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Microbial diversity associated with algae, ascidians and sponges from the north coast of São Paulo state, Brazil

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Summary

Little is known about the microbial diversity associated with marine macroorganisms, despite the vital role microorganisms may play in marine ecosystems. The aim of the present study was to investigate the diversity of bacteria and fungi isolated from eight marine invertebrate and one algae samples. Data derived from ARDRA and sequencing analyses allowed the identification of marine-derived microorganisms isolated from those samples. Microbial strains identified up to the genus level revealed 144 distinct ribotypes out of 256 fungal strains and 158 distinct ribotypes out of 181 bacterial strains. Filamentous fungi were distributed among 24 different genera belonging to Ascomycota, Zygomycota and Basidiomycota, some of which had never been reported in the literature as marine invertebrate-inhabiting fungi (*Pestalotiopsis*, *Xylaria*, *Botrysphaeria* and *Cunnninghamella*). Bacterial isolates were affiliated to 41 different genera, being *Bacillus*, *Ruegeria*, *Micrococcus*, *Pseudovibrio* and *Staphylococcus* the most abundant ones. Results revealed an unexpected high microbial diversity associated to the macroorganisms which have been collected and suggested the selection of certain microbial taxonomic groups according to the host. The combined data gathered from this investigation

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contribute to broaden the knowledge of microbial diversity associated to marine macroorganisms, including as a promising source for the discovery of new natural products.

Introduction

The ocean covers more than 70% of Earth's surface and is considered as a great reservoir of natural resources. However, the extent of marine biodiversity, especially of microorganisms, is barely known. It has been estimated that the biological diversity in marine ecosystems is higher than in tropical rain forests (Larsen et al. 2005). Marine microbial communities are composed of ubiquitous members that can be found not only in the surface waters of the sea, but also in the lower and abyssal depths from coastal to the offshore regions, and from the general oceanic to the specialized niches like blue waters of coral reefs to black smokers of hot thermal vents at the sea floor (Surajit et al. 2006; Schafer et al. 2001).

Marine invertebrates, especially sponges, represent an important source for potential active and biologically functional natural products (Osinga et al. 2001). Many of these compounds exhibit cytotoxic, antibacterial, antifungal, antiviral or anti-inflammatory activities (Blunt et al. 2008; Zhang et al. 2006; Schirmer et al. 2005). Several studies have reported the discovery of new bioactive compounds from marine organisms, focusing mainly on chemistry of secondary metabolites, which include now more than 15,000 structurally diverse bioactive compounds isolated during the last 30 years (Salomon et al. 2004). The secondary metabolism of marine-derived microorganisms started to be investigated much more recently. However, the ecological associations occurring between the microorganisms and the marine substrates have been greatly neglected (Kurtboke 2005).

In the terrestrial and marine environment, fungi are ecologically important intermediaries of energy that flows from detritus to higher trophic levels and play an important role in nutrient regeneration cycles as decomposers of dead and decaying organic matter (Bugni and Ireland 2004; Prasannarai and Sridhar 2001). On the other hand, marine bacteria serve as an important source of food for a variety of marine organisms and may also function as biological mediators through their involvement in the biogeochemical processes (Surajit et al. 2006).

The field of marine mycology has a relatively short history and has yielded so far the classification of two groups of marine fungi on the basis of their ability to grow and to reproduce in seawater (Kohlmeyer and Kohlmeyer 1979). Obligate marine fungi are those that grow and sporulate exclusively in a marine or estuarine habitat, while facultative marine fungi are those from freshwater or terrestrial milieus that are able to grow (and possibly sporulate) in the marine environments. About 800 species of obligate marine fungi have been described, including representatives from the Basidiomycota, Ascomycota, mitosporic fungi and yeasts (Surajit et al. 2006).

Considering that conservation and the sustainable use of biological diversity requires a comprehensive knowledge about the species richness, abundance and distribution and that little is known about the phylogenetic and functional diversity of marine microbial communities, the aim of the present study was to investigate the diversity of filamentous fungi and bacteria from different samples of marine macroorganisms collected at the north coast of São Paulo State, Brazil.

Material and methods

Sampling of marine macroorganisms

Nine samples of marine macroorganisms, including the sponges *Amphimedon viridis* (AV); *Axinella corrugata* (AC); *Dragmacidon reticulata* (DR); *Geodia corticostylifera* (GC); *Mycale laxissima* (ML) and *Mycale angulosa* (MA); the ascidians *Didemnum ligulum* (DL) and *Didemnum* sp. (DSP), and the algae *Sargassum* sp. (AS), were collected in January 2007 in beach areas named Praia Guaecá (23°49S; 45°25W), Ilha Toque-Toque (23°51S; 45°31W) and Ilhota da Prainha (23°51S; 45°24W), in São Sebastião region, São Paulo State, Brazil, at depths between 5 and 10 m. The samples were placed in sterilized polyethylene bags containing seawater and immediately transported to the Center of Marine Biology of São Paulo University (CEBIMar).

Isolation and maintenance of microorganisms

In order to avoid external contamination during the microbial isolation procedure, algae, sponge and ascidian samples were firstly sterilized with 0.001 g l^{-1} mercury chloride in 5% ethanol and then washed twice with sterilized seawater. Two