Costa Rican fungi as potential biomaterials

Hongos costarricenses como potenciales biomateriales

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Keywords

Fungal biomaterials; mangrove; piezoelectric; DNA barcoding.

Abstract

Fungal biomaterials are gaining relevance due to their intrinsic ability of self-repair, higher sensitivity to external conditions and faster growth respective to synthetic materials. This project consists of evaluating and characterizing the physical properties of fungal strains isolated from a Pacific Coast Mangrove in Costa Rica. We identified environmental strains by recording their morphological features and complemented this by ITS-based DNA barcoding, and subsequently, classified three strains based on morphological features and seven strains by molecular analyses. Ongoing work is being done to measure electrical responses of these fungi upon light stimulation; in addition, a protocol for studying their piezoelectric properties is being developed to identify potential candidates to be used in the field of electronics. To the extent of our knowledge, our project is the first one to report piezoelectric properties from microscopic fungi in Costa Rica as means to determine its potential as biomaterials.

Palabras clave

Biomateriales fúngicos; manglar; piezoelectricidad; *barcoding*.

Resumen

Los biomateriales fúngicos han ganado relevancia en la industria debido a su capacidad intrínseca de autorreparación, mayor sensibilidad a las condiciones externas y crecimiento más rápido que los materiales sintéticos. Este proyecto consiste en evaluar y caracterizar las propiedades físicas de cepas fúngicas aisladas de un manglar de la costa del Pacífico en Costa Rica. Identificamos las cepas ambientales mediante el registro de sus características morfológicas y lo complementamos con códigos de barras de ADN basados en ITS y, posteriormente, clasificamos tres cepas en función de las características morfológicas y siete cepas mediante análisis moleculares. Se está realizando un trabajo continuo para medir las respuestas eléctricas de estos hongos ante la estimulación con luz; además, se está desarrollando un protocolo de estudio de sus propiedades piezoeléctricas para identificar posibles candidatos para ser utilizados en el campo de la electrónica. Hasta donde sabemos, nuestro proyecto es el primero en reportar propiedades piezoeléctricas de hongos microscópicos en Costa Rica como medio para determinar su potencial como biomateriales.

Introduction

Fungal organisms are considered cosmopolitans due to its wide range of colonized environments [1]. This feature is dictated by the genetic sequences within the fungal genome, to such an extent that fungi have been exploited by different industries, being recently applied as biomaterials [2] [3]. The latter covers a variety of materials useful in architecture, textile and electronics. For example, companies such as MycoWorks™ use fungal hyphae to create accessories; likewise, Ecovative Design uses fungal hyphae to develop ecological materials for construction [4] [5]. Recently, the idea of using these organisms has been expanded to the field of electronics, thanks to the intrinsic abilities of fungi, for which some authors have demonstrated its potential use in the field [6] [7]. However, to the extent of our knowledge the available reports include only mushrooms, leaving room for research to evaluate microscopic fungi potential.

Costa Rica is known for being one of the most biologically diverse countries in the world [8]; however, there are few studies directed to bioprospecting biodiversity found in different environments such as marine habitats. For this reason, the Applied Bioinformatics Laboratory (LABAP) at Universidad Nacional, focused its efforts on studying fungal strains isolated from such habitats and on utilizing their capabilities to create biotechnological products useful in a plethora of industries (e.g., biosurfactants, biomolecules and biomaterials) from both, bioinformatic and experimental approaches [9]. Herewith, we aim to study, understand and characterize Costa Rican fungal diversity and physical features as potential biomaterials by describing its piezoelectric properties.

Materials and methods

Morphological identification

In order to classify fungal isolates at the lowest possible taxonomic level, we describe fugal morphological features from potato dextrose agar (PDA) cultures. For this, we took a set of photos from every isolate in order to observe and analyze its features such as: color, geometry, texture, sporulation and presence/absence of exudates. In addition, lactophenol cotton blue was used to stain the isolates to observe hyphae septation and the type of conidiophores. This information was used to characterize morphology based on fungal taxonomic keys.

Molecular identification

In order to complement morphological identification, we carried out molecular analysis. For this, we extracted genomic DNA from every isolate using an organic solvent-based method and obtained sequences corresponding to the Internal Transcribe Spacer (ITS) region using ITS1/ITS4 primers [10]. Bidirectional Sanger sequencing was performed by Macrogen Inc. We manually reviewed each sequence using Geneious R 9.1.8 to quality control the sequences and then performed BLASTn [11] analyzes in order to identify each isolate to the genus or species level. Finally, we ran an alignment with Clustal W [12] to infer a phylogenetic tree using IQ-TREE [13], that was visualized with FirgTree [14].

Results and discussion

We have isolated 22 fungal strains from a mangrove ecosystem in the Pacific Coast of Costa Rica; from which 13 have been analyzed so far. Based on morphological traits (color, conidiophores, geometry, and shape) we found that 3 out of 13 strains are classified into *Aspergillus* and *Trichoderma* genera. The morphological classification is being complemented with molecular analysis, and so far, 7 out of 13 isolates has been taxonomically classified (Figura. 1). Identifying the isolates at the species level is vital in order to study their piezoelectric capabilities, as we aim to develop a fungal film to perform electrical measurements in response to light stimulation. To our knowledge, there are only few records which study and describe Costa Rican marine fungi [15] [16]; and therefore, this project is the first one to characterize environmental fungal strains isolated from mangrove ecosystems.

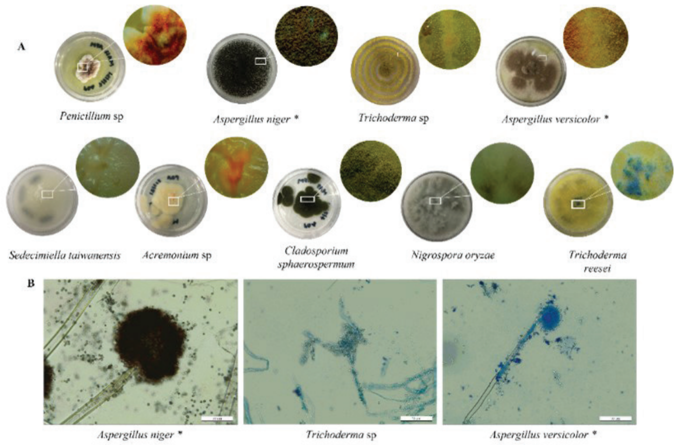

Figura. 1. Morphological record of nine marine fungal strains isolated from a mangrove environment in Costa Rica. A. Fungal strains traits on PDA. B. Fungal structures stained by lactophenol cotton blue. Species with an asterisk (*) were identified only morphologically.

The current lack of ITS sequences on public databases (such as GenBank or Uniprot) from fungal strains isolated from Costa Rican mangrove ecosystems, demonstrates the need and value to report this kind of genetic information, as this sets the required ground to get a better understanding of the organisms' biology, niche and growth requirements leading to better experimental designs. Our results display the high diversity of our isolates, clustering them in three main nodes with a good tree bootstrap (Figura. 2).

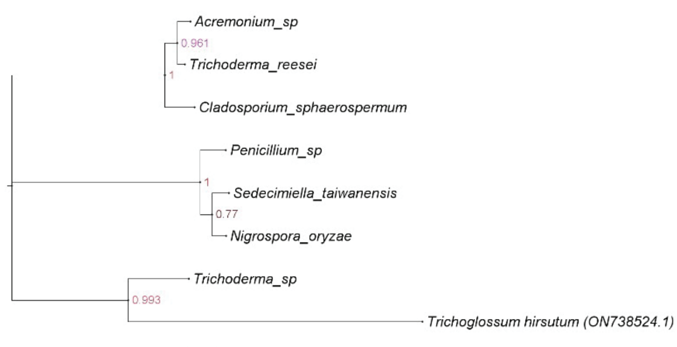

Figura. 2. Phylogenetic relationship inferred by ITS-based DNA barcoding from seven strains isolated from a mangrove ecosystem in Costa Rica. *Trichoglossum hirsutum* (ON738524.1) was used as an outgroup.

Conclusions

We have isolated, characterized, and identified fungal isolates from mangrove environmental samples which have not been reported in Costa Rica, being this the first report. In addition, we identified some methodological issues regarding the use of standard ITS1/ITS4 primers for the sequencing of the ITS region from *Aspergillus* species that consistently did not amplify. To overcome this, we propose to use CMD5/CMD6 primers, which have been tested as efficient barcoding primers for this genus.

Currently, we are testing different growth systems to obtain biofilms to further develop a protocol to evaluate piezoelectric properties of fungal strains based on atomic force microscopy analysis. Research and development of biomaterials is important due to the intrinsic characteristics of organisms that produce them, such as self-repair, higher sensitivity to external conditions and faster growth. In addition, agro-industrial residues can sustain the growth of these microorganisms, which translates into an ecological and sustainable alternative to make a variety of materials using residues as the primary source, contributing to the initiative of circular bioeconomy process. Therefore, fungal biomaterials could satisfy local industries, helping to solve current supply chain disruption related to import-export restrictions on products due to the COVID-19 pandemic.

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