PHOTOSYNTHETIC PIGMENTS OF THE *AZOLLA-ANABAENA* ASSOCIATION AND ITS FREE-LIVING SYMBIONT

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AZOLLA contains a symbiotic nitrogen-fixing cyanobacterium, *Anabaena azollae*, within the cavities in dorsal leaf lobes. Both the host and the symbiont are capable of photosynthesis but the nitrogen requirements of the host are fulfilled by the symbiont. Since a significant amount of combined nitrogen is usually present in the natural ecosystems where *Azolla* plants grow, the aim was to study the effect of combined nitrogen on photosynthetic pigments of the *Azolla-Anabaena* association. Also, an attempt was made to culture the isolated symbiont in a medium free of combined nitrogen in view of some reports[1] that the cyanobacterial symbiont is incapable of independent existence. Photosynthetic pigments of the symbiont were also estimated.

Surface-sterilized fronds of *Azolla pinnata* were grown for 15 days in the nutrient medium[2] (pH 6.0) under fluorescent light of 5000 lux (18 hr light + 6 hr darkness) at 29 ± 2°C. The nutrient medium was supplemented with different levels of $NaNO_3$. The medium was changed every 5th day during the growth of *Azolla* fronds. *A. azollae* was also grown for the same duration in Allen and Arnon's medium[2] enriched with 5 mM $NaNO_3$. Culture vessels were kept under continuous light of 5200 lux from an incandescent bulb at 30 ± 2°C. The pH of the medium was adjusted to 7.5 after sterilization. Unpurified phycobiliproteins of the symbiont were estimated according to the method of Siegelman and Helen Kycia[4] and chlorophylls of the *Azolla-Anabaena* association and the free-living symbiont were calculated quantitatively using the formulae of Jeffrey and Humphrey[5].

Higher proportions of chlorophyll *a* and *b* were observed in *Azolla* fronds on supplementing the medium with 10 and 15 mM $NaNO_3$, indicating about 13% increase over the unsupplemented control (table 1). *Azolla* plants are able to utilize combined nitrogen from the culture medium as well as that which is fixed by the symbiont. The results show stimulatory effect of low levels of combined nitrogen on the *Azolla-Anabaena* pigmentation. Such levels of combined nitrogen do not seem to impair in any way the nitrogen-fixing capacity of the symbiont as is evidenced by the growth of the *Azolla* fronds. Since the isolated symbiont was unable to grow without nitrogen source in the medium, it was regarded as a non-nitrogen-fixing, non-heterocystous strain of *A. azollae*. Of the total phycobiliproteins, C-phycocyanin accounted for about 71%, allophycocyanin about 21% and C-phycoerythrin about 8% (table 2). It may be noted, however, that these values hold good under the above mentioned cultural conditions as light intensity and quality, culture density and combined nitrogen source in the medium may greatly affect the synthesis of photosynthetic pigments. Absorption spectra of the unpurified acetone-soluble pigments of *Azolla* and its free-living symbiont showed similar peaks at 420 and 670 nm. The peak of the symbiont's biliproteins was observed at 610 nm.

Table 2 *Photosynthetic pigments of the free-living symbiont Anabaena azollae (averages of 5 replicates ± SD)*

C-phycocyanin (mg/ml)	0.155 ± 0.02
Allophycocyanin (mg/ml)	0.045 ± 0.009
C-phycoerythrin (mg/ml)	0.017 ± 0.005
Chlorophyll *a* (mg/ml)	0.0076 ± 0.0004

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1. Peters, G. A. and Mayne, B. C., *Plant Physiol.*, 1974, **53**, 820.
2. Watanabe, I., Espinas, C. R., Berja, N. S. and

Table 1 *Chlorophyll pigments of the Azolla-Anabaena association (averages of 5 replicates ± SD)*

Nutrient medium	Chl. *a* (mg/ml)	Chl. *b* (mg/ml)	Total chlorophylls (mg/ml)
Control	0.0073 ± 0.0004	0.0059 ± 0.0003	0.0132 ± 0.0008
+ 5 mM $NaNO_3$	0.0069 ± 0	0.0056 ± 0	0.0125 ± 0
+ 10 mM $NaNO_3$	0.0082 ± 0.0004	0.0067 ± 0.0004	0.0149 ± 0.001
+ 15 mM $NaNO_3$	0.0082 ± 0.0004	0.0067 ± 0.0005	0.0149 ± 0.001
+ 20 mM $NaNO_3$	0.0068 ± 0.0003	0.0054 ± 0.0003	0.0122 ± 0.0008

Alimagno, B. V., IRRI Res. Paper Ser. 11, 1977, 1.
3. Allen, M. B. and Arnon, D. I., *Plant Physiol.*, 1955, **30**, 366.
4. Siegelman, H. W. and Helen Kycia, J., *Handbook of phycological methods* (eds) J. A. Hellebust and J. S. Craigie, Cambridge University Press, Cambridge, 1978, p. 71.
5. Jeffrey, S. W. and Humphrey, G. F., *Biochem. Physiol. Pflanz.*, 1975, **167**, 191.

SEXUALITY AND OXIDASE TESTS OF *STECCHERICIUM SERIATUM* (LLOYD) MAAS GEESTERANUS

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THE type of sexuality is considered by modern mycologists[1–4] as an important criterion for indicating phylogenetic relationship and taxonomic status of the members of Basidiomycetes. Nobles[4] pointed out that the species possessing bipolar type of sexuality are more primitive than those possessing tetrapolar type of sexuality and naturally the bipolar species cannot be congeneric with the tetrapolar ones. Considering the importance attached to the study of sexuality, *Stecchericium seriatum* (Lloyd) Maas Geesteranus has been studied from this point of view and the results are presented.

The sporophore of *S. seriatum* was collected from Santiniketan, Birbhum, West Bengal, India on a living tree of *Ficus bengalensis* L. Twenty monosporous cultures were isolated from the spores of this sporophore following the usual dilution method. When each of the 20 monosporous cultures showed good growth they were checked carefully for clamp connections. The absence of clamp connections was taken as confirmation of their monokaryotic nature. The monosporous cultures were then paired among themselves in all possible combinations by placing the inocula 25–30 mm apart on 2.5% malt agar slants and incubated at room temperature (28–32°C) for a fortnight. The line of contact between the paired mycelia in each tube was then examined under the microscope for the presence of clamp connections. The results of pairings were recorded.

Analysis of the results showed that the single spore cultures from one sporophore of *S. seriatum* fall into four genetic constituents A_1B_1, A_2B_2, A_1B_2 and A_2B_1 on the basis of their ability to form dikaryotic mycelia, recognizable by the presence of clamp connections. Dikaryotic mycelia were formed only in matings between $A_1B_1 \times A_2B_2$ and $A_1B_2 \times A_2B_1$, i.e. between mycelia having no common allele. Therefore, *S. seriatum* is heterothallic and possesses tetrapolar type of sexuality with allelomorph for heterothallism at two loci. The number and distribution of the monosporous cultures in each mating group are:

A_1B_1: 1, 2, 12,
A_2B_2: 3, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20,
A_1B_2: 4, 6, 9,
A_2B_1: 5, 7, 8.

Nobles[5] put forward the hypothesis that the species which possess bipolar type of sexuality are unable to liberate extra cellular oxidase enzymes in culture while the species with tetrapolar type of sexuality liberate extracellular oxidase enzymes in culture. In the present investigation attempts have been made to see whether this hypothesis of Nobles[5] also holds true for *S. seriatum*.

Oxidase tests were carried out by growing the polysporous mycelia of *S. seriatum* for 7 days at room temperature (26 ± 2°C) on 2.5% malt agar media containing 0.5% gallic acid and tannic acid in separate petridishes following the method laid down by Davidson *et al*[6]. The appearance of dark-coloured zones in the media presented positive proof of the production of extracellular oxidase enzymes by the test fungus.

From the results obtained it may be concluded that the hypothesis of Nobles[5] also finds support in *Stecchericium seriatum* (Lloyd) Maas Geesteranus.

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1. Burdsall, H. H. and Lombard, F. F., *Mem. N.Y. Bot. Gard.*, 1976, **28,** 16.
2. Ginns, J. H., *Can. J. Bot.*, 1970, **48,** 1039.
3. Van der Westhuizen, G. C. A., *Can. J. Bot.*, 1963, **41,** 1487.
4. Nobles, M. K., *Can. J. Bot.*, 1958, **36,** 883.
5. Nobles, M. K., In: *Evolution in the higher Basidiomycetes*, (ed.) R. H. Petersen, The