

INHIBITION OF XYLELLA FASTIDIOSA BIOFILM FORMATION VIA METAL CHELATORS

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Pierce's disease (PD) is a lethal disease for a variety of crops caused by *Xylella fastidiosa* (*Xf*). *Xf* is a gram-negative phytopathogen that forms biofilms. One of the twelve genes that regulate exopolysaccharides, a major component of biofilm, is aconitase which seems to respond to intracellular iron levels. It has been reported that lactoferrin can cause deprivation of iron, thus inhibition of biofilm formation in *Pseudomonas aeruginosa*. We have observed that biofilm formation can be blocked using iron chelators such as lactoferrin, EDTA (ethylenediaminetetraacetic acid), and EDDS (ethylenediaminedisuccinic acid). Conalbumin was used in a parallel manner with lactoferrin during a 6.5 day incubation period due to its availability. During our study, incubation of *Xf* in the presence of lactoferrin at 1000 µg/mL for 3.5 days showed the greatest biofilm inhibition of 42%, as well as planktonic (liquid phase bacteria) inhibition of 32%. EDTA at a concentration of 15 mg/mL inhibited 99.7% of biofilm and 98.9% of planktonic in a 24 hour incubation. In contrast, EDDS at a concentration of 38.2mg/mL showed 64.7% inhibition of biofilm and 33.6% inhibition of planktonic. Iron deprivation could serve as a first step towards eradication of PD via blockage of biofilm formation.

SITE-DIRECTED *RPFA* GENE DISRUPTION IN *XYLELLA FASTIDIOSA*: EFFECT ON BIOFILM FORMATION VIA QUORUM-SENSING IN PIERCE'S DISEASE

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The shuttle DNA vector pSP3 was constructed to generate mutations by DNA insertion. This construct can replicate in *E. coli* and in *Xylella fastidiosa* (*Xf*). If a DNA fragment containing part of the *Xf rpfA* gene encoding for aconitase is cloned into pSP3, specific integration of this construct into the *rpfA* gene will be induced. Previous results with the *Xf xpsD* gene, using a pSP3(xpsD600) construct, indicate that this vector is useful in generating gene disruption by homologous recombination. We are currently investigating the potential role of the *rpfA* gene in biofilm production using this gene disruption technique.

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