Effect of endophytic root-associated fungi of Scots pine on seedling growth and polyamines

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Contents

Background and objective
  – Drained peatlands, regeneration and fungal associates
  – Polyamines and fungi (ECM)

Material and methods

Results
  – Growth
  – Polyamines

Conclusion
Drained peatlands - problems after clear cutting and regeneration

Drained peatlands (5 mill. ha) in Finland are **organic soils**, where young tree seedlings after clear cutting and subsequent regeneration are growing under conditions with different abiotic stress due to uttermost variability in temperature, solar radiation, variability in soil ground water level (drought/flood).

There is not much information about tree root-associated fungi in drained peatlands → **what is their role in the survival and growth of the tree seedlings during the most vulnerable early growth stage?**
About fungal associates in drained peatlands - some earlier findings

- In 25-year old Scots pine forest common ECM-morphotypes in the roots were observed (e.g. *Piloderma, Boletus, Cenococcum*) (Sarjala & Kaunisto 2000).
- Ergosterol levels were similar or lower than reported from roots in mineral soils (Sarjala & Kaunisto 2000, Potila et al. 2009).
Minirhizotron view from soil surface in a drained peatland forest

Why are we interested in polyamines?

PA biosynthesis and oxidation in plants. In fungi, ODC (ornithine decarboxylase) synthesizes putrescine instead of ADC.

- Polyamines (PA) play fundamental role in maintenance of viability and growth of cells.
- PAs are tightly involved in the ectomycorrhizal (ECM) symbiotic interaction (Niemi et al. 2006, 2007, Sarjala et al. 2010).
- PAs play a role in abiotic and biotic stress in plants, they act as signal molecules. Free PAs function as scavengers of free radicals, and conjugates are even more efficient antioxidants.

Putrescine
\[ \text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2 \]

Spermidine
\[ \text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2 \]

Spermine
\[ \text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NHCH}_2\text{CH}_2\text{CH}_2\text{NHCH}_2\text{CH}_2\text{CH}_2\text{NH}_2 \]
Polyamines, ECM symbionts and Scots pine

- Some fungal species enhance the growth at the early stage even without forming a mycorrhiza
- Fungal symbionts modulate PA pools of the host plant → related to specific developmental phases of the host plant
- Maybe the modulation of PA pools could help to resist host defence responses at the start of association

**In situ** localization of *ODC* mRNA transcripts in the mycelium of *Suillus variegatus* during mycorrhiza formation.

Our earlier observations (Sarjala et al. 2010, Plant Physiol.Biochem.): 
*P. involutus (PIH) / P. tinctorius (PTI) and P. sylvestris seedlings → both species affected growth (and also Suillus variegatus)*

![Graphs showing the effects of mycorrhizal infection on root length, primary needle length, number of lateral roots, and root/shoot ratio over time.](image-url)
Root associated fungi were isolated from Scots pine growing on a drained peatland 8 years after clear cut and regeneration.
Isolation of pure cultures of fungal strains from Scots pine root tips

Washing the roots

8-year-old Scots pine trees

Short 70% Ethanol → H2O → 30% Hydrogen peroxide (1.5min) → H2O

Surface sterilized root tips on agar plates

Photos: T. Sarjala

PCR ITS1/ITS4 sequencing

Fungal strains on agar plates
Isolation of 191 fungal strains on pure culture

- 105 strains were sequenced (ITS1-ITS4)
- The closest match for the amplified ITS region (NCBI-BLAST): Uncultured (unknown), Phialocephala, Meliniomyces
Inoculation experiment with 11 fungal isolates and Scots pine seedlings

After germination Scots pine seedlings were grown on sterilized peat:vermiculite substrate.

Fungal mycelium growing on filter paper on Hagem agar were placed on pine root tips.

After one month the seedlings were harvested, washed for sampling (microscopy, polyamines) and checked for growth parameters (fresh weight and length of shoots and roots, number of lateral roots).
Root tips

Appearance of the root tips was not similar to ECM.

Photos: T.Sarjala
Inoculated root tips

A few changes in microscopic structure.

Photos: T. Sarjala
No significant effect on biomass or the number of short roots

– Contrary to ECM, which improved early growth of seedlings even without true mycorrhizal formation (Sarjala et al. 2010)
Root polyamines

- Decreasing (3 isolates) or no effect (8 isolates) on free put (ECM increases)

- Increasing (2 isolates) or no effect (9 isolates) on free spd (ECM increases)
Needle polyamines

Free putrescine

- Increasing (1 isolate), decreasing (1 isolate) or no effect (9 isolates) on free put (ECM increases)

Free spermidine

- Increasing effect (1 isolate) or no effect (10 isolates) on free spd (ECM decreases)
Conclusions (I)

- We could isolate a wide diversity of associated fungi from the roots of 8 Scots pine trees (8-years-old) growing close to each other.
- These root-associated fungi did not have similar effects as ECM on early growth or polyamine concentrations of the Scots pine seedlings after inoculation → The role of root-associated fungi (from drained peatland) at early growth stage of the Scots pine seedlings differed from that of ECM.
- Variation in host responses seen in polyamine concentrations between the fungal strains revealed the diversity not only in species but in their effects on host physiology.
Conclusions (II)

Water extracts of these fungus species have antioxidative properties (FRAP, hydrogen peroxide H2O2 scavenging). One of the species protects efficiently human retinal pigment epithelial cells against oxidative stress (Aapola et al. 2011) →


- the host plant benefits from the antioxidative properties of the fungus under oxidative stress conditions.
Thank you