Soil Biology & Biochemistry 57 (2013) 695–705

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Diversity of free-living and lichenized fungal communities in biological soil crusts of the Sultanate of Oman and their role in improving soil properties

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ARTICLE INFO

Article history:
Received 14 May 2012
Received in revised form
25 July 2012
Accepted 26 July 2012
Available online 25 August 2012

Keywords:
Biological soil crusts
Fungi
Lichens
Pyrosequencing
Cultivation
Erosion
Water-holding capacity

ABSTRACT

Biological soil crusts of arid and semiarid regions are one of the least explored habitats with respect to the diversity of their fungal communities and the Arabian deserts, in particular, remains mycologically poorly investigated. Here, we investigate the diversity of free-living and lichen-forming fungal communities associated with crusts at two locations in Oman, using intensive cultivation and pyrosequencing, and their role in improving soil stability and hydrology. A total of 226 fungal isolates were recovered and phylogenetic analysis placed 98% of the isolates within the *Ascomycota* phylum, most of which belonged to *Dothideomycetes* class and *Pleosporales* order. The isolates were phylogenetically affiliated to 101 different species within 44 different genera with >55% of the total isolates belonging to *Alternaria*, *Aspergillus*, *Cladosporium*, *Cochliobolus*, *Fusarium*, *Myrothecium*, *Phoma* and *Ulocladium*. Using pyrosequencing, a total of 26,998 sequence reads were obtained with *Ascomycota*, *Basidiomycota* and *Chytridiomycota* encompassing >96% of the total sequences. In cyanobacterial crusts, between 67.2 and 70.6% of the total fungal sequences belonged to the classes *Dothideomycetes* and *Eurotiomycetes* with the dominance of yeast-like fungi of the genera *Sarcinomyces* and *Aureobasidium*. On the other hand, the sequences obtained from the lichen crusts mainly belonged to the classes *Lichinomycetes*, *Lecanoromycetes* and *Eurotiomycetes*. Among the identified lichens were *Placidium lacinulatum*, *Psora decipiens*, *Peccania fontqueriana*, *Stromatella bermudana*, *Verrucaria chiloensis*, *Pecania arizonica*, *Lempholemma polyanthes* and *Lichinella cribellifera*. Although detected fungi confirmed earlier trends in fungal diversity in other deserts, quite a number of isolates and sequences representing novel taxa were recovered. The presence of lichen in crusts improved their resistance to erosion and increased their water holding capacity. We conclude that desert crusts of Oman harbor a large diversity of fungal communities that varies with crust type and desert ecoregions, and the presence of lichens in these crusts improves soil characteristics.

1. Introduction

Fungi, along with cyanobacteria, heterotrophic bacteria, archaea, mosses, and microalgae, are among the prevalent assemblages that constitute arid and semi-arid biological soil crusts (referred to as crusts hereafter) worldwide (Belnap and Lange, 2001; Soule et al., 2009). Fungi in crusts can be involved in the formation of lichens, which are composite organisms consisting of a symbiotic relationship between a fungus and a photosynthetic partner, usually either cyanobacteria or microalgae (Ahmadjian, 1989; Harper and Pendleton, 1993). Recent studies have also demonstrated the presence of ubiquitous free-living fungi in crusts, however at much lower diversity and biomass contribution (50–400 fold less) compared to their bacterial counterparts (Bates and Garcia-Pichel, 2009). As a component of crusts, lichenized and free-living fungi have been studied in several deserts, including Negev, Chihuahuan, Colorado Plateau as well as grasslands in Wyoming and Utah (States et al., 2001; Grishkan et al., 2006; Green et al., 2008; Bates and Garcia-Pichel, 2009; Bates et al., 2010a,b). These studies showed that *Ascomycota* were the dominant fungal colonizers of all crusts, regardless of their origin. So far there are very few studies that deal directly with diversity and distribution of fungal communities associated with crusts in the large Arabian subtropical deserts (Fathi et al., 1975; Abu-Zinada et al., 1986; Schultz, 1998; Schultz et al., 2000). A recent study on crusts from the Sultanate of Oman showed similarities in bacterial species

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http://dx.doi.org/10.1016/j.soilbio.2012.07.023

composition to other crusts from temperate and cold deserts (Abed et al., 2010). This raises the question whether crusts from the arid deserts of the Arabian Peninsula have also similar fungal biodiversity to those reported in other deserts.

Fungal diversity in crusts has mainly been assessed using cultivation (Grishkan et al., 2006; Green et al., 2008) with the exception of few recent studies where molecular techniques such as denaturing gradient gel electrophoresis (DGGE) and 16S rRNA cloning were employed (Bates and Garcia-Pichel, 2009; Bates et al., 2010b). Although it is well established that isolated strains represent an insignificant proportion of all organisms in the field, and DGGE underestimates the real diversity by detecting only dominant members of the community (Amann et al., 1995; Muyzer and Smalla, 1998), there is a necessity to combine different techniques in order to gain a comprehensive view of cultured and culture-resistant fungal species diversity (Bates et al., 2010a). High throughput 454-pyrosequencing can produce a large number of sequence reads and may provide orders of magnitude more data on the diversity of organisms than DGGE and cloning approaches. However, this approach has not been used to investigate the diversity of fungal communities in crusts. We expect that the use of pyrosequencing will result in a different picture of fungal community structure than by cultivation.

Fungi in crusts contribute to soil stability by aggregating soil particles through their filamentous hyphae. In aridland soils, fungi play a vital role in carbon and nitrogen cycling by their efficient ability to decompose carbon compounds, perform nitrification and denitrification and store nutrients intracellularly (Hawkes, 2003; Crenshaw et al., 2008; Hayatsu et al., 2008; Strauss et al., 2012). Fungal mycelial networks between crusts covering vegetated interspaces and roots of vascular plants have been shown to facilitate the transfer of nutrients to roots (Collins et al., 2008; Green et al., 2008). On the other hand, germination and root penetration of two grass species (i.e. *Bromus tectorum* and *Vulpia microstachys*) was lower in soils covered with lichen-dominated crusts than in bare soils (Deines et al., 2007). Fungi could be utilized as bio-indicators of crust disturbance, for example by livestock activities, and for monitoring rangeland health (Ponzetti and McCune, 2001). Although the role of fungi in improving soil properties has been the focus of many studies, few studies have compared this role between lichen- and cyanobacteria-dominant crusts in a single site (Smith et al., 2004; Belnap, 2006).

This study examines the diversity of lichenized and free-living fungi in six different crusts collected from two desert locations in the north and central region of Oman, using intensive cultivation and pyrosequencing of fungal species. The function of crusts in improving soil stability and hydrology (erosion, evaporation and water holding capacity) was compared in the presence and absence of lichens at one location.

2. Methods

2.1. Site description and sample collection

Crust samples were collected during June and August 2010 from two locations; Wadi Al-Khoud (WK) and Al-Jabal Al-Akhdar (JAK). The sites differ in soil type, presence of vegetation, elevation and general weather patterns. WK is located in northern Oman (coordinates 23°04.595′ N 58°09.055′ E, see map in Abed et al., 2010), at an elevation of 60 m and is surrounded by low limestone hills, where the surface is covered with small to medium gravels with the dominance of *Acacia tortilis* trees. The mean annual rainfall in the area is ca. 86.2 mm from November to April. Temperature ranges between 47 and 50 °C in summer and as low as 12 °C in winter. JAK site is located near Sayh Qatanah, a settlement of the Al-Jabal Al-Akhdar mountains (coordinates 23°05.322′ N 057° 40.441′ E) at an elevation of 2026 m. It is predominated by black limestones and brownish dolomites. Bare rock outcrops and very shallow soils prevail throughout the landscape with sparse vegetation cover. The climatic conditions are arid to semi-arid, with a mean annual rainfall of 318 mm. Temperature reaches a maximum of 34–35 °C in summer but can freeze in winter.

WK site is dominated by cyanobacteria- and lichen-dominated crusts (Fig. 1A) whereas in the JAK site, only lichen-dominated crusts were found (Fig. 1B). The topmost two cm of six crusts were collected, three from WK (two dominated by cyanobacteria and one by lichen) and three from JAK (only lichen crusts). The two cyanobacterial crusts from WK were collected from open (WK-CC1) and shaded areas (WK-CC2) in plastic containers but were then transferred to sterile Petri dishes for the experiments. Approximately one kg of soil was additionally sampled in small sterile plastic bags from each location for the analysis of soil characteristics. The crust samples were stored in the plastic containers at room temperature until analysis and were not moistened prior to any experiment.

2.2. Crust microstructure and physicochemical characteristics

The crust microstructure was studied using scanning electron microscopy (SEM). Crust samples were sputter coated with gold particles using a Bio-Rad SEM coating system and SEM images were recorded using JEOL JSM-5600LV scanning electron microscope with backscattered electron detector (BSE) and secondary electron detector (SEI) operated at ten kV. A standard soil hydrometer method (Klute, 1986) was used to determine the proportion of sand, silt and clay composition of the crust samples. About one kg of air-dried crust samples were crushed and passed through a two-mm sieve. A soil triangle (Brady, 1984) was used to identify soil texture class. The sand portion was further fractionated into smaller particle sizes by separation of dry soil into a standard set of soil sieves as described before (Klute, 1986). Ten grams of soil was thoroughly mixed with 50 ml of deionized water and allowed to stand for 20 min. The solution was re-suspended and the filtrate was collected. Soil pH and electrical conductivity (EC) were measured from the filtrate using calibrated YSI instruments.

2.3. Cultivation of fungi and identification of fungal isolates

Fungi were isolated from all crust samples using dilution plate and soil plate techniques (Al-Sa'di et al., 2008). Dilutions were prepared by mixing 10 g of each soil in 100 ml sterile distilled water followed by a series of dilutions. One ml from each dilution was spread on the surface of 2.5% potato dextrose agar (PDA, OXOID, England) amended with 50 mg l^{-1} rose Bengal. The direct plating involved spreading of 100 mg of the soil sample onto the surface of PDA plates. After three to seven days, emerging fungal colonies were transferred to new PDA plates amended with 200 mg l^{-1} ampicillin and 10 mg l^{-1} rifampicillin to suppress bacterial growth. The isolates were purified using mycelium tip culture. The obtained fungal isolates were identified by sequencing the internal transcribed spacer (ITS) region of the nuclear ribosomal RNA gene. Genomic DNA was purified from mycelia as previously described (Lee and Taylor, 1990; Al-Sa'di et al., 2007). The ITS region of the fungal isolates was amplified by polymerase chain reaction (PCR) using the universal primers ITS1 and ITS4 (White et al., 1990) at an annealing temperature of 55 °C. PCR Products were purified using the Ultra Clean PCR Clean-up Kit (QIAquick, Qiagen) and sequenced at Macrogen Inc. (Korea) using BigDye 3.1 (Applied Biosystems) and the same primers used for the amplification. The forward and reverse