Wilawan Chuaboon 2014: The Flagella Secretion System on Virulence of Soybean Plant by *Xanthomonas axonopodis* pv. *glycines*. Doctor of Philosophy (Plant Pathology), Major Field: Plant Pathology, Department of Plant Pathology. Thesis Advisor: Associate Professor Sutruedee Prathuangwong, Ph.D. 157 pages.

Xanthomonas axonopodis pv. *glycines* (Xag) causes bacterial pustule disease, resulting in severe losses in many soybean growing areas around the world including Thailand. Flagellum has been identified as one of structural components of virulent protein translocation into plant cells for bacterial infection, but not yet for Xag. Also, a recent study has demonstrated that a pectate lyase (Pel) in *xagP* of Xag 12-2 is required to induce a hypersensitive response (HR) on tobacco nonhost plant (*Nicotiana rustica*). Until now its role in pustule disease was unknown and whether the coordinate genetic regulation between flagellum biosynthesis and Pel expression was injected into target soybean host. To better understand of these functions, a genetic and characterization of the pectate lyase (*xagP*), flagella hock (*flgC*), flagellin (*fliC*), and hock associated protein 2 (*fliD*) genes of Xag strain 12-2 were carried out. Mutations in flagella genes *flgC*, *fliC*, and *fliD* resulted in loss of or reduced motility, biofilm formation, development of pustule disease and pathogen growth on soybean. In addition a pectate lyase gene (*xagP*) mutant also showed reduction in biofilm formation and virulence on soybean. The *xagP* mutant lost pectolytic activity whereas the *flgC*, *fliC*, and *fliC&fliD* expressed reduced pectolytic activity on potato slices suggesting the *xagP*-encoded pectate lyase may be secreted through flagella machinery. The flagella mutants were also altered in cellulase, alpha-amylase, endoglucanase, and protease activities again suggesting a role of flagella in secretion of these virulence factors. Complementation of mutants restored all phenotypes to wildtype levels. The flagella gene mutants did not affect development of a hypersensitive response on tobacco whereas the *xagP* mutant was HR-negative. These evidence indicated that flagellum was due in part to secrete pectate lyase resulting disease in soybean development. Since *fliC* and *xagP* decreased three-extracellular enzyme production, RT-PCR and NanoDrop analyses were investigated to examine the gene expression of alpha-amylase, cellulase, and protease. As expected, the lower mRNA levels detected in mutant than wildtype was observed. The relatedness of these genes was focused with two sigma factors *rpoE* and *rpoN* that revealed their responses to extracellular enzyme reaction and pathogenicity regulated by *xagP* and flagellin genes. The expression of *xagP* and *fliC* in *rpoE* and *rpoN* mutants was also reduced in cellulase, alpha-amylase, endoglucanase, and protease; and disease development, suggesting that the two sigma factors (XagPs and FliCs) were also essential for full virulence in Xag 12-2. This is the first report linking a production of effector proteins and their secreted function by these genes to bacterial pustule disease in soybean.

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