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A Comparison Between Cell Antigens in Different Isolates of *Anabaena azollae*

HANNA ARAD[1], AVI KEYSARI[2], ELISHA TEL-OR[1] and DAVID KOBILER[2]

[1] *Department of Agricultural Botany, Faculty of Agriculture, The Hebrew University of Jerusalem, POB 12, Rehovot 76100, Israel Tel. (08) 481211*

[2] *Israel Institute of Biological Research, Ness-Ziona, Israel Tel. (08)481411*

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Abstract

Both ELISA and quantitative immunobinding assays were employed to examine the antigenic relation among cultured isolates of the phycobiont *Anabaena azollae* from three *Azolla* species: *A. caroliniana*, *A. filiculoides* and *A. pinnata*. The isolates from *Azolla caroliniana* and *Azolla filiculoides* were found to be very similar while the isolate from *Azolla pinnata* was antigenically different.

Keywords: *Azolla*, *Anabaena azollae*, symbiotic cyanobacteria, surface antigens, ELISA

1. Introduction

Anabaena azollae is the phycobiont in six different species of the water fern *Azolla* which vary in their geographical distribution and anatomical-morphological properties (Lumpkin and Plucknett, 1980). The available information is not sufficient to determine the taxonomic relationships between *Anabaena* strains from *Azolla*. Immunoassays should provide appropriate tools to establish the degree of homology of antigenic determinants in these microorganisms, as well as to determine the changes occurring in phycobionts freshly isolated from the host fern and after prolonged cultivation *in vitro*. Using the immunofluorescence technique quantitative differences were recently demonstrated in the antigenic structure between fresh isolates of

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Anabaena azollae and the cultured isolates (Gates et al., 1980; Ladha and Watanabe, 1982).

Surface antigens of *Anabaena azollae* are expected to play an active role in the exchange of metabolites between the host fern and the phycobiont (Peters et al., 1982), as well as in the establishment of cell-cell interactions. It was recently suggested that aspects of the *Anabaena-Azolla* association are mediated by an *Azolla* lectin recognizing the *Anabaena* (Mellor et al., 1981) and by an *Anabaena* lectin recognizing the host *Azolla* (Kobiler et al., 1981). Such interactions clearly suggest the importance of cell surface composition in the development of the symbiosis. This report presents a quantitative antigenic comparison of three different isolates of *Anabaena*, from *Azolla caroliniana* (Newton and Herman, 1979), *Azolla pinnata* and *Azolla filiculoides* (Tel-Or et al., 1983). The freshly isolated *Anabaena* from *Azolla filiculoides* was also compared with its cultured counterpart.

2. Materials and Methods

Organisms and cultures

Fresh *Anabaena azollae* cells (fresh cells) isolated from *Azolla filiculoides* were used without further culturing (Tel-Or et al., 1983). The three isolates of *Anabaena azollae* were isolated and axenically cultured as free-living algae. The isolates of *Anabaena azollae* obtained from *Azolla filiculoides* (Arad cells), from *Azolla pinnata* (Pinnata cells) and from *Azolla caroliniana* (Newton cells) were prepared as previously described (Newton and Herman, 1979; Tel-Or et al., 1983). All *Anabaena* isolates were grown in BG 11 medium (Stanier et al., 1971) in 100 ml cultures in 26°C on a rotary shaker, illuminated by white fluorescent light. Samples used for immunization and analytical procedures were taken from 1–3 week old cultures. The *Anabaena* isolates all contained a similar number of cells and similar protein content for a specific cell turbidity (3×10^7 cells/ml were equivalent to absorbance of 0.3 O.D. at 660 nm).

Antisera

Anti-*Anabaena azollae* sera were produced by injecting intact *Anabaena* cells (10^6 cells per immunization) intra-peritoneally into mice and intravenously into rabbits (10^7 cells per immunication), followed by two booster injections at two week intervals. Mouse ascitic fluid (Sartorelli et al., 1966) and IgG fractions of rabbit sera (Hudson and Hay, 1976) were prepared.

Assays

The quantitative immunobinding assay: 100μl of intact cells, or cell wall preparations of equivalent samples (the material obtained by sonication, centrifugation and resuspension of the pellet to the original volume in saline) were incubated with 100μl of diluted serum in Eppendorf microfuge tubes for 10 min, centrifuged and washed three times with 0.5 ml saline. The resuspended preparations were incubated for 30 min with 0.5 ml of dilutes ^{125}I-labelled protein A. The samples were subsequently centrifuged, washed three times with saline and counted in a gamma counter.

The enzyme-linked immunosorbent assay (ELISA): algal cells were sonicated for 2 min with a Brown labsonic sonifier (100 W) and 150μl of diluted sonicate was added to wells of PVC microtiter plates (Dynatech #220–129) and incubated at 37°C for 2 hr. The adsorbed antigens in the wells were incubated for 1 hr at 37°C with serum dilutions and washed three times with saline. The cells were again incubated for 1 hr at 37°C with goat anti-rabbit or rabbit anti-mouse IgG conjugated to horseradish peroxidase (Miles-Yeda, Israel). The wells were washed four times, incubated for 10 min at 20°C with ABTS reagent (Azido-ethyl-benzothiazoline sulphonic acid) and absorbance measured at 405 nm.

3. Results and Discussion

In this work the homology between isolates of the *Anabaena* phycobiont from three different species of *Azolla* was analyzed. An immunological approach was adopted to evaluate the antigenic relationship between these three different *Anabaena azollae* strains.

Antiserum toward intact Newton cells was elicited in mice and antibody binding examined using a quantitative immunobinding assay. The results shown in Fig. 1 define the working parameters used to obtain the optimal interaction (amount of antigen–3 $\times$ 10^6 cells; dilution of antiserum–1:100).

Using these conditions, the binding of mouse anti-Newton serum to intact cells of the three *Anabaena* isolates, Newton, Arad and Pinnata, was tested (Table 1). The results showed an apparent similarity between Newton and Arad cells and differences between these and Pinnata cells. No binding of anti-Newton serum to cells of the non-related algae *Synechococcus* 6311 was observed (data not shown), indicating specificity of the antiserum with the *Anabaena* isolates.

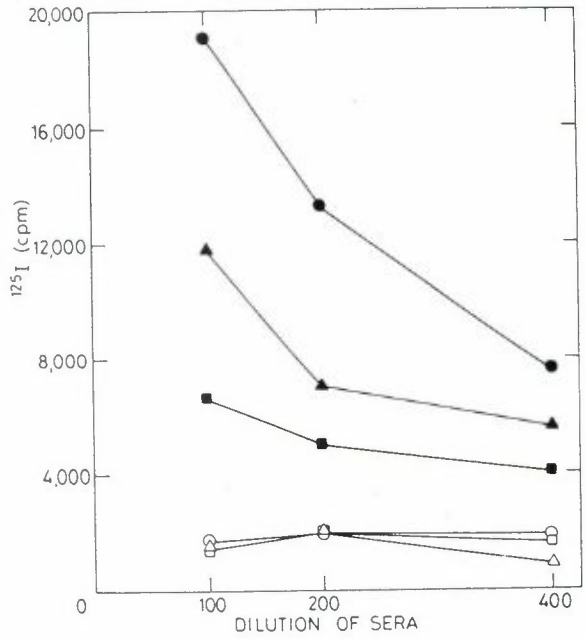

Figure 1. The interaction between intact Newton cells and immune or preimmune serum. Samples of Newton cells containing 3×10^6(●, ○), 7.5×10^5(▲, △) and 2×10^5(■, □) cells per sample were incubated with several dilutions of immune (●, ▲, ■) and preimmune (○, △, □,) mouse ascitic fluid. The numbers represent the CPM of bound ^{125}I-labelled protein A. Input per sample was 68441 CPM of ^{125}I-labelled protein A. Each value is the mean of duplicate determination.

In order to substantiate the apparent difference between Newton and Pinnata cells, antisera toward these two strains were elicited in rabbits and mice. The interaction (Table 2) of Newton and Pinnata cells with the rabbit antisera supported the suggestion of differences between the two cell types. Although there was some crossreaction, each cell type reacted preferentially with its homologous antiserum. A change in the antigen presentation from intact cells to cell walls resulted in no difference in the net interaction of all the sera tested (Table 2); it did however result in reduced background binding (Table 2a). Table 3 shows the interaction of the two mouse antisera with Newton and Pinnata cells. A similar picture emerges; Newton and Pinnata cells bind their homologous antisera well, but only poorly bind heterologous antisera.

Table 1. The binding of anti-Newton serum to whole Newton, Arad and Pinnata cells.

Antiserum	Antigen	Netbinding CPM
	Newton cells	12693
Anti-Newton 1:100	Arad cells	13092
	Pinnata cells	3602

The results represent the netbinding (specific binding with immune serum minus non-specific binding with preimmune serum) expressed in CPM of bound ^{125}I-labelled protein A. Input per sample was 68967 CPM of ^{125}I-labelled protein A. Each sample contained 9×10^6 algal cells. Mouse ascitic fluid was used. Each value is the mean of triplicate determination.

Table 2. Crossreaction between Newton and Pinnata cells.

Antigen presentation	Sera 1:100	Newton cells Netbinding CPM	Pinnata cells Netbinding CPM
Intact cells	Anti-Newton	16715	2820
Intact cells	Anti-Pinnata	4379	9192
Cell walls	Anti-Newton	15192	3246
Cell walls	Anti-Pinnata	5609	7427
Table 2a. Non-specific binding of intact cells and cell walls.			
Intact cells	Preimmune	9820	3531
Cells walls	Preimmune	2191	1855

The results in Table 2 represent the netbinding in CPM. 3×10^6 Newton and Pinnata intact cells or cell wall preparations from the same number of cells incubated with anti-Newton, anti-Pinnata or preimmune serum. IgG fractions of rabbit sera were used. Input was per sample 82120 CPM of ^{125}I-labelled protein A. The results are the mean of duplicate determination.

Table 3. Crossreaction between Newton and Pinnata cells.

Sera 1:100	Newton cells Netbinding CPM	Pinnata cells Netbinding CPM
Anti-Newton	4850	593
Anti-Pinnata	695	9802

The results represent netbinding in CPM. Antigen presentation was as cells walls of 3×10^6 cells. Mouse ascitic fluid was used. Input per sample was 60000 CPM of ^{125}I-labelled protein A. Each value is the mean of duplicate determination.

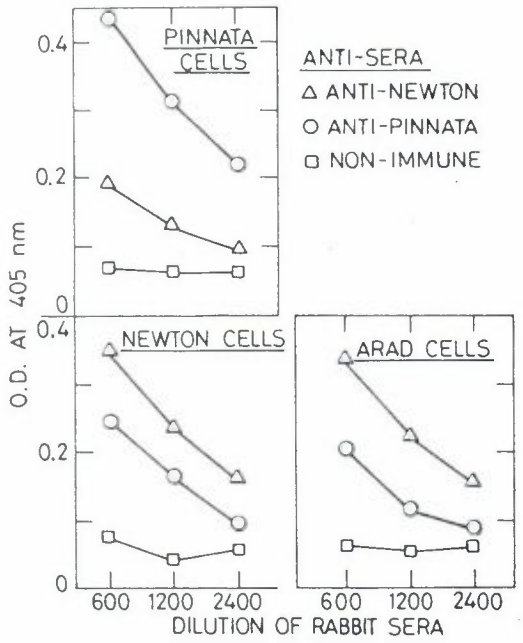

Figure 2. Binding of rabbit antisera and preimmune serum to Newton, Arad and Pinnata cells. Antigens were prepared from cell sonicates of 5×10^5 algal cells/ml. IgG fractions of rabbit sera were used.

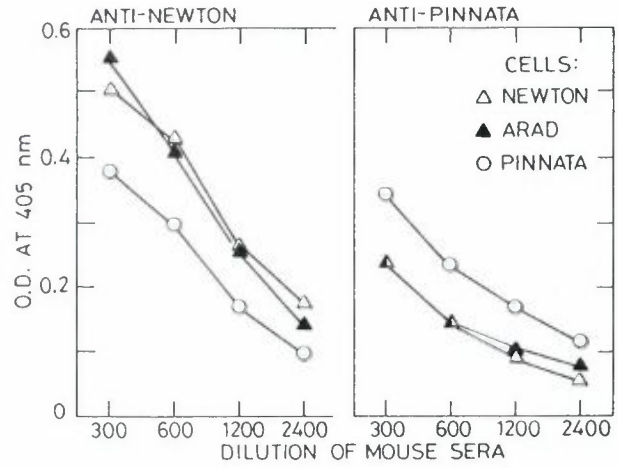

Figure 3. Interaction of anti-Newton and anti-Pinnata mouse ascitic fluids with the three *Anabaena* isolates. Antigens were prepared from cell sonicates of 5×10^5 algal cells/ml.

The overall picture, which emerged from the binding of mouse and rabbit antisera to the *Anabaena* isolates, suggested a clear distinction between Newton and Pinnata cells. This picture was also observed when the ELISA technique was used to study the interaction (Fig. 2 and Fig. 3). In this case solubilized components as opposed to whole cells were compared. Possible differences in the adsorption of antigens to PVC plastic was not considered relevant since all cell types were treated similarly (e.g. harvesting, sonication, immobilization). Thus, detected differences probably reflected quantitative differences in solubilized antigens. Figure 2 demonstrates the similarity between Newton and Arad cells as seen from their binding to both anti-Newton and anti-Pinnata rabbit sera. The distinct interactions of the Pinnata cells with these antisera again suggests clear quantitative differences in the antigenic display of these cells when compared to the other two strains. The same picture can be seen in Fig. 3, in which the interaction of the three isolates with mouse ascitic fluids are shown. Anti-Pinnata serum reacted preferentially with Pinnata cells and to a lesser degree with Newton and Arad cells. On the other hand, anti-Newton serum reacted equally well with Newton and Arad cells and less well with Pinnata cells.

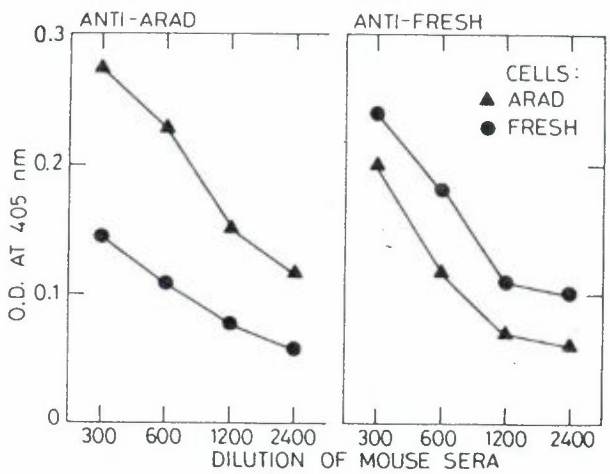

Figure 4. Crossreaction between Arad and fresh cells. Antigens were prepared from cell sonicates of 5×10^5 algal cells/ml. Mouse ascitic fluids were used.

A similar approach was adopted to compare the antigenic relationship between cultured Arad cells and the same cells freshly isolated from the fern. Mouse antisera elicited toward both cells showed preferential binding to the homologous antigen (Fig. 4). The differences shown suggested that antigenic changes occurred during the process of cell line establishment from fresh cells to the axenic Arad culture. Changes occurring in algal cell surface antigens during the establishment of fresh isolates to axenic cultures are a well established phenomenon in lichens (Bubrick and Galun, 1980) as well as in *Azolla* (Gates et al., 1980; Ladha and Watanabe, 1982). On the other hand, these differences could simply result from the disappearance of host-derived antigens. Further efforts should be undertaken to distinguish between these two possibilities.

In conclusion, the similarity between Arad and Newton cells, both in terms of cell surface antigens and soluble antigens, has been demonstrated by immunobinding and ELISA assays. These same assays have also shown that Pinnata cells, representing an Old World *Azolla* phycobiont, were distinct from Arad and Newton cells.

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