Research in Microbiology 167 (2016) 35–45

www.elsevier.com/locate/resmic

Original article

Diversity of yeasts associated with the sea surface microlayer and underlying water along the northern coast of Taiwan

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Received 17 March 2015; accepted 25 August 2015
Available online 3 September 2015

Abstract

Yeast communities inhabiting the sea surface microlayer (SSML) on the northern coast of Taiwan were examined using a cultivation method and compared with those inhabiting the underlying water (UW) at a 50-cm depth. Culturable yeasts were recovered from the SSML and UW samples collected in the morning during 4 field campaigns, and 420 strains were isolated. The 420 isolates were grouped into 43 species using a polyphasic molecular approach, including sequence analysis of the 26S rDNA D1/D2 domain and 5.8S-ITS region. From the SSML samples, 12 genera and 39 species, including 7 new species of *Cryptococcus* sp. (1), *Candida* spp. (4), and *Rhodotorula* spp. (2), were isolated. From the UW samples, 10 genera and 21 species, including one new species of *Rhodotorula* sp. (1), were isolated. *Rhodotorula mucilaginosa* was the most abundant species present in the yeast community in SSML (37.6%) and UW (21.6%) samples. Basidiomycetous yeasts (63.6%) and pigmented yeasts (64.5%) comprised the major yeast population. The yeast community in the SSML had a higher species number and abundance than the UW. Moreover, although the majority of yeast community species were from the SSML, individual species distribution in the SSML was unequal.

Keywords: Sea surface microlayer; Underlying water; Yeast community; New species; Isolation; Characterization

1. Introduction

The sea surface microlayer (SSML), a thin film of liquid at the sea–atmosphere interface, is essential for exchanges between the sea and the atmosphere. The film consists primarily of lipids derived, in the absence of pollution, from external sources such as marine organisms and natural hydrocarbon sinks. Microorganisms are transported to the surface layer with rising gas bubbles or by wind, and those with hydrophobic properties preferentially accumulate there. Conditions in the SSML are harsh; organisms in this microlayer face high levels of visible and ultraviolet radiation (UVR) and high pollutant concentrations [39]. Therefore, the SSML is often considered an extreme environment for microorganisms that may contain uncommon species and taxa [28].

Several studies have reported on the numbers and activity of bacteria living in the SSML [2,13,19,32]; however, information on the composition and distribution of yeast communities in the SSML is scarce. Yeasts are distributed in almost every part of aquatic environments, i.e. oceans and seas, estuaries, lakes, and rivers. Yeast communities differ considerably in different environmental samples. Although yeast communities are supposedly composed of ubiquitous species, the species are geographically or biologically restricted [10]. For instance, some species of *Cryptococcus*, *Rhodotorula* and *Sporobolomyces* were found across various oceanic regions

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http://dx.doi.org/10.1016/j.resmic.2015.08.005

[29]. *Cryptococcus vishniacii* was recognized as an indigenous Antarctic psychrophile, which can grow at −3 °C [40]. Red yeasts isolated from benthic animals at depths of >2000 m were identified as members of *Rhodotorula* and *Sporobolomyces* species, which constituted more than 80% of the yeast communities [30].

A major factor determining the validity of studies in yeast ecology is correct species identification in the ecosystem. Traditionally, yeasts are identified using morphological and physiological criteria [21], but methods using these criteria are generally laborious and time-consuming. Moreover, because of the influence of culture conditions on yeast physiological characteristics, these methods occasionally incorrectly identify species [41]. Molecular methods involving ribosomal RNA (rRNA) and its template ribosomal DNA (rDNA) have been extensively used in yeast taxonomy. Particularly, the D1/D2 domain sequences of the large subunit (LSU) rDNA and the internal transcribed spacer (ITS)-5.8S rDNA have been used [11,12,34]. The D1/D2 domain of the LSU rDNA has been used for determining the taxonomic position of new strains. Strains belonging to separate species generally exhibit >1% sequence divergence in the D1/D2 domain of the LSU rDNA [22]. However, when yeast taxonomy is based only on the LSU rDNA sequences of the D1/D2 domain, closely related yeast species could be incorrectly classified. The D1/D2 domain sequences of *C. magnus*, *C. ater*, *Filobasidium floriforme* and *F. elegans* are indistinguishable, whereas considerable differences exist in the ITS region sequences; *C. ater* and *C. magnus* represent a single species. However, *F. elegans*, *F. floriforme* and *C. magnus* differ significantly in the ITS region sequences, confirming that they are separate species [12]. Furthermore, an ITS-5.8S rDNA sequence was used for confirming relevant yeasts [11,12,34].

In this study, we investigated the biodiversity of yeasts present in the SSML of sea water samples taken from the northern coast of Taiwan. Furthermore, we compared the SSML and the underlying water (UW) yeast communities by using both traditional isolation and molecular identification methods.

2. Materials and methods

2.1. Water samples

All seawater samples were taken near Keelung on the northern coast of Taiwan (25°09′27″N, 120°09′22″E) and were collected in the morning during 4 field campaigns (November 2005, February 2006, May 2006 and August 2006). The SSML samples were collected using a metal screen (300–400-μm thick) [14] and a glass plate (20–100-μm thick) [17], which were vertically introduced across the SSML and vertically withdrawn. The water adhering to the plate was subsequently removed using a Teflon wiper blade and the water adhering to the screen was collected directly. The UW samples were collected by submerging a polycarbonate bottle and opening it at a depth of 0.5 m. All parts of the equipment were thoroughly sterilized before use.

2.2. Isolation and quantitative and phenotypic analysis of yeasts

Isolation of yeast strains was achieved using the method of Chang et al. [5]. After serially diluting the concentrated seawater samples, subsamples (100 μL each) were spread on yeast extract–malt extract (YM) agar plates (1% glucose, 0.5% peptone, 0.3% yeast extract, 0.3% malt extract and 0.2% agar; pH 4.5–5.0) supplemented with 0.01% (w/v) chloramphenicol for inhibiting bacterial growth and then incubated in the dark at 25 °C. All colonies appearing on the plates over 3–5 d were counted and transferred to YM broth. Subsequently, the strains were purified by streaking on YM agar plates without chloramphenicol. To preserve the isolated strains, they were grown on YM agar plates for 3 d at 25 °C, transferred to broth cultures supplemented with 30% (w/v) glycerol as a cryoprotective and stored at −80 °C. Purified yeasts on YM agar plates were also preserved at 4 °C. The isolated strains were morphologically, physiologically and biochemically characterized using standard methods for current yeast taxonomy [20].

2.3. DNA isolation and molecular characterization

The total DNA of the yeast isolates was extracted as previously described [6]. Nucleic acids in the remaining cell suspension were purified using a Genomic DNA Mini Kit (Geneaid Co., Taiwan) according to the manufacturer's protocol. The D1/D2 domain of the LSU rRNA gene and the ITS region of genes were PCR amplified and sequenced using the primer pair F63 (5′-GCA TAT CAA TAA GCG GAG GAA AAG-3′) and LR3 (5′-GGT CCG TGT TTC AAG ACG G-3′) and the primer pair ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) [12,34], respectively. The ITS PCR–restriction fragment length polymorphism (RFLP) method was used, as described by Esteve-Zarzoso et al. [9].

ITS PCR products (10 μL or approximately 0.5–1 μg) were digested with the restriction endonucleases *Cfo*I, *Hae*III and *Hin*fI (Promega, USA) and used without further purification. In particular cases, the endonucleases *Alu*I, *Dde*I, *Scr*FI, or *Taq*I were also used. The PCR products and their restriction fragments were separated using electrophoresis on 1.4% and 3% agarose gels, respectively, with 1 × TAE buffer. After electrophoresis, the gels were stained with ethidium bromide and visualized under UV light, DNA fragments were photographed and the fragment lengths were estimated using a DNA length standard (100-bp ladder, Gibco-BRL, USA).

The purified PCR products were used as templates for sequencing involving a BigDye Terminator cycle sequencing kit and an Applied Biosystems DNA Analyzer (ABI 3730 DNA Analysis System, USA). Sequence comparison was performed using sequences from GenBank (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/) and BLASTN. For phylogenetic analysis, sequences were aligned using CLUSTAL × 1.83 [38]. Phylogenetic and molecular evolutionary analyses were conducted using MEGA