STUDIES ON THE PATHOLOGICAL AND GENETIC CHARACTERIZATION OF RALSTONIA SOLANACEARUM IN MYANMAR

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Title ：STUDIES ON THE PATHOLOGICAL AND GENETIC CHARACTERIZATION OF *RASLTONIA SOLANACEARUM* IN MYANMAR
（ミャンマー産青枯病菌の病理学的及び遺伝学的特性に関する研究）

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Thesis Summary

Ralstonia solanacearum is a causal agent of bacterial wilt disease affecting several hundred-plant species, including solanaceous plants, and causes severe yield losses worldwide. In 2013 and 2014, an extensive survey of bacterial wilt in Myanmar was performed, and 70 strains of *R. solanacearum* were collected from wilting tomato, potato, chili and eggplant plants from seven locations of subtropical and tropical areas of the country. Myanmar strains of *R. solanacearum* were characterized from pathological and genetic points of view by traditional and molecular methods. Whitish fluidal colonies with pink centers were serially streaked on 2, 3, 5-triphenyltetrazolium chloride (TTC) medium plates to obtain purified bacterial cultures with the homogeneity in colony morphology. Polymerase chain reaction (PCR) test then amplified to confirm one specific band (281-bp) from the template DNA of Myanmar strains of *R. solanacearum* by using 759/760 species specific primers. The pathogenicity of the strains was confirmed by individually using stem inoculation on original host plants.

In biochemical determination tests, six biovars (1, 2, N2, 3, 4, and 5) can be differentiated based on utilization of six kinds of sugars and three kinds of hexose alcohols. Biovar determination test showed that 63 % were biovar 3 strains and predominant among all of the Myanmar strains of *R. solanacearum*. Biovar 4 strains occupied seven % which were obtained from both tomato and chili strains, whereas biovar 2 belonged to 30% strains were isolated only from potato. Multiplex-PCR method is applicable to differentiate each phylotype of Myanmar strains of *R. solanacearum* based on differences in the nucleotide sequences of the 16S rDNA-ITS region. The analysis indicated that tomato, eggplant and chili strains belonged to phylotype I, whereas potato strains comprised phylotype I and phylotype II. Strains in phylotype I, which was suggested to be originated from Asia, were the most prevalent in all surveyed areas.

Furthermore, genetic diversity of Myanmar strains of *R. solanacearum* was evaluated by rep-PCR DNA fingerprinting types. BOX, ERIC and REP primer sets expressed 33 fingerprint types and seven clusters among the strains by Dice’s coefficient at 80% similarity. Strains in phylotype I indicated 29 DNA fingerprint types and were divided into six clusters. The clusters were closely correlated with host plant, geographic origin, sequevar and/or biovar of the strains. In contrast, phylotype II strains showed four DNA fingerprint types which contained single cluster 7. The results stated that phylotype II strains were more homogenous than phylotype I strain.

Moreover, hypersensitive reaction of Myanmar strains of *R. solanacearum* also expressed four groups by the results of 72 hrs after infiltration on tobacco leaves. Pathogenicity test to four solanaceous plants

differentiated the strains into six pathogenic groups. Phylogenetic analysis based on the endoglucanase (*egl*) and *hrpB* gene sequences revealed that Myanmar strains of *R. solanacearum* could be partitioned into two major clusters each corresponded to phylotype I and II. Strains in phylotype I were further divided into seven subclusters, each was corresponded to single distinct sequevar (15, 17, 46, 47, 48, unknown 1 or unknown 2). All strains in phylotype II belonged to sequevar 1.

In *hrpB* sequence analysis, phylotype I strain comprised nine subgroups and phylotype II strains contained two subgroups. The subgroups in *hrpB* sequences were mostly equivalent to sequevars of *egl* sequence analysis with slight variations. More diverse groups were observed in *hrpB* sequence analysis than *egl* analysis. The results obviously confirmed high genetic diversity in phylotype I strains in both sequence analyses to confirm that they were indigenous strains of Myanmar. In contrast, phylotype II potato strains were highly homogenous in aspect of same sequevar or subgroups, strongly suggesting that they have invaded to Myanmar.

These are the first detailed studies of Myanmar strains of *R. solanacearum* from diverse regions of Myanmar and the results will support the plant quarantine and management systems to prevent further dissemination of Myanmar strains of *R. solanacearum* to the disease free areas of the country. The exact information of the pathogen will also provide important information to develop resistant varieties in solanaceous crops. Further study underlining on other new hosts such as tobacco, ginger, banana, and groundnut will be preferable to explore the presence of other new strains in the frontier area and to do better management of bacterial wilt prevention in the control strategy of Myanmar agriculture.