Evaluation of Primer Sets for the Analysis of Fungal Root Endophytes in Prairie Grasses

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Introduction

Roots of prairie grasses can associate with septate endophytic (SEF) and arbuscular mycorrhizal (AMF) fungi. It has been demonstrated that these associations can provide nutritional benefits and stress tolerance to plants (Mandyam and Jumpponen, 2005).

Research on grass root endophitic fungi is limited by its diversity and often unculturable nature (Barrow et al 2004). By amplifying fragments of DNA conserved within a taxonomic group with the polymerase chain reaction (PCR) it is possible to obtain specific sequences to identify these root endophytes (Schadt et al 2001).

The choice of PCR primer sets is important. Good primers should amplify a DNA sequence in each member of the target community (good range) but should not amplify DNA from non-members (group specific). Both qualities: range and specificity of PCR primers can be tested on reference microorganisms (Hagn et al 2003).

The objective of this research was to evaluate primer sets for the molecular analyses of septate endophytic (SEF) and arbuscular mycorrhizal (AMF) fungi.

Materials and methods

Pure cultures of fungal endophytes isolated from different grass roots, or microdissected spores were used to test the amplification range of the fungal specific primers EF3-EF4 (Smit et al 1999) and LR1-FLR2 (Gollote et al 2004).

Spores of the AMF *Scutellospora heterogama, Archaeospora trappei, Paraglomus occultum* and *Glomus intraradices* were obtained from international collections. They were used to test the amplification range and specificity of the previously published AMF specific primer sets AMV 4.5NF-AMDGR (Sato et al

2005) 28G1-28G2 (Alves et al 2006) and LR1-FLR4 (Gollote et al 2004). The last two primer sets were also used in a nested PCR after LR1-FLR2.

DNA from roots and leaves of leek (*Allium ampeloprasum* var porrum) trap cultures used to increase the AMF associated with crested wheat grass (*Agropyron cristatum* (Fisch. ex Link) Schult) or western wheatgrass (*Agropyron smithii* Rydb) were also included as positive and negative controls. DNA was extracted from roots and fungal mycelia or AMF spores with the DNeasy plant mini kit (QIAGEN) and Soil DNA extraction kit (MoBio), respectively.

For PCR amplification, a mixture of 10 µL of PCR reaction was prepared using AmpliTaq Gold PCR master mix (Applied Biosystems). The final concentration of each component is as follows: 15mM Tris HCL/pH 8.5; 50 mM KCL; 200 µM each dNTP, 2.5 mM MgCl2, 0.25 U Amplitaq gold DNA polymerase; 0.5 µM each primer. 1 µL of crude DNA was used as a template. The PCR cycle used was as published by each author, except that an initial 10 minutes step at 95 ^{o}C was included to activate the enzyme. PCR products were analysed by electrophoresis in a mixture of Metaphor (2%) and LE (1%) Agarose in 0.5X TBE buffer.

Results

Most of the DNA from SEF was amplified by both primer sets, although DNA bands from microdissected spores were produced only with primers LR1-FLR2 (Table 1).

The analyses of AMF revealed a nonspecific amplification by primers AMV4.5NF–AMDGR, as multiple bands were produced with two samples of roots from leek trap cultures. Bands were also visible when DNA from leek leaves were used as a negative control (Figure 1), suggesting a nonspecific amplification under our experimental conditions.

More specific amplification was observed in the first PCR round with primers LR1-FLR4 and 28G1-28G2, producing individual bands with each DNA sample without amplifying plant DNA.

The detection of bands of the expected length was improved after the use of LR1-FLR2 in the first round, with bands missing only in samples of *A. trappei*. Further analyses revealed that no DNA was present in this sample.

Table 1 Amplification of DNA from fungal cultures of endophytic fungi (1 to 14) or root microdissected spores (15-16) by two primer sets.

Primer set	Fungal DNA sample number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
LR1-FLR2	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
EF3-EF4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-

+ DNA band was present in agrose gel electrophoresis
- Band was absent

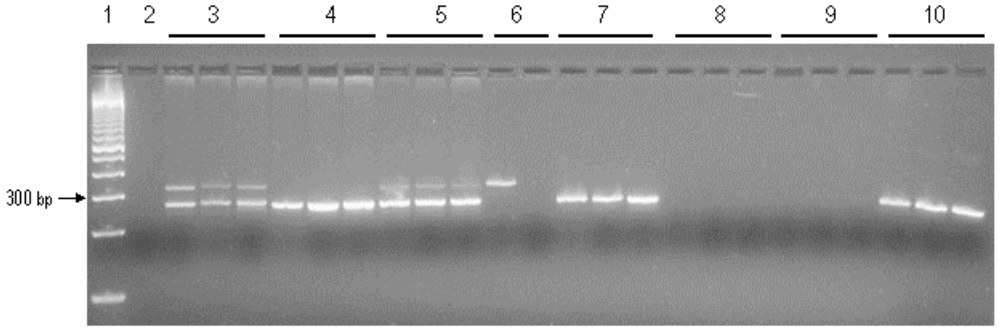

Figure 1. Nonspecific amplification of DNA from AMF by primers AMV4.5NF-AMDGR. Line : 1 100 bp marker, line 2 Blank, lines 3 to 5 leek trap cultures increasing AMF from crested wheatgrass, western wheatgrass and *G.clarum* respectively. Line 6 is DNA from leek leaves, lines 7 to 10 are DNA from *G intraradices, A trappei, P occultum and S heterogama*, respectively.

Table 2 Efficient amplification of DNA from arbuscular mycorrhizal fungi (AMF) by two primer sets in a nested PCR.

Primer set	AMF DNA sample				
	G.claroideum	*G.intraradices*	*A.trappei*[†]	*P.occultum*	*S.heterogama*
	Simple PCR				
LR1-FLR4	+	+	-	-	-
28G1-28G2	+	+	-	+	+
	Nested PCR after LR1-FLR2				
LR1-FLR4	+	+	-	+	+
28G1-28G2	+	+	-	+	+

[†] Further analyses revealed the absence of DNA in this sample
+ DNA band was present in agrose gel electrophoresis
- Band was absent

Conclusions

For AMF molecular analysis, primers LR1-FLR4 or 28G1-28G2 used in a nested PCR with LR1-FLR2 are equally good.

For SEF molecular analysis, primers LR1-FLR2 are the best.

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