

# The Diversity of Endophytic Bacteria in Ectomycorrhizal Root Tips of Scots Pine (*Pinus sylvestris*)

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## BACKGROUND

- Endophytes are the microbes which reside in plant tissues without causing any disease symptoms (Wilson, D. 1995).
- Endophytic bacteria have beneficial effects on host plants in agricultural crops (Hallmann, J. et al., 1997).
- Some bacteria are closely associated with ectomycorrhizas (ECM) (Poole E.J., et al., 2001)

## HYPOTHESIS AND APPROACH OF THE STUDY

### Hypothesis:

Specialised bacteria are present in ectomycorrhizas, and they play a functional role in the symbiosis.

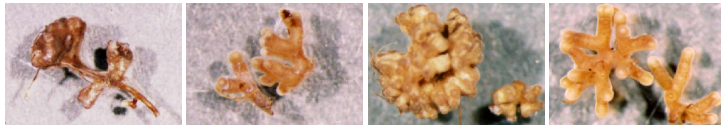
### Approach:

- (1) determine the diversity and community structure of endophytic bacterial populations in Scots pine ectomycorrhizas.
- (2) isolate and characterise endophytic bacteria.

## METHODS

Identification of ectomycorrhizal fungi

Morphotyping of ECM root tips and sequence analysis of fungal ITS regions



p10 morphotype  
*Suillus* sp.

p11 morphotype  
*Russula* sp.

p12 morphotype  
*Suillus* sp.

p14 morphotype  
*Russula* sp.

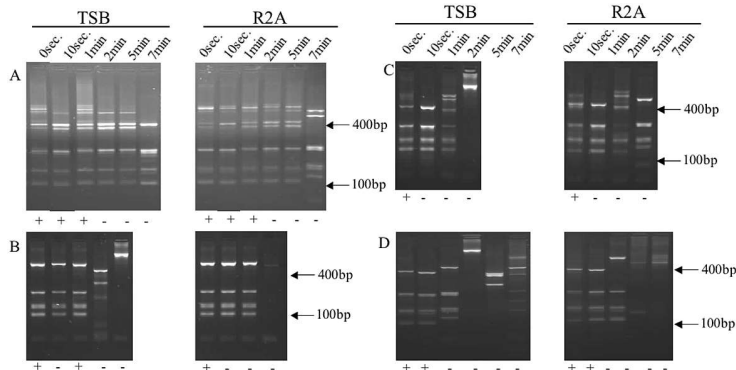
Surface-sterilisation of ECM root tips with different reagents

Sterilisation Method	TSB		R2A	
	Before	After	Before	After
Wash with 0.1% Tween 80	+	+	+	+
1% NaClO (5min treatment)	+	+	+	+
15% H <sub>2</sub> O <sub>2</sub> (1min treatment)	+	-	+	-
30% H <sub>2</sub> O <sub>2</sub> (1min treatment)	+	-	+	-
Water (negative control)	-	NA	-	NA

+ and - indicate presence and absence of bacterial growth in the final rinse water after treatment

## RESULTS

1. PCR-RFLP patterns of cultivable bacterial endophytes following surface-sterilisation with 30% H<sub>2</sub>O<sub>2</sub> for 0 to 7 minutes.



+ and - indicate bacterial growth in final rinse water after sterilisation treatment. (A) p10 (B) p11 (C) p12 (D) p14

2. 16S gene sequence similarities of isolates to database. The species highlighted in blue are reported as nitrogen fixers.

Bacterial isolate	Associated ECM	Most closely related species	Similarity	EMBL accession number
tAp10	p10	<i>Rahnella aquatilis</i>	98.7	AY253921
tDp10	p10	<i>Pseudomonas</i> spp.	99.2	AJ492829
rAp10	p10	<i>Pseudomonas brassicacearum</i>	98.5	AJ29238
rHP10	p10	<i>Burkholderia glatei</i>	97.8	AY154378
tDp11	p11	<i>Pseudomonas</i> spp.	99.4	AJ492829
tKp11	p11	<i>Bacillus psychrosaccharolyticus</i>	99.2	AB021195
tMp11	p11	<i>Paenibacillus kobensis</i>	94.5	AB07336
rGp11	p11	<i>Paenibacillus graminis</i>	97.2	AJ223987
tAp12	p12	<i>Pseudomonas tolaasii</i>	99.5	AF320989
tJp12	p12	<i>Paenibacillus odorifer</i>	98.8	AJ223990
tKp12	p12	<i>Burkholderia glatei</i>	99.1	AY154378
tMp12	p12	<i>Pseudomonas</i> spp.	98.7	AF134704
rDp12	p12	<i>Janthiobacterium</i> spp.	99.0	Y08845
tGp14	p14	<i>Burkholderia glatei</i>	98.8	AY154374
tJp14	p14	<i>Bacillus psychrosaccharolyticus</i>	99.7	AB021195
tMp14	p14	<i>Bacillus</i> sp.	99.4	AY266991
tPp14	p14	<i>Paenibacillus polymyxa</i>	96.5	AY302439
tSp14	p14	<i>Rhodococcus opcus</i>	98.3	AB032565
		<i>Nocardia corynebacteroides</i>	98.3	AY16785
rGp14	p14	<i>Pseudomonas</i> spp.	99.1	AF134704
rJp14	p14	<i>Rhodococcus marinonascens</i>	98.7	X80617
rMp14	p14	<i>Paenibacillus</i> spp.	98.3	AB073363

3. Carbon source utilisation patterns of isolates analysed by Biolog<sup>TM</sup> plates.

Isolate	D-mannitol	Fructose	Sucrose	D-trehalose	D-Glucose
tAp10	+	+	+	+	+
tDp10	+	b	-	+	+
rAp10	+	b	+	b	b
rIp10	+	b	-	-	+
rGp11	+	b	-	+	+
tAp12	+	+	-	+	+
tMp12	+	+	-	+	+
rDp12	-	-	b	b	b
tJp12	b	+	+	+	+
tPp14	b	b	+	+	+
tSp14	-	-	-	b	+
rJp14	+	+	-	-	+
rGp14	+	b	-	-	+

+ and - indicate consumption of given carbon sources. b indicates borderline.

## CONCLUSIONS

- Endophytic bacterial populations within Scots pine ectomycorrhizas are different from those detected on the root surface.
- Sequence analysis of 16S rRNA gene suggests that *Pseudomonas* and *Paenibacillus* are important cultivable endophytic genera.
- Carbon utilisation patterns are diverse among endophytic bacteria in ectomycorrhizas. Isolates which preferentially use fungal carbon sources, rather than those from plants, may be physiologically more closely associated with ECM fungi.