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Taxonomy and systematics

Assessment of non-cultured aquatic fungal diversity from different habitats in Mexico

Estimación de la diversidad de hongos acuáticos no-cultivables de diferentes hábitats en México

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Abstract

With the aim to explore the diversity of aquatic fungi in Mexico we present an investigation using a fragment of the 18S ribosomal DNA as a molecular marker obtained from different water bodies (marine, brackish and fresh water). Ribosomal gene fragments were obtained by DNA amplification, the resulting sequences were compared using multiple alignments against a collection of classified reference fungal sequences and then subjected to phylogenetic clustering allowing the identification and classification of DNA sequences from environmental isolates as fungal down to the family level, provided enough reference sequence were available. From our ensemble of 2,020 sequences identified as fungal, 23.8% were classified at the family level, 48.5% at the order level, 13% at the class/subphylum level and 14.7% of the sequences (all from the same site) could not be unambiguously positioned in any of our reference fungal groups but were closely related to uncultivated marine fungi. The most frequently recovered phylum was Ascomycota (89.1%), followed by Chytridiomycota (8.1%), Basidiomycota (2.8%) and Mucoromycotina (1.3%).

Keywords: Aquatic habitats; Fungi; Taxonomic classification; Fungal populations; 18S ribosomal DNA

Resumen

Con la finalidad de explorar la diversidad de hongos acuáticos en México, se presenta una investigación usando un fragmento del ADN ribosomal 18S como un marcador molecular obtenido de muestras de cuerpos acuáticos con diferentes características (marino, salobre y dulce). Los fragmentos de los genes ribosomales se obtuvieron mediante la amplificación de ADN, las secuencias resultantes se compararon mediante alineamientos múltiples con una selección de secuencias de hongos como referencia y posteriormente se analizaron filogenéticamente, permitiendo la identificación y clasificación de secuencias de ADN provenientes de aislados ambientales hasta la categoría de familia, cuando hubo suficientes secuencias disponibles. De las 2,020 secuencias identificadas como hongos, un 23.8% se clasificaron como familia, un 48.5% como orden, un 13%

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como clase/subphylum y un 14.7% de las secuencias (todas del mismo lugar) no pudieron ser colocadas inequívocamente en alguno de los grupos de hongos que se tomaron como referencia, pero se encontraron muy cercanamente relacionadas a hongos marinos no cultivables. El phylum más representado fue Ascomycota (89.1%), seguido de Chytridiomycota (8.1%), Basidiomycota (2.8%) y Mucoromycotina (1.3%).

Palabras clave: Hábitats acuáticos; Hongos; Clasificación taxonómica; Poblaciones fúngicas; ADN ribosomal 18S

Introduction

Current estimates of fungal diversity based on the plant:fungi ratio found in countries where both populations are sufficiently well studied suggest the existence of 1.5 million species (Hawksworth, 1991, 2001; Mueller & Schmit, 2007). The study of fungal diversity is important because fungi are decomposers of organic matter and comprise a major proportion of microbial biomass. Recently, global climate change and the better known role of fungi in biogeochemical cycles have enforced the importance of studying fungal diversity (Chapin et al., 2000; Wardle & Giller, 1996).

Studies of fungal diversity have been limited by the lack of appropriate microbiological methods (Kimura, 2006; Torsvik & Ovreas, 2002). The application of molecular approaches such as extracting, cloning and amplifying DNA from environmental samples currently allows us to explore biodiversity without the need of culturing. In this regard, 18S ribosomal DNA (rDNA) sequences have been extensively used to explore fungal diversity (Hunt, Boddy, Randerson, & Rogers, 2004; Le Calvez, Burgaud, Mahé, Barbier, & Vandenkoornhuyse, 2009; Monchy et al., 2011; Piquet, Bolhuis, Meredith, & Buma, 2011) and many specific primers have been designed for this purpose (Borneman & Hartin, 2000; Moon-van der Staay, De Wachter, & Vaulot, 2001; Vainio & Hantula, 2000). The capacity of these primers to reveal fungal diversity in environmental samples is based on their specificity in preferentially priming fungal sequences and also their ability to represent all fungal phyla at the same time (Anderson, Campbell, & Prosser, 2003; Hunt et al., 2004). Molecular tools, including 18S rDNA sequence analysis, have been used recently to re-define fungal taxonomy based on multilocus phylogenetic analyses (Hibbett et al., 2007; James et al., 2006). As a consequence, our view of traditional fungal groups has changed drastically.

If terrestrial fungi are still largely under-described, aquatic fungi are even less well known. Most of the cultivated aquatic species belong to the Chytridiomycota and Ascomycota phyla (Mueller & Schmit, 2007; Mueller, Bills, & Foster, 2004; Shearer et al., 2007) and many fungal-related microbes belonging to the straminipiles (oomycetes and hyphochytriomycetes in particular) have been described (Mueller et al., 2004; Van der Auwera et al., 1995). In Mexico, an important effort to explore fungal diversity has been made (Guzmán, 1998). Exploration of aquatic fungal diversity has also been conducted by traditional methods isolating fungi from freshwater and marine environments (González & Chavarría, 2005; González, Hanlin, Herrera, & Ulloa, 2000; González, Hanlin, & Ulloa, 2001; Heredia, Reyes, Arias, Mena-Portales, & Mercado Sierra, 2004). In marine environments, ascomycetes and mitosporic fungi were mainly found, although one basidiomycete was reported (González et al., 2001).

To explore the diversity of aquatic fungi in Mexico and to demonstrate the potential of a classification system based on a single molecular marker, we present an investigation of different water bodies (marine, brackish and fresh water) using a fragment of 18S rDNA sequences and the results of our phylogenetic clustering approach.

Materials and methods

Description of the sampled locations

Zempoala, Morelos (fresh water, 19°01′20″ N, 99°16′20″ W). The Zempoala Lagoons comprises 7 pristine water bodies located in a protected park in the state of Morelos at 2670–3686 masl. The area is surrounded by a temperate forest of pines, firs and oaks. Samples were obtained from one of the permanent lagoons.

Carboneras, Tamaulipas (brackish water, 24°37′41.88″ N, 97°42′59.19″ W). Fishery and leisure town located at 58 km from San Fernando in the state of Tamaulipas. It belongs to the central section of Laguna Madre.

Mezquital, Tamaulipas (sea water, 25°14′55.70″ N, 97°31′05.54″ W). Located in the eastern side of the wider part of Laguna Madre, it is connected to the Gulf of Mexico through an artificial navigation channel.

Media Luna, Tamaulipas (brackish water, 25°09′47.64″ N, 97°40′16.35″ W). Located at the western side of the wider part of Laguna Madre, and due to poor road conditions and to the absence of large settlements, this is one of the less spoiled areas. The distance between the Mezquital and Media Luna sampling places is approximately 12 km.

El Rabón, Tamaulipas (hypersaline sea water, 25°26′23.68″ N, 97°24′34.79″ W). At the northern end of Laguna Madre, this area has suffered serious transformations due to human activity and had become dry. Recently, the wetlands have been restored by pumping in sea water.

Carpintero. Tamaulipas (fresh water, 22°14′01.12″ N, 97°51′20.67″ W). Belonging to the Pánuco River basin and located in a protected natural park covering 7 ha, Carpintero Lagoon is currently used for fish and crocodile breeding grounds. In spite of the urban location of the site in the City of Tampico it is relatively unspoiled.

Vicente Guerrero, Tamaulipas (fresh water, 24°03′43.70″ N, 98°44′13.44″ W). This water body is a dam built in 1971 in the Soto La Marina River basin. It has a surface of 22.1 km^2 at 134 m