The role of sensor kinase GacS involved in production of secondary metabolites and in induced systemic resistance of *Pseudomonas chlororaphis* O6

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Root-associated bacterial populations can influence on plant health in several ways, including altering growth through plant hormone production, inciting as well as protecting plants from disease and environmental stress. *Pseudomonas chlororaphis* O6 is an aggressive root colonizer that improves plant growth and increases tolerance to pathogen challenge and drought stress. However, colonization inhibited germination of certain seeds, such as barley and wheat. To understand how root colonization by this microbe affects plant metabolism, we investigated the mechanisms by which plant-active metabolites are regulated. A sensor kinase GacS controls production of many secondary products that are important in microbial survival in the environment. There was no difference in root colonization ability on cucumber roots between wild-type and the *gacS* mutant. The *gacS* mutant did not inhibit germination of barley and wheat as displayed by the wild-type isolate. Inhibition of germination of barley and wheat also was lacking in another O6 mutant deficient in phenazine but not hydrogen cyanide production suggesting that inhibited germination is correlated with production of phenazines. The sensor kinase GacS induces nonspecific systemic resistance on plants that may have broad-spectrum activity of protection. The *gacS* mutant had abolished induced disease resistance caused by plant pathogens. and did not induced drought resistance in cucumber of *P. chlororaphis* O6. Drought resistance in Arabidopsis but not cucumber also was significantly impaired in plants colonized by the gacS mutant compared to the responses caused by colonization by the wild-type. The alleviation of drought correlated with reduced stomatal openings in Arabidopsis leaves of plants with roots colonized by the wild-type O6. Each of these effects of the *gacS* mutant was restored to the wild-type response

by in trans complementation with the wild-type *gacS* gene. These results suggested that several at present unknown factors involved in changes in plant function are produced by *P. chlororaphis* O6 under control of the global regulator, GacS. Thus, the global regulator *gacS* was required for different elements of interaction between the bacterium and a host plant.

Accumulation of the *gacS* transcript was greatest in late-logarithmic phase and stationary-phase cells. Transcripts from phzI, encoding a regulator of phenazine production, and *phzA*, encoding one of the enzymes in phenazine synthesis, were strongly induced in stationary-phase cells of the wild-type, but were greatly diminished in stationary-phase cells of the *gacS* mutant. Phenotypes and expression of *phz* genes was restored to wild-type levels after complementation of the mutant in trans by the wild-type *gacS* gene. Production of the plant growth regulator indole acetic acid was increased in the *gacS* mutant in comparison to the wild type and complemented mutant when cells were grown on tryptophan-amended medium. Confocal microscopy of bean root tips showed that orange-colored phenazines were produced by the wild-type O6 during colonization but were lacking with the *gacS* mutant.