ABSTRACT

Naglaa Moussa Ahmed Balabel "Persistence of *Ralstonia solanacearum* (Syn. *Pseudomonas solanacearum*) in Differeut Habitats in Egypt" Unpublished Doctor of Philosophy Dissertation. Ain Shams University, Faculty of Agriculture, Department of Agrie. Microbiology, 2006.

Bacterial wilt of solanaceous crops is an important disease in warm climates, though it has been reported in Europe and in the far northern hemisphere. The disease is caused by *Ralstonia (Pseudomonas) solanacearum*. Based on the host range and biochemical tests, five races and five biovars have been identified for the bacterium.

The dominant strain in Egypt is race 3 (biovar II) being characterized by low virulence to tobacco and a lower optimal temperature than other biovars.

From the pathological point of view, the bacterium is found in nature as virulent (vi) and avirulent (av) forms. Both forms may be recovered from diseased plant tissues, though the interrelations between them is not well understood and many questions are still unanswered.

Differentiation between the (vi) and (av) forms can be easily made on media containing 2,3,5 triphenyltetrazolium chloride. Colonies of the avirulent mutants are uniformly round, butyrous and deep red in colour due to the formation of formozan on tetrazolium-containing medium, contrary to the virulent ones. More recently, a Semi Selective Medium of South Africa (SMSA) has been developed for differentiation of virulent and avirulent forms.

The present work reveals the development of large proportion of atypical forms on SMSA medium, from virulent ones stored in water. These forms were phenotypically similar

to the (av) but with strong pathogenic potential on stem inoculation of tomato seedlings. The virulent (vi) and the atypical (at vi) forms were identical in PCR pattern, BOX PCR, Taq-Man and pathogenicity.

Both forms, however, showed considerable differences in fatty acids (FA) profile. The (at vi) forms showed lower content of C12:0 as well as C15:0 ISO and higher content of C15:1 ωC, C15:0 and C17:0 compared to the virulent ones.

The (ty vi) and the (at vi) (previously considered av) based on colony morphology showed distinct differences in nitrate utilization as well. The (ty vi) produced acid in Hugh & Leifson medium containing nitrate, either under aerobic or anaerobic conditions. The (at vi) form, phenotypically avirulent, produced an alkaline reaction under the same conditions with gas evolution anaerobically.

The noticeable differences between the (ty vi) and (at vi) in (FA) profiles and nitrate metabolism may be, in part, attributed to the observed phenotypic differences, on SMSA medium. Such an observation may render colony morphology on SMSA medium, as a sole diagnostic tool for virulence, controversial.

With respect to the origin of *R. solanacearum* isolates, the most pathogenic isolates was recovered from potato tubers and weeds. Soil, water and potato stem isolates were moderate in this regard. *Rumex dentatus* and *Solanum nigrum* were found as alternative hosts for *R. solanacearum* race 3 (biovar II) in Egypt.

Regarding the bacterial survival in the soil, which is of a paramount importance from the pathological and epidemiological viewpoint some unprecedented results have been accumulated. The pathogen has persisted for 6 months in

either loamy sand and clay loam soil under moisture content maintained at 75% WHC and ambient temperature conditions and in dry soil, the pathogen survived in loamy sand soil for 6 months with very high densities in December. On the other hand, densities in dry clay loam soil were extremely low after 5 months (November). This observation(s) on the survival may have a great impact regarding the time of planting potato in Egypt, particularly in view of the failure to detect the pathogen in January & February either under bare fallowing or under controlled soil moisture. These findings may have a great epidemiological value, in considering the disease under Egyptian conditions.

It is interesting to note that the hiofertilization with a biosystem microorganisms product (EM) showed seasoual fluctuation in densities of *R. solanacearum*.

Fluctuation in densities of microbial flora in non-rhizosphere soil shaded with the plaut canopy was studied. The total microbial flora showed gradual decrease in densities, in Spunta and Diamant potato cultivars, up to the middle of June either in clay loam or loamy sand soil. In the latter months however, the brown rot pathogen showed a significant increase, being more pronounced in loamy sand soil.

Key words: *Ralstonia solanacearum*, bacterial wilt disease, typical virulent form (ty vi), atypical virulent form (at vi), virulent (vi), avirulent (av), persistence in different habitat(s), survival in water, survival in soil, preferential host organ effect, bioferti-lization EM product, phenotypic change in colony morphology, effect of organic material on persistence, differences in pathogenic potentials.

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LIST OF ABBREVIATIONS

- at vi form	Atypical virulent form
- av form	Avirulent form
- B_2	Probe used in Taq-Man for detecting only biovar 2 of *R. solanacearum*
- BW	Bacterial wilt
- CFU	Colony forming units
- COX	Fluorogenic probe used in Taq-Man
- CPG	Casamino acids peptone glucose medium
- C. sand	Coarse sand
- Cx	Cellulase
- dNTP	Deoxynucleotide triphosphates
- DTPA	Diethylene triamine penta acetic acid
- EC	Electric conductivity
- EDTA	Ethylene diamine tetra acetic acid
- EG	Endoglucanase
- EM	Effective microorganisms product
- EPS	Extracellular polysaccharide
- EPS1	Exopolysaccharide 1
- FA	Fatty acid
- FAA	Fatty acid analysis
- FITC	Fluorescin isothyocyanate
- FRET	Fluorescence resonance energy transfer
- F. sand	Fine sand
- GNA	Glucose nutrient agar medium
- HOM	Hara and Ono's medium
- IFAS	Immunofluorescence antibody staining
- IFC	Immunofluorescence colony staining
- KB medium	King's medium B
- LED	Light emitting diode
- LPS	Lipopolysaccharide
- NYB	Nutrient yeast broth

- OF	Oxidation/fermentation test
- OLI-1	Specific oligonucleotide primer for *R. solanacearum*
- OM	Organic matter
- PBRP	Potato brown rot project
- PB	Phosphate buffer
- PBS	Phosphate buffer saline
- PC	Phenotype conversion
- PCR	Polymerase chain reaction
- Peh A	Endo polygalacturonase
- Peh B	Exo polygalacturonase
- PFA	Pest free areas
- PG	Polygalacturonase
- PHB	Poly β-hydroxy butyrate
- pJTPS1	Mini plasmid
- PME	Pectin methylestrase
- RS	Probe used in Taq-Man for detecting all biovars of *R. solanacearum*
- SDS-PAGE	Sodium dodecyl sulfate - polyacrylamide gel electrophoresis
- SI	Soil infestation
- So	Isolates of *R. solanacearum* isolated from soil
- So (m)	Mixture isolates of *R. solanacearum* from soil
- SP	Stem puncture
- St	Isolates of *R. solanacearum* isolated from potato stem
- St (m)	Mixture isolates of *R. solanacearum* from potato stem
- SMSA	Selective medium of South Africa
- SUPW	Sterile ultra pure water
- TAE	Tris acetate EDTA
- TSBA	Trypticase soy broth agar medium

- Tu	Isolates of *R. solanacearum* isolated from potato tubers
- Tu (m)	Mixture isolates of *R. solanacearum* from potato tuber
- Ty vi form	Typical virulent form
- TZC	Triphenyl tetrazolium chloride agar medium
- Vi form	Virulent form
- VBNC	Viable but non culturable
- Wd	Isolates of *R. solanacearum* isolated from weeds
- Wd (m)	Mixture isolates of *R. solanacearum* from weeds
- Wt	Isolates of *R. solanacearum* isolated from irrigation water
- Wt (m)	Mixture isolates of *R. solanacearum* from irrigation water
- Y-2	Non specific primer for *R. solanacearum* used in PCR technique
- YPGA	Yeast peptone glueose agar medium