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Short Communication

Cultivar-level genotype differences influence diversity and composition of lettuce (*Lactuca* sp.) phyllosphere fungal communities

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ABSTRACT

Different lettuce genotypes supported significantly different phyllosphere fungal communities. Phyllosphere fungal diversity was low and fungi fell into five similarity groups. These groups were represented in significantly different proportions throughout 26 lettuce accessions indicating cultivar-level variation in the fungal colonization of the lettuce phyllosphere. Significant differences in the proportions of the two dominant groups (with similarity to *Cladosporium* spp. and *Sporobolomyces roseus*) were identified between parental lines of two lettuce mapping populations providing opportunities to further investigate the genetic control of cultivar-level variation in fungal phyllosphere colonisation.

The phyllosphere supports a significant microbial community and both species and cultivar level plant genotype differences have been shown to influence phyllosphere bacterial community composition (Thompson et al., 1993; Kinkel, 1997; Kinkel et al., 2000; Hunter et al., 2010). Culture-independent investigations of phyllosphere fungal communities remain scarce (Whipps et al., 2008), with those studies that have been carried out focused predominantly on trees and woody plants (Balint et al., 2013; Arfi et al., 2012; Cordier et al., 2012a, 2012b; Jumpponen and Jones, 2010, 2009). Whilst these studies have shown plant genetic influence on phyllosphere populations (Balint et al., 2013; Cordier et al., 2012b), particularly at the whole plant level, the influence of plant genotype on phyllosphere fungal communities in crop plants remains largely unknown. This study examined the existence and extent of

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plant genotype influence on the phyllosphere fungal community diversity and composition in lettuce.

Naturally occurring phyllosphere fungal communities (*i.e.* combined phylloplanar and endophytic communities) from a genetically diverse set of 26 lettuce (*Lactuca* spp.) accessions (Atkinson et al., 2013a) were assessed. Communities developed under field conditions until plant commercial maturity, as described by Hunter et al. (2010), and samples were taken contemporaneously with this experiment. rRNA ITS region T-RFLP profiles from each accession and ITS clone libraries from the parental lines of two genetic mapping populations; Saladin (Salinas) × *L. seriola* US96UC23 (Truco et al., 2007) and Saladin × Iceberg (Atkinson et al., 2013b), were analysed (see Supplementary file S1 for methods).

The majority of T-RFs (peaks) in the T-RFLP profiles were present at very low abundance (<0.1% of the population), however, the profiles were dominated by 9 T-RFs indicating the predominance of a few (core) sequences in these communities. ANOVA of the profile data showed significant ($P < 0.05$) differences in both the numbers of T-RFs and the Shannon Diversity Index (Shannon, 1948), which takes into account the relative contribution of each T-RF to the profile (Table S2). Non-parametric multidimensional scaling (NMDS) combined with a multi-response permutation procedure (MRPP) multivariate analysis (Supplementary Fig S3), revealed that the communities formed three groups based on host plant morphology. Communities from the Curly Leaved accessions, the Batavian and Cos types and the remaining lettuce morphotypes were all significantly ($P < 0.05$) different to each other (Fig S3). This is in contrast to bacterial communities sampled from the same experiment (Hunter et al., 2010), which showed that the Curly Leaved and Cos communities were not distinct from each other and formed part of a continuum of diversity which also included the communities from the Batavian type accessions. The fungal diversity (Shannon index) data presented here did not correlate with either bacterial diversity or data on plant factors shown to influence bacterial community diversity from the previous work, suggesting that fungal and bacterial phyllosphere community diversity is influenced by different factors.

Comparisons of the Shannon diversity indices of the clone library sequence profiles obtained from three replicate plants of each parental accession showed a significant ($P < 0.05$) difference between the community from cv. Saladin and those from cv. Iceberg and *L. serriola* (US96UC23), but not between the communities from these latter two accessions, although a comparison of the Cramér von Mises statistics between libraries, according to the method of Singleton et al. (2001), indicated that overall composition of the phyllosphere fungal communities were significantly ($P < 0.001$) different between all three parental accessions. This is a non-parametric rank order test that can be used to provide a measure of difference between two communities based on community structure rather than simple diversity. To relate the sequence data to the whole diversity set, T-RFs predicted from the sequences of the closest database matches for each group (e.g. *Sporobolomyces roseus* AY015438) were identified and screened for in the T-RFLP profiles of the three parent lines. The predicted T-RFs were among the most strongly represented peaks, however, the relative proportions of these T-RFs did not correspond with the relative proportions of the sequences in the similarity groups. Closer inspection revealed several instances of single nucleotide differences in the sequences comprising each group, which resulted in either the insertion or deletion of a terminal restriction site resulting in a different T-RFLP pattern for such sequences. Consequently, a set of unique T-RFs representing each sequence similarity group were determined based on the sequences within the groups and the relative proportions of these T-RFs determined in the T-RFLP profiles from all the lines. The proportions of the T-RF sets specific for sequences similar to *Cryptococcus* spp., *Tilletiopsis* spp. and *Lewia infectoria* were not significantly different between the parental lines, suggesting that not all the sequence variation was captured in the T-RF sets. Significant ($P < 0.05$) differences were detected in the proportions of T-RFs representing sequences similar to *S. roseus* and *Cladosporium* spp. (Table S2), however, and all group-specific T-RF sets were significantly ($P < 0.001$) different across the 26 accessions.

Whilst, T-RFLP (in common with other profiling techniques) inherently underestimates microbial community richness (because low abundance components are often missed), the parallel sequence analysis confirmed the relatively low level of diversity in this study. Although a greater depth of sequencing would likely reveal more diversity, especially since phyllosphere microbial populations appear to be hypervariable in many cases (Cordier et al., 2012b) and may often be under-represented at the OTU level (Unterseher et al., 2011), the fact that the five genera listed in Table 1 represent greater than 80% of the predicted community (at genus level), suggests that they can be considered the core community. This is in keeping with culture-dependant studies, where the filamentous ascomycete genera *Cladosporium, Aureobasidium, Alternaria and Acremonium* and the basidiomycete yeast genera *Cryptococcus, Sporobolomyces* and *Rhodotorula* have been shown to dominate the phyllosphere of crop plants (Thompson et al.,

Table 1 – Percentage representation of the five sequence similarity groups (97% sequence similarity) in 192 fungal sequences from three replicate plants of each of the lettuce mapping population parental lines

Similarity group	% Representation			97% sequence similarity to:
	L. sativa		*L. serriola*	
	cv. Saladin	cv. Iceberg	US96UC23	
Sporobolomyces	35.5	44.0	63.0	AY015438
Cladosporium	51.5	36.0	36.0	AY251074, L25432
Cryptococcus	4.0	8.0	0.0	JN400817, AJ581048
Lewia	1.0	2.0	0.0	AY154692
Tilletiopsis	0.0	2.0	0.0	DQ317635, AY879275
Unidentified	8.0	8.0	1.0	
Coverage[a]	>99 %	83.3 %	>99 %	
Shannon (H) (95 % confidence)	0.660 (±0.146)	0.902 (±0.227)	1.238 (±0.431)	

a Coverage based on Chao1 estimate of population size (Chao, 1987) at 97% sequence similarity.