Examining the Roles of Antisense RNA at pilY1 and tad loci in *Variovorax Paradoxus* EPS Biofilms and Motility

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**Abstract**

*Variovorax paradoxus* EPS is a ubiquitous soil beta-proteobacterium that plays important roles in biotransformation of xenobiotics and plant growth promotion. The genome of *V. paradoxus* EPS has been sequenced. Previous mutagenesis work has identified a number of genes important in motility and surface attachment. Because no clear flagellar locus was annotated in the genome, but flagellar motility is clearly observed in culture, we have examined this phenotype further using transposon mutagenesis and enrichment for non-motile variants. Two loci that were identified in these screens are the type IV pilus (particularly the pilY1 gene) and a putative tad tight adhesion locus. Recent analysis of the *V. paradoxus* EPS transcriptome during log, stationary, and biofilm growth was combined with mutagenesis data to examine the possible role of antisense RNA regulation at these two loci involved in motility. The transcriptome data identified increased levels of antisense corresponding with downregulation of gene expression at each locus during biofilm growth. Because we used Tn5, which contains a promoterless lacZ gene, for our mutagenesis experiments we were able to analyze gene expression patterns directly. We conducted this analysis for multiple insertion mutants within the pilY1 (Varpa_5900) and tad loci (Varpa5148-59) in liquid cultures as well as in surface attached modes of growth. These experiments both confirmed and extended the information from our transcriptome work. Multiple independent insertions in the pilY1 gene, oriented in both directions, were mapped and investigated. From the set of independent unique insertions (10 pilY1 and 5 tad mutans), 2 insertions in the antisense orientation were identified at each locus. Measurement of beta-galactosidase activity in each growth phase in both directions using X-gal, ONPG, and MUG as substrates were used help elucidate the role of the antisense expression in the regulation of these surface structures.

**Background**

**Methods:**

Methods: Expression of β-galactosidase activity was measured during logarithmic and stationary phases of growth using ONPG as substrate. Cultures of each mutant were optically measured and collected at various time points for both growth phases. Samples were chemically treated and concentrations of ONPG were optically measured and then calculated (1).

**Conclusions:**

Conclusions: Sense and antisense expression were detected in both of these loci. The data supports variation with growth phase and in surface growth in both sense and antisense transcripts. Spatial distribution of the expression in biofilms has been demonstrated, and density dependence is an additional possibility.

**Future Work:**

Future Work: Cloning antisense and sense promoters into unstable fluorescent reporter vector. Examine regulation of sense and antisense promoters in wild type background.

**References**