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Molecular studies of photobionts of selected lichens from the coastal vegetation of Brazil

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Abstract

A light microscopic and molecular analysis of photobionts in *Ramalina* and *Cladonia* from coastal habitats of Brazil is presented. A Bayesian phylogenetic analysis of ITS rDNA sequences suggests a *Trebouxia* lineage which is preferentially tropical in geographic distribution. This highly diverse clade also includes the morphological similar species *Trebouxia higginsiae* and *galapagensis*. Within the predominantly tropical clade of *Trebouxia* we distinguish several subclades, three of which are represented in our samples of *Ramalina* species. Since sexuality has not been recognized in coccal lichenised photobionts until recently, we cannot apply a biological species concept, but when compared with the sequence diversity between known species we conclude that several new species need to be described in this clade. The mutually exclusive presence of other *Trebouxia* lineages in temperate samples of *Ramalina* suggests an evolution towards higher selectivity in this genus. A strictly tropical lineage is not conspicuous in the photobionts of the genus *Asterochloris* sampled from *Cladonia* so far.

Keywords: Photobionts; Lichen; *Trebouxia*; *Asterochloris*; *Ramalina*; *Cladonia*; ITS rDNA

1. Introduction

Compared with the mycobiont partner of lichens, still little is known about the geographic distribution and ecology of the associated photobionts. Historically, this can be explained by the general difficulty to recognize the species in the lichen thallus. Previous identifications therefore included the isolation and axenic culture of algal symbionts. With the use of molecular tools, the interest in this symbiotic partner has increased. Friedl and Rokitta [1] showed a clear heterogeneity of trebouxioid photobionts. Species with a chloroplast closely appressed to the cell wall at certain stages and an indistinct pyrenoid, containing regular thylakoids, were found to be distantly related to the core *Trebouxia* cluster. These data agree with the findings of Tschermak-Woess [2] who splitted *Trebouxia* in two subgenera *Trebouxia* and *Eleutherococcus*. Piercey-Normore and DePriest [3] compared the sequences of several photobionts of Cladoniineae, including the subgenus *Eleutherococcus* sensu Tschermak-Woess and the isolated photobionts from *Anzina carneonivea*, i.e. *Asterochloris phycobiontica* [4]. They found 93% of similarity on their ITS sequences, suggesting that all these photobionts belong

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to the same genus *Asterochloris*. Phylogenetic studies of ITS rDNA from *Trebouxia* allowed the assignment of photobionts to certain clades [5,6] or focused on selectivity/specificity of fungi for photobionts [7]. These studies show that there are clear differences in photobiont selectivity in lichens. Only two species of *Trebouxia* were observed in some foliose Physciaceae by Dahlkild et al. [8], while in *Parmelia* and in the lichen genus *Umbilicaria* from Antarctica, several photobionts were found in different thalli of a single species [6,9]. There are few studies dealing with the diversity of photobionts of particular habitats. Beck et al. [5,10] indicated that a green algal species can generally be selected from a more diverse "pool of locally available algae". A similar situation has been found in lichens that are associated with cyanobacteria [11]. The "pool" or guild of photobionts comprise a number of species that are adapted to a certain habitat. It is not clear whether the composition of the "pool" is changing in different geographic regions and still little is known about the biogeography of photobionts. While the systematics of tropical lichens are now increasingly understood taxonomically and floristically, much less is still known about their photobionts. Practically nothing is known about cyanobacterial symbionts of tropical lichens, and the state of knowledge of the very common representatives of Trentepohliaes in lichens is quite limited [12,13]. While morphological data of growth form could assist in the recognition of taxa in the latter group, this is hardly possible in the second large group of eukaryotic tropical lichen photobionts, the coccale green algae of the Chlorophyceae. Phenotypic characters of this group are best studied using cultivated isolates, while molecular sequence data can readily be used to assess their phylogenetic relationships. Photobionts of lichen from tropical regions are included in a clade (Clade IV) in Friedl et al. [14], which includes lichens from Galapagos Islands and Florida – USA [9]. Some tropical members of Physciaceae are also included in this clade [7].

The Brazilian coast is composed of diverse environments. Ecologically outstanding are the "restinga" (sand bank vegetation) and mangrove. Restinga is an assemblage of coastal sandy ecosystems with floristically and physiognomically distinct communities. These plant communities colonize sediments of diverse origin, forming a edaphic vegetational complex that occupies a narrow belt along the coast, including such distinct regions as beaches, dunes and associated depressions, sand ridges, terraces and plains [15]. Although, this environment in Brazil is rich in lichens, detailed information is available only for some few species such as *Ramalina* [16] and *Cladonia* [17–21]. Differently from the restinga, the mangrove represents tropical vegetation developed in areas which are periodically inundated from the tides and are particularly present in areas where riverine habitats border to the sea [22]. However, the lichen flora in the mangrove does not seem to be much different from that in other tropical rain forest close to the coast [23].

In the present paper, we describe the phylogenetic position (ITS rDNA sequence comparison) and light microscopic characters of photobionts from some tropical lichens belonging to the families Ramalinaceae and Cladoniaceae, from different coastal environments of Brazil.

2. Material and methods

2.1. Lichen material

Samples utilized in this study were collected in different environments of Brazil: *Restinga*: *Ramalina sprengelii* 68; *Ramalina gracilis* 63; *Ramalina peruviana* 67 – 25°32′05″S/48°20′30″W; sea level (0 m); Ilha do Mel, Pontal do Paraná – PR, Brazil (February/2004). *Ramalina sorediosa* 59; *Ramalina anceps* 70 – 25°19′52″S/48°25′10″W; 8 m; Ilha Rasa, Guaraquecaba – PR, Brazil (February/2004). *R. gracilis*, *Ramalina complanata* – 27°35′48″S/48°32′57″W, 3 m, Campeche Beach, Santa Catarina Island – SC, Brazil (August/2001). *Cladina confusa* – 25°32′05″S/48°20′30″W; on sandy soil, sea level, Ilha do Mel, Pontal do Paraná – PR, Brazil (August/2001).

Mixed rain forest: R. peruviana 58 – 25°26′15″S/49°03′45″W; 940 m; Piraquara – PR, Brazil (February/2004).

Mangrove: Ramalina sorediosa 60 – 25°19′51″S/48°27′40″W; sea level; Guaraquecaba – PR, Brazil (February/2004). *R. anceps* 78 (6) 25°19′31″S/48°25′49″W; sea level; Guaraquecaba – PR, Brazil (February/2004). *Ramalina dendroides* 79 (8) 25°19′04″S/48°26′41″W; sea level; Guaraquecaba – PR, Brazil (February/2004).

Recovery area of Atlantic rain forest: R. peruviana 81 (5) 25°14′36″S/48°29′38″W; 40 m; Guaraquecaba – PR, Brazil (February/2004).

Caatinga: Cladonia verticillaris – 7°26′S/34°56′W; Alhandra – PB, Brazil.

Atlantic rain forest – Savanna (contact zone): Cladonia crinita; *Cladonia fissidens* – 20°05′S/43°29′W; Sandy Soil, Monastério do Caraça – MG, Brazil.

Some samples from non-tropical environments were included: *Cladonia perforata*, Eric Mendes Biological Station, Florida, USA (subtropical; gift from Rebecca Yahr). *Ramalina fraxinea* FB30 and *Ramalina farinaceae* FB22: Slovenia, 1997.

All the voucher specimens are placed in the UPCB Herbarium (UFPR-Curitiba, Brazil).

2.2. DNA extraction, PCR and sequencing

Total DNA was extracted according to a modified CTAB method [24]. DNA-extracts were used for PCR-amplification of the ITS regions including the 5.8S gene