Serological variability of the *Pellia endiviifolia*-*P. megaspora* complex

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Abstract

Analysis of antigenic proteins in populations of terrestrial and aquatic forms of *P. endiviifolia* and populations from Japan revealed the antigenic differentiation of the examined samples into two distinct groups. *Pellia megaspora* from the eastern part of the USA also exhibits a significant antigenic divergence and bears the most resemblance to the group of populations of the aquatic form of *P. endiviifolia* from Poland and samples from Japan. The observed serological distances between terrestrial and aquatic forms of *P. endiviifolia* are of the same rank as differences between remaining species of the genus *Pellia*. Clarification of the nature of the detected serological differentiation in the *Pellia megaspora*-*P. endiviifolia* complex will require further studies.

Key words: *Pellia endiviifolia* complex, ecological forms, serological differentiation

INTRODUCTION

Studies conducted on biochemical and cytological as well as on anatomical and morphological differentiation of individual taxa of the genus *Pellia* have demonstrated significant intraspecific differentiation. Differentiation observed in the phylogenetically oldest taxon of the genus *Pellia* — in *Pellia endiviifolia* seems particularly interesting. Studies by Schuster (1981) on morphological and anatomical traits of the species, cytological studies (Mendelak 1981, Newton 1981) and biochemical examination (Krzakowa 1981) have demonstrated complex differences between *Pellia endiviifolia* forms from North America, Japan and Europe. One of the forms, the most different one, originating from the eastern part of the USA, was described by Schuster (1981) as a new species — *Pellia megaspora*. Differentiation

observed within the taxon of *Pellia endiviifolia* prompted Schuster to introduce the term *Pellia endiviifolia*-*P. megaspora* complex to include all of the different forms observed within the species. That author regards the different forms of the *Pellia endiviifolia*-complex from the USA, Japan and Europe as representatives of geographic races, sub- or even microspecies. Comparative studies on isoenzymatic proteins of *P. endiviifolia* from Poland have shown that the taxon is differentiated into two enzymatic forms, exhibiting in addition, a relatively clear-cut ecological differentiation (Zieliński, in preparation). One of the forms occurs mainly on solid, caliciferous substrates while the other grows principally in an aquatic environment. The two have been respectively termed the terrestrial and aquatic forms. It should be stressed that the studies performed until now on *P. endiviifolia* differentiation have been conducted only on terrestrial forms, regarded as typical for *P. endiviifolia*.

Since serological techniques employed in earlier studies have proven very suitable for studies on reciprocal phylogenetic relations between taxa of the *Pellia* genus, and for studies on polymorphism of antigens (Szweykowski et al. 1981a, b, Prus-Głowacki and Nowak 1982, Zieliński et al. 1981), they have been applied in the present study to examine antigenic differentiation of terrestrial and aquatic forms of *P. endiviifolia* from Poland and samples of *Pellia endiviifolia* from Japan versus *Pellia megaspora* and the remaining taxa of the genus *Pellia*.

MATERIAL AND METHODS

The plant material used for the analyses was cultured for more than a year in a greenhouse under uniform conditions. Localization of the studied populations of the genus *Pellia* is presented in Table 1. One g of fresh thallii was taken from each culture. Proteins were extracted by grinding the plant material in a mortar in 4 cm³ of a cold extraction buffer (TRIS-boric acid-EDTA), pH 7.4, concentration: 0.01 M, supplemented with PVP (1 part to 4 parts of thallus mass) and 2-mercaptoethanol (0.7 cm³ per 100 cm³ buffer). Next, the homogenate was centrifuged at 2000 × g for 20 min at -4°C. The extracts were analyzed using double immunodiffusion according to Ouchterlony in 1.5% agarose in a veronal-acetate buffer, pH 6.75 and concentration of 0.135 M. Determination of the quantity of antigens precipitated with antiserum against *P. endiviifolia* (terrestrial form) proteins in the studied forms of *Pellia* using rocketimmunoelectrophoretic analysis, was also performed, according to Laurell (1972) with some modifications (Prus-Głowacki et al 1981). The results of immunodiffusion were interpreted according to types of serological reactions (described by Ouchterlony (1967) and listed in the data table, using the following code:

Table 1
Liverwort samples used in serological comparisons

Taxa	Form	Population number	Denotation of samples	Origin	Collectors
<i>Pellia endiviifolia</i>	terrestrial	275	T1	Szczecin, Szmaragdowe Lake Poland	Leg. R.Z. 1980
<i>Pellia endiviifolia</i>	terrestrial	194	T2	Lucień near Oleśnica Poland	Leg. W.K. 1979
<i>Pellia endiviifolia</i>	terrestrial	232	T3	Warszawa Poland	Leg. M.M. 1980
<i>Pellia endiviifolia</i>	terrestrial	298	T4	Diabli Skok near Wałcz Poland	Leg. R.Z. 1981
<i>Pellia endiviifolia</i>	terrestrial	293	T5	Szczecin, Szmaragdowe Lake Poland	Leg. R.Z. 1981
<i>Pellia endiviifolia</i>	aquatic	288	W1	Wałcz Poland	Leg. R.Z. 1981
<i>Pellia endiviifolia</i>	aquatic	395	W2	Tatra Mts. Poland	Leg. R.Z. 1982
<i>Pellia endiviifolia</i>	aquatic	397	W3	Tatra Mts. Poland	Leg. R.Z. 1982
<i>Pellia endiviifolia</i>	aquatic	400	W4	Tatra Mts. Poland	Leg. R.Z. 1982
<i>Pellia endiviifolia</i>	————	—	J1	Honsiu Japan	Leg. H.I. 1980
<i>Pellia endiviifolia</i>	————	—	J2	Honsiu Japan	Leg. H.I. 1980
<i>Pellia endiviifolia</i>	————	—	J3	Honsiu Japan	Leg. H.I. 1980
<i>Pellia endiviifolia</i>	————	—	J4	Honsiu Japan	Leg. H.I. 1980
<i>Pellia megaspora</i>	————	—	M	USA	Leg. R.S. 1980
<i>Pellia neesiana</i>	————	173	N	Tatra Mts. Poland	Leg. J.S. 1978
<i>Pellia epiphylla</i>	————	75	E	Babia Góra Mt. Poland	Leg. I.G. 1975
<i>Pellia borealis</i>	————	169	B	Śnieżnik Mt. Poland	Leg. E.S. 1978

J. S. — J. Szwejkowski; I. G. — I. Gasser; H. I. — H. Inoue; W. K. — W. Kola; R. Z. — R. Zieliński; R. S. — R. Schuster; M. M. — M. Mendelak; E. S. — E. Siudowska.
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0 — absence of antigen, 1 — identical antigens, 0.5 — proteins of partial antigenic identity. The antigens so distinguished were used for calculating coefficients of taxonomic similarity. The Jaccard coefficient of taxonomic similarity (S_{Jac}) was calculated according to the formula (Sneath and Sokal 1973):

$$S_{Jac} = \frac{a}{a+b+c},$$

where "a" is the number of characters (antigens) present in the compared samples and "b" and "c" are the numbers of characters present in one sample but absent in the second samples and vice versa. Basing on coefficients of taxonomic similarity, taxonomic distances (D_{Jac}) were calculated, according to the formula:

$$D_{Jac} = 1 - S_{Jac}.$$

The calculated taxonomic distances (D_{Jac}) were used to construct a dendrite and dendrogram to visualize the reciprocal serological similarities of the studied populations. Quantitative estimation of individual antigens was conducted using rocketimmunoelectrophoresis according to a procedure described earlier (Prus-Głowacki et al. 1981).

Antiserum against proteins of *Pellia endiviifolia* (terrestrial form) was prepared by injecting rabbits 6 times, one injection weekly. The extract used for injections was concentrated twofold using Aquacide to a final protein concentration of about 20 mg per cm³. Due to the relatively low titre of antiserum, antibodies were salted out with ammonium sulfate solution, according to the standard procedure. The isolated antibodies were dissolved in a threefold smaller amount of distilled water.

RESULTS

The antigens distinguished in the individual populations and their identification are presented in Table 2 and Fig. 1. In total, 7 antigens were discerned. The number of detected antigens varied between 2 in *Pellia*

Fig. 1. Example of an immunodiffusion plate of antigenic protein of the studied forms of the genus *Pellia*. Antiserum against terrestrial forms of *Pellia endiviifolia* from Poland. The denotation of samples as in Table 1

Fig. 2. Dendrite (A) and dendrogram (B) based on simple similarity coefficients according to Jaccard ($S_{Jac.}$) showing serological diversity of populations from *Pellia endiviifolia*-*P. megaspora* complex against a background of remaining species from the genus *Pellia*. The denotation of samples as in Table 1

Fig. 3. Diagram of rocketimmunoelectrophoresis of antigens of the studied populations of the *Pellia endiviifolia*-*P. megaspora* complex. The denotation of samples as in Table 1

epiphylla and 4 in most of *P. endiviifolia* samples. Four antigens were observed in aquatic forms, in three Japanese forms (J1, J2, J4), in a terrestrial form (T4) and in *P. neesiana* (N). Three antigens were noted in two terrestrial forms (T1 and T3) in a Japanese form (J3) of *Pellia endiviifolia* as well as in *P. megaspora* (M) and *P. borealis* (B). The data were used for calculating coefficients of similarity (S_{Jac}) and taxonomic distances (D_{Jac}) between the examined samples (Table 3). The dendrite and dendrogram constructed on the basis of taxonomic distances (D_{Jac}) are presented in Fig. 2. In the dendrite (Fig. 2A) the examined samples of *Pellia endiviifolia* form three groups. One of the groups consists of the terrestrial forms (T1, T2, T3), the other consists of the aquatic forms (A1, A2, A3, A4) and forms originating from Japan (J1, J2, J3, J4). *P. megaspora* is positioned

Table 2

Results of immunodiffusion analysis of antigenic proteins of the investigated liverworts. Antisera against proteins of *P. endiviifolia* (terrestrial form). Denotation of samples as in

Table 1

Samples	Antigens							Number of precipitation bands
	1	2	3	4	5	6	7	
T1	1	1	1	0	0	0	0	3
T3	1	1	1	0	0	0	0	3
T4	1	1	1	1	0	0	0	4
W1	0.5	1	1	0	1	0	0	4
W2	0.5	1	1	0	1	0	0	4
W3	0.5	1	0.5	0	1	0	0	4
W4	0.5	1	1	0	1	0	0	4
J1	0.5	0.5	1	0	1	0	0	4
J2	0.5	0.5	1	0	1	0	0	4
J3	0.5	0.5	1	0	0	0	0	3
J4	0.5	0.5	1	0	1	0	0	4
M	0	0.5	1	0	1	0	0	3
N	0	0.5	1	0	1	1	0	4
E	0	0.5	1	0	0	0	0	2
B	0	0.5	1	0	0	0	1	3

close to samples of *P. endiviifolia* from Japan. The position of the examined samples on the dendrogram corresponds to their position in the dendrite (Fig. 2B). The examined samples of *P. endiviifolia* form two groups. One of them is clearly distinct and contains terrestrial forms (T1, T3, T4), the other contains aquatic forms (A1 to A4) and Japanese forms (J1 to J4) resembling them. In the group, one of the Japanese forms is clearly distinct (J3). *P. megaspora* (M) is linked to Japanese forms by the longest distance in the dendrogram.

Rocket immunoelectrophoresis patterns for antigens of the examined *P. endiviifolia* samples and *P. megaspora* are presented in Fig. 3. In the terrestrial forms of *P. endiviifolia* 5 antigenic proteins were observed, when in the Japanese forms 3 (J2 and J4) or 4 antigenic proteins (J1, J3) were found. The aquatic form (A) possesses 4 antigenic proteins and *P. megaspora* exhibited 2 antigens precipitated with antiserum against proteins of the terrestrial form of *P. epiphylla* from Poland. The results of rocket immunoelectrophoresis were used for calculating quantities of proteins precipitated with anti-*P. endiviifolia* (terrestrial form) serum. The quantities of these antigens are presented in histograms on Fig. 4. If we take as 100% the

Fig. 4. Diagram showing the results of quantitative immunoelectrophoresis. Antiserum against proteins of *Pellia endiviifolia* terrestrial forms. The denotation of populations as in Table 1

results of the homological precipitation reaction (sample T2), differences in the content of the antigenic proteins in terrestrial forms amount to approximately 10%, while in the Japanese forms the content of antigens is about 60% lower than in terrestrial forms from Poland. A similar quantity of proteins precipitated with antiserum to a terrestrial form from Poland was

Table 3

Serological similarity coefficients (S_{Jac}) according to Jaccard (Sneath and Sokal 1973) and serological distances (D_{Jac}) for the investigated populations of the genus *Pellia*. The denotation of samples as in Table 1

S_{Jac}																D_{Jac}
	T1	T3	T4	W1	W2	W3	W4	J1	J2	J3	J4	M	N	E	B	
	T1	0	0.25	0.38	0.38	0.50	0.38	0.50	0.50	0.33	0.50	0.63	0.80	0.77	0.75	
	T3	1.0	0.25	0.38	0.38	0.50	0.38	0.50	0.50	0.33	0.50	0.63	0.80	0.77	0.75	
	T4	0.75	0.75	0.50	0.50	0.60	0.50	0.60	0.60	0.50	0.60	0.70	0.83	0.75	0.75	
	W1	0.62	0.62	0.50	0	0.12	0	0.12	0.12	0.38	0.12	0.38	0.60	0.75	0.80	
	W2	0.62	0.62	0.50	1.0	0.12	0	0.12	0.12	0.38	0.12	0.38	0.60	0.75	0.80	
	W3	0.50	0.50	0.40	0.88	0.88	0.12	0.25	0.25	0.50	0.25	0.50	0.67	0.88	0.90	
	W4	0.62	0.62	0.50	1.0	1.0	0.88	0.12	0.12	0.38	0.12	0.38	0.60	0.75	0.80	
	J1	0.50	0.50	0.40	0.88	0.88	0.75	0.88	0	0.25	0	0.25	0.60	0.75	0.80	
	J2	0.50	0.50	0.40	0.88	0.88	0.75	0.88	1.0	0.25	0	0.25	0.60	0.75	0.80	
	J3	0.67	0.67	0.50	0.62	0.62	0.50	0.62	0.75	0.75	0.25	0.50	0.80	0.67	0.75	
	J4	0.50	0.50	0.40	0.88	0.88	0.75	0.88	1.0	1.0	0.75	0.25	0.60	0.75	0.80	
	M	0.37	0.37	0.30	0.62	0.62	0.50	0.62	0.75	0.75	0.50	0.75	0.50	0.67	0.75	
	N	0.20	0.20	0.17	0.40	0.40	0.33	0.40	0.40	0.40	0.20	0.40	0.50	0.67	0.75	
	E	0.33	0.33	0.25	0.25	0.25	0.12	0.25	0.25	0.33	0.25	0.33	0.33		0.50	
	B	0.25	0.25	0.25	0.20	0.20	0.10	0.20	0.20	0.25	0.20	0.25	0.25	0.50		

noted in the aquatic form (W). The quantity of antigens common for terrestrial forms from Poland and *P. megaspora* is approximately only 15%. Rocketimmunoelectrophoresis analysis showing the quantities of common proteins precipitated by antibodies against proteins of *P. endiviifolia* (terrestrial form), showed that the examined samples form two groups and *P. megaspora* shows the lowest amounts of proteins common with *P. endiviifolia* terrestrial forms.

DISCUSSION

Analysis of antigens in *P. endiviifolia* samples including terrestrial and aquatic forms from Poland as well as *P. endiviifolia* samples from Japan has demonstrated a significant antigenic differentiation of the examined forms. The examined samples form two groups. One of the groups is clearly distinct from the remaining samples and includes terrestrial forms from Poland, the other consists of two subgroups of aquatic forms from Poland and samples from Japan. *Pellia megaspora* from the eastern parts of the USA, exhibiting a significant antigenic divergence, is most similar to the latter group (Figs. 1 and 2). The antigenic specificity of aquatic *P. endiviifolia* forms as compared to *P. endiviifolia* terrestrial forms from Poland is interesting (Figs. 2, 3, 4). The obtained results correlate with the isoenzyme analysis of aquatic and terrestrial forms of *P. endiviifolia* (Zieliński, in preparation). Out of 14 analyzed enzymes, 9 showed electrophoretic different mobility for terrestrial and aquatic forms. The result may indicate that the two forms possess two different gene pools. Also the Japanese forms, serologically distinct from aquatic forms, differed from *P. endiviifolia* from Poland from *P. megaspora* in the electrophoretic mobility of peroxidases (Krzakowa 1981) and other enzymes (Zieliński 1985). *P. megaspora* is serologically similar to the whole group of examined *P. endiviifolia* forms but also maintains a significant antigenic specificity. The divergence of this taxon from the *P. endiviifolia*-complex has been indicated by Schuster (1981) for anatomical and morphological traits. Newton (1981) and Mendelak (1981) have pointed out the specific chromosomal characters of the form. Studies of Krzakowa (1981) have shown specificity in peroxidases of *Pellia megaspora*.

In this aspect, serological differentiation of the *P. endiviifolia*-*P. megaspora* complex should be compared with serological differentiation of the remaining three *Pellia* taxa, i.e. *P. neesiana*, *P. epiphylla* and *P. borealis* (Fig. 2).

The serological distance of *P. megaspora* (M) and of one of the Japanese forms (J3) from the remaining forms of the group resembles the distance observed between species i.e. *P. neesiana* and the pairs of species *P. epiphylla*-*P. borealis*. A similar distance has also been observed between terrestrial

forms of *P. endiviifolia* and the remaining examined samples. But even if the observed serological distances between *P. neesiana*, *P. epiphylla*-*P. borealis* and *P. megaspora* are of the same rank, the obtained data do not permit to draw conclusions on the taxonomic status of the distinguished groups. Clarification of the character of the detected serological differentiation in the examined *P. megaspora-endiviifolia* complex samples (i.e. whether the differentiation is of a geographic or ecological type and whether the group also contains other taxonomic units) will require studies on a much broader material.

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Zróżnicowanie serologiczne kompleksu *Pellia endiviifolia*-*P. megaspora*

Streszczenie

Badano zróżnicowanie białek antygenowych u form wodnych i lądowych *Pellia endiviifolia* z Polski oraz kilku prób tego gatunku wątrobowca z Japonii, jak również białka *P. megaspora* z Ameryki Północnej, gatunku wyodrębnionego niedawno z *Pellia endiviifolia*. Przeprowadzone badania wykazały znaczną specyficzność antygenową obu badanych form z Polski, odrębność form japońskich *P. endiviifolia* i największą specyficzność w stosunku do pozostałych prób *P. megaspora*. Porównanie lądowych i wodnych form z innymi gatunkami tego rodzaju wykazało, że różnice występujące między lądową i wodną formą *P. endiviifolia* są takiego samego rzędu, jak różnice między gatunkami.

Wyjaśnienie charakteru wykrytego zróżnicowania, czy ma ono charakter ekologiczny, czy mamy tu do czynienia z dwoma odrębnymi gatunkami, wymaga dalszych badań.