Characterisation of the barley phyllosphere and its interactions with *Rhynchosporium secalis*

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Microbial interactions in the phyllosphere are poorly characterized but could provide a valuable approach to directly or indirectly control diseases. The aim of this work is to develop and use molecular techniques to characterise the dynamics of microbial population on barley leaf. We also focus on the effects of a particular non-host pathogen, *Pectobacterium atrosepticum* (Pba) causal agent of the potato blackleg, on a barley foliar disease *Rhynchosporium secalis* (Rs) ◇.

Material and Methods

Barley cultivars (Optic, Optic Glossy mutant 37-54, Cellar) were grown in field trials at two locations at SCRI and sown in winter and spring. Leaves were sampled at GS30 and stored at -20°C. Epiphytes removal was assessed with three techniques: direct DNA Isolation of Phyllosphere (dDIP)†, sonication and washing. Bacterial communities were determined using Terminal-Restriction Fragment Length Polymorphism (T-RFLP) based on 16S rDNA. Leaf-associated culturable microbes were isolated based on their morphology and identified using BLAST of the rDNA region. Pba detection was done using species specific De Boer and Wart‡ procedure. Effects of Pba on Rs was assessed *in vitro* and under field conditions, where barley was subsequently grown after Pba-infected potato or control crop.

Sonication is a good non-invasive method to remove epiphytes from the leaf surface

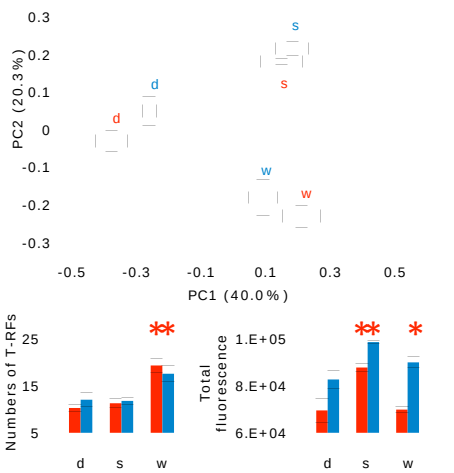

Figure 1: a) Principal Component Analysis (PCA) of T-RFLP profiles of epiphytic bacterial communities isolated with three methods: dDIP (d), sonication (s) and washing (w). T-RFLP were run in four replicates from two DNA concentrations: pure (red) and 1/10 dilution (blue) b) Characteristics of the T-RFLP profiles (ANOVA, F<0.001). Error bars represent standard error.

The phyllosphere is a variable and complex habitat with multiple micro-organisms

Figure 2: Leaf surface SEM of Bowman barley cultivar (left) and one of its wax mutant (right).

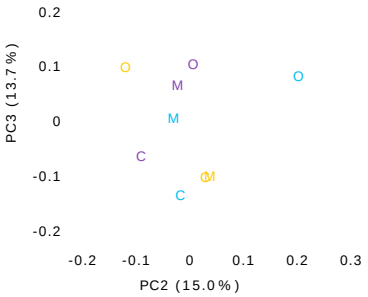

Figure 3: PCA of T-RFLP profiles of leaf epiphytic bacteria isolated by sonication from three barley cultivars (O: Optic, M: Optic Glossy-mutant 37-54, C: Cellar) in triplicate. Barley was grown in field trials (location 1: cyan; location 2: purple and orange) and was either winter- (purple and cyan) or spring-sown (orange).

Bacteria	Beta-proteobacteria	
		Duganella zoogloeoides
	Gamma-proteobacteria	
		Pantoea agglomerans
		Pseudomonas poae
		Pseudomonas syringae
		P. syringae pv. *coryli*
		Pseudomonas veronii
Filamentous fungi	Ascomycota	
		Humicola fuscoatra var. *fuscoatra*
		Dothideomycetes sp.
		Boeremia exigua var. *exigua*
		Penicillium piceum
		Botryotinia fuckeliana
		Arthrinium sp.
	Basidiomycota	
		Leucosporidium scottii

Table 1: Leaf-associated culturable microbe isolated from barley grown in field infected with Rs. Microbes are organised by class for bacteria and phylum for fungi.

Pba was detected on barley leaves and conferred control against Rs by direct interaction

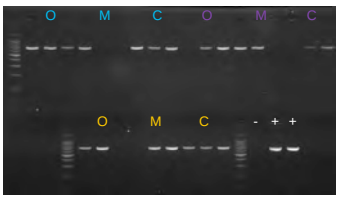

Figure 4: PCR detection of Pba from three field-grown barley leaves pools of various cultivars (O: Optic, M: Optic Glossy-mutant 37-54, C: Cellar).

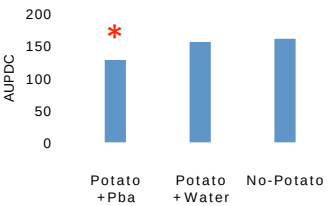

Figure 5: Natural disease severity of Rs under different field conditions. (ANOVA test, P<0.01%).

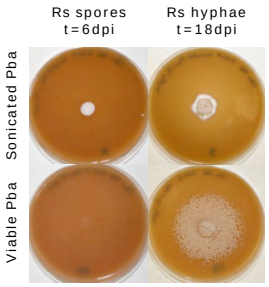

Figure 6: *in vitro* assessment of Rs spores germination (10^5 spores/mL) and Rs hyphal growth in contact with viable or sonicated Pba.

Conclusions

- Sonication is a reliable method to study microbial populations of phyllosphere.
- Location, cultivar and physico-chemical properties of the leaf surface alter microbial communities composition.
- Various micro-organisms live on the phylloplane and can interact with Rs
- Pba is present on leaves of most barley cultivars from field and shows control of Rs

Future work

- Further identify microbes living on the leaf surface with molecular tools.
- Identify effects of treatments (fungicide, elicitor, combination) on microbial dynamics.
- Test potential biocontrol activity of isolated micro-organisms.
- Utilise Pba pathogenicity mutants to identify the mode of action.

Acknowledgement

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References

◇ Newton *et al.* (2004) *New Phytol*, 163: 133-138.
† Suda *et al.* (2008) *Microbes Environ*, 23: 248-252.
‡ De Boer and Ward (1995) *Phytopathology* 85: 854-858.