Characterization of *Ralstonia solanacearum* Isolates in Myanmar

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Abstract

Bacterial wilt caused by *Ralstonia solanacearum* is one of the most devastating diseases in economically important crops in the world. It is also important disease of solanaceous crops in Myanmar. The objective of this study was to identify the pathogenic isolates from different hosts and to characterize their race, biovar and phylotype levels. The disease collection was carried out in Shan State which is highland region (Kalaw, Heho), Mandalay Region (PyinOolwin and NyaungOo) and Naypyidaw Region (Yezin) which are relatively lowland areas during October 2012 and April 2013. Isolation was made on selective media (Tetrazolium chloride media) and identified as *R. solanacearum* based on morphological, pathological and biochemical properties and polymerase chain reaction (PCR) by using the species-specific primers. Total 32 isolates (14 potato isolates from Shan State, six pepper isolates, six tomato isolates and two eggplant isolates from Naypyidaw Region and four tomato isolates from Mandalay Region) were examined and all isolates tested were amplified *R. solanacearum* specific band (281 bp) by PCR. All tomato, eggplant and pepper strains belonged to race 1 biovar 3. Potato strains isolated from Heho area were race 3 biovar 2 and race 1 biovar 3. At Heho area, 33% of the tested strains belonged to race 3 biovar 2. Furthermore, multiplex PCR was conducted for the discrimination of phylotypes. Race 1 biovar 3 strains and race 3 biovar 2 strains belonged to phylotype I and phylotype II, respectively. This is the first report of identification and characterization of Myanmar *R. solanacearum* strains to race, biovar and phylotype.

Keywords: bacterial wilt, *Ralstonia solanacearum*, race, biovar, phylotype

1. Introduction

Ralstonia solanacearum (Yabucchi *et al.*, 1995) a pathogen of bacterial wilt of several crops like potato, tomato, pepper, tobacco, etc. is one of the most important diseases causing organism in tropical, subtropical and warm temperate regions of the world (Hayward, 1991). *R. solanacearum* has a diverse array of

populations that differ in various host range, geographical distribution, pathogenicity, genetic characteristics and physiological properties.

R. solanacearum is traditionally divided into five races (He *et al.,* 1983) based on host range. *R. solanacearum* has six biovars (Hayward, 1964; He *et al.,* 1983) based on the ability of the strains based on utilization of different disaccharides and hexose alcohols.Several molecular-based approaches have been developed to enhance the understanding of the genetic diversity of *R. solanacearum* all over the world (Siri, 2014). In 2005, Fegan and Prior (2005) proposed a new hierarchical classification scheme of phylotypes based on the intergenic transcribed sequence (ITS) of ribosomal RNA genes 16S and 23S rhibosoml DNA. The strains within a phylotype closely correlate with geographic origin.

Phylotype I strains originate from Asia, whereas phylotype II strains are primarily from America. Phylotype III comprises strains from Africa, and phylotype IV includes strains from some Asian countries (Japan, Korea, Philippines, and Indonesia) and Australia (Taghavi *et al.* 1996; Elphinstone 2005; Prior and Fegan 2005; Castillo and Greenberg 2007; Jeong *et al.* 2007; Lewis Ivey *et al.* 2007; Poussier *et al.,* 2000, b; Villa *et al.,* 2005).

Therefore, the objectives of this study were to identify strains of *R. solanacearum* isolated from potato, tomato, pepper and eggplants grown in different growing areas in Myanmar for race, biovar and phylotype levels, to characterize their morphological, pathological and biochemical properties and to assess the molecular characterization of Myanmar *R. solanacearum* strains by using the intergenic transcribed sequence (ITS) of ribosomal RNA genes 16S and 23S rhibosoml DNA.

2. Materials and Methods

Determination of Myanmar *R. solanacearum*Isolates as Race and Biovar

Disease collection

The disease collection was carried out in Shan State, Mandalay Region and Naypyidaw Region during October 2012 to May 2013 in Myanmar. The infected plant samples were checked for oozing out which is the simple method by using sterilized knife and a tube containing sterilized distilled water.

Isolation

Infected tomato, potato, chilli, eggplant stems and cuttings of potato tubers were dipped in 70% ethanol for 30 sec and surface sterilized with 5% sodium hypochlorite (3 min), rinsed in sterilized distilled water for three times. Each piece was placed and dipped in the tubes containing sterilized distilled water. The selected media called 2,3,5-triphenyl tetrazolium chloride (TTC) medium containing

casamino acids 1.0, peptone 10 g, glucose 5.0g and 5ml of TTC per liter (Kelman, 1954) was used to streak the bacterial suspension by using sterilized wire loop.

Checking presence of *R.solanacearum* by using universal primers

Myanmar isolates were confirmed by polymerase chain reaction (PCR) technique by using a pair of universal primers (759 and 760) (Opina *et al.*, 1997). DNA was extracted by heat lyses for 5 min cooling in ice after heating 90 ° C for 10 min. PCR amplification was carried out in a total volume of 25 µl using 2.5 µl of 10 x reaction buffer (supplied) and dNTP mixture (2.5 µl each), 0.5 µl of each of primers 759 and 760, 0.25 µl Blend *Taq* DNA polymerase 2.5 units/ µl (Toyobo, Osaca, Japan) and 1 µl of DNA template and 17.75 µl of Milli Q water. PCR amplifications were done in a Thermal Cycler with an initial denaturing step of 95 ° C for 5 min, followed by 30 cycles of annealing 94 ° C for 15 s, 60 ° C for 30 s and 72 ° C for 1 min, with extension step of 72 ° C for 10 min. The suspensions which showed 281 bp, the universal band of *R. solanacearum* were stored and used for further study.

Pathogenicity tests of Myanmar isolates

Seedlings preparation

The varieties of tomato (*S. Lycopersicum* L.) cv. Sekaiichi, tobacco (*N. tabacum*) cv. Bright yellow, eggplant (*S. melongena*) cv. Hitennaganasu and potato (*S. lycopersicum*) cv. Nishiyukata were used for pathogenicity test. Young seedlings were transplanted in plastic pots containing a horticultural 3:1 soil-vermiculite mixture, and grown in a greenhouse at 27 to 30°C. Three plants of each host were inoculated with each isolate.

Inoculum preparation, inoculation and pathogenicity test

Baceterial cells were incubated for 2 to 3 days at 30°C and were harvested and resuspended in sterilized distilled water containing inoculum levels of ca.10^8cfu/ml. Three plants of each host were inoculated with each isolate. Inoculated plants were placed in the greenhouse at 25 to 30°C under natural light. Appearance of wilting was assessed weekly after inoculation by visual inspection of the inoculated plants.

Biovar determination

Myanmar isolates were determined for biovar check according to the physiological test developed by Hayward (1964; 1991) (Table 1). The test is based on the ability to oxidize three disaccharides (cellobiose, lactose and maltose); three hexose alcohols (mannitol, dulcitol and sorbitol), and the ability to utilize trehalose,

myo-inositol and D-ribose for biovar 2 or N2. The standard biovar test medium (basal medium) (Hayward, 1964) was prepared by adding 1 g of $NH_4H_2PO_4$, 0.2 g of KCl, 0.2 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of DifcoBacto peptone and 80 mg of bromothymol blue into a final volume of 1 l of water (Denny and Hayward, 2001). The pH was adjusted to 7.4 by drop wise addition of 5 N NaOH. Trehalose, mannitol, sorbitol, dulcitol, maltose D-ribose and cellobiose were filter-sterilized. Dulcitol and mannitol were dissolved in boiling water.

Table 1. Reactions of biovars to different alcohols and disaccharides

	Biovars					
	1	2	N2	3	4	5
Carbohydrates						
Utilization of alcohol						
Mannitol	-	-	-	+	+	+
Sorbitol	-	-	-	+	+	-
Dulcitol	-	-	-	+	+	-
Trehalose	+	-	+	+	+	+
Inositol	+	+	+	+	+	+
D-ribose	+	-	+	+	+	-
Utilization of disaccharides						
Maltose	-	+	+	+	-	+
Lactose	-	+	+	+	-	+
Cellobiose	-	+	+	+	-	+

The medium was autoclaved after adding 3 g of agar. After the medium was cooled to 65 °C, carbohydrate solution was mixed with basal medium to obtain a final concentration of 1% of the carbohydate. Five milliliters of the mixed medium for each carbohydrate (test medium) were then dispensed into 15 ml clear culture tubes, and kept at room temperature. A bacterial suspension was prepared in sterilized distilled water at ca. 10^9 CFU ml^{-1} concentration (He *et al.*, 1983). Each tube was inoculated with 3 µl aliquot of the bacterial suspension. The tubes were incubated for three weeks at 28° C and the reactions were weekly observed.

Molecular characterization of *Ralstonia solanacearum* isolates

DNA extraction

Myanmar isolates were cultured on TTC medium (Kelman, 1954) for 3 days at 30°C and total genomic DNA was extracted using the Viogene DNA/RNA extraction Kit (Viogene U.S.A) according to the manufacturer's instructions. Precipitate of DNA was stored at -20°C until further use.

Phylotype-specific-multiplex PCR amplification

Multiplex PCR generated from the ITS region of 16S-23S of the ribosomal DNA (Fegan and Prior, 2005) was performed to identify the phylotype to which an isolate belonged. Phylotype specific primers (Fegan and Prior, 2005) were used in combination with the species specific primers 759/760 (Opina *et al.*, 1997) to determine phylotypes. Each 25-µl reaction mixture containing 2.5 µl of 10 x rection buffer (supplied), 2 µl of dNTP mixture (2.5 µl each); 0.5 µl each of primers Nmult21:1F, Nmult21:2F, and Nmult22: InF; Nmult23: AF; 759 and 760; Nmult22: RR; 0.25 µl of Blend *Taq* DNA polymerase 2.5 units/ µl (Toyobo, Osaca, Japan), 1 µl of extracted DNA and 15.25 µl of milliQ water.

PCR was performed in an automated thermocycler (model T 100™ Thermal Cycler) with an initial denaturation at 95°C for 5 min; followed by 30 cycles of denaturation at 94 °C, 15 s, annealing at 60°C for 30 s, and extension at 72 °C for 1 min; with a final extension at 72 °C for 10 min. The amplified DNA was separated by 2% agarose gel electrophoresis in 1 x TAE buffer solution. Gels were stained with ethidium bromide at 0.5 µg/ml and DNA was examined and photographed under UV light. Primers used in the study were described in table 2.

Table 2. Primers used in phylotype specific multiplex PCR for identification of Myanmar isolates

Primer	Sequence of primer (5' to 3')	Specificity	Amplicon size
Nmult:21:1F	CGTTGATGAGGCGCGCAATTT	Phylotype I	144bp
Nmult:21:2F	AAGTTATGGACGGTGGAAGTC	Phylotype II	372bp
Nmult:23:AF	ATTACSAGAGCAATCGAAAGATT	Phylotype III	91bp
Nmult:22:InF	ATTGCCAAGACGAGAGAAG TA	Phylotype IV	213bp
Nmult:22:RR	TCGCTTGACCCTATAACGAGTA	All Phylotypes	
759	GTCGCCGTCAACTCACTTTCC	*R. solanacearum* species specific	281bp
760	GTCGCCGTCAGCAATGCGGAATCG	*R. solanacearum* species specific	281bp

3. Results

3.1 Bacterial isolates

Ten tomato isolates, six chilli isolates, 14 potato isolates and two eggplant isolates from Myanmar showed typical colonies of *R. solanacearum* which were irregular, smooth, creamy white with pink center and fluidal on TTC medium. Six

tomato isolates, six chilli isolates and two egglant isolates were Yezin isolates. At Kalaw two potato isolates were collected and at Heho, 12 potato isolates were observed. Two tomato isolates were collected in each of PyinOoLwin and NyaungOo fields.

3.2 Confirmation of *R. solanacearum* species by using universal primers

All of 32 isolates were amplified a species specific 281bp band to confirm that the isolates were *R. solanacearum* species by using universal primers 759 and 760.

3.3 Pathogenicity of Myanmar isolates to determine race

In pathogenicity tests, initial symptoms on various plants appeared at 3-4 days after inoculation. Myanmar isolates were found rapid wilting on tomato, tobacco and potato but not in eggplant. The tomato isolates were pathogenic to tomato, and those except YT3-2, YT8-1 and YT8-2 isolates were also virulent to potato and tobacco. All of pepper isolates were pathogenic to tomato, potato and tobacco but not pathogenic to eggplant. Eggplant isolates YE1-1 and YE1-2 were pathogenic to all tomato, potato, eggplant and tobacco. All potato isolates were strongly pathogenic to tomato, potato, tobacco and they were not infective to eggplant except HP2-1 and HP7-1. As Myanmar isolates were pathogenic to solanaceous plants, they could be race 1 or race 3.

3.4 Biovar determination

The biochemical reactions of the isolates were checked weekly and results were confirmed after one month. After biovar determination on different hexose alcohols and disaccharides, some isolates were found as biovar 2 being able to utilize lactose, maltose and cellobiose, but could not change color in mannitol, sorbitol or dulcitol. Most isolates in this study were found as biovar 3 which utilized all of disaccharides and alcohols.

3.5 Race and biovar determination of Myanmar isolates

Race 1 isolates corresponded to biovar 3 and race 3 to biovar 2. We could conclude that most of Myanmar isolates belonged to race 1 biovar 3 and some from Shan State occured race 3 biovar 2. Race 1 biovar 3 was observed in all of tomato isolates (YT3-1, YT3-2, YT8-1, YT 8-2, YZT1-1, YZT 1-2, YNT5-1 and YNT5-2, SKT1-1, SKT1-2) from Yezin, NyaungOo and PyinOoLwin. Eggplant and chilliisolates (YPE3-1, YPE3-2, YPE5-1, YPE5-2, YPE9-1, YPE9-2), eggplant isolates (YE1-1, YE1-2) in Yezin also showed the reaction of race1 biovar 3. The

two potato isolates from Kalaw (KP2-1, KP2-2) were race 1 biovar 3. In Heho area, 33% of the isolates (HP7-1, HP7-2, HP17-1, and HP17-2) tested showed the reaction of race 3 biovar 2 and the other isolates (HP2-1, HP2-2, HP4-1, HP4-2, HP11-1, HP11-2, HP 15-1, and HP15-2) were race 1 biovar 3. This is the first finding of race-biovar identification of Myanmar isolates.

3.6 Molecular characterization of Myanmar isolates (Phylotype Identification)

Multiplex-PCR showed that two out of the four phylotypes were present in Myanmar isolates, namely: the Asian phylotype I, and the American phylotype II (Figure 1). Of these isolates, 87% belonged to phylotype I and 13% belonged to phylotype II. Phylotype II isolates (HP 7-1, HP 7-2, HP 17-1, HP 17-2) were found in Heho region, which is the major potato growing area in Shan State, Myanmar. Among 32 isolates, four isolates were phylotype II from potato isolates and the other 28 isolates from different isolates; potato, tomato, eggplant and chilli were occurred phylotype I.

Among Myanmar isolates, 87% showed race 1 biovar 1 phylotype I and 13% were race 3 biovar 2 phylotype II (Table 3). At potato plantation, we found both phylotype I and phylotype II. Only phylotype I was found in the tomato, eggplant and chilli or field grown chilli fields. Race 1 biovar 3 belonged to phylotype I and race 3 biovar 2 corresponded to phylotype II among Myanmar isolates. Any phylotype III and IV could not be found in this experiment.

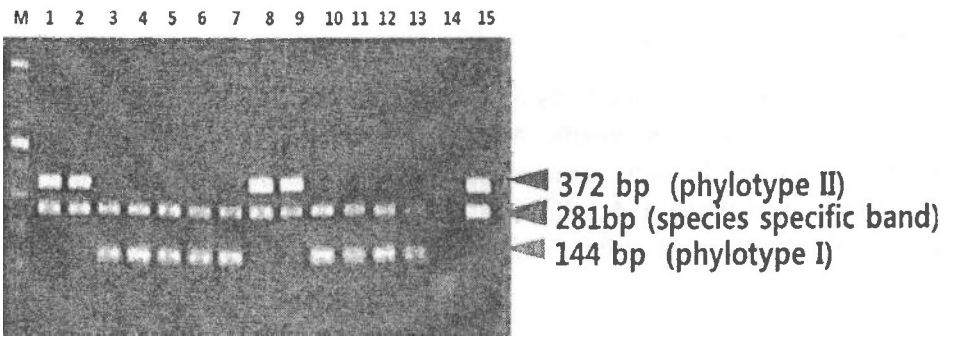

Figure 1. Determination of phylotypes by multiplex PCR (Target region: 16S-23S rDNA internal transcribed spacer region).

4. Discussion and Conclusion

In this study, all of 32 isolates showed the typical colony characters which are mucous and pink-centered on TTC media. The mucoid substance was produced by the accumulation of an exopolysaccharide, to exhibit a typical irregularity of their surfaces (Smith 1920), often with characteristic whorls in the center.

Table 3. Race, biovar and phylotype of Myanmar isolates examined in this study

Isolate	Host	Origin	Race	Biovar	Phylotype
YT3-1	*Solanumlycopersicum*	Yezin	1	3	I
YT3-2	*Solanumlycopersicum*	Yezin	1	3	I
YT8-1	*Solanumlycopersicum*	Yezin	1	3	I
YT8-2	*Solanumlycopersicum*	Yezin	1	3	I
YZT1-1	*Solanumlycopersicum*	Yezin	1	3	I
YZT1-2	*Solanumlycopersicum*	Yezin	1	3	I
SKT1-1	*Solanumlycopersicum*	NyaungOo	1	3	I
SKT1-2	*Solanumlycopersicum*	NyaungOo	1	3	I
YNT1-1	*Solanumlycopersicum*	PyinOoLwin	1	3	I
YNT1-2	*Solanumlycopersicum*	PyinOoLwin	1	3	I
YPE3-1	*Capsicum annuum*	Yezin	1	3	I
YPE3-2	*Capsicum annuum*	Yezin	1	3	I
YPE9-1	*Capsicum annuum*	Yezin	1	3	I
YPE9-2	*Capsicum annuum*	Yezin	1	3	I
YPE5-1	*Capsicum annuum*	Yezin	1	3	I
YPE5-2	*Capsicum annuum*	Yezin	1	3	I
YE1-1	*Solanummelongena*	Yezin	1	3	I
YE1-2	*Solanummelongena*	Yezin	1	3	I
KP2-1	*Solanumtuberosum*	Kalaw	1	3	I
KP2-2	*Solanumtuberosum*	Kalaw	1	3	I
HP2-1	*Solanumtuberosum*	Heho	1	3	I
HP2-2	*Solanumtuberosum*	Heho	1	3	I
HP11-1	*Solanumtuberosum*	Heho	1	3	I
HP11-2	*Solanumtuberosum*	Heho	1	3	I
HP4-1	*Solanumtuberosum*	Heho	1	3	I
HP4-2	*Solanumtuberosum*	Heho	1	3	I
HP15-1	*Solanumtuberosum*	Heho	1	3	I
HP15-2	*Solanumtuberosum*	Heho	1	3	I
HP7-1	*Solanumtuberosum*	Heho	3	2	II
HP7-2	*Solanumtuberosum*	Heho	3	2	II
HP17-1	*Solanumtuberosum*	Heho	3	2	II
HP17-2	*Solanumtuberosum*	Heho	3	2	II

Among all isolates, most isolates belonged to race 1 biovar 3 and some, belonged to race 3 biovar 2. Race 1 biovar 3 isolates were isolated from tomato, potato, eggplant and chilli. Race 3 biovar 2 isolates were isolated from potato.

AtHeho area, 67% of potato isolates were race 1 biovar 3 and 33 % was race 3 biovar 2.

Potato bacterial wilt also known as brown rot of potato, usually caused by the race 3 biovar 2. This subgroup, race3 biovar 2, is reportedly more tolerant than other isolates and is more often foundin tropical highlands and temperate zones where potato is grown (Allen *et al.* 2001, Ciampiand Sequeira1980). The most prevalent isolates, race 3 biovar 2 isolates were observed in Myanmar hilly region Shan State. As Myanmar is one of the South East Asian countries, Asian originated race 1 biovar 3 isolates were obviously prevalent. A distinct group of *R. solanacearum,* race 3 biovar 2, causes a serious disease of potatoes (Hayward 1991, Janse 1996).

Shan State is major potato growing area and source of seed potatoes for all over Myanmar (MAS 2010). It is urgently important to confirm the presence of the most devastating *R. solanaceaum* isolate, race 3 biovar 2 isolates in other regions and prevent to further spread to other hosts or regions in Myanmar.

Our results showed that there were two diverse groups of Myamar isolates, phylotype I and phylotype II, by using multiplex- PCR to discriminate phylotypes, where there were no phylotype III and IV. All of race 1 biovar 3 isolates were corresponded to phylotype I and phylotype II were, race 3 biovar 2 isolates. The 87% was therefore phylotype I and the rest 13 % was found as phylotype II. The American originated phylotype II isolates were observed at Heho, hilly region of Shan State which is the major potato production area and seed source of Myanmar (Maung *et al.* 2006). This result provides the first comprehensive information of race, biovar and phylotype on genetically diverse isolates of *R. solanacearum* in the Naypyidaw region and Mandalay region and Shan State of Myanmar.

6. References

Allen, C., A. Kelman, and E. R. French. 2001. Brown rot of potatoes. in: Compendium of Potato Diseases, 2nd ed. W. R. Stevenson, R. Loria, G. D. Franc, and D. P. Weingartner, eds. American Phytopathological Society, St. Paul, MN:11-13

Castillo, J. A. and J. T. Greenberg. 2007. Evolutionary dynamics of *Ralstonia solanacearum.* Appl. Environ. Microbiol., 73, 1225–1238.

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

Denny TP, AC. Hayward. 2001. In: Schaad NW, Jones JB, Chun W, eds. Laboratory guide for identification of plant pathogenic bacteria. St. Paul, MN: APS Press: 151.

Elphinstone, J. G. 2005. The current bacterial wilt situation: A global overview. Pages 9-28. In: Bacterial Wilt: The Disease and the *Ralstonia solanacearum*

Species Complex. C. Allen, P. Prior, and A. C. Hayward, eds. American Phytopathological Society, St. Paul, MN.

EPPO/CABI. Distribution maps of plant diseases: *Ralstonia solanacearum* 2003-2006. *http://www cabi org/DMPD* 2006.

Fegan, M., and P. Prior. 2005. How complex is the "*Ralstonia solanacearum* species complex"? Pages 449-461, In: Bacterial Wilt Disease and the *Ralstonia solanacearum* Species Complex. C. Allen, P. Prior, and A. C. Hayward, eds. American Phytopathological Society, St. Paul, MN.

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

He, LY, L Sequeira and A. Kelman.1983. Characteristic of *Pseudomonas solanacearum*from China. Plant Disease 67: 1357-1361.

Janse, J. D. 1996. Potato brown rot in Western Europe – History, presence occurrence and some remarks on possible origin, epidemiology and control strategies. Bull. OEPP/EPPO Bull. 26: 679-985.

Jeong Y., J. Y. K. Kim. 2007. Genetic diversity and distribution of Korean isolates of *Ralstonia solanacearum* Plant Dis. 91: 1277-1287.

Kelman A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum*. A literature review and bibliography. Raleigh, N.C.: North Carolina State College.

Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum*to colony appearance on a tetrazolium medium. Phytopathology, 44: 693-695.

Lambert, C. D. 2002. Agricultural Bioterrorism Protection Act of 2002: Possession, Use, and Transfer of Biological; Agents and Toxins; Interim and Final Rule. (7 CFR Part 331). Federal Register 67: 76908-76938.

Lewis-Ivey ML, B.B. McSpadden-Gardener, N. Opina, S.A. Miller. 2007. Diversity of *Ralstonia solanacearum* infecting eggplant in the Philippines. Phytopathology 97:1467–1475

MAS. 1999. Culinary crops production in Myanmar. Myanma Agriculture Service. Yangon, Myanmar. 7p.

Maung MT (Myanmar agriculture service), Maung MM (Yezi agricultural university), Aung H (Department of agricultural research) 2006. Myanmar senerio of potato production, processing and marketing. http://www.unescap. org /tid/mtg/potato_s4myan.pdf

Opina, N, Tavner, F. Hollway, G. Wang, J. F. Li, T. H. Maghirang, R. Fegan, M. Hayward, A.C. Krishnapillai, V. Hong, W. F. Holloway, B. W. and J. Timmins. 1997. A novel method for development of species and strain-specific DNA probes and PCR primers for identifying *Burkholderia*

solancearum (formerly *Pseudomonas solancearum*). Asia Pac. J. Mol. Biol. Biotechnol, 5: 19-30.

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

Siri, M. I., A. Sanabria and M. J. Pianzzola. 2011. Genetic diversity and aggressiveness of *Ralstonia solanacearum* strains causing bacterial wilt of potato in Uruguay. Plant Dis. 95:1292-1301.

Smith EF. 1920. The brown rot of *Solanaceae*. Bacterial diseases of plants. U.S.A.: Saunders Company: 177.

Taghavi M, C. S. L. Hayward, M. Fegan. 1996. Analysis of the phylogenetic relationships of strains of *Burkholderia solanacearum*, *Pseudomonas syzygii* and the blood disease bacterium based on 16 S rRNA gene sequences. International Journal of Systematic Bacteriology 46, 10–5.

Yabuuchi, E. Y. Kosako, I. Yano, H. Hotta, and Y. Nishiuchi. 1995. Transfer of two *Burkholderia*and an *Alcaligenes* species to *Ralstonia* Gen. Nov.: proposal for *Ralstonia pickettii*, *Ralstonia solanacearum* and *Ralstonia eutropha*. Microbiol. Immunol. 39: 897-904.