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Studies on Growth and Respiration of a Mushroom, *Pleurotus ostreatus*

By

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In spite of the increasing investigations on the respiratory metabolism of microorganisms and higher plants, our knowledge of the problem in lower plants, such as lichen and mushroom, is still scanty. It has been the purpose of this study to investigate the nature of respiration of a cultivated mushroom, *Pleurotus ostreatus*. In course of experiment remarkable variations were observed in the respiratory metabolism according to the stages of growth of the mushroom. Simultaneously the chemical constituents of culture media were shown to affect the oxidative metabolism of cultivated fungi.

The formation of fruit bodies was also influenced by the composition of nutrient media. Results of these experiments concerning the relation between respiration and growth of the fungi are reported below.

Material and Method

Pleurotus ostreatus belongs to fungi associated with white rots. Secondary hypha of this fungus grown on culture media were used throughout the experiments. Three kinds of culture media were employed. Their compositions were as follows:

1) Potato-glucose medium

Extracts of boiled potato (200 g)	1000 ml
Glucose	20 g
KH ₂ PO ₄	1.5 g
MgSO ₄ · 7H ₂ O	0.5 g

2) 2% Peptone-glucose medium

{ Peptone	20 g
{ Glucose	20 g
{ KH ₂ PO ₄	1.5 g

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MgSO ₄ ·7H ₂ O	0.5 g
Tap water	1000 ml

3) 1% Peptone-glucose medium

The same as 2), except that 10 g instead of 20 g of peptone.

All the media were adjusted to pH 5.4.

Erlenmyer flasks of 250 ml capacity containing 40 ml nutrient medium were inoculated with pieces of mycelium (about 3 × 3 mm) cut off from the mycelial mat of stock culture on a potato-glucose agar plate. The pieces were cultivated floating on the surface of the liquid. The cultures were incubated at 24°C for about 4–5 weeks.

At intervals, the mycelia were filtered off, rinsed in water, sliced with rasor, and these slices were used for measuring respiratory activity. The amount of growth was determined by weighing completely dried mycelia at 100°C.

Respiratory experiments were run by means of conventional Warburg technique. The oxygen uptake was measured at 25°C in phosphate buffer pH 7.0 and expressed as Q_{O₂}. The concentration of added substrates was M/120. Total volume of fluid in manometer cup was 2 ml.

Results

1. Variations of oxidative activity during mycelial growth

Oxygen uptake was determined applying mycelia grown on three kinds of media, viz. potato-glucose medium, 2% peptone-glucose medium and 1% peptone glucose medium, with or without addition of *p*-phenylenediamine, catechol or hydroquinone. Experimental results obtained are to be seen in the accompanying diagramms (Fig. 1, 2, 3).

The fungus cultivated on different nutrient media behaved in different ways. The most abundant growth occurred in the case of 2% peptone-glucose medium. In the course of growth the formation of pigment was observed in every case. The time of pigment formation was indicated in figures by arrows. In potato-glucose medium, however, only decreased amount of pigment was produced. The pigment seems to appear when the mycelia begin to autolyze, because thereafter the mycelial weight decreases.

The formation of the tubercle, which is the primordial form of the fruit body, was observed after some 15 days in mycelia grown on potato-glucose medium. In the case of mycelia grown on other media no tubercle was produced.

The pH varied from 5.4 to 7.0 in 1% peptone medium and from 5.4 to

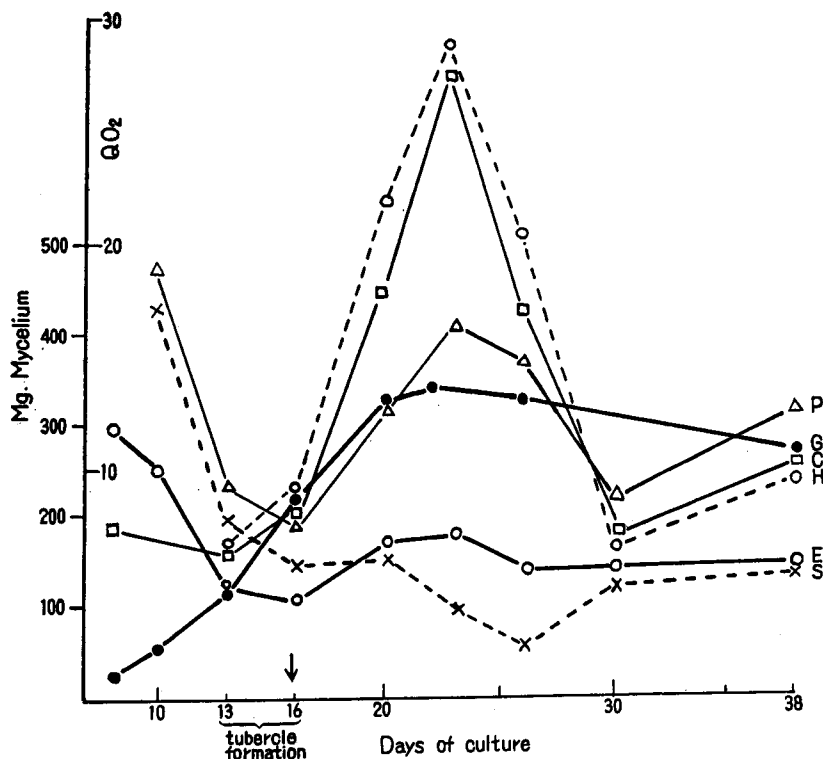


Fig. 1. Oxygen uptake of mycelia grown on potato-glucose medium.

G: Growth; E: Endogenous respiration; P: M/120 *p*-Phenylene diamine-addition; C: M/120 Catechol-addition; H: M/120 Hydroquinone-addition; S: M/120 Succinate-addition.

8.0 in 2% peptone, whereas only a slight variation of pH occurred in potato medium.

As shown in Fig. 1, the rate of endogenous respiration of mycelia grown on potato-glucose medium decreases continuously during the initial 16 days, then it increases slightly. Oxygen uptake in the presence of *p*-phenylenediamine essentially resembles to that of endogenous respiration. The decrease of oxygen uptake during the early stages of growth was observed also in the case of oxidation of succinic acid. In this case, however, the rate of oxidation never increased subsequently. Contrary to the oxidation of *p*-phenylenediamine and succinic acid, the rate of oxidation of catechol and hydroquinone is initially very low, however, after 13 days, it begins to increase rapidly and reaches the maximum value of Q_{O_2} after 23 days. From these data it seems probable to assume that there might be a turning point in respiratory mechanism of

the fungi after about 13 days of growth, because the oxidative activities of *p*-phenylenediamine before 13th day of growth predominate that of catechol and hydroquinone, but, after that time, the oxidative rate of catechol and hydroquinone is far greater than that of *p*-phenylenediamine. The most interesting feature observed from these data is that the time of conversion of oxidative activities for *p*-phenylenediamine to those for diphenol corresponds to the time when tubercle formation from the mycelia occurs. After the tubercle is

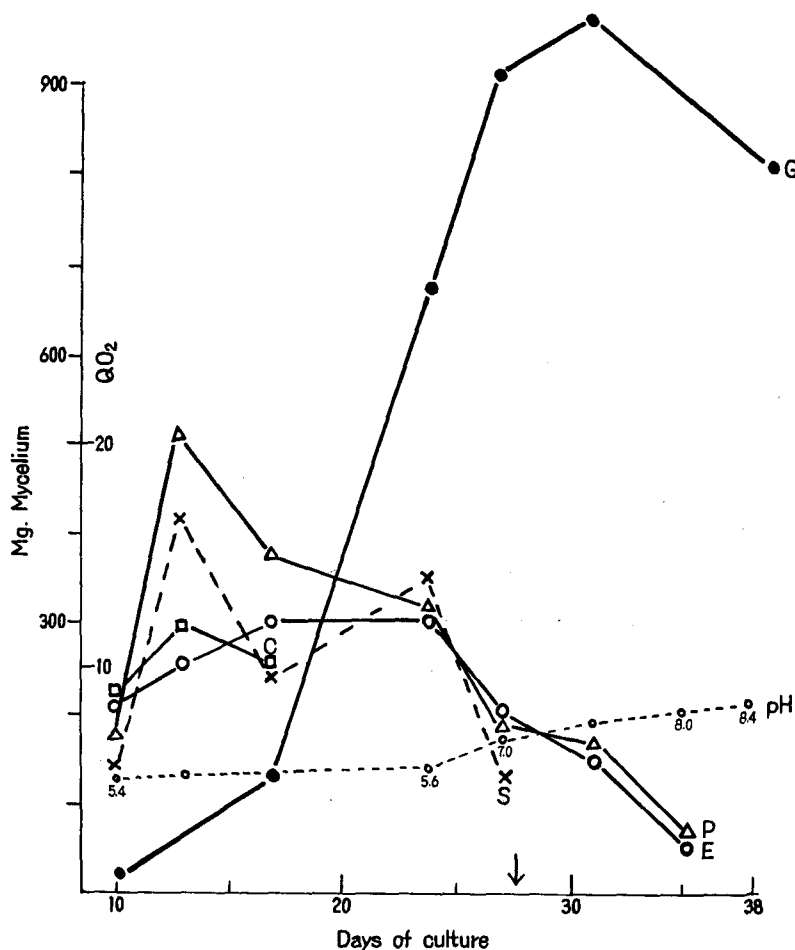


Fig. 2. Oxygen uptake of mycelia grown on 2% peptone-glucose medium.

Signs denote as in Fig. 1.

G: Growth; E: Endogenous respiration; P: M/120 *p*-Phenylenediamine-addition; C: M/120 Catechol-addition; S: M/120 Succinate-addition; pH: pH of medium.

formed the rates of oxidation of both substrates increase continuously. Simultaneously the dry weight of mycelia continues to increase and reaches its maximum level before the arrival of maximum oxidative rate.

Similarly, in the case of 2% peptone medium, as shown in Fig. 2, both endogenous respiration and *p*-phenylenediamine oxidation, following an initial increase, begins to decrease far before the arrival of maximal growth. No subsequent increase of oxidation was observed. The oxidation of succinic acid generally goes parallel with that of *p*-phenylenediamine, though it slightly increases afterwards. Rapid reductions of oxidative activities were seen especially after 25 days, where the value of pH rose to 7.0, then pigment was produced. On peptone media no tubercle was formed.

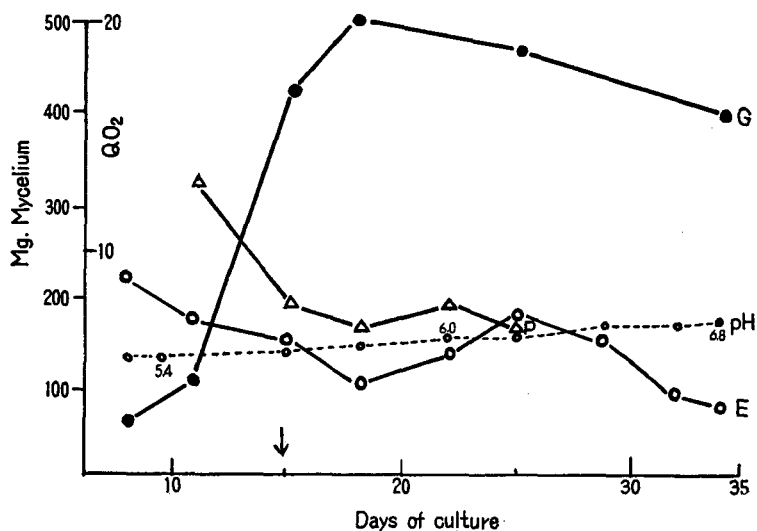


Fig. 3. Oxygen uptake of mycelia grown on 1% peptone-glucose medium.

Signs denote as in Fig. 1.

G: Growth; E: Endogenous respiration; P: M/120 *p*-Phenylenediamine-addition; pH: pH of medium.

Analogous results were obtained with mycelia grown on 1% peptone medium, but in this case the oxidative rate decreased continuously without initial increase and it decreased rapidly after 20 days, where pH reached 6.0. In this case only reduced growth was observed as compared with that in 2% peptone medium. The pigmentation occurred after 15 days.

2. Effect of copper trapping agents upon mycelial respiration

Since it is widely accepted that biological oxidation of phenols are catalyzed

TABLE 1. Effect of KCN and salicylaldoxime on endogenous respiration and catechol oxidation of mycelia grown on potato medium at various stages of growth.

Inhibitors: M/500

"Relative rate of O₂-uptake" denotes the relative rate in per cent of oxygen uptake in the presence of KCN or salicylaldoxime to oxygen uptake in their absence.

Days of cultivation	Endogenous respiration			Catechol oxidation		
	Qo ₂	Relative rate of O ₂ -uptake		Qo ₂	Relative rate of O ₂ -uptake	
		KCN	Salicylaldoxime		KCN	Salicylaldoxime
13	4.8	111	96	6.4	91	88
20	5.8	98	67	17.6	95	56
23	6.0	132	45	27.4	54	35
26	5.5	105	82	16.7	94	79
30	5.8	113	81	7.1	114	83

by copper-containing enzymes, the effect of copper trapping substances on the respiration of the fungus was investigated. Results obtained are shown in Table 1. KCN has no inhibitory effect on endogenous respiration throughout the growth periods. However, the oxidation of catechol by mycelia of 23 day old culture is about 50% inhibited by M/500 KCN. Salicylaldoxime in M/500 concentration inhibits both endogenous respiration and catechol oxidation. It is noteworthy that the mycelia on growth stage of maximum activity in regard to phenol oxidation are most sensitive against inhibitory effect of KCN and salicylaldoxime. Though the rate of inhibition is relatively small, it seems likely that some copper enzyme operates in the respiration of the fungus. It was shown that 8-hydroxyquinoline also suppressed the phenol oxidation.

TABLE 2. Comparison of respirative activities in different parts of fungi.

Part of fungi	Cultivated days	Qo ₂ of the respiration		
		Endogenous	M/120 p-Phenylene-diamine added	M/120 Catechol added
Mycelium	21	6.1	12.9	19.6
	25	6.8	21.0	17.4
Tubercle	21	6.0	15.6	15.8
	25	6.7	13.8	13.0
Fruit body (produced from tubercles)	60	9.6	11.9	9.8

Finally, the comparative studies on the oxidation of *p*-phenylenediamine and catechol by mycelium, tubercle and fruit body were performed. Their results are summarized in Table 2. Fruit body shows the highest rate of endogenous respiration but it oxidizes *p*-phenylenediamine and catechol at more reduced rate than mycelium and tubercle. The oxidative rate of *p*-phenylenediamine of tubercle is higher than that of mycelium at growth stage of 21 days, however, during 25 days the rate of tubercles decreases more rapidly than that of mycelium. Catechol oxidation is lower in tubercle than in mycelium throughout the growth period.

Discussion

The phenol oxidase which have so far been found in hymenomycetes seems to belong to one or the other of two enzyme types, namely tyrosinase and laccase. Studying the phenol oxidases secreted by a number of white rot fungi, FÄHRAEUS¹⁾ found that these enzymes are essentially of the laccase type. *Pleurotus ostreatus* used in this study belongs to white rot fungi. From the experimental results presented above it is highly probable that the enzyme of this fungus is also the laccase type phenol oxidase in agreement with the view of FÄHRAEUS. Tyrosinase seems not to be contained in the mycelia, since tyrosine was not oxidized at all. Furthermore, it is reported (see DAWSON and TAPLEY²⁾), that the rate of catechol oxidation by tyrosinase is constant only for a very short time due to rapid inactivation of the enzyme during the reaction. However, catechol oxidation by the fungus used in this study was not inactivated during one hour. Peroxidase, which is involved in plant materials commonly associated with tyrosinase, could not be confirmed in this fungus by benzidine reaction.

According to the results so far obtained, the oxidative rate of *p*-phenylenediamine does not vary in parallel with that of catechol, and the degree of inhibition by copper trapping agents is different in each stage of growth. These facts support the view that cytochrome oxidase together with laccase may also be operative in the respiration of the fungal mycelia.

The differences of oxidative activities, which were found between the mycelia cultivated on peptone medium and that grown on potato medium may be explained by the differences of phase of maximal enzyme activity, that is, the mycelia on peptone medium produce phenol oxidase at early stage of growth and the activity decreases after about 10 days, however, the mycelia on potato medium forms the enzyme at later phase. It is possible to assume that some nutritional factors in media may affect the enzyme formation, or

the enzyme might be inactivated by certain undetermined factors, which may exist initially in media, or which may be formed during its growth, such as proteinase, suggested by FÄHRAEUS³⁾.

Although LINDBERG *et al.*⁴⁾ have shown that the formation of laccase in fungal mycelia is influenced by the change of pH of media, the decrease of oxidative activities of the fungus used in this experiment is not due to pH change, because pH during initial 11 days of growth remained constant. Rapid fall of oxidative activities after 20–25 days may be explained by the fact of increasing of pH.

While tubercles or fruit bodies are formed by the mycelium in potato medium, they are not produced in peptone medium. In potato medium phenol oxidase activity reaches higher level than that in pepton medium. These facts suggest that an interrelation might exist between tubercle formation and oxidation activity of phenol.

It was shown by LINDBERG and her associates⁴⁾⁻⁶⁾, that the different parts of the fungus form the different phenol oxidase. In the case of *Pleurotus*, cultivated on potato medium, it seems probable from data reported here, that in early stages of growth cytochrome oxidase may be mainly operative, since in this stage *p*-phenylenediamine is predominantly oxidized, but catechol and hydroquinone are not significantly attacked. Subsequently, laccase type phenol oxidase begins to function in the logarithmic phase of growth and its activity increases steadily. Then the oxidation of catechol and hydroquinone predominates that of *p*-phenylenediamine. The highest inhibition rate by copper combining agents is observed in this stage. High activity toward diphenol might in partly be responsible for a slight increase in endogenous respiration at this stage. Therefore, it is possible to assume that phenol oxidase might function as a terminal oxidase. Tubercles are formed in this phase of growth. However, inability to oxidize catechol by fruit body thus formed suggests the absence or insignificant activity of phenol oxidase in fruit body.

The necessity of copper for growth and sporulation of mould and mushrooms occurs in literature. From the experiments reported here, it can be concluded that a function of the copper containing phenol oxidase may be interrelated with the processes of growth and sporulation of the fungi. To determine the nature of phenol oxidase, the inhibition of oxidation by carbon monoxide must be investigated. These investigations together with the oxidability of various substrates by this fungus are now in progress and will be reported in later communication.

Summary

1. The respiratory system of *Pleurotus ostreatus* grown on the peptone-glucose and potato-glucose media was studied.
2. The oxidation rates of *p*-phenylenediamine and diphenols, such as catechol and hydroquinone, were different according to various stages of growth. Tyrosinase could not be detected.
3. On both media, the activity of oxidation decreased after about 10 days. Subsequently, oxidative activities of diphenols by mycelia grown on potato medium increase rapidly in contrast with the oxidation of *p*-phenylenediamine. At that time, it seems probable that some conversions might be performed in the respiratory systems of fungal mycelia.
4. Though the growth of the mycelium on pepton medium is better than that on potato medium, the tubercles which are primordial fruit body were not produced.
5. On potato medium, the tubercles were formed from mycelium after about 13 days, accompanying with the increase of the activity of laccase, but in the fruit body grown from the tubercle only a low activity was observed.

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