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The identification of the mycelia of wood-decay fungi by their oxidation reactions with phenolic compounds

Identifiering av rötsvampsmycel med hjälp av deras produktion av fenoloxidaser

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I. Introduction

The identification of the mycelia of a great number of wood decaying fungi on the basis of their morphological characters is possible with the aid of the excellent descriptions of their cultural characters given by Campbell (1938), Davidson, Campbell & Vaughn (1942), Cartwright & Findlay (1946), Nobles (1948) and a number of shorter studies of various groups or species of such fungi (Maxwell 1954, Nobles 1956, Sarkar 1959, Lombard, Davidson & Lowe 1960, McKay & Lentz 1960, Denyer 1960 and several others). In identifying an unknown fungus one of the first questions is whether this fungus belongs to the white rot or the brown rot fungi. This is usually determined by the well-known Bavendamm test. It is known since a long time ago that the sporophores or mycelia of certain higher fungi contain enzymes which catalyze the oxidation of phenols and related compounds (Bourquelot & Bertrand 1896 and several others, cf. Boidin 1951). Bavendamm (1928 a, b) was the first to point out the difference between the white rot and the brown rot fungi with respect to their oxidative enzymes. When cultivated on nutrient agar containing certain phenolic compounds, as gallic acid or tannic acid, the white rot fungi produce a deeply coloured zone around the mycelium while the fungi causing brown rot do not. On the whole agreement between the type of decay and the type of reaction is good (cf. Davidson, Campbell & Blaisdell 1938, Nobles 1958), but some exceptions are noted. The production and intensity of the coloured zones and the growth rate of the fungi on gallic and tannic acid media are since (Campbell 1938, Davidson, Campbell & Vaughn 1942, Cartwright & Findlay 1946, Nobles 1948 and others) universally used as specific characters of the fungus species.

In the large groups of white rot and brown rot fungi numerous mycelia can be easily identified by their morphological characters alone, but others show only minor differences not easily recognizable. To facilitate the routine identification of such morphologically characterless mycelia, it would be a great help if we could find some physiological characters which were characteristic for the individual species, were easily detectable and could give a basis to divide the mycelia into smaller groups than by the Bavendamm test alone. As mentioned above the production of strongly oxidizing enzymes acting on certain phenolic

compounds is a constant characteristic for a number of wood-destroying fungi. These enzymes are easily detectable and according to the recent works of Boidin (1951), Higuchi & Kitamura (1953), Lyr (1956, 1958 a, b) concerning the identity and substrates of these enzymes, there seems to be a possibility to characterize the mycelia of the white rot fungi and a part of the mycelia of the brown rot fungi by their production of different phenoloxidases acting on different phenolic compounds, thus, together with the morphological characteristics providing a firmer basis for the identification of the mycelia of the wood-decaying fungi.

Since Bavendamm a number of authors has investigated the phenolases of the wood-destroying fungi from several points of view. For identification purpose, Davidson, Campbell & Blaisdell (1938), following Bavendamm's method, tested 210 species of wood-destroying fungi and grouped them on the basis of their type of reaction and characters of growth on gallic and tannic acid agar. For the same purpose Refshauge & Proctor (1935), Preston & Mc Lennan (1948), Jørgensen & Vejlby (1935), Etheridge (1957) and Nobles (1958) tried other ways to differentiate the white rot and the brown rot fungi. Preston & Mc Lennan (l.c.) described a method to separate the white rot and brown rot fungi growing them in test tubes on culture media containing various dyes belonging to different groups, as nitro-, azo-, anthraquinone and other dyes. All the tested 18 dyes were oxidized but of these gentian violet and neutral red gave the most satisfactory results. In the tests described, mycelia belonging to the white rot fungi decolorized the dyes, whereas mycelia belonging to the brown rot fungi did not. Preston & Mc Lennan explained the decolorization of the dyes by an oxidation process due to the production of an extracellular oxidase system by the white rot fungi. In the same way, some years before, Refshauge & Proctor (1935) had used the decolorization of malachite green, belonging to the phenyl methane dyes, for identification of the mycelia of the Australian basidiomycetes, without discussing the cause of the decolorization. Jørgensen & Vejlby (1953) found the results of the Bavendamm test obscure in some cases especially with species not growing on gallic or tannic acid media. They suggested for differentiation between the white rot and the brown rot fungi the use of red-cabbage extract which was added to the medium or to well grown cultures in tubes. Using this method, the extracellular oxidase produced by the white rot fungi caused the change of the original purple colour to yellow whereas the medium was left unchanged by the brown rot fungi. Etheridge (1957), too, noted certain inconsistencies in differ-

entiating between the white rot and the brown rot fungi by Bavendamm's method and he suggested a new method by growing the fungi on casein hydrolyzate medium containing wood meal from which the phenolic substances had been removed. When grown on this substrate, the white rot fungi showed a brownish reaction while the brown rot fungi caused no change in the colour. All these methods work well and have their advantages but also their negative sides. The methods with dyes take as much time as the Bavendamm test and the Etheridge test, although giving results more like those in natural decay than the other methods, is still more complicated and works slower than the Bavendamm test. The fast-working method of Jørgensen & Vejlby shows complications in getting constant indicator material. Thus, these methods are difficult to apply for routine identification purposes. Nobles (1958), searching for a more rapid method for differentiating the white and the brown rot fungi suggested the use of gum guaiac solution in 95 % alcohol, applying drops of this preparation direct to the actively growing mycelium in Petri dishes or in test tubes. The gum guaiac solution has a brown colour which by the white rot fungi is changed to blue by some fungi already after 2—3 minutes. The brown rot fungi do not produce colour change. Nobles compared the gum guaiac reaction with the Bavendamm reaction on gallic and tannic acid media and found that of 133 species tested, 19 gave inconsistent or questionable results with one or both methods. The other 113 species (nearly 85 %) agreed well, giving positive or negative results with both methods. The species giving inconsistent or contradictory results are discussed more thoroughly by Nobles. This is an excellent fast working method for dividing the mycelia into two groups, the reacting and non-reacting, but like all the methods previously metioned, it only indicates the prevalence of strongly acting phenolases and admits no more detailed classification of the enzymes involved. Furthermore, as with all natural products, the qualities of the gum guaiac from different sources are not always constant: some solutions of gum guaiac tested in our laboratory soon lost their reacting capacities, sometimes already after 3—4 weeks. The inconsistency of gum guaiac solutions has already been pointed out by Behrens (1907) (in Bavendamm 1928, Centralbl. Bakt. Parasitenk. p. 188) and Bavendamm (1928, Ztschr. Pflanzenkr. Pflanzenschutz. p. 258).

Meanwhile other authors had been working with crude extracts and purified enzymes of the wood-rotting fungi. They had tried to identify the enzymes catalyzing the oxidation of the dyes and the phenolic substances and discussed their role in lignin decomposition. The

nomenclature of phenol and polyphenol oxidases has been very confusing, as already pointed out by Bonner (1957). Since in recent years in literature concerning the enzymes of the wood-decaying fungi the terms tyrosinase and laccase for the respective enzymes have been used (Lindeberg & Holm 1952, Boidin 1951, Lyr 1956, Rösch 1961), these terms are used in the present paper in the sense of the above authors.

As early as 1928 Bavendamm mentioned that several enzymes of the laccase and tyrosinase type were involved in the oxidase system catalyzing the oxidation of gallic and tannic acids. Law (1950) tried to identify the agent responsible for the oxidation of dyes used by Preston & Mc Lennan (1948). She worked with crude extracts and purified enzymes from different white and brown rot fungi, tested them chemically and compared their action on the dyes and different other media with the action of laccase from *Rhus* tree and peroxidase from horse-raddish. The enzymes isolated from the white rot fungi were of a specific phenol oxidase type the action of which in many respects was similar to that of laccase. Some brown rot fungi were found to produce another phenol oxidase, which resembled tyrosinase. All the white rot fungi tested by her gave furthermore a strong peroxidase reaction. But already Law (1950, p. 76) had found differences in the action of laccase type enzymes from different sources on different phenolic compounds and she says: "It is evident that these related enzymes from different sources show a considerable variation in their substrate specificity". Law found a great variation in the rate of dye destruction in several species of fungi tested, which, according to her, was possibly due to the amount of enzyme produced. The reactions both on agar slope and with purified enzymes were, however, slow and thus the method using the decolorization of dyes by mycelia of wood destroying fungi to distinguish between them has no advantage if used for identification purposes.

Dion (1952) studied the phenol oxidase system produced by *Polyporus versicolor* by testing the visual colour changes when a purified enzyme was added to a range of phenolic materials. He tested several *ortho-* and *para*-substituted phenols as well as the oxidation in side chains and found the colour change to be due to the formation of *ortho-* and *para*quinones and that oxidation as well as polymerization could occur with several substances. He found that the enzyme system under consideration had very strong oxidizing properties and showed that this enzyme was distinguished from other phenol oxidases by its low pH optimum and its stability in acid solution. He, too, thought it likely that in this type of oxidation, several enzyme systems were concerned,

each of which might show its maximum type of activity at different pH values. He found also, as usually occurs with the extracellular enzymes, that high yields of the enzyme were obtained only as a result of good growth of the mycelium. Such growth was only obtained when organic nitrogen sources and readily available carbohydrates were used in the culture medium. Fåhraeus (1949) tested with *Polyporus abietinus* and *Polyporus versicolor* a great number of phenols and aromatic amines by adding them to agar nutrient media, in order to discover their ability to produce coloured zones as oxidation products by fungal enzymes. He showed that dark-coloured zones, which indicated the formation of quinone-like substances, appeared when at least two hydroxy groups in *ortho*-position were present, and further, in monohydric phenols, if the *para*-position was vacant. The *m*-phenols did not appear to be oxidized, but a great number of aromatic amines was readily oxidized. The range of substrates oxidized by *Polyporus versicolor* agreed well with that oxidized by the laccase. Tyrosine and other specific substrates for tyrosinase were not oxidized. Fåhraeus says, however: "Since only growing fungi have been studied, and no purified enzyme system, it is impossible to tell whether the oxidation of all these compounds is the result of the action of one relatively versatile enzyme like 'laccase' or whether there is a mixture of enzymes acting, each with a more restricted range of substrates." Some other white rot fungi which he tested oxidized the same substances as *Polyporus versicolor* but the brown rot fungi did not, or showed only a slight oxidation with some of these substances. Lindeberg (1950), Fåhraeus (1952, 1954), Lindeberg & Fåhraeus (1952), Fåhraeus & Lindeberg (1953) and Lindeberg & Holm (1952) studied the nature and formation of phenol oxidases in some *Agaricaceae* and some *Polyporus* species and came to the result that the phenol oxidases so far found in hymenomycetes belonged to the one or the other of the two enzyme types: tyrosinase and laccase. These enzymes have much in common but differ in their substrate specificity and their sensitivity to carbon monoxide, to elevated temperatures etc. They found that in certain fungi there existed a tendency to form tyrosinase in the sporophores and laccase in the mycelia. Lindeberg & Holm (1952) emphasize that enzymes of the tyrosinase type occur exclusively in the intracellular form whereas enzymes of the laccase type may occur both intra- and extracellularly. Since, according to Lindeberg, the extracellular phenol oxidizing enzymes involved in the Bavendamm and similar tests are of the laccase type. The above authors showed too that if active laccase is to be produced by fungi, great attention must be paid to the compo-

sition of the medium and the period of incubation. With malt extract and other media rich in organic nutrient, laccase is formed and secreted into the medium at an early stage of growth, sometimes after about seven days. If synthetic media were used, after this time usually no activity was observed and the highest activity revealed itself after about 21 days. A high activity was obtained after autolysis of the mycelium. Laccase formation was also found to be induced by a number of specific substances, which may or may not have functioned as enzyme substrates. Fåhraeus (1954) summarizes the conditions in which laccase is formed as follows: it is formed 1) in the presence of certain extracts, as malt or yeast extracts, 2) in the presence of specific enzyme substrates (e.g. phenolic compounds) as an adaptive response and 3) in the presence of certain inducing substances which are not attacked by the enzyme formed (e.g. tyrosine). Certain substituted phenols and amines were found to be very efficient enzyme iducers. Often the production of laccase was stimulated if growth was partially but not completely inhibited. Fåhraeus (1954) characterized laccase as "highly inducible" enzyme.

Lyr, (1956, 1958 a, b) studying the phenoloxidases of wood-decaying fungi, recognized three such oxidases: laccase, tyrosinase and peroxidase. Of the 154 species tested laccase was found in 75 species, tyrosinase in 75 species and peroxidase in 14 species. He found the laccase and the peroxidase to be exoenzymes, wheras tyrosinase was thought to be an endoenzyme. As Lindeberg & Fåhraeus, Lyr (1958 b) found the production of laccase to be highly inducible and highly stimulating to laccase production were tannin, phenol derivatives and organic acids. Most of the laccase inducers are simultaneously growth inhibitors. Amino acids had no effect on laccase production. As Lindeberg & Holm (1952), Lyr (1958 b) suggests that the positive Bavendamm reaction is principally caused by the action of laccase. Lyr describes a method for the detection of the three enzymes in growing cultures by adding the reagent to the underside of actively growing mycelia on malt agar and he suggested the use of his method for diagnostic purposes. Lyr's test is one step forward in using reactions on three enzymes for the identification of fungus mycelia. However, peroxidase was only found in fungi which simultaneously produced laccase, and to distinguish between laccase and peroxidase production according to Lyr's method in fungi with a strong laccase activity is not always easy.

Boidin (1951), Higuchi (1953) and Higuchi & Kitamura (1953) tried to identify the different oxidases in the mycelia of wood-destroying fungi by growing them on malt agar containing specific phenolic

substances. Boidin (l.c.) suggests the use of 0.2 % of tyrosine in malt extract agar for detection of tyrosinase and of 0.2 % of guaiacol for the detection of laccase. Boidin found the optimum pH for tyrosinase activity to be about 7 (Boidin from Miller & Davidson 1941, who found the optimum for tyrosinase activity to be between pH 5.5 to 7.1). The laccase was found to be stable in acidic milieu, acting between 2 to 9.5, with the optimum at about 6.1. Boidin was working with pH 4.5 for the laccase substrate. His results obtained with tyrosine and guaiacol tests agree well with these obtained by the Bavendamm test: of about 130 species studied only 2 species gave inconsistent results. Boidin suggests the use of guaiacol instead of gallic and tannic acids in nutrient media for the detection of laccase. Results with tyrosine were found to be variable, depending on the age of the inoculum and, possibly, on the pH of the medium. *p*-Cresol was found to be more sensitive than tyrosine to the action of tyrosinase, but in the concentrations used it was very toxic to the fungi. Boidin noticed with tyrosine some differences as compared with the results of other authors. He recommends, however, for the identification of fungus mycelia, the use in malt agar media of guaiacol for the detection of laccase and tyrosine, together with *p*-cresol, for the detection of tyrosinase. Of 123 species investigated, 102 were found to be active for guaiacol and 55 for tyrosine. Boidin found certain regularities in the distribution of tyrosine in the different groups of fungi: it was found to occur generally in the genera *Phellinus* and *Xanthochrous* and to be absent in the *Hymenochaete;* it was found to be common in the typical *Stereum* (*Luteola* and *Cruentata*) and rare in the *Merulineae. Peniophora,* especially sect. *Coloratae,* was usually found to produce tyrosinase. Boidin divides the mycelia investigated into four groups: a) not producing tyrosinase nor laccase, b) without tyrosinase but producing laccase, c) producing both laccase and tyrosinase and d) producing tyrosinase but not laccase. In this way, instead of two groups according to the Bavendamm test, four groups are obtained, but principally the same difficulties as with employing the Bavendamm test arise: a long time is required for the growth and reaction on agar media and the toxic action of the reagent in agar to the growth and the enzyme production of the fungi. Nevertheless, this method like the Bavendamm method, gives *inter se* comparable results and can certainly be valuable for the characterization of mycelia.

Rösch (1961) modified Boidin's method by inoculating vertical nutrient agar columns, containing phenolic substances in test tubes with the test fungi. If oxidases were produced by the fungi, the varying

depths of the coloured zones made it possible to measure the enzyme production almost quantitatively. However, Rösch himself was aware of the limitation of such a tests and he used them only for a rough estimation of the enzyme production by fungus mycelia. He considered the results on tyrosinase as an endocellular enzyme to be uncertain by the use of this method. By this method, however, the very soon introduced autolytical processes might well influence the production of other oxidases too.

Like Boidin, Higuchi & Kitamura (1953) employed α-naphthol in the nutrient agar for laccase test and tyrosine or *p*-cresol for tyrosinase test. With this method they detected the production of laccase by the white rot fungi, sometimes together with tyrosinase, whereas the brown rot fungi did not produce laccase, but sometimes produced tyrosinase. They propose the use in nutrient agar of α-naphthol for laccase test and *p*-cresol for tyrosinase test, thus too dividing the mycelia into four groups, instead of two as in the Bavendamm test. Testing the filtrates of culture media, mycelial extracts and mycelial residues for enzyme activities, they found, as opposed to Lindeberg's and Lyr's suppositions, that extracellular tyrosinase was produced in several fungi. However, as a rule, the content of tyrosinase and the degree of secretion were found to be sparse as compared with that of laccase. Thus, for the most part, laccase is responsible for the phenoloxidase reactions if the fungi are growing on substrates containing phenolic compounds, although at least autolytically deliberated tyrosinase might in some fungi (cf. Boidin 1951, tables) be responsible for such oxidation reactions. Recently, Rösch (1961) working with enzyme extracts from mycelia and nutrient solutions has shown that some of the brown rot fungi can produce small amounts of intracellular laccase, which agrees with the results presented in this paper.

Summarizing, it may be said that hitherto, for diagnostic purposes, generally the Bavendamm test, acting on both laccase and tyrosinase, has been used. Some other proposals have been made (Jørgensen & Vejlby 1953, Etheridge 1957, Nobles 1958) but they principally work like the Bavendamm test, dividing mycelia into two groups—those producing oxidizing enzymes and others not producing such enzymes. The investigations with enzyme extracts and purified enzymes (Lindeberg 1950, 1952, Fåhraeus 1949, 1952, 1953, 1954 a.p.) have shown that in these oxidation reactions more than one enzyme system is involved. Boidin (1951), Higuchi & Kitamura (1953) and Lyr (1956, 1958 a, b) have grown fungi on agar media where different phenolic compounds had been added which gave visible reactions if the one or another

enzyme system were present. Thus they were able to separate mycelia which produced laccase, tyrosinase and peroxidase and they suggested the use of their methods for diagnostic purpose. These methods are certainly valuable for diagnostic purposes as they inter se show comparable results, although, as with the Bavendamm test, the results may be not quite the same as with normally growing mycelia. The phenolic substances producing visible colour reactions in the presence of oxidizing enzymes are all more or less poisonous to the fungi, and, as is shown by the investigations of Lindeberg, Fåhraeus and Lyr (1952, 1954, 1958 a respectively), the production of enzymes, especially that of laccase, is highly dependent on the conditions of growth and is inducible in the presence of several compounds, as the phenolic substances, tannin, tyrosine, etc. As with the Bavendamm test, the tests suggested by Boidin (1951), Kitamura (1953) and Lyr (1958 b) require a long period of observation. Thus, the following investigation was undertaken, to find a rapid method for the recognition of the production by fungus mycelia of the different oxidizing enzymes as laccase, tyrosinase, and possibly peroxidase, for diagnostic purposes.

On the basis of this investigation a rapidly working method is suggested for dividing the fungus mycelia in four groups, depending on their production of laccase and tyrosinase. As the fungi often react in different ways to different phenolic compounds used as indicators for laccase and tyrosinase, there are possibilities for further differentiation between the mycelia. In these tests several phenolic compounds may be used which are not applicable in agar tests because of their toxicity.

II. Materials and Methods

The production of different oxidizing enzymes by the mycelia of wood destroying fungi was tested by the addition to the mycelium of drops of different phenolic compounds in alcoholic solution. If the phenolic compounds in the presence of certain oxidative enzymes were oxidized, a marked colour reaction, specific for the substances tested, appeared immediately or after some hours. Colour reactions with a number of phenolic compounds and aromatic amines were tested with a great number of fungi. The reactions produced are seen in Tab. 1—6.

A. Material

The tests were carried out with 587 isolations of fungi, representing 173 species belonging to the *Agaricaceae, Corticiaceae, Stereaceae* and *Polyporaceae*. Most of the strains were isolated at the Laboratory for Wood Mycology of the Royal College of Forestry (formerly Mycological Laboratory of the Forest Research Institute), Stockholm. A part of the mycelia was obtained from CBS, Baarn, and by courtesy of Dr. M. K. Nobles, Ottawa and prof. E. Björkman, Stockholm (cf. Käärik 1963) to whom I wish to express here my sincere thanks.

a) Mycelium. For the phenoloxidase tests the fungi were cultivated on malt extract agar (2.5 per cent malt extract, 1.5 per cent agar) in Petri dishes of 8.5 cm in diameter. The plates were inoculated with one inocolum on one side of the dish about 1.5 cm from the edge of the dish and incubated at 22° C. When the fungi had grown out, tests were carried out as described below.

If possible, several strains of the same fungus were tested (Tab. 1 and 2). Of 25 fungi 5—9 strains were compared and of five fungi from 10 up to 25 strains. Whith the majority of the fungi tests were carried out repeatedly at different ages of the mycelium, with mycelia 10—40 (sometimes up to 120) days old (Tab. 4). In a great number of fungi no changes were observed in the results with mycelia of different ages. In quite a large number of fungi, however, the results were different with the aged mycelia, as with the beginning of autolysis the enzymatical set of the mycelia may be completely changed. It is, therefore, very important that, if comparable results are desired, tests on phenol-

oxidases always be carried out with actively growing mycelia or in other words with mycelia that have just grown over the Petri dish or that are almost reaching the edge, or, with very slowly growing mycelia, as soon as there is enough growth for the test.

On the basis of their rate of growth the mycelia were grouped into three groups: a) very rapidly and rapidly growing mycelia; tests carried out after (8) 10—15 days, b) moderately growing fungi; tests carried out after 16—21 days, with the mycelia extending at least over 3/4 of the dish and c) slowly and very slowly growing fungi; tests carried out after 22 days or more. In tab. 1—3 and 6 the results are shown with mycelia of the mentioned ages. With very young mycelia the results could be weaker and with old mycelia in some fungi quite different results are obtained.

b) Reagents. Of different phenolic substances and organic amines the following compounds were tested: phenol, pyrocatechol, resorcinol, hydroquinone, pyrogallol, phloroglucinol, guaiacol, gallic acid, tannin, ferulic acid, vanilline, thymol, eugenol, *p*-quinone, α-naphthol, aniline, *o*-aminophenol, *p*-phenylenediamine, *o*-anisidine, 2.5-dimethylaniline (= 2.5-xylidine), *o*-toluidine, α-naphthylamine, 8-oxyquinoline, induline, *n*-methylaminophenol (=methol), benzidine, lactophenol and gum guaiac.

With the exception of gum guaiac, which was prepared according to Nobles (1958), the substances were dissoloved in 96 per cent alcohol at a concentration of 0.1 M. Of tyrosine, which is not dissolved in this concentration in alcohol, a suspension in alcohol was prepared and thoroughly shaken before each application. Lactophenol was prepared by taking one part of each, phenol, lactic acid and glycerin to two of distilled water. Gum guaiac solution was prepared by dissolving 0.5 g gum guaiac in 30 cc. of 96 % alcohol and filtering off the insoluble residue. The solutions were controlled after 1, 2 and 4 weeks and after 2, 3 and 4 months after which time they gave similar results. In the tests, solutions not older than 3 months were used.

Of these reagents, *p*-cresol and tyrosine are known to be oxidized only by tyrosinase, the others are oxidized only by laccase or by both laccase and tyrosinase. About the specific substrates of different phenoloxidases, there exist very contradictory reports which are more closely discussed in the last chapter. All the reports, however, agree that in the presence of laccase, *p*-cresol may be oxidized to a yellow substance, probably a dicresol, but in the presence of tyrosinase a brownish-red substance of quinone structure is produced (Fåhraeus 1949, Dion 1952) and that tyrosine can be oxidized only by tyrosinase to a dark-

brown compound. In the present tests only the reaction of *p*-cresol to tyrosinase, not to laccase, is recorded.

The solutions of *p*-phenylenediamine and *o*-aminophenol are after a short time oxidized in the air and they are therefore not suitable for the tests in question. Phloroglucinol, vanilline and *n*-methylaminophenol (methol) when used in tests like those presented here are mostly not oxidized or they are oxidized very slowly with a weak colour reaction. Therefore they are not used in further differentiating tests and results with two of them, vanilline and methol, are not shown in the tables. The solution of gum guaiac was oxidized only in quite fresh solutions. Ferulic acid was oxidized by the laccase-producing fungi but the colour reaction was very weak and not easily recognizable. Results with these two reagents too are left out from the tables.

Of these reagents, pyrocatechol is said to be autoxidized at pH 7 but to be stable in acidic milieu, and pyrogallol to be autoxidized also in acidic milieu (Boidin 1951). In the present tests, probably because of the shorter test time, pyrocatechol and pyrgallol were neither autoxidized nor oxidized by the uninoculated malt agar during the test time.

Attempts were made to detect the production of peroxidase by modifying Lyr's method (1958 a), by applying benzidine + H_2O_2 to the mycelia. Benzidine is readily oxidized by laccase to a dark-brown or black compound, but according to Lyr (l.c.) by the addition of H_2O_2 to benzidine, in the presence of peroxidase the dark colour produced by laccase is still more intensified. In the present tests by the application of benzidine in the presence of laccase, there always appeared almost immediately so deep a brown or black colour that it was impossible to register any intensification by the addition of H_2O_2. In a number of fungi, however, very different reaction rate, was noticed with benzidine with and without the addition of H_2O_2. Possibly the very short reaction time in some fungi after the addition of H_2O_2 to benzidine may account for the presence of peroxidase. As peroxidase is known to be produced almost always in the presence of laccase (Lyr 1956, 1958 a), it was not possible to distinguish with the method used in the present tests between the production of laccase and that of peroxidase. Perhaps, with a closer study of reaction rate it should by possible to distinguish between laccase and peroxidase production also by the present "drop" method. It is possible that the deviating results with some reagents as lactophenol and others, not agreeing with the reactions of the typical laccase and tyrosinase reagents, are partly caused by the action of peroxidase.

B. Tests

When the mycelia were of suitable age, the tests on phenoloxidases were carried out by applying one drop of the reagent to the mycelium about 1—1.5 cm from the edge of the dish or from the edge of the mycelium. As most of the reagents did not interfere with each other, later 4 drops were placed in the same dish, at an equal radius from the inoculum. Only such reagents which rapidly diffused over a large surface, as lactophenol, *o*-toluidine, and 2,5-xylidine, were placed in separate dishes.

The colour reactions may appear with some of the fungi and some of the reagents almost immediately, but with others the reactions appear after some hours or after 1—2 days. The reactions were checked after 24 and 48 hours and after 3, 5 and 7 days. Most of the reagents had produced their maximal colour reaction already after 24 hours but with some of the reagents the maximal colour change appeared after 2—3 days. With most of the fungi the colour produced was stable during a week and more but with some others, the colours of several reagents, especially those of guaiacol, gallic acid, tannin, pyrocatechol, pyrogallol and *o*-aminophenol were bleached after 3—5 days. The degree of bleaching was dependent on the fungus species and was especially striking in *Phlebia radiata, Hypholoma fasciculare, Odontia bicolor* and *Stereum hirsutum*. With some of the fungi, often those producing very intense colour reaction, most of the reactions were entirely bleached after 3—5 days, as in *Polyporus adustus* and *Polyporus benzoinus*. The only exception among the reagents is lactophenol, which is oxidized slowly, usually after 1—3 days to a dark-brown compound. This reaction may be get still more intense during the following days and is never bleached. Even phenol, *p*-quinone and aniline may react slowly. In the following tables 1—6 the colour reactions after 48 hours are shown; only with lactophenol the colour reaction is registered after 3 days. Usually, if there is no lactophenol reaction after 3 days, no reaction or only a very weak reaction will appear later.

III. Results

On the basis of their reactions with the phenolic substances tested, the mycelia of the wood decaying fungi may be divided at first into four large groups:

I. Fungi, producing neither laccase nor tyrosinase.
II. Fungi, producing only tyrosinase.
III. Fungi, producing only laccase.
IV. Fungi, producing both laccase and tyrosinase.

As characteristic reagents for laccase were chosen: α-naphthol, α-naphthylamine, *o*-anisidine, induline, guaiacol, 8-oxyquinoline, *o*-toluidine and xylidine. As characteristic reagents for tyrosinase *p*-cresol and tyrosine were used.

Further the above groups may be subdivided into smaller ones on the basis of the intensity of the colour reactions and on the basis of the specific reactions of the mycelia with reagents listed above. If the different strains of one species show different reactions, these species will be represented in more than one group.

A. Factors which may influence the results

Before the results are presented in tables 1 and 2, some external and internal factors which may influence the results must be discussed.

a) Substrate

As is known, the substrate may have a great influence on the production of phenoloxidases, especially that of laccase (Fåhreaus 1952, 1954, Lyr 1958 b). In the present investigation the oxidation reactions were compared on a standard medium which is generally the same as used for growing fungus mycelia for microscopic examination. Thus the very interesting question, how the enzyme production and the different reactions are influenced by the substrate, is not at all dealt with in the present paper.

From the results of Fåhraeus (1953, 1954) and Lyr (1958 b) it is seen that on malt extract media and other substrates rich in organic nutrients a high laccase activity appears already after 7 days in rapidly growing fungi, and that furthermore a high laccase activity appears

after autolysis of the mycelium. The addition of different substances, especially of some substituted phenols is highly stimulating for the production of laccase (Fåhraeus 1954). After the addition of such inducers to the medium, the maximum laccase production takes place if growth is partially but not completely inhibited. Lyr (1958 b) has further investigated substances inducing laccase production and found that the following groups of substances act as laccase inducers: 1) substances inhibiting oxydative phosphorylation, as substituted phenols, especially pentachlorophenol, 2) poisons of the SH group, 3) acids of the citric acid cycle and 4) inhibitors of the citric acid cycle. As these results show, malt extract agar is a suitable medium for laccase production and with rapidly growing fungi already after 7 days a high laccase activity may be expected. In the present investigation there was no special reason to follow the laccase production as induced by the different substances. So plain malt agar was used for growing the fungi, without the addition of phenolic compounds, as suggested by Madhosingh (1962) and by Fåhraeus (1954) and Rösch (1962 a, b) for a higher yield of the enzymes.

b) Temperature

At the beginning of the present tests, some fungi of the white rot and the brown rot type, viz. *Pleurotus ostreatus, Polyporus abietinus, Polyporus albidus, Polyporus sericeomollis, Polyporus kymatodes, Coniophora cerebella, Merulius lacrymans* and *Lentinus lepideus* were grown at the temperatures 15, 20, 25 and 30° C. With these mycelia the phenoloxidase tests were carried out as described above. No differences or only slight differences in the reactions were observed with the mycelia grown at different temperatures. On the basis of these tests it may be said that in the routine tests, when the fungi are grown at approximately constant temperatures between 20—22° C, temperature variation within these limits has practically no influence on the results.

c) pH of the medium

The acidity of the substrate is known to have a great influence on the action of phenoloxidases (Law 1950, Boidin 1951, Lindeberg & Holm 1952, Rösch 1961). The different phenoloxidases in purified form have been shown to have different pH limits for their action, with a marked pH optimum, varying for various oxidases. Thus, Gregg & Miller (1940) found laccase to be stable between pH 2 to 9.5 with an optimum at pH 6,1. Law (1950) found that the colour reactions catalyzed by laccase

were alike at pH 4—7 and that the optimum action was likely at pH 6. Boidin (1951) says that the optimum for laccase lies in the acidic milieu and he himself used pH 4,5 in his tests for laccase. Lindeberg & Holm (1952) found the optimum laccase action to occur at pH 5. Rösch (1962 a, b) found the optimum pH for intracellular laccase to be lower than that for tyrosinase, or between pH 3—4.5. Concerning intracellular laccase Rösch (1961) showed by photometrical determinations of mycel extracts that a sharply limited pH optimum is only detectable at the maximum enzyme activity and that the pH curves at this time are very sharply pointed. If quantitative analyses at this time are carried out, it is necessary to know an exact pH optimum if comparable results are to be obtained.

Information concerning the pH optimum of tyrosinase is more contradictory. Thus Kubowitz (1937) found the pH optimum for tyrosinase to be very sharply limited, about pH 7.1. Miller & Dawson (1941), on the contrary, noticed in the action of tyrosinase no differences between pH 5.5—7.1. Boidin (1951) worked in his tyrosinase test with pH 7.0 and found that the reactions at pH 4—6 could be delayed or absent. Lindeberg & Holm (1952) showed the tyrosinase from *Marasmius* to have and optimum zone between pH 3.5—5.5 and Rösch (1962 a, b) says that tyrosinase has higher pH optimum than laccase.

As the pH of malt extract from different sources may vary within certain limits and as the fungi themselves may alter the pH of the medium during their growth, there is a possibility that certain inconsistent results in different strains of some fungi were caused by the variations of the pH in the medium. Thus, a test was carried out growing five fungi at different pH levels on malt agar, buffered with 0.1 M KH_2PO_2, 0.1 N HCl and 0.1 N NaOH, from pH 2.5 to 6.5 (Tab. 3). The fungi tested were: *Flammula alnicola, Fomes ulmarius, Hypholoma capnoides, Lenzites betulina* and *Polyporus kymatodes,* showing on standard malt extract agar different reactions to the phenolic compounds.

As appears from Tab. 3, the pH of the medium strongly influences the growth of some fungi. Only *Polyporus kymatodes* grows almost equally well at all pH levels tested, from pH 2.5 to 6.5. *Fomes ulmarius* did not grow at all at pH 2.5 and in the other fungi growth was suppressed at pH 2.5.

The colour reactions at different pH levels did not vary as much as expected. With a number of substances, as tyrosine, ferulic acid, benzidine, α-naphthylamine, 8-oxyquinoline, pyrocatechol, resorcinol, phloroglucinol, *o*-aminophenol, methol and gum guaiac the results were al-

most equal at all pH levels tested. Some of the reagents, however, showed slight differences at the lowest or highest pH value (Tab. 3). Thus, eugenol, hydroquinone and *p*-quinone in some fungi were oxidized only at the lowest pH levels; others, as *o*-anisidine, *o*-toluidine, 2.5-xylidine, tannin, pyrogallol, lactophenol and phenol showed very weak or no oxidation at all at the lowest pH levels. Induline was sometimes not oxidized at either very low or very high pH levels. The variation seemed to be influenced by the fungus species: in *Flammula alnicola* the results were rather variable with different reagents at different pH levels, whereas *Fomes ulmarius* and *Hypholoma capnoides* showed almost equal results at all pH levels. From this test, however, it appears that with the present method testing the reactions on slightly acid media at pH between 4.0 and 5.5, the differences in reactions caused by pH, if occurring, were so slight that they could not influence the general results.

d) Age of the mycelium

The main tests in the tables 1 and 2 were carried out with young, vigorously growing mycelia (10 to 20 days old), and only with very slowly growing mycelia after a longer time. With most of the mycelia separate tests were carried out also with older mycelia (30 to 80, sometimes up to 100 to 120 days old), the plates being inoculated simultaneously with the plates used for the main tests (Tab. 4). It appeared that in a large number of fungi no changes in reaction were observed with the older mycelia but with quite a number of fungi the results with older mycelia were essentially changed. Out of 150 fungi presented in Tab. 4, 85 or 57 % did not change the reaction after 42 days, 29 showed some changes and 21 showed marked changes, the two last groups representing 33 % of all the fungi tested. 15 fungi or 10 % were not followed as long as 42 days. As appears from these results, it is very important that all tests on phenoloxidases be carried out with mycelia which are not too old. In other words, they are to be carried out as soon as the mycelium has grown out over the dish, or, with more slowly growing mycelia, as soon as the mycelium has reached about 1/3 to 1/2 of the dish. A separate experiment on oxidase reactions with four mycelia of different ages is shown in Tab. 5. The mycelia of different ages were inoculated on gallic and tannic acid media and on α-naphthol, guaiacol and tyrosine agar media (according to Boidin 1951) and the reactions were compared with the spot tests on mycelia of the same

ages. All the fungi were of the moderately growing type, *Peniophora gigantea* and *Stereum sanguinolentum* growing faster than the other two fungi. Normally, different strains of these fungi show considerable differences in their reactions.

The results in Tab. 5 show clearly that the phenoloxidase reactions in these fungi vary with the age, more obviously in the spot tests but clearly even in the agar tests. On α-naphthol-agar none of the fungi tested showed any growth nor produced any colour reaction, so this compound is left out from the table. The reactions of the very young mycelia differed both in the drop and in the agar plate tests from those of the middle-aged mycelia and other alterations were observed in quite old mycelia. The relatively fast-growing fungus, *Peniophora gigantea* showed after 8—15 days a stabilized type of reaction in both drop and agar tests, the other relatively fast-growing fungus, *Stereum sanguinolentum*, showed stabilized reactions after 8—15 days in drop tests and after 15—23 days in agar tests. With *Fomes roseus* and *Stereum hirsutum* mycelia up to 15 days old differed in their reaction type from the older ones; this was especially obvious in the drop tests. After this time some stabilization occurred but with mycelia 36—43 days old in all the fungi tested some alterations appeared again both in drop and agar tests. It appears that the drop and agar tests gave different results concerning tyrosinase reactions: *Peniophora gigantea* showed a strong tyrosinase reaction with *p*-cresol in the drop test but none with the agar test. On the basis of the results of the above tests and the unpublished reports of tests with young mycelia of fast-growing fungi, it may be said that an age between 8—15 days for the fast-growing fungi and between 16—23 days for the moderately-growing fungi ought to give comparable results.

Sussman & Markert (1953), studying the tyrosinase production in *Glomerella,* found that tyrosinase activity undergoes rather striking variations during the growth of the fungus. Conidia of this fungus possess a moderate tyrosinase activity but this is lost during germination. After about 5 days growth tyrosinase activity begins to appear and reaches its maximum at about 7 days. The commencement of tyrosinase activity was found to coincide with the end of the growth and with the beginning of the autolysis of the mycelium. A rapid decrease in tyrosinase activity appears after the peak of the activity is reached. The authors suggest that in this case an induced synthesis of tyrosinase occurs, possibly in response to the accumulation of a substance released during autolysis.

This agrees with the results in Tab. 5, where a rich tyrosinase pro-

duction in *Peniophora gigantea* appears in the old mycelium and with results in Tab. 4: the marked alterations in the reaction of the old mycelia were partly due to a strong tyrosinase reaction in old mycelia of fungi producing no tyrosinase in young mycelia and partly due to a strong laccase activity in old mycelia, both types of oxidase activity being induced by autolysis.

e) Variation between different isolates of a fungus species

If the oxidase reactions with different phenolic compounds are intended to be used for the characterization of different fungus species, it is essential to know how great the variations between different isolates of the same species are. From a selection of fungi belonging to both the white rot and the brown rot type, a number of strains was inoculated and tested simultaneously. Of 25 fungi 5—9 strains and of 5 fungi 10 to 25 strains were compared. It was shown that in some fungi, as *Hypholoma fasciculare, Polyporus abietinus, Polyporus betulinus, Polyporus borealis* and *Poria radiculosa* the results of the tests with all the isolations were always perfectly equal; other fungi as *Peniophora gigantea, Polyporus picipes, Polyporus mollis,* and *Stereum sanguinolentum* showed an obvious variation between the individual strains, sometimes so great as to place the different strains of one species in different groups (cf. Tab. 1, 2 and 6). Some of such fungi giving variable results, as *Peniophora gigantea* and *Stereum sanguinolentum* show very variable results with the Bavendamm test as well. With *Stereum sanguinolentum* a test was carried out separately with 12 monosporus strains from two different sporophores, A and B (Tab. 6). The results showed that already newly isolated, vigorously growing monosporous strains from the same sporophore of *Stereum sanguinolentum* could show considerable variation in reactions . In sporophore A four strains of six had identical reactions, one had much weaker and one much stronger reactions, differing in reactions with guaiacol, 8-oxyquinoline, xylidine, *p*-quinone and aniline. The strains from sporophore B showed throughout weaker reactions, five of six showing almost identical reactions and one differing in reactions with xylidine, *p*-quinone and aniline. All the strains of sporophore A showed reaction with pyrogallol whereas those of sporophore B did not. All the strains tested showed a medium laccase reaction and none of the strains showed tyrosinase reaction nor reaction with pyrocatechol or lactophenol, which behaviour is characteristic as well for all the other *Stereum sanguinolentum* strains tested in other connections.

Thus, the monosporous strains isolated from two different sporophores showed some prevalent differences amongst themselves. Although the majority of isolates from one sporophore produced quite similar reactions, some of the strains differed quite markedly which proves that differences between different strains often found in *Stereum sanguinolentum* are most probably fixed genetically. Even the fact that strains from such variable and other species of fungi if tested at different times, always react perfectly in the same way, proves that the existing differences between strains to a great extent are fixed genetically.

As mentioned above, the same isolate of a fungus always produces the same type of reaction, if mycelia of the same age are tested. A number of strains, even such with a very specific type of reaction, were tested several times, at intervals of some months or years and the reactions turned out to be perfectly identical at all repetitions. Such fungi, tested repeatedly were: *Corticium alutaceum* 366, *Fomes roseus* 354, *Fomes pinicola* 65, *Gloeocystidium karstenii* 410, *Hymenochaete tabacina* 389, *Lentinus tigrinus* 31 and 39, *Merulius tremellosus* 438, *Panus torulosus* 374, *Peniophora cinerea* 409, *Peniophora pithya* 405, *Pholiota destruens* 369, *Polyporus cuticularis* 392, *Polyporus guttulatus* 423, *Polyporus kymatodes* 565 and 566, *Polyporus sericeomollis* 202, *Poria candidissima* 430, *Trametes heteromorpha* 434 and several others.

It may be mentioned that the production of phenoloxidases may be lost during the cultivation of the mycelia. Some of the very strikingly degenerated strains of otherwise very active white rot fungi showed no reaction with the phenolic substances at all and as in all probability the ability to synthesize the phenoloxidases, as other enzymes of more or less vital importance is successively lost, we have another reason why some strains of one fungus may differ from others in their reaction capacity.

B. Results of the tests

As appears from the above, if the fungus mycelia are grown on malt agar medium at pH between 4—5,5 and at a temperature between 20—22°C, the oxidation reactions with various phenolic compounds are in a great number of fungi similar or in general simlar for the different isolates of the same fungus and that different fungi may show quite different reactions with a number of phenolic compounds. The reactions depend in some degree on the age of the mycelium but choosing a certain age for the tests the results ought to be comparable. Thus, in

the different reactions to the different phenolic compounds produced by different species of fungi we have at least a means of controling the morphological identification of the mycelia. Of course, fungi are found which show a considerable variation in reaction between different strains and, on the other hand, some of the taxonomically closely related species with very similar mycelia, as *Polyporus versicolor* and *P. zonatus* show quite identical reactions so that the phenoloxidase reactions are of no help in separating these mycelia. In other cases, however, the reactions can provide a good help in identifying more or less similar mycelia as in cases of the *Hypholoma* species, *Polyporus abietinus* and *Odontia bicolor, Fomes igniarius* and *Poria obliqua,* and in the case of *Fomes nigricans* and *F. trivialis,* to name some examples. It is possible that on other, more defined substrates the reactions might show another character, the differences perhaps being more accentuated (c.f. Madhosingh 1962), but in the present work the simple malt extract agar was preferred as the medium on which the fungi are grown for routine identifications.

Simultaneously with the present experiments, Madhosingh (1962) has used a similar method for distinguishing between the mycelia of *Polyporus cinnabarinus, P. coccineus* and *P. sanguineus,* three closely related species with rather similar mycelia. He used as substrate a synthetic nutrient agar medium supplied with 1 % tyrosine. On this medium several phenolic compounds were tested and he found differences in reactions to these compounds in the three species of fungi. However, all these three fungi showed similar reactions when grown on malt extract agar. There seems to be a possiblility that also other closely related species may show differences in reactions to the phenolic compounds, when grown on synthetical media with the addition of some phenolic compounds and that such more detailed investigations must be carried out in more complicated cases.

It is not known at the present time whether the different reactions to different phenolic substances by different fungus species or even strains are caused by chemically different enzymes or whether there are other reactions between the fungus and the substrate that may influence the results. The different reactions with different phenolic compounds cannot be explained with the different sensitivity of these substances to laccase or tyrosinase alone, although in general certain compounds are more sensitive than others. The reactions with different phenolic compounds are in detail described below.

a) Reagents on laccase:

benzidine is a very sensitive reagent to laccase. In the presence of very small amounts of laccase, benzidine solution is oxidized almost instantly to a brown compound which becomes deep brown to black in the presence of strong laccase. Sometimes the brown rot fungi discolour the solution to yellow or yellowish brown (+ to ++ in the tables).

α-naphthol is oxidized in the presence of laccase to violet of different degrees, sometimes nearly to black. It is a very sensitive reagent to laccase, though not as sensitive as benzidine.

α-naphthylamine is oxidized as readily as *α-naphthol* and it produces the same violet to almost black colour.

o-anisidine is oxidized in the same way as *α-naphthol* and *α-naphthylamine* and it produces likewise a violet colour of different degrees, to almost black. These three reagents mostly show the same degree of reaction but quite unexpectedly the results may vary in some fungi (cf. Tab. 5), some of these reagents giving a weak reaction when the others show a strong one or the contrary.

induline gives a reddish-brown to greyish-brown colour in the presence of laccase. It is a weaker reagent than the four mentioned above and only with a number of very strong laccase producers does it show an intense brown colour. Otherwise the colour reaction of induline is medium with most of the fungi that give a strong reaction of the *α-naphthol* type.

guaiacol gives a very beautiful purple red colour in different shades. It is a weaker reagent than the four mentioned above and quite often if these show a strong reaction, only a medium reaction is obtained with guaiacol. Fungi showing a moderate reaction with *α-naphthol* and similar compounds usually do not produce any reaction with guaiacol. There are some exceptions, however.

8-oxyquinoline gives a marked yellow colour which may sometimes be very intense. Like guaiacol, it is a weaker reagent on laccase than *α-naphthol* and similar compounds and it often shows parallel results with guaiacol. In several cases, however, where guaiacol gives a medium to weak reaction, 8-oxyquinoline does not produce any.

2,5-xylidine and *o-toluidine* give rather similar colour reactions with laccase, both quantitatively and qualitatively. They produce a rather light to medium violet colour, sometimes deeper with the one reagent and sometimes with the other.

b) Reagents on tyrosinase

p-cresol produces after oxidation with tyrosinase a rich orange red to brownish red colour, often in the substrate, sometimes in both aerial and substrate mycelium and sometimes in the aerial mycelium only. This colour reaction is known to occur only after oxidation with tyrosinase and it is taken as a specific reaction to this enzyme.

tyrosine is also oxidized only by tyrosinase and it produces a yellowish brown to dark brown colour, but not in all fungi that can oxidize *p*-cresol. Only rarely is it found that tyrosine is oxidized where *p*-cresol is not.

c) Reagents not specific to laccase or tyrosinase

gallic acid and the following reagents are oxidized by both laccase and tyrosinase and they produce colour reactions of varying degrees. Gallic acid is oxidized to yellowish brown or medium brown compound.

tannin produces a rather similar colour reaction to gallic acid, but not always to the same extent.

pyrogallol is likewise oxidized to yellowish brown or dark brown and its colour reaction is often deeper than that of gallic acid or tannin.

hydroquinone is oxidized only by a few fungi and it produces a greyish brown colour.

phloroglucinol is oxidized only in rare cases and it produces a brown colour.

pyrocatechol is a rather sensitive reagent and it produces a brown colour.

p-quinone produces in quite numerous fungi a greyish brown colour which is usually not very intense.

aniline produces a grey colour, sometimes more brownish, sometimes more violet. The colour sometimes becomes very deep and may diffuse over a large surface.

phenol is often not oxidized and it produces a light to medium brown to almost black colour in most of the fungi producing laccase or tyrosinase. The colour produced by pyrocatechol may sometimes be bleached after some days.

lactophenol, which, probably due to its acidity can penetrate better through the fungus cellwalls, gives a very intense, somewhat reddish brown colour, which is produced sometimes both in the substrate and

mycelium but sometimes only in one of them. The colour becomes successively diffused over a large area and gives one of the deepest and most conspicuous colour reactions. One reason for this very strong colour reaction is the high concentration of phenol in this solution, as compared with the concentrations of other reagents, but even in a more dilute solution of phenol with the addition of glycerine and lactic acid or of only glycerine, so that the final concentration of phenol remains constant, the reaction acts faster and the colour becomes much deeper. It is probably the question of better penetration of phenol in this solution into the fungus cells that is the cause of the faster and deeper colour reaction as compared with the solution of pure phenol. Observations on such colour reaction with lactophenol produced by the mycelia of decay fungi were observed by the author in other connections already a long time ago. Thus, tests with this compound were continued in the present investigation to obtain an idea of the consistency of this type of reaction. It seems that this reaction is very characteristic for a number of fungi. In *Stereum sanguinolentum,* e.g., which produces varying amounts of laccase in different strains, this reagent never gives any reaction however strong the laccase reaction is. The same is valid for *Fomes annosus.* On the other hand, fungi producing quite weak or medium reaction with laccase or tyrosinase may give a very strong reaction with lactophenol, as *Merulius lacrymans, Polyporus schweinitzii, Polyporus sericeomollis* and *Radulum orbiculare.*

Besides the listed phenolic compounds and aromatic amines found in tables 1—7, the following substances belonging to the same groups were tested and oxidized by the decay fungi: *ferulic acid,* which produces in the presence of laccase a light yellow colour, *resorcine,* which is sometimes oxidized to give a yellow or medium brown colour, *thymol,* which is very rarely oxidized, producing a light medium brown colour, *eugenol,* which is rather often oxidized to a yellowish brown compound and *methol,* which very rarely produces a greyish brown colour. *O-aminophenol* produces a deep reddish brown colour, but it is very easily oxidized in the air. The brown colour of *o-aminophenol* is completely bleached by some of the brown rot fungi but not by others.

According to Dion (1952) the change of colour observed when the phenols are oxidized enzymatically is often ascribed to the formation of an *ortho*-quinone but he surposes that *para*-quinone formation as well may appear, possibly even in cases where *ortho*-quinone formation is theoretically possible. In some cases the phenols are polymerized as well as oxidized.

d) A general survey of oxidation reactions with the mycelia tested

Tables 1 and 2 show the oxidation reactions with all the fungi and strains tested. The results are quite interesting. It is seen quite clearly that it cannot be expected that all the strains of a given fungus should produce the same reaction pattern, characteristic for the fungus species. However, if all the substances tested are compared, some of the fungi produce a very characteristic pattern, which is rather similar between the individual strains and quite different from other species. Such fungi might well be characterized already by their reactions with different phenolic substances. Such fungi are e.g. *Fistulina hepatica, Corticium alutaceum, Fomes conchatus, Polyporus kymatodes* and *Poria monticola.* Then, a large group of fungi is found, belonging to the very active white rot fungi, which all produce a very similar reaction pattern, giving an intense reaction with almost all of the different reagents except the specific tyrosinase ones. In these fungi the different strains all behave very alike. Such fungi are e.g. *Daedalea unicolor, Hydnum septentrionale, Lenzites betulina, Polyporus abietinus, Polyporus adustus, Polyporus borealis, Polyporus hirsutus, Polyporus versicolor* and *Polyporus zonatus.*

One group of the brown rot fungi never produces reactions, as *Coniophora arida, Grandinia farinacea, Daedalea quercina, Fomes pinicola, Polyporus betulinus, P. fragilis, P. caesius* and *Trametes heteromorpha.* Quite a number of brown rot fungi, however, give some weak laccase or sometimes a weak to strong tyrosinase reaction, as *Coniophora cerebella, Merulius lacrymans, Lenzites sepiaria, Lentinus lepideus,* some *Corticium* species, *Polyporus sericeomollis* as well as several *Poria* and *Trametes* species.

Some of the morphologically related species, as *Fomes nigricans* and *Fomes trivialis* show rather different reactions and furthermore, some non-related fungi, with morphologically similar mycelia may be distinguished by their different reactions, as *Polyporus abietinus* and *Odontia bicolor.* Other taxonomically closely related species as *Polyporus versicolor, Polyporus zonatus* and *Polyporus pubescens* show very similar reactions and thus they cannot be identified by aid of their reactions on phenoloxidases. Some species show rather great variations between the individual strains, as *Armillaria mellea, Stereum sanguinolentum, Stereum hirsutum* and *Peniophora gigantea.*

Among the *Polyporaceae* not many species are found which show tyrosinase reactions with the present method. Tyrosinase production was found in *Fistulina hepatica, Fomes conchatus, F. ulmarius, Poly-*

porus circinatus, P. guttulatus, P. mollis, P. picipes, P. rutilans, P. schweinitzii, P. sericeomollis, Trametes abietis, T. cinnabarina and *T. pini.* More frequent than in other genera among the *Polyporaceae* was tyrosinase production in the genus *Poria,* i.g. in *Poria albobrunnea, Poria cinerescens, Poria monticola, P. obliqua, P. rixosa, P. versipora* and *P. xantha.*

Tyrosinase production is more often met among the *Corticiaceae,* especially among the *Peniophora,* the *Hydnaceae* and very frequently among the *Agaricaceae.* It looks as if the most of the wood-inhabiting *Agaricaceae* hitherto tested produce tyrosinase, as *Armillaria mellea, Flammula gummosa,* all the *Hypholoma* species, *Lentinus lepideus, Panus conchatus, P. stipticus,* almost all the *Pholiota* species tested, *Pluteus cervinus,* but usually not the *Plerotus* species.

Of course, not much is known about the intraspecific variations in reactions in many of the species tested and the number of species showing great variations may increase with future tests. On the other hand, among the very variable fungi listed in table 2 some may prove to be actually more constant. In *Polyporus mollis,* for example, it is most likely that not all the isolates belong to the same fungus species, as the isolates from different sources are morphologically so dissimilar that the individual authors to the isolates must have meant different fungi as *Polyporus mollis.* The isolates of *Peniophora gigantea* vary somewhat morphologically and as the greatest part of them is isolated from decaying wood and as the microscopical characteristics of the mycelia of related *Peniophora* species are not thoroughly known, it is possible that a part of the isolates, hitherto known as *Peniophora gigantea,* belongs to some related species. The majority of mycelia of the fungi and strains tested in the present investigation, however, is isolated from sporophores or obtained from other authentic sources.

In spite of variations sometimes shown by individual strains, such tests easily provide a basis for estimating the production of phenol-oxidases in fungus mycelia both qualitatively and quantitatively. In routine tests in connection with the morphological identification of the mycelia, the number of substances tested may be reduced to leave some more and some less sensitive reagents for laccase, both reagents for tyrosinase and some of the reagents giving more individual results as pyrocatechol, hydroquinone, *p*-quinone, aniline and lactophenol. In the following, the mycelia of the wood decay fungi are divided at first in four large groups on the basis of their production, if any, of laccase and tyrosinase. Subsequently, the groups may be subdivided on the basis of the intensity of their reactions and on the basis of different

reactions with individual substances. Some species, of course, must appear in more than one group, but the same behaviour also is often encountered when grouping the mycelia on morphological basis.

C. The four groups of fungi, based on their production of laccase and tyrosinase

a) A general survey and key to the groups I—IV

Group I. Fungi, producing neither laccase, nor tyrosinase.
II. Fungi, producing no laccase, only tyrosinase.
III. Fungi, producing only laccase, not tyrosinase.
IV. Fungi, producing both laccase and tyrosinase.

These groups are set up on the basis of the reactions of the mycelia with the following substances: benzidine, α-naphthol, α-naphthylamine, *o*-anisidine, induline, guaiacol, 8-oxyquinoline, 2,5-xylidine, *o*-toluidine, gallic acid, tannin, pyrogallol, *p*-cresol, tyrosine.

Key to the groups.

1. No reaction with any of the reagents. Only a very weak reaction with benzidine may occur. Group I.

2. Weak to strong reaction with p-cresol or tyrosine. No reaction with any of the other reagents; only a weak reaction with gallic acid, tannin or pyrogallol or a very weak reaction with benzidine may occur. Group II.

3. Weak to strong reaction with some or all of the following reagents: benzidine, α-naphthol, α-naphthylamine, *o*-anisidine, induline, guaiacol, 8-oxyquinoline, 2,5-xylidine, *o*-toluidine, gallic acid, pyrogallol or tannin. No reaction with *p*-cresol nor tyrosine. Group III.

A. Weak to sometimes medium reaction with some few (never all) of the following reagents: α-naphthol, α-naphthylamine, *o*-anisidine, induline, guaiacol, pyrogallol, gallic acid or tannin. Group III A.

B. Fungi, giving with the above substances a medium or only with some substances a strong reaction. Group III B.

C. Fungi, giving with most or with all of the above substances a strong reaction. Group III C.

4. Weak to strong reaction with some of the above laccase reagents and weak to strong reaction with *p*-cresol or tyrosine. Group IV.

A. Weak reaction with the laccase reagents as listed in III A.
Group IV A.

B. Medium reaction with the laccase reagents as listed in III B.
Group IV B.

C. Strong reaction with laccase reagents as listed III C.
Group IV C.

b) Notes to the fungi belonging to groups I—IV

Group I. No laccase, no tyrosinase. The brown rot fungi.

A. Fungi, not producing any reaction on young mycelia with any of the substanses tested.

a) Fungi, never producing any colour reactions either with young or with old mycelia (cf. Tab. 4):

Collybia velutipes
Coniophora arida
Merulius himantioides
Peniophora cremea
Polyporus betulinus
Polyporus dichrous
„ *sulphureus*
Poria radiculosa
Trametes hetermorpha

aa) Fungi, not producing colour reactions on young mycelia, but giving some, mostly weak, reactions with some of the laccase or tyrosinase reagents on old mycelia (cf. Tab. 4):

Corticium laeve
Grandinia farinacea
Polyporus caesius
Trametes serpens

If the oxidase reaction type of the fungi belonging to these groups is compared with the results found by other authors concerning the same fungi (Tab. 7), it is seen that the results, although obtained by different methods, agree well. None of these fungi, if grown on malt agar media, with or without the addition of phenolic substances, produces any phenoloxidases detectable by the ordinary methods.

B. Fungi, giving on young mycelia in some strains no reaction and in other strains a weak reaction with some of the substances tested.

b) Fungi, not producing colour reactions with typical laccase or tyrosinase reagents, but giving in some strains reaction with some other phenolic compounds:

Coniophora olivacea (medium reaction with lactophenol)

bb) Fungi, giving no reaction or in some strains producing a weak laccase reaction with one or more of the following reagents: benzidine,

α-naphthol, α-naphthylamine, *o*-anisidine, induline, *p*-quinone. Tyrosinase reaction not occurring (These fungi are listed also in group III a):

Coniophora cerebella
Daedalea quercina
Fomes pinicola
Lenzites sepiaria
Merulius silvester
Polyporus mollis
Poria crassa
Poria lenis
„ *radiculosa*
„ *sinuosa*
Stereum frustulosum
Trametes sepium
„ *serpens*

bbb) Fungi, giving no reaction or producing in some of the strains a weak to medium laccase reaction with some (never all) of the following reagents: benzidine, α-naphthol, α-naphthylamine, *o*-anisidine, induline, guaiacol, gallic acid, tannin, pyrogallol, pyrocatechol, *p*-quinone. Tyrosinase reaction not occuring (listed also in group III aa):

Polyporus albidus
Polyporus balsameus
Poria vaillantii
Poria vaporaria
Schizophyllum commune
Trametes serialis

bbbb) Fungi, giving no reaction or producing in some of the strains a weak to medium laccase reaction. A weak to medium tyrosinase reaction may occur. Found also in groups II, III-A-aaa and IV A.

Lentinus lepideus
Merulius lacrymans
Trechispora brinkmanni

Concerning the fungi belonging to the groups I Bb—I Bbbb, even the results of other authors have been rather similar. Generally these fungi do not produce any colour reaction with the Bavendamm test. In *Fomes pinicola* only Higuchi & Kitamura (1953) have shown a clear tyrosinase reaction in the extracts of both the culture medium and mycelium and this reaction was reproduced even in their agar test. *Lenzites sepiaria,* which in the present tests in some strains produces no oxidases and in others gives a weak laccase reaction, according to Boidin (1951) produces no laccase but gives quite a strong tyrosinase reaction. All other reports on the phenoloxidases of *Lenzites sepiaria* have been negative. *Lentinus lepideus,* belonging also to the brown rot fungi, gives in the present tests with most of the strains a weak laccase reaction and with almost all the strains a clear tyrosinase reaction. Accordingly, variable reactions with this fungus or a weak Bavendamm reaction are reported by Davidson et al. (1938), and Nobles (1948) or only a positive tyro-

sinase reaction by Lyr (1958 a). The same may be said about *Merulius lacrymans*. The only positive report on phenoloxidases occurring in *M. lacrymans* is that of Zoberst (1952), showing in an enzyme preparation from the mycelium of this fungus the occurrence of tyrosinase and a very weak laccase. Rösch (1961, 1962 a) has shown the existence of intracellular laccase in *Merulius lacrymans* and *M. silvester*, as well as in *Poria vaporaria* and *Coniophora cerebella*. Other reports on the above fungi are consistently negative with the exception of that of Etheridge (1957) who reported a positive Bavendamm reaction with *Coniophora cerebella* and Law (1950) who found a weak tyrosinase reaction in this fungus. Bavendamm (1928), Davidson et al. (1938), Higuchi & Kitamura (1953), Jørgensen & Vejlby (1953), Lyr (1958 a), Nobles (1958) who sometimes found with their methods a weak polyphenol oxidase reaction in some of the brown rot fungi, did not find any in *Poria vaporaria*.

Schizophyllum commune, usually known as a white rot fungus is reported by Nobles (1958) as giving variable results both with the Bavendamm and gum guaiac tests. Davidson et al. (1938) count the negative reaction with gallic acid and positive with tannin as a species character for this fungus. Boidin (1951) reports for *Schizophyllum* a variable reaction with guaiacol and a negative with gallic acid and tyrosine; Higuchi & Kitamura (1953) found a medium laccase reaction both in the substrate and in the mycelium in this fungus and Lyr (1958 a) notices a variable reaction with gallic acid and weak production of laccase simultanously with negative tyrosinase and peroxidase reactions for it. In the present tests *Schizophyllum commune* showed a negative tyrosinase reaction and a weak, somewhat variable laccase activity, thus agreeing quite well with the previous results.

Thus, if the results with different methods, such as the drop method, agar plate method and methods with enzyme extracts are at all to be compared, the tests with the brown rot fungi of group I give with all these methods more or less consistent results. A number of fungi or strains, previously known to give negative phenoloxidase reactions, showed in the present tests a weak laccase activity, probably due to the production of similar intracellular laccase as shown by Rösch (1961, 1962 a, b) for some other brown rot fungi. Such intracellular laccase production is usually not detected by the agar plate method. The only really diverging results concerning fungi of this group are found for *Fomes pinicola* and *Lenzites sepiaria*, which in the tests of Higuchi & Kitamura (1953) and Boidin (1951), respectively, showed a production of tyrosinase not detected with the present method in any of the strains.

Group II. Fungi, producing no laccase, but only tyrosinase. Fungi, belonging to the brown rot group. Mycelia belonging to this group occur very rarely. Most of the fungi and strains giving tyrosinase reaction produce simultaneously at least a weak laccase reaction.

a) Weak to medium reaction only with *p*-cresol:

Lentinus lepideus *Trechispora brinkmanni*

aa) Medium to strong reaction both with *p*-cresol and tyrosine:

Merulius lacrymans

Not many fungi are found even by others to produce only tyrosinase. Boidin (1951) reports 6 such fungi of 123 tested (*Corticium caeruleum, Stereum abietinum* (x), *Odontia corrugata, Fistulina hepatica* (x), *Lenzites sepiaria* (x) and *Lenzites betulina* (x) (N.B. a very active white rot fungus). Lyr (1958 a) reports the following fungi as producing only tyrosinase: *Anisomyces odoratus, Cereomyces albus, Fistulina hepatica* (x), *Lenzites abietina, Lentinus lepideus* (x), *Polyporus stipticus* (x), *Merulius lacrymans* (x) and *Polyporus schweinitzii* (x). Fungi, marked with (x) have been tested even in the present investigation, but most of them, except some strains of *Lentinus lepideus, Merulius lacrymans* and *Trechispora brinkmanni* showed simultaneously at least a weak laccase reaction. Some of these fungi are found in our group IV A, as fungi producing a clear tyrosinase reaction and a weak laccase reaction and are discussed there.

Group III. Fungi, producing only laccase.

A. Laccase reaction weak. With the exception of *Schizophyllum commune* these fungi belong strictly to the group I, the brown rot fungi.

a) Laccase reaction very weak. A weak or very weak reaction occuring only with one or more of the following reagents: α-naphthol, α-naphthylamine, *o*-anisidine, induline, pyrogallol, *p*-quinone:

Coniophora cerebella (α-naphthol, α-naphthylamine, pyrogallol)
Daedalea quercina (*o*-anisidine)
Fomes pinicola (α-naphthylamine)
Lenzites sepiaria (α-naphtylamine, *o-anisidine*)
Lenzites trabea (α-naphthylamine)
Merulius silvester (induline)
Polyporus mollis, strain Nobles (α-naphthol, *o*-anisidine, α-naphtyhlamine, induline)
Poria aurantiaca (*o*-anisidine, induline, *p*-quinone)

Poria radiculosa (*o*-anisidine, induline)

Poria sinuosa (*o*-anisidine, induline)

Stereum frustulosum (α-naphthylamine)

Trametes sepium (*o*-anisidine, induline)

Trametes serpens (induline)

aa) Laccase reaction somewhat stronger, occurring with a number of the following reagents: α-naphthol, α-naphthylamine, *o*-anisidine, induline, guaiacol, gallic acid, tannin, pyrogallol, pyrocatechol, *p*-quinone. Some strains of these fungi are even found in groups III a or in group I.

Polyporus albidus
„ *balsameus*
„ *tephroleucus*
Poria crassa
Poria vaillantii
„ *vaporaria*
Schizophyllum commune
Trametes serialis

aaa) Fungi, giving very various results with both laccase and tyrosinase reagents. May be found in groups I, II, III and IV, but mostly the reactions, when found, are weak. Belonging strictly to the brown rot fungi.

Lentinus lepideus
Merulius lacrymans
Trechispora brinkmanni

Most of these fungi have been commented upon under the groups I and II. *Polyporus tephroleucus* shows according to Davidson et al. (1938) no reaction in the Bavendamm tests and in the present tests it shows only a weak laccase reaction. Usually the fungi belonging to group III A show a very weak laccase reaction probably due to a similar enzyme as that shown by Rösch (1961, 1962 a, b) for *Merulius lacrymans* and *Coniophora cerebella*. A number of the brown rot fungi belonging to our group III A-aa shows a somewhat stronger laccase activity than those of the group III A-a, and some of these fungi have even previously been reported as producing a negative to weak positive reaction in the Bavendamm or other agar tests, as *Polyporus albidus* (Lyr 1958 a), *Polyporus balsameus* (Nobles 1948), *Schizophyllum commune* (Nobles 1958), which may have been caused by a weak extracellular enzyme system. In *Polyporus albidus*, however, Higuchi & Kitamura (1953) found no phenoloxidase production at all.

B. Laccase reaction medium. The real white rot fungi. Medium reaction with the following reagents: α-naphthol, α-naphthylamine, *o*-anisi-

dine, induline, guaiacol. With some of the reagents the reaction may be strong.

b) no reaction with 2.5-xylidine or toluidine:

Corticium subseriale
Daldinia concentrica
Flammula sapinea
Fomes annosus (most of the strains)
Fomes fomentarius
„ *igniarius*
„ *nigricans*
Omphalia campanella (most of the strains)
Peniophora cornea
„ *gigantea*
„ *hydnoidea*
Pleurotus corticatus
„ *ostreatus* (some strains)
Polyporus albellus
„ *amorphus*
„ *croceus*
„ *fumosus*
„ *kymatodes*
„ *mollis*
„ *lacteus*
Poria weirii
Stereum hirsutum
„ *purpureum*
„ *sanguinolentum*
Thelephora terrestris
Trametes cervina
„ *suaveolens*
Ustulina vulgaris
Xylaria polymorpha

bb) distinct reaction with xylidine or toluidine

Corticium confluens
Flammula alnicola
Gloeocystidium karstenii
Hymenochaete rubiginosa
Irpex lacteus
Peniophora byssoidea
„ *gigantea*
Pholiota flammans
Pleurotus serotinus
Polyporus picipes
Radulum orbiculare
Stereum gausapatum
„ *hirsutum*
„ *rugosum*
Trametes squalens

Fungi of this group, as far as it is possible to compare the results of the present tests with these of other authors, give quite consistent results with different methods, except the disagreeing raports for *Fomes igniarius*. According to Lyr (1958 a) this fungus was found to produce only peroxidase and no laccase. Boidin, however, (1951) finds a strong laccase production in *Fomes igniarius* and according to Higuchi & Kitamura (1953) it produces both an extracellular and intracellular weak tyrosinase.

Fungus mycelia found in the group III B-b and III B-bb show quite

distinct morphological characteristics to separate these mycelia from each other. Otherwise these quite large groups can be divided again on the basis of the reactions of the mycelia with other phenolic compounds, as catechol, hydroquinone, phloroglucinol, *p*-quinone, aniline and lactophenol. With phloroglucinol only two of the mycelia, viz. *Flammula sapinea* and *Ustulina vulgaris* give a reaction, simultaneously not showing any activity with any of the other reagents. Exemples for other fungi, oxidizing only a specific one of the listed compounds are: *Fomes annosus*, giving only catechol-reaction, *Fomes igniarius, Polyporus fumosus, P. kymatodes, Trametes suaveolens* and *Flammula alnicola,* giving only lactophenol reaction and *Omphalia campanella, Polyporus croceus* and *Trametes cervina* giving only a *p*-quinone reaction.

These are merely examples noted here, but as the number of strains of fungi tested hitherto has been too limited for us to be sure of these differences, a further classification based on these reactions is not given in this work. These possibilities are followed in further tests until more material shows whether this will be a reliable basis for differentiation of the mycelia or not. Lactophenol, however, seems to give a constant and characteristic reaction for the species even in fungi showing otherwise quite variable reactions, as in *Armillaria mellea, Peniophora gigantea, Merulius lacrymans, Trechispora brinkmanni* and *Stereum sanguinolentum. Stereum sanguinolentum,* for example, however variable the reactions otherwise may be, never gives any lactophenol reaction and *Merulius lacrymans* always gives a strong lactophenol reaction. The same is seen in numerous other fungi of which a number of strains has been examined, as in *Fomes annosus,* which never gives any reaction with lactophenol. Possibly the production of peroxidase by fungus mycelia may be the cause of this reaction.

C. Strong laccase reaction. The real white rot fungi. Strong reaction with all or most of the following reagents: α-naphthol, α-naphthylamine, *o*-anisidine, induline, guaiacol.

c) No reaction with 2.5-xylidine or toluidine:

Fomes fomentarius
Fomes trivialis
Merulius molluscus
Omphalia campanella
Peniophora septentrionalis
Pleurotus corticatus
Polyporus arcularius
Polyporus cuticularis
Poria albobrunnea
Stereum murrayi
Tricholoma rutilans

cc) Distinct reaction with 2.5-xylidine or toluidine:

Collybia dryophila
Daedalea unicolor
Fomes annosus (some strains)
„ *nigrolimitatus*
Grandinia granulosa
Hydnum septentrionale
Hymenochaete tabacina
Lenzites betulina
Panus stipticus
Peniophora cinerea
„ *gigantea*
„ *incarnata*
Phlebia radiata
Pleurotus corticatus
Pleurotus ostreatus (most of the strains)
Pleurotus ulmarius
Polyporus abietinus
„ *adustus*
„ *albellus*
„ *amorphus*
„ *benzoinus*
Polyporus borealis
„ *brumalis*
„ *frondosus*
„ *hirsutus*
„ *hispidus*
Polyporus lacteus
„ *melanopus*
„ *occidentalis*
„ *picipes*
„ *pubescens*
„ *radiatus*
„ *spumeus*
„ *varius*
„ *versicolor*
„ *vulpinus*
„ *zonatus*
Poria candidissima
„ *ferrugineofusca*
„ *mollusca*
„ *versipora*
Stereum purpureum
Trametes isabellina

In this rather large group of strong white rot fungi, several differences from other authors' results are noted. *Merulius molluscus* is listed by Boidin (1951) as giving entirely negative results with phenoloxidases, contrary to our strains which give a strong reaction both with drop tests and with the Bavendamm test. Nobles (1959, manuscript) lists too this fungus as a positive one with the gum guaiac test. Further Lyr (1958 a) notes for *Daedalea unicolor* a weak tyrosinase reaction, never found in our strains. Otherwise our results with this fungus agree well with those of Davidson et al. (1948), Nobles (1948) and Higuchi & Kitamura (1953).

Lenzites betulina Fr. ex. L., quite astonishingly, Boidin (1951) lists among the brown rot fungi, finding for it totally negative reactions with tannic acid, guaiacol and tyrosine. Still more confusing is his note in the text that some authors, like Hemmi & Kurata (1931) and La Fuze (1937) have found negative reactions with this fungus and that

they, as well as Boidin have been working with the real *Lenzites betulina,* whereas other authors, as Davidson et al. (1938), who found a strong reaction with gallic and tannic acids had, according to Boidin, been working with *Polyporus betulinus* (*Piptoporus betulina* Karst. Bull.) instead. The contrary must be true, as *Lenzites betulina* (L.) Fr. really belongs to the strong white rot fungi, very common on felled birch and always (cf. Davidson et al. 1938, Nobles 1948, 1958, Higuchi & Kitamura 1953, Jørgensen & Vejlby 1953, Lyr 1958 a) giving a strong laccase reaction. On the contrary, *Polyporus betulinus* Bull. ex. Fr. (*Piptoporus betulinus* Karst.), very common on dead standing birches, belongs to the typical brown rot fungi, never producing polyphenol oxidase reaction (Davidson et al. 1938, Nobles 1948, 1958, Higuchi & Kitamura 1953, Jørgensen & Vejlby 1953, Lyr 1958 a, Henningsson 1962, 1964). These fungi are so different indeed that they ought never to be confused.

Concerning other fungi of this group, Lyr (1958 a) has in *Panus stipticus* recognized a weak tyrosinase production which has not appeared in our strains.

In *Peniophora cinerea* and *P. incarnata,* Boidin (1951) has observed a strong tyrosinase production. These fungi were somewhat inconsistent in our tests, *P. cinerea* producing a weak tyrosinase and *P. incarnata* in some isolates no tyrosinase and in others a strong one.

In *Pleurotus ostreatus* too Lyr (1958 a) recognized a weak tyrosinase production, whereas none of our 10 strains produced it.

Polyporus adustus has by Nobles (1958) been characterized as giving inconsistent reactions with gallic and tannic acids and Davidson et al. (1948) give as well varying results for this fungus. Lyr (1958 a) gives a weak laccase reaction and weak tyrosinase and peroxidase reactions for this fungus. Boidin (1951, strain CBS) finds a totally negative reaction with this fungus. Our strains, recently isolated from sporophores, give *inter se* very consistent results, viz. a strong laccase reaction and no tyrosinase. Obviously, this fungus too may be variable in its reactions, although this is not seen from our material.

Polyporus hispidus is reported by Lyr (1958 a) to produce a weak laccase activity but rather strong peroxidase and tyrosinase reactions. Davidson et al. (1938) recognize a strong reaction on gallic and tannic acid agars with this fungus. Our strain from CBS (Cartwright) gives, as many strong white rot fungi, a very strong laccase but no tyrosinase reaction. Similar differences are found between Lyr's results with *Polyporus radiatus* and our strains of this fungus, originating from sporophores. This fungus is listed by Davidson et al. (1938) and Nobles

(1948) as reacting moderately on gallic and tannic acids. In our tests it gives a moderate to strong laccase and no tyrosinase reaction.

In this group a number of white mycelia, not especially characteristic morphologically, is found. These fungi, which give perfectly similar reactions with all the laccase reactives, sometimes react differently to other phenolic compounds, as pyrocatechol, hydroquinone, phloroglucinol, *p*-quinone, aniline and lactophenol. Some of these strong laccase producers do not give a reaction with lactophenol, as *Tricholoma rutilans, Polyporus arcularius, Collybia dryophila, Fomes annosus, Omphalia campanella, Polyporus lacteus, Poria candidissima* and *P. versipora*. The related species of the group *Polyporellus* Karst. viz. *Polyporus arcularis, P. brumalis, P. varius, P. melanopus* and *P. picipes* give rather different reactions. *Daedalea unicolor* and *Lenzites betulina* differ somewhat in their reactions, but *Polyporus pubescens, P. versicolor* and *P. zonatus* give entirely similar results, perhaps somewhat weaker with *P. pubescens* than with others. *Poria candidissima* has a very typical reaction pattern. Only some fungi give a strong reaction with all the substances tested (except phloroglucinol), as *Lenzites betulina, Phlebia radiata, Polyporus borealis, P. frondosus, P. hirsutus, P. hispidus, P. occidentalis* and *Poria mollusca*. Thus, *Polyporus hirsutus* may be distinguished from the *P. versicolor-group* and, if the reactions with the phenolic compounds in the future show the same characteristic pattern for the different strains, it will be easier to distinguish a number of species.

On the basis of the above and similar reactions this rather large group of strong white rot fungi may be divided into smaller ones or individual fungi may be characterized by their typical reactions. (cf. tables 1 and 2).

Group IV. Both laccase and tyrosinase reactions found.

A. Weak reaction with some of the following laccase reagents: α-naphthol, α-naphthylamine, *o*-anisidine, induline, guaiacol. Fungi belonging strictly to the brown rot fungi.

a) Tyrosinase reaction weak:

Poria incrassata

Trechispora brinkmanni

b) Tyrosinase reaction medium to strong:

Fistulina hepatica
Lentinus lepideus
Merulius lacrymans
Polyporus sericeomollis
„ *schweinitzii*
Poria monticola
Trechispora brinkmanni

These fungi show a weak laccase and weak to strong tyrosinase reaction and some strains of these fungi may be found in the groups I, II and III a, the brown rot fungi. In this group, containing the brown rot fungi because of their tyrosinase and weak laccase production are placed here, as in group III A differences are found from other authors' results just because of this registration of the weak laccase reaction with the present method which is not revealed by other methods.

In *Fistulina hepatica,* a very strong tyrosinase producing fungus, Boidin (1951) and Lyr (1958 a) have not found any laccase and Davidson et al. (1938) show entirely negative results with gallic and tannic acids. Even in our tests the laccase production of this fungus is very weak but the tyrosinase production is extremely strong.

For *Poria incrassata* and *Polyporus sericeomollis* Davidson et al. (1938) report entirely negative results as Nobles (1958) does for Poria monticola. In our tests these fungi show weak to strong tyrosinase reaction and a weak laccase reaction.

Polyporus schweinitzii gives an entirely negative reaction according to Davidson et al. (1938); according to Lyr (1958 a) it produces only quite strong tyrosinase. Nobles (1958) has found with this fungus a negative Bavendamm but a strong gum guaiac reaction, and Higuchi & Kitamura (1953) found a strong tyrosinase and a weak laccase action. Our two strains agree best with the results of Higuchi & Kitamura, showing a strong tyrosinase and a clear, almost medium strong laccase activity. Laccase activity in this fungus in our tests is quite strong so that this fungus may nearly be placed in group IV B. *Poria xantha,* according to Nobles (1948, 1958), belongs to the entirely negative fungi; our 2 strains (from CBS and own), react positively to both laccase and tyrosinase.

All these differences can well be explained with the weak intracellular laccase production by these fungi, similar to that reported by Rösch (1961, 1962 a, b) for some other brown rot fungi.

B. Laccase reaction medium (as in group III B). The white rot fungi.

a) Tyrosinase reaction weak:

Stereum hirsutum
Stereum purpureum
Trechispora brinkmanni

b) Tyrosinase reaction medium to strong:

Armillaria mellea
Corticium alutaceum
Fomes conchatus
„ *connatus*
Hypholoma sublateritium
Lentinus tigrinus
Merulius lacrymans
„ *tremellosus*
Panus torulosus
Peniophora cornea
„ *gigantea*
„ *roumegueri*
Pholiota marginata
Polyporus guttulatus
„ *picipes*
„ *rutilans*
„ *vulpinus*
Poria obliqua
„ *xantha*
Radulum orbiculare
Stereum hirsutum
„ *purpureum*
Trametes abietis
Trechispora brinkmanni

This group, including too the true white rot fungi, which produce a medium laccase and weak to strong tyrosinase reactions, again comprises fungi the mycelia of which for the most part may be distinguished already by their morphological characteristics. It is possible, however, to characterize a number of fungi of this group by their specific reactions, i. g. *Poria xantha,* which produces a strong reaction with catechol but a very weak one with lactophenol, and *Radulum orbiculare,* which, on the contrary, produces a strong reaction with lactophenol and none with catechol.

Again, there are some differences from other authors' results. *Lentinus tigrinus* produces according to Lyr (1958 a) a weak laccase and peroxidase and no tyrosinase activity; our fungus shows both strong tyrosinase and laccase activity in the 5 strains tested. *Hypholoma sublateritium, Merulius tremellosus* and *Panus torulosus* according to Boidin (1951) produce strong laccase but no tyrosinase; our two strains of *Hypholoma sublateritium* produced medium laccase and strong tyrosinase reactions and our six strains of *Merulius tremellosus* did the same, whereas both our two strains of *Panus torulosus* showed a strong laccase and weak but clear tyrosinase reaction.

C. Laccase reaction strong (as in III C)

a) Tyrosinase reaction weak:

Pleurotus corticatus
Stereum hirsutum
„ *purpureum*

b) Tyrosinase reaction medium to strong:

Flammula gummosa
„ *ochrochlora*
Fomes ulmarius
Hydnum coralloides
„ *corrugatum*
„ *erinaceus*
Hypholoma capnoides
„ *fasciculare*
Lentinus tigrinus
Lenzites repanda
Merulius molluscus
Odontia bicolor
Panus conchatus
Peniophora gigantea
„ *pini*
„ *pithya*
„ *velutina*
Pholiota adiposa
„ *aurivella*
„ *destruens*
Pholiota mutabilis
„ *squarrosa*
Pleurotus porrigens
Pluteus cervinus
Polyporus circinatus
„ *guttulatus*
Tyromyces guttulatus (CBS)
Polyporus mollis
„ *picipes*
„ *radiatus*
Poria cinerescens
„ *corticola*
„ *rixosa*
Stereum abietinum
„ *chailletii*
„ *hirsutum*
„ *purpureum*
Trametes cinnabarina
„ *pini*
Trechispora brinkmanni

The last large group may be divided up again according to the reactions with different reagents, as catechol, *p*-quinone and aniline. Tyrosinase reaction, if weak, is usually shown by the oxidation of *p*-cresol but if this reaction is strong, usually an oxidation of both *p*-cresol and tyrosine occurs. Sometimes, however, only tyrosine is oxidized, as in the case of *Fomes conchatus, Panus torulosus, Pholiota destruens* and *Poria rixosa. Lactophenol* reaction is variable even in the strong tyrosinase group of fungi and may be useful in differentiating the mycelia.

Fungi of group IV, divided on the basis of tyrosinase reaction alone.

If the mycelia of group IV are classified on the basis of the tyrosinase reaction alone, leaving the laccase reaction out of account, the following groups are obtained:

1. Tyrosinase reaction weak to medium:

Flammula ochrochlora
Fomes conchatus
Hydnum corrugatum
Lentinus lepideus

Panus torulosus
Peniophora pini
„ *pithya*
Pholiota destruens
Pleurotus corticatus
„ *porrigens*
Polyporus vulpinus
Poria incrassata
„ *rixosa*
Radulum orbiculare
Stereum purpureum
Trametes abietis

2. Tyrosinase reaction medium to strong:

Fomes connatus
„ *ulmarius*
Lentinus lepideus
„ *tigrinus*
Odontia bicolor
Panus conchatus
Peniophora gigantea
„ *roumegueri*
Pholiota marginata
Pluteus cervinus
Polyporus picipes
„ *sericeomollis*
Poria cinerescens
„ *monticola*
Trametes pini
Trechispora brinkmanni (a few strains)

3. Tyrosinase reaction very strong:

Fistulina hepatica
Hydnum coralloides
Hypholoma capnoides
Lentinus lepideus
„ *tigrinus*
Lenzites repanda
Peniophora velutina
Pholiota adiposa
„ *aurivella*
„ *squarrosa*
Pleurotus ostreatus (a few strains)
Polyporus circinatus
„ *mollis* (strain CBS)
„ *guttulatus*
„ *rutilans*
„ *schweinitzii*
Poria corticola
„ *obliqua*
Stereum abietinum

In this last group, IV C, again some differences between the results of different authors are noted. *Merulius molluscus* has been discussed already earlier; *Odontia bicolor, Peniophora pini* and *Pholiota mutabilis* give no tyrosinase reaction according to Boidin (1951), whereas these fungi in our tests give a medium to strong tyrosinase reaction. In the case of *Polyporus circinatus* our results agree with those of Davidson et al. (1938), Nobles (1948) and Boidin (1951), whereas Lyr (1956) notes for this fungus only peroxidase reaction.

Polyporus guttulatus is listed by Nobles (1948) and Davidson et al.

(1938) as an entirely Bavendamm-negative fungus, whereas in our tests both the strains, that from CBS and that from Ottawa showed clearly a strong laccase and strong tyrosinase activity, although the colour reactions with these strains were not quite typical. The same applies to *Stereum abietinum*. This fungus produces a clear tyrosinase reaction but reactions with the laccase reagents differ so much in colour — a brown colour instead of violet or red is produced — that some other reaction than that catalyzed by laccase may come into the question.

In *Polyporus cinnabarinus Lyr* (1958 a) has found only laccase activity, whereas in our tests it showed also tyrosinase activity.

Thus, it is seen that some differences in the results are obtained, depending on different methods, but the results for most fungi agree (cf. Tab. 7). Nobles (1958) lists a number (19) of fungi, showing inconsistent reactions with the Bavendamm and gum guaiac tests. Of these, *Peniophora gigantea* and *Fomes roseus* have given highly variable results in the present tests too; *Polyporus adustus, P. fumosus, Collybia velutipes, Schizophyllum commune, Polyporus balsameus, Poria corticola, Polyporus guttulatus* and *P. schweinitzii,* however, in the present tests give constant and characteristic reactions. Of some of these, as *Polyporus fumosus, Collybia velutipes* and *Poria corticola* the number of strains tested was too limited to show the variations if any. Highly variable species in our tests were furthermore *Sterum purpureum, Stereum sanguinolentum, Polyporus mollis, P. picipes,* and, to some extent, *Armillaria mellea, Lentinus lepideus, Merulius lacrymans* and *Coniophora cerebella.*

As appears from the above tables, some of the fungi may occur in several different groups, but most of them give quite constant and characteristic reactions. Such reactions carried out with several phenolic compounds, during a limited age of the mycelia and under constant growth conditions give a more secure basis for classifying the mycelia of the decay fungi than the Bavendamm test alone, which, of course, too may show considerable variation if several strains of certain fungi are tested. Oxidation reactions with phenolic substances, carried out in the way presented here, with the listed or similar substances, may be a help in the identification of the mycelia of the decay fungi.

IV. Discussion

Of the mycelia tested in the present investigation, 81 of 173 fungus species (46 per cent) showed only laccase reaction, 58 (33 per cent) showed both laccase and tyrosinase reactions, 22 (12 per cent) were totally negative and 10 (5 per cent) showed variable reactions. Boidin (1951) found of his 123 mycelia that, 53 (43 per cent) produced only laccase, 49 (39 per cent) produced laccase plus tyrosinase, 25 (12 per cent) were totally negative and 6 (6 per cent) produced only tyrosinase. If different taxonomical groups are compared, it is seen that tyrosinase, together with laccase, is usually produced by fungi belonging to the *Agaricaceae* and to the genera *Peniophora* and *Stereum*. Tyrosinase production is found in the *Polyporaceae* too, but only in a limited number of species. This agrees well with the Boidin's (1951) results, and as in the present work relatively more *Polyporus* species were tested than in that of Boidin, and as even the weak laccase producers were reckoned to the laccase group of fungi, the greater percentage of the laccase fungi found in the present work is easily explained.

If the results obtained by different authors with different methods are compared, a great deal of the fungi gives similar results but quite marked differences are found in some species, as seen in the previous chapter. If compared with Boidin's results (1951), 14 fungi were not in agreement. A better agreement was obtained with the results of Davidson et al. (1938) and Nobles (1948, 1958) with only 7 resp. 3 fungi showing different results.

Different results, however, from employing different methods must be expected. Laccase is highly inducible enzyme, as shown already by Fåhraeus (1952, 1954) and Lyr (1958 b) and even the tyrosinase production is declared to be inducible (Sussman & Markert, 1953). When growing fungi on substrates containing compounds, known to induce laccase synthesis, as tannin or such, inhibiting growth, as gallic acid and tyrosine or being toxic to the fungi, as guaiacol, α-naphthol, and *p*-cresol, it is clear that the results must be influenced by these substances and it may be questionable whether the normal-growing mycelia always produce these enzymes in the same way as on the test media in question. Applying the method with nutrient agar contain-

ing phenolic substances, furthermore, only the production of extracellular enzymes can be detected. By using drop tests as described in the present paper on just grown-out mycelia the effect of the phenolic substances on the enzyme production is eliminated and the oxidizing capacity of the more or less normally grown mycelium is shown. Furthermore, by the method employed in the present tests both extracellular and intracellular enzyme production is shown, as the phenolic substances tested in alcoholic solution obviously can penetrate the cell walls. This is shown by the fact that in the present tests sometimes the reaction is only seen in the substrate, sometimes only in the aerial mycelium and sometimes both in the substrate and mycelium. Lyr's somewhat similar method works only on extracellular oxidases and this too may cause differences in the results registered. It was not the intention of the present work to distinguish between the extracellular and intracellular enzyme activities, as this was sometimes, if the production of aerial mycelium was sparse, difficult to do. These tests show, of course, only the production of different phenoloxidases on a standard medium in culture and it cannot be claimed that the same enzymes are produced in the same relative amounts in the wood. However, correlation between the oxidase production as shown by the present tests and the type of decay is good, the white rot fungi showing medium to strong laccase activity and the brown rot fungi none or a weak laccase production. Tyrosinase activity may be found in both groups of fungi. An unknown mycelium belonging to the decay fungi can with the help of such drop tests be placed into the right group. Correlation between the strength of polyphenoloxidase reactions on malt agar media and the decay capacity of the mycelia in wood is not known, but it seems that such a correlation exists, as all the strong laccase producers belong to the very effective white rot fungi, as *Polyporus versicolor, P. borealis* a. o. and fungi showing moderate laccase reaction are known to have a moderate decay capacity, as *Corticium confluens, C. alutactum, Flammula alnicola* and a number of others.

An important factor influencing the results of the oxidase tests is the age of the mycelium, and even the age of the inoculum in the agar plate tests. Hitherto it has perhaps not been emphasized clearly enough that the mycelia for phenoloxidase tests must be of a comparable age, viz. vigorously growing young mycelia. If older mycelia are used, the results can be different, as it is known that both laccase (Fåhraeus 1952) and tyrosinase (Sussman & Markert, 1953) production may set in through the autolysis of the mycelia.

Sometimes the different results noted for a fungus species by the present method may be explained by the variability of the oxidation capacity between different strains of the same fungus. *Stereum sanguinolentum* and *Peniophora gigantea* are examples of such variable fungi, but they give variable results by the Bavendamm method too, as shown already by Robak (1932) for *Stereum sanguinolentum* and Nobles (1948) for both. In *Stereum* in the present paper such differences were shown to exist already in monosporus mycelia from the same sporophore and these differences are most likely genetically fixed, as the same strains always produce their typical reactions. Koelle (1963) has found the same for the phenoloxidase activity in phanerogams: testing this activity in a number of varieties of tobacco it proved to be a hereditary quality as the differences between different varieties remained during the whole vegetation time and were replicated in the descendants.

In spite of the relatively numerous investigations on the phenoloxidases of the wood-decaying fungi there remain many unsolved problems. Numerous contradictory conceptions concerning these enzymes are met, regarding their nomenclature, identity, occurrence, substrates, inhibitors, pH optimum and function. All the confusing and sometimes rather contradictory conceptions are probably caused by difficulties in their isolation and purification and the sparcity of investigations on such carefully purified enzymes.

The confusing nomenclature of phenoloxidases and polyphenoloxidases has already been pointed out by Bonner (1957) but hitherto concerning the fungus oxidases of these groups more generally two types of oxidases are recognized, viz. the laccase and tyrosinase types. Bonner (1957) classifies tyrosinase as a phenoloxidase and laccase as a polyphenoloxidase, but as Lindeberg & Holm (1952) show, both enzymes may catalyze the oxidation of *o*-dihydric phenols and polyphenols with two hydroxy groups in *ortho* position, thus possessing both a "catecholase" and "polyphenoloxidase" activity. Both, laccase and tyrosinase may occur in the same mycelium but sometimes one of them, mostly laccase, is found in the mycelium and the other, tyrosinase, in the sporophores (Lindeberg & Holm 1952, Lindeberg 1950). Different enzymes in the sporophores and in the mycelium, are found even in the present tests, as the sporophores of some *Polyporus* and *Poria* species belonging to the brown rot fungi which are sometimes found on malt agar plates showed strong oxidase activity, mostly with catechol or lactophenol, whereas their mycelium gave negative results. If such positive reactions were obtained only in sporophores they are

not accounted for in the present tables. Sporophores of other brown rot fungi never showed such activity but information concerning such reactions is too scarce for us to be able to take it as a specific character for the fungus species.

About the extracellular and intracellular occurrence of the two enzymes contradictory information is found. Laccase is commonly characterized as an extracellular enzyme (Lindeberg & Holm 1952, Higuchi & Kitamura 1953, Boidin 1951, Lyr 1958 b) although it is known to occur even intracellularly in the white rot fungi (Lindeberg & Holm 1952, Higuchi & Kitamura 1953) and to be produced intracellularly to some extent even in the brown rot fungi (Rösch 1951, 1952 a, b). More conflicting are reports concerning tyrosinase. Lindeberg & Holm (1952) and Lyr (1958 a) characterize it as an endoenzyme, but Higuchi & Kitamura (1953) and Boidin (1951) claim that in a number of decay fungi even extracellular tyrosinase is produced and their results point without question in this direction.

Tyrosinase is regarded as an enzyme that is readily brought into solution. Bonner (1957), however, has shown that it is present in mitochondrial fractions which explains that it mostly acts as an intracellular enzyme.

Often, if speaking of the fungus oxidases, terms as "laccase type enzyme" or "enzyme of the tyrosinase type" are used. Such enzymes are among others characterized by their substrates and, as seen in the present tests, and in litterature (Law 1950), the laccase type enzymes from different fungi show different oxidizing activity towards different phenolic compounds. Whether this is due to differences in the enzyme itself or to secondary reactions, is not known, as investigations on pure enzymes of this type exist only for a few fungi because of difficulties in large scale production and purification of the enzyme. The purification technique is perhaps not very satisfactory as during this procedure often some of the characters of the enzyme are altered, as compared with the crude enzyme solution. The enzyme is mostly purified by precipitation with ammonium sulphate and dialysis and in pure enzymes obtained by this metod, Law (1950) showed a different copper content in the laccase of different fungi. Mossbach & Fåhraeus (1958) got a better purified product by precipitating the crude laccase solution with ammonium sulphate, dialyzing and lyophilizing it and purifying the product by paper electrophoresis. In the last step two components were obtained and one of them, the main fraction, was studied further. About the minor fraction which showed a considerably lower specific activity, the authors did not know whether this

was a different enzyme or a form of microheterogenity. The existence of such an other component or perhaps other components could maybe explain the different activity of laccase obtained from different fungi.

Laccase and tyrosinase are characterized by their different pH optima, by their different temperature sensitivity and by their partly different substrates and inhibitors. The different results for the pH optima are more closely discussed in chapter 2. Generally laccase is characterized by its low pH optimum and its stability in acid solutions (Dion 1952, Rösch 1961) although Dion suggests that several enzymes may be concerned in the laccase group, each of which may show its maximum activity at different pH values. Tyrosinase is often reported to have a higher pH optimum than laccase (Boidin 1951, Rösch 1961), about pH 7 but others have found wide pH limits for its action (Gregg & Miller 1940, Lindeberg & Holm 1952). Concerning the temperature limits, tyrosinase is inactivated at 60° C; laccase is more thermostabile but it is inactivated too at higher temperatures (Boidin 1951, Lindeberg & Holm 1952). As for the specific substrates of the both types of enzymes, it is shown by Lindeberg & Holm (1952) that laccase catalyzes the oxidation of hydroquinone and *p*-phenylenediamine, which are not attacked by tyrosinase, and tyrosinase was found to catalyze the oxidation of such monophenols as phenol and tyrosine, whereas laccase had no effect on these compounds. Lyr (1956), says that purified laccase most likely has no action on phenol, which on the other hand is readily oxidized by peroxidase. Boidin (1951) lists as substrates for laccase pyrocatechol, guaiacol, hydroquinone, pyrogallol, and *p*-phenylenediamine. Tyrosine is not oxidized by laccase. Substrates for tyrosinase are according to Boidin (1951) *p*-cresol, tyrosine and its derivatives, 3.4 dihydroxyphenylalanine, caffeic acid, protocatechuic acid, pyrogallol, pyrocatechol and adrenaline. Guaiacol, hydroquinone and phenylenediamine were found not to be oxidized by tyrosinase. Law (1950) says that laccase oxidizes potassium ferrocyanide and this action is found even in the white rots (Gregg & Miller 1940). Bonner (1957) says that the relatively impure preparations of laccase will oxidize a number of substrates; it catalyzes the oxidation of *p*-phenylenediamine, hydroquinone and *o*-diphenols but has no activity on monophenolic substances. Ascorbic acid is directly oxidized by laccase, but this ability diminishes with increasing purity of the enzyme. Even Law (1950) emphasizes that the crude enzyme has a much higher activity than the purified one. She explains it by the fact that a complete extraction of the enzyme cannot be achieved and that a great deal of it is lost during purification. Concerning tyrosianase Bon-

ner (1957) says that there is a great diversity of phenolic substances in plant which it is capable of oxidizing. Concerning guaiacol, Boidin (1961), Lyr (1958 b), Graubard (1939) and others have noted that it is oxidized only by laccase, but Rösch (1961) found that guaiacol is oxidized by laccase at low pH values and by tyrosinase at high pH values with an optimum at about pH 6.8—7.2. Bonner (1957) says that it is difficult to distinguish on the one hand between laccase and tyrosinase by means of polyphenol oxidation and on the other side between laccase and cytochrome oxidase by means of ascorbic acid or *p*-phenylenediamine oxidation. Law (1960) emphasizes that laccase from different sources shows a considerable variation in its substrate specificity, which agrees with the results of the present tests.

Concerning inhibitors of both the two enzymes, it is often emphasized that tyrosinase is inhibited by carbon monoxide but not laccase (Lindeberg & Holm 1952, Bonner 1957). Law (1950) shows that laccase is inhibited by potassium cyanide, and Bonner (1957) quotes that laccase, as other copper enzymes, is inhibited by cyanide, azide and dithiocarbamate. Bonner & Wildman (1946) reported *p*-nitrophenol, a compound which may be considered as an analogue of the normal polyphenol oxidase to be a selective, competitive inhibitor to this enzyme system. Bonner (1957) and James (1953) list a number of inhibitors of phenol oxidases which are found to act on all copper proteins, as thiosulphate, thioglycolate, diethyldithiocarbamate a.o. Lyr (1961) has found that laccase was most severely inhibited by substances producing complexes with heavy metals, as gallic acid, 8-oxychinoline, Na-azi benzoic acid, whereas γ-thujaplicine which otherwise very easily gives complexes with heavy metals, and pinosylvinmonomethylester had no inhibitory effect on laccase. Several inhibitors, effective against laccase had no effect on peroxidase and vice versa.

Highly purified tyrosinase from different sources is as well as laccase variable in its action, colour, copper content etc. (Keilin & Mann 1940, Mallette 1950, Bonner 1957). As with laccase, during the purification a part of the activity, especially the ability to oxidize monophenolic substances, is gradually lost. This is explained by Dawson & Tarpley (1951) by the suggestion that both monophenol and *o*-diphenol are oxidized by the same enzyme but due to fragmentation of the protein during the purification process the monophenol oxidizing ability of the enzyme is lost. The ratio of monophenolase to *o*-diphenolase activity in tyrosinase can actually be varied according to the procedure of purification.

Lindberg's work (1950) and Bonner's summarizing facts (1957) about the monophenol and polyphenol oxidases show a close relationship between both the two types of enzymes. Bonner (1957) asserts on the one hand the very close relationship of laccase to ascorbic acid oxidase and, on the other hand, the very close relationship between laccase and tyrosinase. He suggests that the three enzymes are very closely related to each other, if not identical. A free interconversion of laccase to tyrosinase and *vice versa* may be very likely and if the two enzymes are identical, the differences in substrate specificity can be modified by the presence or absence of cofactors.

Summarizing all the known facts about the two phenoloxidases laccase and tyrosinase, it is seen that these enzymes are very closely related, and possibly related even to ascorbic acid oxidase and that different activities between the two enzyme groups as well as between the different enzymes of the laccase and tyrosinase type from different sources may be explained by the presence or absence of cofactors. With the present methods it is extremely difficult to produce these enzymes in purified form for a closer study of their identity and furthermore, their properties are altered during the purification process.

The role of phenoloxidases in plants is not quite clear and many different and sometimes contradictory suggestions have been made. The role of laccase as a principal terminal oxidase has been proposed by Bonner & Wildman (1946), but later, Boulter & Burgess (1954) showed that the terminal oxidation in fungus mycelium is mediated mainly, if not completely, by a cytochrome oxidase system and they found it unlikely that laccase should act as terminal oxidase. Later, Bonner (1957) agrees that the role of laccase in the respiratory system of plants is still unknown. The reports on its functions in plant respiration are very conflicting but it seems likely that by analogy with the ascorbic acid oxidase and tyrosinase the oxygen affinity of laccase is relatively low and that the role of laccase in plant respiration should be sought, not as an enzyme catalyzing the transfer of electrons to oxygen but perhaps as one which utilizes some other acceptor in intracellular electron transfer.

Concerning tyrosinase, in spite of much research, likewise our knowledge of its role in cellular respiration is limited. Tyrosinase is an active catalyst of phenol oxidation, capable of action on a variety of phenolic substances. Because of these considerations and because of its widespread distribution in plants, numerous attempts have been made to implicate this enzyme as a catalyst mediating the final reaction with oxygen in cellular respiration. Sussman & Markert (1953)

however, have shown that this possibility must be eliminated in the case of *Glomerella,* where the tyrosinase-less mutans showed growth and respiration equal to that of tyrosinase-producing strains. Bonner (1957), referring to his previous works, concludes that the efficiency of tyrosinase towards oxygen is almost completely unknown but he suggests that it would appear that the role of phenol oxidation in plant respiration should be evaluated in terms of electron transport in anaerobic systems.

However, the close correlation of laccase occurrence and the decay type of the wood-rot fungi has suggested its possible role in lignin degradation. Many attempts have been made to elucidate the eventual role of phenol-oxidases in lignin degradation, but again, no clear results are seen. So much seems to be clear that polyphenoloxidases have an important role in the formation of lignin. Higuchi, Kawamura & Morimoto (1955) have investigated laccase prepared from the latex of Japanese lac tree and that from the wood rotting fungus *Coriolus versicolor;* preparations from both these sources catalyze the oxidation of coniferyl alcohol, whereas potato tyrosinase did not catalyze the oxidation of this compound. These workers suggested that laccase functions in lignin formation, and not tyrosinase, as previously suggested by Mason (1955) and Mason & Cronyn (1955). Freudenberg, Harkin, Reichert & Fukuzumi (1958) have also shown that laccase has a definite role in the lignin synthesis. According to these authors the phenol-hydroxy groups of the simpler building elements of lignin are enzymatically dehydrated by the enzymes and through dimerization condensed to larger components. A new dehydration may occur now and cause a further polymerization. At the same time the synthesis of lignin may occur in other ways too, over intermediately formed quinones. According to this theory, the synthesis of lignin is possible through the action of only one enzyme system of the phenoloxidase type. The decomposition of lignin seems to be more complicated, and as Lindeberg (1955), Lyr & Ziegler (1959) and Rösch (1961) have suggested, several enzyme systems may be involved and laccase may be one of them. So much is clear, that the white rot fungi are active decomposers of lignin in wood (Cartwright & Findlay 1946, Cambell, 1952).

Contrary to the brown rot fungi which do not attack lignin, the white rot fungi always produce considerable amounts of extracellular phenoloxidases of the laccase type, which is not known to occur in these amounts in any other group of organisms. With lignin preparations, however, even the white rot fungi have difficulties and their ability to decompose these compounds depends largely on the method of isola-

tion of these preparations. Hitherto no entirely satisfactory methods for isolation of pure lignin seems to exist. The best preparation seems to be the so-called Björkman's lignin (Björkman 1954) and after that Brauns "native lignin" (Brauns 1939), whereas phenol-lignin and lignin isolated by acid, alkali or sulphite agents are not suitable substrates for decomposition experiments by fungi, as they often contain degradation and condensation products of carbohydrates and proteins. Accordingly, the white-rot-causing fungi may attack several such preparations, but this attack is not comparable with that of natural wood (Day, Pelczar & Gottlieb 1949, Gottlieb, Day & Pelczar 1950, Pelczar, Gottlieb & Day 1950). Rösch (1961) reported some attack, often after adaption of some white rot fungi on phenol-lignin, but he too emphasizes that the growth of his fungi on lignin preparations can not be compared with that on the wood. The brown rot fungi were not even after adaption able to grow in the various lignin preparations (Rösch 1961). Sometimes it is declared that the lignin remaining after the attack of brown rot fungi is not the same as before the attack but is chemically altered (Apenitis, Erdtman & Leopold 1951, Higuchi, Kawamura & Kawamura 1955). Rösch (1961) explains it by the action of the small amounts of laccase, produced intracellularly and deliberated autolytically by some brown rot fungi.

Other reports exist about the action of more or less purified enzyme laccase on lignin or lignin preparations. Dion (1952) reported that extracellular fungus laccase oxidized lignin, which absorbed O_2 and became more water-soluble than before which he thought to be due to the addition of OH groups by the enzyme action or to a slight depolymerization. He suggested that this enzyme may solubilize the lignin and make it susceptible to the action of other organisms. Other investigators have reported the oxidative attack of fungus oxidases on different lignin preparations, as Gottlieb & Geller (1949), Fåhraeus (1953) and van Vliet (1954). Flaig & Haider (1961) investigated the utilization of different phenolic compounds, some of which are supposed to be components or intermediate products in lignin decomposition, as sole carbon sources for fungi. They found that the white rot fungi could use different phenolic compounds (vanillic acid, protocatechuic acid and *p*-oxybenzoid acid) as a carbon source and that they possessed an enzyme system to break up the cyclic protacatechuic acid to the aliphatic β-keto adipic acid. However, they deny the role of laccase in the decomposition of lignin and, based on the analogy of lignin decomposition by soil organisms, they suggest that the phenoloxidases may have an oxidizing action to lignin or lignin decomposition products

but that this results again in polymeric products firmly bound to the enzymproteid, being of humic acid nature. Several others (Konetzka, Pelczar & Gottlieb 1952, Tabak & Kabler 1959) have shown that lignanes can be decomposed by microorganisms to smaller phenolic units and Fåhraeus (1962) has shown that phenolic compounds, as aniline and p-hydroxybenzoic acid act stimulatingly on the growth of white rot fungi. Further facts on the enzymic decomposition of lignin are, however, lacking. Rösch (1961) gives an interesting theory on the degradation of lignin. He finds is possible that the same oxidases, viz, laccase and peroxidase which lead to the polymerization through dehyration in the lignin formation, may catalyze even the depolymerization of lignin to monomeric units. As hitherto no facts about the capacity of these enzymes to break the aromatic ring are found, probably other enzymes must catalyze such action. This coincides well with the fact that highly purified phenoloxidases of the laccase type give poorer results in the enzymatic breakdown of lignin than the crude enzymes. According to Rösch (1961), in the case of highly purified phenoloxidases only an equilibrium between the oxydative breakdown and a new polymerization is obtained; in the other cases the impurities, containing probably other effective systems catalyze the further breakdown of the depolymerization products. In the same way the poor results with pure lignin preparations might be explained: certain impurities of the lignin preparations, as heavy metals and others, may inhibit the action of other enzymes wich must act together with oxidases to break down lignin entirely. The decomposition of lignin may very likely be an energy-requiring process which cannot set in if other energy supplying carbohydrates are not present at the same time, as occurs in the wood. So, as it is seen, not many facts are known about the decomposition of lignin by fungi and probably much more will not be known until the lignin constitution is definitely cleared up.

Another interesting point in the action of phenoloxidases is their action on the toxic phenolic substances. Higuchi, Watanabe & Tamura (1953) have shown that the white rot fungi and brown rot fungi possess a different resistance against Na-pentachlorophenol. Lyr & Ziegler (1959) could not find such a difference between the white rot and the brown rot fungi, but investigating the influence of several toxic substances on different enzymes, it was found (Lyr 1958 b) that pentachlorophenol had markedly inducing effect on the production of laccase. On the other hand, laccase and in several cases even peroxidase proved to be relatively insensitive to 2.4.5-trichlorophenol, pentachlorophenol, pentachlorothiophenil, urethan, 2.3.5-trijodbenzoic acid, as to

γ-thujaplicin and laccase even to pinosylvinmonomethylether (Lyr 1961 a). Later on, Lyr (1962) showed very interesting results in the enzymatic detoxification of several chlorinated phenols. Some brown rot fungi as *Polyporus betulinus* were shown to be irreversibly damaged by the action of such phenols, whereas in some white rot fungi, as *Polyporus versicolor, P. hirsutus* and *Fomes igniarius* the damage was overcome after a short time. He showed that by the action of laccase and peroxidase the chlorinated phenols were broken down with the deliberation of chlorine. The toxic effect of these compounds to the test fungi was reduced with time and after 13 days 2-chlorophenol had lost its toxic action entirely. The higher chlorinated phenols were more resistant to the action of laccase, but even with pentachlorophenol the toxic effect was only 1/100 after this time. Thus, these oxidases have shown themselves to be very effective in the breaking-down and detoxification of phenolic substances.

From all the facts brought out above it is seen that the phenoloxidases in fungi are of great interest to us not only for identification purposes but also as they probably in some way take part in the degradation of lignin in the wood and as they may have some part in the resistance of the mycelia to different phenolic compounds, as well in the natural substrates as in the wood preservatives. The present rapid method for showing the production of phenoloxidases by the fungus mycelia, making vizible both qualitative and quantitative differences in the enzymes produced might be at least a preliminary means for estimating the production of different enzymes by individual fungus species and strains.

V. Summary

1. For purposes of identification of fungus mycelia the production of laccase and tyrosinase type enzymes in the mycelia of decay fungi is estimated by using several phenolic compounds in a droptest.
2. The influence of some factors, as pH, temperature, age of the mycelium and intraspecific variations on the results is shown.
3. On the basis of their production or not of laccase and tyrosinase the mycelia are divided into four groups: 1) producing neither laccase nor tyrosinase, 2) producing only tyrosinase, 3) producing only laccase, 4) producing both laccase and tyrosinase. On the basis of the intensity of the reactions and taking into account the specific reactions with some substances these groups may be subdivided into smaller ones.
4. The results are commented and compared with results obtained by other authors with different methods.
5. The occurrence and characteristics of laccase and tyrosinase and their possible role in metabolism, especially in lignin degradation, and their action on other phenolic substances is briefly discussed.

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Sammanfattning

Identifiering av rötsvampsmycel med hjälp av deras produktion av fenoloxidaser.

För att underlätta identifiering av rötsvampsmycel har förekomst av olika enzymer av fenoloxidas- och polyfenoloxidas-typ undersökts. En enkel metod, genom användning av s. k. »dropp-test» har utarbetats för att visa förekomsten av enzymer av laccas- och tyrosinas-typ i mycel och substrat. Produktion av dessa olika enzymer kan anses som karakteristisk för arten, fastän några arter visade varierande resultat. Sammanlagt har 587 svampstammar, representerande 173 arter hörande till Agaricaceae, Corticiaceae, Stereaceae och Polyporaceae undersökts. Av några svampar undersöktes 10 till 25 stammar. Svamparna odlades på maltagar i petri skålar och till utvuxna kulturer applicerades droppar av fenoler och fenol-liknande substanser i alkohollösning. Förekomst av de olika enzymerna påvisades genom oxidationsreaktioner med vissa substanser. Faktorer som kan påverka resultat, som temperatur, substratets pH, mycelets ålder och variation mellan olika stammar av samma art undersöktes. Om mycel av en viss ålder, vid standard temperatur och på standard medium testas, är resultat reproducerbara och karakteristiska för samma stam resp. art av rötsvamp.

Efter förekomst av enzymer av laccas- och tyrosinas-typ kan rötsvampsmycel indelas i fyra stora grupper: 1) mycel som producerar varken laccas eller tyrosinas, 2) mycel som producerar endast tyrosinas, 3) mycel som producerar endast laccas och 4) mycel som producerar både laccas och tyrosinas.

Eftersom vissa svampar visar individuella skillnader i sin reaktionsförmåga mot de olika substanserna som oxideras av dessa enzymer, kan dessa fyra stora grupper eventuellt indelas i mindre eller enstaka mycel kan karakteriseras genom sina typiska reaktioner.

Resultat av föreliggande arbete och dessa av andra autorer med andra metoder jämföres och diskuteras. Förekomst av laccas och tyrosinas i svampar, deras egenskaper, deras roll i metabolism, särskilt i nerbrytning av lignin och deras inverkan på andra fenol-liknande substanser diskuteras kort.

APPENDIX

Note to tables 1—6.

+++	reaction strong
++	reaction medium
+	reaction weak
—	no reaction

Table 1. Oxidation of different phenolic compounds by mycelia of the decay fungi.

Fungus	Number of isolates	Strain no	Age of mycelium days	benzidine	α-naphthol	o-anisidine	α-naphthylamine	induline	guaiacol	8-oxyquinoline	2,5-xylidine	o-toluidine	p-cresol	tyrosine	gallic acid	tannin	pyrogallol	hydroquinone	phloroglucinol	catechol	p-quinone	aniline	lactophenol
Agaricaceae																							
Armillaria mellea		Cf. Tab. 2																					
Collybia dryophila	2	678,679	21	+++	+++	++	++	++	+++	+++	+	-	-	-	+	++	++	-	-	+++	++	-	-
Collybia velutipes	1	373	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flammula alnicola	3	377,681,682	25	+++	+++	++	++	++	++	-	-	-	+++	-	+	+	-	-	-	-	-	-	++
Flammula gummosa	1	384	21	+++	+++	+++	+++	++	+++	++	+++	+++	+++	-	++	++	+++	++	-	+++	++	+++	+++
	1	385	21	+++	+++	+++	+++	++	+++	++	+++	+++	+++	++	++	++	+++	++	-	+++	++	+++	+++
Flammula ochrochlora	1	372	21	+++	+++	+++	+++	+++	++	++	+++	+++	++	-	+++	++	++	++	-	+++	++	++	+++
Flammula sapinea	1	394	21	+++	++	++	++	++	++	-	-	-	-	-	-	-	-	-	++	-	-	-	-
Hypholoma capnoides	2	78,497	18	+++	+++	+++	+++	++	++	++	++	++	+++	+	++	++	++	-	-	+++	+	+++	++
Hypholoma fasciculare	5	79,82,92,502, 503	21	+++	+++	+++	+++	++	+++	++	++	++	+++	-	+	++	++	-	-	+++	+	+++	+++
	2	80,81	21	+++	+++	+++	+++	++	+++	++	++	++	+++	++	++	++	++	-	-	+++	++	+++	+++
Hypholoma sublateritium	2	504,505	21	+++	++	+++	++	++	++	++	++	++	+++	-	++	++	++	-	-	+++	++	+++	+++
Lentinus lepideus		Cf. Tab. 2																					
Lentinus tigrinus	4	91,93,94,95	19	+++	+++	+++	+++	++	+	++	-	-	+++	+++	++	++	+++	++	-	+++	++	-	++
	1	92	19	+++	+++	+++	+++	++	+++	++	+	+	+++	+++	++	++	+++	++	-	+++	++	++	++
Omphalia campanella	4	403,461,115,116	19	+++	+++	+++	++	++	++	-	-	-	-	-	+	+	++	-	-	-	+++	-	-
Panus conchatus	4	521,538,619,620	18	+++	+++	+++	+++	++	+++	+++	-	++	+++	+++	++	++	+++	++	-	+++	++	++	++
Panus stipticus	2	376,413	17	+++	+++	+++	+++	+++	+++	+++	++	++	-	-	+++	++	+++	-	-	+++	++	-	++
Panus torulosus	2	374,617	25	+++	+++	+++	+++	+++	+++	++	-	-	-	+++	++	++	++	++	-	-	+	-	+
Pholiota adiposa	1	370	17	+++	+++	+++	++	+++	++	++	++	++	+++	++	++	++	++	++	-	+++	++	+++	+++
Pholiota aurivella	1	371	18	+++	+++	+++	++	++	++	++	-	-	+++	+++	++	++	++	++	-	+++	++	-	+++
Pholiota destruens	1	369	12	+++	+++	+++	+++	+++	++	++	++	++	+	++	++	++	++	-	-	-	++	-	++
Pholiota flammans	2	642,643	12	+++	+++	++	++	++	+++	+	++	+	-	-	++	++	+	+	-	-	-	-	+
Pholiota mutabilis	2	510,11	16	+++	+++	+++	+++	++	+++	++	++	++	+++	-	++	++	++	-	-	+++	++	++	++
Pholiota marginata	1	644	25	+++	+++	+	+	+++	++	+++	+	+	+++	-	+	++	+	-	-	+++	++	++	+++
Pholiota squarrosa	2	141,382	18	+++	+++	+++	+++	+++	+++	++	++	++	+++	++	++	++	++	-	-	+++	++	+++	+++
Pleurotus corticatus	1	391 (Kaufert)	30	+++	+++	++	++	+++	+++	++	-	-	-	-	++	+	++	-	-	+++	++	+++	+++
	4	523,525,528,531	18	+++	+++	+++	+++	+++	+++	+++	+	++	-	-	++	++	+++	++	-	+++	+	-	++
Pleurotus ostreatus	10	142-144,375 415,512-516	14	+++	+++	+++	+++	++	+++	++	+	+	-	-	++	++	++	++	-	++	-	-	+++
Pleurotus porrigens	1	627	28	+++	+++	+++	+++	+++	+++	+++	++	++	++	-	++	++	+++	-	-	+++	+	-	-
Pleurotus serotinus	4	414,517,518,674	14	+++	+++	++	++	++	++	++	+	+	-	-	++	++	++	-	-	+	-	-	+
Pleurotus ulmarius	3	383,614,615	14	+++	+++	+++	+++	++	+++	++	++	++	-	-	++	++	++	-	-	+++	-	-	++
Pluteus cervinus	1	673	28	+++	+++	+++	+++	+++	+	+++	-	-	+++	+++	+	+	+++	+	-	+++	+++	+	++
Schizophyllum commune	2	251,383	17	++	-	++	±	±	-	-	-	-	-	-	-	-	-	-	-	-	±	-	-
Tricholoma rutilans	2	322,323	15	+++	+++	+++	+++	++	+++	+++	-	-	-	-	+++	++	++	++	-	++	++	-	-
Thelephoraceae																							
Coniophora arida	4	335,336,338,440	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coniophora cerebella		Cf. Tab. 2																					
Coniophora olivacea	2	446,346	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Corticium alutaceum	1	336	18	+++	++	++	+++	+++	-	-	+	-	+++	+++	+	+	++	+++	-	-	++	-	++
Corticium confluens	1	661	13	++	+	+	+	+	+	++	+	+	-	-	++	+	++	-	-	-	+	++	++
Corticium laeve	2	26,27	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Corticium subseriale	1	662	14	++	+	+	+	+	+	+	-	-	-	-	-	+	+	-	-	-	++	-	++
Gloeocystidium karstenii	1	410	21	+++	+++	+++	++	++	++	+	++	+	-	-	++	++	++	-	-	-	-	++	++
Hymenochate rubiginosa	1	401	18	+++	++	+++	+++	++	-	-	++	-	-	-	+	++	++	-	-	-	-	-	-

Fungus	Number of isolates	Strain no	Age of mycelium days	benzidine	α-naphthol	o-anisidine	α-naphthylamine	induline	guaiacol	8-oxyquinoline	2,5-xylidine	o-toluidine	p-cresol	tyrosine	gallic acid	tannin	pyrogallol	hydroquinone	phloroglucinol	catechol	p-quinone	aniline	lactophenol
Thelephoraceae																							
Hymenochate tabacina	2	77,389	21	+++	+++	+++	+++	++	++	++	+++	+++	-	-	++	++	++	-	-	-	++	+++	++
Merulius himantioides	1	385	25	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Merulius lacrymans		Cf. Tab. 2																					
Merulius molluscus	2	454,455	19	++	+++	+++	++	+++	++	++	-	-	++	++	++	++	++	+	-	++	++	-	++
Merulius silvester	1	110	21	+	-	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Merulius tremellosus	5	457,458,460,438,439	19	++	++	++	++	++	++	++	-	-	+++	++	++	++	++	-	-	++	++	-	++
	1	459	19	++	++	++	++	++	++	++	-	-	+++	-	++	++	++	-	-	++	++	-	++
Peniophora byssoidea	1	663	13	+++	+++	++	+++	++	+++	+	+	-	-	-	++	+	++	-	-	+++	++	-	-
Peniophora cinerea	1	409	13	+++	+++	+++	++	+	++	++	++	++	+	-	++	++	++	-	-	++	+	++	++
Peniophora cornea	4	118,121	17	+++	+++	+++	+++	++	++ ++	++	-	-	-	-	++	++	++	-	-	++	++	-	+++
Peniophora cremea	1	CBS,492	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Peniophora gigantea		Cf. Tab. 2																					
Peniophora hydnoidea	1	664	14	+++	+++	+	+++	+	+	+++	-	-	-	-	+++	++	++	-	-	-	++	++	+
Peniophora incarnata	3	137,351,507	16	+++	+++	+++	+++	+++	+++	+++	++	++	-	-	++	++	++	-	-	+++	+	++	+++
	1	407	20	+++	+++	+++	+++	+++	+++	++	++	+	+++	+	++	++	++	++	-	+++	+	-	+++
Peniophora pini	4	408,352,259,261	12	+++	+++	+++	+++	+++	+++	++	++	++	+	-	++	++	++	++	-	+++	++	-	+++
Peniophora pithya	1	CBS	10	+++	++	+++	+++	-	++	+++	-	-	-	-	++	++	++	-	-	++	-	-	-
	3	138,139,405	19	+++	+++	+++	+++	++	+++	++	+	+ ++	++	-	+++	++	++	++	+ ++	+++	++	-	++
Peniophora roumeguerrii	1	665	13	+++	++	+	+++	++	++	++	+	+	+++	-	-	+	++	-	-	-	++	+	++
Peniophora septentrionalis	1	406	14	+++	+++	+++	+++	++	+++	++	-	-	-	-	++	++	++	-	-	-	-	-	-
Peniophora velutina	1	666	13	+++	+++	+++	+++	++	+++	+++	++	++	+++	+++	+	+	++	+++	-	+++	+++	++	+++
Stereum abietinum	3	179,356,411	21	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++
Stereum chailletii	1	412	14	++	+++	+++	+++	+++	+++	++	+++	++	++	+++	+++	+++	+++	++	-	+++	++	++	++
Stereum frustulosum	1	254	19	++	-	+	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stereum gausapatum	1	668	14	+++	++	+	++	+	++	+	+	+	-	-	++	+	+	-	-	-	-	-	-
Stereum hirsutum		Cf. Tab. 2																					
Stereum murrayi	1	491	30	+++	+++	+++	+++	+++	+++	++	-	-	-	-	+++	+++	+++	++	++	+++	++	-	+++
Stereum purpureum		Cf. Tab. 2																					
Stereum rugosum	2	586,587	14	++	+	+++	+	+	+	++	+	+	-	-	+	+	+	-	-	-	-	+	++
Stereum sanguinolentum		Cf. Tab. 2																					
Thelephora terrestris	2	266,267	12	+++	+++	+++	++	++	+++	-	-	-	-	-	++	+++	++	++	-	++	++	-	-
Trechispora brinkmanni		Cf. Tab. 2																					
Hydnaceae																							
Grandinia farinacea	4	71,74	25	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grandinia granulosa	1	404	18	+++	+++	+++	+++	+++	+++	++	+	+	-	-	++	++	++	-	-	++	++	-	++
Hydnum coralloides	1	364	30	+++	+++	+++	+++	+++	+++	++	++	+	+++	+++	+++	++	+++	-	-	+++	-	+++	+++
Hydnum corrugatum	2	605,606,607	26	+++	+++	+++	+++	+++	+++	+++	++	-	++ +	-	+++	++	++	+ -	-	+++	++	-	+ -
Hydnum erinaceum	1	400	21	+++	+++	+++	+++	++	+++	++	-	-	+++	++	++	++	++	-	-	+++	+	-	+++
Hydnum septentrionale	1	365	21	+++	+++	+++	+++	+++	+++	+++	++	+	-	-	+++	++	++	++	-	+++	-	-	++
Irpex lacteus	1	490	10	+++	++	+++	+++	++	++	++	++	++	-	-	++	++	-	-	-	++	-	+++	++
Odontia bicolor	4	111,112,113,114	17	+++	+++	+++	+++	++	+++	+++	++	++	+++	- ++	++	++	++	-	-	+++	- ++	+++	++
Phlebia radiata	2	533,535	18	+++	+++	+++	+++	+++	++	+++	++	++	-	-	+	+	+++	++	-	+++	++	+++	+
Radulum orbiculare	1	678	10	+++	++	++	++	+	++	++	++	++	++	-	-	+	+	-	-	-	+	++	+++
Polyporaceae																							
Daedalea quercina	1	35	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Daedalea unicolor	2	519,520	18	+++	+++	+++	+++	+++	+++	++	++	+++	-	-	++	++	++	-	-	+++	-	-	++

Fungus	Number of isolates	Strain no	Age of mycelium days	benzidine	α-naphthol	o-anisidine	α-naphthylamine	induline	guaiacol	β-oxyquinoline	2.5-xylidine	o-toluidine	p-cresol	tyrosine	gallic acid	tannin	pyrogallol	hydroquinone	phloroglucinol	catechol	p-quinone	aniline	lactophenol
Polyporaceae																							
Fistulina hepatica	2	36,611	25	+++	+	+	+++	+	-	-	-	-	+++	+++	-	-	+	+++	-	+++	++	-	+++
Fomes annosus	3	43,40,41	12	+++	+++	+++	+++	++	+++	++	++	+	-	-	++	++	++	-	-	++	-	-	-
	6	37,45,48,39,42 56	12	+++	+++	+++	+++	++	+++	++	-	-	-	-	++	++	++	-	-	++	-	-	-
	2	49,488	12	+++	+++	+++	+++	++	+++	++	-	-	-	-	++	++	++	-	-	++	-	-	++
	3	44,47,38	12	+++	+++	++	++	++	++	++	-	-	-	-	++	++	++	-	-	++	-	-	-
Fomes conchatus	1	427	30	+++	++	++	+++	-	++	-	++	-	-	++	-	-	++	-	-	++	++	-	-
Fomes connatus	2	54,349	24	+++	+++	+	++	+++	-	+	-	+	+++	++	+++	++	+++	-	-	++	-	-	++
Fomes fomentarius	2	26,600	18	+++	+++	+++	+++	+++	+++	+++	-	-	-	-	++	++	++	-	-	+++	++	-	++
Fomes igniarius	2	60,61	14	+++	+++	+++	++	++	++	-	-	-	-	-	-	-	-	-	-	-	-	-	++
Fomes nigricans	2	593,594	16	+++	+++	+++	+++	++	++	-	+	-	-	-	+	+	+++	-	-	++	+	++	+
Fomes nigrolimitatus	2	371,571	18	+++	+++	+++	+++	++	+	++	++	+++	-	-	+	-	++	-	-	+	+	+++	+++
Fomes pinicola	5	63,65,66,130, 62-1	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	64,67	18	-	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fomes roseus		Cf. Tab. 2																					
Fomes trivialis	2	596,597	16	+++	+++	+++	+++	+++	+++	+++	-	-	-	-	+	+	+++	-	++	+++	-	++	++
Fomes ulmarius	1	416	25	+++	+++	+++	+++	+++	++	++	+	+	+++	-	+++	++	+++	-	-	+++	-	-	++
Lenzites betulina	47,365		10	+++	+++	+++	+++	++	+++	+++	+++	+++	-	-	++	++	++	+++	-	+++	++	+++	++
Lenzites saepiaria	2	96,99	17	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	97,98,100		++	-	++	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lenzites repanda	1	453	21	+++	+++	+++	+++	++	+++	++	++	-	+++	++	++	++	++	-	-	+++	-	-	+++
Lenzites trabea	1	355	18	++	-	+	++	+	-	-	-	-	-	-	-	-	-	-	-	++	-	-	++
Polyporus abietinus	10	146-154,463,577	18	+++	+++	+++	+++	+++	+++	++	+++	+++	-	-	++	++	++	-	-	+++	-	+++	+++
Polyporus adustus	5	350,381,464-466	12	+++	+++	+++	+++	++	+++	++	+++	+++	-	-	+	++	++	-	-	++	+	+++	+++
Polyporus albellus	2	357,418	21	+++	+++	+++	+++	+++	+++	++	+	+	-	-	++	++	++	-	-	++	-	++	++
Polyporus albidus		Cf. Tab. 2																					
Polyporus amorphus	4	156,349,467,476	28	+++	+++	+++	+++	+++	+++	++	-	++	-	-	++	++	++	++	-	+++	+	+++	++
Polyporus arcularius	1	361	17	+++	+++	+++	+++	+++	+++	+++	-	-	-	-	++	++	++	++	-	+++	++	++	-
Polyporus balsameus [1]	2	159,161	18	++	-	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++
	1	160	18	++	++	++	++	++	+	-	-	-	-	-	++	-	++	-	-	++	-	-	++
Polyporus benzoinus	4	163,165	16	+++	+++	+++	+++	+++	+++	+++	++	++	-	-	++	++	++	-	-	+++	++	+++	++
Polyporus betulinus	5	469-473	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polyporus borealis	6	170,171,174-176	17	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	-	+++	+++	+++	++	-	+++	++	+++	+++
Polyporus brumalis	2	359,569	14	+++	+++	+++	+++	+++	+++	+++	+	+	-	-	+++	++	+++	+++	-	+++	+++	-	++
Polyporus caesius	2	473,474	30	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polyporus circinatus	3	178,358,475	21	+++	+++	+++	+++	+++	+++	++	++	++	+++	+++	++	++	++	++	++	+++	++	+++	++
Polyporus croceus	1	383	14	+++	+++	+++	+++	++	-	-	-	-	-	-	-	++	++	-	-	-	++	-	-
Polyporus cuticularis	1	292	25	+++	+++	++	++	+++	+++	++	-	-	-	-	++	++	++	++	-	+++	++	-	++
Polyporus dichrous	2	362,396	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polyporus fragilis	3	184-186	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polyporus frondosus	2	187,188	21	++	+++	+++	+++	+++	++	+++	+++	+++	-	-	++	++	+++	++	-	+++	++	+++	++
Polyporus fumosus	3	189,350,352	21	++	++	+++	++	++	-	-	-	-	-	-	+	++	++	-	-	-	-	-	++
Polyporus guttulatus	1	423	21	+++	++	++	++	+++	+	++	++	++	+++	+++	++	++	++	+++	-	+++	+++	+	+++
Tyromyces guttulatus	1	324	16	+++	++	+++	+++	+++	-	-	-	-	+++	+++	-	-	++	-	-	+++	+	-	+++
Polyporus hirsutus	3	191,192,477	21	+++	+++	+++	+++	++	+++	+++	+++	+++	-	-	++	++	++	+	-	+++	++	++	++
Polyporus hispidus	1	381	21	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	-	+++	+++	+++	+++	-	+++	++	++	++
Polyporus kymatodes [2]	2	41,16	18	++	+	++	++	++	-	-	-	-	-	-	++	-	++	-	-	-	-	-	++
Polyporus lacteus	3	46,393	14	+++	+++	+++	+++	+++	+++	++	-	-	-	-	++	++	++	-	-	++	++	+	-
	1	424	14	+++	+++	+++	+++	+++	+++	++	+++	+++	-	-	++	++	++	-	-	+++	+	+++	+++
Polyporus melanopus	1	690	14	+++	+++	+++	+++	+++	+++	+++	+	++	-	-	+++	++	+++	+++	-	+++	+++	+	++
Polyporus mollis		Cf. Tab. 2																					

Fungus	Number of isolates	Strain no	Age of mycelium days	benzidine	α-naphthol	o-anisidine	α-naphthylamine	induline	guaiacol	8-oxyquinoline	2.5-xylidine	o-toluidine	p-cresol	tyrosine	gallic acid	tannin	pyrogallol	hydroquinone	phloroglucinol	catechol	p-quinone	aniline	lactophenol
Polyporaceae																							
Polyporus occidentalis	1	478	10	+++	+++	+++	+++	+++	+++	+++	++	+++	–	–	++	++	++	+++	–	+++	++	+++	++
Polyporus picipes		Cf. Tab. 2																					
Polyporus pubescens	2	358,359	8	+++	+++	+++	+++	++	+++	+++	++	–	–	–	++	++	++	–	–	+++	–	++	++
Polyporus radiatus	2	53,610	18	+++	+++	+++	+++	++	+++	++	++	++	–	–	–	–	++	–	–	+++	–	++	++
Polyporus rutilans	4	196,197,364,592	14	+++	+++	+++	++	++	++	++	–	–	+++	–	++	++	++	–	–	++	++	–	++
Polyporus schweinitzii	2	197,602	12	++	+	++	++	++	–	–	–	–	+++	+++	+	+	+	+++	–	+++	+++	++	+++
Polyporus sericeo-mollis	3	199-201,202	14	++	–	++	++	++	–	–	–	–	+++	±++	–	–	++	±	–	+++	++	–	+++
	1	II-8		++	–	+	+	–	–	–	–	–	+++	–	–	–	–	–	–	–	–	–	–
Polyporus spumeus	2	395,625	18	+++	+++	+++	+++	+++	+++	++	++	++	–	–	++	++	+++	–	–	+++	–	–	+++
Polyporus sulphureus	1	212	8	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Polyporus tephroleucus	1	426	25	++	–	++	++	++	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–
	1	428	25	+	–	–	++	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Polyporus varius	1	388	25	+++	+++	+++	++	+++	+++	++	–	+	–	–	++	++	+++	–	–	+++	–	–	++
Polyporus versicolor	4	213-214,361,362	18	+++	+++	+++	+++	+++	+++	+++	+++	+++	–	–	++	++	+++	–	–	+++	±++	+++	+++
Polyporus vulpinus	4	355,356,357,582	17	+++	++	+++	+++	++	+++	+++	++	++	–	–	+	+	++	–	–	–	–	+++	+++
Polyporus zonatus	3	359,360,479	10	+++	+++	+++	+++	+++	+++	+++	++	++	–	–	++	++	++	+	–	+++	–	+++	++
Poria albobrunnea	1	469	10	+++	+++	+++	+++	+++	+++	+++	–	–	–	–	++	++	++	–	–	+++	++	–	++
Poria aurantiaca	1	608	21	++	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	++
Poria candidissima	1	430	21	+++	+++	+++	+++	++	+++	++	++	++	–	–	++	++	++	–	–	++	–	–	–
Poria cinerescens	1	667	13	+++	+++	+	+++	++	+++	+++	–	–	+++	–	++	+	++	+	–	+	++	–	–
Poria corticola	2	366,367	14	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	–	–	+++	+	+++	–
Poria crassa	1	431	18	++	++	–	–	++	++	–	–	–	–	–	–	–	++	–	–	–	–	–	–
Poria ferruginea-fusca	6	367,368,369,380, 430,483	14	+++	+++	+++	+++	++	+++	++	++	++	–	–	++	++	++	–	–	+++	++	+++	++
Poria incrassata	1	219	16	++	–	+++	–	++	+	+	+	+	+	–	–	–	–	–	–	+	–	++	+
Poria lenis	1	386	14	++	–	++	++	–	–	–	–	–	–	–	–	–	–	–	–	–	++	–	–
Poria mollusca	3	221,222,223	12	+++	+++	+++	+++	+++	+++	+++	+++	+++	–	–	+++	+++	+++	++	–	+++	++	+++	++
Poria monticola	1	432	17	+	+++	+	–	++	–	–	–	–	+++	–	–	–	–	–	–	–	–	–	++
Poria obliqua	4	58,225,226,227,	10	+++	+++	+++	++	++	+++	+++	+	+	+++	++	+++	++	+++	++	–	+++	+	+++	++
Trametes abietis	3	289,292,293,	26	+++	+++	+++	+++	+++	+++	++	++	++	++	–	+	+	+	–	–	++	–	++	+–
	4	288,290,291,294,	16-18	+++	+++	+++	+++	+++	+++	++	++	++	++	–	±++	±	±++	–	–	±++	±	++	–
Trametes cervina	1	669	12	+++	+++	+++	+++	+++	+++	+++	++	++	+++	–	++	++	++	++	–	+++	++	+++	+++
Trametes cinnabarina	1	260	12	+++	+++	+++	+++	+++	+++	+++	++	++	+++	–	++	++	++	++	–	+++	++	+++	+++
Trametes heteromorpha	2	289,346	21	++	–	–	–		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Trametes isabellina	1	390	30	+++	+++	+++	+++	+++	+++	++	+++	+++	–	–	++	++	+++	–	–	+++	++	+++	+++
Trametes pini	5	302-306	18	+++	++	+++	+++	+++	++	–	++	++	++	±++	±++	±++	±++	–	–	++	–	±++	–
Trametes sepium	1	435	20	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Trametes serialis	5	306-308,310,434	14	++	–	++	±++	±++	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Trametes serpens	1	437	35	++	–	–	–	++	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Trametes squalens	1	670	14	++	+++	++	+	++	++	++	+	+	–	–	++	++	++	–	–	–	+	++	++
Trametes suaveolens	1	311		++	–	+++	++	++	++	++	–	–	–	–	++	++	++	–	–	–	–	–	++
Ascomycetes																							
Daldinia concentrica	1	378	21	+++	+++	+++	+++	+	+	++	–	–	–	–	++	++	++	++	–	+	–	–	–
Ustulina vulgaris	1	368	18	++	++	++	++	++	–	–	–	–	–	–	–	–	–	–	++	–	–	–	–
Xylaria polymorpha	1	367	15	+++	++	++	++	++	–	+	–	–	–	–	++	++	++	+	–	++	+	–	–

Table 2. Oxidation of different phenolic compounds by the mycelia of some decay fungi which give varying results with different strains.

Fungus	Number of isolates	Strain no	Age of mycelium days	benzidine	α-naphthol	o-anisidine	α-naphthylamine	indaline	guaiacol	8-oxyquinoline	2,5-xylidine	o-toluidine	p-cresol	tyrosine	gallic acid	tannin	pyrogallol	hydroquinone	phloroglucinol	catechol	p-quinone	aniline	lactophenol
Agaricaceae																							
Armillaria mellea	1	1	30	++	+++	-	++	++	++	-	-	-	+++	+++	-	-	++	++	-	++	++	-	++
	1	3		+++	+++	++	-	++	-	-	-	-	+++	+	-	++	+	++	-	++	+	-	++
	1	18		+++	+++	+	++	++	-	++	-	-	+++	+	++	++	++	++	-	++	++	-	++
	1	2		++	+++	-	-	++	-	++	-	-	-	-	-	-	-	-	-		-	-	++
Lentinus lepideus	3	84,85,87	20	++	-	-	+	+	-	-	-	-	++	-	-	-	-	-	-	++	+	-	++
	1	88	20	++	-	-	+	++	-	-	++	-	++	-	-	-	-	-	-	+	-	-	+
	2	89,90	20	++	-	++	+	+	-	-	++	-	++	-	-	-	-	-	-	-	++	-	+
	1	86	20	++	++	++	++	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	-
	2	326,330	20	+++	-	+	++	+	-	+	++	+	+++	+++	-	-	++	++	+	+++	++	+	++
Thelephoraceae																							
Coniophora cerebella	1	11	14	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1	99	12	-	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1	12	12	-	+	-	++	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
	1	440	14	++	+	-	++	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-
	1	448	14	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++
	1	14	20	+++	+++	-	+++	+	+++	-	-	-	-	-	+	+	++	-	-	-	++	-	-
Merulius lacrymans	3	103,403,107	19	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1	104	19	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+++
	1	109	25	+	-	-	-	-	-	-	-	-	+++	-	-	-	-	-	-	-	-	-	+++
	1	102	21	+	-	-	-	+	-	-	-	-	+++	-	-	-	-	-	-	+	++	-	+++
	2	106,108	17	++	-	++	++	++	-	-	+	+	+++	-	++	++	-	+	-	+++	++	-	+++
	1	332	21	+++	++	++	++	++	++	+	+	++	+++	++	++	++	+	++	+	+++	++	++	+++
Peniophora gigantea	1	123	14	+	++	++	+++	++	++	++	-	-	-	-	-	++	++	-	++	-	++	++	+
	3	134,129,128	14	+++	++	++	++	++	-/++	-/++	-	-	-	-	++	++	++	-	-	-	+	-	++
	3	122,130,124	14	+++	+++	++	+++	++	++	++	+	+	-	-	+	++	++	-	-	++	+	+	++
	1	133	14	+++	++	+++	+++	++	++	++	++	++	-	-	+	++	++	-	-	-	++	++	++
	1	127	14	+++	+++	+	+++	+++	++	++	-	-	++	-	++	++	++	++	-	+++	++	-	++
	2	125,135	14	+++	+++	+++	+++	+++	++	++	-	+	+++	++	++	++	++	-/++	-/++	+++	++	-/++	++
Stereum hirsutum	1	372	17	+++	+++	++	-	++	++	++	-	-	+++	-	-	++	++	-	-	-	++	-	+++
	1	255	17	+++	-	+++	++	++	++	++	++	++	-	-	++	++	++	-	-	-	+	++	-
	1	373	14	+++	+++	+++	+++	++	+++	++	-	-	-	-	++	++	++	-	-	-	++	-	-
Stereum purpureum	2	262,374	14	+++	+++	+++	+++	+++	+++	++	+	+	-	-	++	++	+++	+	-	+++	++	+	++
	1	376	14	++	++	++	++	++	-	-	-	-	-	-	-	++	++	++	-	-	++	-	-
	2	IX,375	14	+++	++	-	-	++	-	-	-	-	++	-	-	++	++	++	-	-	+	-	-
Stereum sanguinolentum	4	7,276,266,380	14	+++	+++	+++	+++	+++	+++	++	+	++	-	-	++	++	++	-	-	+	++	+++	-+)
	12	264,265,270, 271,272,275, 278,279,283, 284,285,487,	14-17	+++	+++	++	+++	++	-	-	-	-	-	-	+	-	+/++	-	-	-	-/++	-	-
	2	273,281	17	++	+	-	++	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	267,269,486		++	++	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	260,277	17	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trechispora brinkmanni	1	314	14	++	-	++	++	+	-	++	-	-	++	-	++	-	+	++	-	++	++	+	++
	2	319,320	12	++	++	++	++	++	-	-	+	-	-	-	-	-	++	-	-	-	++	++	-
	2	313,317	12	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1	321	12	++	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	-	+	-	-
	1	315	12	++	-	+	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-
	1	312	12	++	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	++	++	-	-
	1	318	12	++	-	++	++	+	-	-	-	-	-	-	-	-	++	++	-	++	++	-	-
Polyporaceae																							
Fomes roseus	2	68,69	14	+++	++	+	++	+	-	+	-	-	++	+	+	+	++	++	-	++	++	++	+++
	1	354	12	+++	-	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polyporus albidus	1	402	18	++	++	++	++	++	-	-	-	-	-	-	+	++	++	-	-	-	-	-	-
	3	419,421	18	-	-	-	-	-	-	-	-	++	-	-	-	-	++	-	-	-	-	-	-
	1	422	21	++	++	-	-	-	-	-	-	-	-	-	-	-	++	++	-	-	-	-	-
Polyporus mollis	2	193,399	21	+++	+	++	++	++	+	+	++	++	+++	+++	++	+	++	+++	-	+++	++	++	+++
	1	30-55	21	+++	++	+++	++	++	-	-	-	-	+++	+++	++	-	++	++	-	+++	++	++	+++
	1	425	18	++	+	++	++	++	+	-	-	-	-	-	-	-	-	-	-	-	++	-	-
	1	604	20	+++	+++	+++	+++	+++	+++	+++	++	++	+++	+++	+	+	++	-	-	+++	+++	+	++

Fungus	Number of isolates	Strain no	Age of mycelium days	benzidine	∝-naphthol	o-anisidine	∝-naphthylamine	induline	guaiacol	8-oxyquinoline	2.5-xylidine	o-toluidine	p-cresol	tyrosine	gallic acid	tannin	pyrogallol	hydroquinone	phloroglucinol	catechol	p-quinone	aniline	lactophenol
Polyporaceae																							
Polyporus picipes	1	A x)	20	+++	+++	++	++	+	+++	++	+	++	++	-	-	-	-	++	+	-	-	+++	++++
	1	B	14	+++	++	+	+	+	+	+	+	++	-	-	-	-	++	+	-	+++	-	+	++
	3	C,C-1, C-2	28	+++	++	+	+	++	+	-	-	-	+++	+++	+++	+++	+++	++	-	++	-	-	++
	1	D	21	+++	+++	+++	+++	+++	+++	+	++	++	-	-	-	-	-	-	-	++	+	++	+++
Poria vaporaria	2	242,426	12	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1	241	15	++	-	+	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	244,329	10	++	++	+	++	+	-	-	-	-	-	-	-	-	++	-	-	-	+	-	-

x) growth types

Table 3. Growth and production of phenoloxidases by some fungi at different pH levels.

Fungus	pH	Growth, mm after 10 days	Tests after days	p-cresol	∝-naphthol	o-anisidine	guaiacol	induline	o-toluidine	xylidine	gallic acid	tannin	pyrogallol	hydroquinone	p-quinone	eugenol	lactophenol	phenol
Flammula alnicola	2.5	1	25	-	+++	+	++	-	++	++	+	-	-	-	++	-	++	-
	4.0	10		-	+++	+	++	-	++	++	-	-	-	-	++	-	+	-
	4.8	7		-	+++	++	++	+	++	++	+	+	+	-	+++	-	+	-
	5.5	7		-	++	+++	++	+	++	++	+	+	+	-	++	-	+	-
	6.5	7		++	-	+++	-	-	++	++	+	+	+	-	++	-	+++	++
Fomes ulmarius	2.5	0	25															
	4.0	7		+++	+++	+++	+++	+++	+	+	+++	++	+++	+	++	+	-	-
	4.8	12		+++	+++	+++	+++	+++	+	+	+++	++	+++	+	++	+	+	-
	5.5	18		+++	+++	+++	+++	+++	+	+	+++	++	+++	+	++	-	+	-
	6.5	20		+++	+++	++	+++	+++	+	+	+++	++	+++	+	++	-	+	-
Hypholoma capnoides	2.5	4	18	-	+++	++	+++	++	++	+	++	++	++	-	-	-	++	-
	4.0	25		+++	+++	++	+++	++	++	++	++	++	++	-	-	-	++	+
	4.8	22		+++	+++	++	+++	++	+++	++	++	++	++	-	-	-	++	+
	5.5	24		+++	+++	++	+++	++	+++	++	++	++	++	-	-	-	++	+
	6.5	20		+++	+++	++	+++	++	+++	++	++	++	++	-	-	-	++	+
Lenzites betulina	2.5	26	10	-	+++	+++	+++	+++	++	++	++	++	+++	++	++	+	+++	-
	4.0	49		-	+++	+++	+++	+++	++	++	++	++	+++	++	++	-	+++	+
	4.8	50		-	+++	+++	+++	+++	++	++	++	++	+++	++	++	-	+++	+
	5.5	52		-	+++	+++	+++	+++	++	++	++	++	+++	-	-	-	+++	+
	6.5	47		-	+++	+++	+++	+++	++	++	++	++	+++	-	-	-	+++	+
Polyporus kymatodes	2.5	30	10	-	+	++	-	-	-	-	-	-	-	-	+	-	-	-
	4.0	39		++	+	++	-	+	-	-	-	-	++	-	+	-	++	-
	4.8	34		-	+	++	-	+	-	-	-	-	-	-	+	-	++	-
	5.5	27		-	+	++	-	+	-	-	-	-	-	-	+	-	++	-
	6.5	24		-	+	++	-	+	-	-	-	-	-	-	+	-	++	-

Equal results at all pH levels were produced by: tyrosine, ferulic acid, benzidine, ∝-naphthylamine, 8-oxyquinoline, catechol, resorcinol, phloroglucinol, o-aminophenol, methol and gum guaiac.

Table 4. Oxidase reactions in mycelia of different ages.

Fungus	Reaction		Same type of reaction after ... days	Reaction changed after ... days
	Laccase	Tyrosinase		
Armillaria mellea	++(+)	+++(—)	15, 18, 30	50, 75
Byssocorticium pulchrum	++	—	15, 21, 42	
Collybia velutipes	—	—	15, 21, 42	
Coniophora arida	—	—	14, 21, 32, 42	
» olivacea	—	—	12, 30	
» puteana	—	—	12, 30	
Corticium alutaceum	++	+++	18, 25	63
» laeve	—	—	14, 25	36
Daedalea quercina	—	—	12, 18, 28, 75, 81	
» unicolor	+++	—	18, 20, 41	**)
Daldinia concentrica	++	—	21, 42	
Fistulina hepatica	++	+++	18, 25, 30	
Flammula alnicola	++	—	21, 25, 30	
» gummosa	+++	++	16, 21, 66, 78	**)
» ochrochlora	+++	++	16, 21	
» sapinea	++	—	16, 21	
Fomes annosus	+++	—	12, 21, 25, 32	**)
» conchatus	++	(++)	21, 30	69***)
» igniarius	++	—	13, 35	42
» pinicola	—	—	15, 18, 21, 32, 38, 54	
» roseus	++	(++)	12, 14	
» ulmarius	+++	+++	21, 25, 32	52
Gloeocystidium karstenii	++	—	15, 21, 62	
Grandinia farinacea	—	—	25, 32, 42	66
» granulosa	+++	—	18, 25, 32	
Hydnum coralloides	+++	+++	30, 54, 81, 102	
» erinaceum	+++	+++	21, 69, 81	
» septentrionale	+++	—	21, 69, 81	
Hymenochaete rubiginosa	++	—	18, 25, 32, 46	55
» tabacina	+++	—	21, 26, 52, 66, 74	
Hypholoma capnoides	+++	+++	18, 21, 46, 55, 112	42
» fasciculare	++(+)	+++	21, 34, 44	44, 55, 82
» sublateritium	+++	+++	21, 44	**)
Irpex lacteus	+++	—	10, 21	
Lentinus lepideus	— to ++	—to +++	20, 43	43, 52
» tigrinus	+++	+++	19, 46, 55	41
Lenzites betulina	+++	—	10, 30, 33, 46, 66	**)
» repanda	+++	+++	21, 53	
» sepiaria	—	—	15, 17	38, 52 *)
» trabea	—	—	18, 34, 74	
Merulius himantioides	—	—	25, 32, 55, 80	
» lacrymans	— to ++	(—)+++	17, 18, 21, 42, 46, 55	55, 88
» sylvester	—	—	21, 55	
» tremellosus	++	+++	13, 19, 32, 46, 55	55
Odontia bicolor	+++	+++	17	44, 52
Omphalia campanella	++	—	19, 32	46, 52
Panus stypticus	+++	—	17, 21, 55	44, 52 **)
» torulosus	+++	(++)	30	62 *) ***)
Peniophora cinerea	+++	—	13, 35	
» cornea	++(+)	— to +++	14, 17, 21	44, 52 *)
» cremea	—	—	10, 17, 21	
» gigantea	++(+)	— to +++	14, 28, 36	28, 36, 40 *)

Table 4 (cont.)

Fungus	Reaction		Same type of reaction after ... days	Reaction changed after ... days
	Laccase	Tyrosinase		
Peniophora incarnata....	+++	—	12, 20	33, 37, 44
» pini.....	+++	++	10, 12, 20, 63, 72	63
» pithya.....	+++	++	13, 19, 35, 46, 52	35, 44, 52
,, septentrionalis	+++	—	14, 35	62 *)
Pholiota adiposa.....	+++	+++	17, 35, 69	**)
,, aurivella.....	+++	++	17, 46, 52	**)
,, destruens.....	++	+	12, 21	46, 52 *)
,, mutabilis.....	++	++	16, 37	65
,, squarrosa.....	++	+++	17, 21, 46	46
Pleurotus corticatus.....	++	—	21, 30, 64, 102	**)
,, ostreatus.....	++	—	14, 21, 32, 46, 69, 78	
,, serotinus.....	+++	—	14, 35, 60, 70	**)
,, ulmarius.....	+++	—	28, 32, 57, 80	**)
Polyporus abietinus.....	++	—	12, 14, 18, 28, 34, 42, 66, 73, 80, 108	**)
,, adustus.....	++	—	12, 38	32, 54
,, albellus.....	+++	—	21, 66, 70	58, 69
,, albidus.....	—(++)	—	14, 18, 21, 60, 70	
,, amorphus.....	++	—	25, 28, 43, 52	
,, arcularius.....	++	—	17, 28	50, 58
,, balsameus.....	—(++)	—	17, 32, 52, 80	52, 58
,, benzoinus.....	++	—	16, 30, 52, 82	**)
,, betulinus.....	—	—	14, 35, 60	
,, borealis.....	+++	—	17, 25, 32, 50, 58, 80	**)
,, brumalis.....	++	—	14, 60, 66	
,, caesius.....	—	—	30, 52, 80	
,, circinatus.....	++	+++	14, 21, 39, 52	70
,, croceus.....	+	—	14, 21	54, 68 *)
,, cuticularis.....	++	—	25, 30, 54, 68, 80	**)
,, dichrous.....	—	—	15, 21, 30, 54, 70	
,, frondosus.....	+++	—	21	54, 82 *)
,, fumosus.....	++	—	7, 21, 54	54, 62, 74
,, guttulatus.....	++(+)	+++	21, 25, 74, 88	
Tyromyces guttulatus....	+	+++	16, 23, 38	
Polyporus hirsutus.....	+++	—	17, 21, 54, 82	52, 82 *)
,, hispidus.....	+++	—	21, 54, 70	
,, kymatodes....	++	—	18, 21, 68, 75, 80	
,, lacteus.....	+++	—	14, 22, 54, 68, 72	
,, mollis (Nobles)	(+)	—	21, 62, 74	
,, ,, (CBS) ..	+	+++	21, 35, 54	
,, occidentalis...	+++	—	10, 24, 30, 82	**)
,, pubescens.....	+++	—	8, 42, 54, 82	**)
,, radiatus.....	+++	++	8, 21, 42, 54, 69	54, 82
,, rutilans.....	+++	+++	14, 42, 63	
,, schweinitzii...	+	+++	21, 54, 69	
,, sericeomollis...	+	+++	14, 21, 25, 60, 80	
,, spumeus.....	+++	—	25, 31, 68, 75	**)
,, stipticus.....	+++	—	12, 21	32, 80 *)
,, sulphureus....	—	—	8, 21, 43, 72	
,, tephroleucus ..	++	—	25, 40, 52	30, 42, 80 *)
,, varius.....	+++	—	25, 30, 63	
,, versicolor.....	+++	—	6, 18, 21, 41, 54, 69	**)

Table 4 (cont.)

Fungus	Reaction		Same type of reaction after ... days	Reaction changed after ... days
	Laccase	Tyrosinase		
Polyporus vulpinus.....	+++	—(++)	17, 21, 45	30, 43, 69, 70, 80, *)
„ zonatus.....	+++	—	7, 10, 14, 21, 25, 42, 54, 69, 82	**)
Poria albobrunnea.....	+++	—	10, 14, 21	
„ candidissima.....	+++	—	17, 21, 54, 74	
„ corticola.....	+++	+++	14, 30	
„ crassa.....	(+)	—	14, 21, 62, 74	
„ ferrugineo-fusca....	+++	—	14, 30, 63	**)
„ incrassata.....	+	+	16	45, 63
„ lenis.....	—	—	14, 30, 80	
„ mollusca.....	+++	—	12, 24, 30, 41, 52, 80	**)
„ monticola.....	++	+++	17, 74, 80	
„ obliqua.....	+++	—(+++)	10, 14, 30, 80	80
„ radiculosa.....	—	—	16, 21, 42, 63	
„ rixosa.....	+++	—(++)	14, 21, 35	62, 74
„ sinuosa.....	—(+)	—	21, 25, 45, 70	
„ vaillantii.....	—(++)	—	18, 25, 36, 45, 63	
„ vaporia	—(++)	—	10, 12, 15, 28, 38	
„ weirii.....	+++	—	12, 17, 30, 45	
„ versipora.....	+++	—	17, 25, 34	62, 74
„ xantha.....	+++	+++	12, 21	32, 80 *)
Schizophyllum commune .	—(++)	—	14, 17, 21, 27	24, 38 *)
Stereum abietinum.....	+++	+++	21, 45, 54	
„ chailletii.....	+++	++	14, 21, 35, 68, 74	
„ frustulosum.....	—	—	19, 27, 38, 54	
„ hirsutum.....	+++	—(+++)	14, 17, 45	45 *)
„ murrayi.....	+++	—	25, 30, 37	
„ purpureum	+++	—(++)	8, 14, 30, 38	25, 30, 38 *)
„ sanguinolentum .	++(+)	—	14, 17, 21, 35, 42, 68, 91, 115	110, 120
Thelephora terrestris.....	++	—	12, 13, 30, 40	
Trametes abietis.....	+++	+++	16, 18, 30, 38	27, 38
„ cinnabarina....	+++	+++	12, 38	**)
„ heteromorpha..	—	—	12, 21, 74, 80	
„ isabellina.....	+++	—	30, 35, 43	**)
„ odorata.....	+++	+++	23, 38, 62	38
„ pini.....	+++	+++	14, 18, 25, 38	38
„ sepium.....	(+)	—	20, 70	
„ serialis.....	+(—)	—	14, 21, 30	28, 38 *)
„ serpens.....	+(—)	—	21, 35, 44	81, 92 *)
„ suaveolens.....	++	—	14, 21	30, 38 *)
Trechispora brinkmanni..	—(++)	—(+++)	12, 30, 38	25, 38 *)
Tricholoma rutilans.....	+++	—	15, 21, 25	38 *)
Ustulina vulgaris.....	+	—	18, 25, 38	
Xylaria polymorpha.....	++	—	15, 30	38

Notes:

*) Markedly changed reaction.
**) No changes at all in any of the reactions.
***) Tyrosinase-reaction only with tyrosine; no reaction with p-cresol.

Table 5. Oxidase reactions in mycelia of different ages in drop and agar tests.

Fungus	Age of the mycelium, days.	Drop tests													Agar tests			
		α-naphthol	anisidine	induline	guaiacol	toluidine	p-cresol	tyrosine	gallic acid	pyrogallol	catechol	p-quinone	aniline	lactophenol	gallic acid	tannin	guaiacol	tyrosine
Fomes roseus	8	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	++	+++
	15	-	-	++	-	-	-	-	-	+	+++	++	-	++	-	-	++	+++
	23	-	-	++	-	-	+++	-	-	-	+++	++	-	++	-	-	++	+++
	30	++	-	++	-	-	+++	-	-	-	+++	++	-	++	-	++	-	+++
	36	++	++	++	-	-	+++	+++	-	-	+++	++	++	+++	-	++	-	+++
	43	++	-	++	-	-	++	++	-	-	++	+++	+	+++	++	-	-	+++
	50	++	-	++	-	-	++	++	-	-	-	+++	-	+++	-	-	-	+++
	57	+	-	++	-	-	++	-	-	-	-	++	-	-	-	-	-	+++
Peniophora gigantea	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	15	+++	-	-	++	-	+	-	-	++	-	++	-	+	-	-	++	-
	23	+++	+++	++	++	-	+	-	-	++	+++	++	-	+	-	-	++	-
	30	+++	+++	++	++	-	+	-	-	++	+++	++	-	+	-	++	++	-
	36	+++	+++	+++	++	-	+++	-	+++	+++	+++	++	-	+	-	++	++	-
	43	+++	+++	+++	++	-	+++	-	+++	+++	+++	++	-	++	-	-	++	-
	50	++	+++	+++	-	-	+++	-	+++	+++	+++	++	-	++	-	-	++	-
	57	++	-	+++	-	-	+++	-	+++	+++	-	++	-	++	-	-	++	-
Stereum hirsutum	8	+++	+++	+++	+++	-	-	-	++	+++	-	-	+	+++	++++	++++	+++	-
	15	+++	+++	+++	+++	+	+++	-	++	+++	-	++	+	-	++++	++++	+++	-
	23	++	+++	+++	-	++	++	-	++	+	-	++	++	-	+++	++++	+++	-
	30	++	++	+++	-	-	++	-	-	+	-	++	++	-	+++	+++	++	-
	36	++	+	+++	-	-	++	-	-	+	-	++	-	-	+++	++++	++	-
	43	++	+	+++	-	-	+++	++	-	+	-	++	-	-	++++	++++	-	++
	50	+	+	+++	-	-	+++	-	-	+	-	++	-	-	++++	+++	-	++
	57	+	+	+++	-	-	+++	-	-	+	-	++	-	-	-	-	-	-
Stereum sanguinolentum	8	-	-	-	-	-	-	-	-	-	-	++	-	-	-	++++	-	-
	15	-	-	++	-	-	-	-	-	-	-	++	-	-	-	++++	-	-
	23	++	-	++	-	-	-	-	+	-	-	++	-	-	-	++++	-	-
	30	++	-	+++	-	-	-	-	+	-	-	++	-	-	-	++++	-	-
	36	++	-	+++	-	-	-	-	++	-	-	++	-	-	-	++++	-	-
	43	++	-	+++	-	-	-	-	++	++	-	++	-	-	-	++++	-	-
	50	++	-	+++	-	-	-	-	++	++	-	++	-	-	-	++++	-	-

Table 6. Reactions of twelve monosporous strains of Stereum sanguinolentum from two different sporophores A and B to phenolic substances.

Age of colonies 18 days.

Strains nr.	benzidine	α-naphthol	o-anisidine	α-naphthylamine	induline	guaiacol	8-oxyquinoline	2.5-xylidine	o-toluidine	p-cresol	tyrosine	gallic acid	tannin	pyrogallol	hydroquinone	phloroglucinol	catechol	p-quinone	aniline	lactophenol
a — 1.....	+																			
a — 2, 3, 5, 6	+	+												+				+		
a — 4.....	+	+	+	+	+	+	+	+						+				+		
b — 1, 5.....	+	+	+	+	+	+		+											+	
b — 2, 6.....	+	+	+	+	+													+		
b — 4.....	+	+	+	+	+			+											+	

Table 7. Groups of fungi based on their production of laccase and tyrosinase.

I. Fungi producing neither laccase nor tyrosinase. Some strains of the fungi listed in this group may produce phenoloxidases and are then found even in other groups, but the reaction in these fungi is almost always weak. Only rarely a medium reaction is produced (cf. comments to the groups I—IV in text).

Fungus	Occurring besides in the following groups			Results of other authors	
	II	III	IV	Agreeing with	Not agreeing with
Collybia velutipes				N, L, B	(D)
Coniophora arida					
,, cerebella		A		N, L, D, B, R	
,, olivacea					
Corticium laeve				N, B	
Daedalea quercina		A		D, B	
Fomes pinicola		A		N, L, D, B	H
Grandinia farinacea					
Lentinus lepideus	×	A	A	N, L, D	
Lenzites sepiaria		A		N, L, D, H	B
,, trabea		A		D	
Merulius himantoides				N	
,, lacrymans	×	A	A, B	N, L, H, R	
,, silvester		A			
Peniophora cremea					
Polyporus albidus		A		N, H	
,, balsameus		A		D, N	
,, betulinus				N, L, D, H	
,, caesius					
,, dichrous				N, D	
,, sulphureus				N, L, D, B, H	
Poria crassa		A			
, lenis		A			
,, radiculosa		A			
,, sinuosa		A			
,, vaillantii		A		N, D	
,, vaporaria		A		D, H, R	
Schizophyllum commune		A		(N, D, L, B) H	
Stereum frustulosum		A		B	
Trametes heteromorpha				N, D	
,, sepium		A		N	
,, serialis		A		N, D	
,, serpens		A			
Trechispora brinkmanni	×	A	A, B	B	

II. Fungi, not producing laccase but only tyrosinase.

Fungus	*Occurring besides in* the following groups			Results of other authors	
	I	III	IV	Agreeing with	Not agreeing with
Lentinus lepideus	×	A	A		
Merulius lacrymans	×	A	A, B		
Trechispora brinkmanni	×	A	A, B		

III. Fungi, producing laccase, not tyrosinase.

Fungus	Occurring besides in the following groups			Results of other authors	
	I	II	IV	Agreeing with	Not agreeing with
A. Laccase reaction weak					
Coniophora cerebella	×				
Daedalea quercina	×				
Fomes pinicola	×				
Lentinus lepideus	×	×	A		
Lenzites sepiaria	×				
„ trabea	×				
Merulius lacrymans	×	×	A, B		
„ silvester	×				
Polyporus albidus	×				
„ balsameus	×			(N), D	
„ mollis (strain Nobles)				N	
„ tephroleucus				(D)	
Poria aurantiaca					
„ crassa	×				
„ lenis	×				
„ radiculosa	×				
„ sinuosa	×				
„ vaillantii	×				
„ vaporaria	×				
Schizophyllum commune	×				
Stereum frustulosum	×				
Trametes serialis	×				
„ sepium	×				
„ serpens	×				
Trechispora brinkmanni	×				
B. Laccase reaction medium					
Armillaria mellea			B	N, B, H	
Corticium confluens					
„ subseriale					
Daldinia concentrica					
Flammula alnicola					
„ sapinea					
Fomes igniarius				N, D, B	L, H
„ nigricans					
Gloeocystidium karstenii				N	
Hymenochaete rubiginosa				N, D	
Irpex lacteus				N	
Omphalia campanella				N	
Peniophora cornea			B		
„ gigantea			B, C	(N), B, D	
„ hydnoidea					
Pholiota flammans					
Pleurotus serotinus				N, D	
Polyporus croceus				N, D	
„ fumosus					
„ kymatodes					
„ mollis			C		
„ picipes			B, C		
Poria weirii				N, D	
Stereum gausapatum					

Fungus	Occurring besides in the following groups			Results of other authors	
	I	II	IV	Agreeing with	Not agreeing with
Stereum hirsutum			B	N, L	
„ purpureum			B	N	
„ rugosum				B, N	
„ sanguinolentum					
Thelephora terrestris					
Trametes cervina					
„ squalens					
„ suaveolens					
Ustulina vulgaris				L, (D)	
Xylaria polymorpha					
C. Laccase reaction strong					
Collybia dryophila					
Daedalea unicolor				N, D, H	L
Fomes annosus				N, L, D, H	
„ fomentarius					
„ nigrolimitatus					
„ trivialis					
Grandinia granulosa					
Hydnum septentrionale				N, D	
Hymenochaete tabacina				N, D	
Lenzites betulina				N, L, D, H	B
Merulius molluscus			C		B
Panus stipticus				D	L
Peniophora byssoidea					
„ cinerea				N, D	B
„ gigantea			B, C		
„ incarnata			C	D, B	B
„ septentrionalis				N	
Phlebia radiata					
Pleurotus corticatus			C	D	
„ ostreatus				N, D	L
„ ulmarius				D	
Polyporus abietinus				N, L, D, B, H	
„ adustus				(N), D	L, B
„ albellus				N, D	
„ amorphus				N	
„ arcularius				L, D	
„ benzoinus				N, D	
„ borealis				N, D	
„ brumalis				N	
„ cuticularis				N, D, B	
„ frondosus					
„ hirsutus				N, L, D, B, H	
„ hispidus				D, B	L
„ lacteus					
„ melanopus					
„ occidentalis					
„ picipes			B, C		
„ pubescens				N, D	
„ radiatus				N, D	L
„ spumeus				D	
„ varius					

Fungus	Occurring besides in the following groups			Results of other authors	
	I	II	IV	Agreeing with	Not agreeing with
Polyporus versicolor				N, D, L, H	
„ vulpinus			B	N, D	
„ zonatus				N, L	
Poria albobrunnea					
„ candidissima					
„ ferrugineofusca				N	
„ mollusca					
„ versipora				D	
Stereum murrayi				N, L, B	
„ purpureum			B, C		
Trametes isabellina					
Trioholoma rutilans					

IV. Fungi, producing both laccase and tyrosinase.

Fungus	Occurring besides in the following groups			Results of other authors	
	I	II	III	Agreeing with	Not agreeing with
A. Laccase reaction weak:					
Fistulina hepatica					(B, L, D)
Lentinus lepideus	×	×	A		
Merulius lacrymans	×	×	A		
Polyporus sericeomollis					D
„ schweinitzii				(H, N)	L, D
Poria incrassata					D
„ monticola					N
Trechispora brinkmanni	×	×	A		
B. Laccase reaction medium:					
Armillaria mellea			B	L, D, H	
Corticium alutaceum					
Fomes conchatus				N, L, D, B	
„ connatus					
Hypholoma sublateritium				L, D	B
Lentinus tigrinus					L
Merulius lacrymans	×	×	A		
„ tremellosus				L, D	B
Panus torulosus					B
Peniophora cornea			B		
„ gigantea			B, C		
„ roumegueri					
Pholiota marginata					
Polyporus guttulatus					
„ picipes			B, C		
„ rutilans				B	
„ vulpinus			C	B	
Poria obliqua				N, B	
„ xantha					N

Fungus	Occurring besides in the following groups			Results of other authors	
	I	II	III	Agreeing with	Not agreeing with
Radulum orbiculare					
Stereum hirsutum			B		
,, purpureum			B, C		
Trametes abietis				B, N	
Trechispora brinkmanni	×	×	A		
C. Laccase reaction strong:					
Flammula gummosa					
,, ochrochlora					
Fomes ulmarius					
Hydnum coralloides				D	
,, corrugatum					
,, erinaceus				N, D	
Hypholoma capnoides					
,, fasciculare				L	
Lentinus tigrinus					
Lenzites repanda					
Merulius molluscus			B		B
Odontia bicolor				N	B
Panus conchatus					
Peniophora gigantea			B, C		
,, pini				N, D	B
,, pithya				N, B	
,, velutina					
Pholiota adiposa				N, D	
,, aurivella					
,, destruens					
,, mutabilis				L	B
,, squarrosa				L	
Pleurotus corticatus					
,, porrigens					
Pluteus cervinus					
Polyporus circinatus				N, D, B	L
,, guttulatus					
Tyromyces guttulatus				(N)	D
Polyporus mollis			B		
,, picipes			B, C		
,, radiatus				N, L, D	
Poria cinerescens					
,, corticola				(N)	
,, rixosa					
Stereum abietinum				B	N
,, chailletii				N, B	
,, hirsutum			B		
,, purpureum			B, C	N, D, B	
Trametes cinnabarina				N, D	L
,, pini				N, L, D	
Trechispora brinkmanni	×	×	A		

Abbreviations: B: Boidin, 1951
N: Nobles, 1948, 1958
D: Davidson, Campbell & Blaisdell, 1938
L: Lyr, 1956, 1958 a
R: Rösch, 1962 a, b
H: Higuchi & Kitamura, 1953

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