STUDIES IN THE BIOLOGY OF WOOD-ROTTING FUNGI OF BENGAL

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I. Introduction

The rôle of fungi as agents for the destruction of timber was established by Robert Hartig.[20,21] Wood-rotting fungi are very common in the forests of India, Europe, America and elsewhere. In many cases, a large percentage of timber is found infected. This has led to an increasingly larger number of workers devoting themselves to the study of tree and timber diseases. Among these may be mentioned Ward,[39] von Schrenk,[37,38] Lyman,[25] White,[40] Rhoads,[31] Long and Harsch,[24] Buller,[15,16] Fritz,[19] Baxter[4] and Mounce.[26]

The study of cultural characters for distinguishing wood-rotting fungi is also of great importance. Until recently, identification of the causative organism producing the rot was made from the sporophore found on the external surface. They are, however, not always present on rotten wood, and even when present, may not belong to the fungus actually destroying the internal tissues. In such cases, a pure culture of the fungus from the infected wood can be made.

The most important contribution to the study of this subject in India comes from Bose.[6–13] He has given a systematic account of the Bengal Polyporaceæ and described the life-histories and cultural characters of some of them. But a vast number of wood-rotting fungi still await investigation. The present paper makes a contribution to our knowledge of the life-histories and cultural characters of six wood-rotting polypores of Bengal, namely, *Polyporus brumalis* (Pers.) Fr., *P. friabilis* Bose, *P. rubidus* Berk., *P. ochroleucus* Berk., *Polystictus steinheilianus* Berk. and Lév., and *Merulius similis* B. et Br. Geographical distribution and occurrence of these species have been compiled from the reports of Berkeley,[5] Bresadola[14], Butler and Bisby,[17] Lloyd,[22,23] undkur,[27] Oudemans,[28] Petch,[29] Rabenhorst,[30] Saccardo,[33] Theissen[36] and Bose.[6–10]

II. Cultural Methods and Conditions of Growth

A. *Types of culture*

The initial cultures of all the species under investigation were made either from spores or from the tissue of the sporophore. A spore-culture was, however, preferred to a tissue-culture because in the former case the life-history of the fungus from spore to spore could be studied.

The spore-discharge was obtained from a fresh sporophore on 3% sterile agar contained in the lower lid of a sterile petri-dish, while a trimmed rectangular block of the sporophore was fixed eccentrically to the inner side of its upper lid with the hymenial surface directed downwards and the whole was placed inside a moist chamber for a few hours. The spores, thus deposited, were then transferred aseptically to culture tubes containing *potato-dextrose agar* and within a day or two, several polysporous mycelia were obtained. In *Merulius similis*, polysporous mycelia could not be obtained by this method and monosporous cultures, both by the 'dilution method' and by the 'streak method' were made.

In making tissue-cultures, the technique adopted by Duggar[18] was mainly followed. Fresh, dried sporophores, however, were used, since it was noticed that contaminations, especially bacterial, were large when fresh but water-soaked fruit-bodies were taken. Tissue-cultures were also successfully made from comparatively dry older specimens, but in such cases, a large inoculum was preferred to a small one owing to the likelihood of the former containing a larger percentage of viable mycelia.

B. *Media used*

For the comparative study of cultural characters of these fungi, the following media were used : (1) *potato-dextrose agar* prepared by Fritz's method,[19] (2) *malt-extract agar* (25 gm. agar and 30 gm. malt extract in 1,000 c.c. distilled water), (3) *oat-meal-agar* (25 gm. agar and 50 gm. quaker oats in 1,000 c.c. distilled water), and (4) *Brown's potato-starch agar* (0·2 gm. aspargin, 2·0 gm. glucose, 0·75 gm. magnesium sulphate, 10·0 gm. potato-starch, 1·25 gm. potassium phosphate and 25 gm. agar in 1,000 c.c. distilled water). Eight c.c. of the medium were poured in each culture tube which was then plugged, sterilised and slanted to the same degree in order to provide surface of uniform area. The pH values of the media after sterilisation, were determined by *k*-potentiometric method and found to be 5·2, 5·2, 4·5 and 5·2 respectively.

A new medium for the cultivation of wood-rotting fungi was described by Badcock.[1] According to the author, many wood-rotting fungi which fail to fructify in other commonly used media readily produce fruit-bodies in this medium. This medium was tried successfully but 'pine wood ash' was used instead of 'Scots fir ash' which was not available.

Wood-block cultures were also made both in Roux-tubes and in Erlenmeyer flasks. Normal and healthy pieces of wood of *Mangifera indica*, *Bambusa arundinacea*, *Cocos nucifera*, *Shorea robusta*, *Ficus religiosa* and *Ceriops Roxburghiana* were cut into convenient sizes ($3'' \times \frac{3}{4}'' \times \frac{3}{4}''$), and cultures were made by Bose's method.[12] Though the fungi under consideration were mainly collected from bamboo stumps, blocks of the wood of other monocotyledonous and dicotyledonous plants were used in order to find out whether the fungi could attack these hosts as well.

C. *Light, Temperature and Humidity*

(1) *Light.*—All the cultures were exposed either to the diffused light of the laboratory or to complete darkness. In the former case, cultures were placed in an inclined position on the shelves of an almirah situated at a distance of about 15 feet away from the window and these received diffused light from morning till evening. For complete darkness, the doors of an almirah were covered with thick black papers in such a way that no light entered into it when the doors were closed.

(2) *Temperature.*—The cultures were subjected to three ranges of temperature, namely, constant temperatures of 22° C. and 33° C. and a variable temperature of 23°–27° C. of the laboratory during the months of November and December, 1941. All wood-block cultures were, however, kept only in diffused light and in ordinary room temperatures of the laboratory.

(3) *Humidity.*—The relative humidity at the above temperature conditions, was determined as 69–75% at 22° C., 35–42% at 33° C., and 35–58% at 23°–27° C.

D. *Number of tubes inoculated*

Culture tubes $6'' \times \frac{1}{2}''$ in size made of neutral glass were used for the study. In order to make the comparative study thorough and at the same time avoiding any risk, three tubes were used for each treatment and the results and conclusions were based on the average of these.

E. *Important diagnostic characters in culture*

The following characters were studied in the cultures of the various species : (1) macroscopic characters including rate of growth, texture and colour of the aerial mycelium, staining and decolouration of the medium, appearance and drying up of glistening drops of liquid, etc.; (2) microscopic characters such as types of hyphæ, their septation, branching, colour, clamp-connection, spore-formation and crystalline material. Descriptions of basidia, basidiospores or other anatomical characters in the fruit-bodies in culture have also been given. All observations were made from fresh mounts in water. Preparations of lactophenol, 50% glycerine, staining with lactophenol cotton blue were also made.

III. Descriptions of Species Investigated

1. Polyporus brumalis (*Pers.*) *Fr.*

Geographical distribution

The species is widely distributed over the tropical and temperate regions of the Old World and only in the temperate regions of the New. In North America, it is common in Canada (Manitoba), Lake Superior (Isle Royale), New Hampshire, Michigan, South-West Virginia, Ohio, Iowa, New York, Wisconsin and in the Middle and Upper Carolina. In Europe, it occurs in Great Britain, Spain, France,

Netherlands, Denmark, Czechoslovakia and Esthonia. The fungus is also found in the mountainous forests of Basehberg near Somerset East and Cape of Good Hope in South Africa ; in India, it is found in Bombay, Punjab, Bengal and Orissa. It is also found in Central Asia and Siberia in the North. In Australia, it occurs in New South Wales, Victoria and Queensland.

Occurrence

It has been recorded on several hosts, viz., *Corylus betulus* L., *C. Avellana* L., *Alnus glutinosa* Gaertn., *Betula verrucosa* Ehrh., *Fagus sylvatica* L., *Acer platanoides* L., *Aesculus hippocastanum* L., *Fraxinus excelsior* L., *Tilia cordata* Mill., and *Saccharum munja* Roxb. It has been collected by the authors from suburbs of Calcutta (Behala) where it was growing saprophytically on a dead and fallen branch of a tree.

Fungus in culture

(i) *Habit of growth.*—On *potato-dextrose-agar*, young cultures developed a felty mat in most cases and a sub-felty to felty mat in others. The advancing hyphæ formed a colourless appressed subfelty to felty zone. Irregular condensation of the mycelium in young cultures in darkness at 33° C. made the surface uneven. At 22° C. the cultures exhibited faint zonation. Colourless glistening drops of liquid appeared invariably in cultures 7-days-old. Later on, the mat became compact and uniform. Vigorous growth in darkness at 33° C. was manifested by the evident rolling of the mycelium on the bare glass surface. In most cultures, prominent ridges appeared on the mat making the surface uneven. After 10 days of inoculation, the mat in room temperature became granular at places, though granules in darkness were less than those under diffused light. Later on, however, these granules disappeared. Within a fortnight, the glistening drops of liquid disappeared, growth declined and the mat became thin and appressed. In some cases the mat was covered by a fresh growth of thin, colourless mycelium in old culutres. On *oat-meal-agar*, the mat under all conditions presented a subfelty to felty appearance except at 22° C., where the mat was appressed showing zonation. The advancing zones were distinctly appressed and sodden. The other characteristics as well as the later development of the mat were like that described for the previous medium. On *malt-extract-agar*, the texture was more or less similar to that on oat-meal-agar. In darkness at 33° C. and in diffused light at 22° C. the young hyphæ at the advancing zone were parallel and combed like silk. On *potato-starch-agar*, the growth was poorest in comparison to other media. The mats in most cases were thin, appressed and sodden. This texture also persisted in old cultures.

(ii) *Colour**.—On *potato-dextrose-agar*, colouration appeared after a week's growth, these being shades of cinnamon buff, light vinaceous fawn, pale ochraceous salmon, light ochraceous salmon, sorghum

* According to Ridgeway.[32]

brown and light vinaceous cinnamon. In early stages the colour confined mainly to the inoculum, the hyphæ at the advancing zone remaining colourless. Pigmentation was accentuated in diffused light and tinting in darkness appeared later. After 10-days-growth all the earlier shades disappeared and deeper tint of seashell pink, zinc orange, ochraceous orange, hays russet, mars brown, pale vinaceous drab, light buff, warm buff and orange pink appeared. Colour was best developed in room temperature (23°–27° C.) and for 33° C., pigmentation was denser in diffused light than in darkness. In 22° C. varied colouration was much pronounced in dark than in diffused light, in the latter case, pigmentation developing after a fortnight. Thus, in cold room diffused light definitely retards pigmentation, while darkness accentuates it, but in the other two temperature conditions, reverse was the case. After 21-days-growth, most of the earlier shades persisted, but a deeper shade of liver brown and orange rufus appeared. After a month's time, the shades deepened still further to deep chrome, hæmatite red and vandyke brown. On *oat-meal-agar*, the sequence of colour development as well as the range of colour were like those described above. On *malt-extract-agar*, the intensity and the range of colour were much less. The difference as regards the conditions of light and darkness, if at all present, was very slight. On *potato-starch-agar*, colouration was the poorest and developed after 21-days-growth. The tinting was very light with shades of light buff, pale ochraceous buff, sea-shell pink, pale vinaceous drab, and liver brown. The colour of the medium was changed.

(iii) *Rate of growth.*—The slants were covered with moderate rapidity in all the media, but advance in *potato-starch-agar* was slower in comparison to other media. The rate of growth was highest at 33° C., moderate at room-temperature (23°–27° C.) and comparatively less in 22° C. In general, growth in diffused light was more rapid than growth in darkness.

(iv) *Sporophore production.*—Stalks of fruit-bodies appeared in several cultures under different conditions but the first tube to fructify was on *oat-meal-agar* in diffused light at 33° C. Three stalks arose from the base, two of which were suppressed after some time while the third one developed. Later on, similar stalks appeared in other conditions but comparatively larger number fructified at incubator-temperature. The stalks always had a tendency to branch. In no case, however, pileus was formed. Bits of mycelium from 7-days-old cultures on *potato-dextrose-agar* were transferred to wood blocks in Roux-tubes as well as in flasks. Growth started within a few days with its characteristic colour and zonations as noted on agar media. The mat was thin and appressed. No fruit-bodies, however, developed on wood. Cultures were later grown on the medium advocated by Badcock[1] in Erlenmeyer flasks of 1,000 c.c. capacity each. Normal fruit-bodies with true pilei developed in about a month. A pileus, on sectioning, revealed basidia ($10–12\,\mu \times 5–6\,\mu$), each with four sterigmata bearing basidiospores ($4\,\mu \times 4\,\mu$).

(v) *Mycelium.*—Hyphæ in young and old cultures are of three types, (*a*) thin-walled, hyaline, with dense protoplasmic contents, septate, profusely branched, clamp-connections numerous, 3–4 μ broad ; (*b*) thin-walled, hyaline, with granular protoplasm, septate, sparingly branched, clamp-connections, about 2 μ broad ; and (*c*) thick-walled, hyaline, unbranched and 7–8 μ broad. Chlamydospores abundant both in young and old cultures, terminal and intercalary, with granular contents, 10–12 $\mu \times 10\,\mu$. Abundant crystals of calcium oxalate are present (Text-figs. 1–5).

2. Polyporus friabilis *Bose*

Geographical distribution

Polyporus friabilis is confined to India and is very common in Bengal. Outside Bengal, it occurs in Madras and Orissa.

Occurrence

The fungus usually grows on humus being associated with rotten leaves and grasses. It has been reported to be growing on *Excœcaria agallocha* L. from Madras. The writers collected the material from dead bamboo clumps and palm stems in Calcutta, Howrah and suburbs.

Fungus in culture

(i) *Habit of growth.*—The mycelium spread rather slowly over the agar surface. On the *potato-dextrose-agar*, inoculum, in most cases, was downy or downy to velvety at the beginning and the advancing zone was evident. In 10 days, the mat became compact and felty. Best growth was obtained at 33° C., moderate in room temperature (23°–27° C.) and comparatively poor at 22° C. In the last case, growth appeared as a more or less circular patch with distinct, appressed and sodden advancing zone. In old cultures, growth was vigorous under all conditions. Rolling of the mycelium over the bare glass surface was vigorous at 33° C., less so in room temperature, while no rolling could be noticed in culutres kept at 22° C. After 35 days of inoculation the superficial mat, in all cases, presented a thick matted felt. Colourless glistening drops of liquid appeared in cultures about a month old in diffused light at 33° C. These drops assumed a beautiful pink colouration in about 2-months-old cultures. Later on, the drops dried up. On *oat-meal-agar* the habit of growth was in the main described as above. The advancing zone at room temperature was appressed and like combed hairs. At 33° C., the upper advancing zone was like a pile of velvet. On *malt-extract-agar*, the growth was comparatively poor. In room temperature, the mat was more compact in diffused light than in darkness. The downy texture gave place to cottony mat, and finally to a smooth matted felt. On *potato-starch-agar*, growth appeared to be poorest, while other characteristics were as described for *malt-extract-agar*.

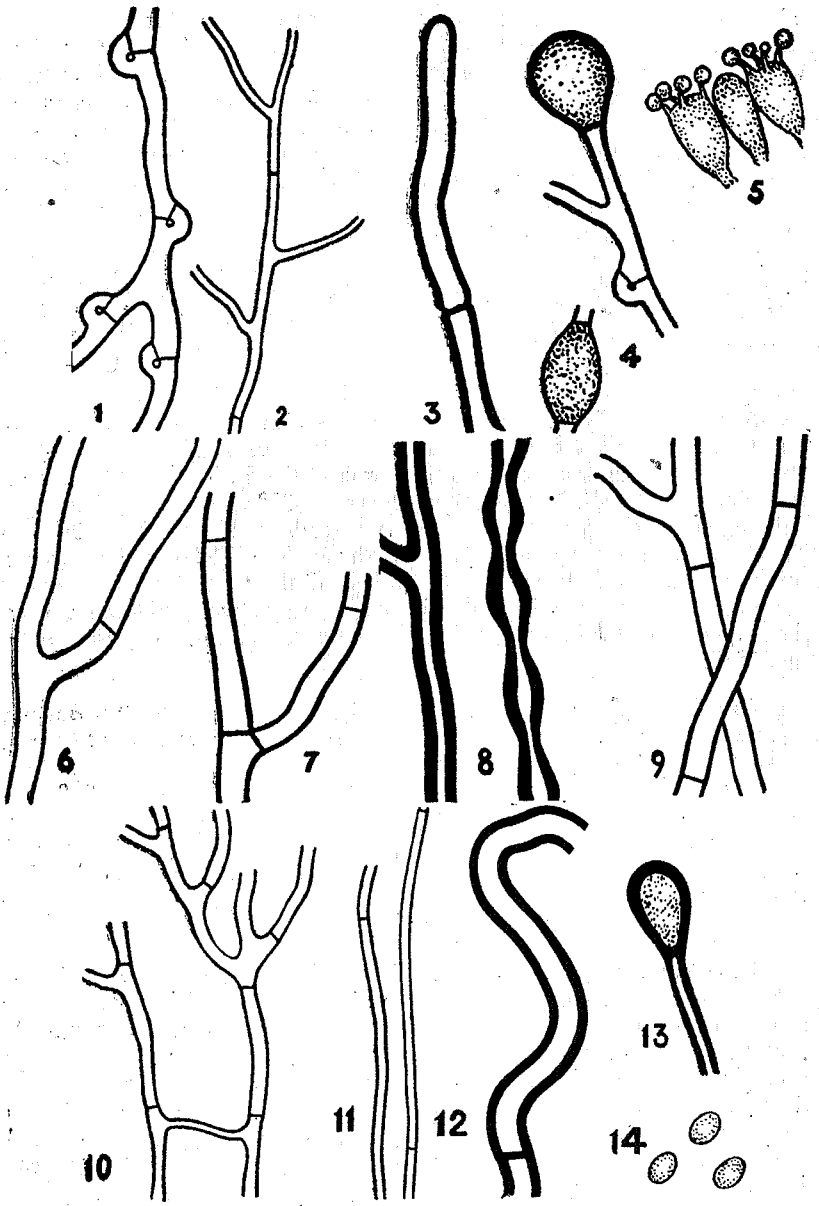
(ii) *Colour.*—On *potato-dextrose-agar*, no colour appeared in 5-days-old cultures except under diffused light at 33° C., where a patch

of deep safrano pink appeared round about the inoculum. In 10 days, colouration appeared in room temperature and at 33° C. but no tinting was noticed at 22° C. This signifies that for this fungus, a low temperature prevents pigmentation, while a higher temperature accentuates it. Again, since colour appeared earlier in diffused light and later in darkness under any condition of temperature, it suggests that light accentuates pigmentation while darkness retards it. Tinting in young cultures was mainly over the inoculum and consisted of shades of light buff, pale flesh colour to flesh colour and pale salmon colour. Other portions of the mat remained colourless, which, later on, developed pigmentation. The earlier shades, later on, deepened to sea-shell pink, salmon buff, pale ochraceous salmon, pale buff, light buff, light ochraceous buff and pale flesh colour. These shades, however, were mixed with white. In 35 days, most of the shades disappeared and deeper tints of salmon orange, light russet, vinaceous, brownish vinaceous, sweet pink and flesh colour appeared. Colour of the medium was considerably changed in old cultures. On *oat-meal-agar* colouration developed late and no colouration was noticed till the 25th day after inoculation except in diffused light at 33° C. where safrano pink, developed on the 5th day, deepened to light buff on the 10th day. The later shades approached nearly those described for the previous medium. On *malt-extract-agar*, tinting of the mycelium was poor as compared to the previous media, while on *potato-starch-agar*, colour developed only in darkness at room temperature and on diffused light at 33° C.

(iii) *Rate of growth.*—The rate of growth was best on *oat-meal-agar* and on *potato-dextrose-agar*, moderate on *malt-extraxt-agar* and least on *pottato-starch-agar*. The advance was rapid in cultures kept at 33° C., moderately high in room temperature, while at 22° C. the advance was very slow. The dual effect of *potato-starch-agar* medium and low temperature had a marked effect on growth, for no sign of growth could be seen in 25-days-old cultures when growth could be evident only in darkness. Growth in darkness was more than growth under diffused light under all conditions.

(iv) *Sporophore production.*—Bits of cultures from 7-days-old cultures on *oat-meal-agar* were transferred to wood-blocks in Roux-tubes and in flasks. Growth started within a fortnight. In Roux-tubes, growth was very vigorous, giving a thick woolly appearance, some mycelium at the lower part penetrated into water and formed a floating mycelial mat. In flasks, the same woolly appearance was seen. The colouration of the mycelia was typically as seen on agar media. Fruit-bodies were developed neither on agar media nor on wood-blocks.

(v) *Mycelium.*—The hyphæ from culture consists mainly of two types, *viz.*, (*a*) thin-walled, branched, septate, 4–4·7 μ broad and (*b*) thick-walled, branched, septate, somewhat beaded in appearance and 4·7–6 μ broad. Crystals of various shapes are present (Text-figs. 6–7).



Text-figs. 1–14—Figs. 1–5. *Polyporus brumalis :* 1. thin-walled hypha with clamp-connections; 2. thin-walled hypha without clamp-connections; 3. thick-walled hypha ; 4. chlamydospores ; 5. basidia with basidiospores. Figs. 6–7. *P. friabilis* **:** 6. thin-walled hypha ; 7. thick-walled hypha. Figs. 8–9. *P. rubidus*: 8. thick-walled hypha ; 9. thin-walled hyphæ. Figs. 10–14. *P. ochroleucus* : 10. thin-walled, much branched hyphæ ; 11. thin-walled, unbranched hyphæ ; 12. thick-walled, unbranched hypha ; 13. terminal chlamydospore ; 14. basidiospores. (×520).

3. Polyporus rubidus (*Pers.*) *Fr.*

Geographical distribution

The fungus has been collected from Brazil and Rio Grande do Sul in S. America. In India, it occurs in Calcutta and suburbs, Sinchul hills (Bengal) and Lokra hills (Assam). It is also found in Burma, Malaya Peninsula, Java, Lang, Alor and Philippine Islands.

Occurrence

It has been reported to grow on dead wood of *Alnus nepalensis* D. Don from Darjeeling. The authors have collected the fungus from dead bamboo clumps in Sonarpur, 24-Parganas, Bengal.

Fungus in culture

(i) *Habit of growth.*—On *potato-dextrose-agar*, and on *oat-meal-agar*, the initial growth of the superficial mycelium produced a subfelty to felty mat with a narrow and appressed advancing zone. Rolling of the mycelium on the bare glass surface in the form of long, weak hyphæ giving a silky appearance began very early in young cultures and is characteristic for the fungus. This rolling was, however, very vigorous at 33° C. Within 10 days after inoculation in room temperature (23°–27° C.) and at 33° C., the mat became compact and felty throughout due to early condensation of the mat with a pile of velvet at the upper advancing zone. At 22° C., there was less condensation of the superficial mat so that it remained loosely felty. The upper advancing zone, moreover, did not exhibit a pile of velvet as stated above. In 35-days-old cultures, the growth characters remained constant under all conditions, each tube exhibiting a semilunar raised velvety region at the upper advancing zone. No glistening drops of liquid were observed during the course of study. As the cultures dried up, the long rolling hyphæ began to recede from the base of the glass tube in the form of dry membranous sheet. On *malt-extract-agar*, the texture of the mat was floccose or floccose subfelty to felty texture in about 10 days after inoculation. At 22° C., condensation was less, so that the mat remained floccose. In 25-days-old cultures, the mat became felty in all cases. The other characteristics, namely, the formation of the pile of velvet at the upper advancing zone, rolling, &c., were like those described above.

(ii) *Colour.*—No colour developed under any condition in 5-days-old cultures. On *potato-dextrose-agar*, colour developed on the 10th day after inoculation, the shades being mainly capucine buff, and pale ochraceous buff towards the inoculum and light buff at the upper advancing zone. No colour developed in darkness at 22° C. Later on, the shades deepened to cinnamon buff, sayal brown, and ochraceous tawny. The colour of the medium was changed to mummy brown. The glass surface was stained due to tinting of the rolling mycelium over the bare glass surface. The tinting on the glass consisted mainly of shades of amber brown, yellow ochre, old gold, pinkish buff. Tinting on the pile of velvet at the upper advancing zone consisted of pale ochraceous buff to light ochraceous buff. On *oat-meal-agar* and on

malt-extract-agar, the intensity of colour was much less than those described above. On *potato-starch-agar*, colour production seemed to be least pronounced. A tinge of sayal brown developed at 33° C. in 25 days. Later on, a shade of tawny developed at 22° C. under diffused light.

(iii) *Rate of growth.*—The rate of growth of the superficial mycelium was best at 33° C., moderate at room temperature and comparatively less at 22° C. The slants were covered within a week in room temperature and at 33° C. and within 10 days at 22° C. Growth in darkness, in general, was more vigorous than that under diffused light.

(iv) *Sporophore production.*—Several wood-blocks were inoculated with the fungus both in Roux-tubes and in flasks as usual. Growth started after about a fortnight and attacked the wood vigorously. After about a month, the mycelium condensed at places forming a buff coloured area. No fructification appeared on agar media or wood-blocks.

(v) *Mycelium.*—Two main types of hyphæ can be seen in cultures, *viz.*, (*a*) thin-walled, much branched, contents hyaline, 2·5–3 μ broad and (*b*) thick-walled, distantly branched, contents hyaline, contents granular and 3–3·7 μ broad (Text-figs. 8–9).

4. Polyporus ochroleucus *Berk.*

Geographical distribution

The fungus is widely distributed, being found in the Bahamas and Brazil in S. America ; Portugal in Europe ; Portuguese Guinea and Eastern Cape Forest conservancy in Africa. In India, it occurs in Bengal, Assam (Lokra hills) and after passing through Malaya Peninsula and Molucca Islands, it extends upto Queensland, New South Wales and Tasmania in Australia. It is also found in Ceylon and Japan.

Occurrence

It has been reported to grow on dead logs at the base of the trunk of *Casuarina equisetifolia* L. and on dead trunks of *Lagerstræmia flos-reginæ* Retz.

Fungus in culture

(i) *Habit of growth.*—On *potato-dextrose-agar*, the young cultures developed a downy sub-felty to felty texture over the inoculum with broadly appressed and sodden texture over the greater part of the slant surface. The advancing zone was distinct and broadly appressed in all cases. In darkness at 22° C., the cultures exhibited faint zonations round the inoculum. On the 10th day after inoculation the mat remained thin, appressed and sodden with a felty inoculum. In 25-days-old cultures, the mat became a thin and powdery felt, this powdery appearance being less pronounced at room temperature. On *oat-meal-agar*, the texture of the superficial mycelium was like that described above, but the mat was more thick. At 22° C., the mat was

more loose. Colourless glistening drops of liquid appeared in old cultures in diffused light at 33° C. On *malt-extract-agar* and on *potato-starch-agar*, the same mat was presented but in the latter medium, growth was very poor.

(ii) *Colour.*—A tint of warm buff on *malt-extract-agar* and patches of chamois and cinnamon buff on *potato-starch-agar* only developed in 35-days-old cultures. No colour developed on other media, the cultures remaining white throughout.

(iii) *Rate of growth.*—The rate of growth was rather slow in comparison to other fungi. On *oat-meal-agar*, the rate of growth was rapid than on other media. Growth was best at 33° C., moderate at room temperature and comparatively less at 22° C. In general, growth was more rapid in darkness than in diffused light.

(iv) *Sporophore production.*—The wood-blocks were inoculated with 10-days-old mycelium on *potato-dextrose-agar*. The growth on the external surface of the blocks was poor and the mat was thin and slightly yellowish in colour. Tiny fruit-bodies developed in several cultures on agar media. A fruit-body, on section, showed basidia and basidiospores. The spores were oval and measured about $7 \cdot 8 \times 13\,\mu$.

(v) *Mycelium.*—Three main types of hyphæ can be distinguished in culture, *viz.*, (*a*) broad, thin-walled, hyaline, branched, closely septate, about $2 \cdot 7\,\mu$ broad ; (*b*) narrow, thin-walled, hyaline, sparingly branched, septate, about $1 \cdot 3\,\mu$ broad and (*c*) thick-walled, sparingly branched, distantly septate, more or less coiled and $2 \cdot 7$–$4\,\mu$ broad. Chlamydospores, both terminal and intercalary, are present. The chlamydospores have thick walls showing striations, contain granular protoplasm and measure about $8\,\mu \times 5 \cdot 4\,\mu$ (Text-figs. 10–14).

5. Polystictus steinheilianus *Berk. and Lév.*

The fungus was identified by Bresadola and at first regarded as synonymous with *Trametes rigida* Mont. and Berk. but later considered as a good species. He also regarded it as synonymous with *Polystictus connexus* Lév.

Geographical distribution

The fungus occurs in Martinique, Brazil and Venezuela in S. America. It is also found in Bengal and Orissa in India.

Occurrence

It grows on dead logs of *Shorea robusta* Gaertn., *Tectona grandis* L. and dead bamboo stem.

Fungus in culture

(i) *Habit of growth.*—On *potato-dextrose-agar*, the initial growth of the superficial mycelium produced a sub-felty to felty mat. The advancing zone at room temperature was narrow, appressed and sodden. No evident advancing zones could, however, be evident in other conditions. Faint zonations were noticed early in young cultures kept at 22° C. Growth seemed to be the best at 33° C.,

moderate at room temperature and comparatively less at 22° C. Vigorous growth at 33° C. was manifested by the rolling of the mycelium on the bare glass surface in 5-days-old cultures. In 10 days after inoculation, the mat invariably became felty with an appressed to sub-felty advancing zone. At 33° C., the upper advancing zone looked like a pile of felt and numerous glistening drops of liquid appeared towards the base of the tube. Rolling of the mat was evident in all the cultures. In old cultures, the felty mat gave place to a woolly growth, and a pile of felt at the upper advancing zone was prominent. On other media the sequence of texture as the cultures developed was like that described for *potato-dextrose-agar*.

(ii) *Colour.*—On *potato-dextrose-agar*, a tint of light buff to shades of isabella colour appeared in 5-days-old cultures under diffused light at 22° C. No colour developed in other conditions. After 10 days of inoculation, colouration appeared in diffused light at room temperature and in darkness at 22° C., and consisted of ochraceous tawny to buckthorn brown. The shades in diffused light at 22° C. depend to pinkish buff, cinnamon buff, tawny olive, Saccardo's umber and drab. The bare glass surface was stained due to tinting of the rolling mycelium. Thus, for this fungus pigmentation was best developed at 22° C. and least at room temperature. At 33° C. the colour appeared very late. Moreover in diffused light, pigmentation developed earlier than in darkness and also consisted of deeper shades. In 25 days, no new shades appeared, but the lighter shades were absent, while the deeper shades only persisted. Pigmentation was noticed at 33° C. In still old cultures, deeper shades consisting of light clay colour, deep sayal brown, tawny olive, etc., only persisted. On *oat-meal-agar*, the same shades of colour as described above were seen but colouration developed late. On *malt-extract-agar*, the shades were deeper than those seen for *potato-dextrose-agar*. The colour over pore-mouths consisted of shades of light cinnamon drab and light drab. These shades, later on, deepened to hair brown, pinkish buff, gull grey and deep gull grey. On *potato-starch-agar*, the intensity and variety of colours were best as compared to other media. Within 5 days after inoculation, pigmentation developed under all conditions and consisted of various shades of ochraceous tawny, cinnamon brown, olive buff, antimony yellow, yellow ochre, buckthorn brown and warm buff. Thus, the shades were much deeper as compared to other media. On the 10th day, the lighter shades deepened to dresden brown, tawny olive, clay colour, saccardo's umber, pinkish buff, cinnamon buff, sayal brown and mikado brown. Colour over pore-tubes consisted of shades of gull grey to deep gull grey. Intensity of colour seemed to be best developed under diffused light at 33° C. and at room temperature. At 22° C. comparatively less pigmentation was noticed, a fact quite contrary to that seen for the previous media.

(iii) *Rate of growth.*—The initial growth was very rapid and the slants were covered within a week in all cases except for *potato-starch-agar* where the growth was comparatively slow and the slants were covered within 10 days of inoculation. The rate of growth was, however, best at 33° C., moderate at room temperature and at 22° C.

The rate of growth in diffused light was more than in darkness under any conditions of temperature.

(iv) *Sporophore production.*—For the early development of fructification *malt-extract-agar* and *potato-starch-agar* proved to be the best. On *potato-dextrose-agar* and *oat-meal-agar* comparatively less number of tubes fructified. The fruit-bodies were resupinate and were mostly formed at the upper advancing zone. The pore-tubes were irregular and consisted of various shades of light cinnamon drab, light drab, hair brown, pinkish buff, gull grey and deep gull grey. The fruit-bodies were peculiar in that the hymenial surfaces after a time were covered by a fresh mycelial growth developed from the germination of the secondary spores. Bits of mycelium from 7-days-old cultures on *potato-dextrose-agar* were transferred to the wood-blocks of all the plants previously mentioned, in Roux-tubes and in flasks. Growth started within a fortnight and in about a month, most of the wood-blocks were thoroughly infected. The mycelium was seen condensing in patches. The colouration of the mycelium was however very slight. In about 2 months time, fruit-bodies developed in all the wood-blocks both in Rouxtubes as well as in flasks. A fruit-body, in section, showed the presence of hyphal pegs and secondary spores. On adding a drop or two of sterile distilled water aseptically to culture tubes, the same fruit-body showed many immature basidia.

(v) *Mycelium.*—The hyphæ in culture consists of three main types, *viz.*, (*a*) thin-walled, branched, hyaline, septate, with clamp-connections, about $2{\cdot}7\,\mu$ broad; (*b*) thin-walled, rarely branched, hyaline, septate, without clamp-connections, about $2\,\mu$ broad, and (*c*) thick-walled, branched, without clamp connections and about $3\,\mu$ broad (Text-figs. 15–20).

6. Merulius similis *B. et Br.*

Geographical distribution

The fungus occurs in Portuguese Guinea, in Africa and Bengal, Assam (Lokra hills), United Provinces, Ceylon and Malaya Peninsula in Asia.

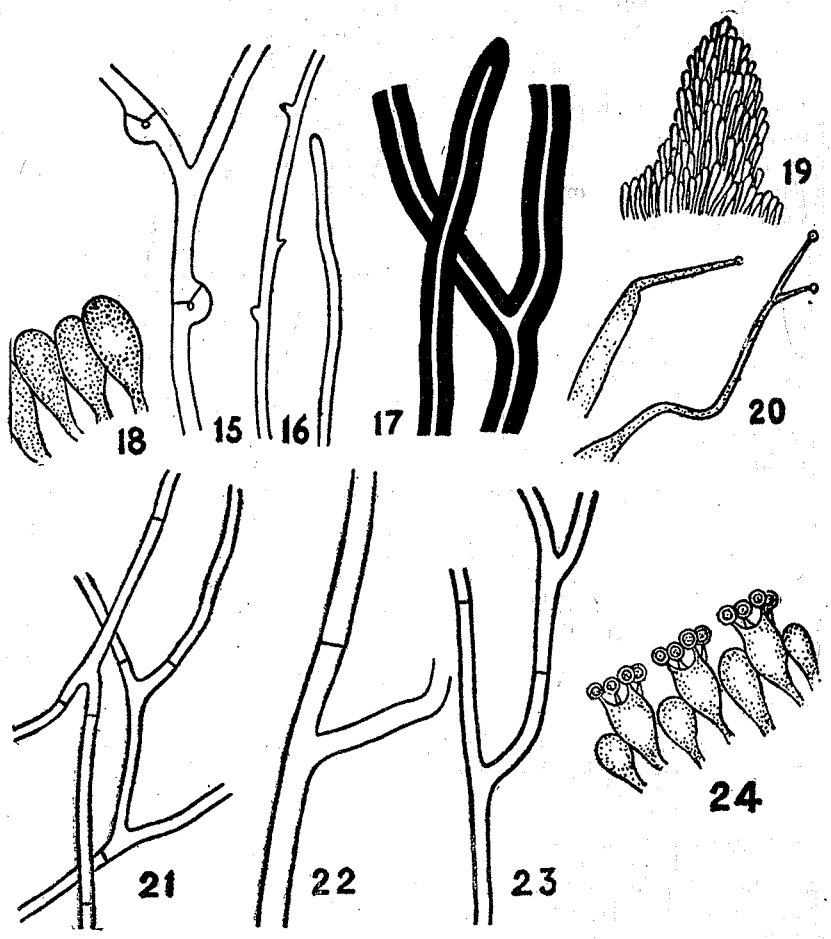
Occurrence

It grows in imbricate clusters on clumps and roots of living and dead bamboos and is very common in Calcutta and suburbs.

Fungus in culture

(i) *Habit of growth.*—On *potato-dextrose* and on *oat-meal-agar*, the initial growth of the superficial mycelium produced a felty mat with appressed advancing zones. The young cultures exhibited faint zonations at 22° C. as well as in room temperature. Within 10 days after inoculation the cultures became faintly granular due to irregular condensation of the mycelium. Rolling of the mycelium was only evident in darkness at 33° C. The cultures under different conditions of light and darkness exhibited no special distinguishing features. As the cultures became old, the mat became coarsely granular and eventually a compact and smooth felt resulted. Numerous colourless

glistening drops of liquid appeared in darkness at 33° C. and 22° C. These drops, later on, dried up. In darkness at 33° C., there was a pile of felt just behind the upper advancing zone. On the other media the mat remained as a thin felt.



Text-figs. 15–24—Figs. 15–20. *Polystictus steinheilianus:* 15. thin-walled, branched hypha with clamp-connections; 16. thin-walled, unbranched hyphæ without clamp-connections; 17. thick-walled hyphæ; 18. immature basidia; 19. hyphal peg; 20. secondary spores. Figs. 21–24. *Merulius similis:* 21. thin-walled, branched narrow hyphæ; 22. thin-walled, branched broad hypha; 23. thick-walled hypha; 24. basidia with basidiospores. (×520).

(ii) *Colour.*—On *potato-dextrose-agar* a shade of light buff developed in the cultures kept at 33° C. in about 10-days-old cultures. This deepened the pale ochraceous buff later on. The mat at the room temperature and at 22° C. remained chalk white throughout. On *oat-meal-agar*, shades of light buff, buff colour and pale ochraceous

buff developed in 10-days-old cultures only under diffused light at 33° C. Later on, these shades deepened to sea-shell pink and maize yellow. On other media, shades of buff, warm buff, mixed with white developed in old cultures at room temperature and at 33° C. under both conditions of light and darkness.

(iii) *Rate of growth.*—The rate of growth was moderately rapid on all the media, being best at 33° C., moderate at room temperature, and a little less at 22° C. Advance was more rapid in darkness than in diffused light.

(iv) *Sporophore production.*—Resupinate patches of fruit-bodies appeared on agar media in about 45 days. The wood-blocks were inoculated with mycelium from 10-days-old cultures on *potato-dextrose-agar*. Within a month the mycelium condensed at places forming a cushion with bright yellow colour. A fruit-body, on sectioning, showed the presence of basidia ($9 \cdot 75$–$10 \cdot 5\,\mu \times 7 \cdot 5$–$8 \cdot 25\,\mu$) with four strigmata and basidiospores ($3 \cdot 75\,\mu \times 3 \cdot 75\,\mu$).

(v) *Mycelium.*—Three main types of hyphæ can be recognised in cultures, *viz.*, (*a*) thin-walled, branched, septate, with granular contents, $1 \cdot 3$–$2 \cdot 7\,\mu$ broad; (*b*) thin-walled, branched, septate, with granular contents, $2 \cdot 7$–$4\,\mu$ broad, and (*c*) thick-walled, branched, hyaline and $1 \cdot 3$–$2 \cdot 7\,\mu$ broad. Calcium-oxalate crystals of various shapes are present in cultures (Text-figs. 21–24).

IV. General Considerations and Conclusions

An attempt has been made to summarise the influence of several external factors which affect the vegetative growth and fruit-body formation in artificial cultures of these fungi. The effect of a single factor has been studied by keeping the others constant.

(1) *Light*

The influence of light on Polypores has been fairly worked out. Long and Harsch[24] allowed direct sunlight to reach the young cultures for one to two hours, but later the amount of direct sunlight was decreased by light screens. This checked the mycelial growth and intensified the colours of the aerial mycelium. Fritz[19] carried on her investigations in complete darkness where the diagnostic characters are accurately manifested. In this investigation the cultures were placed both in diffused light and darkness. Direct sunlight was avoided as its unfavourable action has already been pointed out by Fritz.[19]

The effect of light on the growth and development of each of the fungi has already been described. In the presence of light, the mat becomes more compact due to early condensation and more rich and varied colouration is produced. The writers are of the opinion that although light causes early appearance of pigmentation, it in no way determines the final range of colours, which seems to be constant for each species when grown on a particular medium under definite temperature conditions. Cultures grown in darkness overrun rapidly on the surface of the agar slant.

(2) *Temperature*

Comparative cultural studies were made at ordinary room temperature (23° C.–27° C.) and at constant temperatures of 22° C. and 33° C. This is approximately the same as the range (22° C.–35° C.) used by Fritz.[19] Mounce[26] used a still lower temperature (0° C.–8° C.), which seems to be beyond the usual range.

In general, growth at 22° C. is very unsatisfactory, the mat being thin and loose. Faint zonation is noticed invariably in young cultures of all the fungi. The rate of growth is rather slow. Colour production is delayed and the intensity of pigmentation is much less than that in other temperature conditions. Mycelial growth seems to be best at 33° C., the mat being more compact due to early condensation. The rate of growth is also rapid, pigmentation appears earlier and more intense than in other conditions. At room temperature, the habit and rate of growth are moderate and the range of colour nearly approaches that found at 33° C. These observations agree with those of Fritz[19] and Mounce[26] who also noticed that intensification of colour takes place with increase in temperature. Since during the experiment no intermediate temperature between 27° C. and 33° C. was tried, the writers could not establish the optimum temperature at which growth was at its best but the temperature of 33° C. seemed to be fairly near the optimum.

(3) *Substratum*

(*a*) *Agar-media.*—Bose[12] and other workers found that *malt-extract-agar* was quite suitable for the display of cultural characters as well as for fruit-body formation. The authors, however, obtained better results on *potato-dextrose-agar* medium. Fritz[19] and Zeller[41,42] also found this medium quite suitable. Fritz[19] found that *potato-dextrose-agar* and *malt-extract-agar* were equally good but on the whole the former was more preferable, since mats were closely interwoven and as a consequence presented surfaces of more definite character. For this reason, she based her *key* by growth on *potato-dextrose-agar*. In diffused light and at a temperature of about 33° C., the medium provides the best condition for growth particularly in the case of Polypores. *Oat-meal-agar* medium proved satisfactory for all the fungi. *Potato-starch-agar*, on the whole, proved to be unsatisfactory except for *Polystictus steinheilianus* in which case colour production as well as development of fruit-bodies were found to be the best. Thus, a single medium has been found which is universally good for all fungi and gives a display of cultural characerts of all. Hence, for a comparative study of the cultural characteristics of fungi, it is always advisable to select a number of media, including, at least, one synthetic medium.

(*b*) *Wood-blocks.*—The wood-blocks were inoculated both in flasks and in Roux-tubes. The advantage of flask culture over Roux-tube culture is that in the former case, the mycelium has a good start for growth and attacks the wood earlier than in the latter. In Roux-tube cultures, the inoculum must include a sufficient amount of medium

so that the mycelium may retain its viability for a long time. It has been observed that the specific nature of the wood is not of much importance and growth takes place on all wood under proper conditions, since *Polystictus steinheilianus* fructified on *Bambusa arundinacea*, *Shorea robusta*, *Mangifera indica* and *Ceriops Roxburghiana*. The fact that no sporophore has ever been found in nature on any of these plants except *Shorea robusta* shows that there must be some factor present in the living wood which has been lost during the processes of drying. Another interesting observation was that *Polystictus steinheilianus* formed fruit-bodies on agar media within a week and thus had a remarkably short life-history. The same fungus, however, took two months to fructify on wood proving that life-histories of hard fungi are prolonged on wood-block cultures, as was pointed out by Bose.[13]

(4) *Effects of various factors on sporophore production*

It is known that the production of fruit-bodies in nature in many Hymenomycetes is more or less dependent upon the influence of light. Spaulding,[35] however, found a few Polypores fructifying in clay mines. Long and Harsch[24] observed that certain members of Polyporaceæ could produce fruit-bodies in complete darkness. In artificial cultures, so far as our observations indicate, sporophores were obtained as frequently in darkness as in light.

Detailed observations on the production of true pilei of *Polyporus brumalis* in artificial culture have been published elsewhere.[3] It was observed that the stalks of the fruit-bodies were positively phototropic but had no relation to the influence of gravity. The stalks, however, failed to produce typical pilei on agar media and various treatments of light, temperature and humidity showed that each of these conditions alone had very little effect on pileus-formation. Cultures were later grown on Badcock's medium[1] in Erlenmeyer flasks and it was observed that stalks were formed which reached the bases of the plugs where they remained as such without forming the pilei. As soon as the plugs were opened, the stalks elongated and formed normal pilei. Thus, it appeared that aeration was one of the main factors responsible for the production of normal pilei in *Polyporus brumalis*. In a recent paper Badcock[2] modified his earlier methods and thoroughly discussed the conditions influencing the development of normal fruit-bodies in culture. According to him the following conditions appear to be essential : the provision of a generous supply of a rich, well-aerated medium with plenty of moisture ; moderately high relative humidity, but not a saturated atmosphere, at the surface of the medium and around the developing sporophores : and exposure to light of moderate intensity.

Sections through the hymenial surface of the fruit-bodies of *Polyporus brumalis*, *P. ochroleucus* and *Merulius similis* revealed the presence of basidia and basidiospores. *Polystictus steinheilianus* was remarkable in that the porous surface once formed was later masked by a fresh mycelial growth covering up the entire hymenial surface. This mycelial overgrowth was probably due to germination

of secondary spores which were abundantly found in the sections of such a fruit-body. Bose[13] maintains that a definite percentage of humidity is required for the production of basidia and basidiospores in the fruit-body in artificial culture but with the lowering of humidity, numerous secondary spores are produced. Sections through a part of the fruit-body of *Polystictus steinheilianus* in culture revealed the presence of secondary spores. But when few drops of sterile distilled water were poured aseptically into the culture tube, the same fruit-body, on section, revealed the presence of innumerable immature basidia.

That there is no relation between the appearance of glistening drops of liquid and the formation of fruit-body, was established during the course of the investigation. No doubt in many cases, fruit-bodies appeared immediately after the appearance of liquid drops, but this fact can, on no account, be taken as the cause. In cases of *Polyporus friabilis* and *Polyporus rubidus* glistening drops of liquid appeared and later on dried up, but in no case fruit-bodies developed.

A temperature of about 33° C. in diffused light seemed to be the optimum condition for fruit-body formation. At this temperature, the mat became compact and fructifications developed early.

(5) *Humidity*

To get spore-print from a dried sporophore, the method adopted by Bose[11] was followed. To maintain maximum humidity of the surrounding atmosphere during spore-discharge, moist chamber was made by lining the inside of a bell-jar with moistened blotting paper and placing some water inside the jar. That high humidity provided a favourable condition for vegetative growth was certain but how far the reproduction was retarded or accelerated could not be ascertained. In Roux-tubes, mycelial growth at the lower end of the wood-blocks near the water surface was vigorous and some of them travelled down to the water surface, grew luxuriantly and formed a dense floating mycelial mat. Fruit-bodies when formed on wood in Roux-tubes were seen at the portion of the blocks, away from the water surface.

(6) *Gravity*

The stalks of *Polyporus brumalis* did not respond to the force of gravity. The upward growth of the stalks was purely incidental for in culture tubes kept horizontally it was seen that the vertical stalks arising at the base of the tubes soon turned their apices and grew horizontally parallel to the glass surface.

SUMMARY

1. Six species of Bengal Polyporaceæ, namely, *Polyporus brumalis*, *P. friabilis*, *P. rubidus*, *P. ochroleucus*, *Polystictus steinheilianus* and *Merulius similis* have been studied.

2. The technique of making different types of cultures, preparation of media and the conditions under which the fungi were grown have been described.

3. The descriptions of the fungi including their geographical distribution, occurrence, habit, colour, rate of growth, mycelium in culture, sporophore-production on various media have been given.

4. General observations regarding the effects of light, temperature, substratum, humidity and aeration on vegetative growth and fruit-body formation have been made.

The authors take this opportunity of expressing their deep sense of gratitude to Prof. S. P. Agharkar, Head of the Department, for the facilities given and to Dr. S. R. Bose, Carmichael Medical College, Calcutta, for his valuable suggestions and interest taken in the work.

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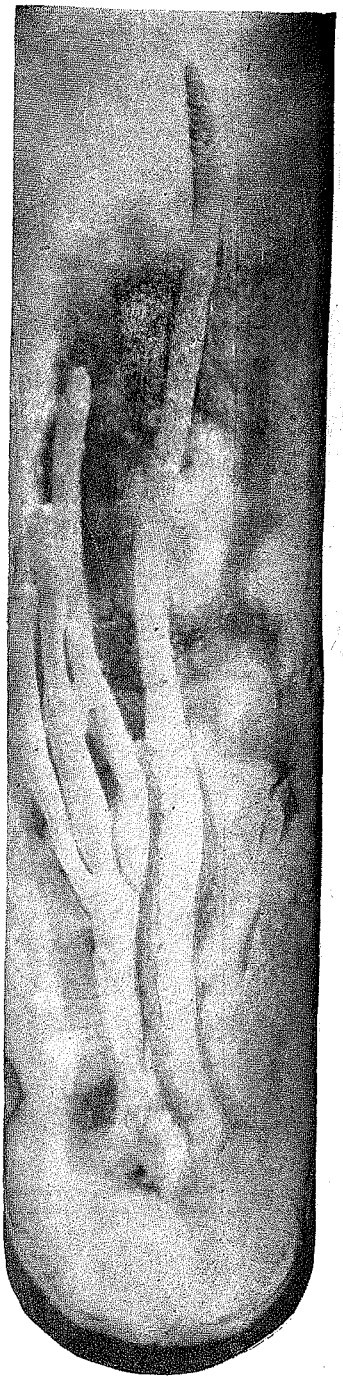

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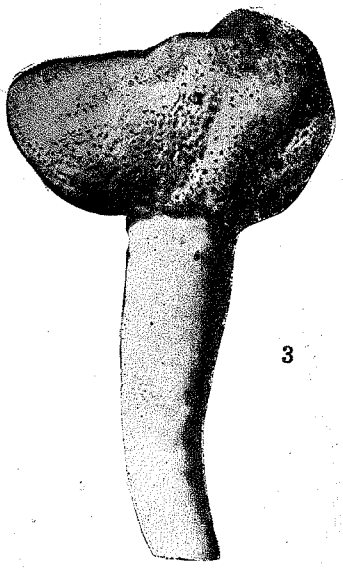

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STUDIES IN THE BIOLOGY OF WOOD-ROTTING FUNGI OF BENGAL

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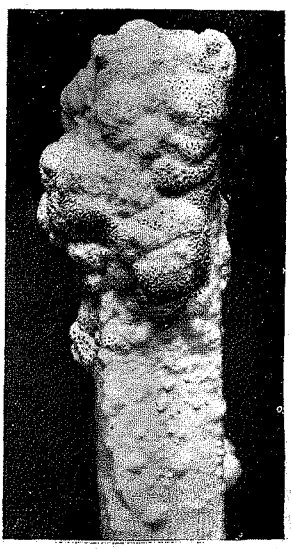

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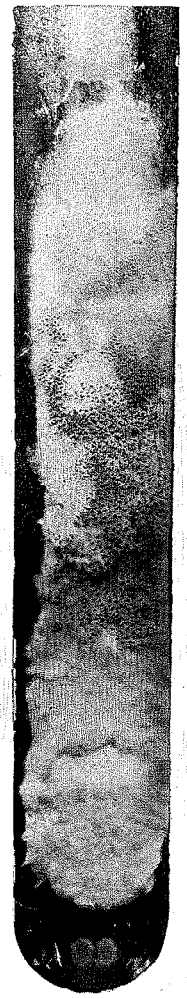

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SACHINDRANATH BANERJEE AND BIMAL KUMAR BAKSHI—

STUDIES IN THE BIOLOGY OF WOODR-OTTING FUNGI OF BENGAL

EXPLANATION OF PLATES

PLATE II

Fig. 1. Stalks of fruit-bodies of *Polyporus brumalis* formed on potato-dextrose-agar about 40-days-old (×3).

Fig. 2. A fruit-body of *P. brumalis* showing typical pileus formed on Badcock's medium (½ Nat. size).

Fig. 3. A pileus of *P. brumalis* showing hymenial surface with well-developed pores on Badcock's medium (×3).

PLATE III

Fig. 4. A resupinate fruit-body of *Merulius similis* formed on Potato-dextrose agar about 30-days-old (Nat. size).

Fig. 5. A resupinate fructification of *Polystictus steinheilianus* formed on malt-extract-agar about 20-days-old (Nat. size).

Fig. 6. A fruit-body of *P. steinheilianus* formed on potato-dextrose-agar about 15-days-old (½ Nat. size).

Fig. 7. A fruit-body of *P. stinheilianus* formed on sterilized wood block of *Mangifera indica* about 2-months-old (½ Nat. size).

Fig. 8. An irregular resupinate fruit-body of *P. steinheilianus* formed on a sterlised wood-block of *Shorea robusta*, about 2-months-old (Nat. size).

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