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Archaeal diversity and community structure in a Swedish barley field: Specificity of the EK510R/(EURY498) 16S rDNA primer

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Abstract

The aim of this study was to analyze a total euryarchaeal community at DNA and RNA levels in a Swedish barley field with relation to soil depth (0–10 and 20–30 cm layers), soil fraction (bulk soil and rhizosphere) and time (August and November sample collection). Amplification of 16S rRNA gene using the archaeal universal A2F and *Euryarchaea* specific EK510R/(EURY498) primer pair, combined with denaturing gradient gel electrophoresis (DGGE), revealed distinct differences between rDNA and rRNA DGGE profiles. The soil depth, time, or rhizosphere effects did not significantly influence *Archaeal* community structure. Surprisingly, sequence analysis of DGGE-derived amplicons revealed the presence of *Euryarchaea* as well as uncultured soil *Crenarchaea* affiliated with group 1. In agreement, sequence comparison analyses showed that the majority of uncultured Crenarchaea group 1 had almost 100% sequence complementarity to the 3′ end of the EK510R/(EURY498) primer. Therefore, we propose that EK510R/(EURY498R) is a universal archaeal primer rather than a *Euryarchaea* specific SSUrRNA primer. Hence, considerable care should be taken during application of this primer in studies of euryarchaeal biodiversity in soil environments.

Keywords: SSUrRNA; *Euryarchaea*; *Crenarchaea*; DGGE; EK510R/(EURY498) primer specificity; Soil

1. Introduction

Since the establishment of the *Archaea* as a distinct domain of life from *Bacteria* and *Eukarya* in late 1970 (Woese and Fox, 1977) intensive use of molecular methods combined with the application of universal and group specific primers targeting the archaeal 16S rRNA genes have contributed to the increase in knowledge of archaeal distribution and diversity in various environments (Baker et al., 2003; Baker and Cowan, 2004; Banning et al., 2005; Crocetti et al., 2006; Nercessian et al., 2004; Skillman et al., 2006). *Archaea* are usually divided into two major phyla, *Euryarchaea* and *Crenarchaea*, and two additional phyla *Korarchaea* and *Nanoarchaea* have also been proposed (Barns et al., 1996; Forterre et al., 2002; Huber et al., 2003; Robertson et al., 2005; Woese et al., 1990).

Within crenarchaeal phyla, uncultured representatives were found to be widespread in a variety of non-extremophilic marine and terrestrial environments (Chaban et al., 2006; Lange et al., 2005; Robertson et al., 2005; Schleper et al., 2005). In terrestrial environments, *Crenarchaea* constitute an active part of the microbial population (Nicol et al., 2003a, 2004, 2005) and account for up to 3% of the total soil microbial community (Buckley et al., 1998; Ochsenreiter et al., 2003). *Crenarchaea* were also identified in the rhizosphere from phylogeneticaly diverse plants in native soil environments (Chelius and Triplett, 2001; Simon et al., 2000, 2005; Sliwinski and Goodman, 2004a). The diversity, abundance, and community structure of soil *Crenarchaea* was shown to depend on environmental and human factors, such as land succession (Nicol et al., 2005), land usage practices (Nicol et al., 2003a), freeze and thaw stress (Pesaro et al., 2003), and heavy metal contamination (Sandaa et al., 1999). On the basis of SSUrRNA sequence analyses, uncultured *Crenarchaea* can be divided into three major phylogenetic lineages 1.1(a–c), 1.2, and 1.3 (DeLong, 1998;

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Jurgens et al., 2000; Ochsenreiter et al., 2003). In many soil environments "group 1.1b" was found to be either the sole or the dominating crenarchaeal lineage (Chaban et al., 2006; Nicol et al., 2003a, 2005; Ochsenreiter et al., 2003; Simon et al., 2000, 2005, Sliwinski and Goodman, 2004a,b). Recently, a close evolutionary relationship between the crenarchaeal non-thermophilic-"group 1.1b" and the archaeal clones from a hot spring has been demonstrated, suggesting that this group is more ecologically diverse than was previously thought (Kvist et al., 2005).

Our understanding of crenarchaeal soil ecology is still hindered by the fact that no soil *Crenarchaea* has yet been obtained in pure laboratory cultures. However, recent improvements in genomic studies, including application of metagenomics in environmental microbiology revealed the presence of several genes important for understanding of both crenarchaeal metabolism and their potential ecological role(s) within soil environment (Quaiser et al., 2002; Riesenfeld et al., 2004; Treusch et al., 2004). In particular, the significance of this group of organisms in the global nitrogen cycle was suggested upon discovery of functional ammonia monooxygenases (*amo*) genes in *Crenarchaea* from various soil environments (Leininger et al., 2006; Nicol and Schleper, 2006; Schleper et al., 2005; Treusch et al., 2005).

In *Euryarchaea*, both novel albeit uncultured representatives and a large numbers of cultured species have been fairly well documented in psychrophilic, mesophilic, hyper/thermophilic, extreme halophilic and alcalophilic environments (Chaban et al., 2006; Conrad et al., 2006; Garcia et al., 2000; Henneberger et al., 2006; Karr et al., 2006; Lange et al., 2005; Oren, 2002; Wu et al., 2006). *Euryarchaea* are usually considered as strict anaerobe and in contrast to *Crenarchaea*, *Euryarchaea* have only been sporadically detected from the upper well-aerated parts of agricultural, forest and grassland soil environments. Enrichment of soil with components essential for archaeal growth was required for detection of *Euryarchaea* at significant levels in such environments (Kasai et al., 2005; Nicol et al., 2003b; Peters and Conrad, 1995). Apart from methanogenesis, *Euryarchaea* have been shown to oxidize methane (Michaelis et al., 2002; Strous and Jetten, 2004), fix nitrogen, reduce nitrate (Cabello et al., 2004; Mehta et al., 2003, 2005; Philippot, 2002, 2005; Raymond et al., 2004; Zehr et al., 2003) and contribute to oxidization of ammonia (Strous and Jetten, 2004).

The aim of this study was to investigate the presence, diversity and community structure at DNA and RNA levels, of *Euryarchaea* in a spring barley field. Moreover, the specificity of the *Euryarchaea* primer EK510R/(EURY498) was reassessed.

2. Materials and methods

2.1. Field descriptions and managements

Soil samples were obtained from a 0.5 ha spring barley (*Hordeum vulgare* L.) field belonging to the Vallgårda Sören AB Company at Läby Kvarnbo Gård, Uppsala, Sweden (map reference N 59°50′; E 17°29′). The field received fertilizer N: P: K (27:3:3) (350 kg ha^{-1}) and treated with Arian S (2.5 l ha^{-1}) to prevent growth of grass, Event Super (0.8 l ha^{-1}), to prevent contamination by oats via air; and Stereo (0.4 l ha^{-1}) against growth of fungi. The field was harvested and plowed in September.

2.2. Soil sampling

Three field plots 8×8 m side located 30 m apart in the field were marked and used for soil sampling in August and November 2004. From each plot, five random soil cores (diameter 2 cm; depth 30 cm) were collected and separated into 0–10 and 20–30 cm depth fractions before sieving with 2 mm mesh to remove stones and dense root material. A final composite sample consisted of 15 pooled 0–10 or 20–30 cm depth core fractions. Rhizosphere samples were collected from the root system of five barley plants within each plot. Roots were shaken to remove loosely attached soil. However, due to the compact soil structure a substantial amount of soil was still left attached to the roots. Upon collection, soil samples were immediately frozen and kept under these conditions for 5 h in a −78 °C pre-cooled container filled with a dry ice/ethanol bath slurry, before transportation to the laboratory and storage at −80 °C in laboratory freezer. Attention was made to constantly keep all samples completely immersed in the dry ice/ethanol slurry. Soil properties from a composite 0–10 and 20–30 cm sample were analyzed at the Department of Soil Sciences, Swedish Agriculture University, Uppsala, Sweden (Table 1).

Two soil samples, representing different soil types, were used as references. One collected from a rice field in China (Wartiainen et al., 2006 unpublished) and the second collected from the upper 0–10 cm of grassland soil outside the Department of Botany, Stockholm University. Both samples were frozen immediately upon collection in liquid nitrogen before transport to the laboratory and storage at −80 °C until use.

2.3. DNA/RNA extraction and purification

Total DNA and RNA were extracted in triplicate from 2 g aliquots of soil according to the procedure described by (Hurt

Table 1
Physical and chemical properties of the barley field soil collected in Uppsala, Sweden

Soil layer (cm)	Clay (%)	Silt (%)	Sand (%)	pH	Humus content (%)	Water content (%)	Carbon content (%)	Nitrogen content (%)	Ammonium mg/kg of dry soil	Nitrate mg/kg of dry soil
0–10	36	23	41	6,5	3,6	12,5	1,85	0,185	2,4	1,7
20–30	40	22	38	6,8	2,4	12,4	1,34	0,141	1,7	1,0