監測和控制樹木褐根病：
香港的經驗和貢獻
Monitoring and control of BRRD:
Hong Kong experience and contribution

關海山

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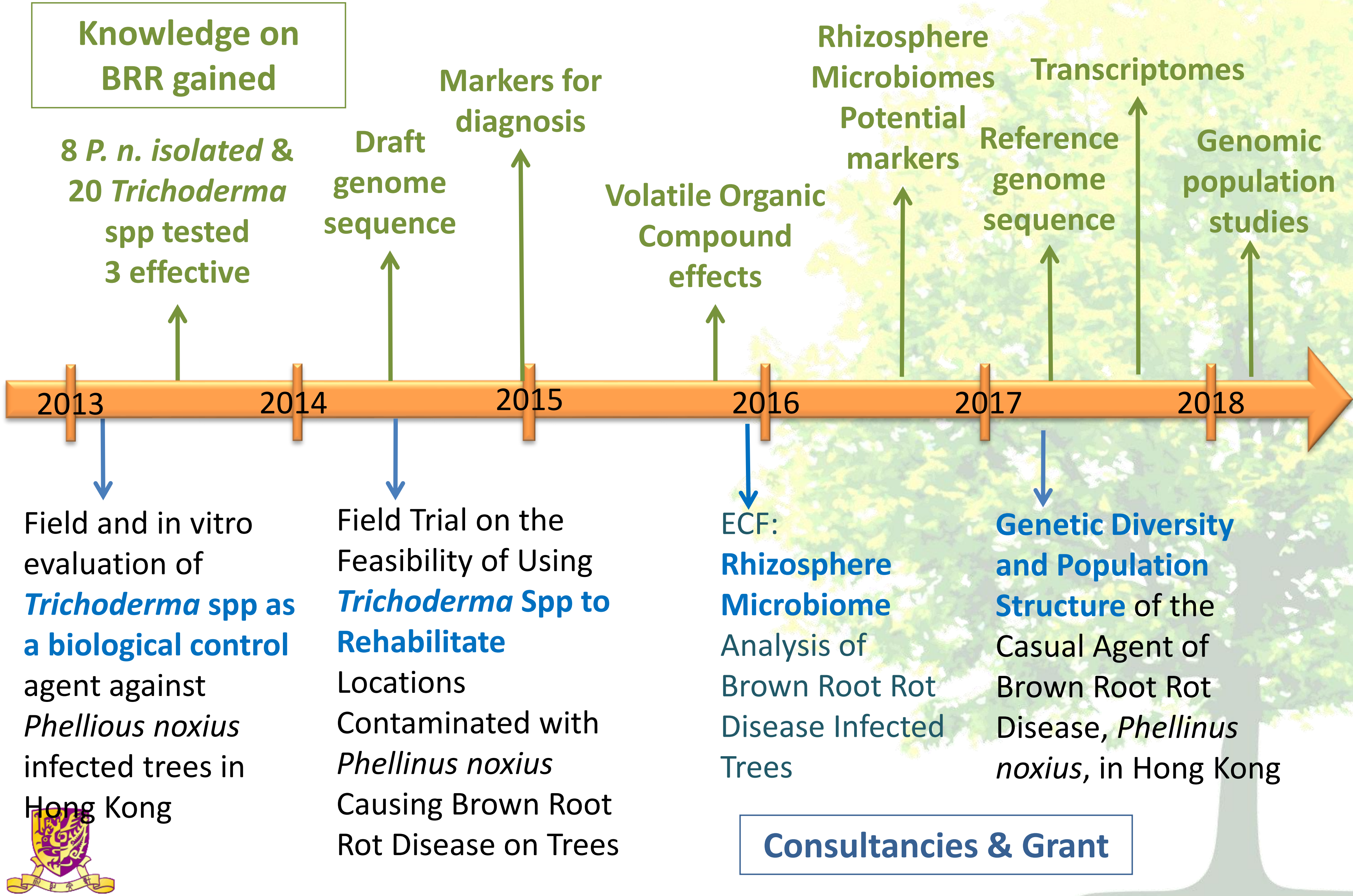
HSK GeneTech Limited

& Mushroom-X Limited

Research & Development in KwanLab on BRR *P. noxius* in Hong Kong
Supported by HKSAR Government contracts & grant



Knowledge on BRR gained

8 *P. n. isolated* & 20 *Trichoderma* spp tested 3 effective

Draft genome sequence

Markers for diagnosis

Volatile Organic Compound effects

Rhizosphere Microbiomes Potential markers

Reference genome sequence

Transcriptomes

Genomic population studies

Field and in vitro evaluation of ***Trichoderma* spp as a biological control** agent against *Phellious noxius* infected trees in Hong Kong

Field Trial on the Feasibility of Using ***Trichoderma* Spp to Rehabilitate** Locations Contaminated with *Phellinus noxius* Causing Brown Root Rot Disease on Trees

ECF: **Rhizosphere Microbiome** Analysis of Brown Root Rot Disease Infected Trees

Genetic Diversity and Population Structure of the Casual Agent of Brown Root Rot Disease, *Phellinus noxius*, in Hong Kong

Consultancies & Grant

Trichoderma Species as a Biological Control Agent against *Phellinus noxius*

Biological Control

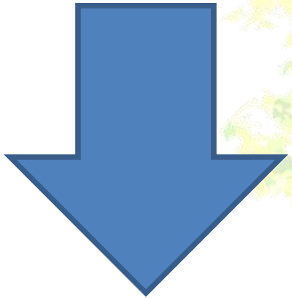
- Involves the use of one or more organisms to control a target pest or disease organism
- Microorganisms as biological control agent to rehabilitate locations contaminated with biological contaminants

Advantages of biological control

- High specificity
- Minimal or no negative impact on people and environment (including immediate, long-term)
- Suppression of plant disease (including development & spread)

What’s more….

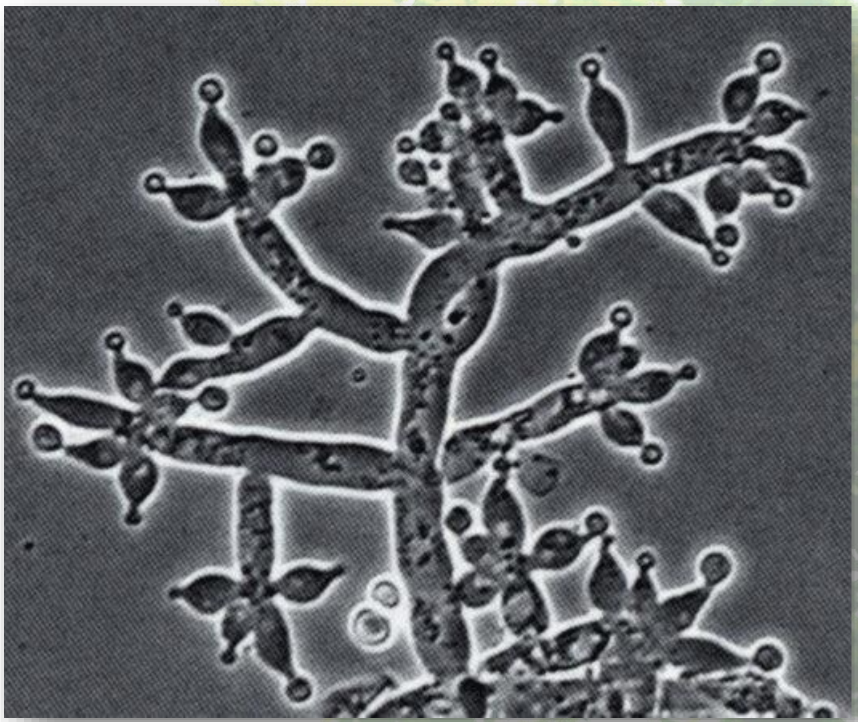
- Enhancement of soil microbial population
  - Develop disease-suppressive soil

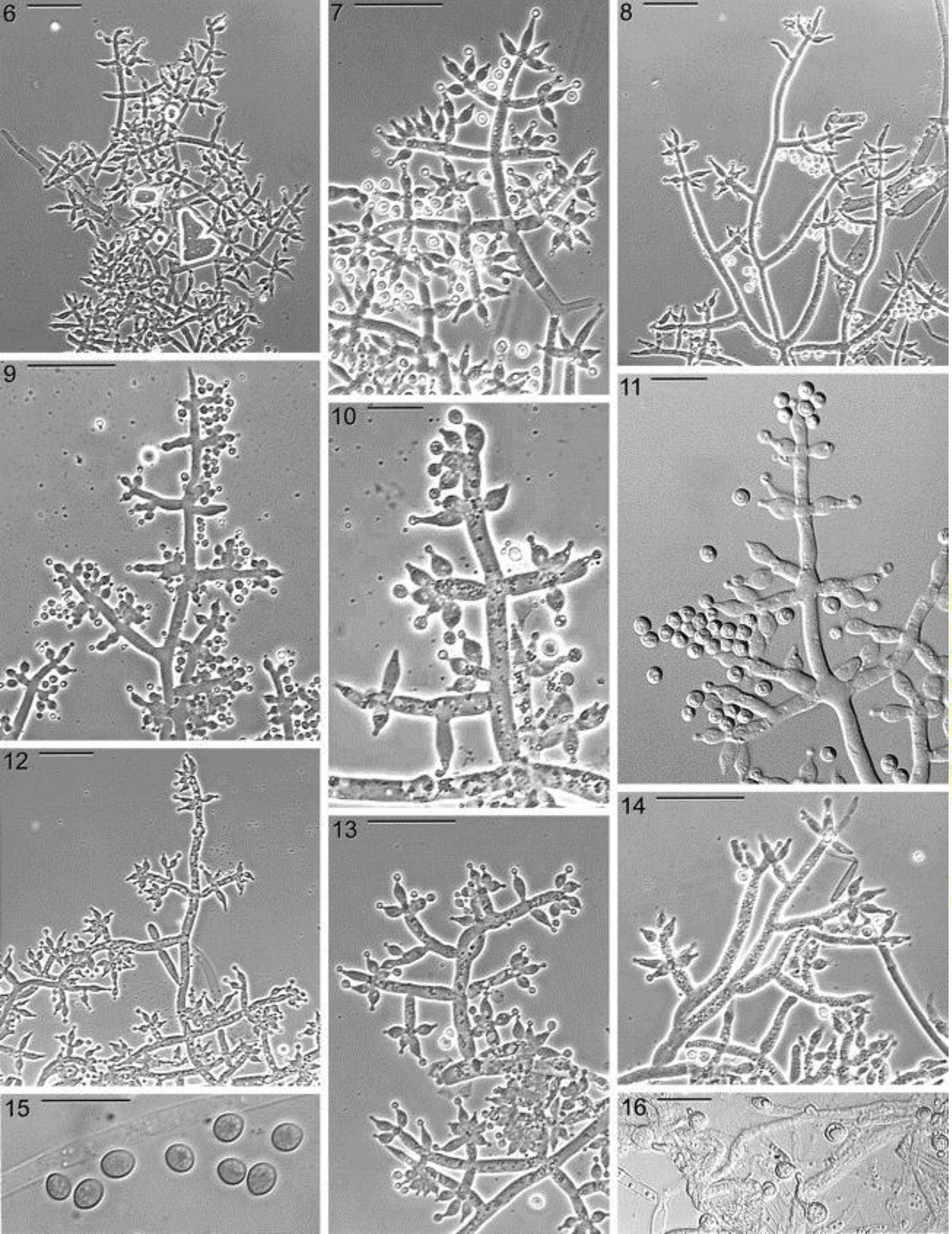


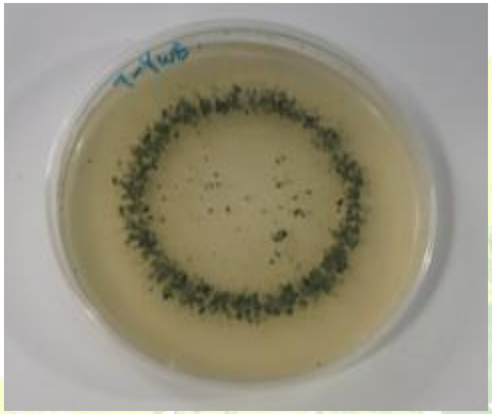
Enhancement of Soil Health

What is *Trichoderma*?

- One of the most frequently isolated **soil fungi** that occur on or in the vicinity of roots of plants
- Has been known as biological control agent **since 1930s**
- **Beneficial in agriculture system** has long been demonstrated

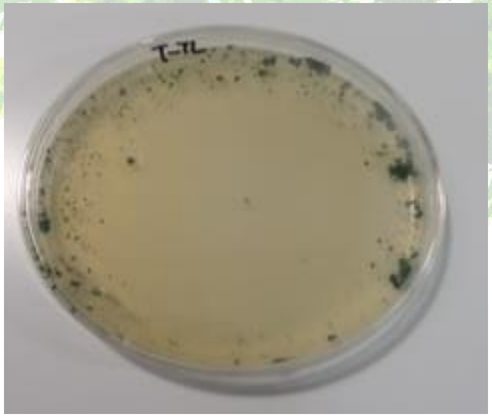






T-YWE

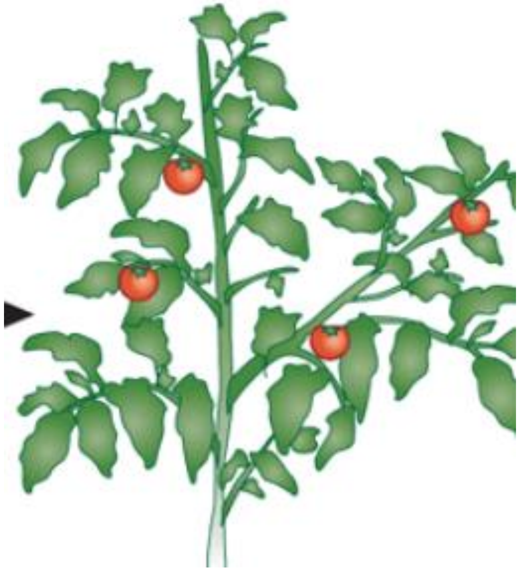
T-TMS



T-TL

Potential mechanisms of *Trichoderma*

Fight against Pathogen



Enhance Host Resistance

Potential mechanisms of *Trichoderma*

- Fight against Pathogen
- Mycoparasitism
- Antibiosis
- Competition for nutrients or space
- Inactivation of pathogen enzyme

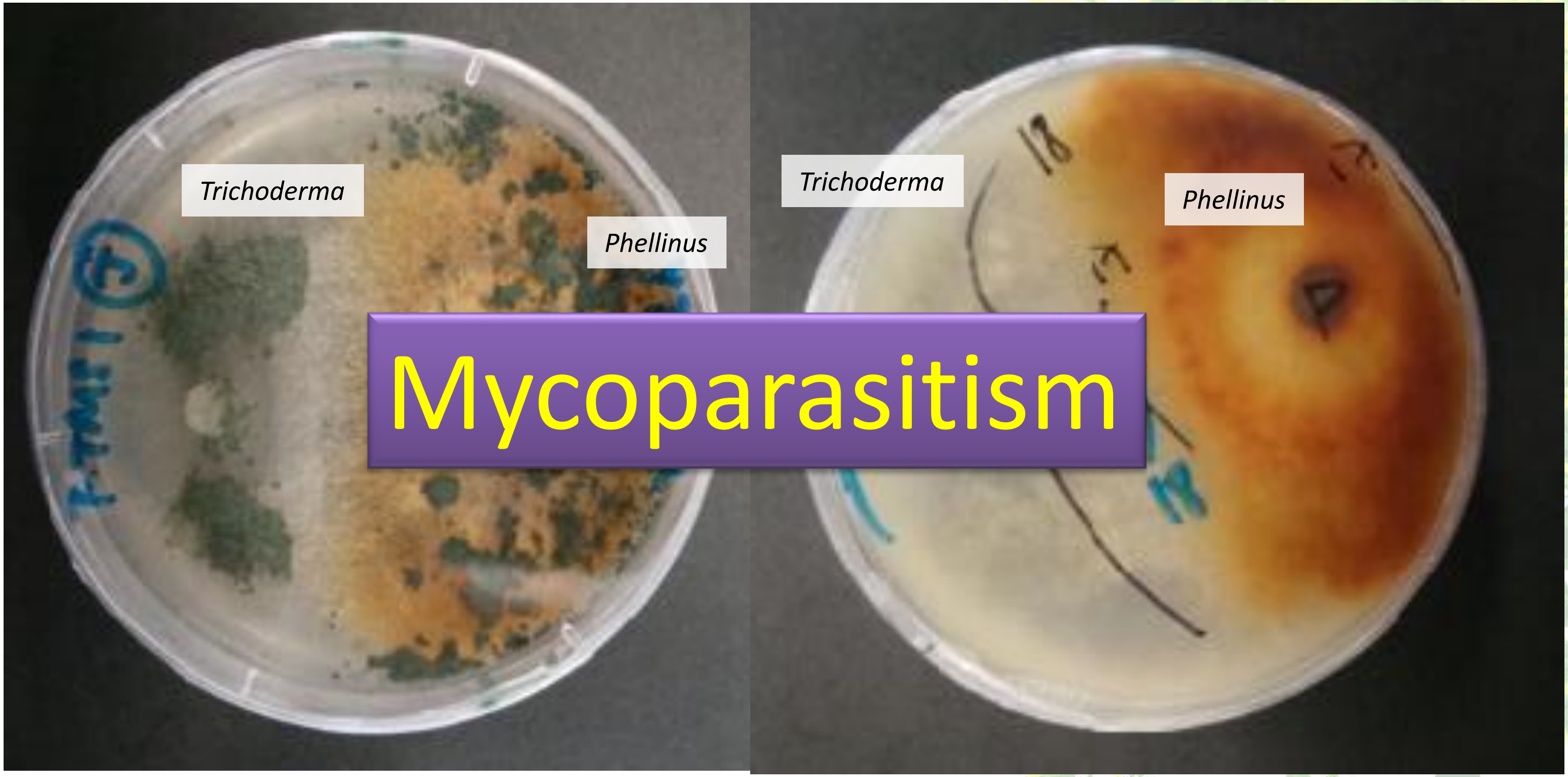
- Enhance Host Resistance
- Localized or systemic induced resistance
- Enhanced uptake and use of nutrients

- Three *Tricholderma species* tested *in vitro*
  - T. gamsii T-TL
  - T. harzianum T-YWE
  - T. harzianum T-TMS1

Strong mycoparasitism capability

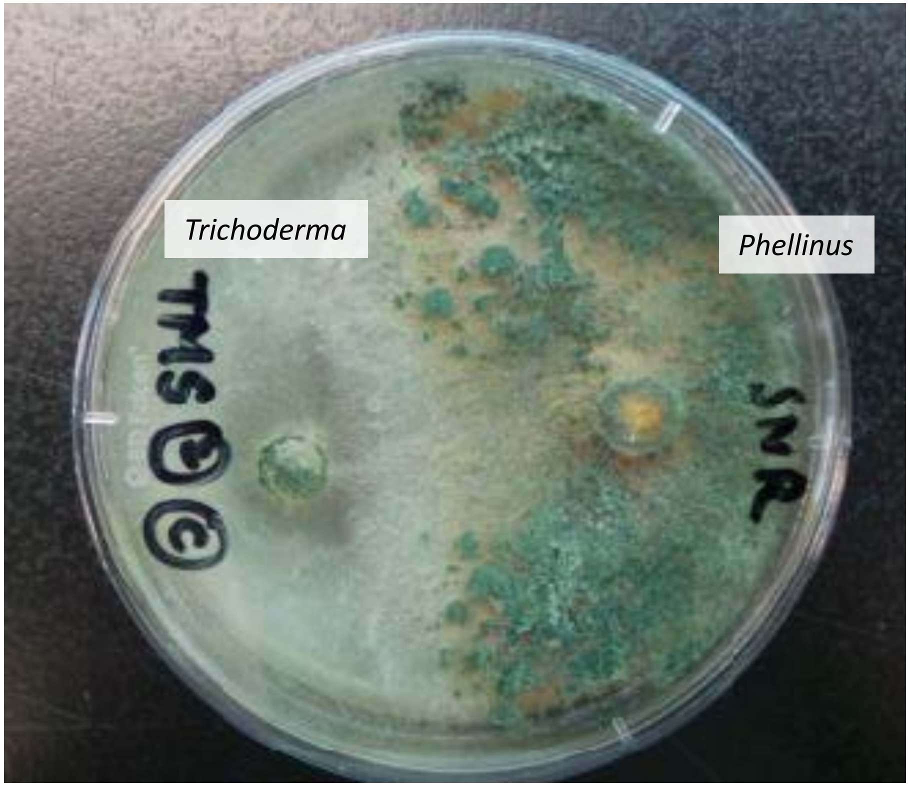
How to fight against pathogen?

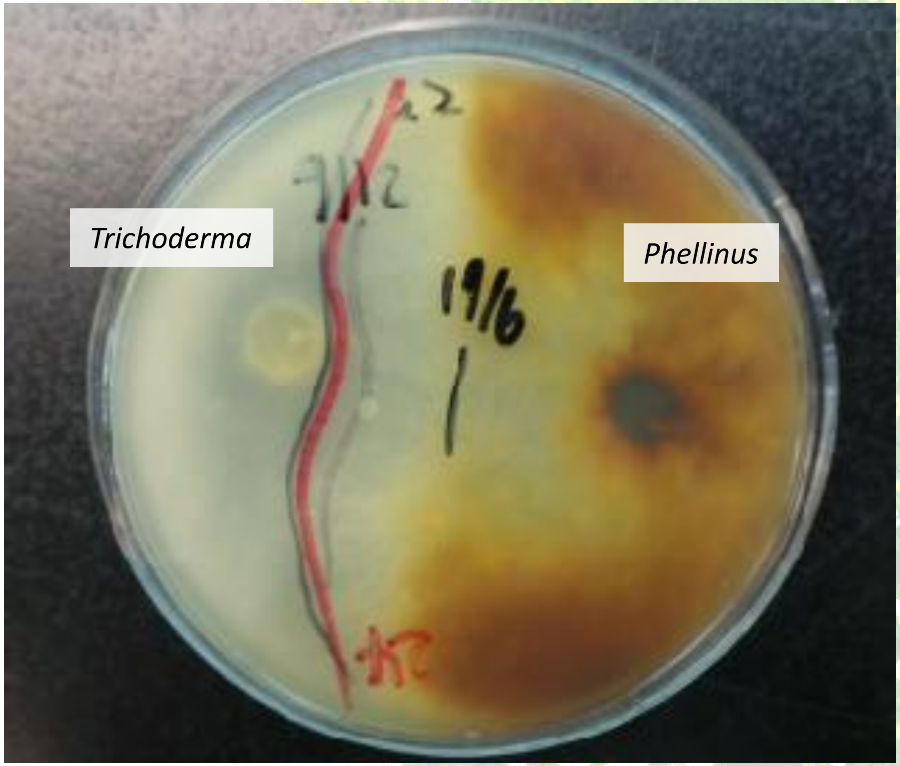
Trichoderma T-TMS1 vs. *Phellinus* YTM65



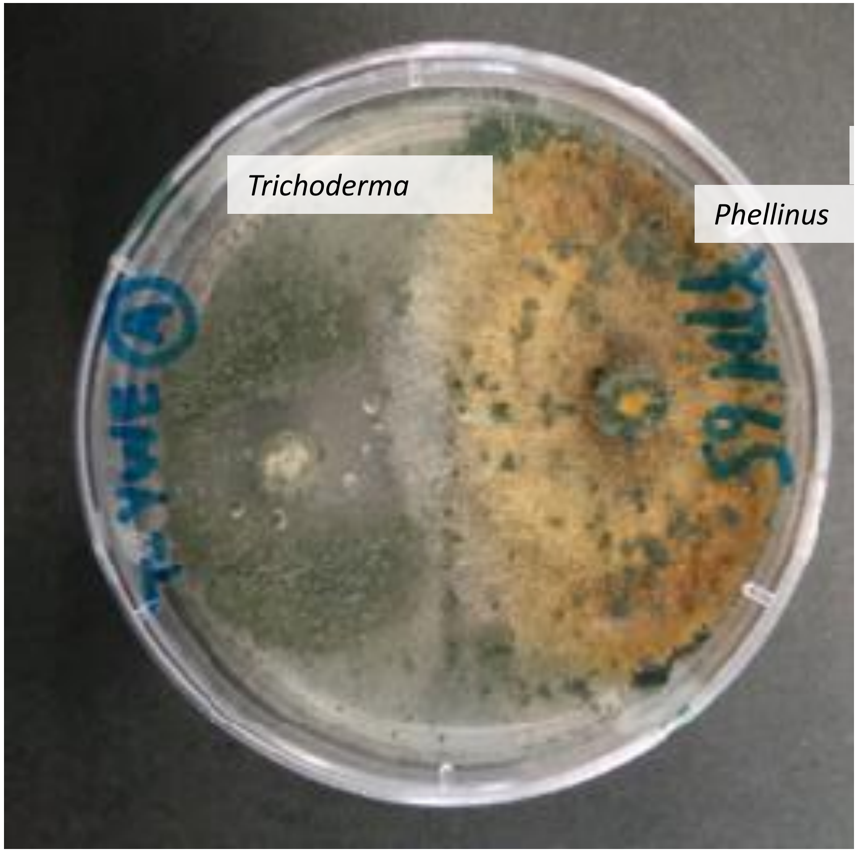
Mycoparasitism

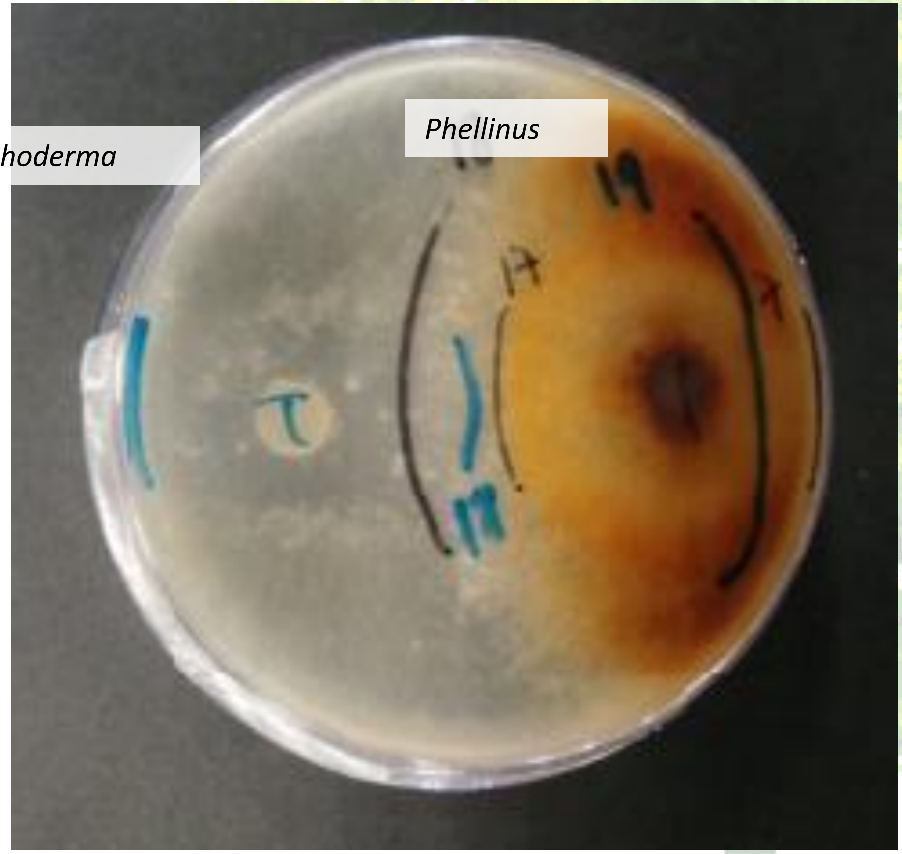
Trichoderma T-TMS1 vs. *Phellinus noxius* SNRP





Trichoderma T-YWE vs *Phellinus* YTM65

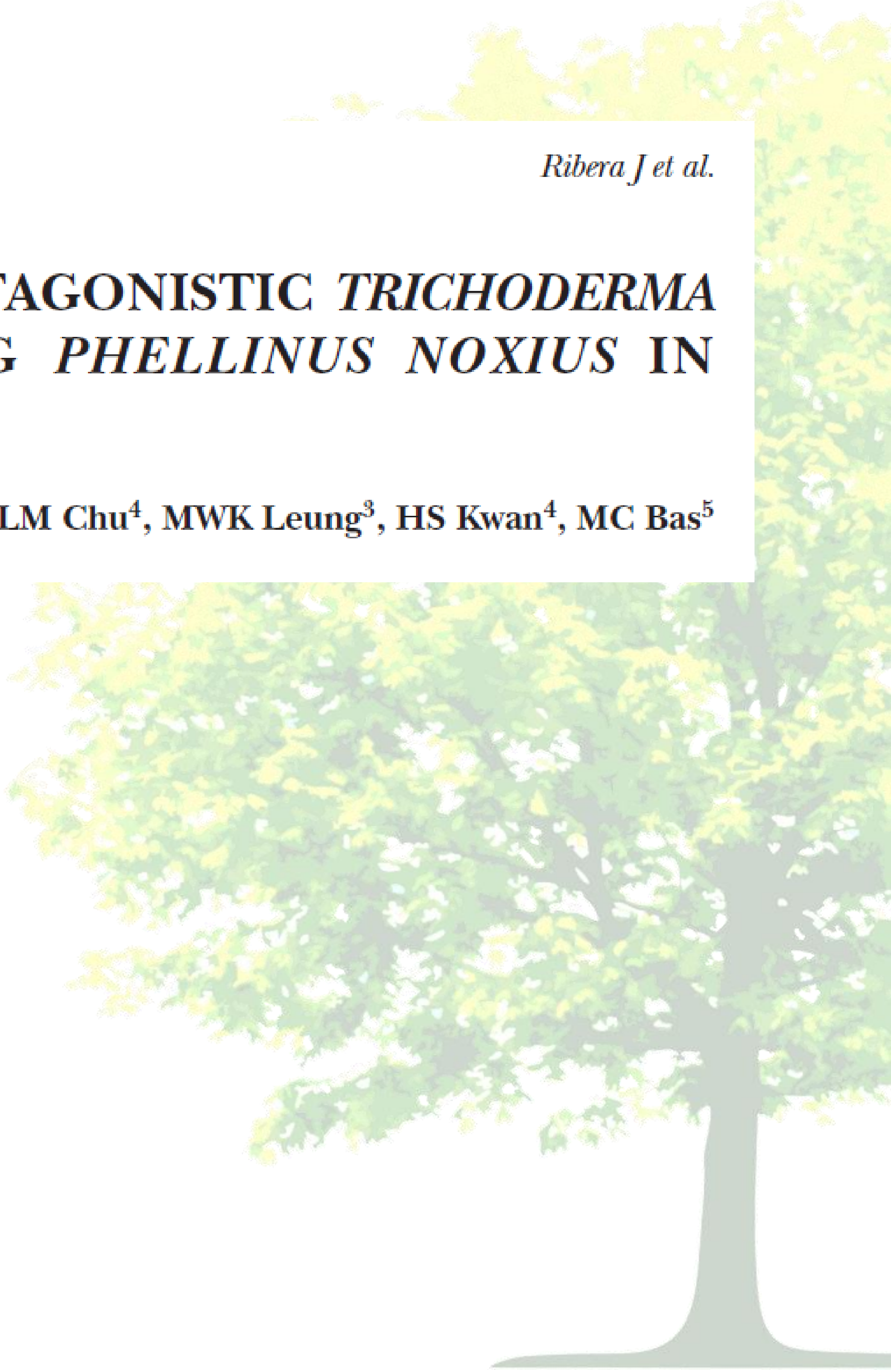




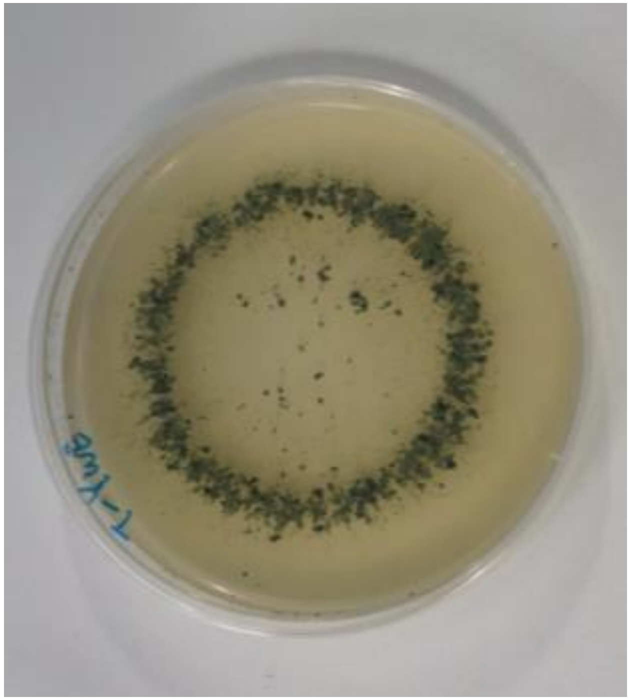
Journal of Tropical Forest Science 28(4): 457–468 (2016) *Ribera J et al.*

IN-VITRO EVALUATION OF ANTAGONISTIC *TRICHODERMA* STRAINS FOR ERADICATING *PHELLINUS NOXIUS* IN COLONISED WOOD

J Ribera[1, *], AMC Tang[2], M Schubert[1], RYC Lam[3, 4], LM Chu[4], MWK Leung[3], HS Kwan[4], MC Bas[5] & FWMR Schwarze[1]



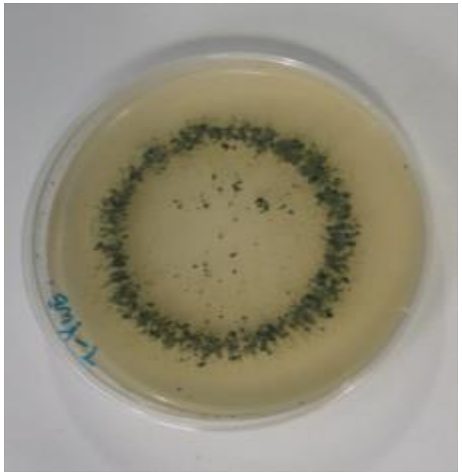
Preparation of *Trichoderma* inoculum



- Locally isolated strains
  - T. gamsii T-TL
  - T. harzianum T-YWE
  - T. harzianum T-TMS1

- 3 Trichoderma strains (*T. gamsii* T-TL, *T. harzianum* T-TMS1, *T. harzianum* T-YWE) were compatible to each other
- Significant overgrowth of mycelium and sporulation of *Trichoderma* over *P. noxius* isolates.

Preparation of *Trichoderma* inoculum



Trichoderma seed culture

Inoculation of *Trichoderma* to carrier substrates (sawdust / rice)

Incubation of *Trichoderma* inoculum

Monitoring parameters

- Physical parameter
  - Bulk density
- Chemical parameters
  - pH
  - Electrical conductivity
  - Available nitrogen
  - Available phosphorus
  - Available potassium
- Biological parameters
  - Microbial concentration
  - *Trichoderma* concentration
  - *Phellinus noxius* concentration

The Provision of Consultancy Services for Field and In Vitro Evaluation of *Trichoderma* Species as a Biological Control Agent against *Phellinus noxius* Infected Trees in Hong Kong

WQ/054/12

Research objectives

- Conduct an initial **laboratory and field trials**
- Use commercially available and **locally isolated *Trichoderma* species**
- **As biological control agent** against trees infected with *P. noxius* in Hong Kong
- To supplement our existing introductory guidelines on brown root rot disease with an evidence-based practical **management strategy** for managing and maintaining the **long-term health of trees** infected with *P. noxius*

Methodology

- Isolation of native *Trichoderma* species and molecular identification
- Evaluation of antagonistic effect of *Trichoderma* species against *P. noxius*
- Preliminary field trials on the applicability, effectiveness and practicality of using *Trichoderma* species as a biological control agent to suppress *P. noxius*

Methodology

- **In-vitro Test**
  - 20 strains of local *Trichoderma* spp.
  - Against Hong Kong *P. noxius* strains
  - 3 local isolated *Trichoderma* sp. showed strong control over *P. noxius*

Methodology

- Field Trials
  - Three BRRD infected trees:
    - 1) LCSD Tree A
    - 2) LCSD Tree B
    - 3) LCSD Tree C

LCSD Tree C
(*Ficus microcarpa*)

Application Period
July 2014- May 2015

LCSD Tree C (*Ficus microcarpa*)

- Application Period
  - Jul 2014 – May 2015
- Infection Stage
  - Insignificant root rot
- Tree Condition
  - Fair

LCSD Tree C

New Root Growth

07 Aug 2014

16 Aug 2014

24 Sep 2014

Quantity of *P. noxius* of tree C in soil

	Number of Gene Copy (per 0.25g)						
	2014-07	**2014-09**	**2014-10**	**2014-12**	**2015-02**	**2015-03**	**2015-05**
N	83.71	Not Detectable	Not Detectable	Not Detectable	Not Detectable	Not Detectable	Not Detectable
E	59.87	Not Detectable	Not Detectable	Not Detectable	Not Detectable	Not Detectable	Not Detectable
S	63.39	Not Detectable	Not Detectable	Not Detectable	Not Detectable	Not Detectable	Not Detectable
W	54.96	Not Detectable	Not Detectable	Not Detectable	Not Detectable	Not Detectable	Not Detectable

Quantity of *P. noxius* of tree C in plant tissue

	Number of Gene Copy (per 0.2g)			
	N	**E**	**S**	**W**
C	Not Detectable	Not Detectable	Not Detectable	Not Detectable

*Sampled on the last day of inspection

Observed changes after application of *Trichoderma* inoculum

- Stimulation of new roots growth
- Reduction of population density of BRR in root and soil uncertain

Summary

- Comparison of the 4 trees after field trials

	1	C	B	A
Change of tree condition	5	2	3	1
Change of infection stage	5	1	3	1
Change of quantity of *P. noxius* in soil	2	1	5	3
Emergence of *P. noxius*	5	1	3	1
Mycoparasitism of *Trichoderma*	1	5	2	5
New Root Growth	2	3	4	5
Total	**20**	**13**	**20**	**16**

*1- best 5 –worst

Results

- *In vitro* evaluation provided some information about *Trichoderma* spp. for biocontrol of *P. noxius*
  - No *P. noxius* were detected from soil and plant samples of LCSD Tree A and LCSD Tree C after treatment
  - But *P. noxius* was detected from LCSD Tree B
  - Reduction of BRR is uncertain
  - Tree responses were variable, but mostly improved

Conclusion

- *Trichoderma spp.* controlling *P. noxius in vitro* could be isolated
- *Trichoderma* treatment effective in some cases
- But not effective in all cases
- More field tests needed to design effective treatment protocols

Consultancy Services for Field Trial on the Feasibility of Using Trichoderma Species to Rehabilitate Locations Contaminated with Phellinus noxius Causing Brown Root Rot Disease on Trees

WQ/013/14

Field Trial on Using *Trichoderma* Species to Rehabilitate *P. noxius* Contaminated Locations

- Field Trials
- Three locations
- 1) Kowloon Park
- 2) Lei Yue Mun Park
- 3) North District Park

What do we expect on the effectiveness of biological control in soil?

- Inhibit plant pathogens in plant tissues
- Rehabilitate soil contaminated with plant pathogens
- Protect plants from disease attack

Factors affecting the effectiveness of *Trichoderma* application?

- Application-related issues:
- Application depth
- Inoculum carrier
- Application frequency
- Application dosage
- *Trichoderma*-related issues
- Abundance of *Trichoderma* in particular time
- Proliferation and survival of Trichoderma

Factors affecting the effectiveness of *Trichoderma* application?

- Environment-related issues:
- Temperature and moisture
- Soil texture and bulk density
- Soil chemical properties
- Supply of available nutrients
- Competition from other microbes
- Predation by other organisms (insects and microbes)
- Pathogen-related issues
- Abundance of pathogen
- Virulence of pathogen
- Proliferation and survival of pathogen

Objectives

- To conduct initial field trials using locally isolated *Trichoderma species* to rehabilitate selected locations with *P. noxius*

- To enrich TMO's existing Guidelines on Brown Root Rot Disease for management of Brown Root Rot disease infected locations

Methodology

- 3 Trichoderma strains used:
- *T. gamsii* T-TL
- *T. harzianum* T-TMS1
- *T. harzianum* T-YWE)

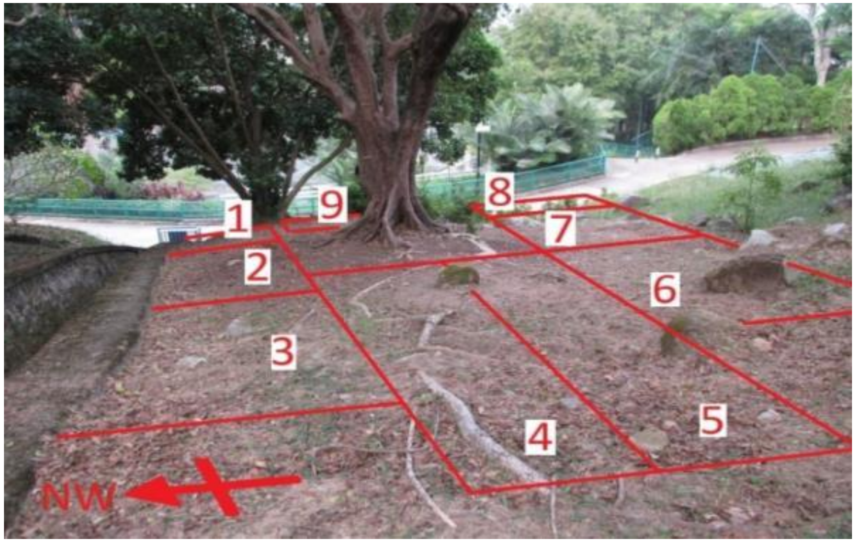
Preparation

Mature *Trichoderma*

Soil excavation

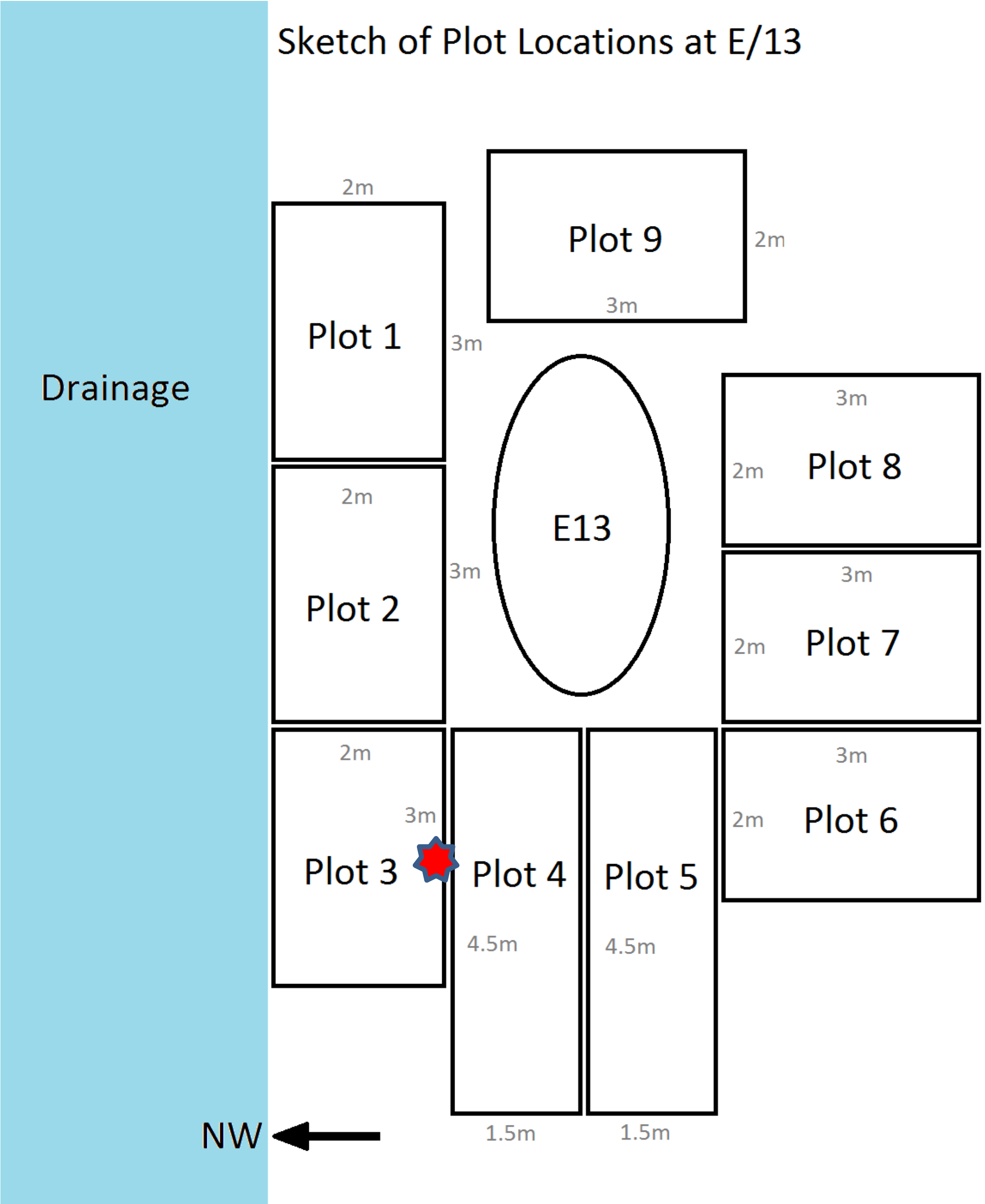
Mixing Compost

Field Trials- Location 1



- Tree D
- Duration: Jan 2015 – Feb 2016

Sketch of Plot Locations at E/13



Experimental Design

Orthogonal design

Factor	Factor level	
Depth (cm) [a]	0	10
Dosage (# of inoculum bags)[b]	4	10
Carrier form[c]	Rice	Sawdust
Application frequency (per year)[d]	2	4

[a] "Depth" represents the application depth of *Trichoderma* in two levels, including application of *Trichoderma* on soil surface with compost (0) and 10 cm belowground in soil with compost (10).

[b] "Dosage" represents the number of *Trichoderma* inoculum bags used with each peat moss pack in the production of the '*Trichoderma*-compost mix'. Each *Trichoderma* inoculum bags weighted 250g (dry weight).

[c] "Carrier form" represents different types of carrier forms, including **rice** + rice bran and **sawdust** + oat meal + bark.

[d] "Application frequency" represents application of *Trichoderma* inoculum two or four times during field trial in each plot.

Experimental treatments

Treatment	Depth below ground (cm)	Dosage (# of inoculum bag)	Carrier form (1: Rice 2: Sawdust)	Application frequency (per year)
Treatment 1 (S4R2)	Surface	4	1	2
Treatment 2 (S4R4)	Surface	4	1	4
Treatment 3 (S10S2)	Surface	10	2	2
Treatment 4 (S10S4)	Surface	10	2	4
Treatment 5 (B4S2)	10	4	2	2
Treatment 6 (B4S4)	10	4	2	4
Treatment 7 (B10R2)	10	10	1	2
Treatment 8 (B10R4)	10	10	1	4
Control	Surface	4	2	2

Monitoring parameters

- Physical parameter
  - Bulk density
- Chemical parameters
  - pH
  - Electrical conductivity
  - Available nitrogen
  - Available phosphorus
  - Available potassium
- Biological parameters
  - Microbial concentration
  - Trichoderma concentration
  - Phellinus noxius concentration

Summary

Control of *P. noxius* in soil by *Trichoderma* application

- Propagules of *P. noxius* were not detected in all three field areas initially
- Only a few positive detections of *P. noxius* with low density levels along study periods
- Application of *Trichoderma* could effectively boost the microbiome in soil
  - Creating unfavorable condition for pathogenic propagules

Best combination of Trichoderma application

Orthogonal analysis

Parameter \ Factor		Depth			Dosage			Carrier Form			Application Time		
		Effectiveness*	Surface	-10cm	Effectiveness*	4 bags	10 bags	Effectiveness*	Rice	Saw dust	Effectiveness*	2 times	4 times
Soil nutrients	**Available nitrogen (mg/kg)**	++			+++			++++	✓		+		
	Available phosphorus (10^{-4} *M*)	+			+			+			+		
	Available potassium (10^{-4} *M*)												
Colony forming units (10^4 CFU/g)	**Total microorganisms**												
	Total *Trichoderma*												
qPCR result	**Mean CT values of *P.noxius***	+			+			+					

-10 cm

10 bags

Rice

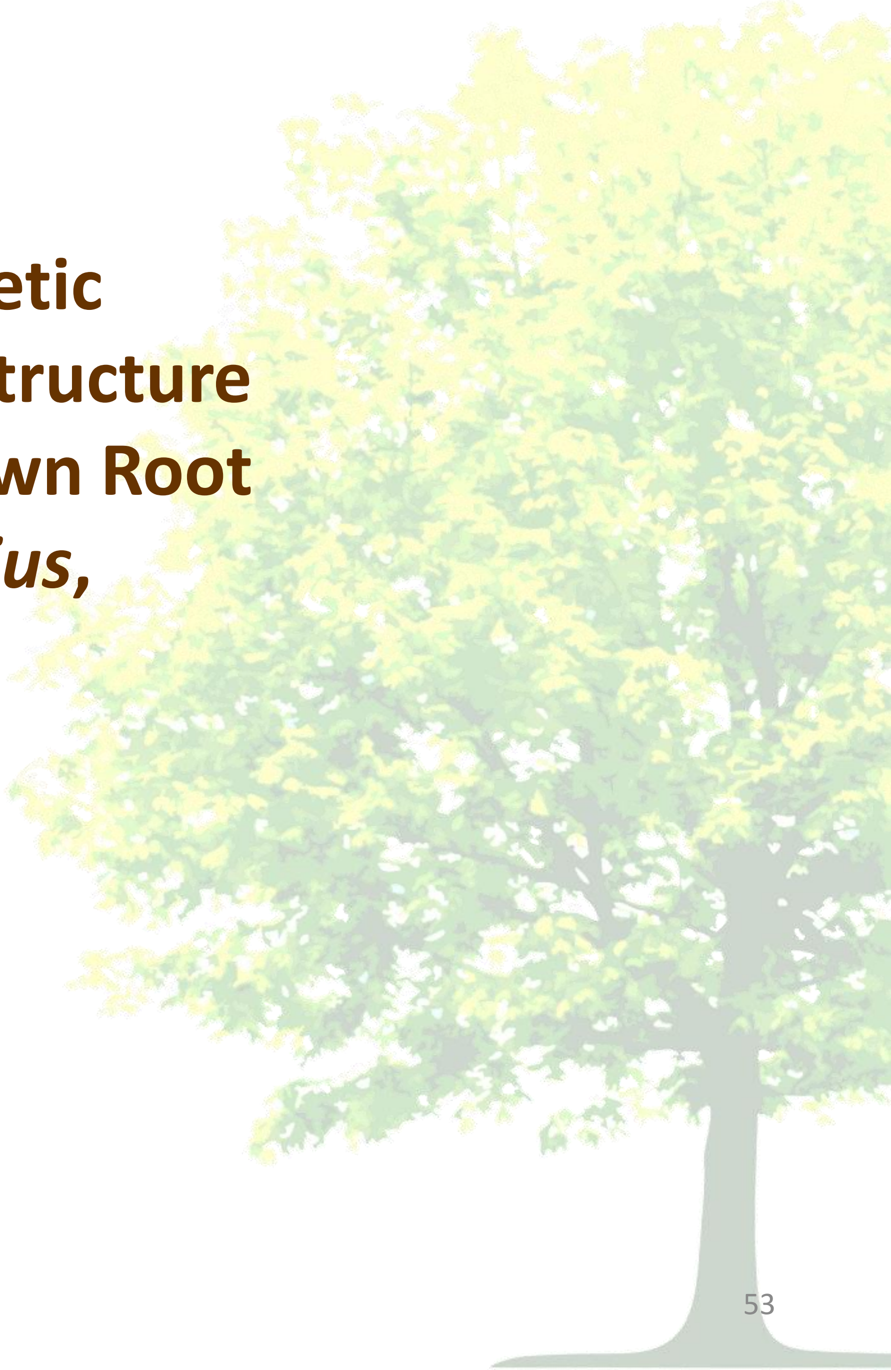
4 times/y

Recommendations

- Apply *Trichoderma* every 3 months
- Apply at 10 cm below ground level
- Apply high dosage
- Use rice / rice-bran carrier

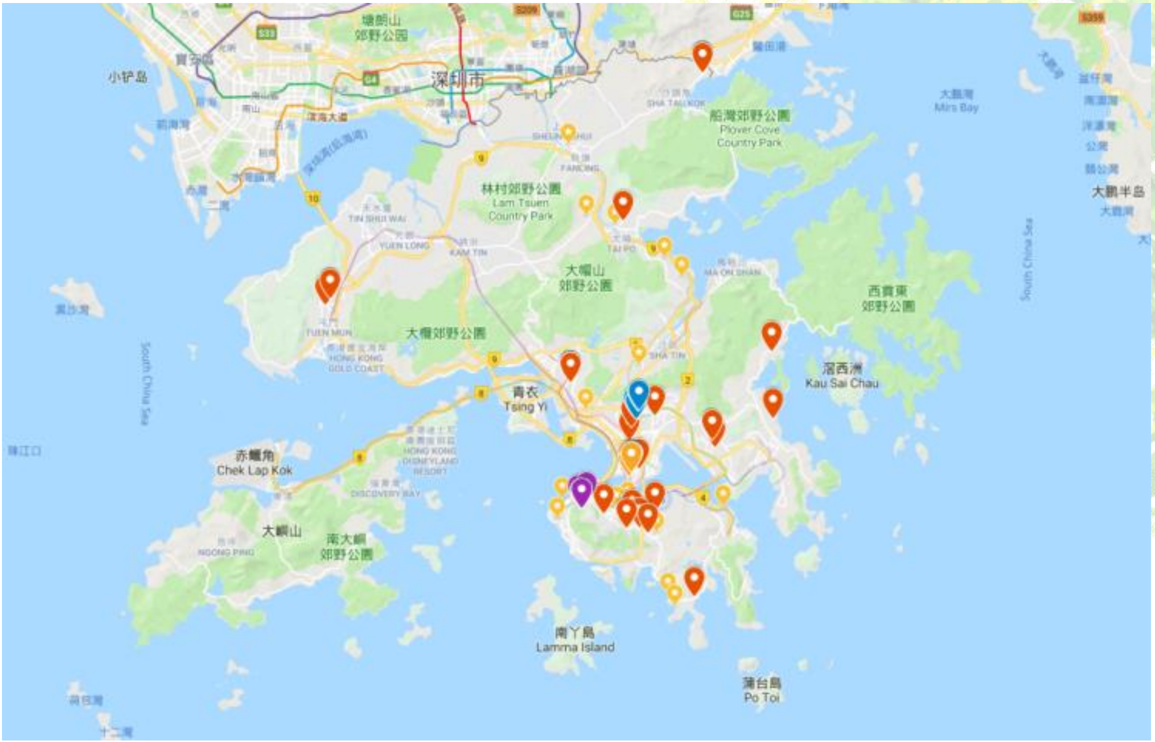
Consultancy Study on Genetic Diversity and Population Structure of the Casual Agent of Brown Root Rot Disease, *Phellinus noxius*, in Hong Kong

WQ/046/17



Investigation of genetic diversity of *Phellinus noxius* in Hong Kong

- Genomes of 50 samples from Hong Kong and 10 samples from overseas will be compared



Genomics & Transcriptomics of *P. noxius*

Supported by Food Research Centre, CUHK

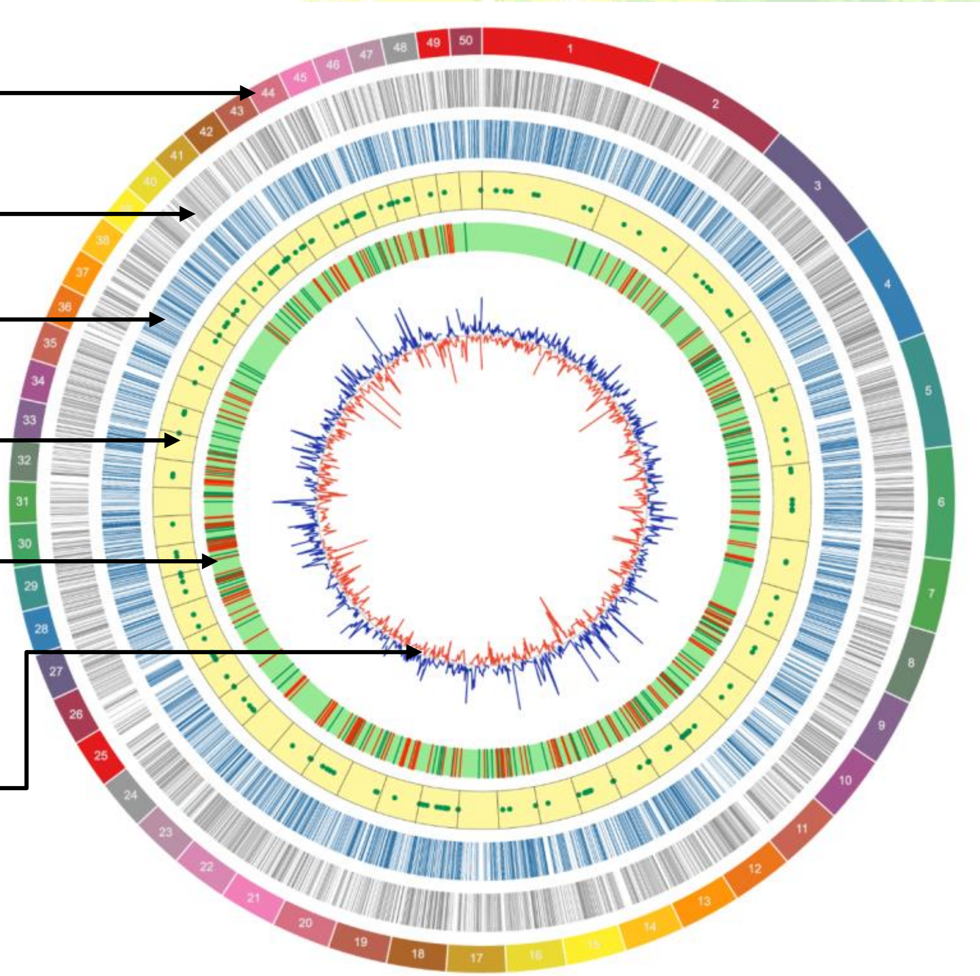
P. noxius Genome Assembled

	YTM97	YTM65	SSP14	S39	KPN57
Total length (Mbp)	31.2	30.9	32.0	31.6	30.9
Number of contigs	**221**	5,240	4,719	4.466	827
GC (%)	41.55	41.52	41.51	41.5	41.54
N50 (Kbp)	302.5	8.2	10.5	11.0	119.7
N75 (Kbp)	190.7	4.3	5.2	5.5	51.6
Max contig length (Kbp)	1,232	165	158	161	816
# Ns per 100 kbp	0	0	0	0	0.98
Estimated fold coverage	40x	40x	50x	46x	35x
Total protein-coding genes	9,411	10,911	11,101	10,962	10,182
Estimated genome completeness	**96.20%**	69.60%	81.10%	75.80%	78.60%

Circular representation of the genome assembly

Showing 50 largest contigs (21 Mb) out of a complete 31 Mb assembly.

From outside to inside –



1. Sizes of the 50 largest contigs
2. CDS forward strand
3. CDS reverse strand
4. Transposable elements (TEs)
5. Secreted proteins
    - green – forward strand
    - red – reverse strand
6. GC skew (10 kb sliding window)

P. noxius Genome

- *P. noxius* has the genes to:
  - Degrades lignin
  - Uses cellulose and xylans extensively
- Opportunistic pathogen
  - many cell wall and hyphal developmental components and degradation capabilities
  - virulence against plant hosts

Carbohydrate-active enzyme (CAZyme) analysis reveals differences between *P. noxius* and other white rot fungi

Some AA1_1 laccases

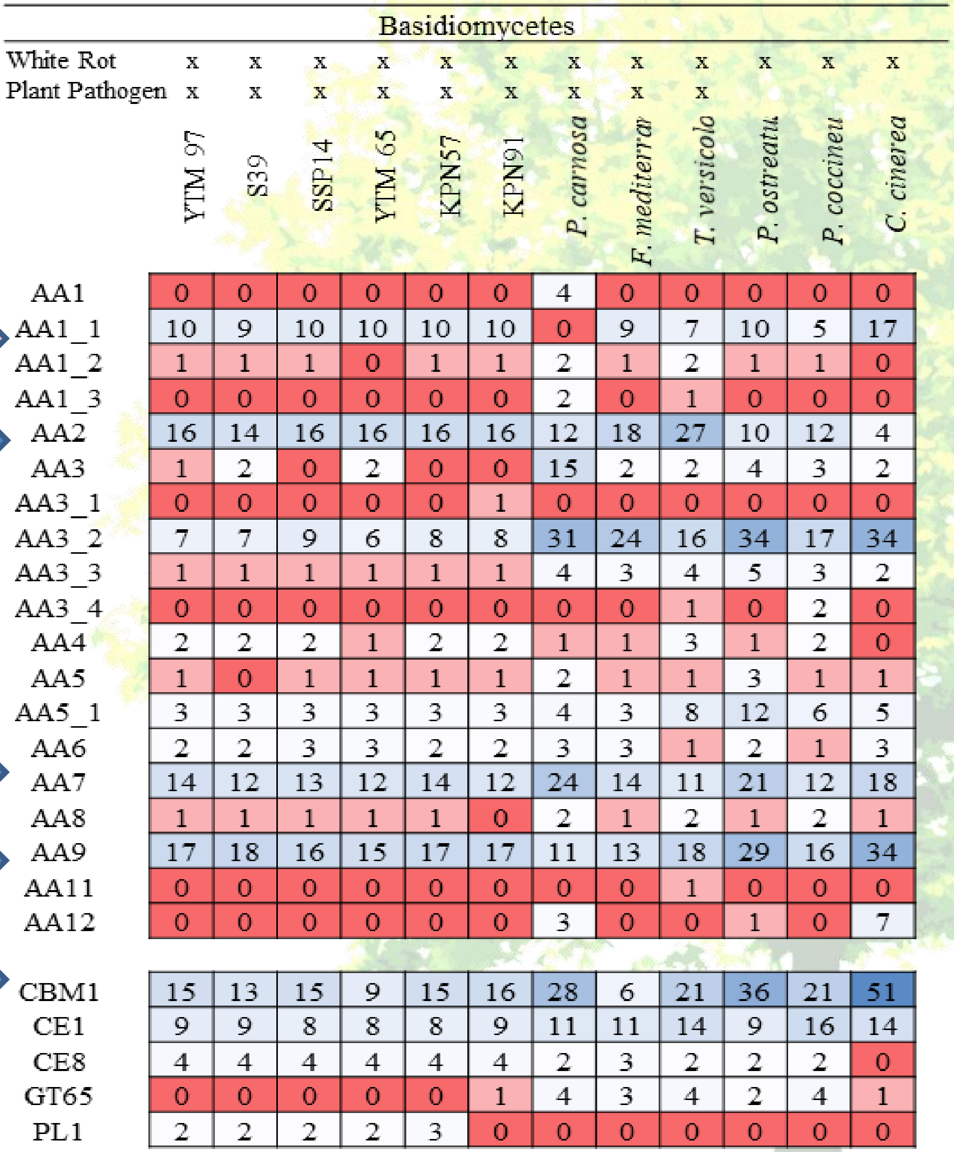
Many

- AA2 Lignin-modifying peroxidases
- AA7 Glucooligosaccharide oxidases
- AA9 Cu Monooxygenases
- CBM1 Carbohydrate binding module

P. noxius uses xylans and cellulose efficiently

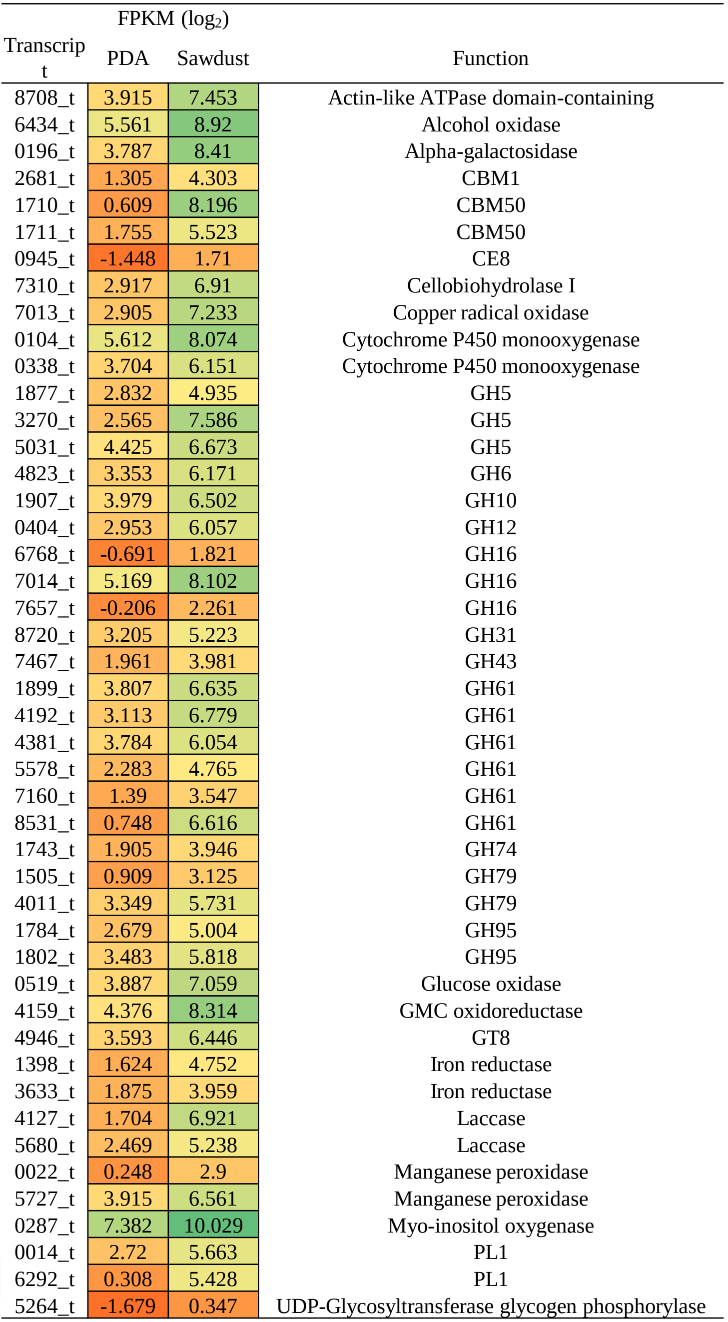
→ a simultaneous white rot fungus

→ virulent



	Basidiomycetes											
White Rot	x	x	x	x	x	x	x	x	x	x	x	x
Plant Pathogen	x	x	x	x	x	x	x	x	x			
	YTM 97	S39	SSP14	YTM 65	KPN57	KPN91	*P. carnosa*	*F. mediterra*	*T. versicolo*	*P. ostreatu*	*P. coccineu*	*C. cinerea*
AA1	0	0	0	0	0	0	4	0	0	0	0	0
AA1_1	10	9	10	10	10	10	0	9	7	10	5	17
AA1_2	1	1	1	0	1	1	2	1	2	1	1	0
AA1_3	0	0	0	0	0	0	2	0	1	0	0	0
AA2	16	14	16	16	16	16	12	18	27	10	12	4
AA3	1	2	0	2	0	0	15	2	2	4	3	2
AA3_1	0	0	0	0	0	1	0	0	0	0	0	0
AA3_2	7	7	9	6	8	8	31	24	16	34	17	34
AA3_3	1	1	1	1	1	1	4	3	4	5	3	2
AA3_4	0	0	0	0	0	0	0	0	1	0	2	0
AA4	2	2	2	1	2	2	1	1	3	1	2	0
AA5	1	0	1	1	1	1	2	1	1	3	1	1
AA5_1	3	3	3	3	3	3	4	3	8	12	6	5
AA6	2	2	3	3	2	2	3	3	1	2	1	3
AA7	14	12	13	12	14	12	24	14	11	21	12	18
AA8	1	1	1	1	1	0	2	1	2	1	2	1
AA9	17	18	16	15	17	17	11	13	18	29	16	34
AA11	0	0	0	0	0	0	0	0	1	0	0	0
AA12	0	0	0	0	0	0	3	0	0	1	0	7
CBM1	15	13	15	9	15	16	28	6	21	36	21	51
CE1	9	9	8	8	8	9	11	11	14	9	16	14
CE8	4	4	4	4	4	4	2	3	2	2	2	0
GT65	0	0	0	0	0	1	4	3	4	2	4	1
PL1	2	2	2	2	3	0	0	0	0	0	0	0

AA = auxiliary activities
CBM = carbohydrate binding module
CE = carbohydrate esterases
GT = glycosyltransferases
PL = polysaccharide lyases



Transcript	FPKM ($\log_2$)		Function
	PDA	Sawdust	
8708_t	3.915	7.453	Actin-like ATPase domain-containing
6434_t	5.561	8.92	Alcohol oxidase
0196_t	3.787	8.41	Alpha-galactosidase
2681_t	1.305	4.303	CBM1
1710_t	0.609	8.196	CBM50
1711_t	1.755	5.523	CBM50
0945_t	-1.448	1.71	CE8
7310_t	2.917	6.91	Cellobiohydrolase I
7013_t	2.905	7.233	Copper radical oxidase
0104_t	5.612	8.074	Cytochrome P450 monooxygenase
0338_t	3.704	6.151	Cytochrome P450 monooxygenase
1877_t	2.832	4.935	GH5
3270_t	2.565	7.586	GH5
5031_t	4.425	6.673	GH5
4823_t	3.353	6.171	GH6
1907_t	3.979	6.502	GH10
0404_t	2.953	6.057	GH12
6768_t	-0.691	1.821	GH16
7014_t	5.169	8.102	GH16
7657_t	-0.206	2.261	GH16
8720_t	3.205	5.223	GH31
7467_t	1.961	3.981	GH43
1899_t	3.807	6.635	GH61
4192_t	3.113	6.779	GH61
4381_t	3.784	6.054	GH61
5578_t	2.283	4.765	GH61
7160_t	1.39	3.547	GH61
8531_t	0.748	6.616	GH61
1743_t	1.905	3.946	GH74
1505_t	0.909	3.125	GH79
4011_t	3.349	5.731	GH79
1784_t	2.679	5.004	GH95
1802_t	3.483	5.818	GH95
0519_t	3.887	7.059	Glucose oxidase
4159_t	4.376	8.314	GMC oxidoreductase
4946_t	3.593	6.446	GT8
1398_t	1.624	4.752	Iron reductase
3633_t	1.875	3.959	Iron reductase
4127_t	1.704	6.921	Laccase
5680_t	2.469	5.238	Laccase
0022_t	0.248	2.9	Manganese peroxidase
5727_t	3.915	6.561	Manganese peroxidase
0287_t	7.382	10.029	Myo-inositol oxygenase
0014_t	2.72	5.663	PL1
6292_t	0.308	5.428	PL1
5264_t	-1.679	0.347	UDP-Glycosyltransferase glycogen phosphorylase

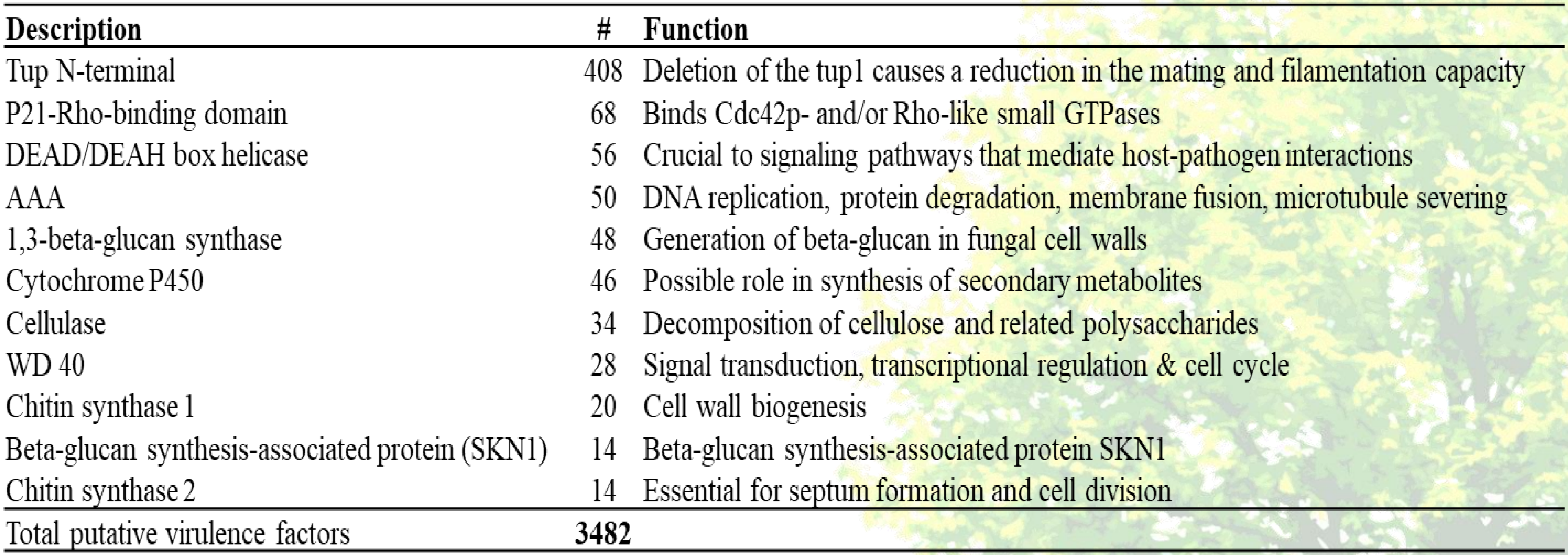
Heatmap showing FPKM ($\log_2$) values of differentially expressed carbohydrate related genes which were upregulated at least 4-fold in sawdust ($p < 0.05$)

Essential virulence factor analysis

- Many cell wall formation and hyphal development components

Virulence Factors	# of hits	Function
Chitin synthase 1	29	Cell wall biogenesis
Chitin synthase 2	45	Essential for septum formation and cell division
Glucan synthase	34	Generation of beta-glucan in fungal cell walls
SKN1	7	Beta-glucan synthesis-associated protein SKN1
P21-Rho binding domain	52	Binds Cdc42p- and/or Rho-like small GTPases
AAA proteins	46	DNA replication, protein degradation, membrane fusion, microtubule severing
WD40	78	Signal transduction, transcriptional regulation & cell cycle
SNF2 family N-terminal domain	16	Hyphal formation
Tup protein N-terminal domain	186	Deletion of the tup1 gene causes a drastic reduction in the mating and filamentation capacity
Total putative virulence factors	**938**	

Virulence factor analysis



Description	#	Function
Tup N-terminal	408	Deletion of the tup1 causes a reduction in the mating and filamentation capacity
P21-Rho-binding domain	68	Binds Cdc42p- and/or Rho-like small GTPases
DEAD/DEAH box helicase	56	Crucial to signaling pathways that mediate host-pathogen interactions
AAA	50	DNA replication, protein degradation, membrane fusion, microtubule severing
1,3-beta-glucan synthase	48	Generation of beta-glucan in fungal cell walls
Cytochrome P450	46	Possible role in synthesis of secondary metabolites
Cellulase	34	Decomposition of cellulose and related polysaccharides
WD 40	28	Signal transduction, transcriptional regulation & cell cycle
Chitin synthase 1	20	Cell wall biogenesis
Beta-glucan synthesis-associated protein (SKN1)	14	Beta-glucan synthesis-associated protein SKN1
Chitin synthase 2	14	Essential for septum formation and cell division
Total putative virulence factors	**3482**	

	FPKM ($\log_2$)			
Transcript	PDA	Sawdust	$p < 0.05$	Predicted Function
92_t	8.273	8.734		Hypothetical protein
395_t	4.419	6.545	x	Cerato-platanin
460_t	1.092	3.409	x	Hypothetical protein
1710_t	0.609	8.196	x	CBM50
1711_t	1.755	5.523	x	CBM50
2249_t	4.504	3.944		Expansin family
2806_t	-1.991	4.438	x	Hypothetical protein
2947_t	5.497	7.389	x	Expansin family
2951_t	-2.514	-1.855		Hypothetical protein
3488_t	3.274	2.625		Hypothetical protein
3687_t	-0.729	-2.088		Hypothetical protein
4164_t	-0.495	1.075		Hypothetical protein
5885_t	-0.741	2.284		Hypothetical protein
6015_t	6.092	5.963		Yos1-like protein
7225_t	6.902	6.550		Expansin family
7593_t	8.458	7.875	x	FK506 binding family
7853_t	9.616	6.782	x	Expansin family
8458_t	0.484	0.871		Hypothetical protein
8784_t	0.000	0.000		Hypothetical protein
8785_t	-2.365	0.000		Hypothetical protein
8786_t	0.000	-1.894		Hypothetical protein
9221_t	4.612	9.110	x	Thaumatin-like protein

Heatmap FPKM ($\log_2$) values of the expression levels of putative effector genes of YTM97 grown on PDA and sawdust

- I wonder if the presentation of statistical testing is the best here. Are there any better ways?

Volatile organic compounds control of *Phellinus noxius*

Supported by United College, CUHK, Research Fund

Volatile organic compounds control of *Phellinus noxius*

- In vitro test
  - Incubate with VOCs from wasabi
- Killing
- Suppressing growth

Result highlights:

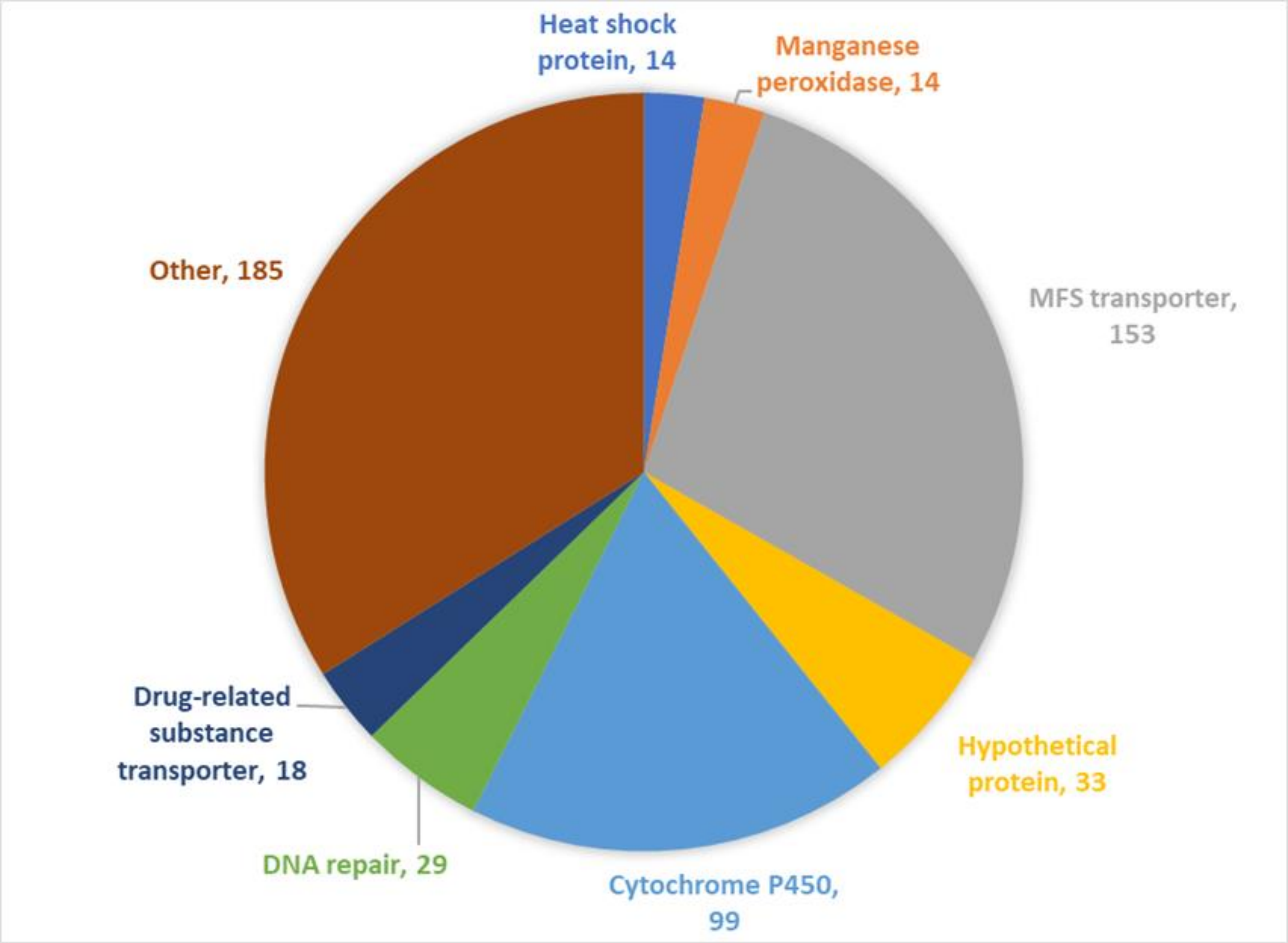
- Volatile organic compounds of **T-TMS1** caused the highest inhibition of growth of *P. noxius*
- **T-TMS1** showed high antagonistic potential
- against 4 *P. noxius* isolates **JCHS, SNRP, YTM97& E35** in dual cultures
- **T-YWE** --high antagonistic potential
  - against 5 *P. noxius* isolates **JCHS, SNRP, YTM97, E35 & SSP14**
- After 24 weeks of incubation, *P. noxius* was completely eradicated in balsa wood blocks containing **T-TMS1**

Volatile organic compounds control of *P. noxius*--gene expression changes

- After treatment to suppress growth
- RNA-sequencing
  - 9411 genes mapped
  - 545 (5.79%) stress related genes
  - 379 stress related genes were affected by VOCs

Volatile organic compounds control of *Phellinus noxius*

- Identified stress-related gene of *P. noxius*



Volatile organic compounds control of *Phellinus noxius*

- Stress-related gene and its responsible stress

Factor1	**Factor2**	**No. of Gene**
Genotoxic stress		66
Oxidation Stress		42
Oxidation Stress	Antimicrobials	256
Oxidation Stress	Heat Stress	1
Heat Stress		17
Heat Stress	Calcium signaling	2
Cell Wall Stress		14
Antimicrobials		15
Other		132
		545

P. noxius Genomics & Transcriptomics

- Provided the reference genome sequence for population studies
- Allow better understanding

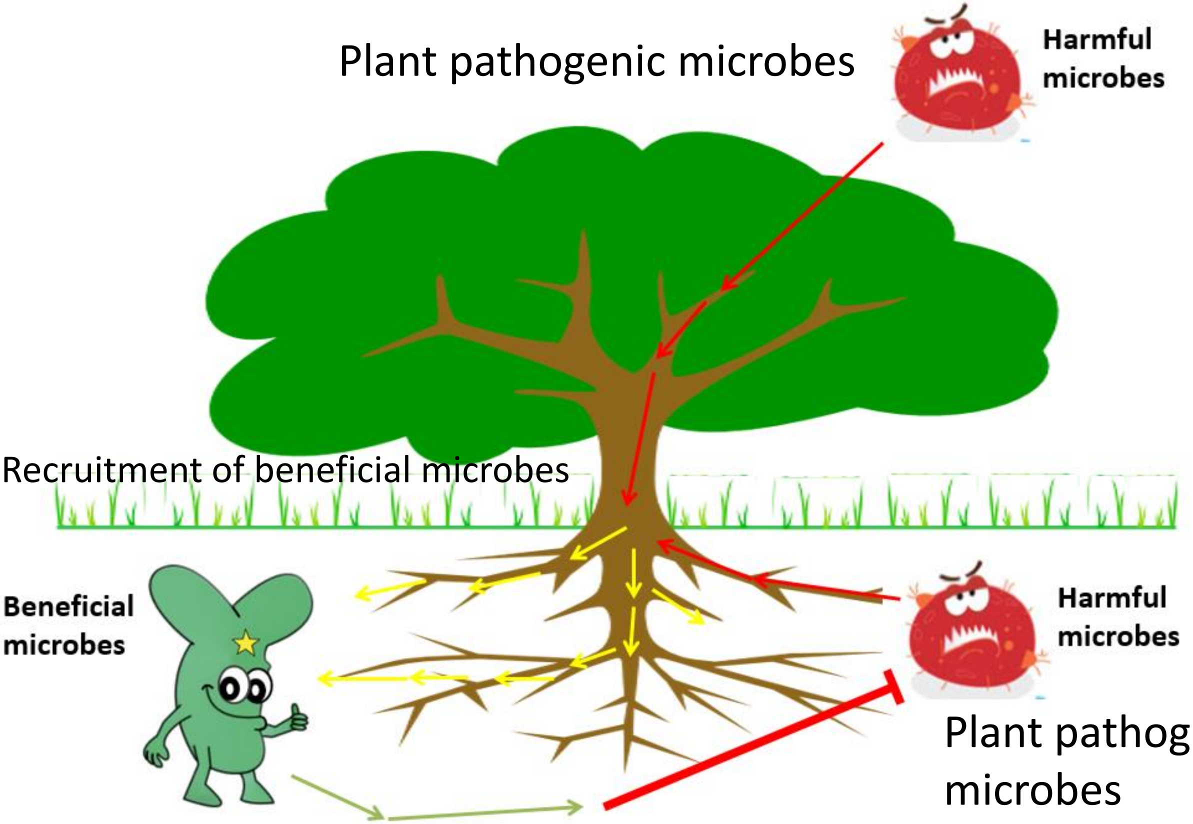
Rhizosphere Microbiome Analysis to Discover Potential Bioindicators for the Early Detection of Brown Root Rot Disease

Supported by Environmental Conservation Fund

Importance of an early detection method

- Current research in field focused on late stage curative measures
- Crucial for the prognosis of infected trees
- Need to find novel ways of detecting disease in addition to current methods

Rhizosphere Microbiome



Plant pathogenic microbes

Recruitment of beneficial microbes

Plant pathogenic microbes

Activation of beneficial microbes

Release of pathogen inhibitory compounds

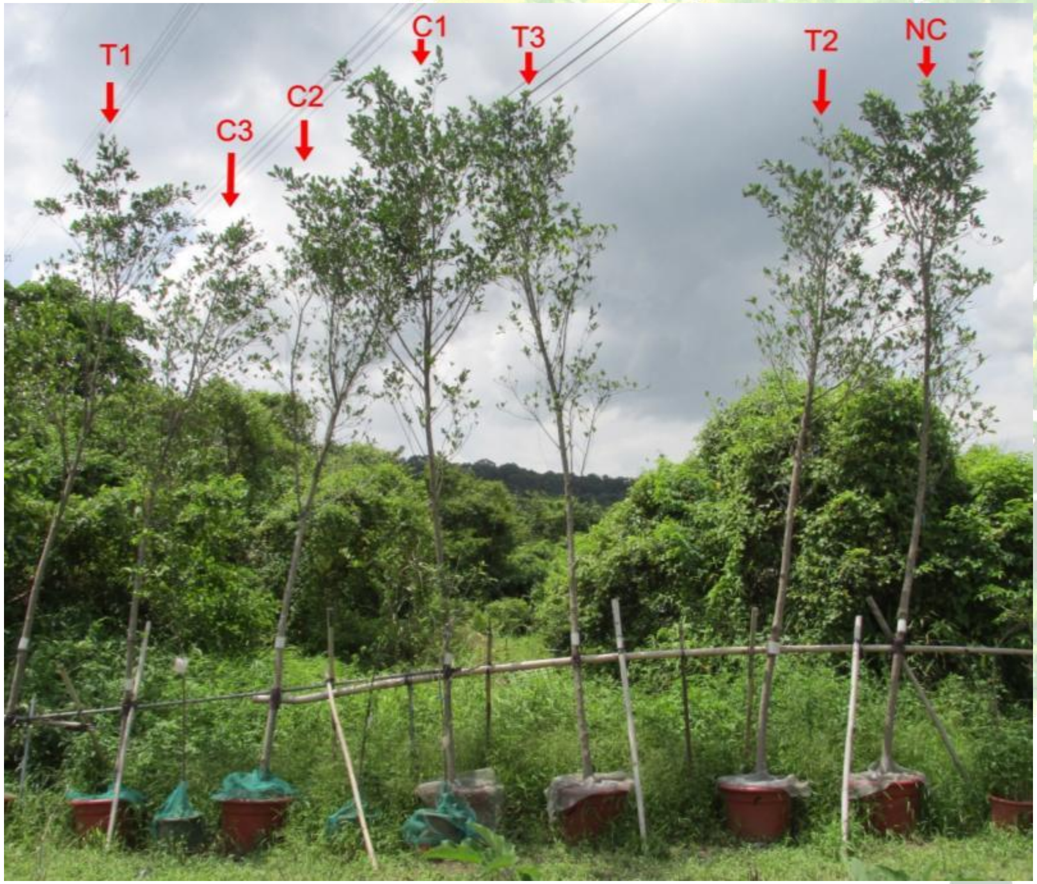
- Symbiotic
- Crucial to plant health

Project Objectives

- Perform a baseline study on rhizosphere microbial diversity in healthy trees and infected trees
- Identify the effect of a *P. noxius* infection on rhizosphere microbial communities
- Isolate potential bioindicators that indicate occurrence of disease
- Test bioindicators using artificially infected trees

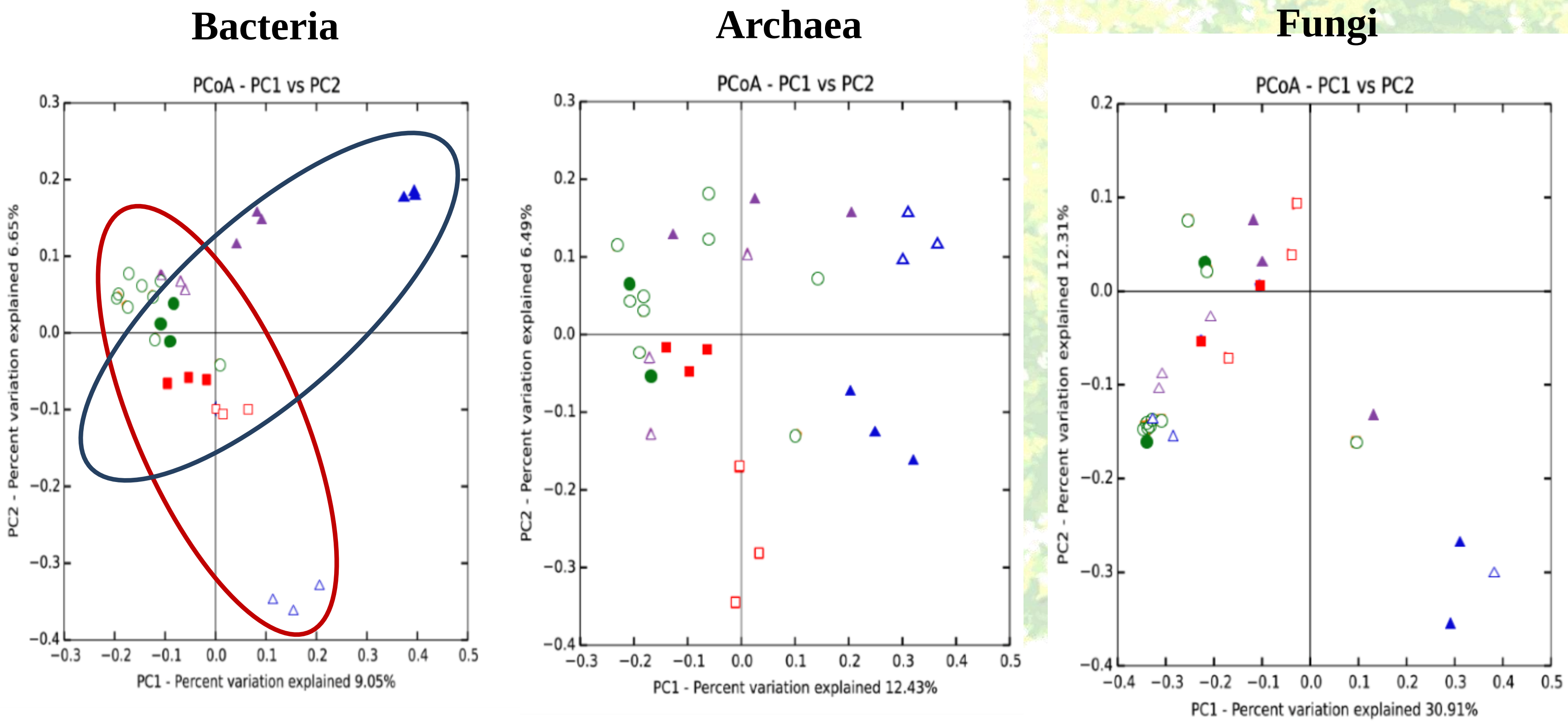
Research Plan

- *Ficus microcarpa*
- 3 infected trees
- 3 control trees
- 4 - 5 m tall



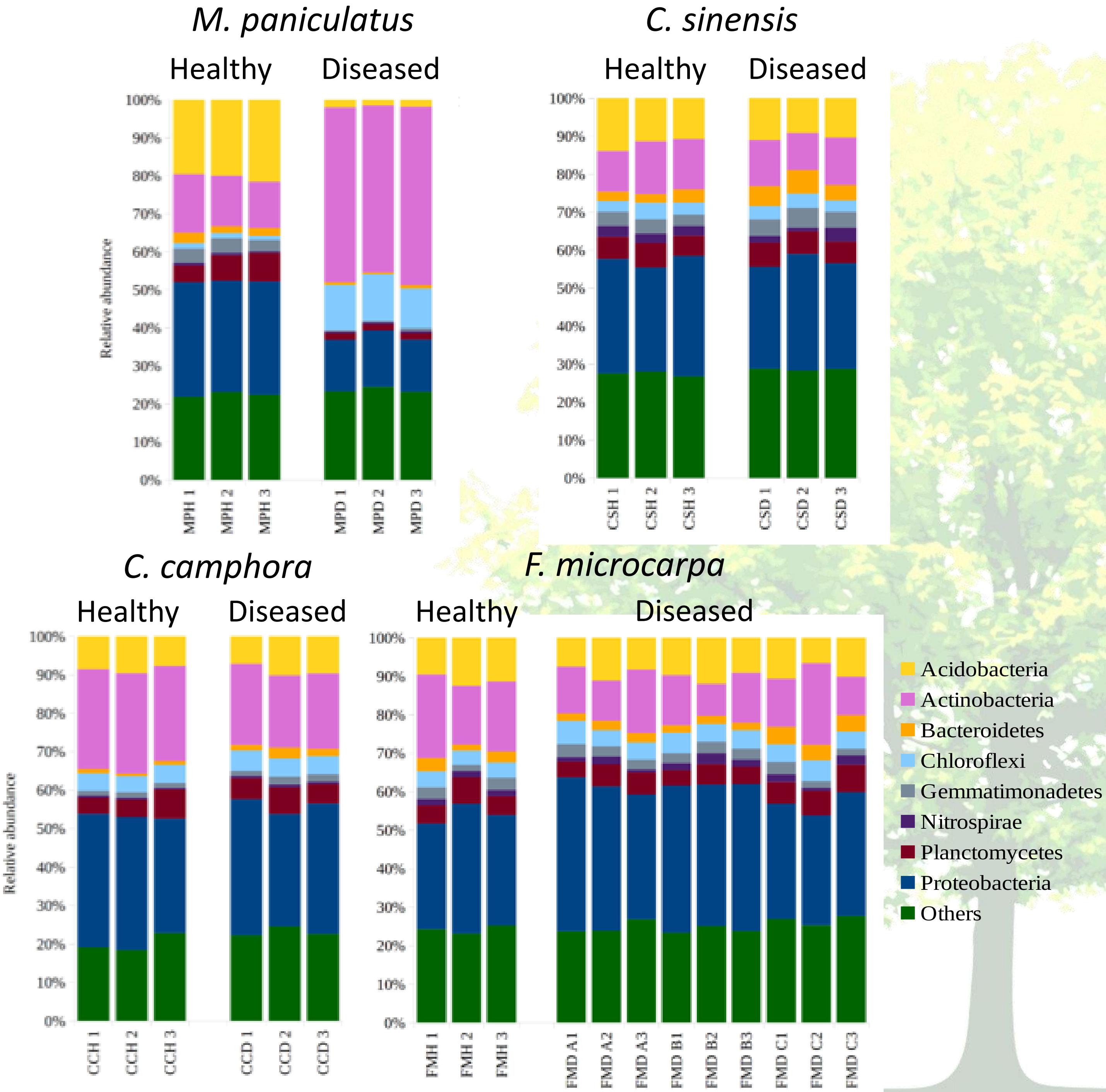
Principle Component Analysis

Unweighted UniFrac distances for bacteria and archaea
Bray-Curtis distances for fungi



- ▲ *M. paniculatus* (Healthy)
- △ *M. paniculatus* (Diseased)
- ▲ *C. sinensis* (Healthy)
- △ *C. sinensis* (Diseased)
- ■ *C. camphora* (Healthy)
- □ *C. camphora* (Diseased)
- ● *F. microcarpa* (Healthy)
- ○ *F. microcarpa* (Diseased)

Relative abundances of dominant (>1%) bacterial phyla in the four species of trees in healthy and diseased states

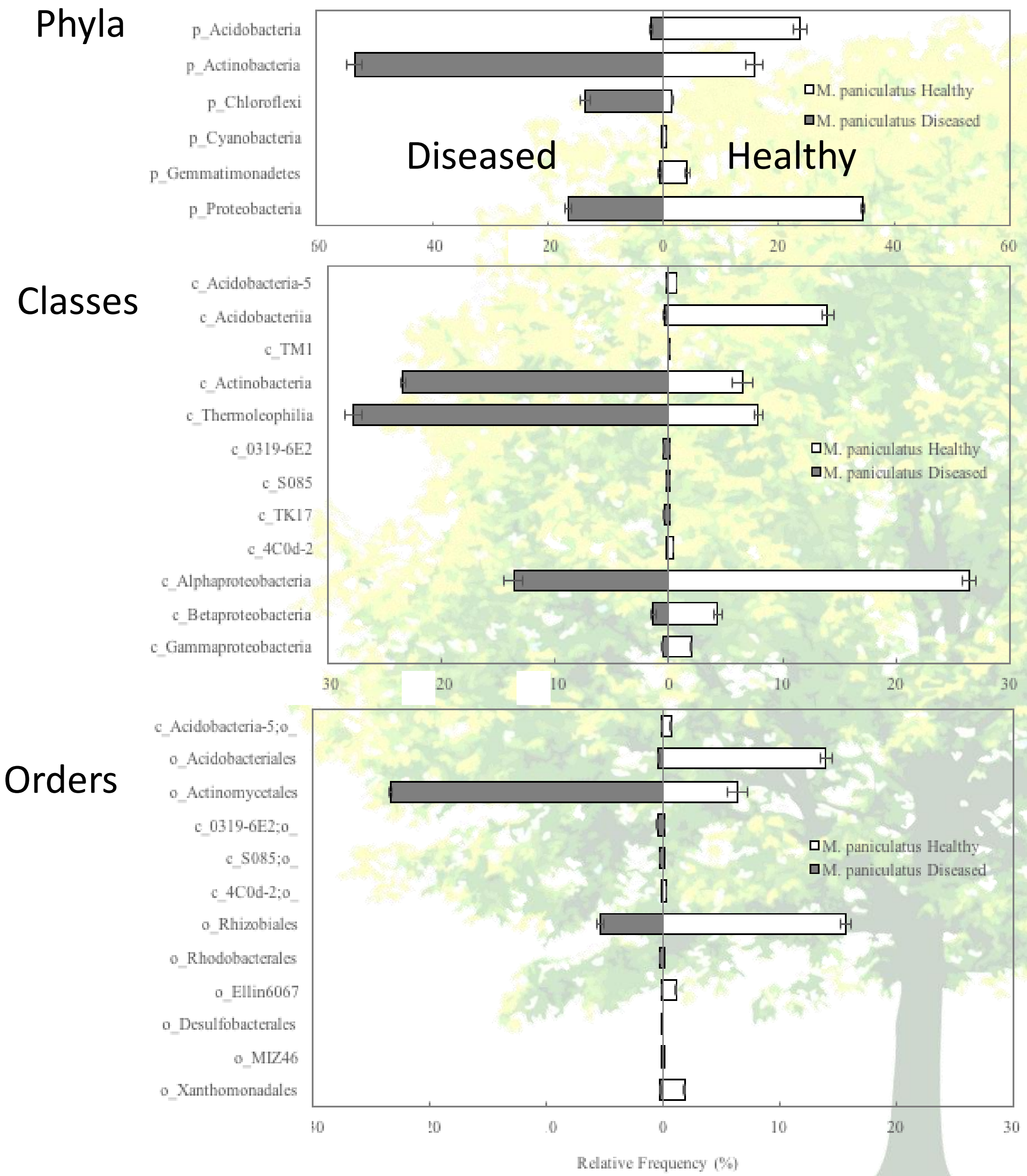


Significant changes ($p < 0.05$) between overall diseased and healthy rhizospheres in bacterial

- phyla
- classes
- orders
- families

Significant changes ($p < 0.05$) between diseased and healthy rhizospheres in *M. paniculatus* trees at

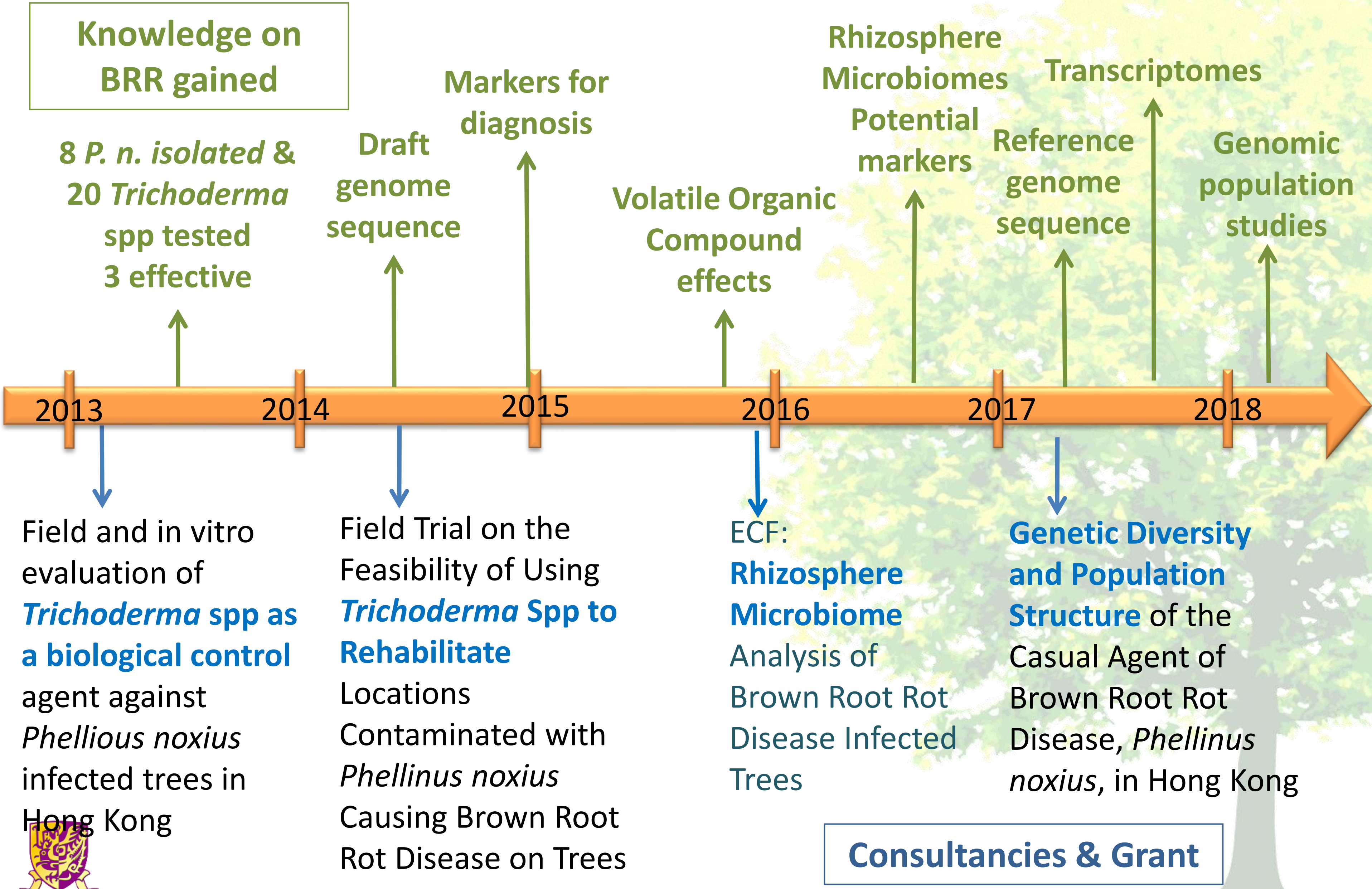
- phyla
- classes
- orders



Summary

- Examined the effects of *P. noxius* on the diversity and composition of bacterial, archaeal and fungal rhizosphere microbiomes of four species of trees.
- Infection did not affect the overall diversity of the rhizosphere microbiomes
- Health status related to the changes in community structure of all 3 kingdoms
- Between healthy and diseased:
  - Statistically significant changes in the bacterial community composition
  - But not archaeal and fungal communities.

Research & Development in KwanLab on BRR P. noxius in Hong Kong Supported by HKSAR Government contracts & grant



Knowledge on BRR gained

- 8 *P. n. isolated* & 20 *Trichoderma* spp tested 3 effective
- Draft genome sequence
- Markers for diagnosis
- Volatile Organic Compound effects
- Rhizosphere Microbiomes Potential markers
- Reference genome sequence
- Transcriptomes
- Genomic population studies

Consultancies & Grant

Field and in vitro evaluation of ***Trichoderma*** **spp as a biological control** agent against *Phellious noxius* infected trees in Hong Kong

Field Trial on the Feasibility of Using ***Trichoderma*** **Spp to Rehabilitate** Locations Contaminated with *Phellinus noxius* Causing Brown Root Rot Disease on Trees

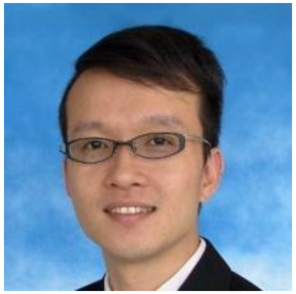
ECF: **Rhizosphere Microbiome** Analysis of Brown Root Rot Disease Infected Trees

Genetic Diversity and Population Structure of the Casual Agent of Brown Root Rot Disease, *Phellinus noxius*, in Hong Kong

Prof. Hoi Shan Kwan
School of Life Sciences
The Chinese University of Hong Kong

Prof. Lee Man Chu
School of Life Sciences
The Chinese University of Hong Kong

Dr. Alvin Tang
Muni Arborist Limited/
HK Baptist University



Mr. Mike Leung

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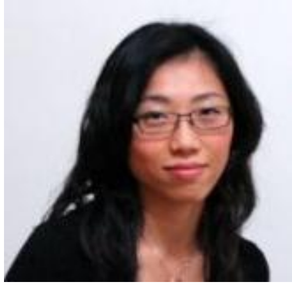

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- United College Research Fund
- Food Research Centre, CUHK

Thank You