Sensitivities of Four Non-target Agronomic Chemicals against *Ralstonia solanacearum* and Its Metabolic Reaction under Chemical Pressures

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Oct 1, 2014

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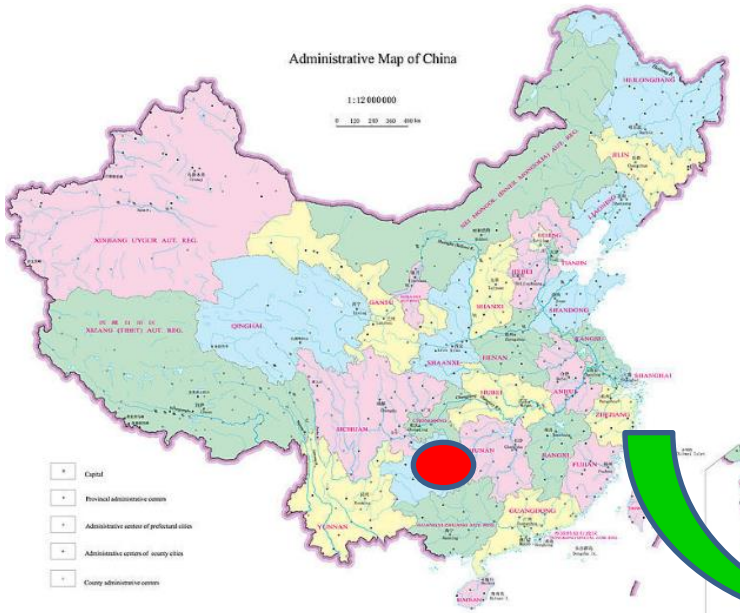
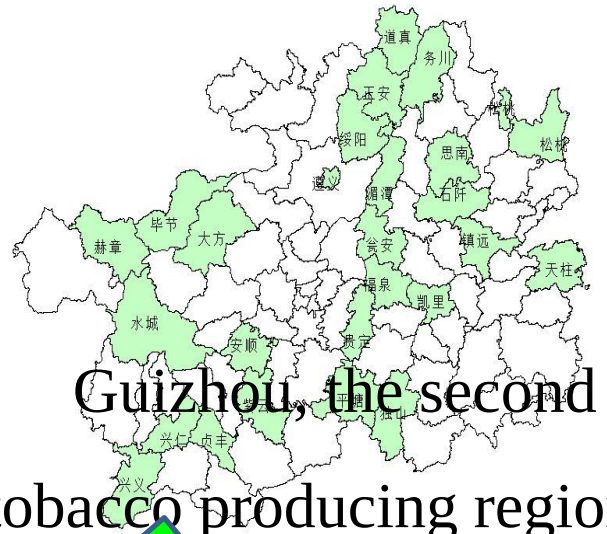
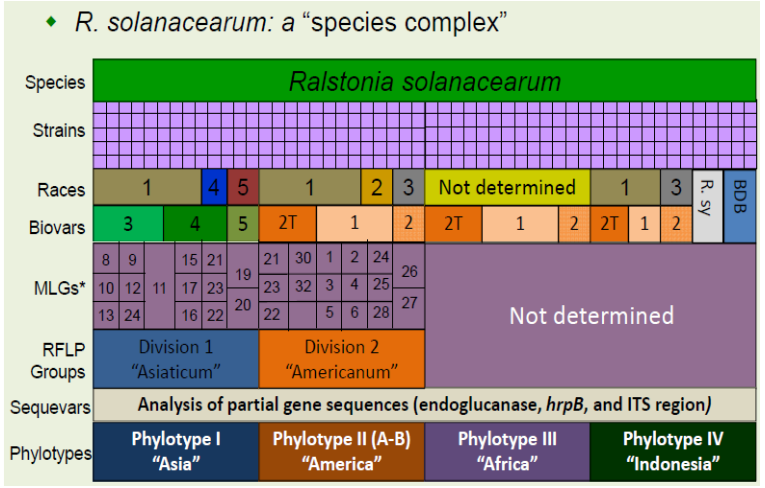
China is the biggest single tobacco market in the world, and accounts for more than **39.6%** of the total global tobacco production and **40%** of the global tobacco consumption.





Guizhou, the second largest tobacco producing region in China



（*Ralstonia solanacearum*）

In **China**： Race 1, Biovars 3 and 4

Integrated bacterial wilt management program in China

- Resistant cultivars: Yunyan 85/87/97; still lack real resistant cultivars.
- Crop rotation: widely recommended, but uneconomical to growers.
- Cultural practices: difficult to deal with.
- Chemicals: streptomycin (efficacy is quite limited).
- Bio-control: Efficacy is instability and limited.

Use dosage of four agronomic chemicals in flue-cured tobacco production seasons in Southwest China

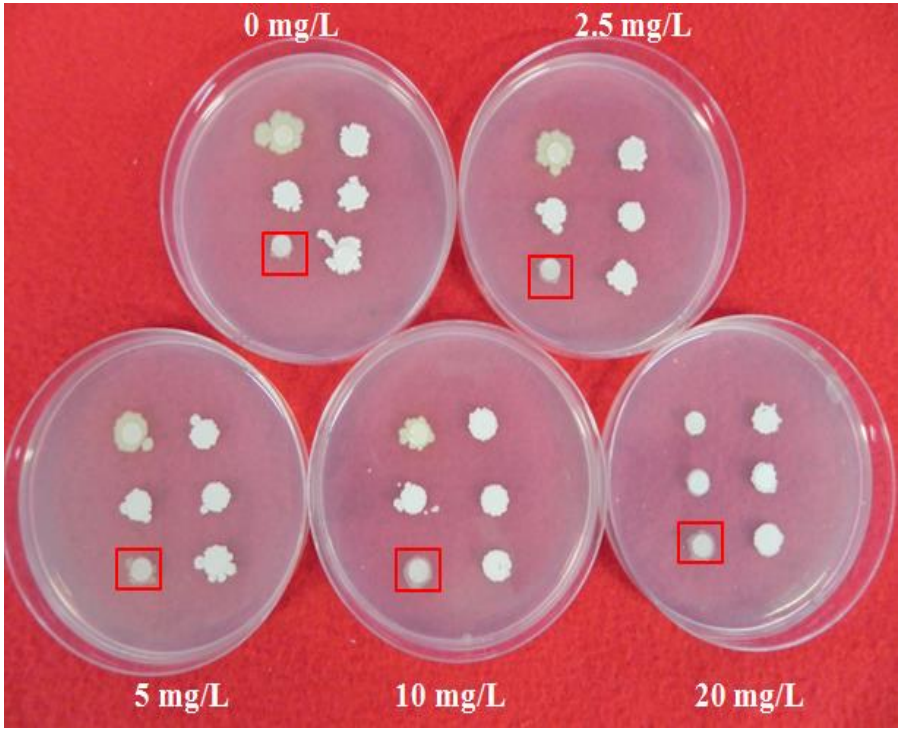
Chemicals	Chemical use rate		Target diseases
	Per application	Per season	
Streptomycin	210-420 g/ha	≥3 applications	tobacco bacterial wilt
Mancozeb	2000-2500 g/ha	≥3 applications	tobacco black shank
Calcium oxide	750-3000 kg/ha	1 application	tobacco bacterial wilt tobacco black shank
Tobacco specific synthetic fertilizer	750 kg/ha	1 application	None

Questions

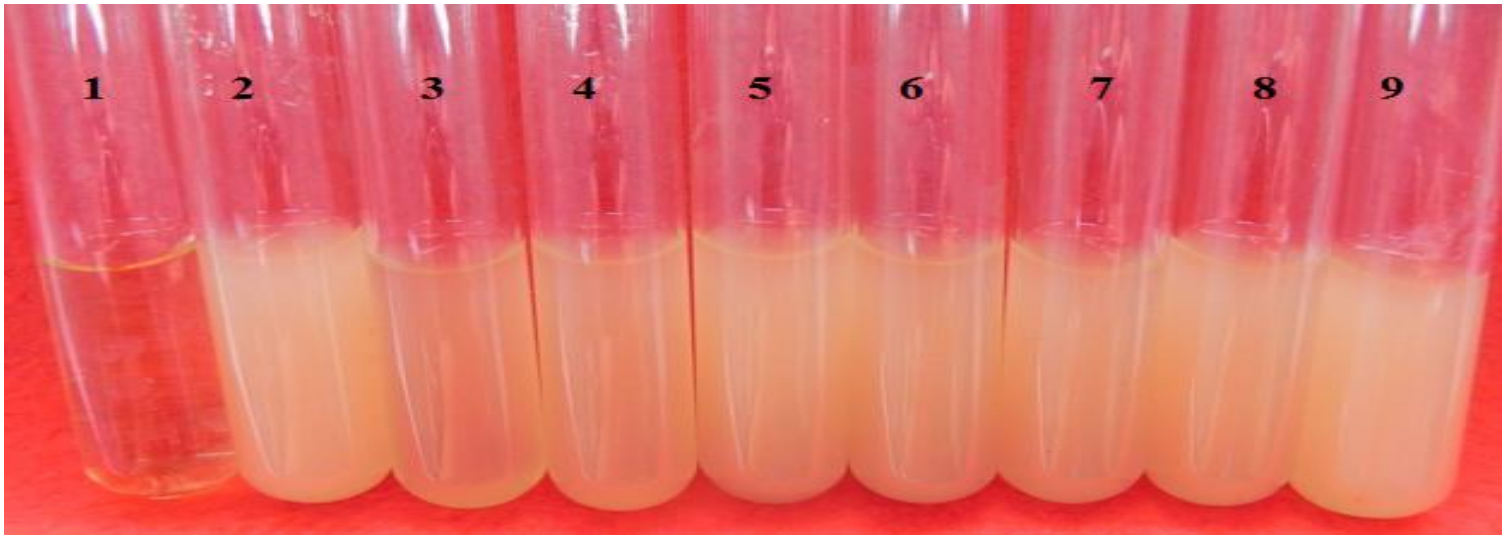
1. Investigate the in vitro activities of four agronomic chemical (streptomycin, calcium oxide, mancozeb, and synthetic fertilizer) against *R. solanacearum*?

2. Evaluate the metabolic reaction of R. solanacearum under pressures of those chemicals from phenomics.

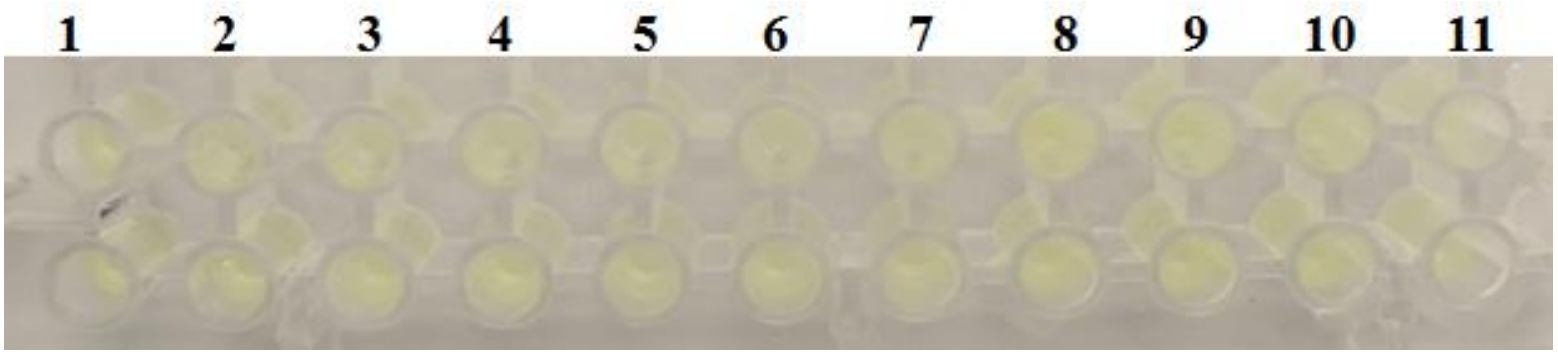
In vitro activity study-streptomycin



R. solanacearum growth inhibition tests by **streptomycin**

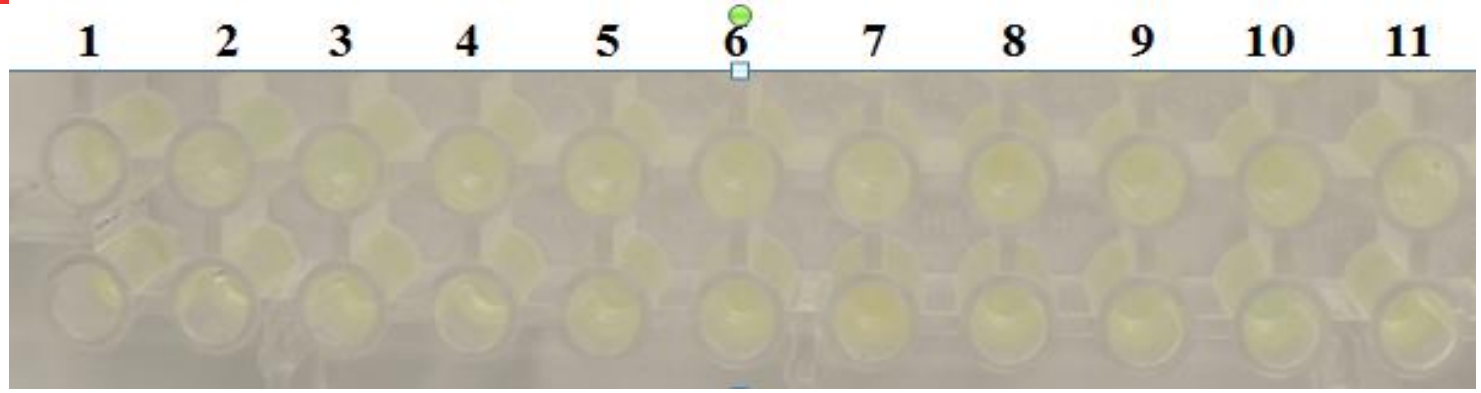


Streptomycin against *R. solanacearum* （1-9：ck-1.56-3.13-6.25-12.5-25-50-100 mg/L）



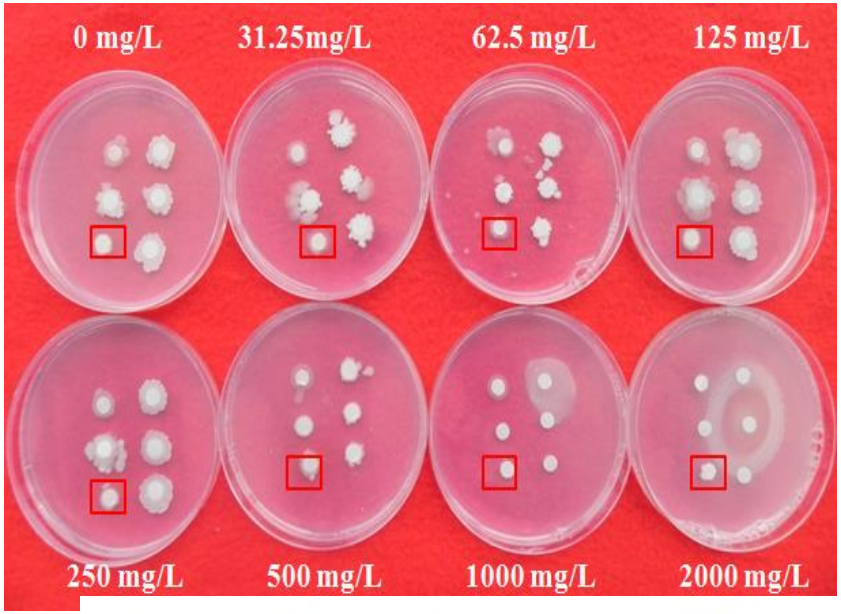
R. solanacearum growth inhibition tests by **streptomycin** on 96-well plate（1-11:200 mg/L -100-50-25-12.5-6.25-3.13-1.56-0.78-0.39-ck）

In vitro activity study-synthetic fertilizer



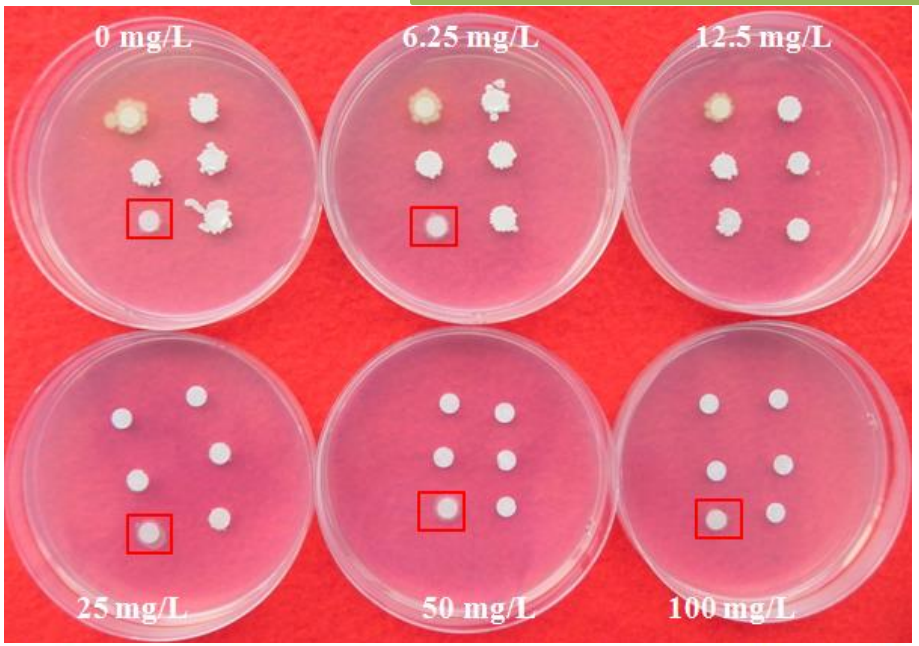
R. solanacearum growth inhibition tests by **synthetic fertilizer** on 96-well plate（1-11:2000 mg/L -1000-500-250-125-62.5-31.3-15.6-7.8-3.9-ck）

In vitro activity study-calcium oxide



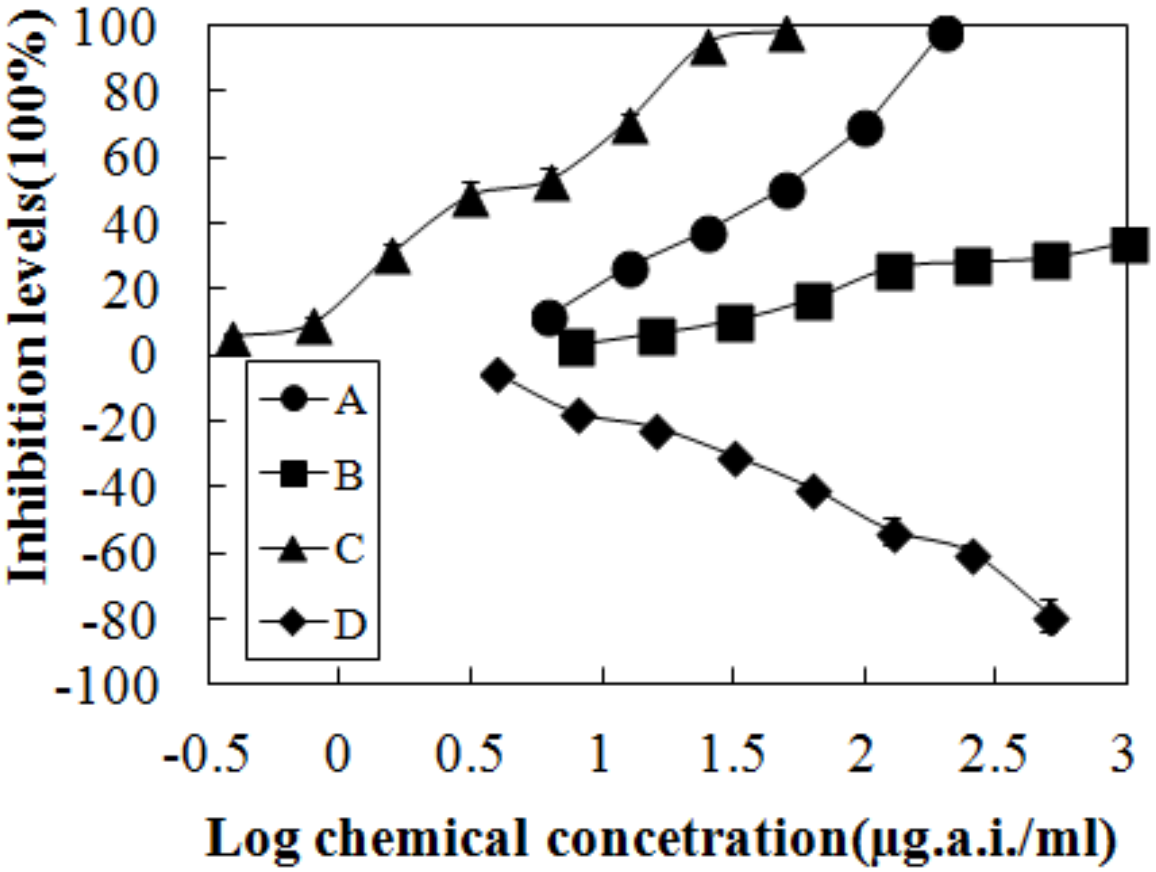
R. solanacearum growth inhibition tests by **calcium oxide** on 96-well plate（1-11:1250 mg/L -625-313-156-78-39-18.5-9.25-4.6-2.3-ck）

In vitro activity study-mancozeb



R. solanacearum growth inhibition tests by **mancozeb**
on 96-well plate（1-11:100 mg/L -50-25-12.5-6.25-3.13-1.56-0.78-0.39-0.2-ck）

Result I



Influence of dose of streptomycin (A), calcium oxide (B), mancozeb (C) and synthetic fertilizer (D) on inhibition of the growth of *R. solanacearum*

EC_{50}, EC_{90} and MIC values of four agronomic chemicals against the growth of *R. solanacearum*

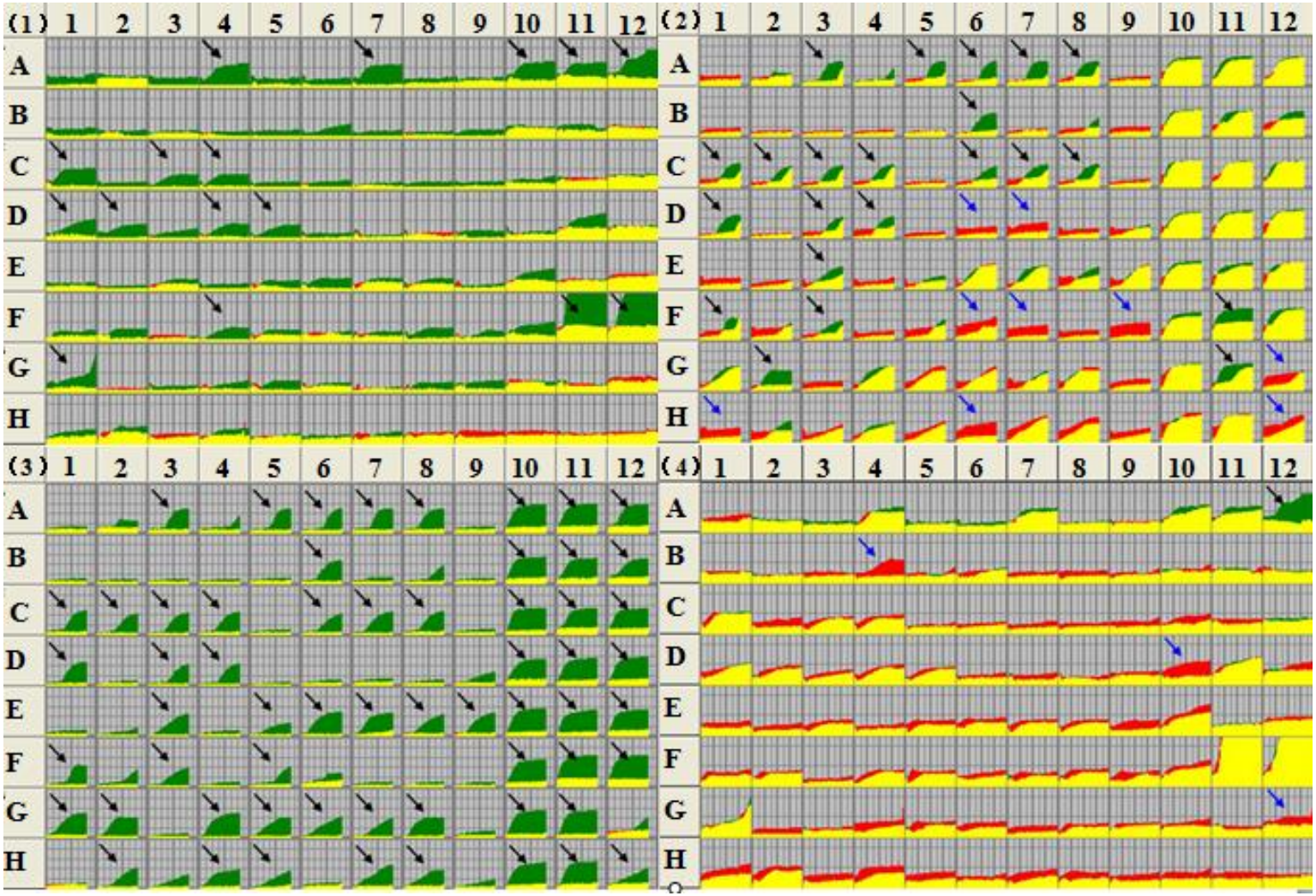
EC_{50}, EC_{90} and MIC values (μg.a.i/ml)	Chemicals			
	Streptomycin sulphate	Calcium oxide	Mancozeb	Synthetic fertilizer
EC_{50}	32.06	>1000	3.80	—
EC_{90}	151.32	>1000	21.48	—
MIC	200	>2000	50	—

Metabolic reaction of *R. solanacearum* under pressures of chemicals

A1 Negative Control	A2 Dextrin	A3 D-Maltose	A4 D-Trehalose	A5 D-Cellobiose	A6 Gentiobiose	A7 Sucrose	A8 D-Turanose	A9 Stachyose	A10 Positive Control	A11 pH 6	A12 pH 5
B1 D-Raffinose	B2 α-D-Lactose	B3 D-Melibiose	B4 β-Methyl-D-Glucoside	B5 D-Salicin	B6 N-Acetyl-D-Glucosamin	B7 N-Acetyl-β-D -Mannosami	B8 N-Acetyl-D-Galactosami	B9 N-Acetyl Neuraminic	B10 1% NaCl	B11 4% NaCl	B12 8% NaCl
C1 α-D-Glucose	C2 D-Mannose	C3 D-Fructose	C4 D-Galactose	C5 3-Methyl Glucose	C6 D-Fucose	C7 L-Fucose	C8 L-Rhamnose	C9 Inosine	C10 1% Sodium Lactate	C11 Fusidic Acid	C12 D-Serine
D1 D-Sorbitol	D2 D-Mannitol	D3 D-Arabitol	D4 myo-Inositol	D5 Glycerol	D6 D-Glucose-6-PO_4	D7 D-Fructose-6 -PO_4	D8 D-Aspartic Acid	D9 D-Serine	D10 Troleandomycin	D11 Rifamycin SV	D12 Minocycline
E1 Gelatin	E2 Glycyl-L-Proline	E3 L-Alanine	E4 L-Arginine	E5 L-Aspartic Acid	E6 L-Glutamic Acid	E7 L-Histidine	E8 L-Pyroglutamic	E9 L-Serine	E10 Lincomycin	E11 Guanidine HCl	E12 Niaproof 4
F1 Pectin	F2 D-Galacturonic	F3 L-Galactonic Acid Lactone	F4 D-Gluconic Acid	F5 D-Glucuronic Acid	F6 Glucuronamide	F7 Mucic Acid	F8 Quinic Acid	F9 D-Saccharic Acid	F10 Vancomycin	F11 Tetrazolium Violet	F12 Tetrazolium Blue
G1 p-Hydroxy-Phenylaceti	G2 Methyl Pyruvate	G3 D-Lactic Acid	G4 L-Lactic Acid	G5 Citric Acid	G6 α-Keto-Glutaric	G7 D-Malic Acid	G8 L-Malic Acid	G9 Bromo-Succinic	G10 Nalidixic Acid	G11 Lithium Chloride	G12 Potassium Tellurite
H1 Tween 40	H2 γ-Amino-Butyric Acid	H3 α-Hydroxy-Butyric Acid	H4 β-Hydroxy-D LButyric	H5 α-Keto-Butyric	H6 Acetoacetic Acid	H7 Propionic Acid	H8 Acetic Acid	H9 Formic Acid	H10 Aztreonam	H11 Sodium Butyrate	H12 Sodium Bromate

Fig. 1. Layout of assays in Biolog GEN III MicroPlate

metabolic reaction of *R. solanacearum* under pressures of chemicals



Metabolic reaction comparisons (1) control and 100 µg/ml streptomycin; (2) control and 2000 µg/ml calcium oxide; (3) control and 20 µg/ml mancozeb; (4) control and 2000 µg/ml synthetic fertilizer

Result II

Carbon sources	Chemicals				
	Control	Streptomycin sulphate	Calcium oxide	Mancozeb	Synthetic fertilizer
Dextrin	±	±	±	±	±
D-Maltose	+	+	±	−	+
D-Trehalose	−	−	−	−	+
D-Cellobiose	+	+	−	−	+
Gentiobiose	+	+	−	−	+
Sucrose	+	−	−	−	+
D-Turanose	+	+	−	−	+
Stachyose	−	−	−	−	−
D-Raffinose	−	−	−	−	−
α-D-Lactose	−	−	−	−	−
D-Melibiose	−	−	−	−	−
β-Methyl-D-Glucoside	−	−	−	−	−
D-Salicin	−	−	−	−	−
N-Acetyl-D-Glucosamine	+	+	−	−	+
N-Acetyl-β-D-Mannosamine	−	−	−	−	−
N-Acetyl-D-Galactosamine	±	±	±	−	±
N-Acetyl Neuraminic Acid	−	−	−	−	−
α-D-Glucose	+	−	±	−	+
α-D-Glucose	+	+	±	−	+
D-Fructose	+	−	±	−	+
D-Galactose	+	−	±	−	+
3-Methyl Glucose	−	−	−	−	−
D-Fucose	+	+	±	−	+

Result II-continued

Carbon sources	Chemicals				
	Control	Streptomycin sulphate	Calcium oxide	Mancozeb	Synthetic fertilizer
D-Galactose	+	−	±	−	+
3-Methyl Glucose	−	−	−	−	−
D-Fucose	+	+	±	−	+
L-Fucose	+	+	±	−	+
L-Rhamnose	+	+	±	−	+
Inosine	−	−	−	−	−
D-Sorbitol	+	−	±	−	+
D-Mannitol	−	−	−	−	−
D-Arabitol	+	+	±	−	+
myo-Inositol	+	−	±	−	+
Glycerol	−	−	−	−	−
D-Glucose- 6-PO_4	−	−	±	−	−
D-Fructose-6-PO_4	−	−	±	−	−
D-Aspartic Acid	−	−	−	−	−
D-Serine	±	±	±	−	±
Gelatin	−	−	±	−	−
Glycyl-L-Proline	−	−	−	−	−
L-Alanine	+	+	±	−	+
L-Arginine	−	−	−	−	−
L-Aspartic Acid	±	±	±	−	±
L-Glutamic Acid	+	+	+	−	+
L-Histidine	+	+	+	−	+
L-Pyroglutamic Acid	+	+	+	−	+
L-Serine	+	+	+	−	+
Pectin	+	+	±	−	+
D-Galacturonic Acid	±	±	±	−	±

Result II-continued

Carbon sources	Chemicals				
	Control	Streptomycin sulphate	Calcium oxide	Mancozeb	Synthetic fertilizer
D-Gluconic Acid	−	−	−	−	−
D-Glucuronic Acid	+	+	+	−	+
Glucuronamide	±	±	±	±	±
Mucic Acid	−	−	±	−	−
Quinic Acid	−	−	−	−	−
D-Saccharic Acid	−	−	±	−	−
p-Hydroxy-Phenylacetic Acid	+	−	+	−	+
Methyl Pyruvate	+	+	−	−	+
D-Lactic Acid Methyl Ester	−	−	−	−	−
L-Lactic Acid	+	+	+	−	+
Citric Acid	+	+	+	−	+
α-Keto-Glutaric Acid	+	+	+	−	+
D-Malic Acid	+	+	+	−	+
L-Malic Acid	+	+	+	−	+
Bromo-Succinic Acid	−	−	−	−	−
Tween 40	±	±	±	±	±
γ-Amino-Butyric Acid	+	+	+	−	+
α-Hydroxy-Butyric Acid	±	±	+	−	±
β-Hydroxy-D,L-Butyric Acid	+	+	+	−	+
α-Keto-Butyric Acid	+	+	+	−	+
Acetoacetic Acid	±	±	+	±	±
Propionic Acid	+	+	+	±	+
Acetic Acid	+	+	+	±	+
Formic Acid	±	±	±	±	±

Result II-continued

Chemical sensitivities	Chemicals				
	Control	Streptomycin sulphate	Calcium oxide	Mancozeb	Synthetic fertilizer
pH 6	+	−	+	±	+
pH 5	+	−	+	±	+
1% NaCl	+	+	+	±	+
4% NaCl	+	+	+	±	+
8% NaCl	+	+	+	±	+
1% Sodium Lactate	+	+	+	−	+
Fusidic Acid	+	+	+	±	+
D-Serine	+	+	+	±	+
Troleandomycin	+	+	+	±	+
Rifamycin SV	+	+	+	±	+
Minocycline	+	+	+	±	+
Lincomycin	+	+	+	−	+
Guanidine HCl	+	+	+	−	+
Niaproof 4	+	+	+	±	+
Vancomycin	+	+	+	±	+
Tetrazolium Violet	+	−	+	±	+
Tetrazolium Blue	+	−	+	±	+
Nalidixic Acid	+	+	+	−	+
Lithium Chloride	+	+	+	−	+
Potassium Tellurite	±	±	+	±	+
Aztreonam	+	+	+	±	+
Sodium Butyrate	+	+	+	±	+
Sodium Bromate	±	±	+	±	±

Summary

- Bacterial growth of *R. solanacearum* was more sensitive to mancozeb, less sensitive to streptomycin, and not sensitive to calcium oxide and synthetic fertilizer.
- Metabolic reaction of *R. solanacearum* on Biolog GEN III Microplates was not or poorly affected by synthetic fertilizer or calcium oxide; while significantly inhibited by mancozeb and poorly inhibited by streptomycin.

Summary

- Substrates utilization of sucrose, α-D-glucose, D-fructose, D-galactose, D-sorbitol, myo-inositol and p-Hydroxy-phenylacetic acid were inhibited by streptomycin and chemical sensitivities of pH 6, pH 5, tetrazolium violet and of tetrazolium blue were also changed by this chemical.
- None carbons could be utilized by the pathogen under the pressure of mancozeb.
- So, streptomycin had limited efficacy against *R. solanacearum*, while mancozeb could be used for bacterial wilt management.

Acknowledgements

Dr. Jin Wang

Dr. Ning Lu

Dr. Maosheng Wang

贵州省科学技术厅
GUIZHOU SCIENCE AND TECHNOLOGY DEPARTMENT

中国烟草
CHINA TOBACCO