

Characterization of a Bacterial Contaminant in Loblolly Pine Tissue Culture

M. J. Cook¹, J. J. Clark¹, J. W. Anderson², A. D. Junkins³,
C. K. Karr⁴ and M. R. Becwar¹

Poster Abstract

Bacterial contamination of immature seed explants can decrease the efficiency in initiating and establishing pine embryogenic cultures. A type of contaminant commonly observed surrounding the megagametophyte explant of immature loblolly pine (*Pinus taeda* L.) appears as a white to creamy halo on the surface or within the culture medium. This contaminant appears within 24-48 hours after culturing the immature seed. The severity of the contamination varies by year and family. The pattern of contamination among seeds pooled from several cones did not suggest operator technique as the source of contamination. Tests with seeds separated by cone during sterilization and culture suggest that the source of the contamination was from seeds of specific cones. To better understand the causal bacterium and provide insight to help prevent or decrease the occurrence of contamination, several isolates taken from different contaminated explants were identified. This identification was done by PCR amplification of a 500bp sequence of a 16S ribosomal RNA gene. The closest match in terms of sequence similarity was *Erwinia amylovora*. *Erwinia* species are the causal agents of several economically important diseases. For example, fire blight (*E. amylovora*) in the *Roseaceae* family that affects apple and pear trees, and soft rot (*E. carotovora*) in several crops including potato. Our results suggest that *Erwinia* may also infect immature loblolly pine seeds and is a probable causal agent of this type of bacterial contamination in pine tissue culture. We have been able to significantly reduce the negative impact of this contamination and increase culture establishment efficiency by separating seeds by cone for sterilization and culture.

1 ArborGen, Summerville, South Carolina 29484-8401; ² Marion County Medical Center, Marion, South Carolina 29571; ³ Dept. of Pathology, University of Iowa, Iowa City, Iowa 52242-1181; ⁴ Medical University of South Carolina, Office of Research Integrity, Charleston South Carolina 29425.