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Ultrastructural Deterioration of *Xanthoria parietina* (L.) Th. Fr. Phycobiont Induced by a *Xanthoria* Lectin

By

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With 5 Figures

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Summary

Molina M. C. & Vicente C. 1996. Ultrastructural deterioration of *Xanthoria parietina* (L.) Th. Fr. phycobiont induced by a *Xanthoria* lectin. – Phyton (Horn, Austria) 36 (2): 197–208, with 5 figures. – English with German summary.

A full description of ultrastructural modifications of the lichen *Xanthoria parietina* produced by a lectin (or algal-binding protein) isolated from the same lichen species is given. When algal-binding protein enters algal cells lacking its cell wall receptor, phycobionts are completely disorganized except in their cell walls. New structures very similar to large vacuoles are also found as well as a large bacterial development. The occurrence of a specific receptor for this protein in the cell wall reduces these changes to a reorientation of pyrenoglobuli in an ellipsoid or moon-like shape.

Zusammenfassung

Molina M. C. & Vicente C. 1996. Störungen in der Ultrastruktur des Phycobionten von *Xanthoria parietina* (L.) Th. Fr. durch ein *Xanthoria* Lektin. – Phyton (Horn, Austria) 36 (2): 197–208, 5 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Es wurden hier ultrastrukturelle Veränderungen der Flechte *Xanthoria parietina*, welche durch ein Lektin (oder algenbindendes Protein) hervorgerufen wurden, beschrieben; das Lektin wurde von derselben Flechtenart isoliert. Sobald das algenbindende Protein in die Algenzellen, welchen der Zellwandrezeptor fehlt, gelangt, erscheinen die Phycobionten bis auf ihre Zellwand total verändert. Neue

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Strukturen, großen Vakuolen ähnlich, können ebenso gefunden werden, wie eine starke bakterielle Entwicklung. Das Auftreten eines spezifischen Rezeptors für dieses Protein in der Zellwand reduziert diese Veränderungen auf eine Neuanordnung von Pyrenoglobuli in eine elliptische oder mondförmige Form.

Introduction

Lectins from lichens, showing phytohaemagglutinin activity, have been described from the fifties (ESTOLA & VARTIA 1956). However, BUBRICK & al. 1981 adjudicate for the first time the category of "recognition-type protein" to one of these proteins, isolated from the lichen *Xanthoria parietina*. This protein seems to be produced by the fungal symbiont and it is located at the fungal cell surface (BUBRICK & GALUN 1980, BUBRICK & al. 1981). This protein can be involved in the recognition mechanism of compatible phycobionts (GALUN & BUBRICK 1984). Moreover, this protein develops arginase activity (MOLINA & al. 1993), related to a fungal attenuated parasitism (MOLINA & VICENTE 1995 a). In a similar way, a lot of lectins for *Rhizobium*-legume symbiosis have been described as enzymes (SHANNON & HANKINS 1981).

On the other hand, structural modifications of a lichen thallus or its algal biont, effected by extreme conditions (GALUN & al. 1970) or environmental variations (ASCASO & GALVAN 1976, HOLOPAINEN 1982) have often been described, although the knowledge about ultrastructural or physiological changes in the phycobiont induced by a protein synthesized by the mycobiont is lacking.

In this paper, the algal binding protein described by BUBRICK & al. 1985 has been isolated and used to study some ultrastructural modifications of *X. parietina* thalli produced by this enzyme. Results have been examined in relation to the putative, parasitic role of this enzyme (or some secreted isoform) and to its implication in defense-attack mechanism between symbionts.

Abbreviations. – DTT: dithiothreitol; PBS: phosphate saline buffer.

Material and Methods

Plant material

Xanthoria parietina (L.) Th. Fr., growing on *Robinia pseudoacacia* L. was collected in Pedraza (Segovia, Spain). Thalli were air-dried and stored at 4 °C in the dark, no longer than two weeks.

Partial purification of *Xanthoria* ABP

ABP (Algal-Binding-Protein) was partially purified according to BUBRICK & al. 1985. Air-dried thalli (5.0 g dry weight) were disrupted by hand in a mortar with 50 ml PBS buffer (1.5 mM KH_2PO_4, 3.0 mM Na_2HPO_4, 2.7 mM KCl, 0.4 mM $MgSO_4$ and 1.34 mM NaCl), pH 7.4, containing 5.0 mM phenyl methylsulphonyl fluoride, 2.32 mM DTT and 15.0 sodium azide. Homogenate was stored overnight at 4 °C and then

centrifuged at 30,000 × g at 2 °C for 45 min. The pellet was re-extracted with PBS for 2h at 4 °C, centrifuged and the two supernatants were combined. The solution was brought out to 40% saturation with ammonium sulfate, stored for 2h at 4 °C and then centrifuged at 30,000 × g for 45 min at 2 °C. Supernatant was brought up to 60% saturation with ammonium sulfate and newly centrifuged. The supernatant was discarded and the pellet was dissolved in 4.0 ml PBS and dialyzed overnight against 5.0 l distilled water. After dialysis, the content of bags was microfuged and the pellet was dissolved in PBS. When indicated, this partially purified extracts was filtered through a column of Sephadex G-150 (32 cm × 2.5 cm) equilibrated with PBS buffer. The void volume was estimated as about 45 ml by filtering through the column 5.0 ml 0.5% (p/v) Dextran blue 2000. Protein was eluted in fractions of 5.0 ml with PBS buffer and monitored by their absorbance at 280 nm.

Bacterial analysis

X. parietina samples (1.0 g dry weight) were superficially washed with distilled water and aliquots of this wash water were cultured on agar plates for 24h at 37 °C. After obtaining the first colonies, bacteria were assayed by using PanthoTec-U test.

Transmission electron microscopy

Samples of 1.0 g of *X. parietina* thalli were floated on 10 ml 100 mM urea in PBS buffer (pH 7.4) for 2h at 26 °C in the dark to induce urease. When indicated, samples were previously loaded with 28 mM arginine in 10 ml PBS buffer (pH 7.4) for 30 min. After washing two times with distilled water, samples were floated on 5.0 ml of ABP solution (74 μg ml^{-1}) for 18h at 25 °C. Finally, untreated samples were floated on PBS buffer for time periods identical to those used for the incubation with arginine, urea and ABP. Then, samples were dehydrated and fixed as described by Ascaso & Galvan 1976. Small pieces of thallus, from 2–3 mm behind the lobe edge, were fixed at 4 °C for 3h with 3% (v/v) glutaraldehyde in 50 mM phosphate buffer (pH 7.1). Samples were washed overnight with phosphate buffer and then post-fixed with 1% (w/v) osmium tetroxide in 50 mM phosphate buffer (pH 7.1), at room temperature in the dark for 3h. Material was dehydrated in graded ethanol solutions, embedded in Spurr's resin (Spurr 1969) and sections stained with lead citrate (Reynolds 1963). Sections were examined in a Philips EM 300 electron microscope.

Results

The ultrastructure of *X. parietina* thalli, recently collected and untreated, is shown in Fig. 1. The chloroplast of the trebouxoid phycobiont, *Trebouxia* and *Pseudotrebouxia*, according to Ahmadjian 1982, showed a central pyrenoid with abundant well-defined pyrenoglobuli (lipid-containing droplets). Thylakoids were associated in groups within the chloroplast (Fig. 1.1). The tonoplast became evident. Big vesicles forming a complex were located in the surrounding zone of plasmalemma, close to some storage bodies very dense to electrons (Fig. 1.2). The ultrastructure of the mycobiont seemed to be very complex, including several nuclei and

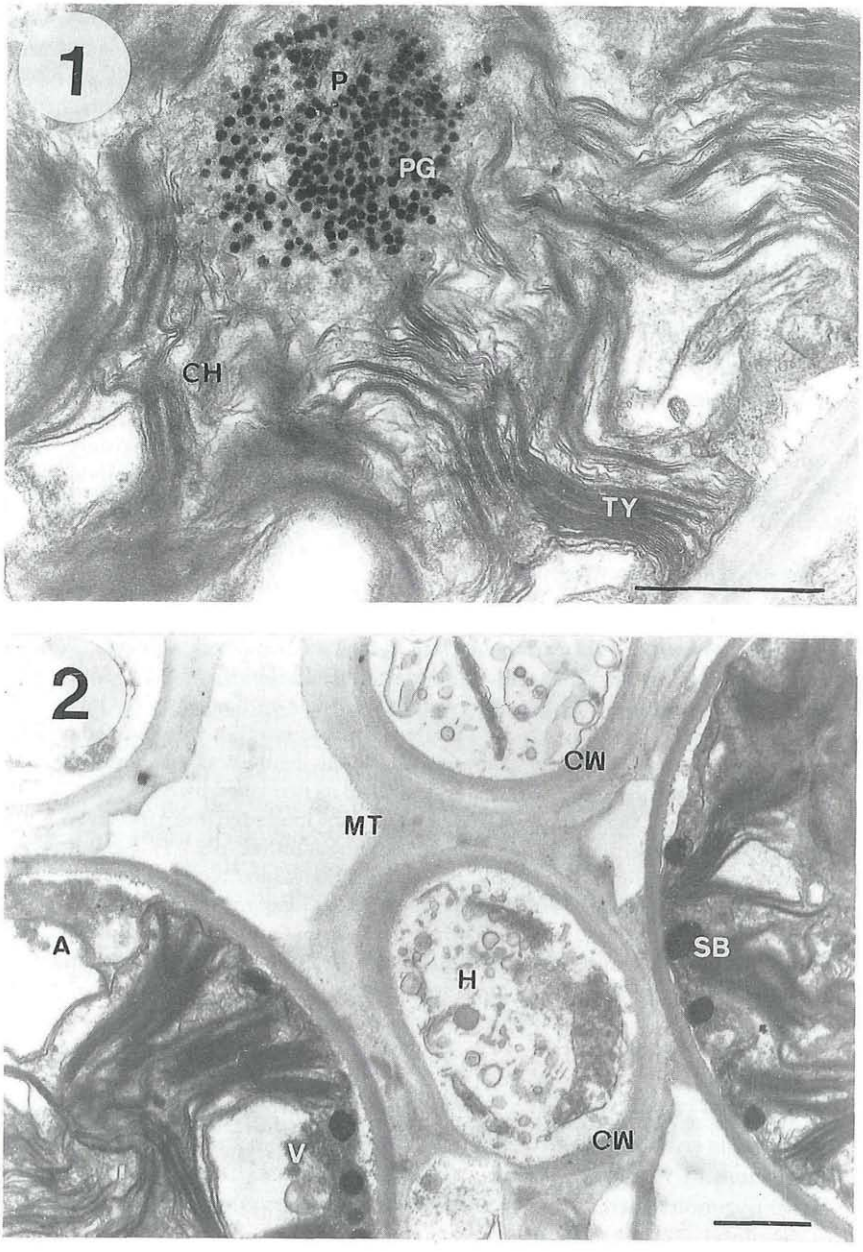

Fig. 1. Transmission electron micrographs of *X. parietina* thalli recently collected and untreated. 1) Phycobiont cell, 2) alga-fungus contact zone. Bar = 1.0 µm. A = Algal cell; CH = Chloroplast; CW = cell wall; H = hypha; MT = extracellular matrix; P = pyrenoid; PG = pyrenoglobuli; SB = storage bodies; V = vacuole; TY = thylakoid.

membrane organelles. The mycobiont-phycobiont interface showed an extracellular matrix and no haustorial prolongations were found (Fig. 1.2).

Incubation of lichen thalli with 28 mM arginine in PBS buffer for 30 min produced strong structural changes. Both bionts were collapsed and the contact areas between symbionts became irregular (Fig. 2.1). Satellite pyrenoids (Fig. 2.2) were observed, appearing some pyrenoglobulli without a clear perimeter. Samples incubated with 100 mM urea for 2h did not suffer significant modifications at a ultrastructural level, neither in the cell shape (Figs. 3.1 and 3.3) nor at the contact zone (Fig. 3.1). However, pyrenoid acquired an ellipsoid form (Fig. 3.2).

Symbiotic equilibrium was apparently maintained and hyphae did not show changes with respect to the control samples when thalli recently collected were loaded with arginine for 30 min, incubated with 100 mM urea for 2h and then floated on a solution of ABP for 18h (Fig. 4.1). Pyrenoid was centrally placed within the chloroplast but pyrenoglobulli were oriented in an ellipsoid or moon-like shape (Figs. 4.2 and 4.3), as well as the thylakoids (Fig. 4.2). However, relationship between both bionts was broken when thalli was loaded with arginine and then incubated with ABP solution. Phycobiont cells were completely disorganized excluding their cell walls that remained surrounding rests of membranes as well as some cell wall structures (Figs. 5.2 and 5.3). New structures, not yet described for lichen symbionts, very similar to large vacuoles usually were found (Fig. 5.1 and 5.3), as well as many different bacteria (Fig. 5.1). Previous studies about the natural bacterial population living on untreated thalli revealed that they are generally pseudomonad-like microorganisms. This was confirmed in this study by using standard PanthoTec-U test for *Pseudomonas* genus.

Discussion

Partially purified ABP from *X. parietina* behaves as a glycosylated protein developing arginase activity (Molina & al. 1993). When this protein enters algal cells, chlorophyll concentration decreased (Molina & Vicente 1993). ABP seems to be able to break the equilibrium between *X. parietina* symbionts, as observed in Fig. 5. As a consequence, bacterial contamination seems to be promoted. However, ABP does not enter the algal cells and symbiosis is preserved when glycosylated urease, described as a receptor for ABP (Molina & al. 1993, 1994) and induced after incubation of lichen thalli on 100 mM urea, occurs in the algal cell wall (Fig. 4). Nevertheless, dramatic pyrenoidal modifications are observed, probably produced by the high concentration of urea in the incubation media (Figs. 3 and 4).

Moreover, thalli loaded with arginine contain three times more amino acid (Molina & Vicente 1993) and, consequently, a loss of the ultrastructural integrity is observed for the phycobiont cell (Fig. 2) with respect

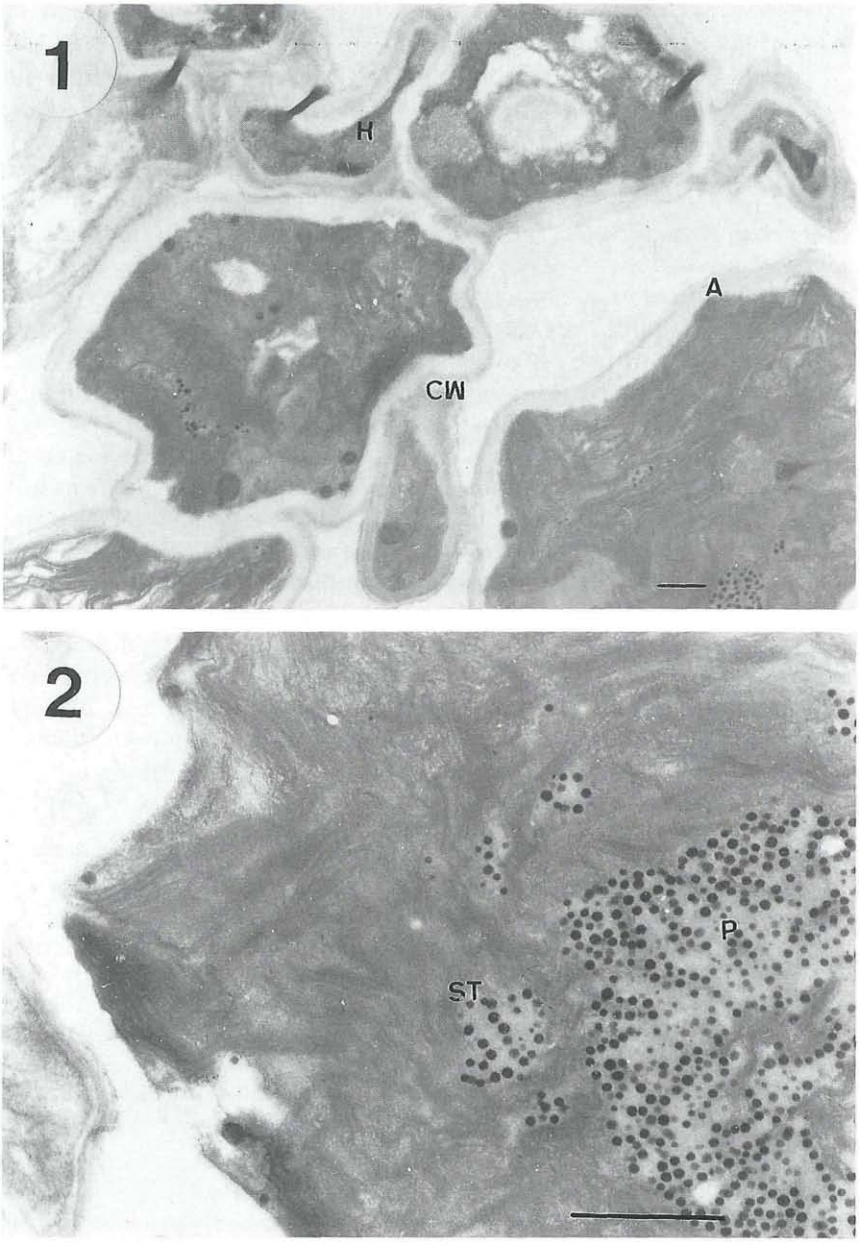

Fig. 2. Transmission electron micrographs of *X. parietina* thalli recently collected and floated on 28 mM arginine for 30 min at 26 °C in the dark. 1) Alga-fungus contact zone, 2) phycobiont cell. Bar = 1.0 μm. A = algal cell; CW = cell wall; H = hypha; P = pyrenoid; ST = satellite pyrenoid.

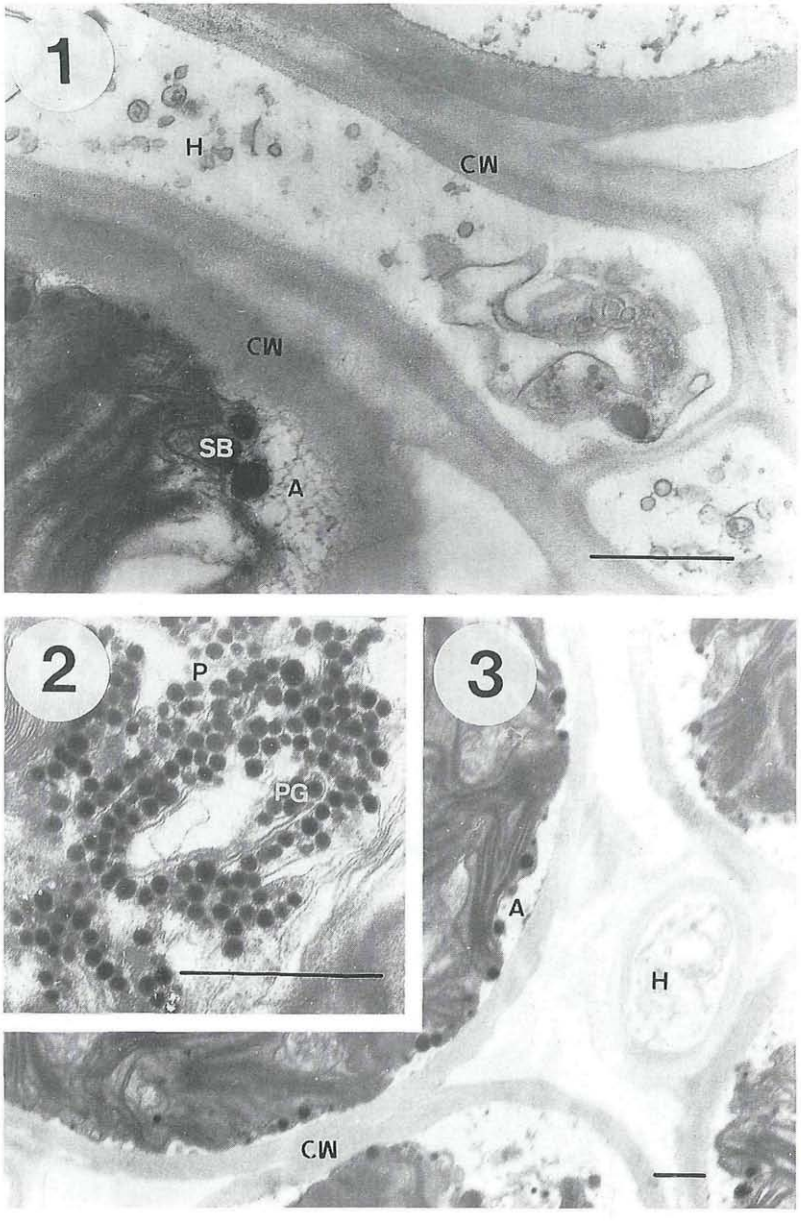

Fig. 3. Transmission electron micrographs of *X. parietina* thalli recently collected and floated on 100 mM urea for 2h at 26 °C in the dark. 1) Alga-fungus contact zone, 2) pyrenoid, 3) an overview to show the normal shape of both algal and fungal cells. Bar = 1.0 μm. A = algal cell; CW = cell wall; H = hypha; P = pyrenoid; PG = pyrenoglobuli; SB = storage bodies.

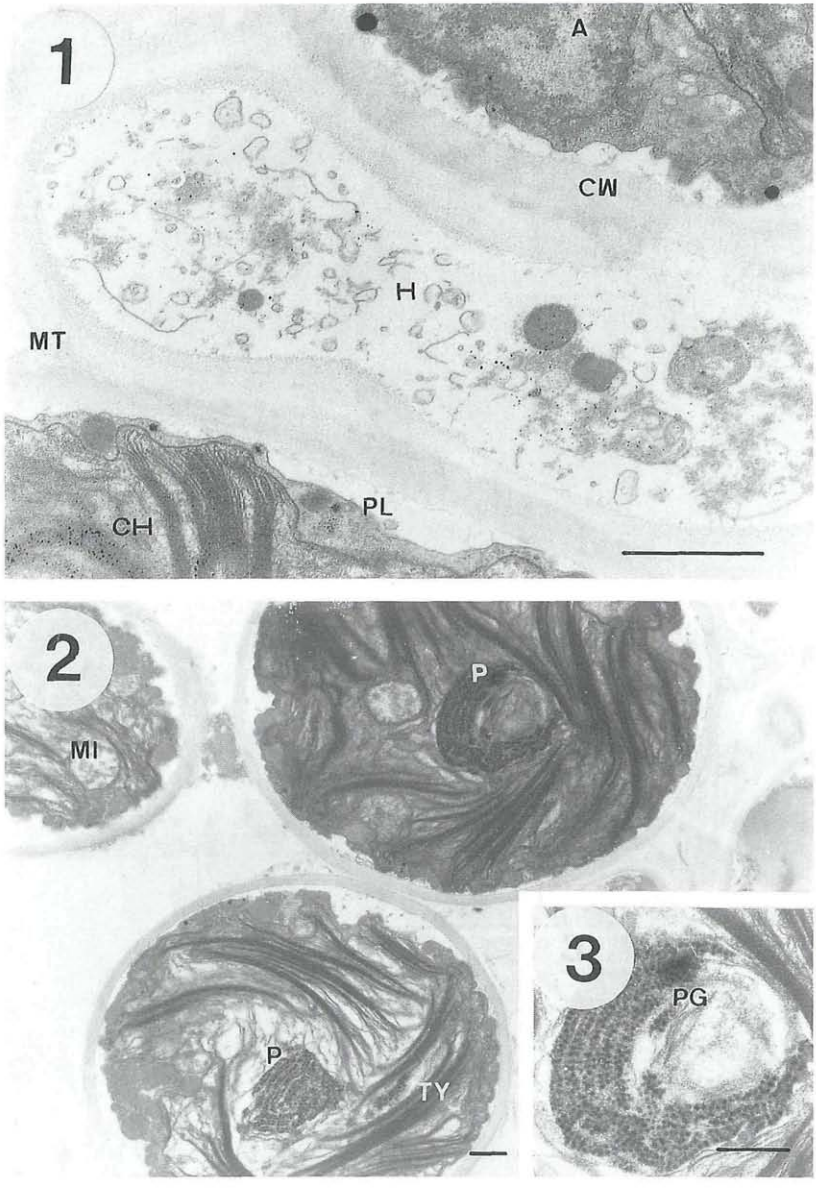

Fig. 4. Transmission electron micrographs of *X. parietina* thalli recently collected and loaded with 28 mM arginine for 30 min, later incubated on 100 mM urea for 2h and then floated on 5.0 ml of an ABP solution containing 74 mg protein per ml, for 18h at 25 °C in the dark. 1) Alga-fungus contact zone, 2) cell overview, 3) pyrenoid. Bar = 1.0 μm. A = Algal cell; CH = Chloroplast; H = hypha; MI = mitochondria; MT = extracellular matrix; P = pyrenoid; PG = pyrenoglobuli; PL = plasmalemma; TY = thylakoid.

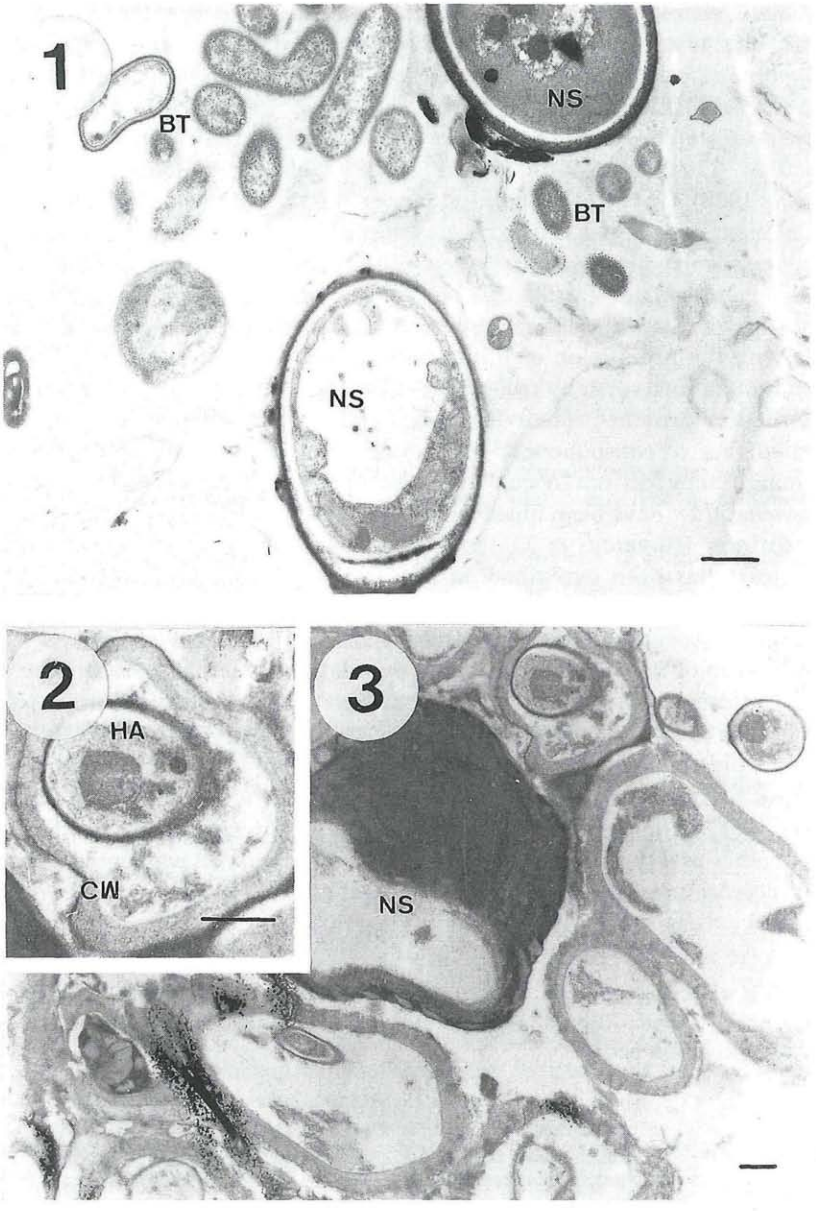

Fig. 5. Transmission electron micrographs of *X. parietina* thalli recently collected loaded with 28 mM arginine for 30 min, and later incubated on 5.0 ml of ABP solution at 25 °C in the dark. 1) Bacterial infection, 2) haustorial prolongation, 3) cell overview. Bar = 1.0 μm. BT = bacteria; CW = cell wall; HA = haustorial prolongation; NS = new vacuole-like structures.

to the control sample (Fig. 1). The increase of concentration of the intracellular arginine and the parallel uptake of glycosylated arginase by phycobiont cells could push putrescine synthesis by hydrolysis of the amino acid (LEGAZ 1991, VICENTE & LEGAZ 1985). Putrescine clearly accelerates chlorophyll degradation and induces lethality in some cyanobacteria (GUARINO & COHEN 1979) and lichen phycobionts (VICENTE & LEGAZ 1983). On the other hand, it is possible that increased concentration of putrescine in algal cells, or even the lectin itself, activated hydrolytic enzyme involved in the degradation of both plasmalemma and cell wall. In this way, MOLINA & VICENTE 1995 b reported an increase of algal glucanase activity when arginase isolectins were incorporated into the phycobiont, allowing the production of protoplasts. Moreover, invertase from *Ricinus communis* is activated by several plant lectins (VATTUONE & al. 1991). So, an interaction protein-protein in the glucanase activation cannot be discarded. As a consequence, haustorial prolongations appear (Fig. 5), although they do not occur in *X. parietina* under natural conditions. However, they have been observed in thalli resynthesized under laboratory conditions (BUBRICK & GALUN 1986). Development of intracellular haustoria has been explained on the basis of the parasitic behaviour of the mycobiont (HONEGGER 1991). The occurrence of molecules with phycocide action, produced by the mycobiont, could be considered as a mechanism of regulation of the algal population. The biological activity of these molecules, in which lectins could be included, would explain the difference of size and shape of photobionts from cyano- (BOISSIERE 1982) as well as phycolichens (HILL 1992) in axenic culture and in symbiosis.

As a conclusion, ABP could be involved in an "ancestral parasitic behaviour" of lichen symbiosis (AHMADJIAN 1987) in such a manner that the occurrence of a specific receptor for ABP in the algal cell wall could be seen as a defense mechanism against the fungal attack.

Acknowledgements

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