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STUDIES ON THE SEXUALITY IN HIGHER FUNGI: *I. POLYPORUS AGARICEOUS BERK.*

BY

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The present investigation on *Polyporus agariceous* Berk., a member of the family Polyporaceae, has been undertaken in order to study the nature of sexuality in the fungus. The experiment has been carried out in the laboratory by making pairing experiments with large numbers of monosporous cultures, obtained from a single sporophore. It has been observed that the fungus is 'heterothallic' and sexually 'tetrapolar'. The primary mycelia can be grouped into four distinct categories on the basis of their ability to form dikaryophasic state, recognised only by the presence of clamp-connexions.

INTRODUCTION

Polyporus agariceous Berk. is, a member of the family Polyporaceae, characterised by the presence of simple stipe, sub-umbilicate, somewhat brown-coloured pileus and polygonal decurrent pore-tubes. The fungus attacks the common Sal (*Shorea robusta* Gaerten.f.) and varies considerably in colour, in degree of roughness of the pileus and also in size and shape of the pore-tubes.

Upto this date very little is known about the biology of this fungus. In this present investigation, the phenomena of heterothallism has been investigated. After extensive studies on the condition of sexual reproduction in Mucorales, Blakeslee (1904) coined the term "heterothallism". Whitehouse (1949a, b), Quintanhilha and Pinto-Lopes (1950) and Raper (1954, 1959, 1960, 1966), Ahmed (1954), Burnett (1956) and Esser and Kuenen (1965) have reviewed the literatures on sexuality on basidiomycetes. Among fungi, a number of distinct basic patterns of sexuality have been described as responsible for heterothallism. It is true that very little work has been done in this direction, so far as Indian Hymenomycetes are concerned. In India, Bose (1934) and Banerjee and his collaborators (1954, 1957, 1964) and a few others have worked in this direction.

So far, no work has yet been done on this species in India, although Vandendries (1936) reported that *Leucoporus arcularius* (Fr. ex. Batsch) Quel is heterothallic and tetrapolar in nature. Berkeley (1847) and Bose (1922) also reported that *Leucoporus arcularius* and *Polyporus arcularius* and *Polyporus agariceous* are synonymous.

MATERIALS AND METHODS

Twenty-five monosporus cultures were made from a single sporophore collected from Dum Dum log-yard, Dum Dum, West Bengal. The fungus

was found to grow on logs of *Shorea robusta*. The monosporous cultures were obtained according to the usual laboratory procedures. The cultures were then examined thoroughly maintained as stock cultures for further experimental works. Of these, fifteen were paired among themselves in all possible combinations at 2.5% *malt-agar* slants and allowed to grow for three weeks under normal laboratory conditions.

After three weeks, the paired cultures were carefully examined. It was clearly observed at this stage that certain reactions were exhibited at the junctions of the two interacting mycelia. The types of reactions were carefully recorded and the presence or abscence of clamp-connexion have been considered as the criteria for compatibility or incompatibility of a testing pair respectively.

Results

The results have been given in the Table 1 and Table 2. Compatible reactions are shown by+signs, while all sorts of incompatibility by—signs.

Table 1. *Showing the compatible and incompatible pairings by the fifteen monosporous mycelia derived from a single sporophore of* Polyporus agariceous.

		AB				ab			Ab						aB	
		1	3	6	7	10	12	13	2	4	5	11	14	15	8	9
Ab	1	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−
	3	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−
	6	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−
	7	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−
ab	10	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	12	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	13	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
Ab	2	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	4	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	5	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	11	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	14	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	15	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
aB	8	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−
	9	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−

From Table 1, it is observed that mycelia of 1, 3, 6 and 7 when paired with any of 10, 12 or 13 form clamp-connexion and mycelia of 2, 4, 5, 11, 14 and 15 only form clamp-connexion when they were paired with 8 and 9 mycelia. Therefore, it is clear that *Polyporus agariceous* is heterothallic and sexually tetrapolar, and as such agrees with *Leucoporus arcularies* as shown by Vandendries (1936).

The monosporus cultures obtained from a single fruit body fall into four distinct sex group based on their ability to produce clamp-connexions in some of the combinations. In a tetrapolar species the four spores of a basidium have nuclei of different genetic constituents, designated as *AB*, *Ab*, *aB* and *ab*. The mycelia resulting from them consequently contain the same genetic constitution and therefore, they were fundamentally different from another. As regards the mating of mycelia and the process of diplodization resulting in typical secondary mycelia with clamp-connexion. The necessary condition in this case

Table 2. *The different types of reaction shown by the fifteen interacting monosporous mycelia derived from a single sporophore of* Polyporus agariceous.

		AB				ab			Ab						aB	
		1	3	6	7	10	12	13	2	4	5	11	14	15	8	9
AB	1	N	N	N	N	C	C	C	I	I	I	I	I	I	A	A
	3	N	N	N	N	C	C	C	I	I	I	I	I	I	A	A
	6	N	N	N	N	C	C	C	I	I	I	I	I	I	A	A
	7	N	N	N	N	C	C	C	I	I	I	I	I	I	A	A
ab	10	C	C	C	C	N	N	N	A	A	A	A	A	A	I	I
	12	C	C	C	C	N	N	N	A	A	A	A	A	A	I	I
	13	C	C	C	C	N	N	N	A	A	A	A	A	A	I	I
Ab	12	I	I	I	I	A	A	A	N	N	N	N	N	N	C	C
	4	I	I	I	I	A	A	A	N	N	N	N	N	N	C	C
	5	I	I	I	I	A	A	A	N	N	N	N	N	N	C	C
	11	I	I	I	I	A	A	A	N	N	N	N	N	N	C	C
	14	I	I	I	I	A	A	A	N	N	N	N	N	N	C	C
	15	I	I	I	I	A	A	A	N	N	N	N	N	N	C	C
aB	8	A	A	A	A	I	I	I	C	C	C	C	C	C	N	N
	9	A	A	A	A	I	I	I	C	C	C	C	C	C	N	N

C—compatible, N—neutral, A—antagonistic, I—inhibitory.

is that a double heterozygosity is to be restored as in the combinations, $AB \times ab$ and $Ab \times aB$. Failure of production of typical heterozygotic condition can also be observed in the combinations $AB \times Ab$, $aB \times ab$, $AB \times aB$ and $Ab \times ab$ and the peculiar phenomena of 'antagonism' and 'inhibition' are to be noted. However, no clamp-connexion is expected in pairing between the mycelia of the same sexual phase.

In Table 2, different types of reactions that have been shown by the interacting primary mycelia of *Polyporus agariceous* are given and they are as follows :

Compatible : Two interacting mycelia (haploid) intermingle freely, lose their identity and ultimately form an almost homogenous mat, looking like that of polysporus culture.

Microscopic examination of such mats reveal the presence of typical secondary mycelia with abundant clamp-connexions. Agar medium below the place of contact however, does not show any discolouration Such a reaction in the combination where the double heterozygosity is fulfilled as in $AB \times ab$ and $Ab \times aB$.

Neutral : Two interacting primary mycelia intermingle completely, lose their identity in course of time and eventually form a homogenous mat, but there is never formation of any secondary mycelia recognised by the presence of clamp-connexion. Sometimes, however, the mycelia remain slighty separated from each other at line of contact and thus appear to be somewhat antagonastic. But careful examination of these cultures show the presence of submerged intermingled hyphae at the juncture of the two mycelia. There is, however, no remarkable discoloration of the medium below the line of contact. Such cultures have been found to contain no clamp-connexions and occur in combinations $AB \times AB$, $Ab \times Ab$, $aB \times aB$ and $ab \times ab$.

Antagonistic : In this case, two primary mycelia do not intermingled and leave a distinct line separating the aerial mats. A narrow line of aversion is also noted in some cases. But in no case, secondary mycelia with clamp-connexion can ever be found. The colour of the medium below the line of aversion is slightly changed. Antagonistic reactions have been found in the reactions $AB \times aB$ or $Ab \times ab$, where the double heterozygosity is hampered by the presence of the same recessive factor B or b.

Inhibitory : This reaction presents an extreme case of antagonism, as the reacting haploid cultures never intermingle with each other, thus maintaining respective individualitis and as such

no secondary mycelia with typical clamp-connexions can be expected from these cultures. The colour of the medium, however, changed to dark brown below the space of aversion. Inhibitory reactions have been observed in the combinations $AB \times Ab$, or $aB \times ab$, where the heterozygosity is perturbed due to the presence of the dominant factor A or a.

Discussions

It has been found that monosporus cultures of the fungus are devoid of clamp—connexion, while polysporus cultures have them. It has also been noted during the experimental period that mycelium from a single spore sometimes produce stalk-like structures, but never true pilei when grown in culture, while that of polysporous origin almost always produce typical fertile sporophores under identical conditions. This indicates that the former type of mycelia is in the primary state, while the latter type with clamp-connexion reveal the secondary nature. Thus, the heterothallic nature of the fungous is evident. as suggested by Mounce (1921).

Similar reactions on antagonism and inhibition on sexual barrages and compatibility have been noted in case of other Basidiomycetes by Vandendries (1932, 1933), Vandendries and Brodie (1933), Verral (1934), Qaufert (1936 and many others.

According to Whitehouse (1949), instances are not uncommon where species have been recorded as bipolar by one author and tetrapolar by another. It is not wise, therefore, that a fungus is strictly bipolar or tetrapolar. Whitehouse (1949) also reported that of the heterothallic species so far investigated in Hymenomycetes, about 61 % are tetrapolar and 39 % bipolar.

The two allele system of heterothallism limits compatibility to 50 % of random matings, not only between the progeny of a single fruit body but also in the population as a whole. In Hymenomycetes, compatibility is controlled by multiple alleles at one locus. A in the bipolar and A and B in the tetrapolar species. In many tetrapolar basidiomycetes, the two mating type loci A and B control different parts of the process of dicaryon formation.

Parag (1962) and Fulton (1950) reported that the formation of clamp-connexions is controlled by the A locus, since only when this locus is heterozygous are clamp or false clamp are formed. Nuclear migration is controlled by the B locus, it only occur when the mated mycelia possess different B alleles. Takemaru (1961) has claimed that a similar functional differentiation between the A and B mating type loci in eight other tetrapolar Hymenomycetes.

Basing upon the above ideas, the experimental data of the present investigation can be interpreted. If the data of the Table 2 is analysed, it can be stated that in the present case also the A locus controlled the nuclear migration and the B locus controlled the formation of clamp-connexion. Further study is in progress to find out the real nature of role of these two locus A and B in this tetrapolar species.

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