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TECHNICAL NOTE

Microsatellite markers for *Dictyochloropsis reticulata* (Trebouxiophyceae), the symbiotic alga of the lichen *Lobaria pulmonaria* (L.)

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Abstract We isolated and characterized eight microsatellite markers for *Dictyochloropsis reticulata*, the primary photosynthetic partner of the epiphytic lichen *Lobaria pulmonaria*. These are the first microsatellite loci reported for a lichen symbiotic alga. These polymorphic markers will be useful for investigating spatial genetic structure, biogeography and dispersal of this eukaryotic alga and will generally shed light on the coevolution of the green-algal lichen symbioses.

Keywords Coevolution · *Lobaria pulmonaria* · Photobiont · Population genetics · SSR

Introduction

The foliose tripartite lichen *Lobaria pulmonaria* has become an important model species for studies on the biological conservation of epiphytic lichens, because of its wide geographical distribution (Yoshimura 1998) and its value as a bioindicator of long ecological continuity (Gauslaa 1994). While *L. pulmonaria* is widespread and locally common in boreal North America (Brodo et al. 2001), it suffered a serious decline in many parts of Central Europe during the twentieth century (Wirth et al. 1996). Despite the amount of available biological and genetic information for the fungal partner of this symbiosis (e.g. Walser et al. 2005; Werth et al. 2007), little is known about its primary photosymbiotic partner, namely the eukaryotic green alga *Dictyochloropsis reticulata* (Trebouxiophyceae; Geitler 1966), primarily because of a lack of informative genetic markers. In order to develop efficient strategies to conserve and enhance *L. pulmonaria* populations, more information is needed about patterns of genetic variation at different spatial scales for the algal part of the symbiosis. In this paper, we describe the isolation and characterization of eight informative microsatellite markers for *D. reticulata*, to provide adequate tools for corresponding population-level studies. To our knowledge, these are the first ones for a lichen symbiotic alga.

Following the method of Beck and Koop (2001), an axenic culture of *D. reticulata* was obtained and grown from a single algal cell isolated from a specimen of *L. pulmonaria* from Pamplona, Spain; voucher specimen 6577 (herbarium of the Swiss Federal Research Institute WSL, Birmensdorf), algal culture AB06.006A2 (algal culture collection of the Botanische Staatssammlung, Munich). Genomic DNA was extracted from 20 mg of vegetative cells of *D. reticulata* using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. An enriched library was made by ecogenics GmbH (Zurich, Switzerland) from size selected genomic DNA ligated into SAULA/SAULB-linker (Armour et al. 1994) and enriched by magnetic bead selection with biotin-labelled $(GT)_{13}$, $(CT)_{13}$, $(TAC)_{10}$ and $(AAC)_{10}$ oligonucleotide repeats (Gautschi et al. 2000a, b). Of the 754 recombinant colonies screened, 127 gave a positive signal after hybridization. Plasmids from 104 positive clones were sequenced and primers were designed for 20 microsatellite inserts using PRIMER3 (Rozen and Skaletsky 2000). The 20 primer pairs were tested for PCR-

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Table 1 Primer pairs and characteristics of eight polymorphic microsatellite loci for *Dictyochloropsis reticulata*

Locus	Repeat motif	Primer sequence (5′–3′)	Dye label	T_a (°C)	Size (bp)	*A*	Gene diversity[b]	GenBank accession no.
LPh1	$(AT)_3(GT)_{23}$	F: GTCTCAGGTGACCACTTGATTG[a]	VIC	62	252–494	41	0.991	FJ754261
		R: GCAATGGATATGATGCTTGTTC						
LPh2	$(CT)_{16}$	F: GACAGCTGTTCCAGTGCATC	6FAM	62	155–159	3	0.370	FJ754262
		R: GCAGAGGAAGTGCATGACG[a]						
LPh3	$(CA)_{15}$	F: TGCAGTAGGTGTCATATGTGT[a]	NED	62	106–112	4	0.610	FJ754263
		R: GAAGGCGCATCTTGATATAC						
LPh4	$(AG)_{27}AT(AG)_5$	F: GTGGTGGTACAACATGCTCA[a]	NED	62	183–199	8	0.687	FJ754264
		R: ACGACCACGTGGGATATCTA						
LPh5	$(TG)_{24}TA(TG)_2$	F: TGGTGTTAGTAAGAATCGGCATC[a]	PET	62	143–151	4	0.447	FJ754265
		R: GTGTATGTCGGCCCCAATAA						
LPh6	$(TGT)_{10}$	F: GAATCCTGCCTGCCTACAAG[a]	6FAM	62	121–127	3	0.076	FJ754266
		R: AGCAACCCATTTCAACCAAC						
LPh7	$(TTG)_{20}TTT(TTG)_4TTT(TTG)_6$	F: TGTGACAGGTGAAACACCAA[a]	VIC	62	150–201	8	0.598	FJ754267
		R: TATGGTCCCTCATGGCAAAT						
LPh8	$(TG)_7TA(TG)_6TA(TG)_3$	F: GGAAGGTGGTGTGATTGATT[a]	6FAM	59	151–153	2	0.061	FJ754268
		R: TGCTCCACATTATCACAAACA						

F forward, *R* reverse; T_a optimized annealing temperature, *A* the observed number of alleles. Sample size, $N = 60$

[a] Primer labelled with dye

[b] Gene diversity was calculated after Nei (1987)

amplification with the genomic DNA obtained from the axenic culture of *D. reticulata* mentioned above. Six primer pairs failed to amplify, and fourteen were then used to screen for polymorphism with a sample of 60 specimens of *L. pulmonaria* (North Carolina, USA). Total genomic DNA was extracted from the lichen thalli using the DNeasy 96 Plant Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. PCR reactions for the alga-specific microsatellites were performed on an Applied Biosystems Veriti thermal cycler (Applied Biosystems, Foster City, CA) in a 10 µl reaction mixture containing 1–10 ng of template DNA, 0.2 µM of each primer and 1× Multiplex PCR Kit (QIAGEN, Hilden, Germany). PCR conditions were as follows: 15 min at 95°C, 25 cycles of 30 s at 94°C, 90 s at 62°C (or 59°C for LPh8), 60 s at 72°C and a final elongation for 30 min at 60°C. Genotyping was performed on an ABI 3730 Genetic Analyser (Applied Biosystems, Foster City, CA) and electropherograms were analyzed with GENEMAPPER v3.7 using LIZ500 size standard (Applied Biosystems, Foster City, CA).

Eight of the fourteen tested loci were polymorphic (Table 1). In all individuals, a single allele was recovered per locus. This indicates that *D. reticulata* is haploid. Gene diversity per locus (Nei 1987) ranged from 0.061 to 0.991, and the number of alleles per locus varied from 2 to 41 alleles, resulting in 52 unique multilocus genotypes. The primer sequences and other characteristics for the microsatellite loci are given in Table 1. Linkage disequilibrium in pairwise combinations of the loci was tested using GENEPOP 4.0 ON THE WEB (Raymond and Rousset 1995). After Bonferroni correction, no significant linkage disequilibrium was detected.

These eight microsatellite loci are currently used for studying the spatial population structure, phylogeography and dispersal of *D. reticulata*. Of particular interest is their application to investigate whether different lichen-forming fungi share the same photobiont. When used in combination with the fungus-specific microsatellites for *L. pulmonaria* (Walser et al. 2003; Widmer et al. in prep.), these markers are a promising tool for studying coevolution of the green-algal lichen symbiosis.

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