Supot Kasem 2007: Isolation and Characterization of Tn5-Induced Mutants of *Xanthomonas axonopodis* pv. *glycines* Deficient in Pathogenicity. Doctor of Philosophy (Tropical Agriculture), Major Field: Plant Pathology, Department of Plant Pathology. Thesis Advisor: Associate Professor Sutruedee Prathuangwong, Ph.D. 130 pages.

Xanthomonas axonopodis pv. *glycines* (Xag), the causal agent of bacterial pustule was subjected to transposon mutagenesis with random Tn5 insertion to generate mutant defective in pathogenesis through an effect on the production of virulence factors. A novel Xag mutant strain KUMNTP2 which was intermediate and delayed in disease induction on host plant soybean (*Glycine max* cv. SJ4), and failed to induce visible hypersensitivity response on tobacco (*Nicotiana tabacum* cv. Xanthi), but still induced cell death on tomato (*Lycopersicon esculentum* cv. Sridatip-II), was selected for study. Sequence analysis of 2.37 kb *Sac*I DNA fragment contained Tn5 element in which a mutation chromosome revealed the transposon was inserted in phosphoenal pyruvatesyntase (*ppsA*) genes with homolog to *ppsA* of *X. axonopodis* pv. *citri* (99%). Xag mutant was unable to grow on non-sugar, NaAC as a sole carbon source unless glucose was added to the growth medium exhibiting once the transposon disrupted *ppsA* function. A mutant knockout *ppsA* protein secretion KUMNTP2 showed delayed growth and reduced multiplication in host plant and was restored by supplemented with 10%(v/v) glucose that seemed to revive automously with secretion compromised in its ability to induce a few necrotic lesions formed. Furthermore, the production of extracellular polysaccharide was significantly decreased and the secreted protein elicitor of extracellular cellulase, but not amylase or protease, virulent factors of Xag, was illustrated defecting in the *ppsA* mutant. Comparison among wild type, mutant and complementation strains showed that enhanced cellulase secretion requires induction from *ppsA* and found to be involved in a feedback mechanism that modulates *ppsA* expression. Taken together, our data suggest the possibility that mutation in the *ppsA* genes of Xag may affect, either directly or indirectly, the transport of certained secreted proteins from the cytoplasm to the critical factor for Xag pathogenesis and full virulence in soybean. We further demonstrated that KUMNTP2 mutant had ability to trigger induced systemic resistance of soybean and linked to the production of defense related-enzyme or protein expression against pathogenic Xag wildtype infection. The β-1,3-glucanase and phenylalanine ammonia lyases were found to accumulate with greater amount in mutant pretreated-plant challenged with pathogen wildtype at one day after pathogen challenge. These two chemicals reached maximum at the 3rd and 4th day after pathogen challenge respectively. The pathogen-inoculated plants, the accumulation started with lesser amount at the 2nd day and drastically decreased 3, and 4 days after pathogen inoculation respectively. Activities of phenols and peroxidase also increased in an equivalent amount in all treatments tested. The coinoculation of soybean leaves with mutant and Xag wildtype challenge also revealed a tenfold reduction in the number of infected pustule lesions at 7 days after infection. Moreover, the growth of wildtype in inoculated plants was significantly smaller in mutant pretreated-plants after 4 days of inoculation, suggesting that pathogen growth was mediated by difference in enzyme defense responses. This work has generated an interest in resistance strategies exploiting weak or non pathogenic strains as a tool for defense mechanism in plant, and might be model contribution to the development of novel strategies for disease control.

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