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Short communication

Adhesion of photobiont cells to mycobiont hyphae of the lichen, *Xanthoria parietina*

PAUL BUBRICK

Department of Plant Pathology, University of California
Riverside, CA 92521, USA
Tel. (714) 787-4120

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Abstract

Adhesion of the *Xanthoria parietina* photobiont to cultured mycobiont hyphae is illustrated using an *in vitro* adhesion assay. A previously characterized, lichen produced algal-binding protein (ABP), or antibody against ABP, blocked adhesion. In addition, a soluble protein extracted from the cultured *X. parietina* mycobiont also inhibited adhesion.

Keywords: *Xanthoria parietina*, photobiont, adhesion, algal-binding protein

Abbreviations: SD = standard deviation; n = number of fungal colonies scored in each experiment (exp.); PBS = phosphate buffered saline; BSA = bovine serum albumin; ABP = algal-binding protein; MYC = soluble protein extract from the *in vitro* cultured *X. parietina* mycobiont

1. Introduction

Lichens are a biotrophic association between algae (photobionts) and fungi (mycobionts). The range of possible combinations between symbionts is limited; a mycobiont can form a fully developed lichen with only certain types of photobionts. Such a selective (Bubrick et al., 1985a; Galun and Bubrick, 1984) interaction between cell types suggests that mycobionts possess mechanisms for the discrimination between potential photobionts. These mechanisms probably function throughout the developmental cycle of lichens, from the early stages of a algal-fungal contact through the later stages of metabolic integration.

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Attempts to study symbiont selectivity at various stages of lichen development have been undertaken in several laboratories (reviewed in Bubrick, 1988; Galun and Bubrick, 1984; Jahns, 1988). In cases of the incorporation of cephalodial bionts into established thalli, selective interactions appear to take place during contact between symbionts (e.g. Brodo and Richardson, 1978; Jahns, 1972, 1973; Renner and Galloway, 1982). For example, mycobionts can discriminate between epiphytic cyanobacteria, or cyanobacteria normally found in cephalodia. Hyphae respond to normal cyanobionts with a proliferation of hyphae which envelop the cyanobiont and eventually incorporate it into the thallus (Jahns, 1973). On the other hand, some lichen mycobionts have the remarkable ability to temporarily associate with a non-preferred photobiont, and then to exchange this photobiont with a more preferred one when it is detected (Friedl, 1987; Ott, 1987a,b). Such a range of developmental behaviors emphasizes the selective nature of lichen mycobionts and places less emphasis on single molecular recognition events as determinants of specificity or morphogenesis.

We have been studying the contribution of the contact phase to selectivity in *Xanthoria parietina*. We have isolated and partially characterized a fungal-produced, cell-wall associated algal-binding protein (ABP) which can bind to cell walls of the *X. parietina* photobionts as well as to several photobionts from closely related lichens (Bubrick, 1984; Bubrick et al., 1985a,b). Here, a cell adhesion assay was developed to further study the role of ABP in the adhesion of algal cells to mycobiont hyphae.

2. Materials and Methods

Photobionts were isolated and cultured as previously described (Bubrick and Galun, 1980). Polyspore cultures of the *X. parietina* mycobiont were isolated and cultured on agarized (1.5%) tap water.

An adhesion assay was developed using 3–5 week old cultures of the mycobiont. Segments (0.5 ca) from 1 ml glass disposable pipettes (approximately 0.4 cm in diameter) were flame sterilized and placed over fungal colonies. The glass tube was then pressed into the agar forming an isolated "well". Each well contained 5–20 fungal colonies. Algae (10^5 in 10–20 μl) were pipetted into the well and incubated 2–3 hr at room temperature. During this time, algae settled to the bottom of the well, but the liquid inside the well was retained. The glass segments with agar wells were then removed, and agar plugs transfered to depression slides containing PBS (KH_2PO_4, 1.5 mM; Na_2HPO_4, 3.0 mM; KCl, 2.7 mM; $MgSO_4$, 0.4 mM; NaCl, 137.9 mM; pH 7.4).

Plugs were washed by manual agitation until algae adhering to the agar surface devoid of fungi were largely removed (Fig. 1). Adherent cells were then counted under low magnification in a microscope.

Algae or fungi were also pretreated with various reagents prior to assay. Algal cells were preincubated with PBS, bovine serum albumin (BSA, 1% w/v) (Sigma), an unrelated hyperimmune serum against a free-living cyanobacterium, or isolated and purified ABP (2 μg/μl) for 30 min at room temperature, followed by washing (3×) in PBS.

Photobionts were also pretreated with a protein extract from the cultured *X. parietina* mycobiont. Fungal colonies on agar were removed, excess agar trimmed and the hyphae ground in a chilled mortar in PBS containing dithiothreitol (3.23 mM) (Sigma), phenylmethylsulphonyl fluoride (5 mM) (Sigma) and sodium azide (15 mM); protein was extracted into 40 ml for 18 hr at 4°C. The extract was centrifuged (30,000×g, 45 min) and the supernatant dialyzed against distilled water (2 changes, 4 liters total) overnight at 4°C. The extract was lyophylized, redissolved in PBS, insoluble material removed by centrifugation, and soluble protein stored in small aliquots at –20°C. Protein concentration was estimated with fluorescamine (Roche) (Castell et al., 1979) using BSA as a standard. Approximately 300 μg of protein was recovered from approximately 0.4 g fresh weight hyphae plus agar. This extract was used to pretreat cells at 2 μg/μl total protein as described above.

Fungal hyphae in wells were pretreated with antiserum to ABP (Bubrick, 1984). Antiserum was diluted in PBS (1/400) and added to wells for 30 min at room temperature followed by washing (4 × 10 min) in PBS. An unrelated hyperimmune serum directed against a free-living cyanobacterium was used as a negative control.

3. Results

The photobiont of *X. parietina* adhered to hyphal cell walls of the ***in vitro*** cultured mycobiont (Fig. 1). This adherence was strong enough to withstand washing until most algae were removed from the surrounding agar surface. Under similar conditions, photobionts from *Caloplaca aurantia, Ramalina duriaei* and *R. pollinaria* did not adhere to mycobiont hyphae. These 4 photobionts are taxonomically related, and have roughly the same cell shape and diameter.

Cells of the *X. parietina* photobiont were pretreated with various reagents and then tested for adherence to fungal hyphae (Table 1). Preincubation with BSA or an unrelated hyperimmune serum did not affect adherence.

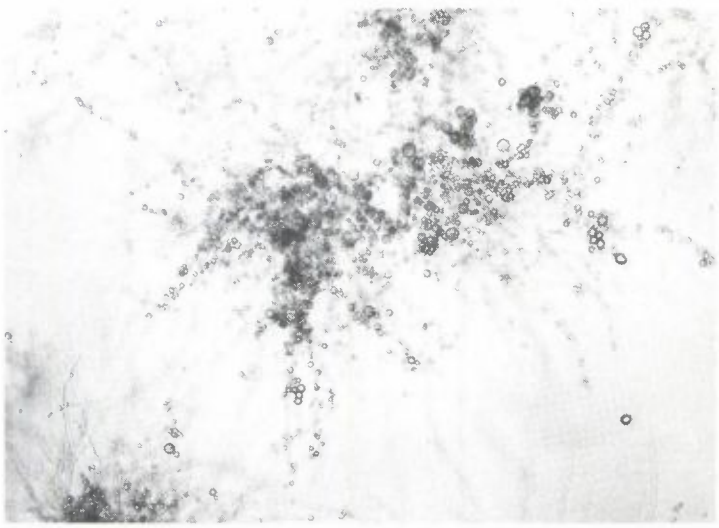

Figure 1. Adhesion of untreated *X. parietina* photobiont cells (spheres) to cultured mycobiont hyphae (threads). A low degree of "background" binding of algae to agar is observed (×100).

Table 1. Adhesion of photobiont cells to fungal hyphae

	Trial number (cells + S.D.; n)[1]		
Cells pretreated with	1	2	3
PBS	24 ± 5; 14	21±4; 10	23±5; 10
BSA	28 ± 4; 10	26±3; 7	25±6; 9
ABP	4 ± 2; 16	10±3; 10	10±4; 7
MYC	11 ± 4; 13	9±4; 13	8±3; 12
Hyphae pretreated with anti-ABP	7 ± 2; 13	9±4; 8	8±3; 15

[1] Algal cells bound to fungal hyphae ± standard deviation: Number of fungal colonies screened.

However, preincubation of algae with purified ABP reduced the numbers of adhering cells by at least half (Table 1). When preincubated with a soluble protein extract from the cultured *X. parietina* mycobiont, algal cell adherence was also dramatically reduced (Table 1) suggesting that a component similar to ABP may be produced by the cultured mycobiont.

The cultured mycobiont was pretreated with antiserum to purified ABP or with an unrelated hyperimmune serum and then tested in the assay (Table 1). Anti-ABP greatly reduced the numbers of adhering cells clearly suggesting that ABP or an immunologically related molecule plays a role in the observed adhesion to fungal hyphae.

4. Discussion

The described assay demonstrates that the cultured *X. parietina* mycobiont hyphae can bind suitable algal cells. Of the 4 tested photobionts, only the normal *X. parietina* photobiont adhered to the hyphae. The adherence was blocked by pretreatment of cells with purified ABP, or pretreatment of hyphae with antiserum to ABP. In addition, a component(s) of soluble protein extracts from the cultured mycobiont blocked algal cell adhesion to hyphae. It is not known whether this component and ABP are identical although they appear to be immunologically cross-reactive (Bubrick, 1984). These data suggest that ABP or a related molecule may be involved in the binding and immobilization of potential photobionts onto mycobiont hyphae *in vitro*. The role of this molecule in nature has yet to be examined.

Xanthoria parietina appears to utilize a number of reproductive strategies in nature, ranging from resynthesis of separated bionts to aggressive colonization of other lichen thalli. Ott (1987a) demonstrated that the *X. parietina* mycobiont can invade nearby *Physcia* thalli and appropriate algae. Bubrick (1984) observed that in pure stands of *X. parietina*, mycobiont hyphae passed through colonies of free-living algae without response, but enveloped "trebouxoid" algae. Young primordia of less than 20 algal cells were often detected which already possessed lichen acids characteristic of thalli. As suggested by Jahns (1988) it appears that selective interactions as well as the type of interaction during early stages of lichen development are in part dependent upon environmental conditions. This plasticity of behavior can be viewed as a further advantage to lichens such as *X. parietina* which primarily uses spores as a means of dispersal and colonization of new habitats.

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REFERENCES

Brodo, I.M. and Richardson, D.H.S. 1978. Chimeroid associations in the genus *Peltigera*. *Lichenologist* **10:** 157–170.

Bubrick, P. 1984. Cell wall properties, cell adhesion molecules, and resynthesis in the lichen, *Xanthoria parietina* (L.) Th.Fr. Ph.D. thesis, Tel Aviv University, Tel Aviv, Israel.

Bubrick, P. 1988. Biont selectivity in lichens. In: *Recent Topics in Experimental and Conceptual Plant Pathology.* R.S. Singh and U.S. Singh, eds. IBH Publ. Co., Oxford, New Delhi (in press).

Bubrick, P. and Galun, M. 1980. Symbiosis in lichens: differences in cell wall properties of freshly isolated and cultured phycobionts. *FEMS Microbiol. Lett.* **7:** 311–313.

Bubrick, P., Frensdorff, A., and Galun, M. 1985a. Selectivity in the lichen symbiosis. In: *Lichen Physiology and Cell Biology.* D.H. Brown, ed. Plenum Publ. Corp., New York, pp. 319–334.

Bubrick, P., Frensdorff, A., and Galun, M. 1985b. Proteins from the lichen *Xanthoria parietina* (L.) Th.Fr. which bind to phycobiont cell walls. Isolation and partial purification of an algal-binding protein. *Symbiosis* **1:** 85–95.

Castell, J.V., Cervera, M., and Marco, R. 1979. A convenient micro-method for the assay of primary amines and proteins with fluorescamine. A re-examination of the conditions of reaction. *Anal. Biochem.* **99:** 379–391.

Friedl, T. 1987. Thallus development and phycobionts of the parasitic lichen *Diploschistes muscorum*. *Lichenologist* **19:** 183–191.

Galun, M. and Bubrick, P. 1984. Physiological interactions between the partners of the lichen symbiosis. In: *Encyclopedia of Plant Physiology*, N.S. Vol. 17. H.-F. Linskens and J. Heslop-Harrison, eds. Springer-Verlag, Berlin, pp. 362–401.

Jahns, H.M. 1972. Die Entwicklung von Flechten-Cephalodien aus *Stigonema*-Algen. *Ber. Deutsch. Bot. Ges.* **85:** 615–622.

Jahns, H.M. 1973. Anatomy, morphology and development. In: *The Lichens.* V. Ahmadjian and M.E. Hale, eds. Academic Press, New York, pp. 3–58.

Jahns, H.M. 1988. The establishment, individuality and growth of lichen thalli. *Bot. J. Linn. Soc.* **96:** 21–29.

Ott, S. 1987a. Sexual reproduction and developmental adaptations in *Xanthoria parietina*. *Nord. J. Bot.* **7:** 219–228.

Ott, S. 1987b. Reproductive strategies in lichens. *Bibl. Lichenol.* **25:** 81–93.

Renner, B. and Galloway, D.J. 1982. Phycosymbiodemes in *Pseudocyphellaria* in New Zealand. *Mycotaxon* **16:** 197–231.