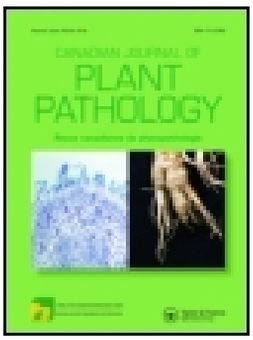

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Bacteria and phytoplasmas/Bactéries et phytoplasmes

Effect of temperature on phenotype characterization of *Ralstonia solanacearum* from tobacco

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Abstract: The incidence of tobacco bacterial wilt, caused by *Ralstonia solanacearum*, is significantly affected by temperature. However, the effect of temperature on the metabolism of carbon and amino acid substrates used in phenotypic characterization of *R. solanacearum* is still unknown. In order to evaluate the effect of temperature on metabolic phenotypes, the phenotypes of the pathogen were characterized and compared with a BIOLOG Phenotype MicroArray (PM) at 20, 24, 30 and 35°C. The results showed that phenotypes of *R. solanacearum* at all four temperatures were mostly similar. However, a few differences were observed, such as in the ability to metabolize L-proline. The bacterium was able to metabolize more than 30% of the tested carbon sources and 83% of amino acid nitrogen substrates. For carbon sources, the most informative utilization patterns were organic acids and carbohydrates; for nitrogen sources, the most informative were amino acids. The bacterium showed much higher metabolic ability at 35°C than at 20, 24 and 30°C, and was more adaptable to osmolytes and pH environments at 30 and 35°C than at 20 and 24°C. It presented no deaminase activity, had high decarboxylase activity at 35°C and low decarboxylase activity at 20 and 24°C. When the phenotypic data were subjected to comparative phenomic analyses, temperature did have a significant effect on the characterization of the pathogen. The results provide clues to possible new methods to manage tobacco bacterial wilt.

Keywords: metabolic phenotype, phenomics, *Ralstonia solanacearum*, temperature, tobacco bacterial wilt

Résumé: L'incidence de la flétrissure bactérienne du tabac, causée par *Ralstonia solanacearum*, est considérablement influencée par la température. Toutefois, l'effet de la température sur le métabolisme du carbone et des substrats d'acides aminés utilisés pour la caractérisation phénotypique de *R. solanacearum* est encore mal compris. Afin d'évaluer l'effet de la température sur les phénotypes métaboliques, les phénotypes de l'agent pathogène ont été caractérisés et comparés à l'aide d'un microréseau de phénotypage BIOLOG à 20, 24, 30 et 35°C. Aux quatre températures, les résultats ont indiqué que les phénotypes de *R. solanacearum* étaient en général similaires. Toutefois, quelques différences ont été observées, comme la capacité de métaboliser la L-proline. La bactérie a pu métaboliser plus de 30% des sources de carbone testées et 83% de la partie azotée des substrats d'acides aminés. Quant aux sources de carbone, les modes d'utilization les plus révélateurs étaient les acides organiques et les hydrates de carbone; en ce qui a trait aux sources d'azote, les plus révélateurs étaient les acides aminés. La bactérie a affiché une capacité métabolique beaucoup plus élevée à 35°C qu'à 20, 24 et 30°C et était plus adaptable aux osmolytes et aux différents pH à 30 et 35°C qu'à 20 et 24°C. Elle n'a affiché aucune activité déaminase, avait une forte activité décarbolyxase à 35°C et une faible à 20 et 24°C. Quand les données phénotypiques ont été soumises aux analyses comparatives basées sur la phénomique, la température a eu un effet significatif sur la caractérisation de l'agent pathogène. Les résultats fournissent des indices quant aux méthodes éventuelles visant à gérer la flétrissure bactérienne du tabac.

Mots clés: flétrissure bactérienne du tabac, phénomique, phénotype métabolique, *Ralstonia solanacearum*, température

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Introduction

Ralstonia solanacearum (E. F. Smith 1896) Yabuuchi et al. is a soil-borne bacterial pathogen (Hayward 1995) distributed worldwide with hosts in 200 different plant species belonging to 50 families (Hayward 1964; Xue et al. 2011). It causes bacterial wilt and damage to many important plants, particularly the Solanaceae crops: tomatoes, tobacco, potatoes, etc. (French and Sequeira 1970; He et al. 1983). Recently, tobacco wilt caused by *R. solanacearum* has become widespread throughout China (Chen et al. 2013). Losses could reach more than 60% in some cropping regions (Peng et al. 2007). Traditionally, *R. solanacearum* strains have been classified into five races based on host range (Buddenhagen et al. 1962) and six biovars based on the metabolism of disaccharides (Hayward et al. 1990), and they have been further subdivided into sequevars based on the endoglucanase gene (Prior and Fegan 2005) and phylotypes based on 16s rRNA gene sequences (Fegan and Prior 2005). In China, most strains of *R. solanacearum* on tobacco belong to Race 1 Biovar 3 (R1Bv3) (Deng et al. 2004; Zou and Xiao 2008).

Ralstonia solanacearum infects tobacco through the roots and ultimately blocks the xylem, resulting in plant wilting. Its infection process is quite complex, and the mechanisms of invasion are still unknown. Many factors affect the disease process, including the physiological state of the pathogen, the host and environmental conditions (Schell 1987; Hayward 1991). Early studies demonstrated that injury of the host, the inoculum load of *R. solanacearum*, soil pH value, type of soil, moisture, and temperature all affected wilt incidence (Singh et al. 2014; Bittner et al. 2016). In addition, temperature is an important factor that affects the growth, motility, aggressiveness and toxic enzyme production of the pathogen and the expression of symptoms in the plant (Schell et al. 1988; Liu et al. 2001; Kang et al. 2002). Although *R. solanacearum* is able to survive at relatively low temperatures for long periods of time (Van Elsas et al. 2000; Milling et al. 2009), most strains of *R. solanacearum* are non-pathogenic below 20°C (Thurston 1963; Ciampi and Sequeira 1980). The aggressiveness decreases when temperature either exceeds 35°C or falls below 18°C (Ciampi and Sequeira 1980; Fajinmi and Fajinmi 2010). Wilt symptoms caused by this pathogen on tobacco rarely appear below 18°C and are most severe when temperature ranges between 25 and 35°C (Thurston 1963; Milling et al. 2009; Wang et al. 2017). Tobacco wilt severity increases with an increase of the temperature into the range of 30–35°C. It is also difficult to maintain host resistance in environments with high temperature and humidity (Hayward 1991). Tobacco plants resistant to *R. solanacearum* at moderate temperatures became more susceptible at higher temperatures (Bittner et al. 2016). Even though the effects of temperature on *R. solanacearum* have been widely studied, how it affects the metabolism of carbon and amino acid substrates used in phenotypic characterization are still unknown.

Traditional cellular metabolic phenotypes were tested one at a time. It was time-consuming and the phenotypes were sometimes not easy to define. Recently, a Phenotypic MicroArray/OmniLog system (PMs), with high throughput, has been developed by Biolog In. (Hayward, CA, USA). The system can simultaneously detect around 1000 metabolic phenotypes at a time (Bochner et al. 2001). This PMs is easy to use and can rapidly deduce the phenotype of a tested cell (Bochner 2003). In this system, phenotypes of the utilization of carbon, nitrogen, phosphorus and sulphur substrates; biosynthetic pathways; and survival in osmotic, ionic and pH conditions can all be determined. Phenotypes of many microorganisms have been analysed with the PMs, such as *Phytophthora parasitica* (Wang et al. 2015a), *Alternaria alternata* (Wang et al. 2015b), *Escherichia coli* (Bochner et al. 2001) and *Bacillus subtilis* (Gusarov et al. 2009).

In this study, the effect of temperature on the phenotype of *R. solanacearum* was characterized and compared with a BIOLOG Phenotype MicroArray (PM) at 20, 24, 30 and 35°C. The temperature 20°C is the minimum detectable temperature of the PMs, and 30 and 35°C are associated with serious bacterial wilt in commercial tobacco fields. Although few management methods are available to control bacterial wilt in tobacco, a detailed understanding of the phenotypes of *R. solanacearum* is valuable in developing management practices to decrease the impact of tobacco bacterial wilt.

Materials and methods

Pathogen, media and chemical preparation

Three R1Bv3 strains of *R. solanacearum* were collected in 2011 from infected tobacco stems with typical bacterial wilt symptoms from a commercial tobacco field in Guizhou province of China. They were identified in our earlier study (Wang et al. 2015c) and utilized here throughout the experiment. These bacteria were incubated in nutrient media with or without agar (beef extract [0.3%] and peptone [0.5%] in water [wt/vol]) (NA or NB) (Wang et al. 2012). For phenotypic characterization testing, they were incubated in Biolog Universal Growth Medium with blood (BUG+B) (Wang et al. 2015c). All materials, reagents and media for the phenotypic study were purchased from Biolog (Hayward, CA, USA).

Effect of temperature on phenotype characterization of R. solanacearum

Phenotype characterization of the *R. solanacearum* R1Bv3 strains was conducted at 20, 24, 30 and 35°C. These temperatures were chosen based on the temperatures in the field that led to symptom development and the available temperature range (20–40°C) of the Biolog PM Omnilog system (Bochner et al. 2008). The strains were incubated on (BUG+B) plates at 30°C in darkness. After 48 h incubation, bacterial cells on the surface of the plates were scraped with a sterile cotton swab, and re-suspended in the medium containing Dye Mix A to make an 85% transmittance cell suspension. Afterwards, 100 µl of cell suspension was added to each well of the Biolog PM plates. A total of 950 different growth conditions were analysed, including 190 different carbon substrates, 95 nitrogen substrates, 59 phosphorus substrates, 35 sulphur substrates, 94 biosynthetic pathways, 285 nitrogen pathways, 96 osmotic and ionic conditions, and 96 pH environments. Plates 1–8 were utilized to test the phenotypes of metabolizing carbon (PM 1–2), nitrogen (PM 3, 6–8), phosphorus (PM 4), sulphur (PM 4) and biosynthetic pathways (PM 5), and plates 9–10 were used to test the phenotypes of the bacterium under various ion/osmotic (PM 9) and pH (PM 10) conditions. Additionally, IF-0 GN Base was used for PM 1–2. The mixture of IF-0 GN Base, 20 mM sodium succinate (pH 7.1), and 0.002 mM ferric citrate were used for PM 3–8. The mixture of IF-10 GN Base and Dye Mix A was used for PM 9–10. After inoculation, the plates were placed in an OmniLog incubator and incubated for 96 h at 20, 24, 30 and 35°C. Data were obtained every 15 min by the Biolog software. The phenotypes of *R. solanacearum* at the different temperatures were compared. Each isolate was examined twice, and the average metabolic phenotype characterization in each well is presented.

Data analyses

Data analysis for the phenotype characterization of *R. solanacearum* was conducted using Kinetic and Parametric software (Biolog). Phenotypes were determined based on the area under the kinetic curve of dye formation (Bochner et al. 2001). Heat maps of phenotype analysis were prepared with the software of HemI (Heatmap IIIlustrator, version 1.0) (Zhao et al. 2017).

Results

Effect of temperature on phenotype characterization of R. solanacearum

Phenotype characterization of *R. solanacearum* at temperatures of 20, 24, 30 and 35°C was conducted using the Biolog PM plates 1–10. All three isolates showed a similar metabolic fingerprint at each test temperature. The results of the phenotypic characterization of *R. solanacearum* R1Bv3 at all four temperatures were similar. However, a few differences were observed (Fig. 1). For the utilization of carbon sources, *R. solanacearum* showed the highest use ratio (54%) at 35°C, followed by 24°C (39%) and 20°C (36%), and the lowest use ratio (30%) was found at 30°C (Table 1). For amino acid nitrogen substrate metabolization, the highest use ratio (96%) was found at 35°C, while the other temperatures all showed a use ratio of 83%. For peptide nitrogen substrate metabolization, the highest utilization ratio (100%) was found at 35° C, followed by 20°C (80%) and 24°C (73%), and the lowest utilization ratio (60%) was recorded at 30°C. For phosphorus substrate metabolization, the highest utilization ratio (100%) was found at 30 and 35°C followed by 20 and 24°C (85%). For sulphur substrate metabolization, *R. solanacearum* at all four temperatures presented 100% utilization. Meanwhile, all four biosynthetic pathway tests showed 100% utilization. In the osmotic and ionic adaptability tests, *R. solanacearum* metabolized in more than 13% of the tested conditions. The highest use ratio (74%) was found at 35°C followed by 30°C (25%), and the lowest use ratio was recorded at 20 and 24°C (12.5% and 17%). For pH environment adaptability tests at the four temperatures, the metabolization ratio ranged from 18% to 44%. The highest ratio was found at 35°C (44%), followed by 30°C (20%) and 24°C (19%), and the lowest ratio was recorded at 20°C (18%) (Table 1).

Using data from PM1 and PM2 (carbon sources), *R. solanacearum* was able to use more than 57 different carbon sources (Tables 2, and (3). In addition, approximately 34 compounds were effectively utilized by the pathogen at all four temperatures, including N-acetyl-D-glucosamine, L-proline, D-trehalose, glycerol, D-glucuronic acid, DL-malic acid, L-asparagine, sucrose, L-glutamine and fumaric acid (Tables 2 and 3). In comparison, approximately 83 compounds could not be metabolized at any of the four tested temperatures, including D-serine, L-fucose, D-galactonic acid-γ-lactone,

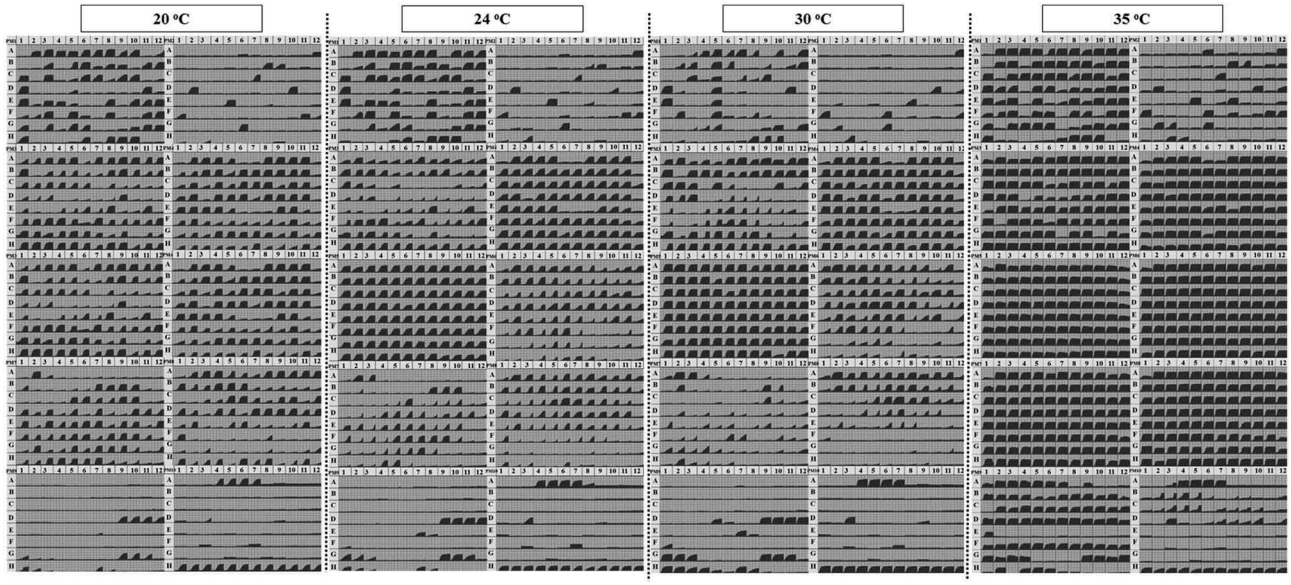

Fig. 1 Data for the Biolog Phenotype MicroArray PM 1–10 plates for *Ralstonia solanacearum*. The phenotypes of *R. solanacearum* were tested at 20, 24, 30 and 35°C, respectively. The PM 1 to PM 10 plates for each isolate are presented at the first row, first column; first row, second column; second row, first column; second row, second column; third row, first column; third row, second column; fourth row, first column; fourth row, second column; fifth row, first column; and fifth row, second column, respectively. Numbers 1–12 on the horizontal axis and letters A–H on the vertical axis denote the layout of the tested Biolog MicroPlate. Utilization by the isolate of *R. solanacearum* at the four different temperatures is indicated by the green areas of the growth curve for each substrate.

Table 1. Ratios of substrate metabolization by *Ralstonia solanacearum* at four different temperatures in Biolog PM 1 to 10 MicroPlates.

Temperature (°C)	Carbon substrate (%)[a]	Amino acid nitrogen substrates (%)	Peptide nitrogen substrates (%)	Phosphorus substrates (%)	Sulphur substrates (%)	Biosynthetic pathways (%)	Osmotic and ionic conditions (%)	pH condition (%)
20	36	83	80	85	100	100	13	18
24	39	83	73	85	100	100	17	19
30	30	83	60	100	100	100	25	20
35	54	96	100	100	100	100	74	44

[a]Percentages listed in each column means the number of substrates metabolized by *R. solanacearum* in each kind of Biolog microplate divided by the total number of the substrates in each kind of Biolog microplate.

1,2-propanediol, D-threonine, propionic acid, tricarballylic acid, and acetoacetic acid (Tables 2 and 3). For the PM analysis at four different temperatures, the carbon utilization level in several substrates was different, especially for the sources of L-proline, D-trehalose, dulcitol, D-ribose, adonitol, glycogen, laminarin, pectin, D-melezitose, etc. (Tables 2 and 3). *Ralstonia solanacearum* presented higher carbon substrate utilization at 35°C than at the other three temperatures.

Using the PM3 plate, *R. solanacearum* was tested for its ability to grow on 95 different nitrogen sources (amino acids). Approximately 33 compounds were effectively utilized by the pathogen at all four temperatures, including ammonia, L-alanine, L-asparagine, L-glutamic acid, L-glutamine, L-histidine, L-proline and L-threonine (Table 4), while other compounds could not be used or were poorly utilized (Fig. 1, Table 4). Phenotype characteristics of *R. solanacearum* R1Bv3 at all four temperatures were mostly similar. However, a few differences were observed such as the ability to metabolize glycine, D-asparagine, D-lysine, D-serine, D-valine, N-amylamine, agmatine, tyramine, adenine, etc. (Table 4). *Ralstonia solanacearum* at 35°C presented higher amino acid nitrogen substrate utilization than those at the other three temperatures.

Using the PM4 plate, *R. solanacearum* was tested for its ability to grow on 59 different phosphorus

Table 2. Metabolic profiling of *Ralstonia solanacearum* at four different temperatures in PM 1 MicroPlate substrates.

Cond.[a]	Substrate	Temperature °C				Cond.[a]	Substrate	Temperature °C			
		20	24	30	35			20	24	30	35
A1	Negative Control	-	-	-	-	C1	D-Glucose-6-Phosphate	++	++	-	+++
A2	L-Arabinose	++	++	-	+++	C2	D-Galactonic Acid-γ-Lactone	-	-	-	-
A3	N-Acetyl-D-Glucosamine	+++	+++	+++	+++	C3	D,L-Malic Acid	++	+++	+++	+++
A4	D-Saccharic Acid	++	++	++	+++	C4	D-Ribose	-	++	-	+++
A5	Succinic Acid	++	++	++	+++	C5	Tween20	+	++	++	+++
A6	D-Galactose	++	+++	++	++	C6	L-Rhamnose	++	+++	-	+++
A7	L-Aspartic Acid	++	+++	+++	+++	C7	D-Fructose	++	++	++	+++
A8	L-Proline	+++	+++	+++	+++	C8	Acetic Acid	+	+	+	+++
A9	D-Alanine	++	-	-	++	C9	α-D-Glucose	++	+++	+++	+++
A10	D-Trehalose	++	+++	+++	+++	C10	Maltose	++	+++	-	+++
A11	D-Mannose	-	++	-	+++	C11	D-Melibiose	-	-	-	++
A12	Dulcitol	++	+++	+++	+++	C12	Thymidine	-	-	-	+++
B1	D-Serine	-	-	-	-	D1	L-Asparagine	+++	+++	+++	+++
B2	D-Sorbitol	-	+	++	+++	D2	D-Aspartic Acid	-	-	-	++
B3	Glycerol	++	+++	++	+++	D3	D-Glucosaminic Acid	-	-	-	+++
B4	L-Fucose	-	-	-	-	D4	1,2-Propanediol	-	-	-	-
B5	D-Glucuronic Acid	+++	+++	+++	+++	D5	Tween40	+	++	++	+++
B6	D-Gluconic Acid	++	+++	+++	+++	D6	α-Keto-Glutaric Acid	-	-	-	+++
B7	D,L-α-Glycerol Phosphate	+	+	-	+++	D7	α-Keto-Butyric Acid	-	-	-	+
B8	D-Xylose	++	++	-	+++	D8	α-Methyl-D-Galactoside	-	+	-	++
B9	L-Lactic Acid	+	++	+	+++	D9	α-D-Lactose	-	+	-	++
B10	Formic Acid	-	-	-	++	D10	Lactulose	-	-	-	++
B11	D-Mannitol	++	+++	++	+++	D11	Sucrose	+++	+++	+++	+++
B12	L-Glutamic Acid	+++	+++	+++	+++	D12	Uridine	-	-	-	+++

Cond.[a]	Substrate	Temperature °C				Cond.[a]	Substrate	Temperature °C			
		20	24	30	35			20	24	30	35
E1	L-Glutamine	+++	+++	+++	+++	G1	Glycyl-L-Glutamic Acid	++	-	-	+++
E2	M-Tartaric Acid	-	-	-	++	G2	Tricarballylic Acid	-	-	-	-
E3	D-Glucose-1-Phosphate	++	++	-	+++	G3	L-Serine	++	+++	+	+++
E4	D-Fructose-6-Phosphate	++	++	-	-	G4	L-Threonine	-	+	++	+++
E5	Tween80	-	++	++	+++	G5	L-Alanine	+++	+++	+++	+++
E6	α-Hydroxy Glutaric Acid-γ-Lactone	-	-	-	-	G6	L-Alanyl-Glycine	++	++	-	+++
E7	α-Hydroxy Butyric Acid	-	-	-	+	G7	Acetoacetic Acid	-	-	-	-
E8	β-Methyl-DGlucoside	+++	+++	-	+++	G8	N-Acetyl-β-D-Mannosamine	-	-	-	+
E9	Adonitol	-	-	-	++	G9	MonoMethyl Succinate	-	-	+	++
E10	Maltotriose	++	+	-	+++	G10	Methyl Pyruvate	+	++	++	+++
E11	2-Deoxy-Adenosine	++	++	-	+++	G11	D-Malic Acid	++	++	-	+++
E12	Adenosine	++	+++	-	+++	G12	L-Malic Acid	++	++	++	+++
F1	Glycyl-L-Aspartic Acid	+	-	-	+	H1	Glycyl-L-Proline	+++	-	-	+++
F2	Citric Acid	+	++	++	++	H2	p-Hydroxy PhenylAcetic Acid	-	-	-	-
F3	M-Inositol	+++	+++	-	+++	H3	m-Hydroxy PhenylAcetic Acid	-	-	-	-
F4	D-Threonine	-	-	-	-	H4	Tyramine	-	-	-	-

(*Continued*)

Table 2. (Continued.)

Cond.[a]	Substrate	Temperature °C				Cond.[a]	Substrate	Temperature °C			
		20	24	30	35			20	24	30	35
F5	Fumaric Acid	+++	+++	+++	+++	H5	D-Psicose	-	+	-	-
F6	Bromo Succinic Acid	+	-	+	+++	H6	L-Lyxose	++	++	-	-
F7	Propionic Acid	-	-	-	-	H7	Glucuronamide	-	-	+	++
F8	Mucic Acid	++	++	++	+++	H8	Pyruvic Acid	++	+++	+++	+++
F9	Glycolic Acid	-	-	+	+++	H9	L-Galactonic Acid-γ-Lactone	++	+++	+++	+++
F10	Glyoxylic Acid	-	-	-	-	H10	D-Galacturonic Acid	++	+++	+++	+++
F11	D-Cellobiose	++	+++	-	+++	H11	Phenylethylamine	-	-	-	-
F12	Inosine	+++	+++	-	+++	H12	2-Aminoethanol	-	-	-	-

[a]Cond. is short for the assay conducted on the Biolog PM 1 plate. '–', '+', '++' and '+++' means that *R. solanacearum* could not utilize the tested substrate, utilized poorly, moderately and effectively, respectively in Biolog PM 1 Microplate after 8 days incubation at 20, 24, 30, 35°C, respectively.

compounds (Wells A2 to E12) and on 35 different sulphur substrates (Wells F2 to H12). *Ralstonia solanacearum* was very efficient in utilizing these substrates at all four temperatures. More than 46 phosphorus compounds were effectively utilized (Table 5). *Ralstonia solanacearum* metabolized all tested phosphorus substrates at both 30 and 35°C. There were nine compounds that could not be utilized by the pathogen at either 20 or 24°C, including triethyl phosphate, hypophosphite, adenosine-3′,5′-cyclic monophosphate, guanosine-3′,5′-cyclic monophosphate, uridine-3′,5′-cyclic monophosphate, phosphonoacetic acid, 2-aminoethyl phosphonic acid, methylene diphosphonic acid and thymidine 3′,5′-cyclic monophosphate (Table 5). In comparison, all sulphur substrates were metabolized by *R. solanacearum* at all four test temperatures. A higher utilization efficiency was found for most sulphur substrates at elevated temperatures (Table 5).

Using the PM5 plate, *R. solanacearum* presented 95 different biosynthetic pathways at all four temperatures (Fig. 1). Meanwhile, using the PM6, PM7 and PM8 plates, *R. solanacearum* was tested for its ability to grow on 285 different peptide nitrogen substrates. The pathogen metabolized more than 60% of the tested compounds (Fig. 1, Table 1). At 35°C, it metabolized all tested peptide nitrogen substrates, while fewer substrates were utilized at 20, 24 and 30°C. For all four temperatures, approximately 116 peptide nitrogen substrates were metabolized efficiently, including Ala-Ala, Ala-Arg, Ala-Asn and Met-Gln (Fig. 1).

Plates PM9 and PM10 were used to test bacterial growth under various stress conditions. For the tests at 20, 24 and 30°C, *R. solanacearum* showed similar metabolic fingerprints on plates PM9 and PM10. It showed active metabolism with up to 20% ethylene glycol, 2% urea, 100 mM sodium phosphate (pH 7.0), 50 mM ammonium sulphate (pH 8.0), 80 mM sodium nitrate and 20 mM sodium nitrite. In comparison, the metabolic fingerprint of the bacterium at 35°C was quite different (Fig. 1, Tables 6 and 7). The pathogen showed active metabolism with up to 7% sodium chloride, 6% potassium chloride, 5% sodium sulphate, 20% ethylene glycol, 1% sodium formate, 12% sodium lactate, 200 mM sodium phosphate (pH 7.0), 100 mM ammonium sulphate (pH 8.0), 100 mM sodium nitrate and 20 mM sodium nitrite (Table 6). When combined with different osmolytes and a treatment of 6% sodium chloride, *R. solanacearum* exhibited active growth in all tests (plate PM9, Wells B1 to C12), except for potassium chloride (plate PM 9, Well C1). The *R. solanacearum*

Table 3. Metabolic profiling of *Ralstonia solanacearum* at four different temperatures in PM 2 MicroPlate substrates.

Cond.[a]	Substrate	Temperature °C				Cond.[a]	Substrate	Temperature °C			
		20	24	30	35			20	24	30	35
A1	Negative Control	-	-	-	-	C1	Gentiobiose	-	-	-	-
A2	Chondroitin Sulphate C	-	-	-	-	C2	L-Glucose	-	-	-	-
A3	α-Cyclodextrin	-	-	-	-	C3	Lactitol	-	-	-	-
A4	β-Cyclodextrin	-	-	-	-	C4	D-Melezitose	-	-	-	-
A5	γ-Cyclodextrin	-	-	-	-	C5	Maltitol	-	-	-	-
A6	Dextrin	-	+	+	+++	C6	α-Methyl-D-Glucoside	-	-	-	-
A7	Gelatin	-	-	-	-	C7	β-Methyl-D-Galactoside	++	++	-	+++
A8	Glycogen	-	-	-	-	C8	3-Methyl-Glucose	-	-	-	-
A9	Inulin	-	-	-	-	C9	β-Methyl-D-Glucuronic Acid	-	-	-	-
A10	Laminarin	-	-	-	-	C10	α-Methyl-D-Mannoside	-	-	-	-
A11	Mannan	-	-	-	-	C11	β-Methyl-D-Xyloside	-	-	-	-
A12	Pectin	++	++	+++	+++	C12	Palatinose	-	-	-	-
B1	N-Acetyl-D-Galactosamine	-	-	-	-	D1	D-Raffinose	-	-	-	-
B2	N-Acetyl-Neuraminic Acid	-	-	-	-	D2	Salicin	++	++	-	+++
B3	β-D-Allose	-	-	-	-	D3	Sedoheptulosa	-	-	-	-
B4	Amygdalin	-	-	-	-	D4	L-Sorbose	-	-	-	-
B5	D-Arabinose	-	-	-	-	D5	Stachyose	-	-	-	-
B6	D-Arabitol	-	-	-	-	D6	D-Tagatose	-	-	-	-
B7	L-Arabitol	-	-	-	-	D7	Turanose	-	-	-	+
B8	Arbutin	++	++	-	+++	D8	Xylitol	-	-	-	-
B9	2-Deoxy-DRibose	++	++	-	+++	D9	N-Acetyl-DGlucosaminitol	-	-	-	-
B10	I-Erythritol	-	-	-	-	D10	γ-Amino Butyric Acid	+++	++	++	+++
B11	D-Fucose	-	-	-	+	D11	δ-AminoValeric Acid	-	-	-	-
B12	3–0-β-D-Galacto-pyranosyl-D-Arabinose	-	+	-	+	D12	Butyric Acid	-	-	++	++

Cond.[a]	Substrate	Temperature °C				Cond.[a]	Substrate	Temperature °C			
		20	24	30	35			20	24	30	35
E1	Capric Acid	-	-	-	-	G1	Acetamide	-	-	-	-
E2	Caproic Acid	-	-	+	-	G2	L-Alaninamide	-	-	+++	+++
E3	Citraconic Acid	-	-	-	-	G3	N-Acetyl-LGlutamic Acid	-	-	-	++
E4	Citramalic Acid	-	-	-	-	G4	L-Arginine	-	-	-	-
E5	D-Glucosamine	++	+++	+	+++	G5	Glycine	-	-	-	-
E6	2-Hydroxy Benzoic Acid	-	-	-	-	G6	L-Histidine	+++	+++	+++	+++
E7	4-Hydroxy Benzoic Acid	-	-	-	+	G7	L-Homoserine	-	-	-	-
E8	β-Hydroxy Butyric Acid	-	+	+++	+++	G8	Hydroxy-L-Proline	-	-	-	-
E9	γ-Hydroxy Butyric Acid	-	-	-	-	G9	L-Isoleucine	-	-	-	-
E10	α-KetoValeric Acid	-	-	-	-	G10	L-Leucine	-	-	-	-
E11	Itaconic Acid	-	-	-	-	G11	L-Lysine	-	-	-	-
E12	5-Keto-D-Gluconic Acid	-	-	+	+	G12	L-Methionine	-	-	-	-
F1	D-Lactic Acid MethylEster	++	++	+++	+++	H1	L-Ornithine	-	-	-	-
F2	Malonic Acid	-	-	-	-	H2	L-Phenylalanine	-	-	-	-
F3	Melibionic Acid	-	-	-	-	H3	L-Pyroglutamic Acid	+	++	+++	++

(*Continued*)

Table 3. (Continued.)

Cond.[a]	Substrate	Temperature °C				Cond.[a]	Substrate	Temperature °C			
		20	24	30	35			20	24	30	35
F4	Oxalic Acid	-	-	-	-	H4	L-Valine	-	-	+	++
F5	Oxalomalic Acid	-	-	-	-	H5	D,L-Carnitine	-	-	-	-
F6	Quinic Acid	-	+	+++	+++	H6	Sec-Butylamine	-	-	-	-
F7	D-Ribono-1,4-Lactone	-	-	-	-	H7	D,L-Octopamine	-	-	-	-
F8	Sebacic Acid	-	-	+	++	H8	Putrescine	-	-	-	-
F9	Sorbic Acid	-	-	-	-	H9	Dihydroxy Acetone	-	-	-	-
F10	Succinamic Acid	-	-	+	+	H10	2,3-Butanediol	-	-	-	-
F11	D-Tartaric Acid	++	++	-	+++	H11	2,3-Butanone	-	-	-	-
F12	L-Tartaric Acid	+	++	++	+++	H12	3-Hydroxy2-Butanone	-	-	-	-

[a]Cond. is short for the assay conducted on the Biolog PM 2 plate. '–', '+', '++' and '+++' means that *R. solanacearum* could not utilize the tested substrate, utilized poorly, moderately and effectively, respectively in Biolog PM 2 Microplate after 8 days incubation at 20, 24, 30, 35°C, respectively.

isolates showed good growth between pH 5.0 and 8.0, with an optimal pH of approximately 6.0. While the pathogen at 35°C still presented active growth at a pH of 4.5, it also exhibited no growth at 20°C when the pH value was 8.0. In the combination of different amino acids with a pH value of 4.5, *R. solanacearum* at 20, 24 and 30°C presented no growth in most tests (plate PM10, Wells B1 to D12) except for when combined with L-norvaline (plate PM10, Well D3). Meanwhile, for the test at 35°C, approximately 12 amino acids were metabolized effectively. In comparison, the pathogen exhibited no growth at all four temperatures with the combination of different amino acids and a pH of 9.5 (plate PM10, Wells E1 to F12). When combined with amino acids at pH 4.5 and pH 9.5, wells B1-D12 and E1-G12 (PM 10) investigated the decarboxylase and deaminase activities of *R. solanacearum*, respectively. *Ralstonia solanacearum* showed no deaminase activity at all four temperatures and showed active decarboxylase activity at 35°C (Fig. 1, Table 7). Additionally, *R. solanacearum* at all four temperatures presented similar metabolic fingerprints under pressure from other compounds in plate PM 10 (Wells H1 to H12).

Discussion

Temperature is a key factor for disease occurrence and development. While numerous studies have been performed on the effect of temperature on the growth of *R. solanacearum* (Bocsanczy et al. 2012) and on the disease incidence of tobacco bacterial wilt (Bittner et al. 2016; Wang et al. 2017), the effect of temperature on the phenotype of *R. solanacearum* is still poorly explored. The metabolic phenomics of the R1Bv3 strain of *R. solanacearum* at four different temperatures was systematically analysed using the PMs.

A previous study with *R. solanacearum* from tomato showed that the lowest pathogenic temperature was 22°C, and the disease latent period significantly decreased with the increase in temperature (Zhuo 2005). The most suitable temperature for pepper bacterial wilt development was 30°C (Tran and Kim 2010). Our early studies showed that temperatures needed for disease development on detached leaves ranged from 20 to 35°C, whereas at 18°C the strain was non-pathogenic (Wang et al. 2017). All these findings agree with previous reports revealing non-pathogenicity of most strains of *R. solanacearum* below 20°C (Ciampi and Sequeira 1980; Bocsanczy et al. 2012). The aggressiveness of the pathogen increased greatly when temperature increased from 25 to 35°C. This might be due to the

Table 4. Metabolic profiling of *Ralstonia solanacearum* at four different temperatures in PM 3 MicroPlate substrates.

Cond.[a]	Substrate	Temperature °C				Cond.[a]	Substrate	Temperature °C			
		20	24	30	35			20	24	30	35
A1	Negative Control	++	+	+++	+++	C1	L-tyrosine	++	+++	+++	+++
A2	Ammonia	++	++	+++	++	C2	L-valine	++	++	+++	+++
A3	Nitrite	++	+	++	++	C3	D-alanine	++	+	+++	+++
A4	Nitrate	++	+	+++	+++	C4	D-asparagine	++	-	-	+++
A5	Urea	++	+	++	++	C5	D-aspartic acid	++	+	+++	+++
A6	Bluret	+	+	+++	+++	C6	D-glutamic acid	++	-	++	++
A7	L-alanine	++	++	+++	+++	C7	D-lysine	-	-	-	++
A8	L-arginine	++	+	+++	+++	C8	D-serine	++	-	-	+++
A9	L-asparagine	++	++	+++	+++	C9	D-valine	+	-	-	+++
A10	L-aspartic acid	++	+	+++	+++	C10	L-citrulline	+	+	+++	+++
A11	L-cysteine	++	+	+++	+++	C11	L-homoserine	-	-	-	+++
A12	L-glutamic acid	++	++	+++	+++	C12	L-ornithine	+	++	+++	+++
B1	L-glutamine	+++	++	+++	+++	D1	N-acetyl-D,L-glutamic acid	+	+	++	+++
B2	Glycine	-	++	-	+++	D2	N-phthaloyl-L-glutamic Acid	+	++	++	+++
B3	L-histidine	++	++	+++	+++	D3	L-pyroglutamic acid	++	+	+++	+++
B4	L-isoleucine	++	++	+++	+++	D4	Hydroxylamine	-	-	-	-
B5	L-leucine	++	+	+++	+++	D5	Methylamine	-	+	+	+++
B6	L-lysine	+	+	++	+++	D6	N-amylamine	-	-	+	++
B7	L-methionine	+	+	+++	+++	D7	N-butylamine	-	+	+	++
B8	L-phenylalanine	++	++	+++	+++	D8	Ethylamine	+	+	+	++
B9	L-proline	+++	+++	+++	+++	D9	Ethanolamine	++	-	+	++
B10	L-serine	++	+	+++	+++	D10	Ethylenediamine	-	+	-	+++
B11	L-threonine	++	++	+++	+++	D11	Putrescine	-	+	-	+++
B12	L-tryptophan	++	++	+++	+++	D12	Agmatine	-	-	-	+++

Cond.[a]	Substrate	Temperature °C				Cond.[a]	Substrate	Temperature °C			
		20	24	30	35			20	24	30	35
E1	Histamine	+	+	+	+	G1	Xanthine	++	+	+++	+++
E2	ß-phenylethyl-amine	-	-	-	+++	G2	Xanthosine	++	++	+	+++
E3	Tyramine	-	-	-	+++	G3	Uric acid	+	++	+++	+++
E4	Acetamide	+	+	+	+++	G4	Alloxan	++	++	++	+++
E5	Formamide	+	+	+	+++	G5	Allantoin	++	++	+++	+++
E6	Glucuronamide	++	++	+++	+++	G6	Parabanic acid	++	+	+++	+++
E7	D,L-lactamide	+	+	+	+++	G7	D,L-α-amino-N-butyric acid	+	-	+	-
E8	D-glucosamine	+++	+++	+	+++	G8	γ-amino-N-butyric acid	+	++	+++	+++
E9	D-galactosamine	-	+	+	+++	G9	ε-amino-N-caproic acid	++	+	++	+++
E10	D-mannosamine	-	+	+	+++	G10	D,L-α-amino-caprylic acid	-	-	-	-
E11	N-acetyl-D-glucosamine	+++	+++	++	+++	G11	δ-amino-N-valeric acid	++	+	+	+++
E12	N-acetyl-D-galactosamine	-	-	-	+++	G12	α-amino-N-valeric acid	+	-	-	++
F1	N-acetyl-D-mannosamine	++	++	+	+++	H1	Ala-asp	+++	++	+++	+++
F2	Adenine	++	++	-	-	H2	Ala-gln	+++	+++	+++	+++
F3	Adenosine	+++	++	+	+++	H3	Ala-glu	+++	++	+++	+++

(*Continued*)

Table 4. (Continued.)

Cond.[a]	Substrate	Temperature °C				Cond.[a]	Substrate	Temperature °C			
		20	24	30	35			20	24	30	35
F4	Cytidine	++	++	++	++	H4	Ala-gly	++	++	++	++
F5	Cytosine	++	+	++	++	H5	Ala-his	++	++	++	++
F6	Guanine	+	+	++	+	H6	Ala-leu	++	++	+	++
F7	Guanosine	++	++	++	++	H7	Ala-thr	++	++	+	++
F8	Thymine	++	+	+	++	H8	Gly-asn	++	++	++	++
F9	Thymidine	++	++	+	++	H9	Gly-gln	++	++	++	++
F10	Uracil	++	++	+	++	H10	Gly-glu	++	++	+	++
F11	Uridine	++	++	+	++	H11	Gly-met	++	++	++	++
F12	Inosine	++	++	++	++	H12	Met-ala	++	++	++	++

[a]Cond. is short for the assay conducted on the Biolog PM 3 plate. '–', '+', '++' and '+++' means that *R. solanacearum* could not utilize the tested substrate, utilized poorly, moderately and effectively, respectively in Biolog PM 3 Microplate after 8 days incubation at 20, 24, 30, 35°C, respectively.

growth rate, motility and toxic enzyme production of the pathogen being much higher at higher temperatures. An earlier study demonstrated that a tobacco plant resistant to *R. solanacearum* at moderate temperatures became more susceptible at high temperatures (Katawczik and Mila 2012). Meanwhile, previous studies also showed that tobacco cultivars at all resistance levels could be infected by the bacterium at 35°C (Bittner et al. 2016). Therefore, it was important to choose some typical standard temperatures (20–35°C) for the study of *R. solanacearum* here. However, some more temperatures, such as 18 and 40°C, should be selected for phenomics study of *R. solanacearum* in future work.

Ralstonia solanacearum can infect several hundred host plant species worldwide and can survive in many kinds of soil that are distributed in various conditions (Hayward 1991; Schell 2000). This suggests significant adaptability of the pathogen. At four different test temperatures, a large amount of carbon, nitrogen, sulphur and phosphorus substrates was metabolized by *R. solanacearum*. More compounds were utilized at 35°C than at 20, 24 and 30°C. Carbon (PM 1–2) and nitrogen (PM 3) sources, as well as osmolyte (PM 9) and pH conditions (PM 10), were the most informative for the phenotype analysis of the R1Bv3 strain of *R. solanacearum*. Corresponding findings have also been reported in other microorganisms, including *Hypocrea atroviridis* (Friedl et al. 2008) and *Pseudomonas syringae* (Guo et al. 2017). For carbon substrates, organic acids and carbohydrates provided the most informative phenotypes for *R. solanacearum*. For nitrogen substrates, amino acids and peptides were the most informative. Some of these compounds are detected commonly in soil, root exudates and plant tissues (Wu et al. 2015; Li et al. 2017). Additionally, they have been shown to help infection by *R. solanacearum* of the host root, development of symptoms in plant tissues, and survival of the pathogen under different conditions (Wu et al. 2015; Li et al. 2017).

Additionally, compared with the metabolic characteristics of soil-borne tobacco phytopathogen *P. parasitica* (Wang et al. 2015a), some common carbon and nitrogen substrates were utilized by both pathogens. These common sources belong to the root exudates of tobacco and affect the growth of the two soil-borne pathogens. However, the amount of carbon and nitrogen substrates metabolized by *P. parasitica* was higher than that of *R. solanacearum*. It appears that *R. solanacearum* presents poorer carbon substrate use ability, especially for chemicals tested on PM1. The reason for this difference

Table 5. Metabolic profiling of *Ralstonia solanacearum* at four different temperatures in PM 4 MicroPlate substrates.

Cond.[a]	Substrate	Temperature °C				Cond.[a]	Substrate	Temperature °C			
		20	24	30	35			20	24	30	35
A1	Negative Control	-	-	+	+	C1	Phosphoenol pyruvate	+	+++	+++	+++
A2	Phosphate	++	+++	+++	+++	C2	Phospho-glycolic acid	++	++	+++	+++
A3	Pyrophosphate	++	+++	+++	+++	C3	D-glucose-1-phosphate	+	+++	+++	+++
A4	Trimeta-phosphate	++	+++	+++	+++	C4	D-glucose-6-phosphate	++	++	++	+++
A5	Tripoly-phosphate	++	+++	+++	+++	C5	2-deoxy-D-glucose 6-phosphate	++	++	+++	+++
A6	Triethyl phosphate	-	-	++	++	C6	D-glucosamine-6-phosphate	+++	+++	+++	+++
A7	Hypophosphite	-	-	+	++	C7	6-phospho-gluconic acid	+++	+++	+++	+++
A8	Adenosine-2′-monophosphate	+++	++	+++	+++	C8	Cytidine-2′-monophosphate	++	+++	+++	+++
A9	Adenosine-3′-monophosphate	+++	+++	+++	+++	C9	Cytidine-3′-monophosphate	+	+++	+++	+++
A10	Adenosine-5′-monophosphate	++	+++	+++	+++	C10	Cytidine-5′-monophosphate	++	++	+++	+++
A11	Adenosine-2′,3′-cyclic monophosphate	+++	+++	+++	+++	C11	Cytidine-2′,3′-cyclic monophosphate	++	++	+++	+++
A12	Adenosine-3′,5′-cyclic monophosphate	-	-	+	+++	C12	Cytidine-3′,5′-cyclic monophosphate	+	+	+++	+++
B1	Thiophosphate	+	+++	+++	+++	D1	D-mannose-1-phosphate	+	++	+++	+++
B2	Dithiophosphate	++	++	+++	+++	D2	D-mannose-6-phosphate	++	++	+++	+++
B3	D,L-α-Glycerol phosphate	++	+++	+++	+++	D3	Cysteamine-S-phosphate	-	-	++	+++
B4	β-Glycerol phosphate	++	+++	+++	+++	D4	Phospho-L-arginine	++	+++	+++	+++
B5	Carbamyl phosphate	++	+++	+++	+++	D5	O-phospho-D-serine	++	+++	+++	+++
B6	D-2-phospho-glyceric acid	++	++	+++	+++	D6	O-phospho-L-serine	++	++	++	+++
B7	D-3-phospho-glyceric acid	++	++	+++	+++	D7	O-phospho-L-threonine	++	+++	+++	+++
B8	Guanosine-2′-monophosphate	++	+++	+++	+++	D8	Uridine-2′-monophosphate	++	+++	+++	+++
B9	Guanosine-3′-monophosphate	++	+++	+++	+++	D9	Uridine-3′-monophosphate	++	++	+++	+++
B10	Guanosine-5′-monophosphate	++	+++	+++	+++	D10	Uridine-5′-monophosphate	++	+++	+++	+++
B11	Guanosine-2′,3′-cyclic monophosphate	++	++	+++	+++	D11	Uridine-2′,3′-cyclic monophosphate	++	++	+++	+++
B12	Guanosine-3′,5′-cyclic monophosphate	-	-	+	+++	D12	Uridine-3′,5′-cyclic monophosphate	-	-	+	+++

Cond.[a]	Substrate	Temperature °C				Cond.[a]	Substrate	Temperature °C			
		20	24	30	35			20	24	30	35
E1	O-phospho-D-tyrosine	++	+++	+++	+++	G1	N-acetyl-L-cysteine	++	+++	+++	+++
E2	O-phospho-L-tyrosine	++	++	+++	+++	G2	S-methyl-L-cysteine	++	+++	+++	+++
E3	Phosphocreatine	++	+++	+++	+++	G3	Cystathionine	+	+++	+++	+++
E4	Phosphoryl choline	++	+++	+++	+++	G4	Lanthionine	++	+++	+++	+++
E5	O-phosphoryl-ethanolamine	+	+++	+++	+++	G5	Glutathione	++	+++	+++	+++
E6	Phosphono acetic acid	-	-	+	+++	G6	D,L-ethionine	++	++	++	++
E7	2-aminoethyl phosphonic acid	-	-	+	+++	G7	L-methionine	++	+++	+++	+++
E8	Methylene diphosphonic acid	-	-	+	++	G8	D-Methionine	++	+++	+++	+++
E9	Thymidine-3′-monophosphate	++	++	+++	+++	G9	Glycyl-L-methionine	++	+++	+++	+++
E10	Thymidine-5′-monophosphate	++	++	+++	+++	G10	N-acetyl-D,L-methionine	++	+++	+++	+++
E11	Inositol hexaphosphate	+	++	+++	+++	G11	L-Methionine sulphoxide	+	++	+++	+++
E12	Thymidine 3′,5′-cyclic monophosphate	-	-	+	+++	G12	L-methionine sulfone	++	++	+++	+++
F1	Negative control	+	++	++	++	H1	L-djenkolic acid	+	+++	+++	+++
F2	sulphate	++	+++	+++	+++	H2	Thiourea	+	+++	+++	+++
F3	Thiosulfate	+	++	+++	+++	H3	1-thio-β-D-glucose	+	+++	+++	+++

(*Continued*)

Table 5. (Continued.)

Cond.[a]	Substrate	Tempreature °C				Cond.[a]	Substrate	Temperature °C			
		20	24	30	35			20	24	30	35
F4	Tetrathionate	+	+++	+++	+++	H4	D,L-lipoamide	+	++	+++	+++
F5	Thiophosphate	+	+++	+++	+++	H5	Taurocholic acid	+	+++	+++	+++
F6	Dithiophosphate	+	+++	+++	+++	H6	Taurine	+	+++	+++	+++
F7	L-cysteine	+	+++	+++	+++	H7	Hypotaurine	++	+++	+++	+++
F8	D-cysteine	++	+++	+++	+++	H8	p-amino benzene sulphonic acid	+	++	+++	+++
F9	L-cysteinyl-glycine	+	++	+++	+++	H9	Butane sulphonic acid	++	++	+++	+++
F10	L-cysteic acid	++	++	+++	+++	H10	2-hydroxyethane sulphonic acide	++	++	+++	+++
F11	Cysteamine	+	+	+++	+++	H11	Methane sulphonic acid	++	++	+++	+++
F12	L-cysteine sulfinic acid	+	+	+++	+++	H12	Tetramethylene sulfone	+	++	++	+++

[a]Cond. is short for the assay conducted on the Biolog PM 4 plate. '–', '+', '++' and '+++' means that *R. solanacearum* could not utilize the tested substrate, utilized poorly, moderately and effectively, respectively in Biolog PM 4 Microplate after 8 days incubation at 20, 24, 30, 35°C, respectively.

is still unclear. More work should be conducted in the future to investigate the details. Additionally, the characterization of a wide range of nutrient substrate utilization phenotypes at different temperatures may aid in studies of plant–microbe interactions.

Moreover, *R. solanacearum* at all four temperatures was able to adapt to a wide range of osmolytes and pH conditions. More osmolytes were metabolized by the bacterium at 35°C than at 20, 24 and 30°C. Typical osmolytes metabolized at 35°C included 6% sodium chloride and 1% sodium formate. This difference might be due to the physiological state of *R. solanacearum* varying at different temperatures. A previous study showed that low temperature could affect the viable but non-culturable state of *R. solanacearum* (Imazaki and Nakaho 2009). Additionally, at all four test temperatures, the metabolic fingerprints of *R. solanacearum* with regard to pH were mostly similar, although there were some differences. At a higher pH value of 8.0, *R. solanacearum* at 24, 30 and 35°C presented good growth, whereas it did not at 20°C. At a lower pH value of 4.5, the bacterium still showed good growth at 35°C but not at 20, 24 and 30°C. To the best of our knowledge, this is the first report that temperature affects the pH adaptability of *R. solanacearum*. Moreover, when incubated at 35°C and pH of 4.5, *R. solanacearum* metabolized many amino acids. Decarboxylases of microorganisms produce alkaline amines by catabolism of amino acids, which help to counteract an acidic pH. On the other hand, a high pH can be counteracted by deaminases that generate acids (Durso et al. 2004; Maurer et al. 2005). In this study, *R. solanacearum* presented no deaminase activities at any of the four test temperatures. It showed high decarboxylase activities at 35°C while exhibiting lfewer decarboxylase activities at 20, 24 and 30°C. Decarboxylase activities of *R. solanacearum* might play an important role in supporting the metabolization and pathogenicity of the pathogen in acidic soil, and help to initiate disease. The metabolic data on the PM 10 plate suggested that *R. solanacearum* preferred an acidic soil environment. Tobacco bacterial wilt normally is more severe in acidic versus alkaline soils. A previous study with other Solanaceous crops also showed that an acidic pH environment enhanced bacterial wilt incidence (Hacisalihoglu et al. 2009). In our early study of the same pH test condition on detached leaves, bacterial wilt severity at 35°C was much greater than at other tested temperatures (Wang et al. 2017). The reason might be due to higher temperatures enhancing the decarboxylase activities of *R. solanacearum* and thus also enhancing the pathogenicity of the pathogen. The

Table 6. Metabolic profiling of *Ralstonia solanacearum* at four different temperatures in PM 9 MicroPlate conditions.

Cond.[a]	Substrate	Temperature °C				Cond.[a]	Substrate	Temperature °C			
		20	24	30	35			20	24	30	35
A1	1% NaCl	-	-	-	+	C1	6% NaCl + KCl	-	-	-	-
A2	2% NaCl	-	-	-	+++	C2	6% NaCl + L-Proline	-	-	-	+++
A3	3% NaCl	-	-	-	+++	C3	6% NaCl + N-Acetyl-L-glutamine	-	-	-	+++
A4	4% NaCl	-	-	-	+++	C4	6% NaCl + β-Glutamic Acid	-	-	-	+++
A5	5% NaCl	-	-	-	+++	C5	6% NaCl + γ–Amino-N-butyric Acid	-	-	-	+++
A6	5.5% NaCl	-	-	-	+++	C6	6% NaCl + Glutathione	-	-	-	+++
A7	6% NaCl	-	-	-	++	C7	6% NaCl + Glycerol	-	-	-	+++
A8	6.5% NaCl	-	-	-	+	C8	6% NaCl + Trehalose	-	-	-	+++
A9	7% NaCl	-	-	-	++	C9	6% NaCl + Trimethylamine-N-oxide	-	-	-	+++
A10	8% NaCl	-	-	-	-	C10	6% NaCl + Trimethylamine	-	-	-	+++
A11	9% NaCl	-	-	-	-	C11	6% NaCl + Octopine	-	-	-	+++
A12	10% NaCl	-	-	-	-	C12	6% NaCl + Trigonelline	-	-	-	+++
B1	6% NaCl	-	-	-	+++	D1	3% Potassium Chloride	-	-	-	+++
B2	6% NaCl + Betaine	-	-	-	+++	D2	4% Potassium Chloride	-	-	-	+++
B3	6% NaCl + N-N Dimethyl glycine	-	-	-	+++	D3	5% Potassium Chloride	-	-	-	+++
B4	6% NaCl + Sarcosine	-	-	-	+++	D4	6% Potassium Chloride	-	-	-	+++
B5	6% NaCl + Dimethyl sulphonyl propionate	-	-	-	++	D5	2% Sodium Sulphate	-	-	++	+++
B6	6% NaCl + MOPS	-	-	-	+	D6	3% Sodium Sulphate	-	-	+	+++
B7	6% NaCl + Ectoine	-	-	-	+++	D7	4% Sodium Sulphate	-	-	-	+++
B8	6% NaCl + Choline	-	-	-	+++	D8	5% Sodium Sulphate	-	-	-	+++
B9	6% NaCl + Phosphorylcholine	-	-	-	+++	D9	5% Ethylene Glycol	++	++	+++	+++
B10	6% NaCl + Creatine	-	-	-	++	D10	10% Ethylene Glycol	++	++	+++	+++
B11	6% NaCl + Creatinine	-	-	-	+++	D11	15% Ethylene Glycol	++	++	+++	+++
B12	6% NaCl + L-Carnitine	-	-	-	+	D12	20% Ethylene Glycol	++	+++	+++	+++

Cond.[a]	Substrate	Temperature °C				Cond.[a]	Substrate	Temperature °C			
		20	24	30	35			20	24	30	35
E1	1% Sodium Formate	-	-	-	+++	G1	20 mM Sodium Phosphate pH 7	+	++	+++	+++
E2	2% Sodium Formate	-	-	-	-	G2	50 mM Sodium Phosphate pH 7	-	++	+++	+++
E3	3% Sodium Formate	-	-	-	-	G3	100 mM Sodium Phosphate pH 7	-	+	+++	+++
E4	4% Sodium Formate	-	-	-	-	G4	200 mM Sodium Phosphate pH 7	-	-	-	+++
E5	5% Sodium Formate	-	-	-	-	G5	20 mM Sodium Benzoate pH 5.2	-	-	-	-
E6	6% Sodium Formate	-	-	-	-	G6	50 mM Sodium Benzoate pH 5.2	-	-	-	-
E7	2% Urea	-	+	+++	-	G7	100 mM Sodium Benzoate pH 5.2	-	-	-	-
E8	3% Urea	-	-	+	-	G8	200 mM Sodium Benzoate pH 5.2	-	-	-	-
E9	4% Urea	-	-	-	-	G9	10 mM Ammonium Sulphate pH 8	++	++	+++	+++
E10	5% Urea	-	-	-	-	G10	20 mM Ammonium Sulphate pH 8	++	++	+++	+++
E11	6% Urea	-	-	-	-	G11	50 mM Ammonium Sulphate pH 8	++	++	+++	+++
E12	7% Urea	-	-	-	-	G12	100 mM Ammonium Sulphate pH 8	-	-	+	+++
F1	1% Sodium Lactate	-	-	++	+++	H1	10 mM Sodium Nitrate	++	++	+++	++
F2	2% Sodium Lactate	-	-	-	+++	H2	20 mM Sodium Nitrate	+	++	+++	+++
F3	3% Sodium Lactate	-	-	-	+++	H3	40 mM Sodium Nitrate	-	+	+++	++

(*Continued*)

Table 6. (Continued.)

Cond.[a]	Substrate	Temperature °C				Cond.[a]	Substrate	Temperature °C			
		20	24	30	35			20	24	30	35
F4	4% Sodium Lactate	-	-	-	+++	H4	60 mM Sodium Nitrate	-	-	+++	+++
F5	5% Sodium Lactate	-	-	-	+++	H5	80 mM Sodium Nitrate	-	-	++	+++
F6	6% Sodium Lactate	-	-	-	+++	H6	100 mM Sodium Nitrate	-	-	+	++
F7	7% Sodium Lactate	-	-	-	+++	H7	10 mM Sodium Nitrite	++	++	+++	++
F8	8% Sodium Lactate	-	-	-	+++	H8	20 mM Sodium Nitrite	+	+	++	+
F9	9% Sodium Lactate	-	-	-	+++	H9	40 mM Sodium Nitrite	-	-	-	-
F10	10% Sodium Lactate	-	-	-	+++	H10	60 mM Sodium Nitrite	-	-	-	-
F11	11% Sodium Lactate	-	-	-	+++	H11	80 mM Sodium Nitrite	-	-	-	-
F12	12% Sodium Lactate	-	-	-	+++	H12	100 mM Sodium Nitrite	-	-	-	-

[a]Cond. is short for the assay conducted on the Biolog PM 9 plate. '–', '+', '++' and '+++' means that *R. solanacearum* could not utilize the tested substrate, utilized poorly, moderately and effectively, respectively in Biolog PM 1 Microplate after 8 days incubation at 20, 24, 30, 35°C, respectively.

relationship between temperature and decarboxylase activities of *R. solanacearum* should be analysed in future studies.

For the tobacco host, the R1Bv3 and R1Bv4 strains of *R. solanacearum* have been detected in China (Liu et al. 2008; Xue et al. 2011), with the R1Bv3 strain being the major pathogen. Meanwhile, strains of *R. solanacearum* in different regions have been assigned to R1Bv1, R1Bv2, R2Bv1, R3Bv2, etc. (Bocsanczy et al. 2012). This study investigated the phenotype of the R1Bv3 strain from tobacco. However, the relationship between other biovars of *R. solanacearum* is still unclear, and further analysis using more isolates from different biovars is needed. There may be many different phenotypes' characteristics among the different races and biovars of *R. solanacearum*. More R1Bv3 strains from other crops in different regions of China should also be tested in future studies. Meanwhile, strains of *R. solanacearum* from different races and biovars should also be compared for phenotype characterization. Additionally, our findings would provide some useful information for some scientists characterizing biovars or doing race typing on different host cultivars, as well as breeders looking for sources of resistance. The outcome would provide valuable profiles for developing practical ideas and methods for bacterial wilt management.

Important discoveries from analysing the comparative effects of temperature on metabolic characteristics of *R. solanacearum* provided us with some ideas of how to find potential novel approaches for tobacco bacterial wilt management. Altering transplantation time to avoid periods of high temperature or covering fields with straw to decrease soil temperature may efficiently reduce bacterial wilt disease incidence in tobacco. This disease control hypothesis has been verified on the bacterial wilt of tomato (Wei et al. 2015). Meanwhile, enhancing the amount of some nutritional sources that could not be metabolized by *R. solanacearum*, or changing the osmolytes and pH conditions of the soil to make it unsuitable for *R. solanacearum* might decrease the growth and pathogenicity of the pathogen, and subsequently decrease tobacco bacterial wilt. In this study, *R. solanacearum* at 35°C could not grow at osmolytes of 8% sodium chloride, or at conditions of pH value over 8.5. Thus, perhaps a high percentage of sodium chloride could be used for tobacco bacterial wilt management. A previous study showed that a high percentage of calcium nutrition had some effect against *R. solanacearum* and could be used for tomato bacterial wilt management (Jiang et al. 2013). In China, lime is frequently added to the soil before tobacco

Table 7. Metabolic profiling of *Ralstonia solanacearum* at four different temperatures in PM 10 MicroPlate conditions.

Cond.[a]	Substrate	Temperature °C				Cond.[a]	Substrate	Temperature °C			
		20	24	30	35			20	24	30	35
A1	pH 3.5	-	-	-	-	C1	pH 4.5 + L-Methionine	-	-	-	-
A2	pH 4	-	-	-	-	C2	pH 4.5 + L-Phenylalanine	-	-	-	+
A3	pH 4.5	-	-	-	++	C3	pH 4.5 + L-Proline	-	-	-	++
A4	pH 5	+++	+++	+++	+++	C4	pH 4.5 + L-Serine	-	-	-	++
A5	pH 5.5	+++	+++	+++	+++	C5	pH 4.5 + L-Threonine	-	-	-	++
A6	pH 6	+++	+++	+++	+++	C6	pH 4.5 + L-Tryptophan	-	-	-	-
A7	pH 7	+++	+++	+++	+++	C7	pH 4.5 + L-Tyrosine	-	-	-	+
A8	pH 8	-	+	+	+	C8	pH 4.5 + L-Valine	-	-	-	+
A9	pH 8.5	-	-	-	-	C9	pH 4.5 + Hydroxy-L-Proline	-	-	-	+
A10	pH 9	-	-	-	-	C10	pH 4.5 + L-Ornithine	-	-	-	+
A11	pH 9.5	-	-	-	-	C11	pH 4.5 + L-Homoarginine	-	-	-	-
A12	pH 10	-	-	-	-	C12	pH 4.5 + L-Homoserine	-	-	-	+
B1	pH 4.5	-	-	-	-	D1	pH 4.5 + Anthranilic acid	-	-	-	-
B2	pH 4.5 + L-Alanine	-	-	-	++	D2	pH 4.5 + L-Norleucine	-	-	-	-
B3	pH 4.5 + L-Arginine	-	-	-	++	D3	pH 4.5 + L-Norvaline	+	+++	+++	+++
B4	pH 4.5 + L-Asparagine	-	-	-	+++	D4	pH 4.5 + α- Amino-N-butyric acid	-	-	-	-
B5	pH 4.5 + L-Aspartic Acid	-	-	-	++	D5	pH 4.5 + p-Aminobenzoate	-	-	-	-
B6	pH 4.5 + L-Glutamic Acid	-	-	-	+	D6	pH 4.5 + L-cysteic acid	-	-	-	+
B7	pH 4.5 + L-Glutamine	-	-	-	-	D7	pH 4.5 + D-Lysine	-	-	-	++
B8	pH 4.5 + Glycine	-	-	-	+	D8	pH 4.5 + 5-Hydroxy Lysine	-	-	-	++
B9	pH 4.5 + L-Histidine	-	-	-	+	D9	pH 4.5 + 5-HydroxyTryptophan	-	-	+	+
B10	pH 4.5 + L-Isoleucine	-	-	-	-	D10	pH 4.5 + D,L Diamino-Pimelic acid	-	-	-	++
B11	pH 4.5 + L-Leucine	-	-	-	-	D11	pH 4.5 + Trimethylamine-N-oxide	-	-	-	-
B12	pH 4.5 + L-Lysine	-	-	-	+	D12	pH 4.5 + Urea	-	-	-	++

Cond.[a]	Substrate	Temperature °C				Cond.[a]	Substrate	Temperature °C			
		20	24	30	35			20	24	30	35
E1	pH 9.5	-	-	-	-	G1	pH 9.5+ Anthranilic acid	-	-	-	-
E2	pH9.5 + L-Alanine	-	-	-	-	G2	pH 9.5 + L-Norleucine	-	-	-	-
E3	pH9.5 + L-Arginine	-	-	-	-	G3	pH 9.5 + L-Norvaline	-	-	-	-
E4	pH9.5 + L-Asparagine	-	-	-	-	G4	pH 9.5+ Agma tine	-	-	-	-
E5	pH9.5 + L-Aspartic Acid	-	-	-	-	G5	pH 9.5 + Cadaverine	-	-	-	-
E6	pH9.5 + L-Glutamic Acid	-	-	-	-	G6	pH 9.5+ Putrescine	-	-	-	-
E7	pH9.5 + L-Glutamine	-	-	-	-	G7	pH 9.5+ Histamine	-	-	-	-
E8	pH9.5+ Glycine	-	-	-	-	G8	pH 9.5+ Phe nylethy lamine	-	-	-	-
E9	pH9.5 + L-Histidine	-	-	-	-	G9	pH 9.5+ Tyra mine	-	-	-	-
E10	pH9.5 + L-Isoleucine	-	-	-	-	G10	pH 9.5+ Crea tine	-	-	-	-
E11	pH9.5 + L-Leucine	-	-	-	-	G11	pH 9.5+ Trimethylamine-N-oxide	-	-	-	-
E12	pH9.5 + L-Lysine	-	-	-	-	G12	pH 9.5+ Urea	-	-	-	-
F1	pH9.5 + L-Methionine	-	-	-	-	H1	X-Caprylate	+++	+++	+++	+++
F2	pH9.5 + L-Phenylalanine	-	-	-	-	H2	X-α-D-Glucoside	++	+++	+++	+++
F3	pH9.5 + L-Proline	-	-	-	-	H3	X-β-D-Glucoside	+++	+++	+++	+++

(*Continued*)

Table 7. (Continued.)

Cond.[a]	Substrate	Temperature °C				Cond.[a]	Substrate	Temperature °C			
		20	24	30	35			20	24	30	35
F4	pH9.5+ LL-Serine	-	-	-	-	H4	X-α-D-Galactoside	+++	+++	+++	+++
F5	pH9.5 + L-Threonine	-	-	-	-	H5	X-β-D-Galactoside	+++	+++	+++	+++
F6	pH9.5 + L-Tryptophan	-	-	-	-	H6	X-α-D-Glucuronide	+++	+++	+++	+++
F7	pH9.5 + L-Tyrosine	-	-	-	-	H7	X-β-D-Glucuronide	+++	+++	+++	+++
F8	pH9.5 + L-Valine	-	-	-	-	H8	X-β-D-Glucosaminide	++	++	+++	+++
F9	pH9.5 + L-Hydroxy-L-Proline	-	-	-	-	H9	X-β-D-Galactosaminide	++	+++	+++	+++
F10	pH9.5 + L-Ornithine	-	-	-	-	H10	X-α-D-Mannoside	++	+++	+++	+++
F11	pH9.5 + L-Homoarginine	-	-	-	-	H11	X-PO4	++	+++	+++	+++
F12	pH9.5 + L-Homoserine	-	-	-	-	H12	X-SO4	++	++	++	++

[a]Cond. is short for the assay conducted on the Biolog PM 10 plate. '–', '+', '++' and '+++' means that *R. solanacearum* could not utilize the tested substrate, utilized poorly, moderately and effectively, respectively in Biolog PM 10 Microplate after 8 days incubation at 20, 24, 30, 35°C, respectively.

transplantation, and losses caused by tobacco bacterial wilt normally decrease slightly (Wang et al. 2015c). The control provided by lime might be due to an increase of the soil pH. However, it is very difficult to modify the soil pH value to 8.5 in the field. Tobacco bacterial wilt still frequently occurs, and losses are still great each year.

In conclusion, the effect of temperature on the phenotype of *R. solanacearum* was characterized and compared with a BIOLOG Phenotype MicroArray (PM) at 20, 24, 30 and 35°C. The most informative utilization patterns for carbon sources of *R. solanacearum* R1Bv3 were organic acids and carbohydrates, and for nitrogen the most effective sources were amino acids. The bacterium showed much higher metabolic ability at 35°C than at 20, 24 and 30°C, and appeared to be more adaptable to osmolytes and pH environments at 30 and 35°C than at 20 and 24°C. *Ralstonia solanacearum* R1Bv3 presented no deaminase activity, had high decarboxylase activity at 35°C and low decarboxylase activity at 20 and 24°C. The comparative phenomics of the R1Bv3 strain of *R. solanacearum* showed that temperature significantly affected its phenotype.

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Author contributions

Conceived and designed the experiments: H. Wang and W. Ding. Performed the experiments: H. Wang, H. Guo, J. Yu, L. Cai, L. Cai and Y. Guo. Wrote and revised the paper: H. Wang, H. Guo and W. Ding. All authors approved the final version of the manuscript.

References

Bittner RJ, Arellano C, Mila AL. 2016. Effect of temperature and resistance of tobacco cultivars to the progression of bacterial wilt, caused by *Ralstonia solanacearum*. Plant Soil. 408:299–310.

Bochner BR. 2003. New technologies to assess genotype-phenotype relationships. Nat Rev Genet. 4(4):309–314.

Bochner BR, Gadzinski P, Panomitros E. 2001. Phenotype microarrays for high-throughput phenotypic testing and assay of gene function. Genome Res. 11:1246–1255.

Bochner BR, Giovannetti L, Viti C. 2008. Important discoveries from analyzing bacterial phenotypes. Mol Microbiol. 70(2):274–280.

Bocsanczy AM, Achenbach UCM, Mangravita-Novo A, Yuen JMF, Norman DJ. 2012. Comparative effect of low temperature on virulence and twitching motility of *Ralstonia solanacearum* strains present in Florida. Phytopathology. 102:185–194.

Buddenhagen IW, Sequeira L, Kelman A. 1962. Designation of races in *Pseudomonas solanacearum*. Phytopathology. 52:726.

Chen Y, Yan F, Chai YR, Liu HX, Kolter R, Losick R, Guo JH. 2013. Biocontrol of tomato wilt disease by *Bacillus subtilis* isolates from natural environments depends on conserved genes mediating biofilm formation. Environ Microbiol. 15:848–864.

Ciampi L, Sequeira L. 1980. Influence of temperature on virulence of race 3 strains of *Pseudomonas solanacearum*. Am Potato J. 57:307–317.

Deng ZP, Kuang CF, Zhou ZC, Huang SY, Luo K. 2004. Identification of the race of tobacco bacterial wilt in Hunan. J Hunan Agric Univ. 30:47–49.

Durso LM, Smith D, Hutkins RW. 2004. Measurements of fitness and competition in commensal *Escherichia coli* and *E. coli* O157: H7strains. Appl Environ Microbiol. 70(11):6466–6472.

Fajinmi AA, Fajinmi OB. 2010. An overview of bacterial wilt disease of tomato in Nigeria. Agric J. 5:242–247.

Fegan M, Prior P. 2005. How complex is the *Ralstonia solanacearum* species complex? Bacterial wilt: the disease and the *Ralstonia solanacearum* species complex. Allen C, Prior P, Hayward AC, editors. St Paul (MN): American Phytopathological Society Press; p. 449–461.

French R, Sequeira L. 1970. Strains of *Pseudomonas solanacearum* from central and south America: a comparative study. Phytopathology. 60:506–512.

Friedl MA, Kubicek CP, Druzhinina IS. 2008. Carbon source dependence and photostimulation of conidiation in *Hypocrea atroviridis*. Appl Environ Microbiol. 74(1):245–250.

Guo YS, Su XK, Cai LT, Wang HC. 2017. Phenotypic characterization of *Pseudomonas syringae* pv. *Tabaci*, the causal agent of tobacco wildfire. J Plant Pathol. 99(2):499–504.

Gusarov I, Shatalin K, Starodubtseva M, Nudler E. 2009. Endogenous nitric oxide protects bacteria against a wide spectrum of antibiotics. Science. 325:1380–1384.

Hacisalihoglu G, Momol MT, Wen A, Olson S. 2009. Effect of pH on bacterial wilt incidence and plant growth in hydroponic tomato. Int Symp Tomato Dis. 808(1):301–305.

Hayward AC. 1964. Characteristics of *Pseudomonas solanacearum*. J Appl Bacteriol. 27:265–277.

Hayward AC. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annu Rev Phytopathol. 29:65–87.

Hayward AC. 1995. Pseudomonas solanacearum. In: Singh US, Singh RP, Kohmoto K, editors. Pathogenesis and host specificity in plant disease: histopathological, biochemical, genetic and molecular bases, Vol. 1. Tarrytown: Elsevier; p. 139–151.

Hayward AC, El-Nashaar HM, Nvdegger U, De Lindo L. 1990. Variation in nitrate metabolism in biovars of *Pseudomonas solanacearum*. J Appl Bacteriol. 69:269–280.

He LY, Sequeira L, Kelman A. 1983. Characteristics of strains of *Pseudomonas solanacearum* from China. Plant Dis. 67:1357–1361.

Imazaki I, Nakaho K. 2009. Temperature-upshift-mediated revival from the sodium-pyruvate-recoverable viable but nonculturable state induced by low temperature in *Ralstonia solanacearum*: linear regression analysis. J Gen Plant Pathol. 75(3):213–226.

Jiang JF, Li JG, Dong YH. 2013. Effect of calcium nutrition on resistance of tomato against bacterial wilt induced by *Ralstonia solanacearum*. Eur J Plant Pathol. 136:547–555.

Kang Y, Liu H, Genin S, Schell MA, Denny TP. 2002. *Ralstonia solanacearum* requires type 4 pili to adhere to multiple surfaces and for natural transformation and virulence. Mol Microbiol. 46:427–437.

Katawczik M, Mila AL. 2012. Plant age and strain of *Ralstonia solanacearum* affect the expression of resistance of tobacco cultivars to Granville wilt. Tob Sci. 49:8–13.

Li SL, Xu C, Wang J, Guo B, Yang L, Chen JN, Ding W. 2017. Cinnamic, myristic and fumaric acids in tobacco root exudates induce the infection of plants by *Ralstonia solanacearum*. Plant Soil. 412 (1–2):381–395.

Liu H, Kang Y, Genin S, Schell MA, Denny TP. 2001. Twitching motility of *Ralstonia solanacearum* requires a type IV pilus system. Microbiology. 147:3215–3229.

Liu X, Xia XQ, Yao G, Gu Y, Du WM. 2008. Studies on the strains and epidemiology of bacterial wilt of tobacco. Southwest China J Agric Sci. 21:1587–1590.

Maurer LM, Yohannes E, Bondurant SS, Radmacher M, Slonczewski JL. 2005. pH regulates genes for flagellar motility, catabolism, and oxidative stress in *Escherichia coli* K-12. J Bacteriol. 187:304–319.

Milling A, Meng FH, Denny TP, Allen C. 2009. Interactions with hosts at cool temperatures, not cold tolerance, explain the unique epidemiology of *Ralstonia solanacearum* race 3 biovar 2. Phytopathology. 99:1127–1134.

Peng X, Zhou G, Deng Z, Kuang C, Luo K, Hongyuan L. 2007. Screening, identification and control efficacy of tobacco antagonistic endophytic bacteria against *Ralstonia solanacearum*. Acta Phytopathol Sin. 37:670–674.

Prior P, Fegan M. 2005. Recent developments in the phylogeny and classification of *Ralstonia solanacearum*. Acta Hortic. 695:127–136.

Schell MA. 1987. Purification and characterization of an endoglucanase from *Pseudomonas solanacearum*. Appl Envioron Microbiol. 53:2237–2241.

Schell MA. 2000. Control of virulence and pathogenicity genes of *Ralstonia solanacearum* by an elaborate sensory network. Annu Rev Phytopathol. 38:263–292.

Schell MA, Roberts DP, Denny TP. 1988. Analysis of the *Pseudomonas solanacearum* polygalacturonase encoded by *pgl*A and its involvement in phytopathogenicity. J Bacteriol. 170:4501–4508.

Singh D, Yadav DK, Sinha S, Choudhary G. 2014. Effect of temperature, cultivars, injury of root and inoculums load of *Ralstonia solanacearum* to cause bacterial wilt of tomato. Arch Phytopathol Plant Prot. 47(13):1574–1583.

Thurston HD. 1963. Bacterial wilt of potatoes in Colombia. Am Potato J. 40:381–390.

Tran NH, Kim BS. 2010. Influence of temperature, pathogen strain, inoculum density, seedling age, inoculation method and varietal resistance on infection of pepper seedlings by *Ralstonia solanacearum*. Hortic Environ Biotechnol. 51(2):95–100.

Van Elsas JD, Kastelein P, Van Bekkum P, Van Der Wolf JM, De Vries PM, Van Overbeek LS. 2000. Survival of *Ralstonia solanacearum* biovar 2, the causative agent of potato brown rot, in field and microcosm soils in temperate climates. Phytopathology. 90:1358–1366.

Wang HC, Huang YF, Xia HQ, Wang J, Wang MS, Zhang CQ, Lu HX. 2015b. Phenotypic analysis of *Alternaria alternata*, the causal agent of tobacco brown spot. Plant Pathol J. 14(2):79–85.

Wang HC, Li WH, Chen QY, Huang YF, Li K, Xia HQ, Wang M, Cai L, Shang S, Shi J. 2012. A rapid microbioassay for discovery of

antagonistic bacteria for *Phytophthora parasitica* var. *nicotianae*. Phytopathology. 102:267–271.

Wang HC, Wang J, Xia HQ, Huang YF, Wang MS, Jia MO, Yu ZH. 2015c. Sensitivities of *Ralstonia solanacearum* to streptomycin, calcium oxide, mancozeb and synthetic fertilizer. Plant Pathol J. 14(1):13–22.

Wang HC, Yu J, Cai LT, Lu N. 2017. Effect of temperature, relative humidity, inoculum amount and pH on pathogenicity of *Ralstonia solanacearum* on tobacco. Chin Tob Sci. 38(5):8–12.

Wang MS, Wang HC, Huang YF, Wang J, Zhang CQ, Lu HX. 2015a. Phenotypic analysis of *Phytophthora parasitica* by using high throughput phenotypic microarray. Acta Microbiol Sin. 55 (10):1356–1363.

Wei Z, Huang JF, Hu J, Gu YA, Yang CL, Mei XL, Shen QR, Xu YC, Friman VP. 2015. Altering transplantation time to avoid periods of high temperature can efficiently reduce bacterial wilt disease incidence with tomato. PLoS One. 10:e0139313.

Wu K, Yuan SF, Xun GH, Shi W, Pan B, Guan HL, Shen B, Shen Q. 2015. Root exudates from two tobacco cultivars affect colonization of *Ralstonia solanacearum* and the disease index. Eur J Plant Pathol. 141:667–677.

Xue QY, Yin YN, Yang W, Heuer H, Prior P, Guo JH, Smalla K. 2011. Genetic diversity of *Ralstonia solanacearum* strains from China assessed by PCR-based fingerprints to unravel host plant- and site-dependent distribution patterns. FEMS Microbiol Ecol. 75:507–519.

Zhao HN, Cai K, Qin J, Wu SJ, Ren Z, Pan WJ, Lei B. 2017. Variance analysis of main metabolites in tobacco leaves before and after curing. Tob Sci Technol. 50(9):61–67.

Zhuo GH. 2005. Infection effect of temperature and inoculum concentration of *Ralstonia solanacearum* to tomato. Plant Quarantine. 19 (3):143–144.

Zou Y, Xiao CG. 2008. Preliminary identification of physiological race of *Ralstonia solanacearum* in Chongqing. Tob Sci Technol. 5:60–64.