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# ON THE BIOLOGY OF *TRAMETES LACTINEA* BERK. IN CULTURE

By

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(With Plates I–III and 3 Text-figures)

*Trametes lactinea* Berk., a common wood-rotting polypore, has been taken up for studying some aspects of its life-history. It attacks the logs and stumps of various economically important trees in India and abroad.

A host-list has been compiled which includes 30 species of dicotyledons and monocotyledons of India and other countries of the world. Only one gymnospermous host has been recorded so far.

The geographical distribution of the fungus has also been noted. It is of wide occurrence particularly in the tropics.

The morphology and hyphal anatomy of the fructifications have been thoroughly described. The fungus is conspicuous for its large, applanate and dimidiate fruit-bodies, tough and corky texture, white to creamish white colour, and lustrous hymenial surface with regular pore-mouths.

The basidiospores obtained from fresh fructifications germinate readily in water and also in other media. Carbohydrate-media have been found to be the most suitable for spore-germination. Germ-tube is generally produced from the apicular end of the spore and its further growth and development depend upon the suitability of the medium.

Oxidase tests show that *T. lactinea* is a 'white-rot' fungus. It exhibits oxidase reactions when grown in *malt-agar* media containing 0.5% gallic or tannic acid.

The growth characteristics of the fungus have been studied in detail. Acidity and alkalinity of the media have been found to exert definite influence upon the growth characteristics of primary and secondary mycelia. Both types of mycelia are acid-loving and grow best on a slightly acidic medium having the *p*H range 5.0–6.0. Higher alkalinity of the medium is injurious for the fungal growth.

Cultural characteristics of primary and secondary mycelia on four different agar-media have been studied under identical conditions. It has been found that the fungus grows best in *potato-dextrose-agar.*

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## Introduction

*Trametes lactinea* Berk., a member of the family Polyporaceae, is well-known for its activities as a wood destroyer in India. It grows profusely on logs and stumps of various trees, some of which are commercially important for their timber and hence it is directly linked with the national economy. The fungus is widely distributed in the tropical countries of the Pacific coasts and those of the Indian Ocean. The large symmetrical, applanate, greyish or yellowish white fructifications of the fungus are very conspicuous during the monsoon and like many other species of Polyporaceae, it seems to have the capacity of surviving through a relatively drier climate. New basidiocarps, however, can appear even up to the late months of winter (January to February). Reports on its occurrence and distribution are frequent throughout the literature, but strange enough, no attempt has so far been made for a complete documentation of its biology. The present investigation has, therefore, been undertaken with a view to throw some light on its life-history.

## History and Synonymy

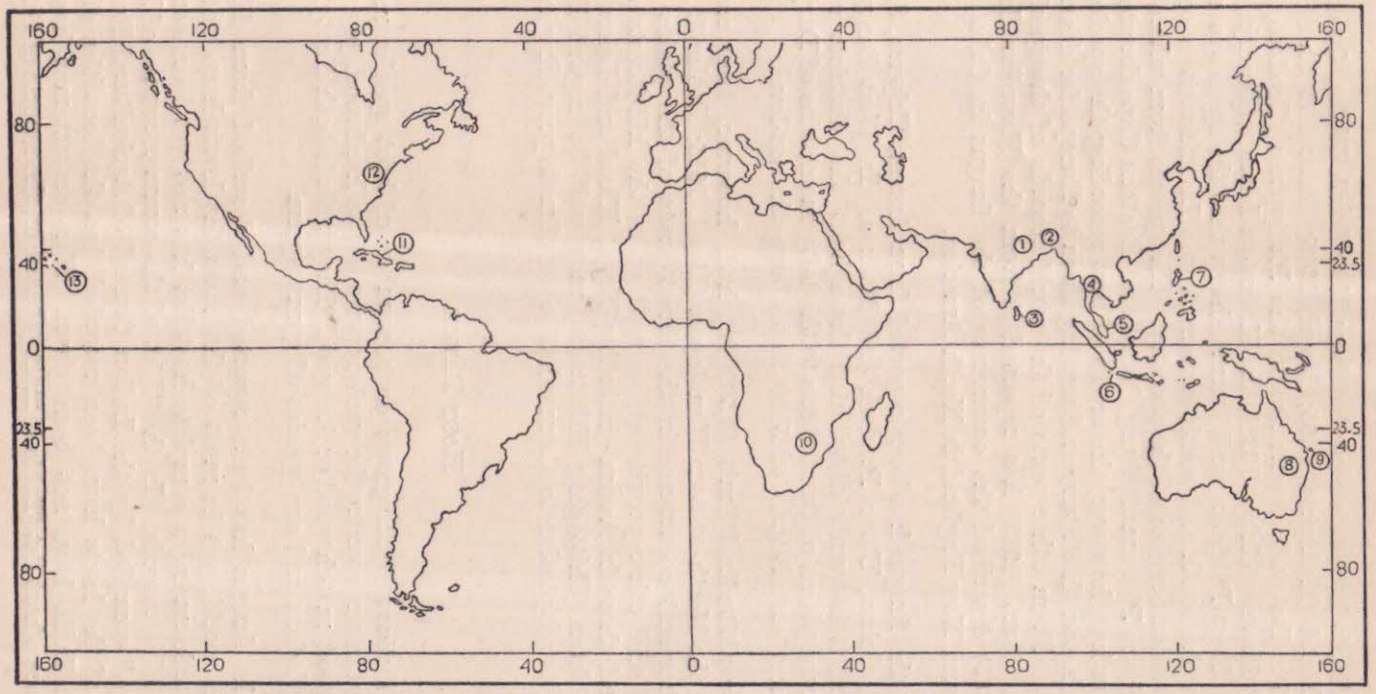
*Trametes lactinea* Berk. was first described by the leading British mycologist, M. J. Berkeley (1842) to whom the right of authorship of the species is due.

It appears from the available literature that different forms and varieties of the test fungus have been found in other parts of the world. In the Kew Herbarium one specimen of the fungus has been marked as '*Trametes lactinea* var.' by Berkeley while another specimen of the same has been marked as 'co-type' by Lloyd (Petch, 1916). Doubts have also been expressed regarding the validity of *T. levis* Berk. as a separate species, which according to some authors (Petch, 1916; Petch & Bisby, 1950) is actually a form of *T. lactinea* Berk. From the Philippines Teodoro (1937) reports a specimen as *Trametes lactinea* forma *conchata* Berk. which indicates that the Philippine specimen described by Berkeley (1878) as *T. conchatus* is also a form of *T. lactinea*. Inspite of such reports, no synonym of the fungus has so far been traced from any literature. Extreme form variation of the specimens growing on different hosts at different localities have also been experienced by the writers during their field studies. No definite conclusion, can, however, be made at present regarding the existence of different 'forms' and 'varieties' of the fungus pending detailed investigations with special reference to inter-fertility phenomena.

From the collections of Irani, Saxton and Cave the fungus was first reported from India by Lloyd (1913, 1915, 1920), but subsequently Bose (1920) redescribed the same and included it in his works on Bengal Polyporaceae.

## Geographical Distribution

The distribution of *Trametes lactinea* Berk., so far recorded from different parts of the world, clearly shows the tropical origin and distribution of the fungus (Text-fig. 1). It is mainly restricted to Australia,




TEXT–FIG. 1. MAP SHOWING THE DISTRIBUTION OF TRAMETES LACTINEA BERK.

1. India; 2. East Pakistan; 3. Ceylon; 4. Burma; 5. The Malay Peninsula; 6. The Krakatau Islands; 7. The Philippines; 8. Australia; 9. Fraser Island; 10. Africa; 11. Cuba; 12. New England; 13. The Hawaiian Islands.

Asia and Africa being mainly confined to the coasts of the Pacific and Indian Oceans.

In Australia, it has been recorded from New South Wales, Queensland and Fraser Island (McAlpine, 1895; Lloyd, 1915; Cleland & Cheel, 1923; Cleland, 1935). Its wide occurrence in the Philippines (Teodoro, 1937), the Malaya Peninsula (Chipp, 1921), the Krakatau Islands (Boedijn, 1940), Ceylon, India, East Pakistan, and Burma shows that the species is mainly distributed in the south and south-eastern Asia. In Africa it is confined to the south eastern region of the country. The places from where it has been recorded are Pietermaritsburg, Natal and Eastern Cape forests (Bijl, 1922). Ceylon has, however, the credit of first recording the species very early in the nineteenth century and sending them to Europe for description (Petch, 1916). From other parts of the world records of the species are very meagre. Burt (1923) has reported it from the Hawaiian Islands. Saccardo (1888) described the occurrence of the fungus in Cuba in the West Indies and New England in North America.

In India* the fungus has been reported from Assam, Tripura, Sikkim, West Bengal, Orissa, Uttar Pradesh and Madras. In West Bengal and East Pakistan, it is evenly distributed throughout the districts ranging from the alluvial plains of Sunderbans to the lofty mountains of Darjeeling district reaching an altitude of 5000 feet above the sea-level.

The upper altitudinal limit (6,500 ft.) had, however, been recorded by Saxton in the North-western Himalayas (Lloyd, 1915; Butler & Bisby, 1931). Its frequent occurrence in the forest areas of the plains (U.P., West Bengal and other provinces), as well as in the hill-forests of the Himalayas and those in Assam (including Naga, Khasi and Lokra Hills) and East Pakistan (Chittagong Hill tracts) may well be regarded as an indication of its adaptation to a somewhat cooler climate prevailing in those regions.

## Host Range

*Trametes lactinea* Berk. grows on a number of host species most of which are used in our country for various purposes as hardwood, ply wood, ornamental wood, match wood or fire wood. They include both dicotyledons and monocotyledons and one species of gymnosperm.

Though the fungus has been reported and described from different regions of the world, yet in most of the cases the hosts have not been recorded. Furthermore, a complete host list has not yet been compiled with the available records. In the "List of the common names of Indian Plant Diseases" compiled by the Indian Council of Agricultural Research (1950) some of the hosts have been recorded, but the localities of their occurrence in India have not been given. Here an attempt has been made to prepare a host-list (Table 1) mainly based on the reports of Banerjee

* From the records in S. R. Bose's "Polyporaceae Herbarium", R. G. Kar Medical College, Calcutta.

(1947), host list compiled by I.C.A.R. (1950), records in the "Polyporaceae Herbarium" of S. R. Bose at R. G. Kar Medical College, Calcutta and the field collections of the writers themselves. The species are arranged under the respective families according to Bentham and Hooker's system.

It has been reported that the fungus may cause white-rot or spongy-rot leading to the decay of the sapwood only in most of the cases but sometimes both sap-wood and heartwood may also be decayed. There are reports in the "Polyporaceae Herbarium" of Bose that the fungus also grows on living trees, but nothing is known about its parasitic activity.

Table 1. *Host-list of* Trametes lactinea Berk.

| Host | Locality | Collected or reported by |
|---|---|---|
| DICOTYLEDONES | | |
| Dipterocarpaceae | | |
| *Shorea robusta* Gaertn. f. | Calcutta, Jalpaiguri, Kamrup Division. | Anonymous (1950)** Bagchee (1953). |
| *Dipterocarpus turbinatus* Gaertn. f. | Calcutta | The Authors (1957) |
| Sterculiaceae | | |
| *Heritiera minor* Roxb. | Sunderban, Khulna (E. Pakistan). | Bose (1921)* |
| Meliaceae | | |
| *Carapa obovata* Bl. | Sunderban | Bose (1921)* |
| Sapindaceae | | |
| *Acer oblongum* Wall. | | Anonymous (1950)** |
| *Aesculus indica* Colebr. | | ,, ,, |
| Anacardiaceae | | |
| *Mangifera indica* L. | Tripura, Barisal (E. Pakistan). | Bose (1923)* |
| *Spondias mangifera* Willd. | Calcutta | ,, |
| Leguminosae | | |
| *Dalbergia latifolia* Roxb. | | Anonymous (1950)** |
| *Schotia latifolia* Jacq. | Africa | Bijl (1922) |
| *Acrocarpus fraxinifolius* Wight. | | Anonymous (1950)** |
| *Ougeinia dalbergioides* Benth. | | ,, |
| *Acacia* sp. | Calcutta | Banerjee (1957) |
| Combretaceae | | |
| *Terminalia catappa* L. | Shibpur, Howrah Dt. | The Authors (1958) |
| *T. belerica* Roxb. | | Anonymous (1950)** |
| *T. arjuna* W. & A. | Shibpur, Howrah Dt. | The Authors (1957) |
| *T. procera* Roxb. | ,, ,, | ,, ,, |
| Myrtaceae | | |
| *Melaleuca leucadendron* L. | Calcutta | Bose (1921)* |
| *Eugenia malaccensis* L. | Barisal Dt. (E. Pakistan) | |
| *Psidium guyava* L. | Khulna, Sunderbans, (E. Pakistan) | |
| Rubiaceae | | |
| *Hymenodictyon excelsum* Wall. | Rajabhatkhawa, Jalpaiguri Dt. | Bose (1925)* |

| Host | Locality | Collected or reported by |
|---|---|---|
| Sapotaceae | | |
| *Sideroxylon ferrugineum* H. & A. | Indian Botanic Garden, Howrah Dt. | Banerjee (1947) |
| Amarantaceae | | |
| *Amarantus rohituca* W. & A. | | Anonymous (1950)** |
| Euphorbiaceae | | |
| *Excoecaria agallocha* L. | Sunderbans, Khulna, (E. Pakistan). | Bose (1921)* |
| Urticaceae | | |
| *Ficus bengalensis* L. | Chittagong Hill Tracts (E. Pakistan). | Bose (1920)* |
| *F. infectoria* Roxb. | Calcutta | The Authors (1957) |
| *Artocarpus integrifolia* L. | Calcutta | ,, (1958) |
| Casuarinaceae | | |
| *Casuarina equisetifolia* Forst. | Calcutta | Bose (1923)* |
| GYMNOSPERMAE | | |
| Pinaceae | | |
| *Pinus longifolia* Roxb. | | Anonymous (1950)** |
| MONOCOTYLEDONES | | |
| Plamae | | |
| *Cocos nucifera* L. | Calcutta | Bose (1919)*; Banerjee (1947) |
| *Phoenix sylvestris* Roxb. | 24-Parghanas | Bose (1921)* |

## Source of Material

With a view to collect the material for the present investigation field studies in different timber-yards of Calcutta and its suburbs, were done at every week end during and after the rainy seasons of 1957–61. The habit and habitat of the fungus and also the nature of its growth were studied in the field (Plate I, fig. 1). The fungus has been found to be of sporadic occurrence in those timber-yards which are located by the side of the river Hooghly and mainly on those logs which get a regular supply of rain as well as tidal water. In order to have a clear understanding of the morphological and anatomical peculiarities, fully mature basidiocarps were collected from a number of infected poles, viz., *Terminalia procera* Roxb., *Shorea robusta* Gaertn. f., *Artocarpus integrifolia* L. and *Ficus infectoria* Roxb.

Fresh and mature fruit-bodies were brought to the laboratory and the present investigation was started. Like many woody species of polypores the dried fructifications when soaked with distilled water, readily revived and liberated viable spores. Again, the ability of the fungus to withstand the dry and cold months of winter was of great help for this investigation. Whenever necessary, new basidiocarps were collected from the field as late as February every year.

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** Host-list compiled by I.C.A.R., Anonymous (1950).

* From the records in S. R. Bose's "Polyporaceae Herbarium".

## The Basidiocarp

(Plate I, Figs. 1–4)

### A. *EXTERNAL MORPHOLOGY*

*Fructifications:*—Annual, surviving up to winter months of the year; usually sessile, occasionally effuso-reflexed or even resupinate; when sessile either broadest at the base or attached to the substratum by comparatively narrow base; usually single, sometimes several growing laterally in an imbricate cluster with pilei fused with one another, often becoming more or less circular in form and attaining huge dimension; usually dimidiate and applanate; stiff and corky; becoming hard and woody on drying; sessile fructifications about 22 cm.—40 cm. across, about 13–15 cm. deep and about 3–5.5 cm. in thickness; in effuso-reflexed fructifications the dimension of the resupinate portion 19–24 cm.×6–7.5 cm. and the pilei about 23 cm. across, 3 cm. deep and 2 cm. in thickness; margin smooth, straight, thick, semicircular, whitish, Old Bronze, Syrup with whitish patches here and there.

*Upper Surface :*—Rough due to formation of innumerable postules throughout, particularly throughout the base; broadly zonate towards the margin but azonate behind, zones unicolourous; whitish or cream coloured throughout or whitish, Buckthorn brown, Syrup or Bronze coloured behind, sometimes shades of Drab near the margin evident (Plate I, fig. 2).

*Context :*—Thick, tough and corky; whitish when fresh, becoming slightly cream coloured towards the upper surface on drying; zonate; about 2–3 c.cm. thick (Plate I, fig. 4).

*Hymenial surface :*—Smooth, in some cases faintly zonate towards the margin; distinctly poroid (Plate I, fig. 3); whitish with a shining lustre, Amber White or cream coloured, near the margin Old Bronze, Syrup here and there; pore-mounths regular, circular or slightly angular, about 123–525 (986) $\mu$ in diameter; dissepiments about 135–550 (815) $\mu$ thick; pore-tubes long deeping unequally into the context, about 9–15 mm. long; whitish in fresh condition (Plate I, fig. 4).

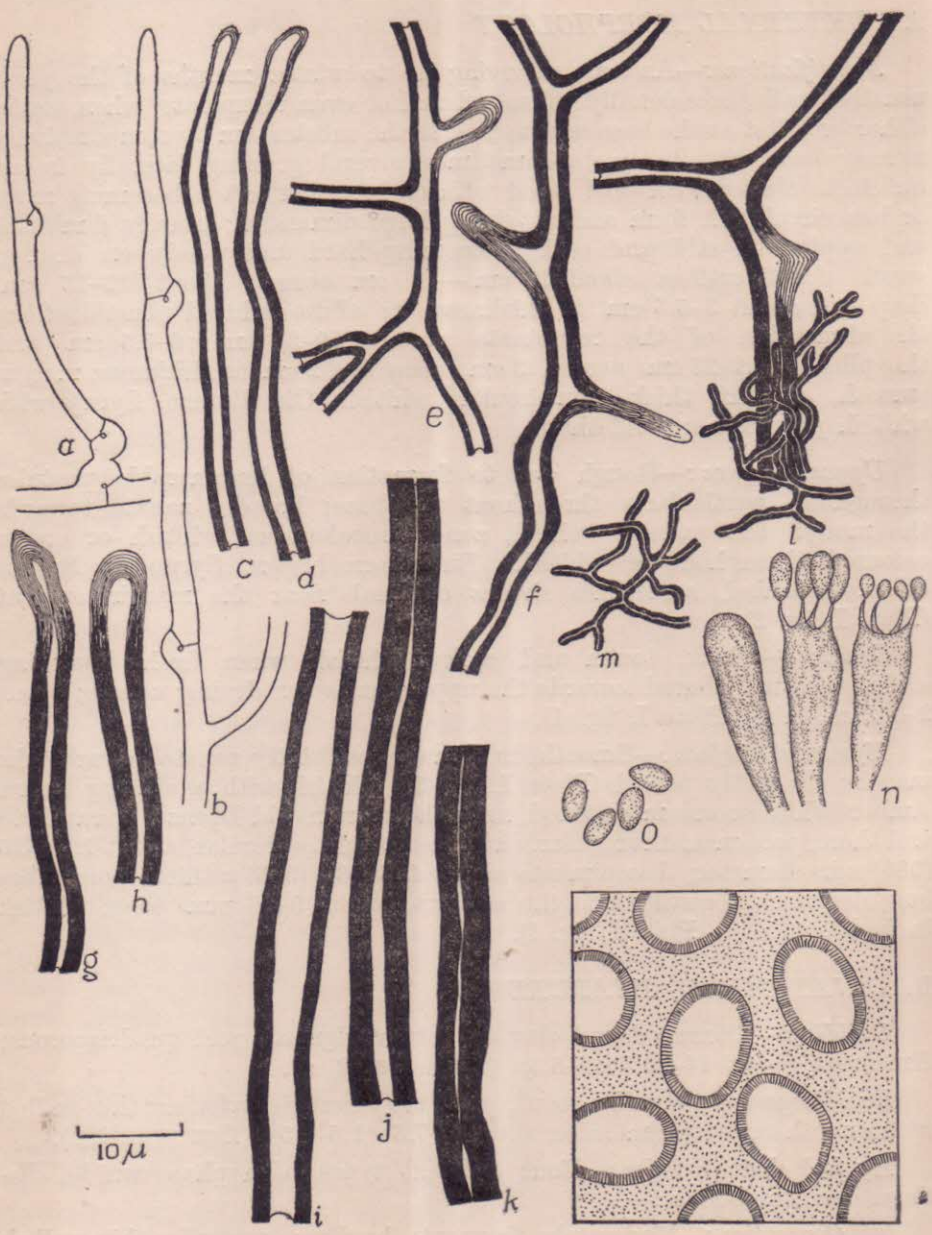
### B. *ANATOMICAL FEATURES*

*Basidia :*—Elongated and clavate; tetrasterigmatic and quadrisporous; dimension about 14–24.5×2–5.5$\mu$ (Text-fig. 2, *n*).

*Basidiospores :*—Hyaline, oval, with an excentric apiculus; thin-walled with smooth surface; dimension about 5–7.5×1.5–2.5$\mu$ (Text-fig. 2, *o*).

*Hyphal characteristics :*—Four different types of hyphae can be distinguished as follows :—

(a) *Generative :*—Moderately frequent; hyaline; generally thin-walled; sparingly branched; closely or distantly septate; longitudinal or interwoven; usually with simple clamp-connexion at each septum, sometimes plain septa also present; with abundant protoplasmic contents; about 1.5–3.5$\mu$ wide (Text-fig. 2, *a-b*).




TEXT-FIG. 2. Hyphal system (a-m), basidia and basidiospores (n-o), and a rectangular portion of the transverse section through the hymenial surface showing the pore-mouths of *Trametes lactinea* Berk. (vide Text).

(b) *Skeletal :*—Most abundant; always thick-walled, wall thickness very variable, either comparatively narrow and uniform throughout leaving a wide lumen or the lumen becoming merely obliterated and interrupted at places due to extreme thickening of the walls; unbranched; straight or slightly flexuous, more or less longitudinal; usually aseptate; pale yellow in colour; without clamp-connexions; empty or with a few granular contents; about 3.5–7$\mu$ wide; apical portion also thick-walled, pointed or rounded, hyaline (TEXT-FIG. 2, *g–k*).

(c) *Mediate :*—Frequent; always thick-walled and sparingly branched; wall thickness very variable; lumen usually continuous and with few granular contents; usually distantly septate, without clamp-connexions; branches straight or flexuous, interwoven; pale yellow in colour; about 2–4$\mu$ wide; apical portions of the branches also thick walled, rounded and hyaline (TEXT-FIG. 2, *c–f*).

(d) *Binding :*—Frequent; always thick-walled and much branched; highly flexuous; aseptate; without clamp-connexions; intertwined or twisting around skeletal or mediate hyphae; lumen almost obliterated; pale yellow to hyaline; about 1–2.5$\mu$ wide; apices more or less rounded (TEXT-FIG. 2, *l–m*).

The above description is based on large number of basidiocarps collected from different hosts, viz., *Terminalia procera*, *Shorea robusta* and *Artocarpus integrifolia* during 1957 to 1961. The various colours mentioned in the description are according to Merz and Paul (1950). In describing the different types of hyphae the writers have followed the nomenclature adopted by Corner (1932).

## SPORE GERMINATION

In order to have a clear understanding of the process of germination in *Trametes lactinea* and the requirement associated with it experimental work was undertaken. Spore deposits on dry sterilized slides were obtained both from fresh basidiocarps and dried fruit-bodies previously soaked with distilled water. The spores were sown aseptically in hanging-drops of different media and were incubated under the ordinary conditions of light and temperature (30°C.) of the laboratory. The *p*H of the medium, where necessary, was adjustded to 7. A few sterile dry slides with spore-deposits were also kept under identical conditions. All the test media were sterilized at 15 lbs pressure for fifteen minutes in an autoclave. After a fixed interval of time, the percentage of germination was counted by a clinical Haemocytometer. The results of these observations are given in Table 2.

From Table 2 it is evident that adequate moisture supply is of primary importance for the germination of spores. About 5–10% of spores germinate in distilled water within 6 hours after sowing. In distilled water containing trace of alcohol germination is still more favourable. It is interesting to note that even the spores on dry slides, when kept in slightly humid

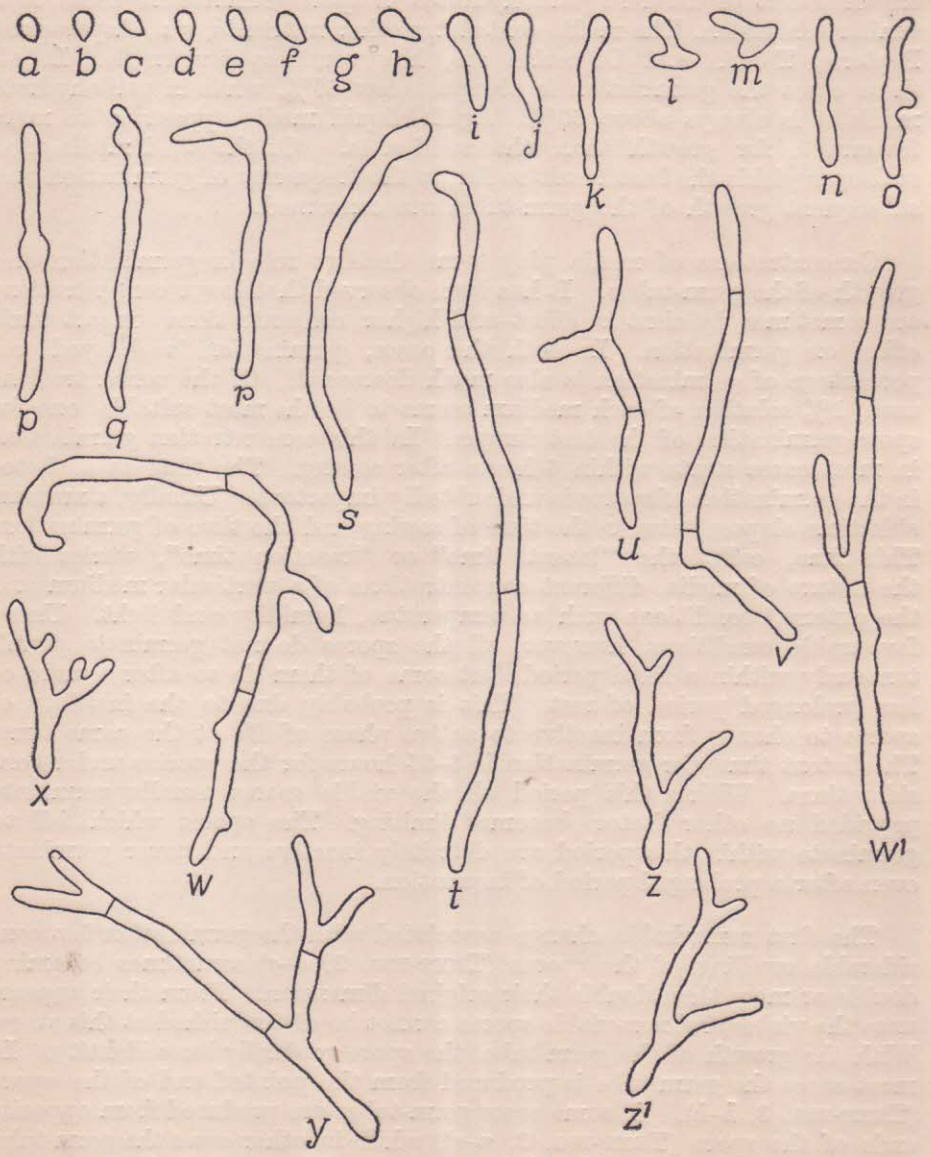
Table 2. *Data showing rate of spore-germination of* Trametes lactinea Berk. *on different media and its percentage after 24 hours at the most suitable concentrations*

| Media | Conc. (%) | Rate of Spore-germination after 6 hrs. | 9 hrs. | 12 hrs. | 24 hrs. | (%) of germination after 24 hours at the most suitable concentration |
|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Spores on dry slide | — | + | + | + | + | |
| Distilled water | — | + | + | + | ++ | 20 |
| Host-wood decoction | 5 | + | + | ++ | +++ | 32 |
| Dextrose solution | 0.5 | o | + | ++ | ++++ | |
| ,, ,, | 1.0 | o | + | ++ | ++++ | 56 |
| ,, ,, | 1.5 | o | + | ++ | ++++ | |
| ,, ,, | 2.0 | o | o | + | +++ | |
| Levulose solution | 0.5 | o | + | ++ | ++++ | |
| ,, ,, | 1.0 | o | ++ | +++ | ++++ | 60 |
| ,, ,, | 1.5 | o | + | ++ | ++++ | |
| ,, ,, | 2.0 | o | o | + | +++ | |
| Sucrose solution | 0.5 | + | ++ | +++ | ++++ | |
| ,, ,, | 1.0 | + | ++ | +++ | ++++ | 65 |
| ,, ,, | 1.5 | ++ | +++ | ++++ | ++++ | |
| ,, ,, | 2.0 | + | ++ | +++ | ++++ | |
| Maltose solution | 0.5 | o | + | ++ | +++ | |
| ,, ,, | 1.0 | + | + | ++ | ++++ | 57 |
| ,, ,, | 1.5 | o | + | ++ | +++ | |
| ,, ,, | 2.0 | o | + | + | +++ | |
| Asparagine solution | 0.5 | o | + | + | +++ | |
| ,, ,, | 1.0 | + | + | + | +++ | 43 |
| ,, ,, | 1.5 | o | o | + | ++ | |
| ,, ,, | 2.0 | o | o | + | ++ | |
| Peptone solution | 0.5 | o | + | + | +++ | |
| ,, ,, | 1.0 | + | + | ++ | +++ | 46 |
| ,, ,, | 1.5 | o | + | ++ | +++ | |
| ,, ,, | 2.0 | o | o | + | ++ | |
| Tannin solution | 0.5 | o | + | + | ++ | |
| ,, ,, | 1.0 | o | + | ++ | +++ | 38 |
| ,, ,, | 1.5 | o | + | + | ++ | |
| ,, ,, | 2.0 | o | o | + | ++ | |
| Glycerine solution | 0.5 | o | + | + | ++ | |
| ,, ,, | 1.0 | + | + | + | +++ | 35 |
| ,, ,, | 1.5 | o | + | + | ++ | |
| ,, ,, | 2.0 | o | o | + | ++ | |
| Sodium chloride solution | 0.5 | + | + | + | + | 8 |
| ,, ,, ,, | 1.0 | + | + | + | + | |
| ,, ,, ,, | 1.5 | o | o | + | + | |
| ,, ,, ,, | 2.0 | o | o | o | o | |
| Ordinary agar | 2.0 | o | + | ++ | ++++ | 50 |
| *Malt-agar* | 2.5 | + | + | ++ | ++++ | 62 |
| Potato-dextrose-agar (Fritz, 1923). | — | o | + | ++ | ++++ | 58 |

(o) = No germination
(+) = Very few germination (5–10%)
(++) = A few germination (10–20%)
(+++) = Moderate germination (20–50%)
(++++) = Profuse germination (50–70%)

atmosphere, germinate quickly by producing short germ-tubes. In these cases, however, no subsequent growth of the germ-tubes takes place, mainly due to lack of nutrition. Thus, it becomes evident that water alone is

any branch (TEXT-FIG. 3, *s*, *t* & *v*). In host-wood decoction the germ-tubes put forth branches at an early stage. The formation of septa is somewhat delayed and it takes place at a later stage of development of the germ-tubes (TEXT-FIG. 3, *t*–*z*$^1$). Further development leads to the formation of a richly branched primary mycelium.




TEXT-FIG. 3. Basidiospores and stages of germination of basidiospores of *Trametes lactinea* Berk. (× 725). (vide Text).

## Fungus in Culture

A. *Oxidase tests*

Following the method of Bavendamm (1928), experiments were carried out to determine whether *Trametes lactinea* is a 'white-rot' or a 'brown-rot' fungus. Both primary and secondary mycelia of *T. lactinea* were allowed to grow on 2.5% *malt-agar* medium containing 0.5% gallic acid or tannic acid in Petridishes. For each acid and with each type of mycelium a set of three Petridishes were inoculated. Uniform discs (diam. 4 mm) of inoculum were cut out from the advancing zones of 5-day-old Petridish culture and used for the purpose. The inoculated Petridishes were then incubated for 24 hours in complete darkness at 30°C. In all the cases positive reactions were noted and dark brown rings were formed around the inoculum within 24 hours. The intensity of reactions was, however, greater in the medium containing gallic acid than in that containing tannic acid in cases of both primary and secondary mycelia of the test fungus.

The results are given in Table 3 and the reactions are described following the terms and symbols adopted by Nobles (1948).

Table 3. *Results of oxidase tests with primary and secondary mycelia of* Trametes lactinea Berk.

| Types of mycelia | Reactions in 2.5% *malt-agar* | |
|---|---|---|
| | 0.5% gallic acid | 0.5% tannic acid |
| Primary mycelium | Very strong (+++++); diameter 18 mm.; diffusion zone very intense, dark brown, opaque, forming a wide corona around the inoculum; no growth above the inoculum. | Strong (++++); diameter 14 mm.; diffusion zone dark brown, opaque, extending considerably beyond margin; no growth above the inoculum. |
| Secondary mycelium | Very strong (+++++); diameter 22 mm.; diffusion zone very intense, dark brown, opaque, forming a wide corona around the inoculum; slight growth above the inoculum. | Strong (++++); diameter 16 mm.; diffusion zone dark brown, opaque, extending considerably beyond margin; no growth above the inoculum. |

It is evident from the foregoing table that *T. lactinea* is a 'white-rot' fungus.

B. *Effect of variation in hydrogen-ion concentration of the medium* (Plate II, figs. 5–16).

The effect of variation of hydrogen-ion concentration on the test fungus was performed with *potato-dextrose-agar* as the medium (Fritz, 1923). By using acetate and phosphate buffers, the following seven different grades of hydrogen-ion concentration were prepared. The grades of *p*H are 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0. Petridishes containing the sterile media of the different grades of *p*H were then inoculated by standard discs (diam.

4mm.) of inoculum of the mycelia of the test fungus. The Petridishes were then incubated at 30°C. and under diffused light for 30 days. Sufficient Petridishes were incubated in order to have three replicates for each treatment during the experimental period. One set of control was also kept for each treatment. The *p*H values of the medium before and after the experimental period were also noted.

The following growth characteristics of the fungus have been recorded on the sixth day of inoculation and the rate of growth in diameter of the colony is also given. In all the cases *p*H values, have been noted with the help of a *p*H-meter.

It is evident from Tables 4 and 5 that the fungus grows better in the acidic than in the alkaline media. Even in a high degree of acidity (*p*H 4.0) growth is better than that in any alkaline grade used. It has also been found that the growth rates of both types of mycelia are greater in the acidic media. On the contrary, none of the mycelia, primary or secondary, can grow in a higher alkaline medium than with a *p*H 8.0. It is thus concluded that *T. lactinea* is definitely remarkable for its tolerance of a long range of acidity, while it can tolerate very little of alkalinity of the medium.

Table 4. *Cultural data showing the effects of variation in hydrogen-ion concentration of the medium for primary mycelium of* Trametes lactinea Berk.

| *p*H Values | Habit of growth | Growth in diameter (in mm.) |
|---|---|---|
| 4.0 | Over the inoculum plush-like; aerial mat raised, velvety, radially widely furrowed; margin even, raised, but appressed at places, not uniformly appressed throughout; white. | 48 |
| 5.0 | Over the inoculum compact, felty; aerial mat throughout raised, even at the centre but uneven towards the periphery, felty throughout but at the margin becoming sub-felty, outermost zone entirely appressed and about 4–5 mm.; margin even and of unequal width (4–6 mm.); white. | 76 |
| 6.0 | Over the inoculum felty; aerial mat irregularly condensed to form a broad circular felty area which becomes thinner towards the periphery; advancing zone appressed with even margin (5–6 mm. wide); white. | 83 |
| 7.0 | Over the inoculum felty-velvety; aerial mat raised and felty forming a broad, central, circular zone, bayed at periphery; advancing zone appressed and of variable width (6–8 mm.); white. | 78 |
| 8.0 | Over the inoculum felty; aerial mat smooth, felty throughout, more or less circular, surrounded externally by a broad appressed zone of advance (3 mm. wide). | 40 |
| 9.0 | No appreciable growth even on the inoculum evident. | |
| 10.0 | same. | |

Table 5. *Cultural data showing the effect of variation in hydrogen-ion concentration of the medium for secondary mycelium of* Trametes lactinea Berk.

| *p*H Values | Habit of growth | Growth in diameter (in mm.) |
|---|---|---|
| 4.0 | Over the inoculum growth poor; aerial mat uniformly raised, downy to sub-felty throughout, with narrow radial depressions; zone of advance not in evidence; somewhat chamoise-like around the inoculum, with broad concentric zonations throughout; white. | 60 |
| 5.0 | Over the inoculum thin; mat uniform, thin, chamoise-like throughout; a narrow middle depressed circular zone evident; similar zone also present around the inoculum; zone of advance not in evidence; white. | 83 |
| 6.0 | Over the inoculum felty; a broad, circular appressed zone around the inoculum present; outer region farinaceous to chamoise-like; zone of advance not in evidence; white. | 90 |
| 7.0 | An irregular, more or less circular zone around the inoculum, smooth and felty; surrounding it a narrow, circular appressed zone, about 4–5 mm. wide; outer zone farinaceous to chamoise-like, but ultimately becoming appressed at the zone of advance; white. | 80 |
| 8.0 | Over the inoculum growth very poor; aerial mat flat, adpressed to the substratum; a faint and narrow farinaceous zone in between the two appressed areas present; faintly zonate; white. | 53 |
| 9.0 | No appreciable growth even on the inoculum evident. | |
| 10.0 | same. | |

It will also be evident from Tables 4 and 5 that the variation in acidity and alkalinity of the medium have also some remarkable effects upon the habit of growth. The primary and secondary mycelia, however, differ in many aspects. In the primary mycelium condensation of the mat is conspicuous. In the acidic grades (as in *p*H 4.0 & 5.0) the margin of the mat becomes increasingly irregular and zone of advance becomes gradually decreased. The texture of the mats becomes felty to a somewhat velvety in appearance with the gradual increase in acidity of the medium. In the alkaline grade (*p*H 8.0) on the other hand, the mat becomes smooth, uniform and circular in appearance.

Further studies of the cultures reveal that the mats gradually become more compact and thick. They secrete minute drops of transparent liquid on the surface. Colouration appears first in the secondary mycelium in *p*H 5.0 after 10 days of inoculation and also in other grades gradually. Light-Buff to Buff colour becomes more distinct in the secondary mycelium. In the primary mycelium, however, the appearance of colour on the mats is somewhat delayed.

C. *Growth characteristics of primary and secondary mycelia on different media* (Plate III, figs. 17–24)

Effect of various media on the culture characteristics of *Trametes lactinea* was studied with *potato-dextrose-agar* (Fritz, 1923), 2.5% *malt-agar*, *Czapeck's synthetic agar* (as modified by Fritz, 1923) and *wood-decoction-agar*. The *wood-decoction-agar* was prepared from healthy sapwood sawdust of *Terminalia procera*. Fifty grams of oven-dried sawdust was mixed with 500 ml. of distilled water in an Erlenmeyer's flask and was autoclaved at 5 lbs pressure for 30 minutes. The decoction thus obtained was filtered, mixed with 500 ml. of 5% melted agar and the whole medium was made upto 1000 ml. by adding required amount of hot distilled water.

The *p*H values of all the media were then adjusted to 6.0 by using acetate and phosphate buffers and finally sterilized. Approximately 40 ml. of each of the media were poured in a Petridish. Sufficient Petridishes were employed to prepare three replicas of each medium for each type of mycelium. The plates were inoculated separately with standard discs (4 mm. diam.) of 5-day-old cultures of primary and secondary mycelia of the test fungus and incubated for 5 days at 30°C.

The cultural characteristics of the fungus are summarised as follows :—

(a) Primary mycelium

(i) *Potato-dextrose-agar*.—The mycelium starts its growth within twentyfour hours of inoculation. On the first day little growth occurs around the inoculum only. The aerial mycelium becomes prominent on the second day, and it becomes somewhat uniformly matted. On the following days the superficial mat almost covers the entire surface of the medium in about 5 days. Growth characters on the 5th day show an aerial felty mat with peripheral radiating lines, particularly behind the zone of advance. Condensed postulates also appear here and there. The central region remains more or less smooth. The mycelium over the inoculum is felty. Advancing zone surrounding the mat is narrow and entirely appressed. The mat remains white throughout (Plate III, fig. 17).

(ii) *Malt-agar*.—Growth of the mycelium starts within twenty-four hours of inoculation. The mycelium grows mainly over the inoculum and extends very little on the surface of the media. Considerable growth in diameter of the aerial mycelium follows from the second day after inoculation. On the fifth day, the aerial mat becomes mostly but irregularly thin and felty. The peripheral region behind the zone of advance develops radiating lines. Zone of advance is narrow, appressed and of variable width (3–6 mm). Over the inoculum growth is poorer, somewhat sub-felty to felty. The mat remains white throughout (Plate III, fig. 18).

(iii) *Czapeck's synthetic agar*.—General growth characteristics and growth rate is extremely poor in this case. On the first day after inoculation, the mycelium grows only over the inoculum. On the second day growth on the medium becomes somewhat visible but remains entirely appressed throughout. Faint radiating lines appear over the appressed mat after

the third day of inoculation. Finally, the aerial mat remains appressed, but radiating lines of thin felty mycelium become evident. Growth over the inoculum is poor and somewhat patchy. Mat remains white throughout (Plate III, fig. 19).

(iv) *Wood-decoction-agar.*—On this medium growth is also poor. Growth rate is very slow at first but better growth takes place from the second day after inoculuation. Subsequently, the aerial mat becomes uniform and shows a thin sub-felty appearance throughout excepting the zone of advance. Faint radiating lines are noticeable. The advancing zone is appressed and sodden. Over the inoculum growth is somewhat compact and felty in appearance. The mat remains white throughout (Plate III, fig. 20).

Further observations were continued for a period of thirty days. The superficial mycelium becomes more condensed on *potato-dextrose-agar* and *malt-agar*. Irregular mycelial lumps appear here and there. Colour of the mat changes to Light-Buff or Buff. Glistening drops of liquid appear here and there but ultimately dry up. On the other two media, *Czapeck's synthetic agar* and *wood-decoction-agar*, the mat remains thin and appressed throughout. No fructification was, however, produced in any case.

(b) Secondary mycelium

(i) *Potato-dextrose-agar.*—Within twentyfour hours of inoculation considerable growth around the inoculum takes place. The growth rate of the aerial mycelium is rapid and it covers the entire surface of the medium within five days after inoculation. The mat remains somewhat uniform at first but zonation appears from the third day and later becomes distinct. Over the inoculum growth remains thin and poor throughout. Around the inoculum a circular but entirely appressed zone is evident, which in turn is surrounded by concentric layers of chamoise-like farinaceous mat with continuous or somewhat patchy radiating lines particularly behind the broad and appressed zone of advance. The advancing zone is, however, interrupted by the radiating lines from behind. The mat remains white throughout (Plate III, fig. 21).

(ii) *Malt-agar.*—Growth starts within twentyfour hours of inoculation and continues with moderate rapidity. The mat at first remains uniform but zonation around the inoculum appears on the third day. Within five days the surface of the medium is almost covered by the growth of aerial mycelia. A broad, appressed and circular zone is formed around the inoculum. Surrounding this, the aerial mat is more or less uniform and sub-felty in appearance. Faint concentric rings and broad radiating lines are also noticeable. Advancing zone is narrow (2–4 mm.) and appressed. Growth over the inoculum is very poor. The mat remains white throughout (Plate III, fig. 22).

(iii) *Czapeck's synthetic agar.*—Growth is extremely poor, mycelial growth becomes distinctly visible after two days of incubation. From the

very beginning growth of the mat remains entirely appressed, and growth rate is also very slow. On the fifth day no change in the appressed nature of the mat is observed. A somewhat patchy and sub-felty growth of the mycelium over and just around the inoculum is also noticeable (Plate III, fig. 23).

(iv) *Wood-decoction-agar.*—Growth starts within twentyfour hours. Though entirely appressed at the beginning, the aerial mat becomes somewhat evident on the third day after inoculation. The aerial mat, however, remains thin and adpressed to the medium upto the 5th day. A very narrow, circular zone of appressed mycelium is evident a little away from the inoculum. In between this zone and zone of advance a faint circular ring is also noticeable. The mat remains white throughout (Plate III, fig. 24).

Further observations for a period of thirty days reveal greater condensation of the mycelia in all cases except in the synthetic medium. Condensation was, however, maximum in *potato-dextrose-agar*, where colouration also appeared first within 12 days of inoculation. Certain irregular compact areas are also formed where the colour becomes deeper. With age Light-Buff and Buff colour appear over these compact areas and spread throughout. The mats on *potato-dextrose-agar* and *malt-agar* exude transparent liquid drops over their surface. Mat on *Czapeck's synthetic agar*, on the other hand, remains appressed throughout and exhibits no further change. In no case fructification was produced within the period of observation.

The daily increment of growth in diameter of primary and secondary mycelia of *T. lactinea* on different media upto the 5th day of inoculation has been recorded in Tables 6 and 7.

Table 6. *Data showing growth of the primary mycelium of* Trametes lactinea Berk. *on different media*

| No. of days after inoculation | Growth in diameter (in mm.) | | | |
|---|---|---|---|---|
| | Potato-dextrose-agar | Malt-agar | Czapeck's synthetic agar | Wood-decoction-agar |
| 1 | 13 | 10 | 8 | 6 |
| 2 | 32 | 30 | 22 | 18 |
| 3 | 51 | 50 | 35 | 40 |
| 4 | 67 | 68 | 49 | 55 |
| 5 | 76 | 74 | 57 | 66 |

A comparative study of the cultural characteristics of the primary and secondary mycelia grown on the different media reveal some interesting facts. The following general conclusions can be drawn from this study :—

*Habit of Growth* :—Of the four media used, *potato-dextrose-agar* and *malt-agar* seem to exhibit typical cultural characteristics of the fungus. Zonations and radiating lines of mycelia are the most characteristic features of the mycelia in culture. The mats formed by the secondary mycelium

Table 7. *Data showing growth of the secondary mycelium of* Trametes lactinea Berk. *on different media*

| No. of days after inoculation | Growth in diameter (in mm.) | | | |
|---|---|---|---|---|
| | Potato-dextrose-agar | Malt-agar | Czapeck's synthetic agar | Wood-decoction-agar ] |
| 1 | 14 | 11 | 7 | 5 |
| 2 | 35 | 32 | 23 | 23 |
| 3 | 56 | 53 | 39 | 44 |
| 4 | 80 | 75 | 57 | 60 |
| 5 | 86 | 83 | 65 | 70 |

appear to be more condensed, compact and uniform in comparison with those of the primary mycelium. *Potato-dextrose-agar* seems to be the best for the growth of both primary or secondary mycelia. The mat on this medium is more thick and compact. The two other media, *Czapeck's synthetic agar* and *wood-decoction-agar* appear to be less favourable for the growth of the test-fungus. The mats remain uniformly thin and somewhat appressed throughout. The radiating lines are only evident in case of primary mycelium, while circular zonations are noticeable in case of secondary mycelium. The secondary mycelium on *Czapeck's synthetic agar* remains entirely appressed throughout and almost invisible.

*Colour* :—At first the mat remains white throughout on all the media used. Colouration, however, appears later in *potato-dextrose-agar* and subsequently also in *malt-agar* medium. Light-Buff and Buff colour appear which gradually become more distinct over the more condensed and patchy areas.

*Rate of Growth* :—The growth rate of the secondary mycelium is better than that of the primary one in all the media used. It has also been observed that the rate of growth of the said fungus is most rapid in *potato-dextrose-agar* in comparison to other test-media (Tables 6 and 7).

## Discussion

The investigation on the biology of *Trametes lactinea* Berk., a common saprophytic fungus, has led the authors to discuss some salient features of its life-history. The fungus has been reported to be of wide occurrence in the tropical world, but its adaptation to different altitudes and different environmental conditions of the hill areas and forest ranges of the Indian territory is very significant. Again, the fungus has been seen to survive remarkably through the winter months of our country. When all these facts are taken into consideration, its present distribution only in the tropics seems to be more apparent than real. Explorations in the temperate regions would possibly throw more light about its wider distribution in the world.

The fungus grows on quite a large number of angiospermic hosts. This might well be a reason for the reported occurrence of different 'forms'

and 'varieties' of the fungus in nature. Form variation of basidiocarps have also been noticed by the authors in different localities and on different hosts.

Experiments have shown that adequate supply of moisture is of primary importance for the germination of spores of *T. lactinea*. So far the nutrients are concerned, the carbohydrates serve best for the purpose. This again is definitely influenced by the percentage of the nutrient materials available in the media (1% in this case). Nutrients in the media are obviously necessary for enhancing the process of further growth and differentiation of the germ-tubes. All the spores that are liberated by a basidiocarp are not viable as was evident from the fact that even in the best medium about thirty percent of the spores did not germinate. Always a measureable time will elapse between the time of sowing of the spores and the time of germination. This is the 'latent time' or 'reaction time' for germination.

Extensive cultural studies have shown that *T. lactinea* is distinctly acid-loving and always prefers a slightly acidic medium for its growth (near *p*H 5.0–6.0). Tolerance of alkalinity in the medium is very low. With considerable increase of alkalinity in the medium growth is completely checked, whereas even in more acidic medium, the fungus is capable of continuing its growth.

The fungus is of a fast-growing type and grows best on *potato-dextrose-agar*. *Malt-agar*, *wood-decoction-agar* and *Czapeck's synthetic agar* are less favourable for growth. The less effectiveness of the wood-decoction indicates that the cellulosic and lignin constituents of the wood, which constitute the main food of the fungus, are not present in the decoction.

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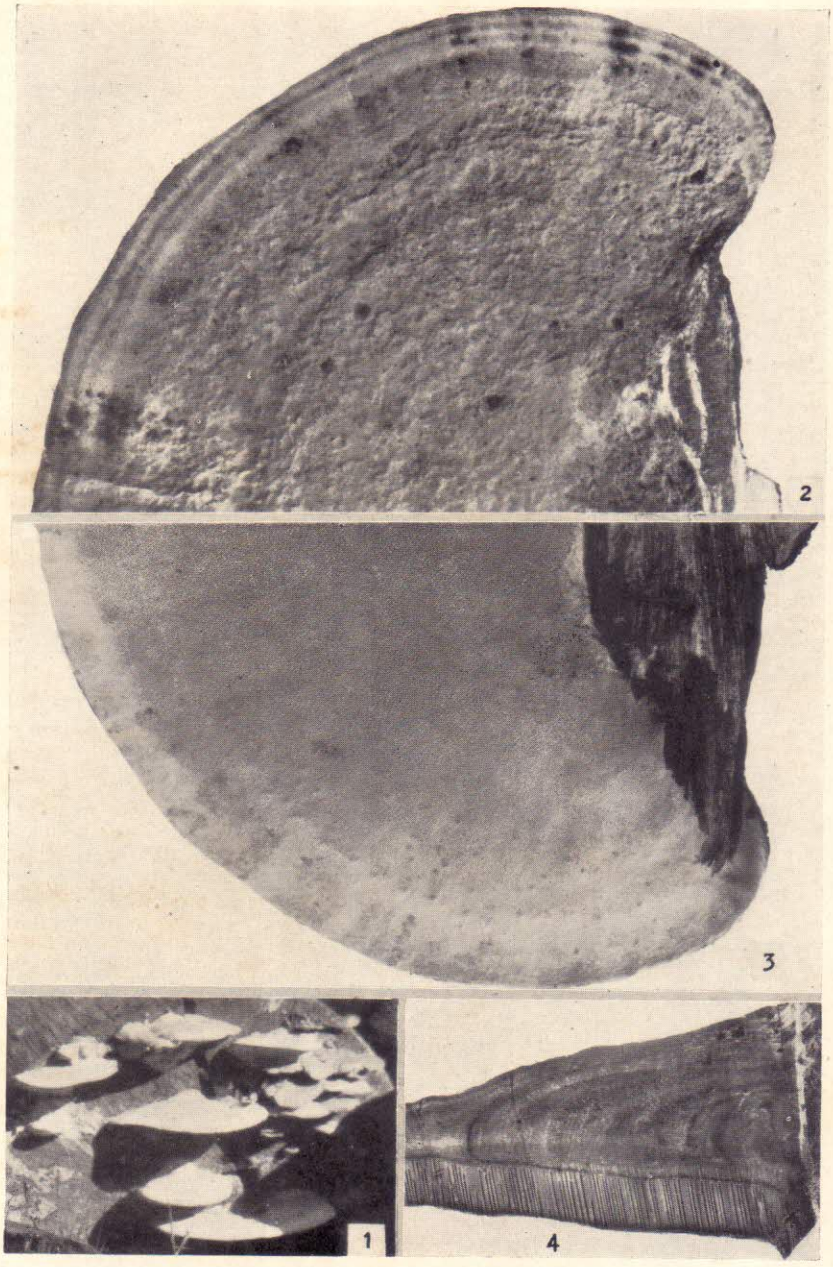


On *Trametes lactinea* Berk. S. N. Banerjee & N. C. Banerjee