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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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PRODUCTION OF GIANT FORM IN *WESTIELLOPSIS PROLIFICA* JANET UNDER HETEROTROPHIC CULTURE CONDITIONS

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THE effect of different light intensities on the growth and morphology of *Westiellopsis prolifica* Janet on a liquid medium with organic carbon sources was investigated and the present report embodies the results of the investigation. The procedure for the determination of growth was described earlier[1]. Allen and Arnon's nitrogen free, growth medium was used[2]. Sugars and sodium salts of organic acids to the final concentration of 15 mM were added and the pH adjusted to 7.5. The experiments had three variants: the first contained conical flasks exposed to full illumination with 2,200 lux, the second had flasks wrapped in wax paper and received approximately 1,100 lux (i.e. 50% of maximum) and the third contained flasks covered with black paper so that they received no light. All the culture flasks were incubated at 24–26°C after inoculation with equal amounts of exponentially growing material (equivalent to 1 mg dry weight) into 25 ml of culture medium and harvested after 20 days of growth.

Of the various carbohydrates tested, glucose, galactose, fructose, lactose and sucrose were superior because they supported better growth of *Westiellopsis* (table 1). The highest growth increase in different light intensities and in dark was obtained when the cultures were supplemented with fructose. This agrees with earlier work on *Chlorogloea fritschii*[3], *Tolypothrix tenuis*[4] and *Anabaena* sp[5–6], where carbohydrates were shown to be the best substrates for heterotrophic growth. Although in the present study, various organic substrates supported the heterotrophic growth of the organism, the induced growth of the substrate in dark was only a fraction of the autotrophic growth of the cyanobacterium. Light was highly stimulatory for the utilization of organic substrates and at low light intensity there was almost no autotrophic growth. While it is difficult to precisely explain the reasons for this differential growth response it is possible that light and dark treatments produce alterations in the membrane permeability and transport properties for assimilation of exogenous substrates. The sugars viz mannose, the pentose and the organic acids were practically ineffective to support the growth of *Westiellopsis* in dark as well as under various conditions of illumination. The inability of various strains of cyanobacteria to grow in mannose and various pentose sugar-supplemented cultures has been reported earlier[7]. The failure of *W. prolifica* to grow on the organic compounds which are probably intermediates or products of metabolic cycles may be due to their inability to penetrate the cell membrane for assimilation[8, 9].

In the mineral medium incubated in darkness, *Westiellopsis* did not show profuse branching. However, when the medium was supplemented with glucose, galactose, fructose, lactose or sucrose and grown in dark, giant form of the organism was produced. In the giant form, the heterocysts were not observed and the cells of the prostrate and the erect

Table 1 *Growth (dry weight in mg) of W. prolifica under various conditions in a mineral medium with the addition of 15 mM concentration of a sugar (each value represents the mean of three closely concordant determinations).*

	Illumination intensity		
Sugar added	0%	50%	100%
AA medium (control)	1.5	2.4	18.1
Glucose	3.9 (+)	14.4	36.5
Galactose	4.87(+)	9.96	22.9
Fructose	5.4 (+)	16.2	39.9
Mannose	2.19	2.2	15.5
Xylose	1.8	2.65	14.7
Ribose	1.93	2.21	15.8
Arabinose	1.88	3.99	17.0
Rhamnose	2.5	4.15	17.5
Sorbose	3.0	9.32	24.0
Lactose	3.36(+)	12.49	37.6
Sucrose	3.99(+)	14.0	37.6
Maltose	2.41	13.87	28.4
Acetate	2.61	3.82	17.97
Pyruvate	1.53	2.75	14.42
Succinate	2.19	2.88	17.3

(+) = Giant form of the cyanobacteria produced in cultures supplemented with glucose, galactose, fructose, lactose or sucrose in the dark (0% illumination).