Pathogenic and ice nucleation active (ina) bacteria causing damages to energy forestry plantations (Willow, Poplar): from problem assessment, characterisation, identification to solution.

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To find out whether bacteria isolated from diseased plant parts can be the main causal agent for the dieback appearing in *Salix* energy forestry plantations in Sweden during the last few years, and if the joint effects of bacteria and frost injury are synergistic, extensive sampling of shoots from diseased *Salix* plants was performed. We performed several laboratory and greenhouse investigations and used evaluation techniques on the functions of the Ice-Nucleation Active (INA) bacteria.

We carried out a comparison between spring and autumn bacterial communities isolated from within (endophytically) and surface (epiphytically) plant tissues of *Salix viminalis*. Seasonal variation of bacteria in willow clones with different levels of frost sensitivity and symptoms of bacterial damage was also investigated. We further focused on possible effect of fertilisation and nutrient availability on the bacterial community in relation to plant dieback in Estonian willow plantations.

The identification and detection of INA bacteria which cause damage in combination with frost to willow (*Salix* spp) plants in late fall, winter and spring was performed using BIOLOG® MicroPlate, biochemical tests, selective INA primers and 16S rDNA analysis. To distinguish the character for differentiation between these bacteria morphologically and with respect to growing ability different culture media were used. We studied the temperature, at which ice nucleation occurred for individual bacteria, estimated the population of INA bacteria, effect of growth limiting factors, and evaluated the effect of chemical and physical agents for disruption and possible inhibition of INA among individual bacterial strains. The concentration of carbon, nitrogen and phosphorus on INA is discussed.

We demonstrate that among the bacterial isolates recovered from the willow plantations, there were many that were capable of ice nucleation at temperatures between -2℃ and -10℃, many that were capable of inducing a hypersensitive reaction in tobacco, as well as causing necrotic symptoms on willows exposed to frost treatment.

The most frequently isolated types were found to be non-fluorescent *P. fluorescens* (biotype A, B, C, F, G) and/or *Sphingomonas* spp. *Erwinia* spp, *P. fluorescens*, *Xanthomonas* spp and *P. syringae* however, were considered to be the most important pathogens in the field. We conclude that diseases caused by INA bacteria in relationship with frost are a limiting factor in willow and poplar plantations in Sweden and most likely also in other temperate regions in the world.

Possible solutions: Bacterial/rust fungus resistance in current clonal material, testing for pathogenicity, certified cuttings — sterilisation, studying factors influencing pathogens (chemical/physical, growth temperatures, growth limiting factors) and Quorum sensing (QS) and regulation of biological functions.

Key words: *Salix viminalis*, INA bacteria, frost damage, ina gene primer, BIOLOG®MicroPlate, nutrient starvation.