Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Plant Pathology Journal

ISSN 1812-5387
DOI: 10.3923/ppj.2016.102.107

Research Article
Phenotypic Fingerprints of *Ralstonia solanacearum* Under Various Osmolytes and pH Environments

[1]Xingjiang Chen, [2]Licui Li, [1]Hancheng Wang, [3]Yanfei Huang, [1]Maosheng Wang and [3]Changqing Zhang

[1]Key Laboratory of Molecular Genetics, Guizhou Academy of Tobacco Science, 550081 Guiyang, People's Republic of China
[2]College of Agriculture, Yangtze University, 434025 Jingzhou, People's Republic of China
[3]College of Life Science, Yangtze University, 434025 Jingzhou, People's Republic of China

Abstract

Background: Tobacco bacterial wilt caused by *Ralstonia solanacearum* is a devastating disease in Solanaceae crops and the pathogen is a notorious pathogen worldwide. **Methodology:** The phenotypic characterization of *R. solanacearum* under various osmolytes and pH environments were analyzed by using biolog Phenotype Microarray system (PMs). Using PM plates 9 and 10, 192 different assays were tested, including 96 different osmolytes and 96 pH environments. **Results:** Result presented that all four isolates of *R. solanacearum* exhibited similar phenotypic fingerprints. They had wide range adaptabilities in osmolytes with up to 4% sodium chloride, up to 20% ethylene glycol, up to 4% urea, up to 2% sodium lactate, up to 200 mM sodium phosphate (pH 7), up to 50 mM ammonium sulfate (pH 8), up to 100 mM sodium nitrate and up to 80 mM sodium nitrite. The bacterium did not grow at other osmolytes, including sodium chloride ranging from 5.5-10%, sodium formate ranging from 3-6%, urea ranging from 5-7%, sodium lactate ranging 7-12% and sodium benzoate (pH 5.2) ranging from 100-200 mM. They also exhibited active metabolism under pH values between 5 and 10, with an optimal pH value of around 6. The *R. solanacearum* showed active deaminase activity, while no decarboxylase activity in the presence of various amino acids. **Conclusion:** This study increased understanding on the bacterium, especially in survivability of the bacterium in the environment and would provide valuable profiles in developing practical ideas and methods for the disease control.

Key words: Biolog phenotype microarray, metabolic fingerprint, bacterial wilt, osmolytes

Received: January 15, 2016 Accepted: March 29, 2016 Published: June 15, 2016

Citation: Xingjiang Chen, Licui Li, Hancheng Wang, Yanfei Huang, Maosheng Wang and Changqing Zhang, 2016. Phenotypic fingerprints of *Ralstonia solanacearum* under various osmolytes and pH environments. Plant Pathol. J., 15: 102-107.

Corresponding Author: Hancheng Wang, Key Laboratory of Molecular Genetics, Guizhou Academy of Tobacco Science, 550081 Guiyang, People's Republic of China Tel: +86-851-84116941 Fax: +86-851-84116909

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tobacco bacterial wilt caused by *Ralstonia solanacearum* (Smith) causing is a notorious soil-borne bacterial disease. Its pathogen has a large host range of more than 200 species in 50 families and a worldwide distribution[1]. It is a major threaten in the production of several important economy crops, including tobacco, tomato and potato in the world[2]. Recently, *R. solanacearum* is one of the wide spread and most important bacterial phytopathogens in flue-cured tobacco[3]. Symptoms of bacterial wilt include leaf wilting, yellowing and necrosis[4]. The bacterium spreads through contaminated irrigation water and infected planting material and survives in infested soil, infected plants, susceptible weed hosts and volunteer crops[5].

Ralstonia solanacearum presents great diversities in geographical distribution, host range, physiological properties and pathogenicity[6,7]. Most bacterial wilt in host of tobacco in China caused by *R. solanacearum* belongs to Race 1 biovar 3 (R1Bv3)[8-10]. With environment variation of soil in the growing season, soil osmolytes and pH environments may change greatly. This fluctuation may influence the survival of the soil pathogen *R. solanacearum*. However, to the best of our knowledge, the survival ability of *R. solanacearum* under various osmolytes and pH environments is still unclear and will also affect the control of bacterial wilt.

Recently, a high throughput Phenotypic Microarray (PM)/Omnilog system, was developed by the company Biolog (Hayward, CA) to assay osmotic and pH metabolic phenotypes of many microorganism[11]. In the system, microorganism cells in test medium including a tetrazolium dye are inoculated into 96-well plates, where the plates include various substances to analyze for varying effects of osmotic, ionic and pH environments. A software sketches kinetic data of color formation against time for each well and ascribes artificial color to each plot.

In this study, phenotypic characterizations of *R. solanacearum* under various osmolytes and pH environments were characterized by phenotypic microarray system. The characterizations were also compared between different strains to explore their phenotypic diversities. Though nearly no effective management methods are available to control bacterial wilt in tobacco, a detailed understanding of the phenotypes of *R. solanacearum* will be valuable to develop management practices to decrease the impacts of the disease.

MATERIALS AND METHODS

Origin and collection of *Ralstonia solanacearum* isolates: Four isolates (R1, R2, R3 and R4) of *R. solanacearum* used in this study were collected from infected tobacco stem with typical bacterial wilt symptoms in 2011 during the disease epidemic season in Guizhou province of China. They were incubated on a semi-selective medium (SMSA)[12] at 30°C in the dark. All strains were stored as suspensions in sterile water at 25°C for long-term storage and retrieved at 28°C using the YPGA medium (peptone 5 g L^{-1}, yeast extract 5 g L^{-1}, glucose 10 g L^{-1} and agar 16 g L^{-1}, pH 7.2).

Phenotypic characterization of *Ralstonia solanacearum* under various osmolytes and pH environments: Four isolates (R1, R2, R3 and R4) of *R. solanacearum* from tobacco were detected for this study. All materials, reagents and media for the phenotypic study were bought from Biolog. Isolates were streaked on Biolog Universal Growth medium plus blood agar (BUG+B) plates and incubated at 30°C in darkness for 48 h. Cells were scraped from the surface of the plates and re-suspended in appropriate medium containing Dye Mix A, 120 µL of a 1:200 dilution of an 85% transmittance cells suspension were added to each well of the PM plates. Plates 9-10, which test for phenotypes of varying effects of osmotic, ionic and pH environments, were used in this study. The IF-10 GN Base plus Dye Mix A was utilized for plates 9-10. After inoculation under clean beanth, plates were incubated in the OmniLog incubator for 72 h. Data were collected every 15 min by the Biolog incubator and analyzed using kinetic and parametric software (Biolog). Phenotype diversities were performed based on the area difference under the kinetic curve of dye formation.

RESULTS

Phenotypic characterization of *R. solanacearum* under various osmolytes and pH environments was conducted by using the PM plates 9 and 10. A total of 192 different growth conditions were analyzed, including 96 different osmolytes and 96 pH environments. All tested isolates of *R. solanacearum* from tobacco had nearly the same metabolic fingerprints. The *R. solanacearum* were able to utilize 53.13% of tested osmolytes, including sodium chloride ranged from 1-4%, ethylene glycol ranged from 9-20%, urea

Table 1: Metabolic profiling of *Ralstonia solanacearum* on Biolog PM 9 plate*

Well	Substrate	R	Well	Substrate	R	Well	Substrate	R	Well	Substrate	R[a]
A1	NaCl 1%	+	C1	NaCl 6%+KCl	-	E1	Sodium formate 1%	+	G1	Sodium phosphate pH 7 20 mM	+
A2	NaCl 2%	+	C2	NaCl 6%+L-proline	-	E2	Sodium formate 2%	+	G2	Sodium phosphate pH 7 50 mM	+
A3	NaCl 3%	+	C3	NaCl 6%+N-acethyl L-glutamine	-	E3	Sodium formate 3%	-	G3	Sodium phosphate pH 7 100 mM	+
A4	NaCl 4%	+	C4	NaCl 6%+ß-glutamic acid	-	E4	Sodium formate 4%	-	G4	Sodium phosphate pH 7 200 mM	+
A5	NaCl 5%	+	C5	NaCl 6%+γ- amino-n -butyric acid	-	E5	Sodium formate 5%	-	G5	Sodium benzoate pH5.2 20 mM	+
A6	NaCl 5.5%	-	C6	NaCl 6%+glutathione	-	E6	Sodium formate 6%	-	G6	Sodium benzoate pH 5.2 50 mM	-
A7	NaCl 6%	-	C7	NaCl 6%+glycerol	-	E7	Urea 2%	+	G7	Sodium benzoate pH 5.2 100 mM	-
A8	NaCl 6.5%	-	C8	NaCl 6%+trehalose	-	E8	Urea 3%	+	G8	Sodium benzoate pH 5.2 200 mM	-
A9	NaCl 7%	-	C9	NaCl 6%+trimethylamine -N-oxide	-	E9	Urea 4%	+	G9	Ammonium sulfate pH 8 10 mM	+
A10	NaCl 8%	-	C10	NaCl 6%+trimethylamine	-	E10	Urea 5%	-	G10	Ammonium sulfate pH 8 20 mM	+
A11	NaCl 9%	-	C11	NaCl 6%+octopine	-	E11	Urea 6%	-	G11	Ammonium sulfate pH 8 50 mM	+
A12	NaCl 10%	-	C12	NaCl 6%+trigonelline	-	E12	Urea 7%	-	G12	Ammonium sulfate pH 8 100 mM	+
B1	NaCl 6%	-	D1	Potassium chloride 3%	+	F1	Sodium lactate 1%	+	H1	Sodium nitrate 10 mM	+
B2	NaCl 6%+betaine	-	D2	Potassium chloride 4%	+	F2	Sodium lactate 2%	+	H2	Sodium nitrate 20 mM	+
B3	NaCl 6%+N-N dimethyl glycine	-	D3	Potassium chloride 5%	+	F3	Sodium lactate 3%	-	H3	Sodium nitrate 40 mM	+
B4	NaCl 6%+sarcosine	-	D4	Potassium chloride 6%	+	F4	Sodium lactate 4%	+	H4	Sodium nitrate 60 mM	+
B5	NaCl 6%+dimethyl sulphonyl propionate	-	D5	Sodium sulfate 2%	+	F5	Sodium lactate 5%	+	H5	Sodium nitrate 80 mM	+
B6	NaCl 6%+MOPS	-	D6	Sodium sulfate 3%	+	F6	Sodium lactate 6%	+	H6	Sodium nitrate 100 mM	+
B7	NaCl 6%+ectoine	-	D7	Sodium sulfate 4%	+	F7	Sodium lactate 7%	-	H7	Sodium nitrite 10 mM	+
B8	NaCl 6%+choline	-	D8	Sodium sulfate 5%	+	F8	Sodium lactate 8%	-	H8	Sodium nitrite 20 mM	+
B9	NaCl 6%+ Phosphoryl choline	-	D9	Ethylene glycol 5%	+	F9	Sodium lactate 9%	-	H9	Sodium nitrite 40 mM	+
B10	NaCl 6%+creatine	-	D10	Ethylene glycol 10%	+	F10	Sodium lactate 10%	-	H10	Sodium nitrite 60 mM	+
B11	NaCl 6%+creatinine	-	D11	Ethylene glycol 15%	+	F11	Sodium lactate 11%	-	H11	Sodium nitrite 80 mM	+
B12	NaCl 6%+L-carnitine	-	D12	Ethylene glycol 20%	+	F12	Sodium lactate 12%	-	H12	Sodium nitrite 100 mM	+

*: '+' and '-' means the substrate was metabolized and not metabolized by *Ralstonia solanacearum* examined using the Biolog PM 9 plate, respectively, [a]R is short for pathogen *R. solanacearum*

ranged from 2-4%, sodium lactate ranged 1-2%, sodium phosphate (pH 7) ranged from 20-200 mM, ammonium sulfate (pH 8) ranged from 10-50 mM, sodium nitrate ranged from 10-100 mM and sodium nitrite ranged from 10-80 mM. However, it could not grow at some other osmolytes, including sodium chloride ranged from 5.5-10%, sodium formate ranged from 3-6%, urea ranged from 5-7%, sodium lactate ranged 7-12% and sodium benzoate (pH 5.2) ranged from 100-200 mM (Table 1).

The pH range where *R. solanacearum* exhibited active growth was between 5 and 10, with an optimal pH value of around 6.0. When combined with various amino acids at the pH value of 4.5, *R. solanacearum* did not grow in all tests (plate PM10, Well B01-B12, C01-C12 and D01-D12). In comparison, when combined with various amino acids at the pH of 9.5, *R. solanacearum* presented actively growth in all tests (plate PM10, Well E01-E12, F01-F12 and G01-G12). The PM 10, wells B1-D12 and E1-G12, tested the decarboxylase and deaminase activities of the bacterium in the presence of amino acids at pH 4.5 and pH 9.5, respectively. In the presence of most amino acids, *R. solanacearum* showed active deaminase activity, while no decarboxylase activity (plate PM 10). Meanwhile, in the well of H01-H12 in plate PM 10, *R. solanacearum* also exhibited active growth under pressures of other compounds, including X-caprylate, X-α-D-glucoside, X-ß-D-glucoside, X-α-D-galactoside, X-ß-D-galactoside, X-α-D-glucuronide, X-ß-D-glucuronide, X-ß-D-glucosaminide, X-ß-D-galactosaminide and X-α-D-mannoside and X-PO_4 and X-SO_4 (Table 2).

DISCUSSION

Despite many molecular biology, genetic and some phenotypic characters studies have been conducted to the sequenced pathogen *R. solanacearum*, the phenotypic characterization of this bacterium under various osmolytes and pH environments is still unclear. Direct high-throughput testing of phenotypes using the Phenotype Microarray (PM) system (Biolog)[11] has provided a much easy way for biology, genomic and population studies of microorganisms[13-16] and was used here to detect the metabolic abilities of

Table 2: Metabolic profiling of *Ralstonia solanacearum* on Biolog PM 10 plate*

Well	Substrate	R	Well	Substrate	R	Well	Substrate	R	B	Well	Substrate	R[a]
A1	pH 3.5	-	C1	pH 4.5+L-methionine	-	E1	pH 9.5	+	+	G1	pH 9.5+anthranilic acid	+
A2	pH 4	-	C2	pH 4.5+L-phenylalanine	-	E2	pH 9.5+L-phenylalanine	+	+	G2	pH 9.5+L-norleucine	+
A3	pH 4.5	-	C3	pH 4.5+L-proline	-	E3	pH 9.5+L-arginine	+	+	G3	pH 9.5+L-norvaline	+
A4	pH 5	+	C4	pH 4.5+L-serine	-	E4	pH 9.5+L-asparagine	+	+	G4	pH 9.5+Agmatine	+
A5	pH 5.5	+	C5	pH 4.5+ L-threonine	-	E5	pH 9.5+L-aspartic acid	+	+	G5	pH 9.5+cadaverine	+
A6	pH 6	+	C6	pH 4.5+L-tryptophan	-	E6	pH 9.5+L-glutamic acid	+	+	G6	pH 9.5+putrescine	+
A7	pH 7	+	C7	pH 4.5+L-tyrosine	-	E7	pH 9.5+L-glutamine	+	+	G7	pH 9.5+histamine	+
A8	pH 8	+	C8	pH 4.5+L-valine	-	E8	pH 9.5+glycine	+	+	G8	pH 9.5+phenylethylamine	+
A9	pH 8.5	+	C9	pH 4.5+hydroxy-L-proline	-	E9	pH 9.5+L-histidine	+	+	G9	pH 9.5+tyramine	+
A10	pH 9	+	C10	pH 4.5+L-ornithine	-	E10	pH 9.5+L-isoleucine	+	+	G10	pH 9.5+creatine	+
A11	pH 9.5	+	C11	pH 4.5+L-homoarginine	-	E11	pH 9.5+L-leucine	+	+	G11	pH 9.5+trimethyl amine -N-oxide	+
A12	pH 10	+	C12	pH 4.5+L-homoserine	-	E12	pH 9.5+L-lysine	+	+	G12	pH 9.5+urea	+
B1	pH 4.5	-	D1	pH 4.5+anthranilica acid	-	F1	pH 9.5+L-methionine	+	+	H1	X-caprylate	+
B2	pH 4.5+L-alanine	-	D2	pH 4.5+L-norleucine	-	F2	pH 9.5+L-phenylalanine	+	+	H2	X-α-D-glucoside	+
B3	pH 4.5+L-arginine	-	D3	pH 4.5+L-norvaline	-	F3	pH 9.5+L-proline	+	+	H3	X-ß-D-glucoside	+
B4	pH 4.5+L-asparagine	-	D4	pH 4.5+α-amino-N-butyric acid	-	F4	pH 9.5+L-serine	+	+	H4	X-α-D-galactoside	+
B5	pH 4.5+L-aspartic acid	-	D5	pH 4.5+p-aminobenzoate	-	F5	pH 9.5+L-threonine	+	+	H5	X-ß-D-galactoside	+
B6	pH 4.5+L-glutamic acid	-	D6	pH 4.5+L-cysteic acid	-	F6	pH 9.5+L-tryptophan	+	+	H6	X-α-D-glucuronide	+
B7	pH 4.5+L-glutamine	-	D7	pH 4.5+D-lysine	-	F7	pH 9.5+L-tyrosine	+	+	H7	X-ß-D-glucuronide	+
B8	pH 4.5+glycine	-	D8	pH 4.5+5-hydroxy lysine	-	F8	pH 9.5+L-valine	+	+	H8	X-ß-D-glucosaminide	+
B9	pH 4.5+L-histidine	-	D9	pH 4.5+5-hydroxy tryptophan	-	F9	pH 9.5+hydroxy-L-proline	+	+	H9	X-ß-D-galactosaminide	+
B10	pH 4.5+L-isoleucine	-	D10	pH 4.5+D,L-diamino pimelic acid	-	F10	pH 9.5+L-ornithine	+	+	H10	X-α-D-mannoside	+
B11	pH 4.5+L-Leucine	-	D11	pH 4.5+trimethyl amine-N-oxide	-	F11	pH 9.5+L-homoarginine	+	+	H11	X-PO_4	+
B12	pH 4.5+L-Lysine	-	D12	pH 4.5+urea	-	F12	pH 9.5+L-homoserine	+	+	H12	X-SO_4	+

*: '+' and '-' means the substrate was metabolized and not metabolized by *Ralstonia solanacearum* examined using the Biolog PM 10 plate, respectively, [a]R is short for pathogen *R. solanacearum*

R. solanacearum under various osmolytes and pH environments. Significant metabolic diversity was found.

Ralstonia solanacearum is a notorious bacterium that has a fair ability to colonize both plants and soils[17]. Bacterial wilt caused by *R. solanacearum* is a great threat to many important crops in China, especially for tobacco and tomato[18,19]. Huge economic losses happened each year for tobacco and tomato throughout the country[18-20]. Due to high prevalence and varies of the pathogen, many strategies for bacterial wilt management showed very limited success. Phenotypic diversities of *R. solanacearum* might be one of the reasons for the difficulty. The results in this study may help us to know the great survival ability of the pathogen in various soil conditions. The phenotypic character of wide range of osmolytes and pH environments found in this study has great adaptive value. These characters indicate a general view of the lifestyle of the pathogen. Meanwhile, since the characters tested including different environmental stressors, it becomes possible to determine whether it has a chance for bacterial wilt management in agriculture. Changing the pH values of the soil or irrigating tobacco plant with sodium chloride or sodium lactate to get more than 5.5 or 7% osmotic pressure may decrease the survival ability of *R. solanacearum* and thus may reduce the damage caused by bacterial wilt in the field. These imagines could be proved in the next study in the near future.

In this study, the soil pathogen *R. solanacearum* had wide range adaptabilities in osmolytes and pH conditions. These adaptabilities can be rationalized as adaption of the bacterium to the variation in soil pH and osmolytes due to varying conditions of the season. Decarboxylase of the bacterium generates alkaline amines by the 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[21]. The *R. solanacearum* showed effective deaminase activity while no decarboxylase activity. Consequently, phenotypic characters for widerange adaptabilities of *R. solanacearum*

increased understanding on the bacterium, especially in plant-microbe interaction and survivability of the bacterium in the soil of Solanaceae crops and would provide valuable profiles in develop practical ideas and methods for the disease control.

CONCLUSION

Four isolates of *R. solanacearum* exhibited similar phenotypic fingerprints in 192 different assays concerning osmolytes and pH environments. They had wide range adaptabilities in osmolytes with up to 4% sodium chloride, up to 20% ethylene glycol, up to 4% urea, up to 2% sodium lactate, up to 200 mM sodium phosphate (pH 7), up to 50 mM ammonium sulfate (pH 8), up to 100 mM sodium nitrate and up to 80 mM sodium nitrite. The bacterium did not grow at other osmolytes, including sodium chloride ranging from 5.5-10%, sodium formate ranging from 3-6%, urea ranging from 5-7%, sodium lactate ranging 7-12% and sodium benzoate (pH 5.2) ranging from 100-200 mM. They also exhibited active metabolism under pH values between 5 and 10, with an optimal pH value of around 6. The *R. solanacearum* showed active deaminase activity while no decarboxylase activity in the presence of various amino acids. Phenotypic characterizations of *R. solanacearum* increased understanding on the survivability of the bacterium in the environment.

ACKNOWLEDGMENT

This study was supported by National Natural Science Foundation of China (31360448, 31501679) and Guizhou Tobacco Company (201305, 201336, 201436). We also thank the anonymous reviewers for critical reviews of the manuscript.

SIGNIFICANCE STATEMENT

Tobacco bacterial wilt is difficult to control in the world. Various osmolytes and pH environments might influence the survival ability of the pathogen *R. solanacearum.* Various studies have shown the metabolic characterization of this bacterium. Unfortunately, such researches are not systemic and lots are still unclear. The present study provides dynamic data relating metabolic ability of *R. solanacearum* in 192 different osmolytes and pH environments. It helps to know the survival ability of the bacterium.

REFERENCES

1. Hayward, A.C., 1995. *Pseudomonas solanacearum*. In: Pathogenesis and Host Specificity in Plant Disease: Histopathological, Biochemical, Genetic and Molecular Bases, Singh, U.S., R.P. Singh and K. Kohmoto (Eds.). Vol. 1, Elsevier Science Ltd., London, UK., pp: 139-151.
2. French, E.R. and L. Sequeira, 1970. Straine of *Pseudomonas eolanacearuni* from central and South America: A comparative study. Phytopathology, 60: 506-512.
3. Peng, X., G. Zhou, Z. Deng, C. Kuang, K. Luo and H.Y. Liu, 2007. Screening, identification and control efficacy of tobacco antagonistic endophytic bacteria against *Ralstonia solanacearum*. Acta Phytopathologica Sinica, 37: 670-674.
4. Swanson, J.K., J. Yao, J. Tans-Kersten and C. Allen, 2005. Behavior of *Ralstonia solanacearum* race 3 biovar 2 during latent and active infection of geranium. Phytopathology, 95: 136-143.
5. Hayward, A.C., 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev. Phytopathol., 29: 65-87.
6. Horita, M. and K. Tsuchiya, 2001. Genetic diversity of japanese strains of *Ralstonia solanacearum*. Phytopathology, 91: 399-407.
7. Poussier, S., P. Vandewalle and J. Luisetti, 1999. Genetic diversity of African and worldwide strains of *Ralstonia solanacearum* as determined by PCR-restriction fragment length polymorphism analysis of the *hrp* gene region. Applied Environ. Microbiol., 65: 2184-2194.
8. Deng, Z.P., C.F. Kuang, Z.C. Zhou, S.Y. Huang and K. Luo, 2004. Identification of the race of tobacco bacterial wilt in hunan. J. Hunan Agric. Univ. (Nat. Sci.), 30: 47-49.
9. Xue, Q.Y., Y.N. Yin, W. Yang, H. Heuer, P. Prior, J.H. Guo and K. Smalla, 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.
10. Zou, Y. and C.G. Xiao, 2008. Preliminary identification of physiological race of *Ralstonia solanacearum* in Chongqing. Tobacco Sci. Technol., 5: 60-64.
11. Bochner, B.R., P. Gadzinski and E. Panomitros, 2001. Phenotype microarrays for high-throughput phenotypic testing and assay of gene function. Genome Res., 11: 1246-1255.
12. Engelbrecht, M.C., 1994. Modification of a semi-selective medium for the isolation and quantification of *Pseudomonas solanacearum*. Bacterial Wilt Newslett., 10: 3-5.
13. Guard-Bouldin, J., C.A. Morales, J.G. Frye, R.K. Gast and M. Musgrove, 2007. Detection of *Salmonella enterica* subpopulations by phenotype microarray antibiotic resistance patterns. Applied Environ. Microbiol., 73: 7753-7756.

14. Mols, M., M. de Been, M.H. Zwietering, R. Moezelaar and T. Abee, 2007. Metabolic capacity of *Bacillus cereus* strains ATCC 14579 and ATCC 10987 interlinked with comparative genomics. Environ. Microbiol., 9: 2933-2944.
15. Viti, C., F. Decorosi, E. Tatti and L. Giovannetti, 2007. Characterization of chromate-resistant and -reducing bacteria by traditional means and by a high-throughput phenomic technique for bioremediation purposes. Biotechnol. Prog., 23: 553-559.
16. Wang, H.C., Y.F. Huang, J. Wang, M.S. Wang, H.Q. Xia and H.X. Lu, 2015. Phenotypic fingerprints of *Ralstonia solanacearum* biovar 3 strains from tobacco and tomato in China assessed by phenotype microarray analysis. Plant Pathol. J., 14: 38-43.
17. Wei, Z., X.M. Yang, S.X. Yin, Q.R. Shen, W. Ran and Y.C. Xu, 2011. Efficacy of *Bacillus*-fortified organic fertiliser in controlling bacterial wilt of tomato in the field. Applied Soil Ecol., 48: 152-159.
18. Lu, T., 1998. Advances in research of *Pseudomonas solanacearum* of crop in China. Fujian J. Agric. Sci., 13: 33-40.
19. Xu, J., Z.C. Pan, P. Prior, J.S. Xu and Z. Zhang *et al*., 2009. Genetic diversity of *Ralstonia solanacearum* strains from china. Eur. J. Plant Pathol., 125: 641-653.
20. Li, G.C., L.P. Jin, K.Y. Xie and D.Y. Qu, 2004. Advances in research on bacterial wilt of ginger in China. Chin. Potato, 18: 350-354, (In Chinese).
21. Maurer, L.M., E. Yohannes, S.S. Bondurant, M. Radmacher and J.L. Slonczewski, 2005. pH regulates genes for flagellar motility, catabolism and oxidative stress in *Escherichia coli* K-12. J. Bacteriol., 187: 304-319.