American Journal of Biology and Life Sciences
2015; 3(1): 17-21
Published online January 20, 2015 (http://www.openscienceonline.com/journal/ajbls)

Some aspects of the lichen colonization and its interaction with soil particles

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To cite this article

Peña-Contreras Z. C., Dávila-Vera D., Rojas-Fernández J. A., Balza-Quintero J. A., Marcano V., Mendoza-Briceño R. V., Palacios-Prü E. L.. Some Aspects of the Lichen Colonization and Its Interaction with Soil Particles. *American Journal of Biology and Life Sciences*. Vol. 3, No. 1, 2015, pp. 17-21.

Abstract

In this study, an analysis of the soil participation in the genesis of lichens, was practiced using photonic microscopy and transmission electron microscopy. In addition, the role of lectins and polysaccharides in the primary interaction was also revised. *Cladonia rappii* was used in this study to understand also the role of lectins, phenolic carboxylic acids and polysaccharides of the cell wall of the symbiont cells and their exchanges with the soil particles. The species showing lectins exhibited a matrix constituted by organic polymers having mineral crystals immersed in it. The difference in the composition of the polysaccharides of the cell wall could be not important in the cell/mineral interaction. Thus, the results revealed that the presence of agglutinant proteins (lectins) could lead primarily the binding of the soil crystals to the cell wall of the lichen.

Keywords

Lichen, *Cladonia rappii*, Microenviroment, Venezuela

1. Introduction

Interactions that occur at the interface lichen-soil are strongly affected by biochemical processes, which are mediated largely by reactions of organic and inorganic products (1). That interface provides an ideal microenviroment for modeling interactions between communities of microorganisms and minerals of the soil, such as kaolinites, micaceous and quartz crystals.

In is thought that interaction among minerals and cell wall of the symbiont cells would allow the availability of essential substances by the dissolution, leaching or weathering of the soil elements. These interactions may also stimulate the secretion of mucous substances to facilitate the gluing of the soil minerals particles. This may be incidental or can be induced by the effect of lichens that accelerate the degradation of minerals (2).

Hyphae produce physical and chemical actions in concert with chemical weathering mechanisms such as lichen acids (3-5), penetrate mineral cleavages and grain boundaries, leading to accumulation of substratum-derived mineral fragments within the lower thallus (6,7). Lichen polysaccharides moreover can produce an effect of dissolution of minerals, which can vary depending on the different concentrations and the orientations of carboxylic acids functional groups. Polysaccarides having a low molecular weight can increase the extent of mineral weathering by complexing with ions in solution (2).

Glycoconjugates have an important role in cellular recognition and it is thought that in the case of lichens participate the establishments of symbiosis (8, 9). These molecules can also participate allowing the adhesion of the substratum derived mineral fragments and its role in such sense is not perfectly known (10).

The objective of this paper is microscopically illustrate the interaction of the cell wall of the symbiont cells with soil particles and minerals, essential interrelationship to the formation of mycobionts and photobionts constituting the *Cladonia rappii.*

The *Cladonia rappii* has a cosmopolitan global distribution; in our country it has been reported that *Cladonia rappii* is distributed in Lara, Mérida, Táchira and Trujillo states, in mountainous woodlands and highlands, at higher altitudes to 1400 m (11). It grows on soil and other substrata; preferably shaded microsites to open (forests, rangelands, in roadsides, secondary forest and wilderness).

Usually, genus Cladonia belonging to the *Cladoniaceae* family, are characterized by having a crusty surface and fruiting bodies borne in simple or branched podetia. The thallus is dimorphic, with fruticose podetium, the primary thallus is crusty, and is gray to yellowish green, with simple or branched soft and cylindrical podetium, which during growth have sharply to truncated forms, and acquire aspect cup or dish when fully developed (12).

2. Materials and Methods

2.1. Study Area

The study area comprises a highly oligotrophic open zone ecologically characterized by old human disturbance which was located at sector Los Pinos (2250 m), San Javier El Valle in proximity to the Mérida city, Mérida state (8º39.183'N,71º7.686'W), which is part of mountainous formation La Culata at the Venezuelan Andes. This area is isothermal (22°C) during daylight hours and at very low temperatures overnight. The soil where *Cladonia rappii* grow is mostly metamorphic and sedimentary composed by very fine clay, brownish to reddish or orange red, with a high content of iron oxide and silica.

2.2. Material

A total number of 32 specimens *Cladonia rappii* were collected at study area described in the previous section. Samples were washed thoroughly with distilled water Milli-Q quality. Complete specimens intended for light microscopy study were placed in a vacuum chamber until the time of observation; while selected for analysis with electron microscopy were immediately processed.

2.3. Photonic Microscopical Analysis

For the study of photonic microscopy, samples were removed from the vacuum chamber and observed, stereoscopically, in a Reitcher Jung Polyvar microscope, through which the photographic record was made.

2.4. Ultramicroscopical Analysis

The ultrastructural aspect was evaluated by transmission electron microscopy. Samples were fixed using 3% paraformaldehyde plus 3% glutaraldehyde mixed in 0.1 M sodium cacodylate buffer, pH 6.3 (13). After 12h, tissue plugs were cut from different sites of the colonies and postfixed in 1% osmium tetroxide. The samples were dehydrated in a series of graded ethanol and propylene oxide, and finally infiltrated with epoxy resin. Ultrathin sections were made using a Porter-Blum Sorvall MT2-B ultramicrotome, were contrasted with uranyl acetate and lead citrate (14, 15), and observed through a transmission electron microscope Hitachi H-7000.

3. Results

3.1. Photonic Microscopical Analysis

Observations with photonic microscopy allow visualizing in four stages, the way elements as will establishing the relationship between the symbiont cells and soil particulates elements. In the first phase, isolated fungal hyphae are seen mixed with soil microcrystal of kaolinites, micaceous and quartz that compose the surface of the area subject of this study (Fig. 1). In the second stage small propagules are identified on soil microcomponents; also begins to observe the formation of small thallus (Fig. 2).

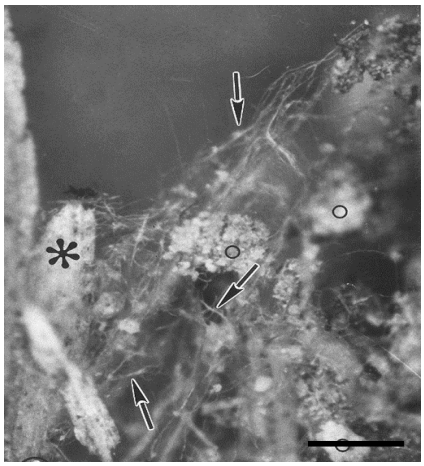

Figure 1. *Small fragment of the soil were Cladonia rappii lichen grow. Among the elements composing this soil are vegetal fragments (asterisk) and numerous isolated hyphae (arrows). The small circles indicate the presence of associated mineral elements. Bar=2.5µm.*

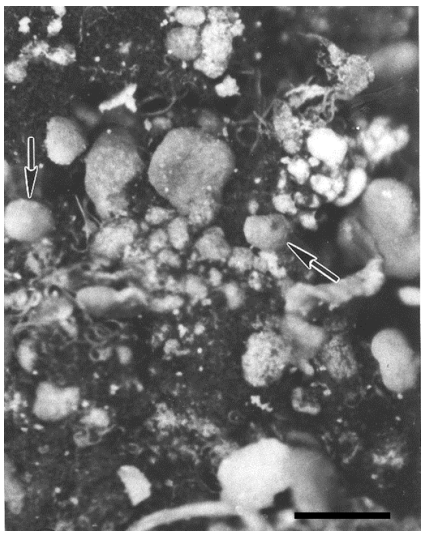

Figure 2. *In a second stage over the surface of the soil spheric or irregular small propagules (arrows) can be clearly observable, and begin to appear the horizontal thallus. Bar= 2.5 µm.*

The presence of few and small thallus and propagules, characterize the third stage that is necessary for the final establishment of the symbiotic interrelationship (Fig. 3).

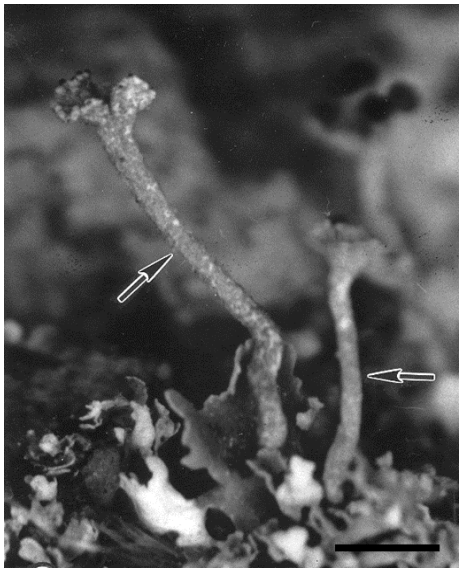

Figure 3. *During the thirst stage, from the propagule single shaft (arrows) of lichen Cladonia rappii began to be seen. Bar=8.0 µm.*

Finally, the fourth stage is when the *Cladonia rappii* reaches full development characterized by the presence of well-developed thallus and with multiple branches formed by tubular segments and germinal discoidal segments (Fig. 4).

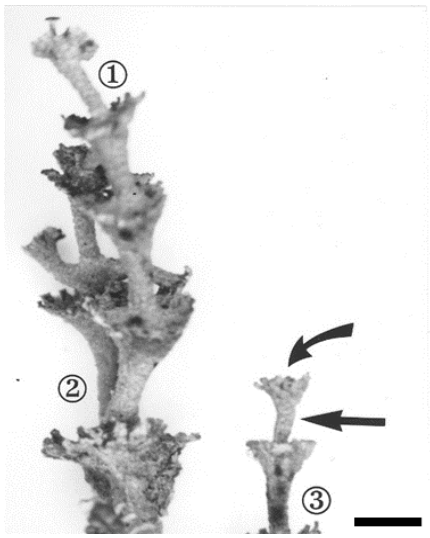

Figure 4. *When Cladonia rappii reach full development from the same original shaft give origin to branches (1, 2, and 3) subdivided in tubules (straight arrow) and discoidal segments (curved arrow). Bar=4.0 µm.*

3.2. Ultramicroscopical Analysis

The interface between the cell wall of the symbiont cells and soil minerals in the case of the hyphae of *Cladonia rappii,* can be determined by the presence of binding glycoproteins (Fig. 1).

In hyphae cells revealed the presence of electron dense crystals immersed in a complex external matrix consisting of organic polymer type substance and which may correspond to non-lichen polysaccharides; while in cell wall small amounts of another compound type lectin are observed (Fig. 5). It is considered that the glycoproteins produced by the hyphae are capable of binding to soil crystals during the forming process the lichen to be used as support material, while glycoproteins are nutrient source (10).

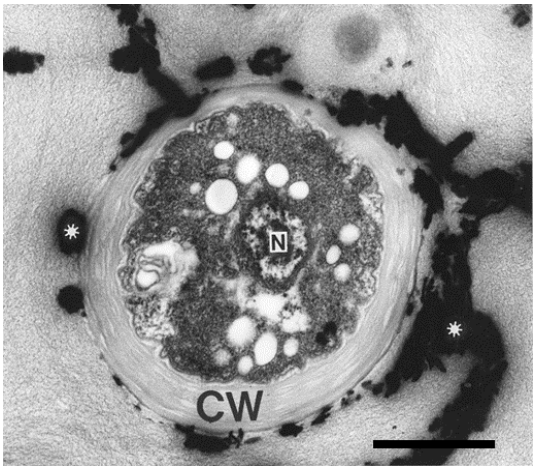

Figure 5. *Initial stages when mineral element (asterisks) are still associated to the aggregating hiphae. N, nucleus; CW, cell wall. Bar= 0.6 µm.*

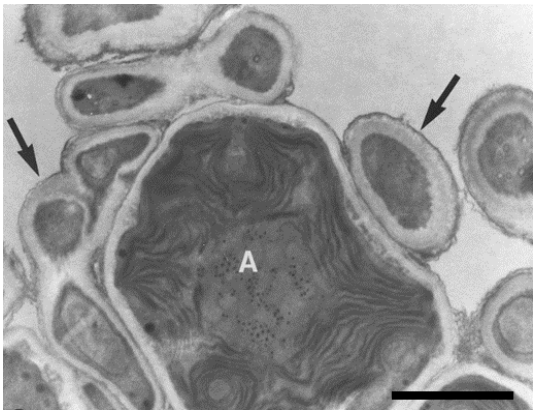

Figure 6. *From the propagules to the adult stage of the Cladonia rappii the algae began to participate in the symbiosis, and the mineral component seen during the early stage disappears to allow the association between hyphae (arrows) and the green algae (A). Bar=0.35 µm.*

Lichen hyphae are seen colonizing and penetrating soil surfaces. Mineral microcrystals accumulated commonly around the thallus are seen coated by extracellular mucopolysaccharides associated with fungi, and with endolithic algae. In these zones of intimate contact between hyphaes and minerals, the presence of extracellular biological substances and compounds is a selective and complex media to start the process of lichenization.

Specialized microenvironments initially produce microcrystals from constituents derived from soil mineral surfaces. Therefore, depending on the presence or not of glycoproteins having an agglutinating function could be expected to originate the beginning of the matrix to give rise the association of the mycobiont with the photobiont (Fig. 6).

4. Discussion

In the formation of the symbiotic structure originating lichen is undisputable paper played by the substances of chemical nature produced by the biological elements, whether fungi, algae, or both structures simultaneously (16-20). In forming lichens mucopolysaccharides play a major role while meeting three basic functions: provide the nutrients necessary for the survival of biological components of lichen, binder pickup serve structure and soil microcrystals, and in conjunction with other soil elements providing physical support for the development and growth of lichen.

In the species analyzed was conclusively determine that the photobionts are not identified at early stage, but in the areas having less free hyphal and you propagules observed already photobionts or green algae, as has been reported by other researchers (21-24).

Moreover, it has been referred to the more soluble polysaccharides in water, such as isolicheninae and licheninal (25) have been detected in the lichen that was reliable to exhibit by transmission electron microscopy to cell wall of the symbiont cells/mineral interaction. This fact may suggest that the presence of lichenin can be controlling this interaction. Moreover, it is evident that the association of algae to the fungus complex / mineral is delayed, since at the initial stages algae cells were not seen.

The way cellular organisms recognize each other and interact to form lasting associations, is one of the most important questions for the understanding of intercellular communication in the lichens formation; and is also necessary to determine how the interrelationship of these biological structures with soil inorganic elements is established, which are essential for the origin and growth lichen.

Lichens are a symbiotic exceptional in nature for its ability to confront the most extreme and harsh environmental conditions, and they have deserved reputation as pioneer plants in the vegetable succession. Are precursors in terrestrial habitats from the Arctic and Antarctic to tropical areas; its adaptation to xeric environment allows them to dominate in most habitats where competition with other plants is negligible (26).

Lichens play a very important role in the process of photosynthesis and containing cyanobacteria (blue-green algae) are nitrogen fixers; and they constitute bioindicators of degrees of environmental pollution, climate change and nitrogen fixers and producers.

In this partnership fungi, called mycobionts, generally are responsible for shaping the talin structure or vegetative body of moss and algae or photobionts, photosynthetic constituents. The union of the two symbionts is done through a complex process of lichenification, which performed in the following stages: (a) pre-contact stage, stimulation by the alga, and tigmotrophic response of the fungus; (b) contact stage, recognition and binding; (c) alga sheath by mycobionte stage, haustoria development; (d) integration stage, formation of a common matrix by both symbionts; and (e) differentiation stage, final thallus formation (27).

The thalli are fixed to the substrate by special structures, and they can also take in the ciliary margin or fibrils; there thalli adhering through its entire surface others do it just for the central region.

Through this attachment to the substrate, lichens acquire a high proportion substances and chemical elements for initial development and permanence in their adult stage, because the basic elements to metabolize their biochemical products are extracted mainly from the soil; hence the importance of lichen/substrate interaction, which has allowed lichens colonize extreme areas where neither algae or fungi individually had survived.

Lichens are organisms of great importance as pioneers in the colonization of bare soils and rocks exposed, that meet ecological functions very particular within ecosystems, such as soil formation. Probably, lichens represent the group of organisms of greater wealth and chemical diversity and the substances they produce are of potential use in various branches of medicine and environmental sciences.

The substrate is a discriminating factor in the lichen colonization, because it can be influenced by means of their physical and chemical features. This is how the texture or physical nature of the soil is a limiting factor for the genesis of lichens, being a selection factor for a species or another is generated, depending on whether the soil is sandy, clay, hard, mobile or stable, to allow a better Exchange between the symbiont cells and soil particulates elements (28-31).

5. Conclusions

In this study, we sought to know the different phases of the interaction fungi/algae/soil particles to start the symbiotic association known as lichens. The role that chemicals substances produced by biological structures have in the interaction of these structures soil with microcrystals produced from a process not biogenic weathering is shown. The presence of the binding protein allows both lichens adhesion between cells (fungi and algae), also uptake crystals in order to participate in the thallus formation.

Acknowledgements

This work was supported by grants M-689-00-03-A from the Council for Scientific, Humanistic, Technological and Art (CDCHTA), University of Los Andes, Mérida, Venezuela. The authors wish to thank for the skillful technical assistance to Francisco Durán-Montilla, Emilitza Labarca-Villasmil, and José Ramírez for his photographic assistance.

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