The GacS or Lon mutant was found to be deficient in the production of phenzines, exoenzymes, and the acylhomoserine lactone. These mutants were not complemented by *phz* operon and addition of exogenous AHL. These results indicate that the GacS global regulatory systems controls phenazine production at multiple levels. Future research will focus to identifying the GacS-mediated regulatory cascade involving in production of phenazine in *P. chlororaphis*.

### 2-28. Transcriptional regulation and mutational analysis of a *dctA* encoding organic acid transporter protein from *Pseudomonas chlororaphis* O6.

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A *dctA* gene encoding a protein with identity to a C4-dicarboxylate/$H^+$ was cloned from a beneficial biocontrol bacterium, *P. chlororaphis* O6. Expression of the *dctA* was induced in minimal medium by several organic acids and was repressed by glucose. Highest expression was observed in early-log cells grown on fumarate and succinate with decline as cells approached late-log phase. The *dctA* transcript accumulated weakly when cells were grown on malate but strong expression was observed with benzoate. Expression of the *dctA* transcript was repressed in early-log cells upon addition of glucose to fumarate, but was detected as the cell culture aged. A *dctA*-deficient mutant of O6, constructed by marker exchange mutagenesis, did not grow on minimal medium containing succinate, benzoate, or fumarate, and growth on malate was delayed. The *dctA* mutant and wild type grew equally on glucose. The *dctA* mutant on cucumber roots in sterilized potting soil was colonized at levels comparable to those of the wild type, but induction level of disease resistance by the mutant against target leaf spot disease was decreased. These results may indicate that the *dctA* is essential for utilization of certain organic acids and its expression is controlled by the availability of sugars. In addition, the *dctA* is not essenitial for cucumber root colonization, but important for induction of disease resistance.

### 2-29. The global regulator GacS of a biological bacterium *Pseudomonas chlororaphis* O6 regulates expression of the stationary-phase sigma factor *rpoS* and reduces survival in oxidative stress.

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The global regulator, GacS (global antibiotic and cyanide sensor kinase), was required for the increased resistance to hydrogen peroxide occurring as cultures of the rhizobacterium, *P. chlororaphis* O6, matured. Specific stationary-phase peroxidase and catalase isozymes were absent in the GacS mutant, whereas a manganese-superoxide dismutase isozyme was expressed earlier and to a great extent than wild type. In the wild type cell, transcript accumulation of *rpoS* was higher in late logarithmic-phase cells than cells from mid logarithmic- or stationary-phase. Transcripts from

*rpoS* in the GacS mutant were reduced in each of these growth phases compared to the wild type expression. The down stream sequence from *rpoS* lacked sequences encoding a small RNA, *rsmZ*, found in other pseudomonads and implicated in control of genes activated by the GacS system. These findings suggest that GacS-mediated regulation of RpoS plays role in control of oxidative stress in *P. chlororaphis* O6 by as yet an unknown mechanism.

**2-30. Isolation and characterization of induced disease resistance (ISR)-deficient mutants of a biocontrol bacterium *Pseudomonas chlororaphis* O6.**

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Lipopolysaccharide, siderophore, and cyclic dipeptide have been shown to be necessary for ISR induction by pseudomnads. However, there is no report on cloning of genes or generating specific mutants involving in ISR activity. A biological control bacteium *P. chlororaphis* O6 induces resistance to *Erwinia carotovora* subsp. carotovara SCC1 in tobacco and induces drought resistance in *Arabidopsis*. To isolate genes involved in ISR activity and induction of drough resistance of O6, we constructed Tn5 mutants and were used to screen for ISR activity and drought resistance activity using microtiter assay with tobacco and *Arabidopsis*. Thirty-three ISR-deficient mutants were selected, and the nine ISR-deficient mutants were also lost activity of drought resistance. The flanking sequence analysis of the ISR and drought resistance-deficient mutants showed that a *gacS* gene encoding a two-component sensor kinase, and a *mce* gene encoding a protein involved in mycobacterial cell entry were mutated. The flanking sequence of each Tn5 mutant altered ISR activity is currently under investigation. These results indicate that *gacS* and *mce* are important genes in induction of ISR activity and drought resistance of *P. chlororaphis* O6. Our works will open opportunities for identification of bacterial genes or traits that are involved in ISR activity and induced drought resistance of *P. chlororaphis* O6.

**2-31. Bacterial determinants involved in the induction of systemic resistance and plant growth promotion in tobacco by *Pseudomonas chlororaphis* O6.**

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The ability of *P. chlororaphis* O6 to induce resistance to *Erwinia carotovora* subsp. carotovara SCC1 and to promote growth in tobacco was demonstrated in microtiter assays on plants pre-inoculated at the root level with the bacteria before challenge with the leaf pathogen. To identify th bacterial determinants involved in induced systemic resistance and plant growth promotion, cell culture of O6 grown in King's medium B was fractionated with organic solvents and purified using various columns. *In vivo* and *in vitro* assays with samples from successive fractionation steps of the O6 supernatant led to the conclusion that antibacterial compounds were