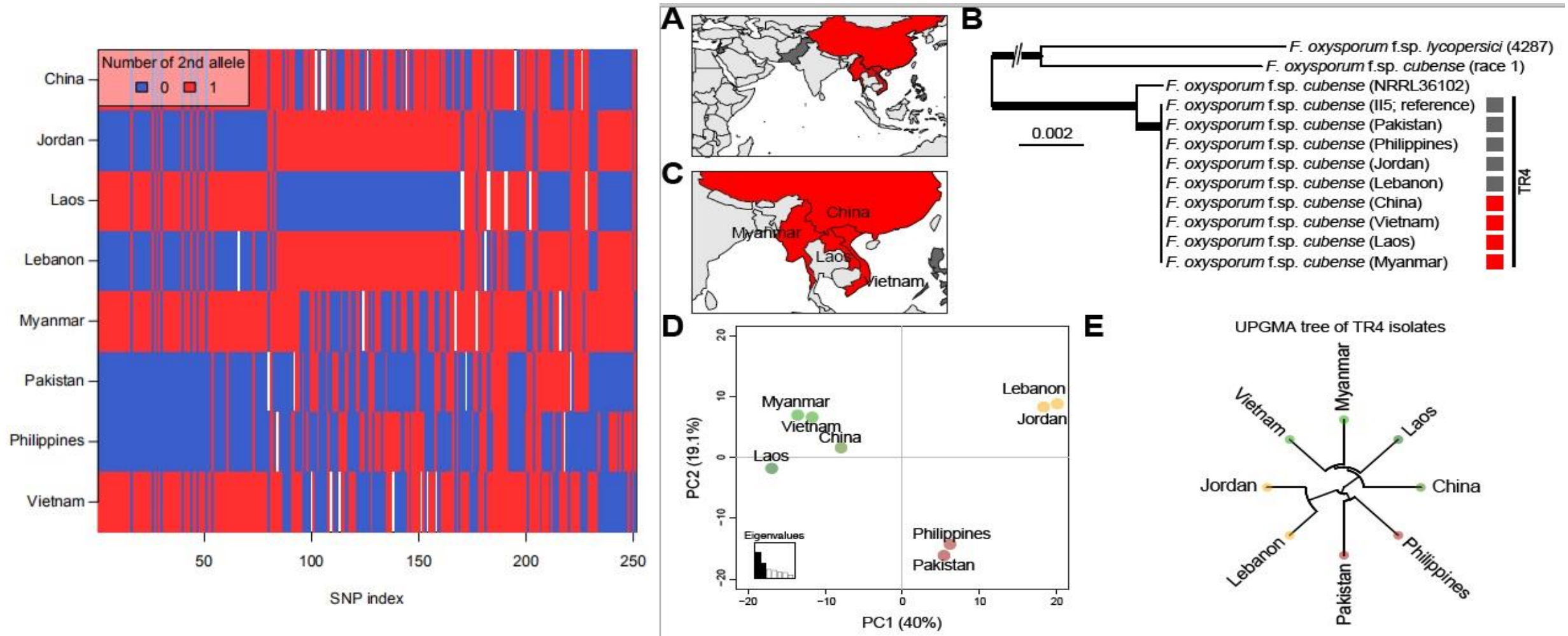
TR4 spreading in Asia



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Pathogenicity of TR4 isolates and its possible geographical expansions



Zheng et al. (2018) *Frontiers In Plant Science* (fpls.2018.00457)



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More than 300 banana hectares without Fusarium wilt disease although with initial more than 200 plants with TR4 symptom during 2008-2009 in Jiangcheng, Yunnan

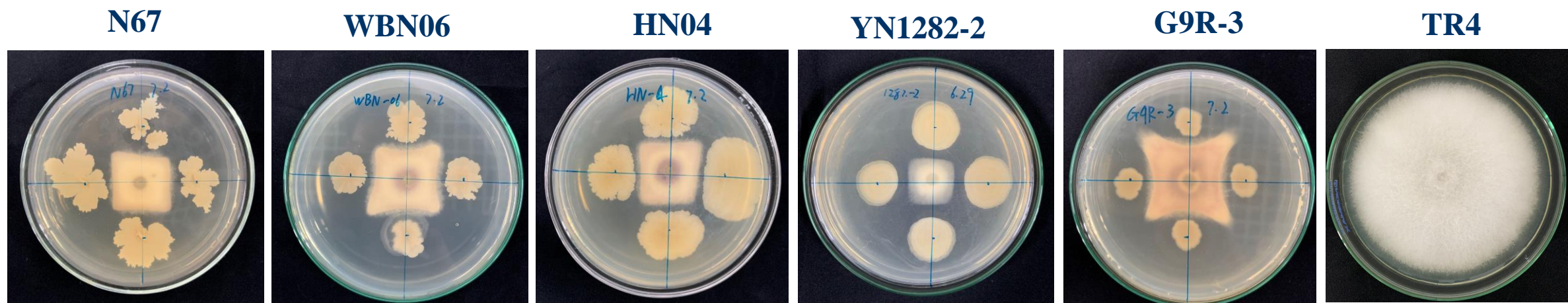
**Application of
organic fertilizer
and beneficial
microorganisms:
livestock + banana
system**



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From practice to mechanism in China: *Bacillus velezensis*, secretes a variety of antibiotics, is considered as an important group of biocontrol agents against plant disease



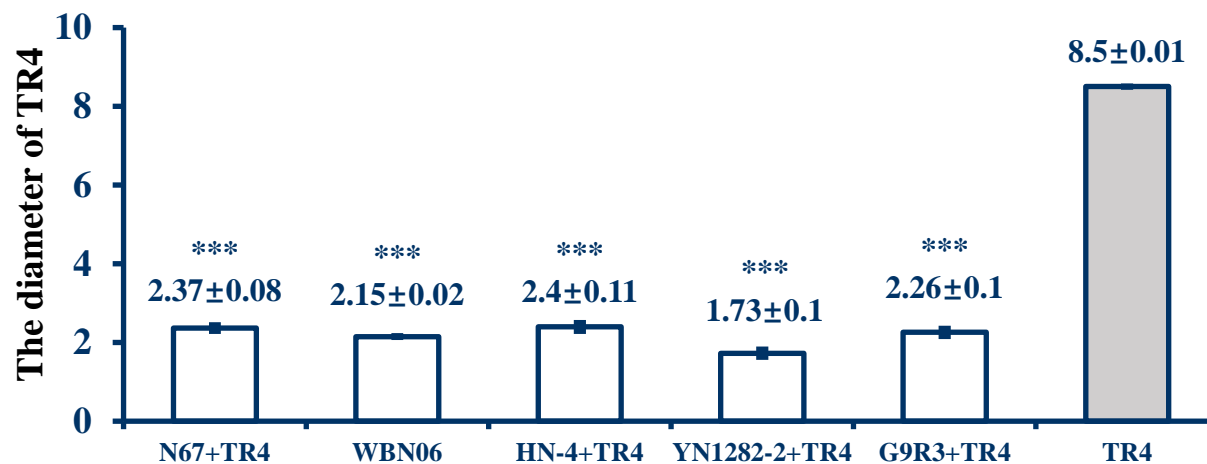
Inhibition rate: 72.12%

Inhibition rate: 74.71%

Inhibition rate: 72.53%

Inhibition rate: 79.63%

Inhibition rate: 74.11%



Article

A Real-Time Fluorescent Reverse Transcription Quantitative PCR Assay for Rapid Detection of Genetic Markers' Expression Associated with Fusarium Wilt of Banana Biocontrol Activities in *Bacillus*

Shu Li ^{1,†}, Ping He ^{1,2,†}, Huacai Fan ¹, Lina Liu ¹, Kesuo Yin ¹, Baoming Yang ¹, Yongping Li ¹, Su-Mei Huang ³, Xundong Li ¹ and Si-Jun Zheng ^{1,4,*}

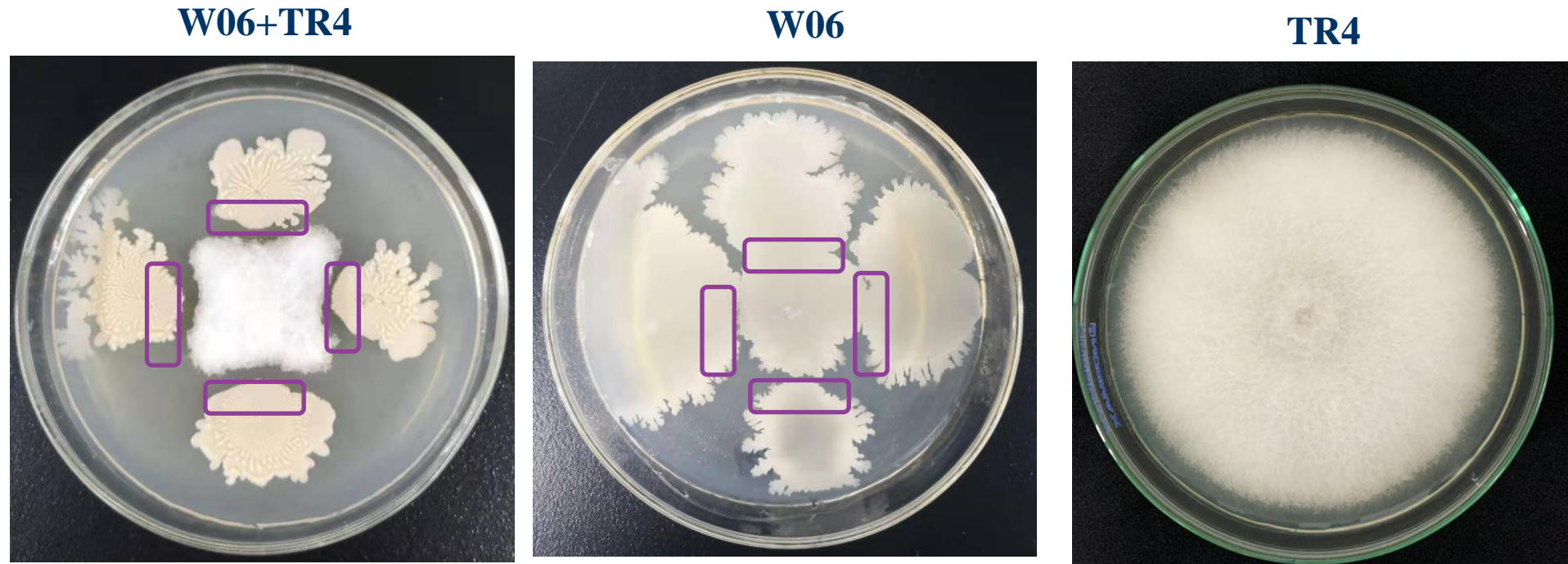
2021



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Expression of genes associated with *Foc* TR4 biocontrol activities in 5 strains of *Bacillus velezensis*.



Sampling location. 5 *Bacillus velezensis* strains and TR4 were single or dual cultivated respectively using dual-culture test, and samples were taken after 7 days when mycelia of TR4 filled the whole petri dish. sampling position were circled in green ink. single cultured of each strain use as control.

Primers design

category	metabolites	Synthesis gene	Primers (5'-3')
Non-ribosomal peptide synthetases (NRPS) lipopeptide	surfactin	<i>srfAA</i>	AGCGTAAACGGCATTTCAGGAG
			TATGAGACGGCAGTGTTTCGG
	fengycin	<i>fenD</i>	CATTTTAACCAGTCCGTCATGC
			TCTTTTTTGCAGACAAGGCGC
	iturin	<i>ituC</i>	AACGAATACGGGCCTACAGAG
			CTTCATGCTCTTATCCAGCACG
	bacillomycin e D	<i>bamD</i>	ATTGGCGAAACGAAACATCTGC
			AACATCTGATTGTGCTCACGTTC
	YNGG	<i>yngG</i>	CAGAGCGACAGCAATCACATC
			ATTGCTCGGCAGGATCATACG
Polyketide synthetases (PKS)	macrolactin	<i>mln</i>	CTGATGAACTGATAACAACCGAG
			ACGTGCCGAAACAACGATTGG
	bacillaene	<i>bae</i>	TGT GCG GTC GTG TAT GAA CAG
			AAC GGT CTG TAT AAA TGC CGA TG
	difficidin	<i>dfn</i>	TAT CTC AAT CGG ATC GCC GAG
			ATA CGG TGC CTA ATC CGG AAG
	bacilysin	<i>bac</i>	TGAAGGGACAAGTAGTGAGTAC
			AGGCACAATTGTGTATTCCAGC
siderophore	bacillibactin	<i>dhbA</i>	CAGTGAAATCGAGCCGATCC
			TCTGAAACGGCTTTACAGCATG
vitamins	biotin	<i>bioA</i>	GTCGCCGAAAAATCAAAAACGG
			ACAAGCTCTATGCCGCACATG

Housekeeping gene

RopB-F	AGTATCCCGTTGAAGAGTCAA AAGA
RopB-R	CAAGCTGAGATACGATAACAC GTTC

Kwan *et al.* 2004

Target genes and primers used in this study.

The target primers design followed by requirements imposed by qPCR using [Primer Express \(version 3.0; Applied Biosystems, Foster City, CA\)](#). All primers had a single peak for dissolution curve and the slope of all standard curve are greater than 0.99.

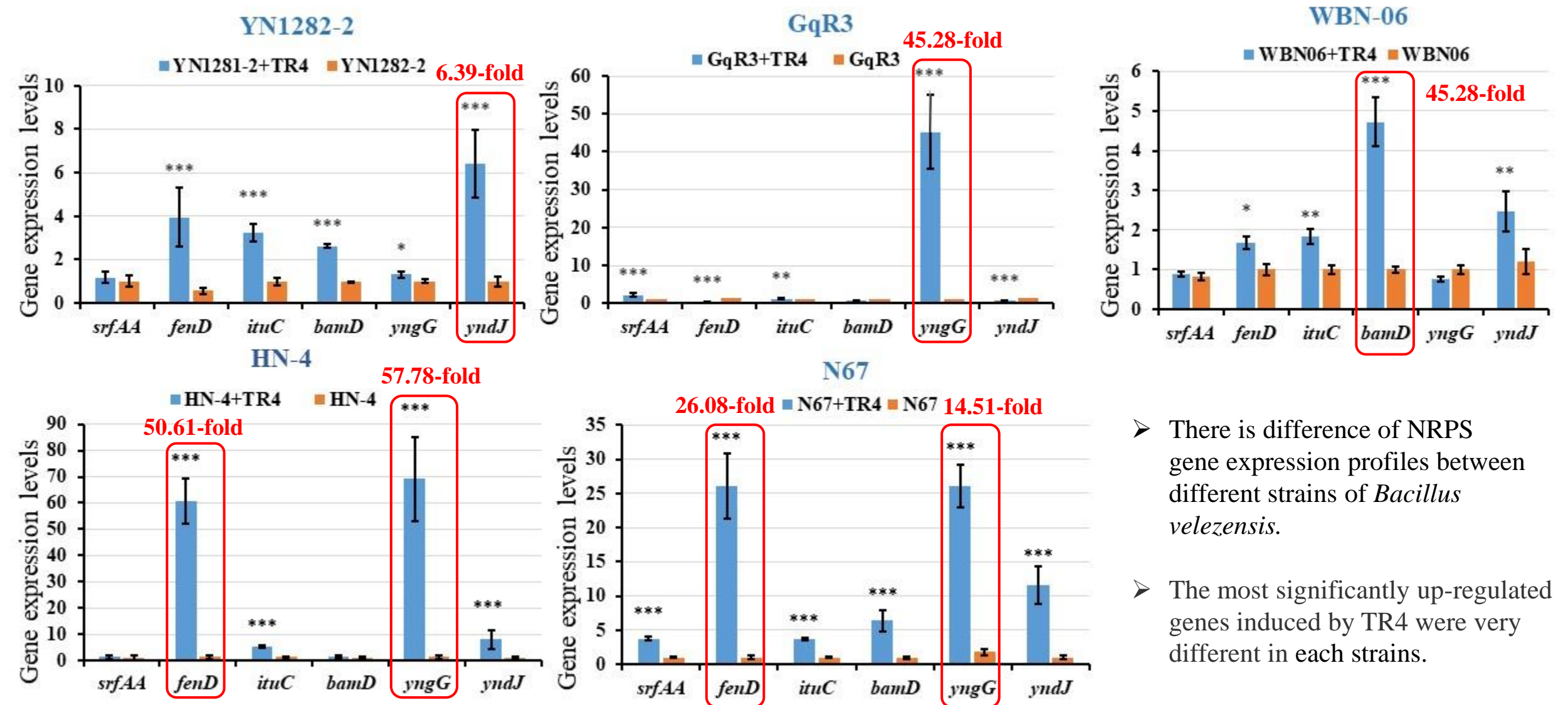
At least six samples were used for each treatment, and each reaction was run in triplicate.



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Biocontrol NRPS gene expression profiles of 5 *Bacillus velezensis* strains



- There is difference of NRPS gene expression profiles between different strains of *Bacillus velezensis*.
- The most significantly up-regulated genes induced by TR4 were very different in each strains.

Isolation and Screening of Antagonistic Endophytic Bacteria against *Foc* TR4 from Yunnan

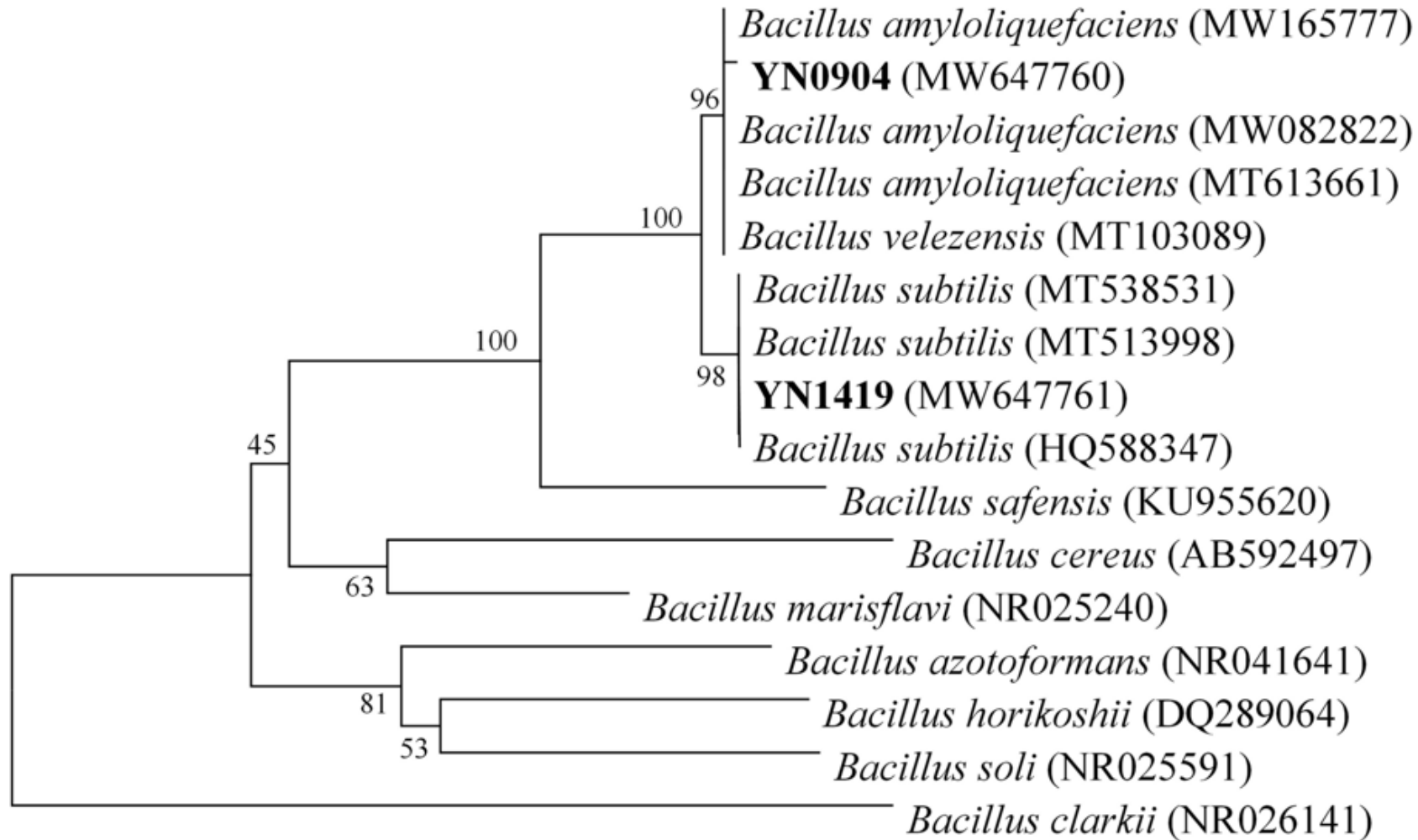
Collection location	Collection time	Sample type	Samples	Strains	Strains primary screening	Strains secondary screening
Xishuangbanna	2009.07-2014.07	Symptomless pseudostem samples already infected with FWB and bacterial soft rot	36	197	6	1(YN0904); 1(YN1419)
	2010.07-2011.07	Healthy banana plants pseudostem	11	30	0	0
Honghe	2011.07-2013.07	Symptomless pseudostem samples already infected with FWB and bacterial soft rot	14	44	0	0
Yuxi	2013.07-2016.07	Symptomless pseudostem samples already infected with FWB and bacterial soft rot	18	67	1	0
Wenshan	2014.09	Symptomless pseudostem samples already infected with FWB and bacterial soft rot	1	2	0	0
Baoshan	2014.07-2017.07	Symptomless pseudostem samples already infected with FWB and bacterial soft rot	11	20	2	0
Dehong	2016.01	Symptomless pseudostem samples already infected with FWB and bacterial soft rot	5	22	0	0
Total			96	382	9	2



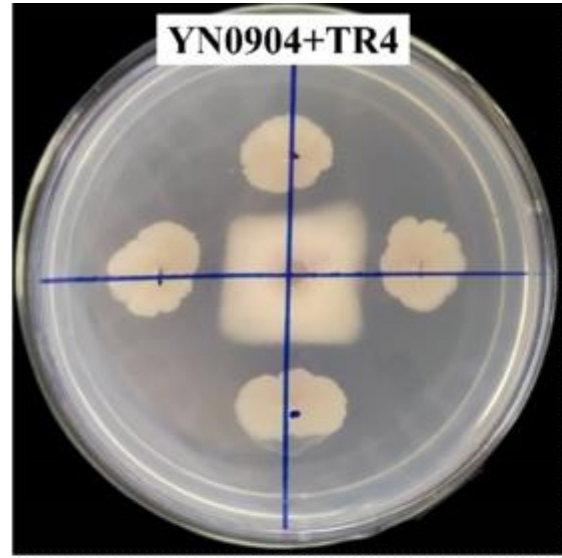
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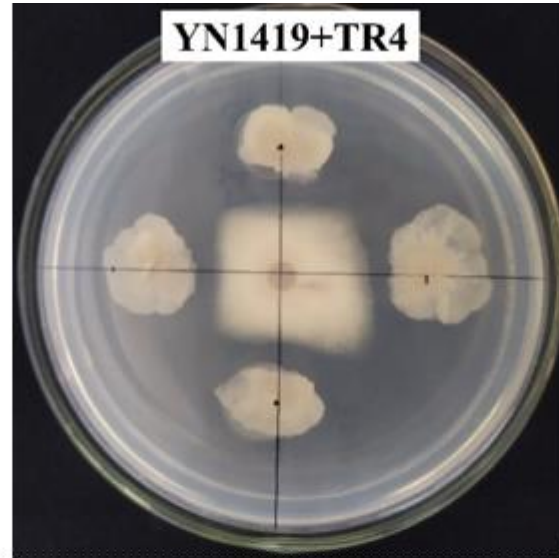
Phylogenetic tree based on 16S rRNA gene sequences of the antagonistic strain YN0904 and YN1419



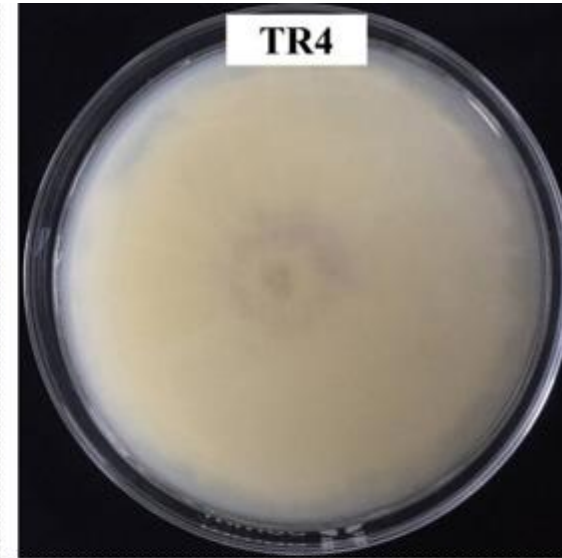
The antagonistic effect of strains YN0904 and YN1419 on TR4



(a)↵



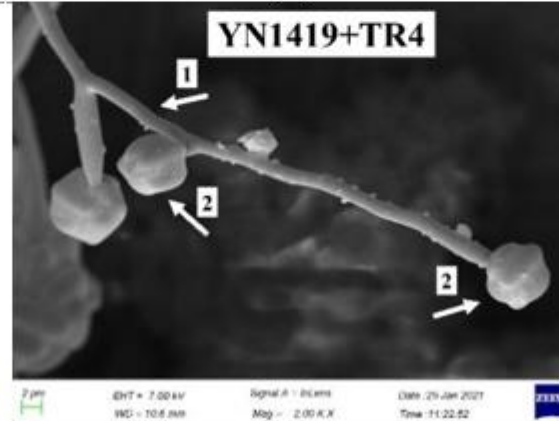
(b)↵



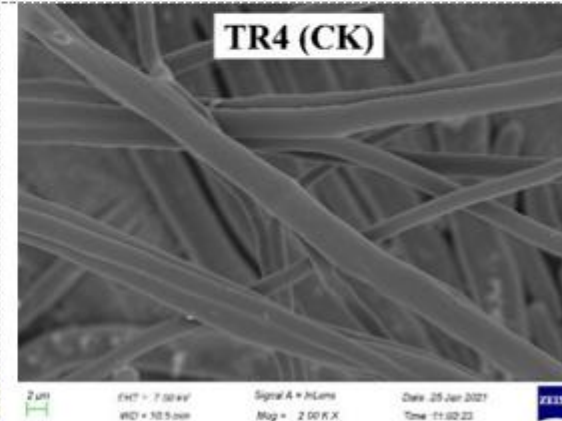
(c)↵



(d)↵



(e)↵



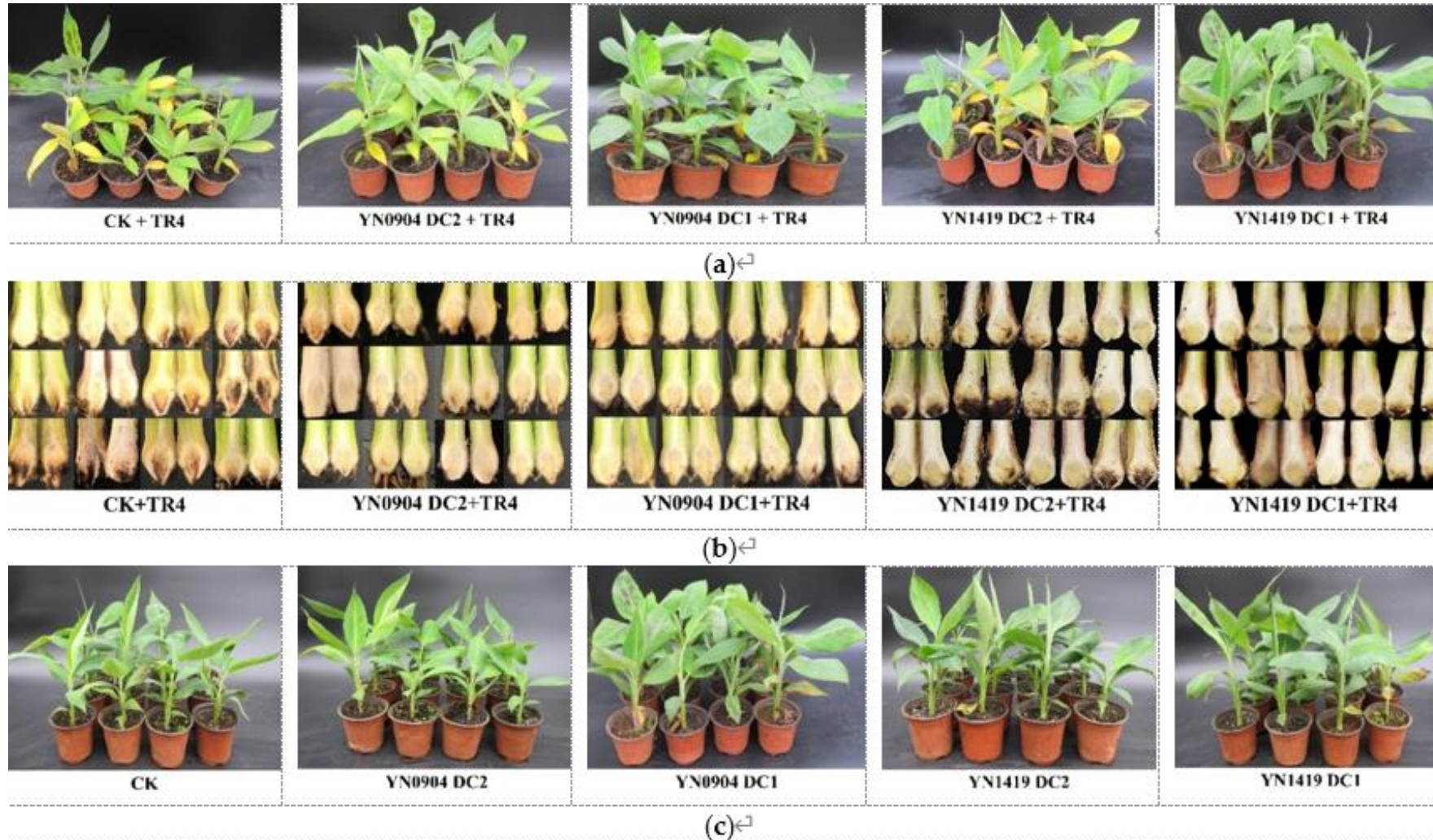
(f)↵



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Biocontrol effects and growth-promoting of antagonistic strains YN0904 and YN1419



Biocontrol effects of the antagonistic strains YN0904 and YN1419 on the TR4 in pot experiment

Treatment	Disease Index		Control Effect (%)	
	Corm	Leaf	Corm	Leaf
YN0904 DC1 + TR4	14.58 ± 2.08 c	12.50 ± 3.61 c	74.26 ± 2.27 a	82.58 ± 4.61 a
YN0904 DC2 + TR4	18.75 ± 3.61 c	20.83 ± 2.08 bc	67.22 ± 4.33 a	70.71 ± 2.02 ab
YN1419 DC1 + TR4	16.67 ± 2.08 c	10.42 ± 4.17 c	70.09 ± 4.41 a	85.61 ± 5.30 a
YN1419 DC2 + TR4	37.50 ± 6.25 b	31.25 ± 6.25 b	32.87 ± 11.46 b	56.30 ± 7.34 b
CK + TR4	56.25 ± 3.61 a	70.83 ± 2.08 a		

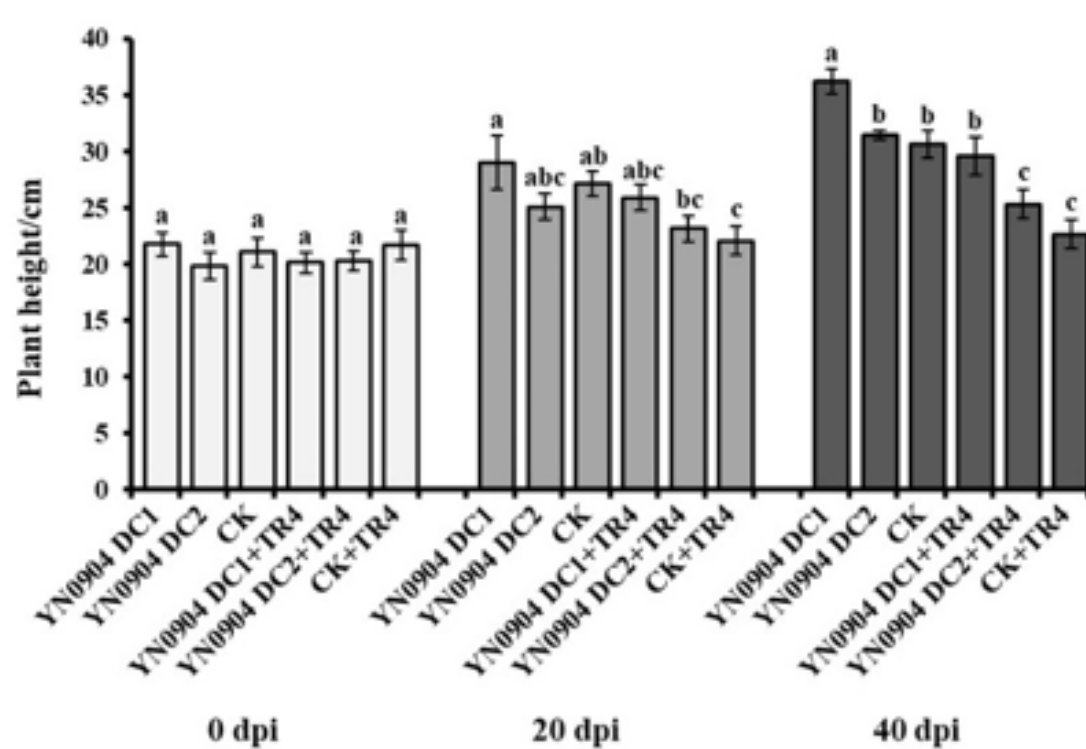
Data are presented as means ± standard error. Data with different lowercase letters indicate a significant difference at the 0.05 level.



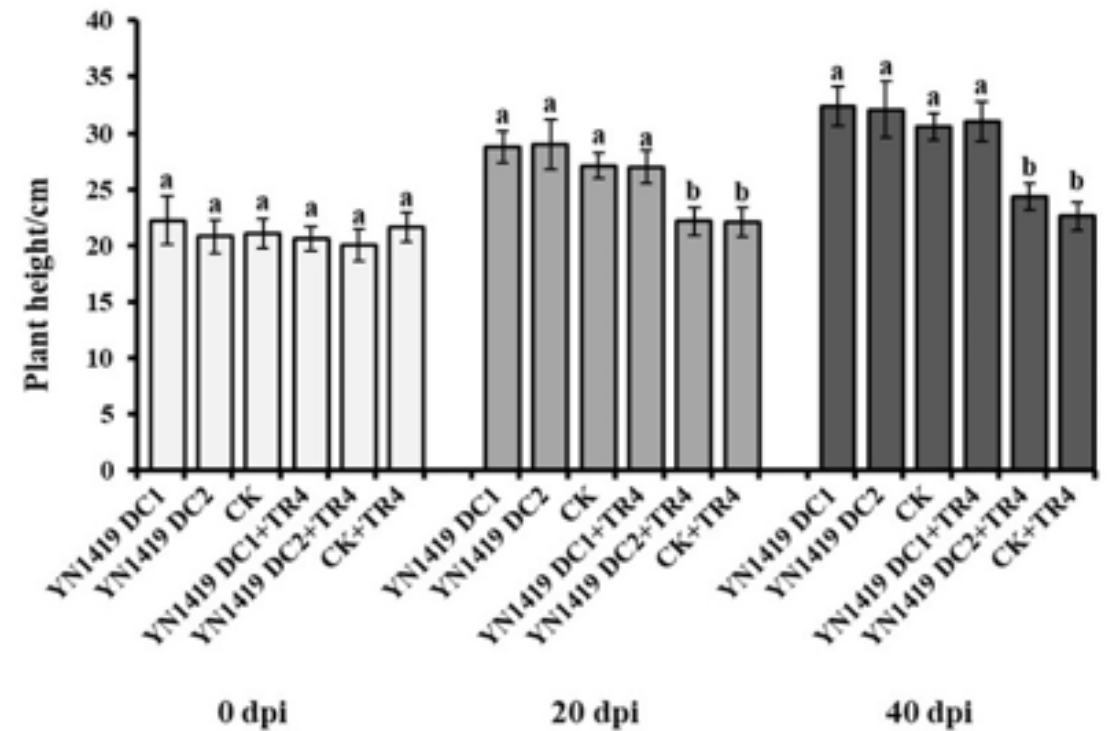
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Growth-promoting effects of antagonistic strains YN0904 and YN1419 on banana plant height

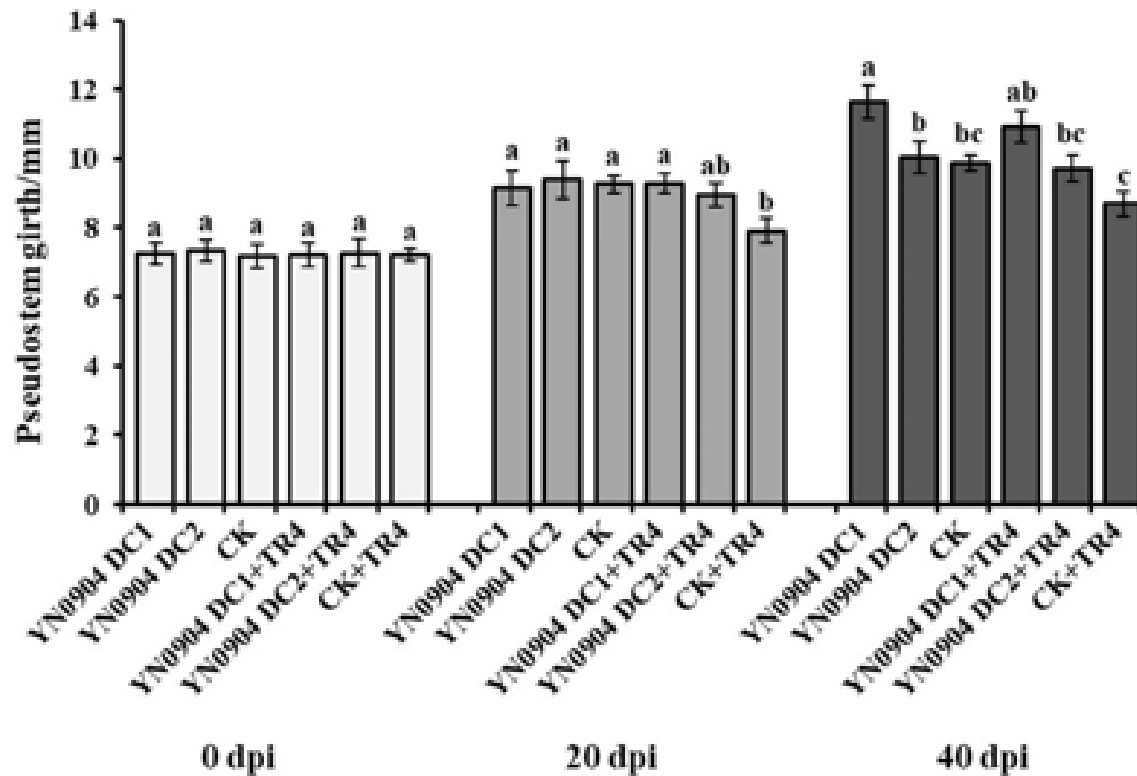


(a)

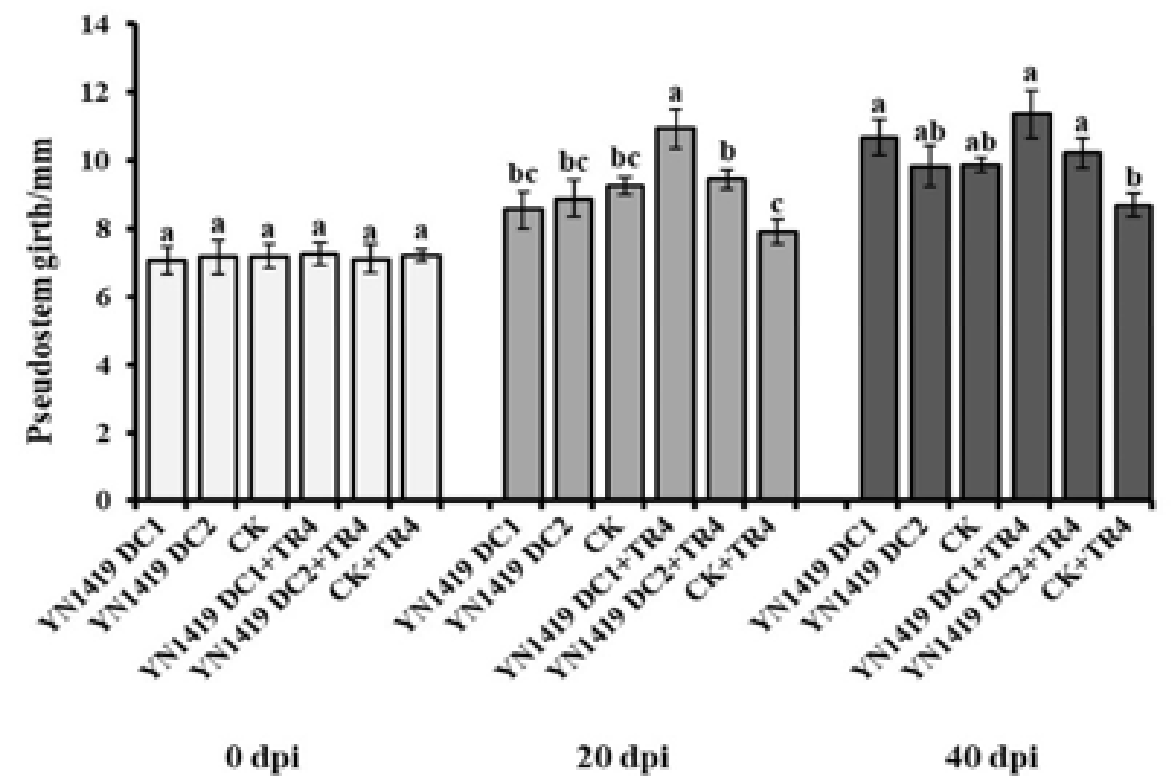


(b)

Growth-promoting effects of antagonistic strains YN0904 and YN1419 on pseudostem girth



(a)



(b)

Future perspective for banana sustainable production

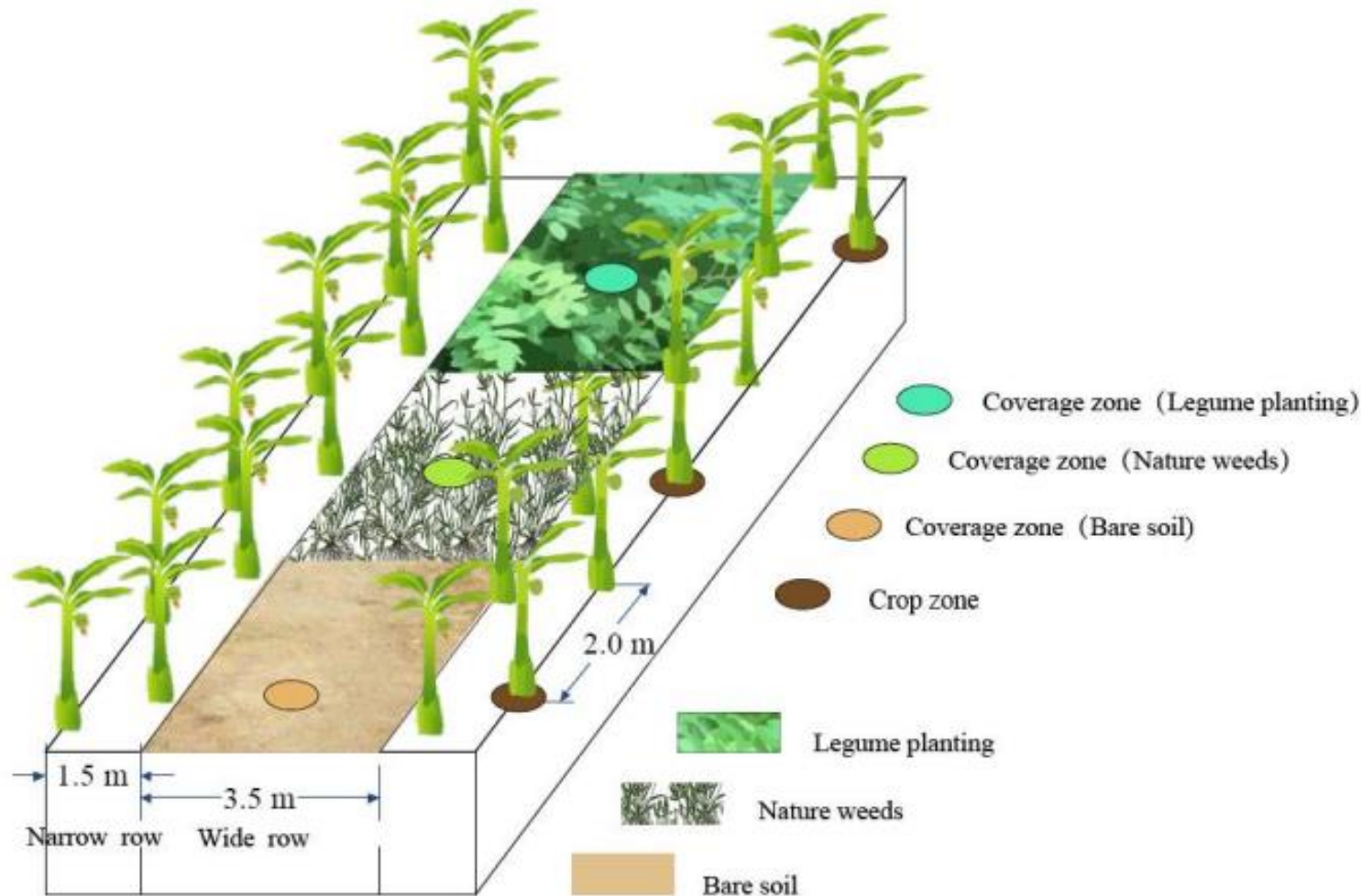
- Re-inoculating novel endophytes into plantlets against deadly Fusarium wilt in field condition
- Novel planting and intercropping system to combat TR4 as agroecological intensification for sustainable production in the presence of TR4



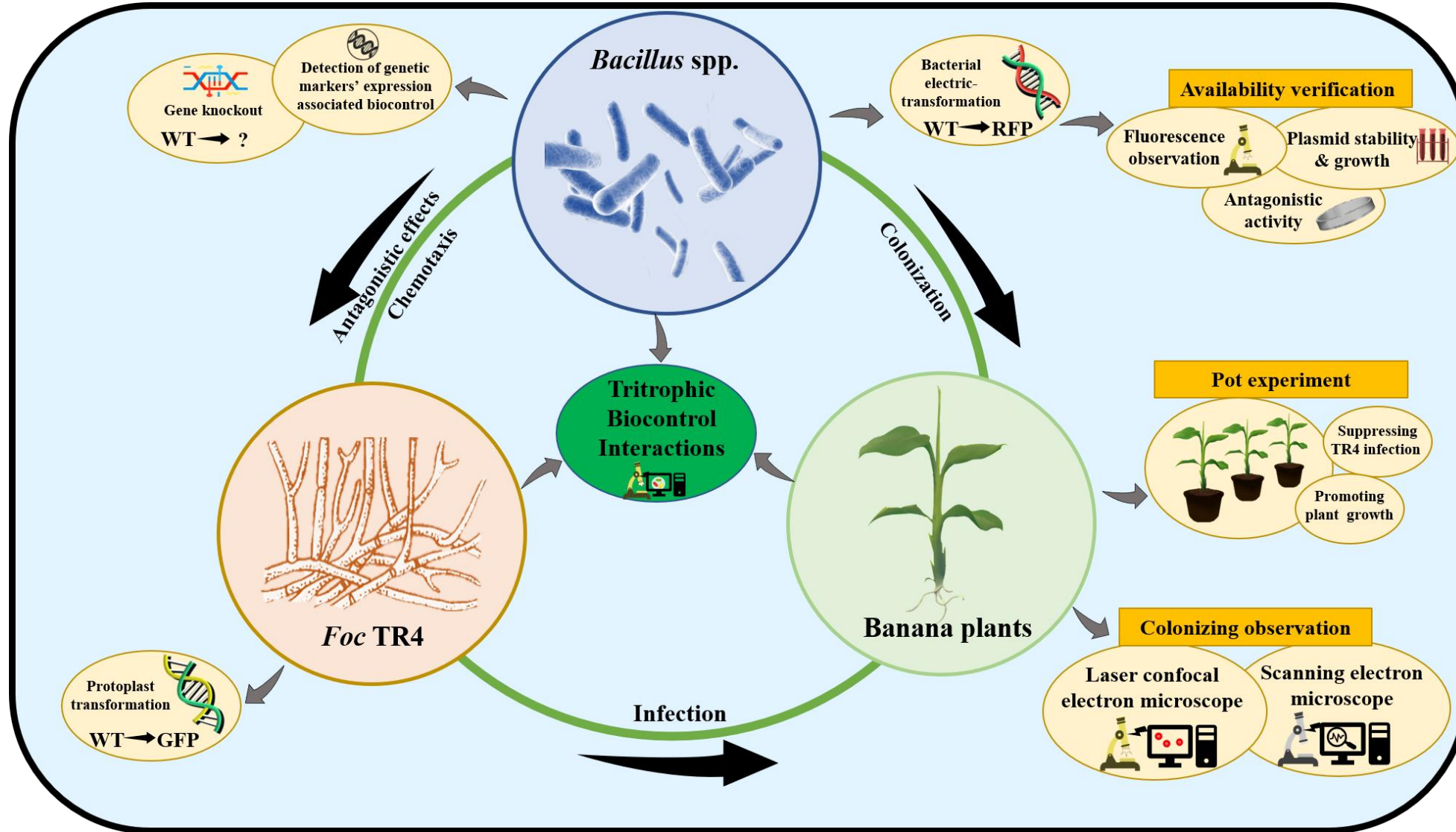
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Novel planting and intercropping system with inoculated plantlets with beneficial micorganisms to combat TR4 as agroecological intensification for sustainable production in the presence of TR4

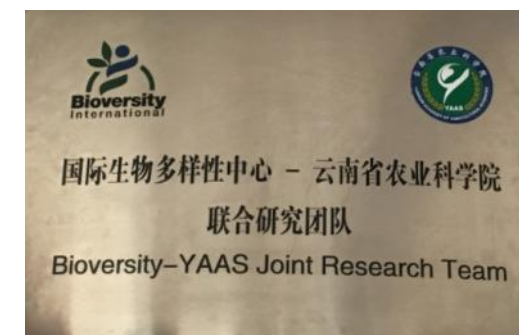


Tritrophic interactions of biocontrol agent-pathogen-plants



- Further screening and identifying natural variations of various types of beneficial microbes
- Functional microbes application in the field

Alliance of Bioversity International and CIAT and YAAS research team



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Thank you!



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