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Research paper

Resource utilization of microalgae from biological soil crusts: Biodiesel production associated with desertification control

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ABSTRACT

With the continuing consumption of resources and increasingly prominent environmental issues, microalgal resource utilization has received extensive attention. In this study, based on the microalgal investigation in desert biological soil crusts (BSCs) using pyrosequencing technology, the cultivated crust microalgae were further isolated in order to obtain high quality microalgae for resource utilization. The results showed that with crust development and succession, microalgal diversity gradually decreased, including the number of operational taxonomic units (OTUs) and genus, although *Microcoleus* always was the dominant genera. Pyrosequencing obtained 630 OTUs of cyanobacteria, 25 OTUs of green algae and 9 OTUs of diatom; however, part of cultivated microalgae still could not yet be detected due to the DNA extraction preferences and errors caused by PCR amplification. After isolation, four strains were purified and cultivated, including two filamentous cyanobacteria *Microcoleus vaginatus* BSC-06 and *Scytonema javanicum* BSC-39, and two unicellular green algae *Chlorella* sp. BSC-24 and *Monoraphidium dybowskii* BSC-81. The two green algae grew fast (> 250 mg L^{-1} d^{-1}), and achieved high lipid productivity up to 75–85 mg L^{-1} d^{-1}, with lipid content of 28.7–39.0%, thus was considered as promising feedstock for biodiesel production. In addition, the two crust cyanobacteria could be used to construct artificial cyanobacterial soil crusts in desertification control, although their biomass accumulation was not as high as that in the green algae. Ultimately, combining biodiesel production with desertification control would not only improve desert environments, but also provide ideal places for the local microalgal resource exploitation, further promoting desert socioeconomic development.

1. Introduction

With the increasing depletion of non-renewable resources and prominent environmental issues, microalgal (for simplicity including cyanobacterial) resource utilization has recently received a great deal of attention [1,2]. Particularly, as an alternative important bioenergy feedstock, microalgae have been considered as a promising lipid source for biodiesel production [3]. At present, although some lipid-producing microalgal species have been studied, most of the microalgae come from culture collection libraries, such as the Culture Collection of the University of Texas [4], Freshwater Algae Culture Collection at the Institute of Hydrobiology [5], Microbial Culture Collection, National Institute for Environmental Studies [6], CSIRO Algal Culture Collection [7], and culture collection of algae of Göttingen University [8]. Lots of the lipid-producing microalgae in the culture collection libraries have undergone long-time moderate environments, and it is difficult to adapt well to the field changeable environmental conditions when they are cultivated on a large scale [9,10]. Therefore, it becomes an important issue to directly isolate excellent lipid-producing microalgae from harsh environments, so that the microalgae can adapt well to the cultivation environmental conditions.

In arid and semi-arid desert regions, the environments are generally characterized by a series of harsh conditions, such as poor soil, extreme drought, high salinity, pH and radiation, large temperature variation and accustomed wind and sand storm [11,12]. In such extreme evil environments, many types of organisms are restricted, while biological soil crusts (BSCs) can be widely distributed there because of their unique physio-ecological characteristics, and even occupy more than 70% of the living coverage in some areas [13,14]. BSCs are the complex biological soil mosaic layers within the uppermost millimeters of the soil, generally first colonized by microalgae [15,16]. As the pioneer, microalgae not only play an irreplaceable role in crust formation, development and succession, but also have important ability to adapt to the field environmental conditions [16,17]. Therefore, isolating lipid-producing microalgae from desert BSCs may provide more high quality microalgal species for large scale cultivation.

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Table 1
Physicochemical characteristics of different successional biological soil crusts.

	Cyanobacterial soil crusts	Lichen soil crusts	Moss soil crusts
Thickness (mm)	3.80 ± 0.81 a[a]	8.10 ± 1.72 b	16.24 ± 2.87 c
Cyanobacterial coverage (%)	> 95	< 20	0
Lichen coverage (%)	0	> 70	0
Moss coverage (%)	< 5	< 10	100
Dominant species	*Microcoleus vaginatus*	*Collema* sp.	*Bryum* sp.
Chl-*a* content (μg cm^{-2})	2.83 ± 0.20 a	6.18 ± 1.11 b	16.20 ± 2.09 c
Polysaccharides content (μg cm^{-2})	42.55 ± 16.54 a	84.17 ± 6.77 b	478.84 ± 30.74 c

[a] For a given crust parameter, values with different letters are significantly different at 0.05 level ($P < 0.05$).

Desertification has brought a series of threatens to the local environment and socio-economic development. Isolating lipid-producing microalgae in desert regions not only provides the possibility for biodiesel production to promote local economic development, some microalgal species could also be used to accelerate the development and succession of BSCs for desertification control [14,17]. Therefore, combining desertification control and biodiesel production together would further promote the socio-ecological development in desert regions. Generally, high lipid-producing microalga are eukaryotic, but at present most of the investigations on crust microalgae are still concentrated in prokaryotic cyanobacteria [15–17]; while there has been very little work investigating on crust eukaryotic microalgae [18,19]. A comprehensive study on the composition of crust microalgae is important because it will not only help us understanding the development, succession and ecological functions of BSCs in deep, but also have great value in microalgal resource utilization in desert regions.

In this study, on the basis of comprehensive microalgal investigation in the different developmental and successional BSCs in the Shapotou region (the Tengger Desert), the cultivated crust microalgae were isolated and purified. Then from the point of view of microalgal lipid content, biodiesel production associated with artificial cyanobacterial soil crust construction, the potential of microalgal resources from BSCs were explored, and the results would provide significant guidance for the resource utilization in desert regions.

2. Materials and methods

2.1. Sampling

BSCs including cyanobacterial, lichen and moss soil crusts (Table 1) were sampled from the Shapotou region (a part of Ningxia Hui Autonomous Region), located at the southeast edge of the Tengger Desert (37°32′ N and 105°02′ E). The BSCs were collected into the sterilized Petri dishes with a sharp shovel to make sure the crust samples were in their natural thickness. The sampling was conducted randomly from the interspaces between shrubs (0.2 m away from the shrubs), and all the samples were carried to the laboratory as soon as possible for subsequent analysis. Each type of BSCs was sampled at three different sites as repetition.

2.2. Physicochemical characteristics

Crust thickness was measured using a Vernier caliper. Crust coverage of cyanobacteria, lichens, mosses and dominant species were visually assessed and identified under a microscope with charge-coupled device (CCD, LY-WN-SUPER HP CCD, China) according to the description of Wu et al. [20]. Chlorophyll-a (Chl-a) content was measured in the ethanol extract using a spectrophotometry [21], and polysaccharides content was determined using the phenol-sulfuric acid method [19].

2.3. Crust pyrosequencing data analysis

Total DNA was extracted from the BSCs with Mag-Bind Soil DNA Kit (OMEGA, USA) following the manufacturer's instruction, and 16 S and 18 S rRNA gene segments were PCR amplicated from each sample DNA according to the method of Zhang et al. [22]. The amplicons were used for pyrosequencing analysis on a Roche GS FLX Titanium machine (Roche, USA), which was carried out by Majorbio Biotech Co. Ltd. (Shanghai, China). All the sequences then were submitted to the NCBI database under the accession numbers SRP063082 and SRP063545. The low quality sequences were discarded and the trimmed sequences (primers and adaptors were removed) were clustered into different operational taxonomic units (OTUs) at 97% similarity level. The taxonomic annotation information of each OTU were then extracted from the SILVA SSU rRNA database. Although the microbial community in the BSCs has been analyzed at phylum level by Zhang et al. [22], microalgal composition is still unknown. Therefore, in this study the same pyrosequencing data were used to analyze microalgal composition in the BSCs from Shapotou region. According to the number of sequences in each OTU, microalgal abundance in genera level was calculated and those microalgae with more than 5% abundance were considered as the dominant.

Table 2
Microalgal diversity and the dominant genus in the different successional biological soil crusts.

	Cyanobacterial soil crusts	Lichen soil crusts	Moss soil crusts
Number of cyanobacterial OTUs	630	235	87
Number of cyanobacterial genus	25	16	13
Dominant cyanobacterial genus	*Crinalium, Microcoleus, Oscillatoria, Phormidium, Symploca*	*Microcoleus, Nostoc*	*Calothrix, Crinalium, Microcoleus, Nostoc, Symploca, Tolypothrix*
Number of green algal OTUs	25	10	7
Number of green algal genus	13	6	5
Dominant green algal genus	*Chlorosarcinopsis, Enallax*	*Chloromonas, Chlorosarcinopsis, Enallax, Prasinoderma, Pyramimonas*	*Gungnir, Hafniomonas, Lobosphaera, Neochlorosarcina, Pyramimonas*
Number of diatom OTUs	9	4	1
Number of diatom genus	5	3	1
Dominant diatom genus	*Campylodiscus*	*Campylodiscus*	*Nitzschia*