

Final Report for DOE-NABIR

1. *Pseudomonas fluorescens* Pf0-1: Discovery of AdnA

P. fluorescens Pf0-1 is a soil bacterium isolated by this laboratory from sandy loam soil (4). Because of the importance of adhesion for persistence in natural environments, we utilized adherence to sand as an assay to screen a library of Pf0-1 mutants for defects in adhesion. Three adhesion defective mutants, Pf0-5, Pf0-10, and Pf0-15 were recovered. Pf0-5 and Pf0-10 had different insertions in the same gene, which we called *adnA*, and also showed motility defects (3). Pf0-15 was motile, but was hyper-flagellated. The insertion was in a different gene, *adnB*, which shows similarity to *mot* genes involved in flagella functions (Strain and Levy, unpublished). These early studies demonstrated the important but separable requirements for flagella and motility in adherence.

In a field study, the *adnA* mutant Pf0-5 was less able to persist than the wildtype Pf0-1 and did not spread as fast or as far from the point of inoculation as did Pf0-1 (7), linking adhesion and soil fitness. DNA sequencing revealed that AdnA shares 82% identity with the flagella regulator FleQ from *P. aeruginosa* (3). FleQ is required for adhesion of *P. aeruginosa* to respiratory mucin, which is important for pathogenesis (1, 2).

Using a gene fusion approach, seven loci that are expressed in an AdnA-dependent manner were identified (8). The loci were called “*aba*”, for affected by AdnA. We uncovered genes involved in motility, chemotaxis, LPS synthesis, and two genes of no known function. Four of the *aba* genes were not reported to be in the FleQ regulon (5).

2. Expression of fitness genes in soil

We recently began using the IVET (*in vivo* expression technology) promoter-trap to identify genes whose expression is upregulated in soil. We identified 22 sequences (termed *iiv* for induced *in vivo*) that are upregulated in sterile soil (9). Ten of these genes are similar to sequences present in genbank, and two sequences are classed as 'hypothetical'. We also found ten *iiv* genes that are antisense to known genes, providing new insight into genome organization (10). We have called these sequences “cryptic” because they were not detected during annotation of the *P. fluorescens* Pf0-1 genome sequence. We have detected open reading frames for each of the 10 cryptic genes.

To test the importance of soil-induced genes in survival or persistence, we constructed mutations in three *iiv* genes, and tested them in soil growth assays. Relative to the wildtype, these mutants had no growth defect in laboratory culture, but the mutations did increase the time needed to establish the population in sterile soil (9). These data provide support for our hypothesis that IVET will reveal genes involved in environmental (soil) survival. The gene *iiv2* which has a colonization defect in sterile soil, has no similarity to known sequences and thus is a novel sequence with a role in soil colonization. In live soil microcosms, wildtype and *iiv2* mutant bacteria were inoculated in a central “core”. Persistence within the core and spread from the core were quantified, using a specific competitive PCR (6) approach which we developed and optimized. The *iiv2* mutant showed reduced persistence within the microcosm core, and was impaired in its ability to spread out and colonize soil. These data further support the IVET approach for finding previously uncharacterized genes that are critical to fitness in soil.

To directly confirm transcription, we have carried out RT-PCR analysis on cryptic *iiv* genes, and the sequences on the opposite DNA strand. Primers that permitted the amplification of a region internal to both cryptic and opposite gene were used. Reverse transcription used a single gene-specific primer, so that transcripts from both strands to be detected separately. The RT-PCR experiments have confirmed expression of eight cryptic genes, and the genes encoded on the opposite strand. The relative cryptic:opposite gene expression level varies between loci. For example, *iiv14* is expressed more than its opposite gene, while both *iiv5* and *iiv23* appear to be expressed at lower levels than the opposite genes.

The *iiv* genes show increased expression in soil relative to laboratory media. We have begun to isolate mutants in which *iiv* promoter activity is increased to levels that can be detected in laboratory culture. We have successfully generated mutants that upregulate 15 different *iiv* genes, including five cryptic genes. In the case of the cryptic gene *iiv14*, de-repression resulted in an 18.3 fold increase, while for *iiv23* the increase was 6.3 fold. The mutation that led to de-repression of *iiv23* was found to lie in the *dsbD* precursor gene. Cloned *dsbD* precursor complemented the mutation, i.e. repression of *iiv23* was restored.

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