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Invited Article

Studies on Some Aspects of Karyological Phenomenon of Wood Rotting Fungi

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Abstract : *A forest is vital to the national economy. Forest checks soil erosion and curbs environmental pollution which is one of the serious head ache of the present century. In West Bengal (a state of India) forests constitutes only about 14% of the total area and are distributed rather unevenly. One of the valuable materials from the forests in any developing country is timber. Timbers form one of the important resources in national economy. Certain karyological aspects of the timber / wood rotting fungi has been discussed herewith.*

Key words: *Forest Pathology, Karyological Analysis, Shorea robusta Gaertn. f. (Sal), Basidiomycetes*

Introduction

Forest pathology originated in the study of tree diseases and wood decay. In urban park and recreation sites, heartwood decay appears to be a major problem. It has been ascertained that the variety of factors bring about destruction and deterioration of valuable trees and timbers, the main causes being mechanical wear, physical and chemical decomposition, insect damage and decay by fungi. It is obvious that anything, which may have some influence to reduce the annual output of timber production or destruction of timbers, should receive immediate attention. It is well-known that reduction of timber output in forests may happen due to several causal factors whereas destruction of timber is primarily accomplished by disintegration or decay of host tissues. However, tissue disintegration is known to be a primary mode of pathogenesis in forest pathology.

In India, investigations on fungi causing decay of important timbers, belonging mainly to the members of Basidiomycetes, have been carried out by Bose (1930); Banerjee and his co-workers (1943, 1944, 1945, 1954, 1955, 1957, 1959, 1960, 1962 1965 and 1966); Bagchee and others (1951, 1953, 1954a, 1954b, 1960); Bakshi et al. (1961); Bakshi (1971) and Puri (1960). Though Sal (*Shorea robusta* Gaertn. f. of the family Dipterocarpaceae) tree forms one of the chief forest resources in India yet no detailed elaborative works on destruction of Sal by higher fungi and other microorganisms have been done extensively. Sal is mainly timber yielding plant. From the economic point of view, it is used as a construction material for bridge, railway sleepers, houses etc. and

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is also widely used in the timber industries. Millions of rupees worth of timber is annually lost due to decay of Sal tree. Sal trees of all ages are prone to attack of various heart-rot fungi. This phenomenon, i.e. susceptibility to fungal attack, is interesting from the point of silviculture of Sal and for the study of the Sal diseases. There are at least 24 species of fungi belonging to the Hymenomycetes which behave as facultative parasites of Sal. These fungi can be described as parasites of the second order to distinguished them from the primary parasites which produce active root and stem rot. It has been experimentally proved in several cases that these fungi enter the living trees through dead sapwood in the collar or butt region or through broken branch stubs or wounds deep enough to contact the heartwood where they are finally established. The dynamic response of sapwood to infection is more than heartwood. The inhibitory materials that effect resistance to infection by fungal pathogens and metabolic shunts that occur after infection are lacking in sapwood.

Wood destroying basidiomycetes normally occur in nature as dikaryotic mycelia and almost all laboratory investigations conducted on or with them use dikaryotic mycelial cultures. Few attempts appear to have been made to compare the physiological behaviour of monokaryotic mycelia with that of related dikaryotic mycelia. These questions became of considerable interest to us following the discovery that, in the presence of wood preservatives and other fungitoxic agents, dikaryotic mycelia of wood destroying basidiomycetes often reverted to the monokaryotic conditions. As such in the present investigation an attempt has been made to study the effect of some environmental factors on the growth and the decaying capabilities of monokaryotic and dikaryotic mycelia of *Lenzites abietina* Fr. and *Polyporus shoreae* Wakef. of Sal wood as these two fungi are very common in association with Sal wood in West Bengal. During rainy season both the fungi are found to grow abundantly on Sal timbers in timber yards in and around Calcutta and cause heavy economic losses every year.

The findings of the present investigation on the decaying capabilities of wood of *Shorea robusta* Gaertn. f. by the four monokaryotic (AB, ab, Ab and aB) and two dikaryotic (AB+ab; Ab+aB) mycelia of two wood rotting fungi namely *Lenzites abietina* Fr. and *Polyporus shoreae* Wakef. make it possible to discuss some salient features in a general way.

A comparison of nature of growth, linear growth rate and oxidase activities using two dikaryotic mycelia (AB+ab and Ab+aB) and their respective four monokaryotic mycelia (AB. ab and Ab; aB) indicated clearly genetic variation in all the responses exhibited by both the fungi namely *L. abietina* and *P. shoreae.*

The effect of temperature on the growth of the fungi revealed that 30°C was the optimum temperature for growth of all the four monokaryotic and dikaryotic mycelia of *L. abietina* and *P. shoreae*. Here also there was clear indication of the existing genetic variational responses exhibited by the test fungi.

The effect of hydrogen-ion-concentrations on the growth all the types of mycelia of the test fungi revealed that all of them acid-loving though they differed widely among themselves

with regard their individual responses against each pH grade. The optimum pH for the growth of both the test fungi was found to be 6.0. This was found to be best for all the monokaryotic and dikaryotic mycelia of the test fungi. It was further found that all the types of mycelia of test-fungi grew well within the range of pH 5.0 and 7.0. Similar nature of optimum pH requirement for growth was recorded in different species of fungi (Maechum, 1918; Montgomery, 1936; Rennerfelt and Paris, 1953; and Etheridge, 1955). The evidence so far obtained show convincingly that pH is an environmental factor of enormous importance in modifying their metabolic activity. These effects are always intricately correlated. In exhibiting growth responses at different pH, the four monokaryotic and two dikaryotic mycelia of both the test fungi showed wide variation which indicated their variation in genetic constituents.

After evaluating the role of different sources of carbon on the growth of all the types of mycelia of the fungus. It was observed that glucose was the best sources among the sugars and the organic acids used. Next to glucose, maltose appeared to be the best source of carbon followed by sucrose, sorbitol, xylose, sodium acetate, sodium succinate, mannitol and starch. Glucose was taken up by fungi for further metabolism. Maltose was broken down into two molecules of glucose and sucrose was broken down into fructose and glucose. The growth data suggested that the required enzymes, namely b-fructofuronosidase and a-glucosidase were produced by all the types of mycelia of the test fungi. Xylose and starch were not broken down appreciably due to lack of the specific enzymes in required amount. Among the organic acids, all the types of mycelia of test-fungi were able to utilize the two acids of Tricarboxylic acid cycle as source of carbon. This fact indicated that all the test fungi could possibly use the TCA-cycle for metabolism of organic acids. After detecting the role of nitrogen on the growth of the test fungi, it was found that ammonium tartrate, and peptone were the best sources among the inorganic and the organic nitrogen respectively. Among the Inorganic sources, sodium citrate came next and then urea. of the organic sources, asparagine came next to peptone.

One of the main uses of nitrogen is the synthesis of proteins with the exception of certain amino acids (primary amino acids) and ammonia, most nitrogen sources undergo modification before entering the synthetic metabolic pathways. Nitrates are presumably reduced to ammonia before assimilation. Those amino acids (secondary amino acids) which do not enter directly into the metabolic pathways leading to the synthesis of proteins are probably deiminated. The process of deamination releases nitrogen in the form of ammonia, which is then utilized by fungi. Ammonia enters and leaves the cell by passive diffusion of the undissociated NH_3 molecule. It is believed that the major and primary reactions of ammonium assimilation is the formation of glutamic acid. Other amino acids can be formed from glutamate by transamination (Cochrane, 1958). Asparaginc has been found to be very active as amino group doner to transamination reaction (Jennison et. al., 1955).

After studying the data on the decay resistance tests, it has been found that the sapwood of *Shorea robusta* has been appeared to be very much susceptible to all the monokaryotic and

the dikaryotic mycelia of both the test-fungi, while the heart wood has been found to be resistant to all of them. The under lying reasons for this is not, however, known. It may be possible that the heart wood contains some substances which have inhibitory effect on growth of both the fungi. This may also be the lignin content of the cell wall or the nitrogen content of the heart wood. Of the two types of mycelia, the dikaryotic mycelia have been found to be more active than the monokaryotic ones of both the test fungi. It is possibly due to its better ability to produce the required enzyme, which is again definitely genetically controlled. The changes in the chemical composition of the sapwood of *Shorea robusta* due to the attack of the test fungi have revealed that all of them have utilized lignin of the host wood. *L. abietina* is able to utilize more lignin than *P. shoreae*. The ability of the dikaryotic mycelia cause more decay of wood, as found in the present investigation, has also been reported in *Lentinus edodes* and in *Lenzites tabea* although there are reports that monokaryotic are more destructive than dikaryotic ones Aoshima (1953, 1954).

The cellulose and lignin component of the Sal wood are found to be decomposed by the four monokaryotic mycelia and two dikaryotic mycelia of both the test-fungi of which the mycelia of *L. abietina* are more active than *P. shoreae*. Lignin degradation is a very complicated one and the white rot fungi are only capable of degrading the lignin effectively. In the present investigation it has been found that all the mycelia of this test fungi are able to release vanillic acid and syringic acid from the lignin of heart-wood of Sal. These two compounds are probably released by the enzymatic splitting of arylglyceral-b-aryl ether bounds in lignin. These could also have been released from the side groups than from the proper polymer.

The native lignin is soluble in more polar organic solvent while the acid lignin is insoluble in organic solvents. However, the alkaline lignin is soluble in methanol, ethanol, dioxane, pyridine, sodium hydroxide and ammonium solution, but insoluble in acetone, chloroform, acetic acid, petroleum ether, ether, benzine and water. These observations tally with report on lignin of white-scott pine wood.

The lignin decomposition has revealed that it is a relatively slow process and conditioned by some critical cultural parameters in cases of both the test fungi. These types of lignin decomposition might be the same for all the white rot fungi in general (Kirk et. al., 1973).

The effect of the different carbon sources on the lignin decomposition by the monokaryotic and dikaryotic mycelia of both the fungi clearly indicate the views that growth substrates support ligr.in metabolism (Kirk et. al., 1973). The specific role played by the growth substrates like glucose in the decomposition of lignin is very difficult to interpret as because lignin is potentially one of the important and most abundant sources of carbon and energy for the fungi in nature. But the phenomenon may be assumed to be possibly due to two reasons: (a) that the energy released during decomposition of lignin is not sufficient at all to support the growth of the fungi and (b) that in absence of the growth substrates, the level of ligninolytic activity is too low to allow the lignin decomposition in required rate.

The optimum temperature for lignin decomposition by all the monokaryotic and dikaryotic mycelia of the test fungi was found to be 30°C which was also the optimum for their vegetative growth. Similar observation was reported by Lindegreen (1933). It was also found in the present investigation that pH (5.0) had a marked effect on the lignin decomposition. The results further revealed that the strong effect of pH was not due to the amount of vegetative growth as the optimum pH for growth was 6.0.

The effect of nitrogen on the lignin decomposition by the monokaryotic and dikaryotic mycelia of both the fungi revealed that high nitrogen content decreased the lignin decomposition whereas low nitrogen content increased the lignin decomposition. There were several reports that nitrogen amendments exhibited in either increased or decreased lignin decomposition by fungi depending on sources of nitrogen used i.e. amount of N2 availability whether low or high. In the present case the inhibition of high nitrogen for the lignin decomposition might be due to their inabilities to activate their ligninolytic system at a high concentration of nitrogen. These might be also for the following reasons namely (1) high N favours rapid utilization of the growth substrate (2) high N competes for its metabolism with lignin metabolism for the same required co-factor(s) and N regulates the synthesis of one or more components of the lignin-degrading system.

The effect of concentration of lignin on its decomposition by the monokaryotic and dikaryotic mycelia of both the fungi revealed that even at 5mg lignin/10ml culture, the methoxy degrading activities were more than the ring and side chain degrading activities. The similar observation was reported on another white rot fungus, *Phanerochaete chrysosporium*.

The data on the ligninolytic system of both the fungi revealed that the ligninolytic system is synthesized in the medium in absence of lignin and the system was not induced by Lignin. It clearly indicated the nonspecific nature of the system. Similar observations were also reported on *Phanerochaete chrysosporium* and *Coriolus* versicolor other two white rot type wood destroying fungi. The non-inducing nature of the ligninolytic system and the diversity of lignin subunit structures also suggested the nonspecific or low specificity of the ligninolytic system of the basidiomycetous fungi in general. It revealed that lignin did not serve as a growth substrate for both the fungi and also was possibly a consequence of low ligninolytic system and low specificity.

The data on the effect of nitrogen on the ligninolytic system of both the fungi suggested that synthesis of the ligninolytic system was associated with nitrogen starvation and with a shift to secondary metabolism. Moreover, with the availability of high nitrogen there was production of large biomass in expense of the utilization of the available carbon sources. The ligninolytic system of both the fungi were stimulated by monosaccharide and other major carbon sources in the natural substratum of the ligninolytic basidiomycetes were polysaccharides of the wood i.e. cellulose and hemicellulose. These polysaccharides were not degraded at high rates for sufficient production of the ligninolytic system. Moreover, the fungi were found to operate the ligninolytic system in a wood substratum environment which was basically nitrogen poor woody tissues.

A comparison of morphological growth characters, decaying capabilities and ligninolytic enzyme system of the four monokaryotic and two dikaryotic mycelia of *L. abietina* and *P. shoreae* indicated a significantly higher genetic variation in both the fungi. It was further evident from the present study that monokaryotic mycelia of the same parental strain exhibited some variations in the responses with regarded to the above-mentioned characters.

The data on the ligninolytic activities of the test fungi revealed that the enzyme production could be increased by the hybrid strains produced by crossing compatible monokaryotic strains. The genetic regulation of the synthesis of phenoloxidases and laccase structure had also been reported. It was suggested that sympatric speciation might be common in Basidiomycetes. In the present study the physiological and morphological differences and the production of the ligninolytic enzymes in the monokaryotic and dikaryotlc mycella of *L. abietina* and *P. shoreae* revealed the typical Mendelian segregation pattern.

The experimental findings further revealed that the ligninolytic activities leading to wood decaying capabilities and lignin decomposition of both the test fungi are genetically controlled possibly at a multigenic level. These genes are probably arranged in close sequence and are not separated during genetic exchange and consequent segregation to off springs from parents. The expression of these genes also remains unimpaired in the different states of A and B factors which show clearly almost identical capabilities of the two monokaryotic mycelia, Ab and aB.

The increased activities in the dikaryotic mycelia are due to the presence of two sets of such gene sequence i.e. double dosing effect. Such double doses lead to accelerated action up to a certain level due to gene interaction.

Conclusion

In the present Investigation the decaying capabilities of four monokaryotic and two dikaryotic mycelia of two white root wood rotting fungi namely *Lenzites abietina and Polyporous shoreae* were studied using the wood of Shorea robusta Gaertn. It revealed that all the four monokaryotic and two dikaryotic mycelia of *L. abietina* were more responsive than those of *P. shoreae* in all the cases.

The growth rate of the dikaryotic mycelia of *L. abietina* varied from 0.44 to 0.52 cm/day whereas the same for the monokaryotic mycelia varied from 0.28 to 0.34 cm/day. The oxidase reaction was much more in the dikaryotic mycelia than those of the monokaryotic mycelia. The same was true of *P. shoreae*. The growth rate of dikaryotic mycelia of *P. shoreae* varied from 0.37 to 0.46 cm/day and the same for monokaryotic was 0.20 to 0.30 cm/day. In liquid culture the growth of the dikaryotic and monokaryotic mycelia of *L. abietina* and *P. shoreae* varied from 118-136, 68-84, 110-112, 62-72 mg/50 ml respectively.

It was found that 30° C was the optimum temperature for all the monokaryotic and dikaryotic of *L. abietina* and *P. shoreae*. The optimum pH for growth of all the monokaryotic and dikaryotic mycelia of both the test fungi was found to be 6.0.

Glucose and ammonium tartrate were the best sources of carbon and nitrogen for the growth of all the four monokaryotic and dikaryotic mycelia of *L. abietina* and *P. shoreae*.

The monokaryotic and dikaryotic mycelia of *L. abietin*a caused more decay of both the sapwood and heartwood of Shorea robusta than those of *P. shoreae*. The decaying capabilities of the dikaryotic and monokaryotic mycelia varied from 38.68 to 44.54% and 29.16-33:69% In sapwood and 7.60-8.13% and 4.34-5.32% in heartwood whereas the same was 30.14-36.22% and 25.16-28.68% in sapwood and 5.10-5.80% and 3.92-4.35% in heartwood in case of *P. shoreae* respectively.

Chemical analysis of sound and decayed wood (both sap and heart) also correlated with the results of "Decay resistance test". Both the fungi were lignin-destroyer and cellulose decomposer; hence, they were "white rot" type of fungi.

The lignin decomposing capability of the monokaryotic and dikaryotic mycelia of *L. abietina* varied from 33-42 and 46-54 and the same for *P. shoreae* 26-38 and 38-46 respectively.

It was also found 30° C and pH 5.0 were the optimum for the decomposition of lignin by all the monokaryotic and dikaryotic mycelia of *L. abietina* and *P. shoreae*.

Glucose and sodium nitrate were the best carbon and nitrogen sources respectively for the decomposition of lignin by the monokaryotic and dikaryotic of *L. abietina* and *P. shoreae*. It was further revealed that 1.00 mM of glucose and low nitrogen level were the best sources for the ode composition of lignin by both the test fungi.

The effect of lignin concentration on the decomposition of lignin revealed that at 5 mg lignin/ 10 ml the ring- and side chain decomposition almost became saturated whereas the methoxyl decomposition was still continued by both the test fungi.

It further revealed that Ligninolytic system of both the test fungi was nonspecific and non-dividable. It was revealed that the maximum Ligninolytic activities were found in low nitrogen level in both the fungi.

It has been discussed and compared in detail with the help of the available information of other scientists in a separate chapter under discussion and it was assumed that the decaying capabilities and Ligninolytic enzyme system were genetically controlled and the same was segregated in the Mendelian segregation type.

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