

SMALL RNAS IN *XYLELLA FASTIDIOSA* AND THEIR EPIDEMIOLOGICAL APPLICATIONS

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ABSTRACT

Bacterial non-coding small RNAs (sRNAs) have attracted considerable attention due to their roles in regulating numerous cellular processes including survival, adaptation and pathogenesis. Sequence variation in sRNA genes reflect a previously unrecognized source of genomic diversity in bacteria. *Xylella fastidiosa* (*Xf*) is an important bacterial pathogen causing many economically important diseases such as almond leaf scorch, citrus variegated chlorosis and Pierce's disease of grapevine. Little is known about sRNAs in this bacterium. Therefore, a research project was initiated to search for sRNAs in *Xf*. The complete genome sequences of four *Xf* strains (9a5c, M12, M23, and Temecula1) representing three *Xf* subspecies were selected and scanned for sRNA genes with established computer programs. Candidate sRNA genes were identified in all of the four *Xf* strains (46 in strain 9a5c, 50 in strain M12, 49 in strain M23, and 47 in strain Temecula1). Candidate sRNA genes ranged in size from 40 to 350 bp. Expression of sRNA genes was proved using a procedure involving quantitative reverse transcriptase PCR (qRT-PCR) and confirmed with negative detection with primers from regions flanking the predicted sRNA genes. BLAST analysis showed that 34 sRNA genes were shared by all three *Xf* subspecies. To test for epidemiological application of variable sRNA genes, four sRNA genes in strain M23 were selected to design PCR primers. A total of 22 different bacterial strains were cultured in PW broth at 28 °C for 14 days. DNA was extracted and used as templates for RT-PCR with the four sRNA primer sets. Both inter- and intra- subspecies variation of *Xf* strains was observed.

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