

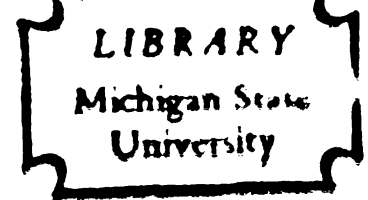


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ENZYMATIC ACTIVITIES DURING THE
BASIDIOCARP FORMATION OF
PLEUROTUS OSTREATUS (Fr) KUMMER

Thesis for the Degree of M. S.
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ABSTRACT

ENZYMATIC ACTIVITIES DURING THE BASIDIOCARP FORMATION OF PLEUROTUS OSTREATUS (Fr) KUMMER

by Chester James Michalski

The determination of certain enzymatic changes were made during the development of the basidiocarp of Pleurotus ostreatus (Fr) Kummer (oyster mushroom). Comparisons were also made between the enzymatic activities of the basidiocarps in culture and those growing naturally. Specific activities of α -D-galactosidase, β -D-glucosidase and acid phosphatase were determined during the mycelial, young basidiocarp stages of this fungus as it developed in culture.

Fructifications of Pleurotus ostreatus developed on 2.5% malt extract containing 1.5% agar, while mycelial pellets were cultured in 2.5% malt extract liquid medium on a rotary shaker. Assays were performed on the cell free extracts of the indicated growth stages after a 30 minute incubation with the chromogenic substrates p-nitrophenyl- α -D-galactopyranoside, p-nitrophenyl- β -D-glucopyranoside and p-nitrophenylphosphate.

Experimental results showed that as the basidiocarp matured, there was a threefold increase in α -D-galactosidase

activity with only a slight increase in β -D-glucosidase activity. Acid phosphatase activity, although very low, showed a slight decrease as maturation was attained.

Studies on basidiocarps of two naturally growing strains of Pleurotus ostreatus revealed enzymatic differences with respect to acid phosphatase. Basidiocarps of this organism found growing on dead elm produced a much greater acid phosphatase activity than the fruiting bodies obtained from fallen aspen, a strain similar to that used in the cultural studies.

ENZYMATIC ACTIVITIES DURING THE BASIDIOCARP
FORMATION OF PLEUROTUS OSTREATUS (Fr)

KUMMER

By

Chester James Michalski

A THESIS

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To my parents

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I. INTRODUCTION

In a study on the α -D-galactosidase of higher fungi, Li and Shetlar (1964) found appreciable activity of this enzyme in several species of Calvatia and Lycoperdon as well as in representatives of the Agaricales namely Agaricus sp., Lepiota sp., Marasmius sp. and Psathyrella sp.

Bulmer and Li (1966), in their recent work on Calvatia cyathiformis, were able to demonstrate high β -D-glucosidase and acid phosphatase activities in addition to that of α -D-galactosidase. In attempting to correlate enzymatic activity with the development of the fruiting body, the authors noted a considerable increase in acid phosphatase activity in the meiotic tissue. The activity tapered off greatly in postmeiotic tissue leading the authors to believe that the phosphatase may be associated with the meiotic process.

There is also the possibility that a similar relationship might exist in the mushroom as well as in the puffball. Pleurotus ostreatus (Fr) Kummer with the ability to fruit in culture, serves as an excellent representative among the mushrooms since its various stages of development may be readily obtained under laboratory conditions.

The objectives of this investigation were to detect any changes in α -D-galactosidase, β -D-glucosidase and acid phosphatase activities that occur during fructification and to compare the enzymatic activities of the cultured basidiocarps with those of naturally growing Pleurotus ostreatus.

II. LITERATURE REVIEW

Although many studies have been made on the α -D-galactosidases, β -D-glucosidases and acid phosphatases occurring in both plants and animals, relatively little has been done with respect to the higher fungi, especially the mushrooms or the Agaricales.

Extensive investigations have been made on the β -D-galactosidases, however, α -D-galactosidases remain relatively unexplored. Wallenfalls and Mahorta (1961) report on α -D-galactosidase activity in certain yeasts, filamentous fungi and higher plants. Recently α -D-galactosidase activity was found in certain bacteria according to the findings of Bailey (1962 and 1963) in his work on Streptococcus bovis and Li and Shetlar (1963) in their report on Diplococcus pneumoniae.

In a later report Li and Shetlar (1964) found the Basidiomycetes to be an excellent source of α -D-galactosidase. Mushrooms such as Agaricus sp., Lepiota sp., Marasmius sp. and Psathyrella sp. as well as the puffballs, Calvatia sp. and Lycoperdon sp. proved to be high in activity. These authors were also able to isolate and purify an α -D-galactosidase from Calvatia cyathiformis by means of column chromatography.

The occurrence of β -D-glucosidase is widespread ranging from barley (Anderson, 1964) to the cockroach, Periplaneta americana (Newcomer, 1954). Studies on the β -D-glucosidase activity in the fungi have been limited mainly to a few of the Deuteromycetes and Ascomycetes such as Aspergillus niger (King and Smibert, 1963 and Li and King, 1963), Trichoderma koningii (Li, Flora and King, 1963) and Neurospora crassa (Eberhart, 1961 and Eberhart and Mahadevan, 1965). Jermyn (1953) noted an increase in β -D-glucosidase activity as the conidia of Strachybotrys atra developed.

Among the Basidiomycetes, the puffball proved to be an excellent source of β -D-glucosidase. Powning and Irzykiewicz (1962) found appreciable activity in Lycoperdon perlatum, while Bulmer and Li (1966) investigated the β -D-glucosidase of Calvatia cyathiformis.

Studies on acid phosphatase, although numerous in human and animal tissues, seem to be limited to the bacteria and yeasts among the lower forms of life. Rogers and Reithel (1960) and Porath and Von Hofsten (1962) separated and purified acid phosphatase from Escherichia coli in their studies of the general behavior of the enzymes. A purified phosphomonoesterase was obtained from Neurospora crassa by Kuo and Blumenthal (1961). Yeast acid phosphatase activity on glucose-1-P was observed by Heredia, Yen and Sols (1963). Weinberg and Orton (1965) were able to elute this enzyme

from the cell surface of Saccharomyces mellis using potassium chloride.

In a more recent publication by Gezelius (1966), acid phosphatase activity was noted in the slime mold, Dictyostelium discoideum, while Bulmer and Li (1966) used Calvatia cyathiformis as a source for this enzyme.

In most of the above studies, the investigators were primarily concerned with either the properties, specificities, behavior or purification of the enzymes. Bulmer and Li (1966), however, attempted to correlate the enzymatic activities with process of meiosis by studying the α -D-galactosidase, β -D-glucosidase and acid phosphatase activities during the stages of development of Calvatia cyathiformis. Their experiments revealed that acid phosphatase activity greatly increased in meiotic tissue, while α -D-galactosidase and β -D-glucosidase activities remained fairly constant.

Although numerous studies have been conducted on the nutrition, growth conditions and cultivation of Pleurotus ostreatus and related species, it is not in the scope of this thesis to discuss these results. However, certain pertinent references with respect to the fructification of Pleurotus ostreatus in culture should be noted.

As early as 1929 Etter was able to induce the formation of sporophores of Pleurotus ostreatus by adding powdered pine, spruce or juniper wood to a medium of malt extract, corn starch, sphagnum moss and corn meal. Badcock

(1943) utilized sawdust while Block, Tsao and Han (1959) used balsa wood sawdust fortified with oatmeal to cultivate Pleurotus ostreatus. Bano, Zakia and Srinivasan (1963) grew Pleurotus sp. on undercomposed paddy straw at high humidity to obtain fructifications which were analyzed for their protein content.

For the nutritional studies of certain species of the Agaricales, Volz (1966) isolated several strains of Pleurotus ostreatus which readily produced basidiocarps in culture when grown on malt extract agar.

III. MATERIALS AND METHODS

Collection and Maintenance of Cultures

A culture of Pleurotus ostreatus designated as strain P-64-10 was isolated from the basidiocarps of fresh material by Volz in 1964. Small pieces of mycelium from inside the pileus were removed with a sterile needle, placed on agar slants and allowed to develop. Unifungal cultures of these as well as of future isolates were maintained on 2.5% malt extract agar slants at 24°C.

Three stages of Pleurotus ostreatus development studied, the mycelial, the young basidiocarp and the mature basidiocarp stages, were obtained under the following conditions:

1. Mycelial Stage.--Mycelium from the stock slant culture was transferred to malt extract plates and allowed to grow for 20-24 days. At this time about 2-2.5 sq. in. of the mycelial growth was transferred from the plate into 55 ml of sterile distilled water and blended in a Waring blender for 30 seconds. Five ml volumes of the mascerated mycelium were then added, with a sterile pipette, to 100 ml of 2.5% malt extract medium contained in 250 ml shaker flasks. The flasks were then placed on a rotary shaker

(180 rpm) and the mycelial pellets were allowed to develop for 7-9 days before checking for enzymatic activity (Plate I).

2. Young Basidiocarp Stages.--In order to grow Pleurotus ostreatus on a larger scale, transfers from the malt extract agar slants were made to one quart Mason jars containing 180 ml of 2.5% malt extract and 1.5% agar which were slanted to increase the surface area.

After about 2-3 weeks at a constant temperature of 24°C, the organism reached the stage designated as the young basidiocarp stage (Plate II).

3. Mature Basidiocarp Stages.--In about 4-6 weeks at constant temperature and with slight aeration, small, fully mature basidiocarps developed (Plate III) and produced the typical shaped basidiospores of naturally growing Pleurotus ostreatus (Plates IV and V).

Collection and Storage of Fresh Basidiocarp Material

Naturally growing strains P-67-1 (Plate VI) and P-67-2 of Pleurotus ostreatus were collected from the woods and the basidiocarps were stored at -20°C until they were used for the enzymatic assays.

Media Employed

2.5% malt extract agar:

Malt Extract

25.0 g

Agar	15.0 g
Distilled Water	1000.0 ml

Malt extract medium for shaker cultures:

Malt extract	25.0 g
Distilled Water	1000.0 ml

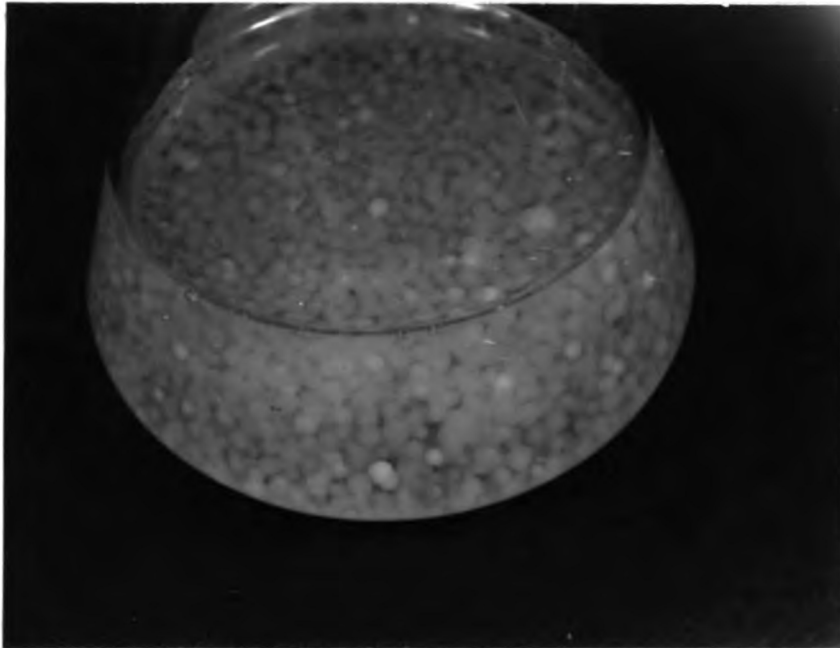


Plate I

Mycelial pellets of Pleurotus ostreatus grown at 24°C for 7 days in 2.5% malt extract on a rotary shaker (180 rpm).



Plate II

Formation of young basidiocarps of Pleurotus ostreatus
grown for 2-3 weeks on 2.5% malt extract agar at 24°C.



Plate III

Small, mature basidiocarps of Pleurotus ostreatus grown for 4-6 weeks at 24°C on 2.5% malt extract agar.



Plate IV

Cross section of a gill of a mature basidiocarp of Pleurotus ostreatus produced in culture showing basidia and basidio-
spores. X500.

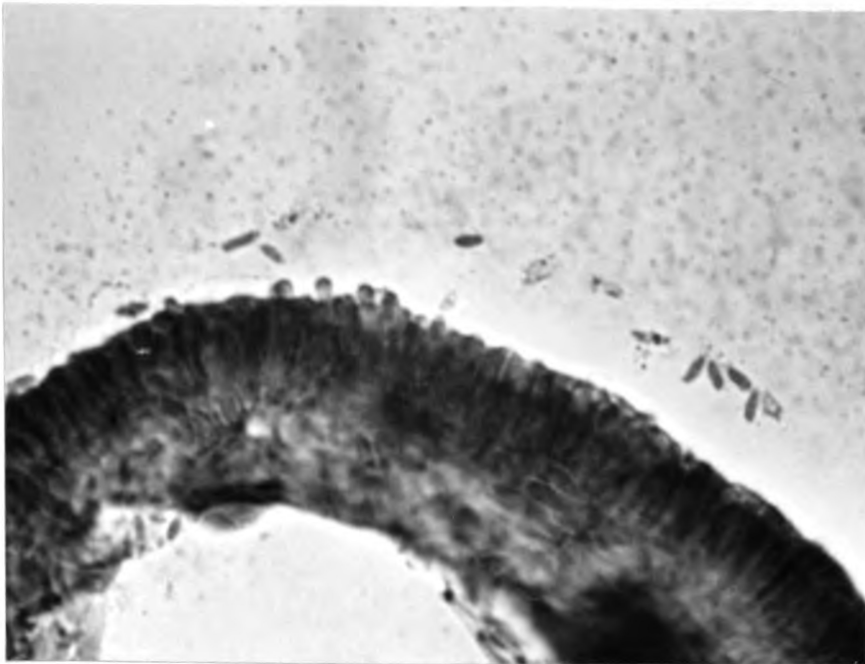


Plate V

Cross section of a gill from a mature basidiocarp of fresh Pleurotus ostreatus found growing on dead aspen showing basidia and basidiospores. X500.



Plate VI

Basidiocarps of naturally growing Pleurotus ostreatus on fallen aspen.

Experimental Procedure

Cell Free Extracts.--The method was the general procedure used by Li and Shetlar (1964) with slight modifications. All operations were performed at 0 to -5°C.

About 5 gm of basidiocarp material was crushed in a Waring blender (with ice jacket) containing 50.0 ml of acetate buffer, pH 4.5, ionic strength 0.05 and about 15.0 g of glass beads (3M Company size 110). After blending for 8-10 minutes, a loopful of material was examined microscopically to insure sufficient cell breakage.

Cellular debris and glass beads were removed by centrifugation at 0°C for 10 minutes at 20,000 g. This cell free extract was then used for all enzyme assays.

Enzyme Assay.--p-nitrophenyl- α , D-galactopyranoside, p-nitrophenyl- β , D-glucopyranoside and p-nitrophenylphosphate from Sigma Chemical Company were used as substrates. The reaction mixture contained 1.5 μ moles of chromogenic substrate (1.5 ml volume) and 1.5 ml of the buffered extract. This mixture was incubated at the desired temperature for 30 minutes after which 1.5 ml of 1N NaOH was added. The liberated nitrophenolate ion was determined by means of a Bausch and Lomb Spectronic 20 colorimeter at 450 m μ . The standard curve for the sodium p-nitrophenol standard appears in Figure 1.

General Properties of the Enzymes

Effect of pH.--The pH optimum for each enzyme was determined by adding 1.5 ml of extract to 1.5 μ moles (1.5 ml volume) of the chromogenic substrate dissolved in a buffer of 0.20 ionic strength at various pH levels. After a 30 minute incubation time at 40°C, the assay was performed. Buffers used were acetate (pH 2.0-3.5), glycine-HCl (pH 4.0-5.5) and imidazole (pH 6.0-7.0), (Colowick, 1955).

Effect of Temperature.--The normal reaction mixture (1.5 μ moles of substrate and 1.5 ml of buffered extract) was used with the reaction tubes being placed in a heated water bath at temperatures ranging from 20-70°C at 10°C intervals. Once again the assay was performed after 30 minutes incubation.

Effect of Enzyme Concentration.--In determining the dependency of enzyme activity on the amount of enzyme present, the concentration of enzyme was expressed in terms of the amount of protein present. The method of Lowry et al. (1951) was used in determining the amount of protein present per ml of extract and appropriate dilutions were made in order to obtain an increasing series of protein concentrations. The protein standard was a bovine plasma crystallized albumin with Figure 2 representing the standard curve. The reaction mixture consisted of 1.5 μ moles of substrate (1.5 ml volume) and 1.5 ml of extract at increasing protein

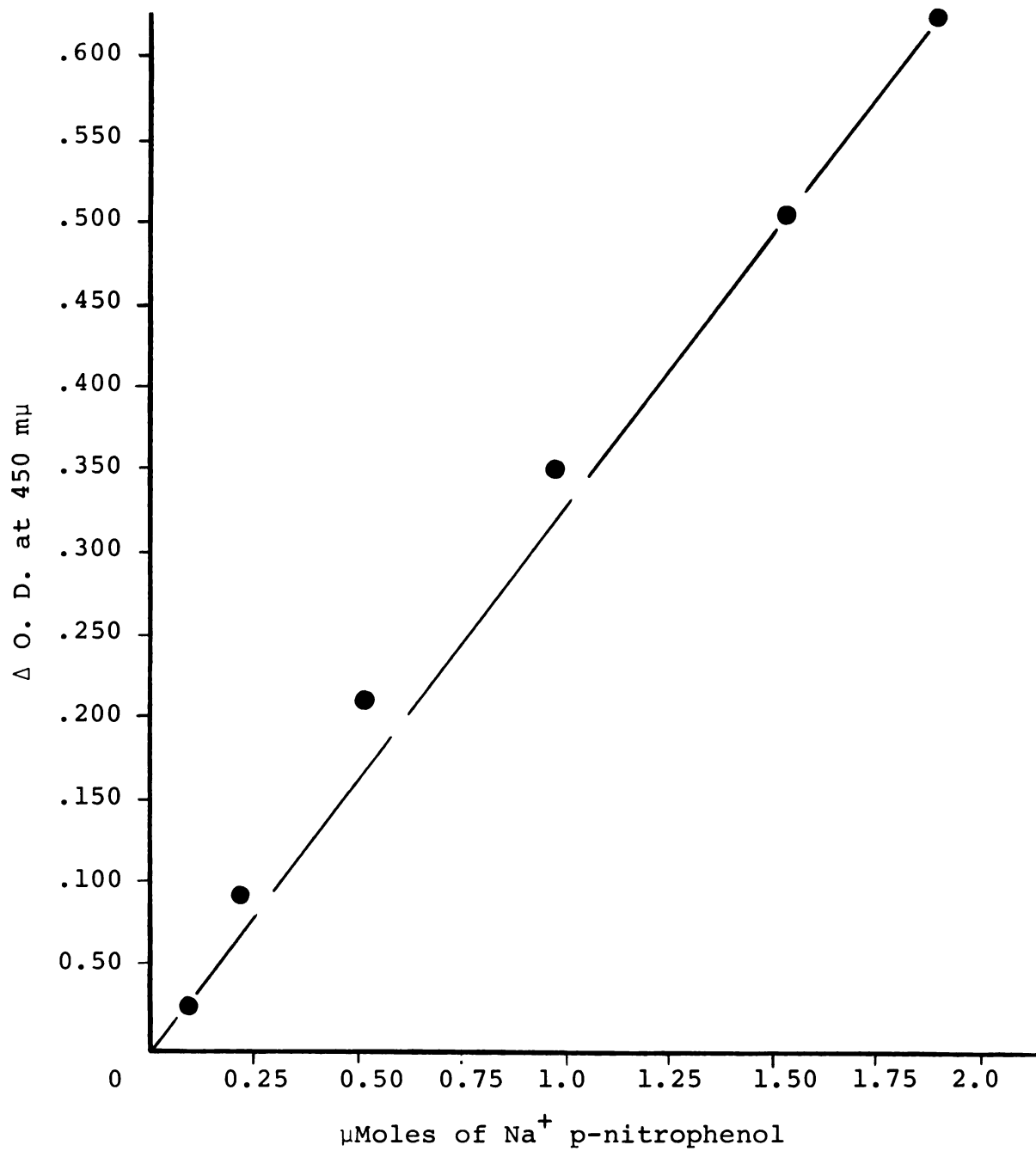


Figure 1.--Standard curve for sodium p-nitrophenol at 450 mμ.

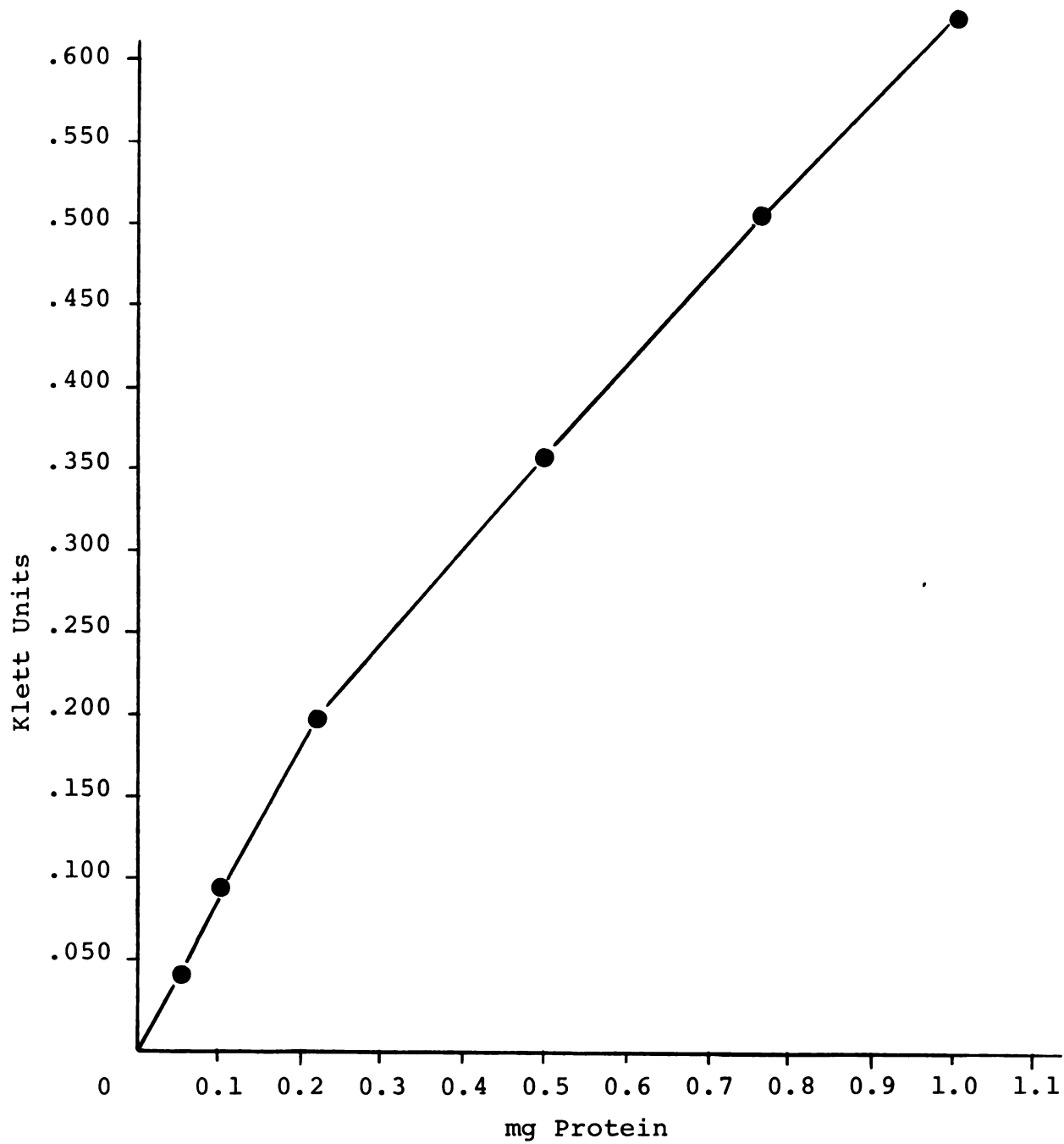


Figure 2.--Standard curve for protein determination using bovine plasma crystallized albumin.

concentrations. One and five-tenths ml of 1.0 N NaOH was added after 30 minutes at 40°C and the colorimetric reaction was read spectrophotometrically.

Specific Activities During the Stages of Basidiocarp Development

The specific activity in this case is defined as the number of units per milligram of protein in solution. One unit of enzyme is defined as the amount of enzyme which will liberate 0.1 μ mole of nitrophenol per 30 minutes under the conditions stipulated.

Cell free extracts of the mycelial, young basidiocarp and mature basidiocarp stages along with extracts of two strains of fresh basidiocarp material were assayed and the specific activity for each enzyme at each stage was determined. The extracts were buffered at pH 4.5, ionic strength 0.05 and the reaction mixture was allowed to incubate at 40°C for 30 minutes. Protein determinations of each extract were made using the Lowry method with bovine plasma crystallized albumin as the protein standard.

VI. RESULTS

General Properties of the Enzymes

Effect of pH.--The effect of the pH on the three enzymes tested is demonstrated in Figures 3, 4 and 5. Optimums for α -D-galactosidase, β -D-glucosidase and acid phosphatase are in the pH ranges of 4.0-5.0, 3.5-4.5 and 4.5-5.5 respectively.

Effect of Temperature.--Figures 6, 7 and 8 give an indication on the behavior of these enzymes at various temperatures. α -D-galactosidase and β -D-glucosidase show an optimum at 45-50°C while acid phosphatase reaches its peak near 40°C.

Enzyme Concentration.--The activity of each enzyme increases linearly with the enzyme concentration as shown in Figures 9, 10 and 11.

Enzymatic Activities During the Developmental Stages in Culture

Table I summarizes the results obtained from the assays of cell free extracts of the mycelial pellets, young basidiocarps and mature basidiocarps grown on 2.5% malt extract agar. It is noted that although both α -D-galactosidase and β -D-glucosidase activities increased as the

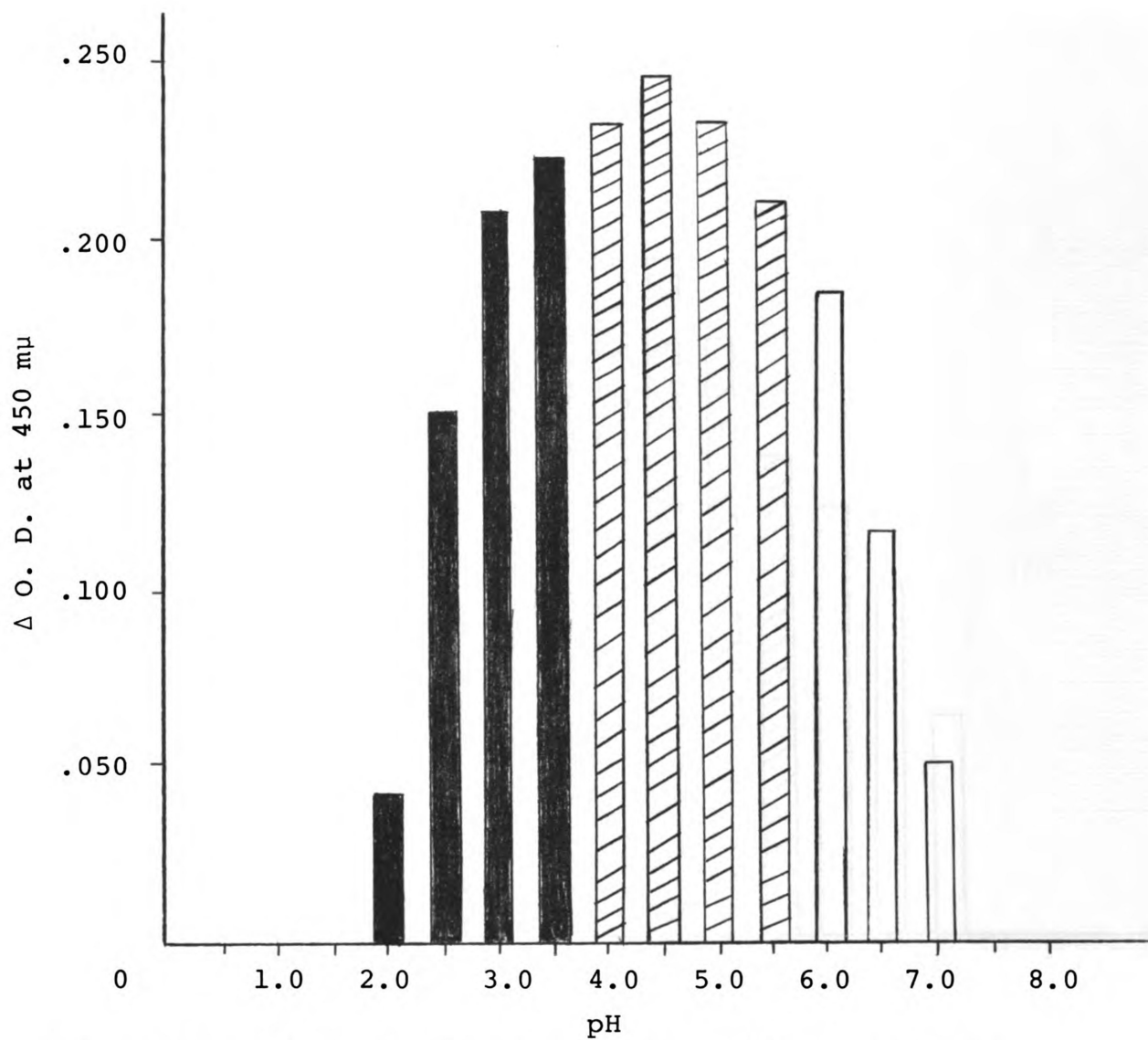
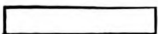


Figure 3.--Effect of pH on α -D-galactosidase activity.

-  - Glycine-HCl Buffer
-  - Acetate Buffer
-  - Imidazole Buffer

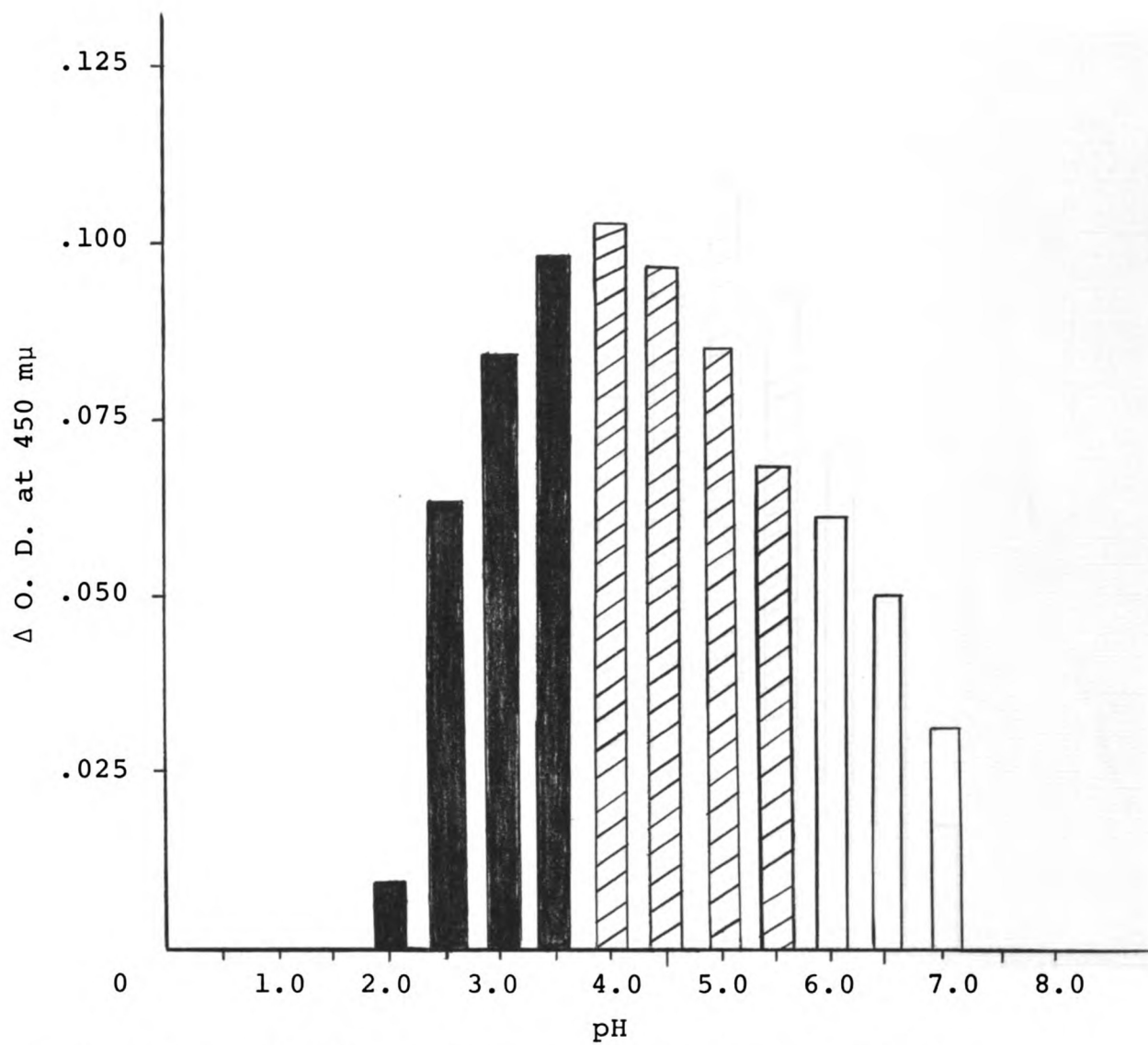


Figure 4.--Effect of pH on β -D-glucosidase activity.

- Glycine-HCl Buffer
- Acetate Buffer
- Imidazole Buffer

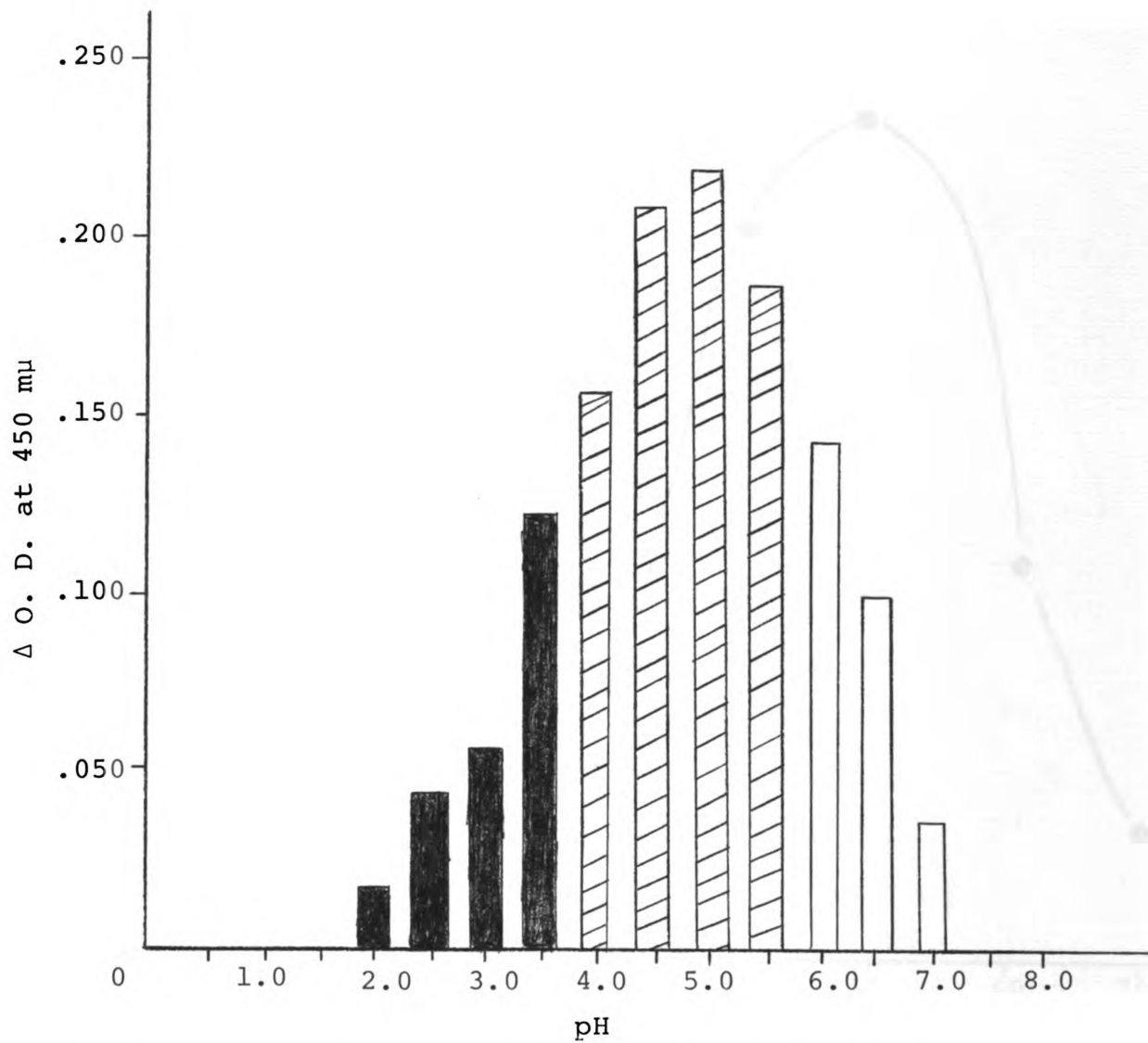
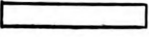


Figure 5.--Effect of pH on acid phosphatase activity.

-  - Glycine-HCl Buffer
-  - Acetate Buffer
-  - Imidazole Buffer

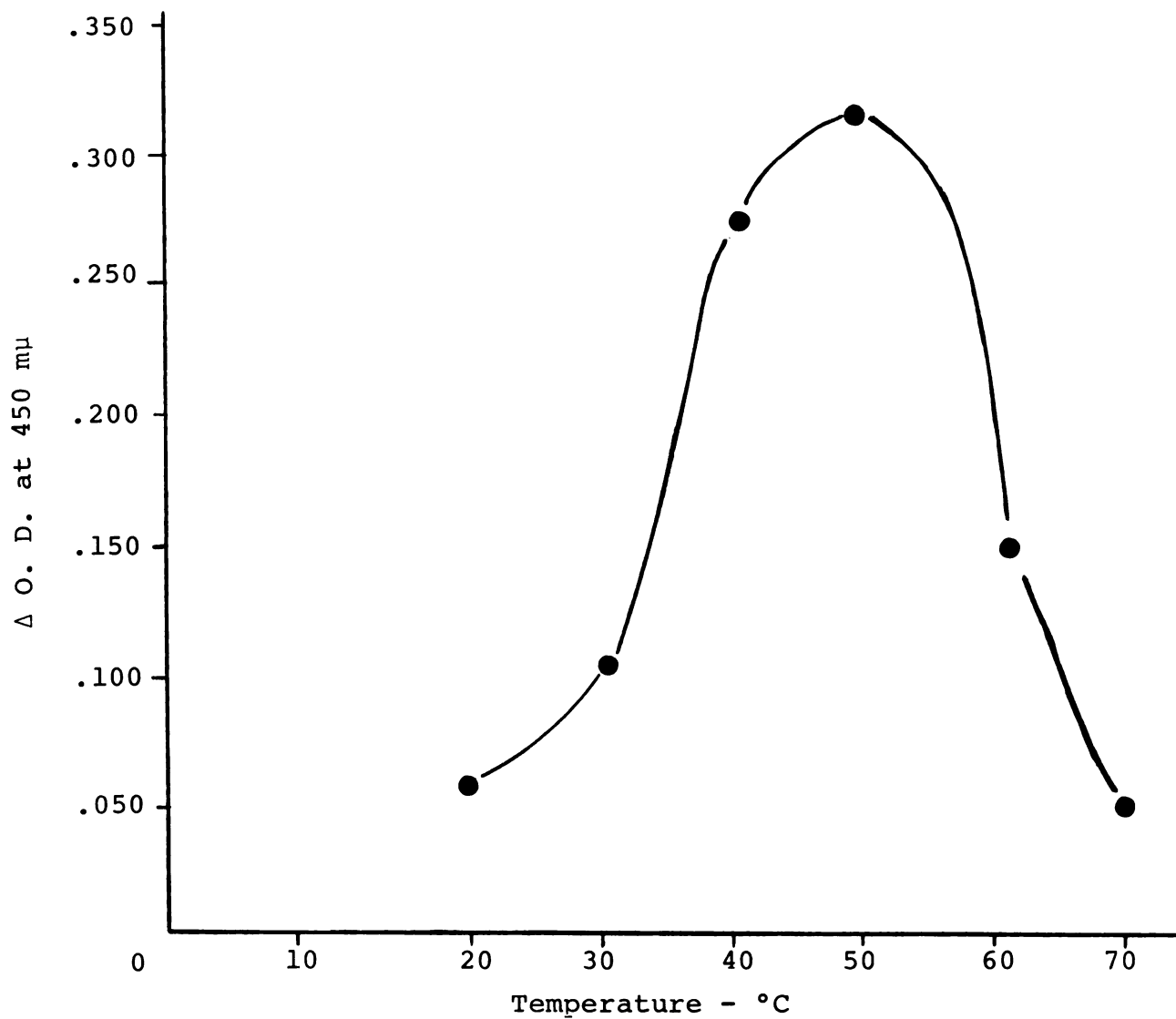


Figure 6.--Effect of temperature on α -D-galactosidase activity.

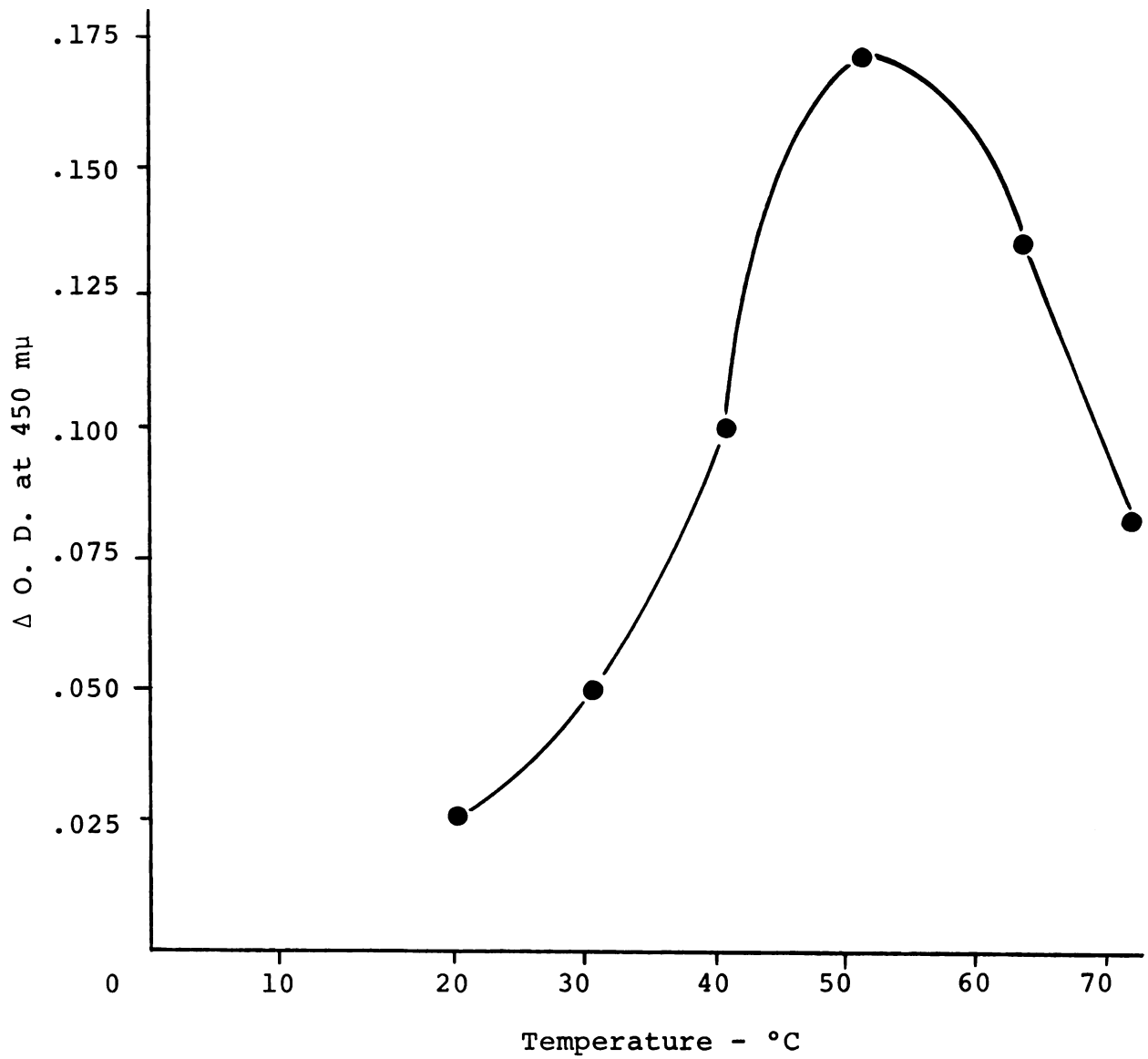


Figure 7.--Effect of temperature on β -D-glucosidase activity.

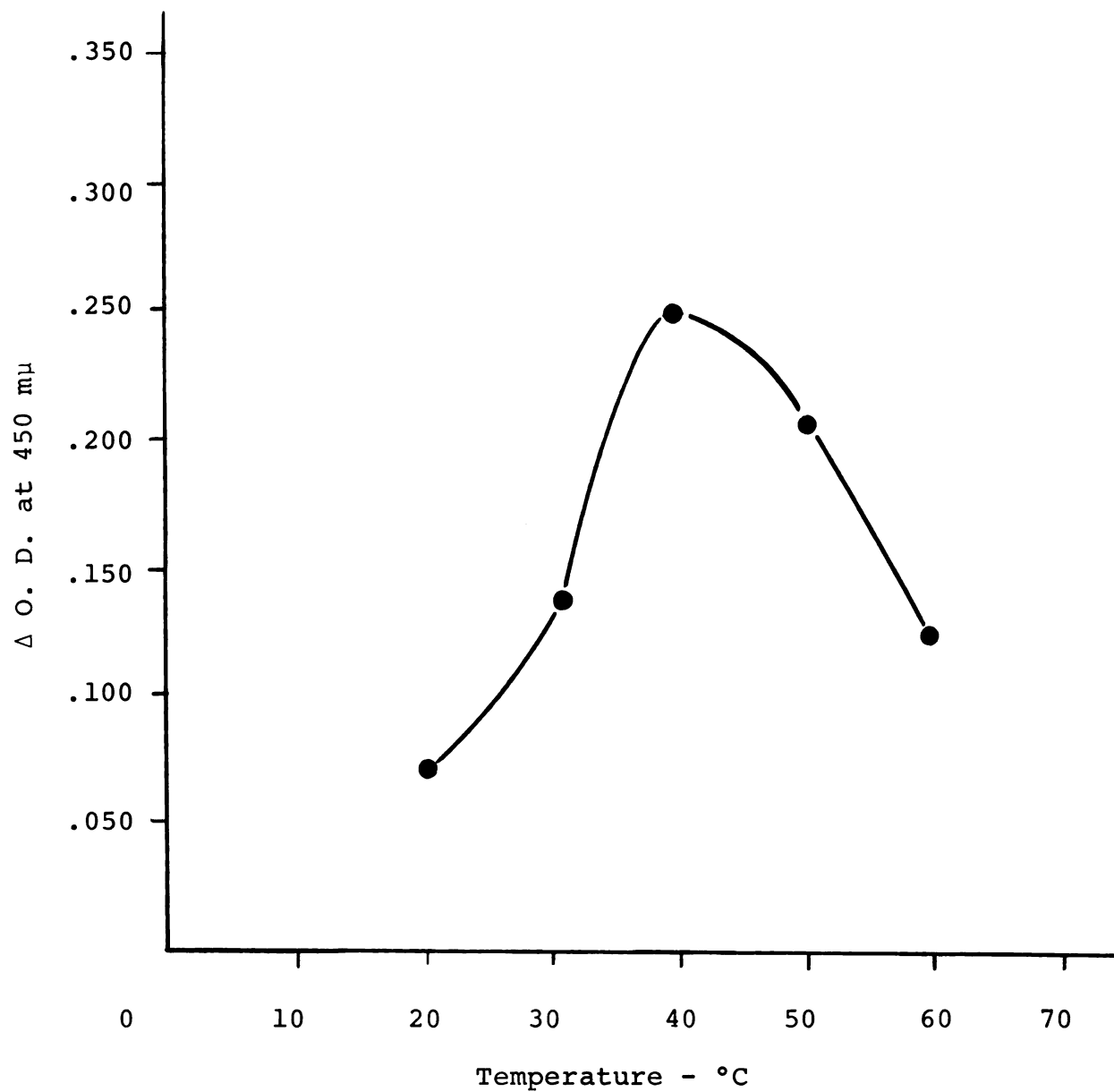


Figure 8.--Effect of temperature on acid phosphatase activity.

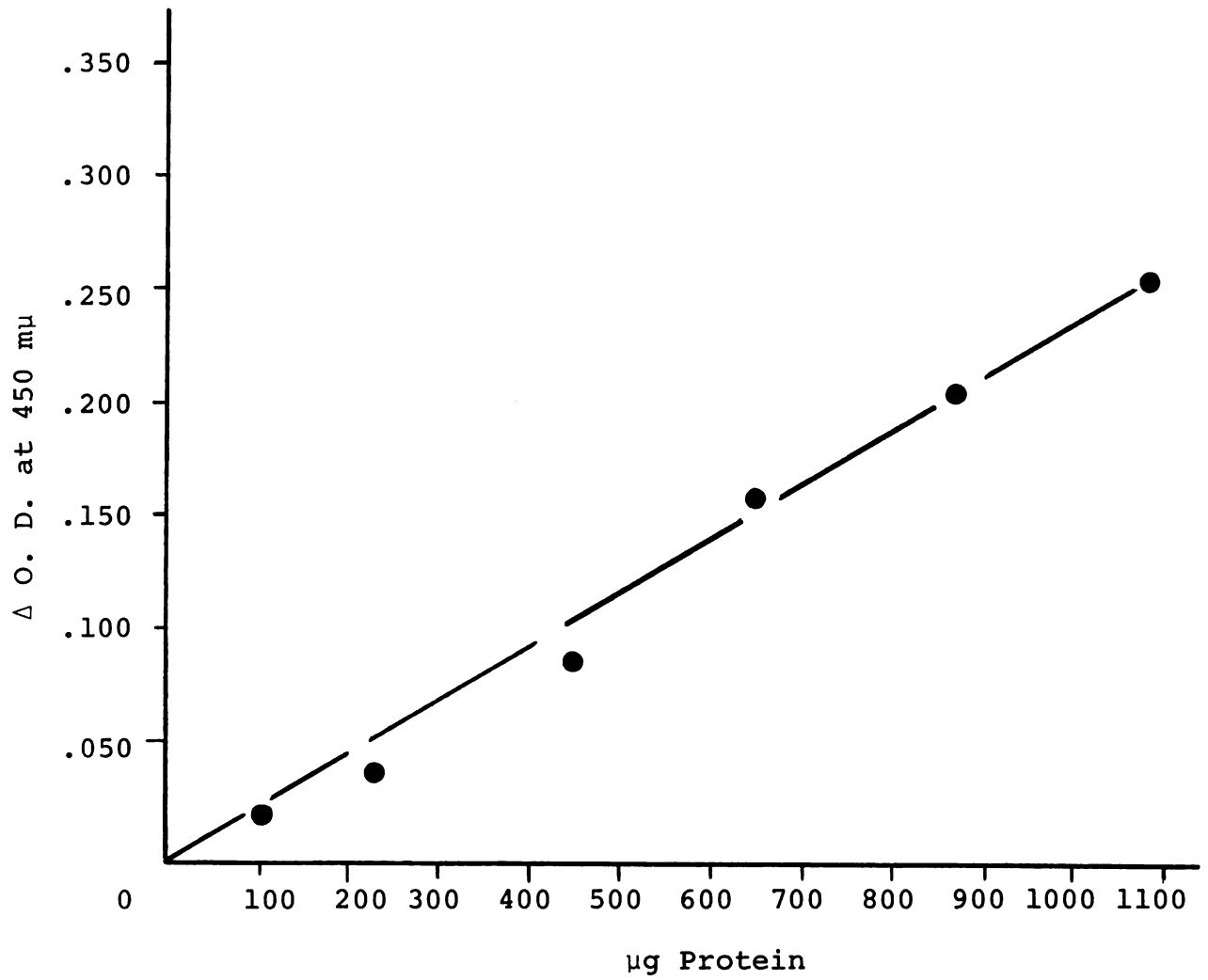


Figure 9.--Effect of enzyme concentration on α-D-galactosidase activity.

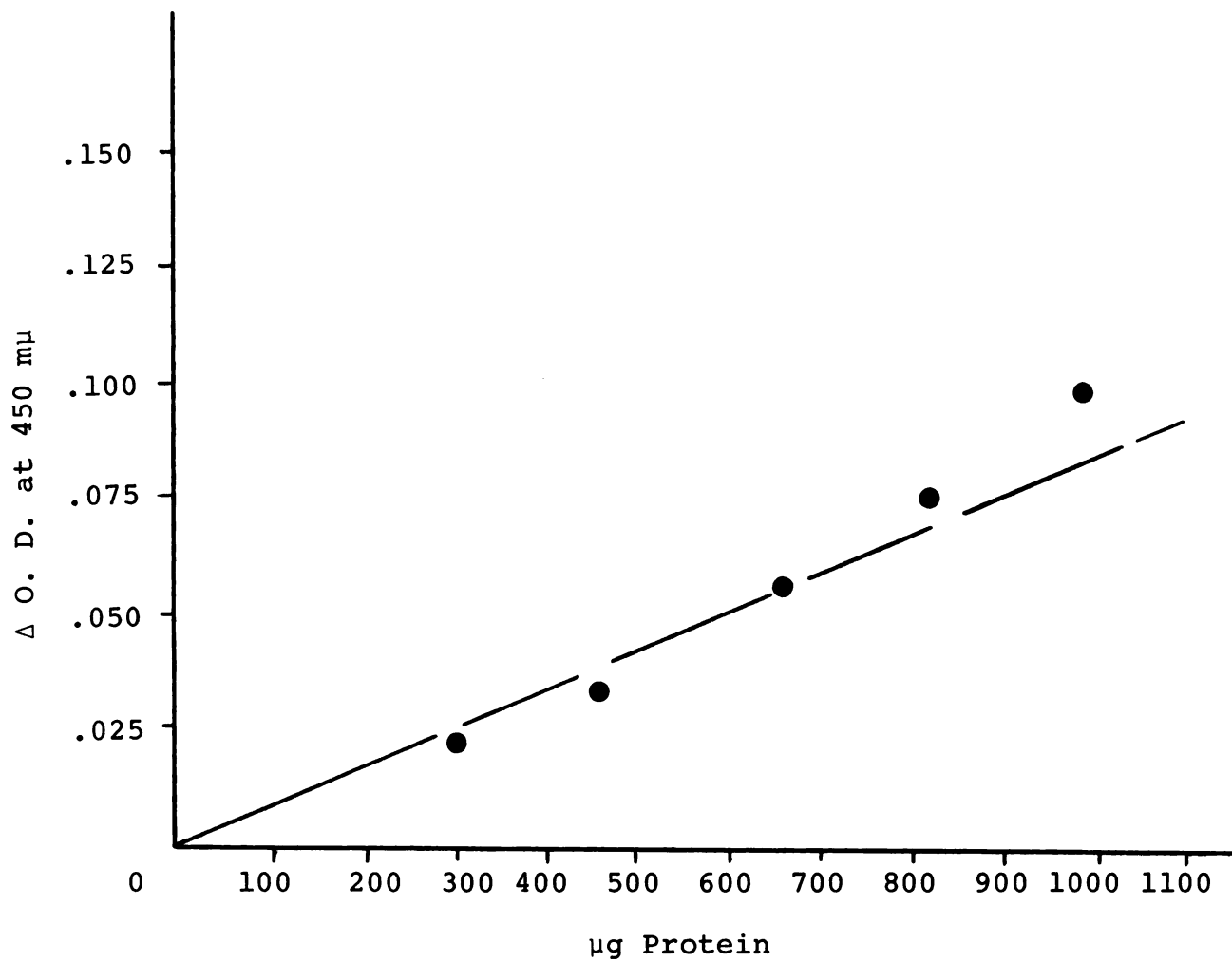


Figure 10.--Effect of enzyme concentration on β -D-glucosidase activity

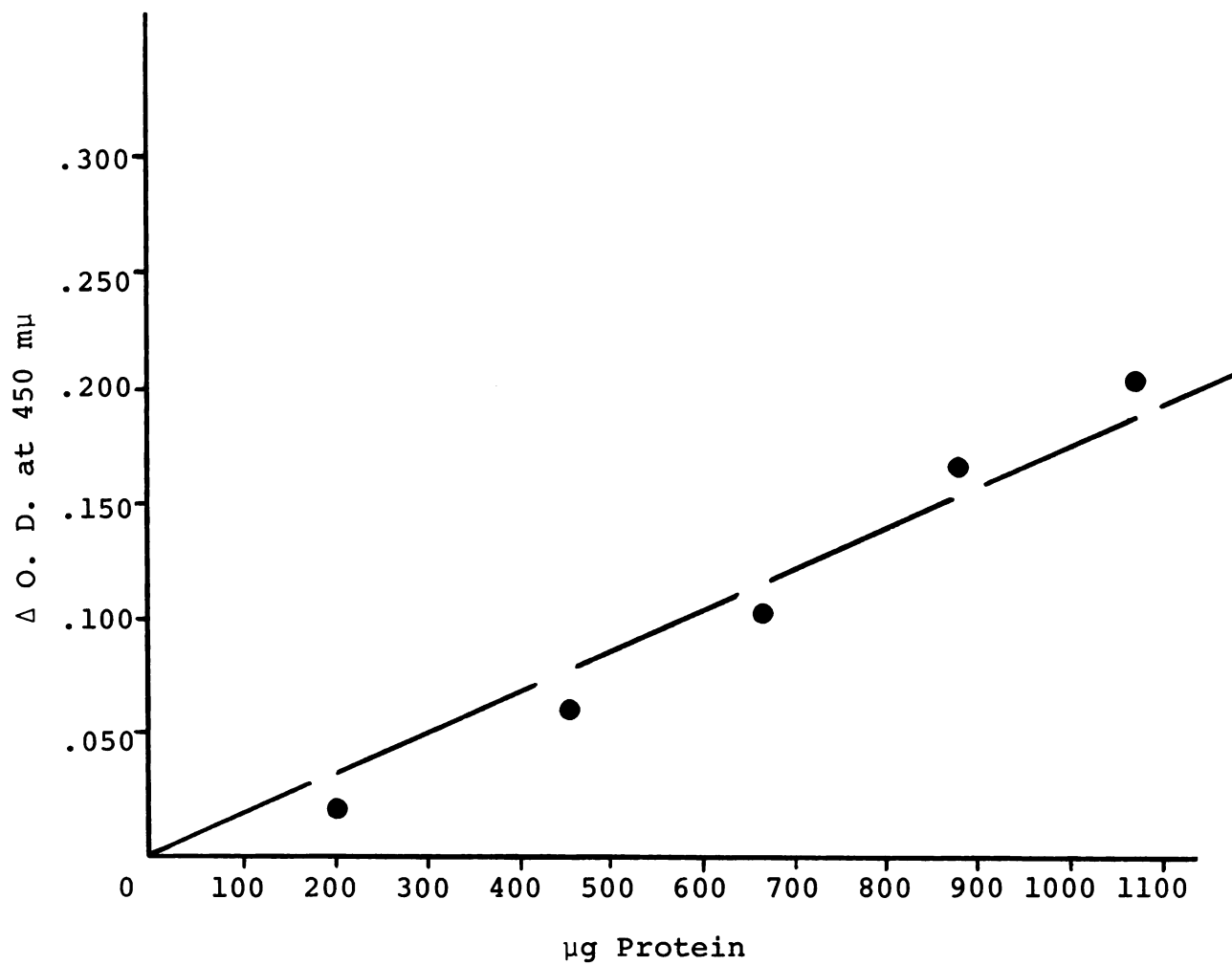


Figure 11.--Effect of enzyme concentration on acid phosphatase activity.

basidiocarp matured, the only significant change appeared to be in the specific activity of α -D-galactosidase. The mycelial and young basidiocarp stages showed specific activities of 13.6 and 15.6 respectively, while a threefold increase of 44.3 was obtained in the mature basidiocarp. Acid phosphatase, on the other hand, slowly decreased in activity as the fruiting developed.

TABLE I.--Enzymatic activities of extracts from different stages of basidiocarp development of Pleurotus ostreatus strain P-64-10 in culture with values expressed as units of specific activity.*

Stages of Development			
Enzyme	Mycelial	Young Basidiocarp	Mature Basidiocarp
α -D-galactosidase	13.60	15.60	44.30
β -D-glucosidase	9.72	12.06	19.04
Acid phosphatase	4.15	3.66	2.12

*Above data is based on an average of five assays run in duplicate.

Enzymatic Activity of *Pleurotus ostreatus* Basidiocarps Taken from Decaying Trees.

A comparison was also made between the enzymatic behavior of mature basidiocarps developed in culture and fresh basidiocarps found growing on decaying trees under natural conditions. Two strains designated as P-67-1 and P-67-2 were collected and the specific activities determined.

Strain P-67-1 found growing on fallen aspen trunks (Appendix) was cream to white in color and occurred in fleshy clusters about 3-6 in. in diameter (Plate 6). The other strain, P-67-2, obtained from a deal elm tree (Appendix) had a leathery texture and a brownish colored pileus 3-6 in. in diameter. Both strains produced white gills which bore the typical oblong-shaped basidiospores, 7-10 x 3-4 μ , of Pleurotus ostreatus (Plate V).

The results in Table II indicate that not only do these two strains differ in texture and color but also in enzymatic activity. Although strains P-67-1 and P-67-2 were almost identical with respect to α -D-galactosidase and β -D-glucosidase activities (9.56 to 10.21 and 5.41 to 4.02 respectively), there was a striking difference in the specific activities of the acid phosphatase. Strain P-67-1 had a low specific activity of 1.15 and P-67-2 had a higher specific activity of 14.89.

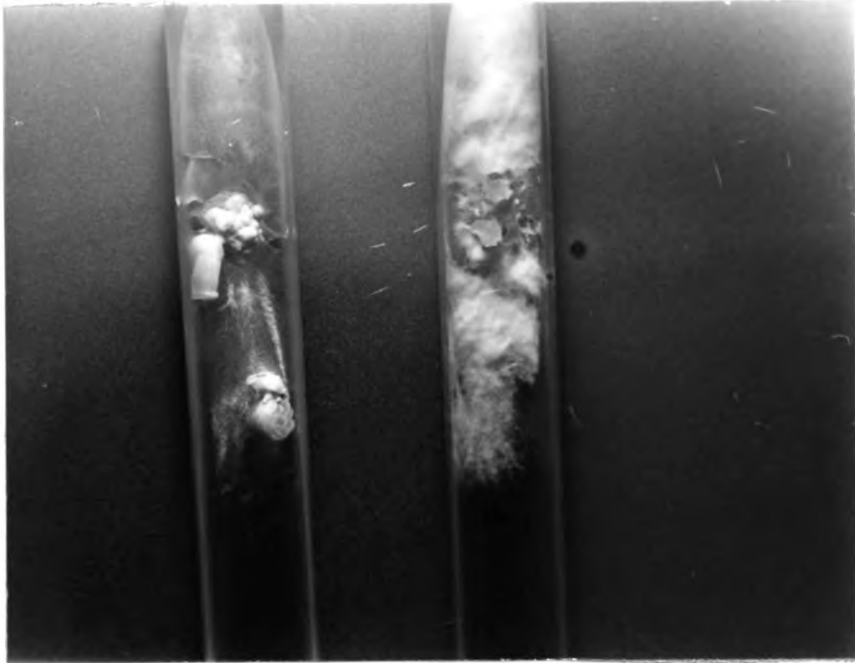
Table II.--Enzymatic activities of extracts of basidiocarps of Pleurotus ostreatus taken from decaying trees with values expressed in units of specific activity.*

Enzyme	P-67-1	P-67-2
α -D-galactosidase	9.56	10.21
β -D-glucosidase	5.41	4.02
Acid phosphatase	1.15	14.89

*Data based on an average of five assays run in duplicate.

It would appear that strain P-67-1 is very close to the strain P-64-10 isolated by Volz (1966) in culture since each was found growing on fallen aspen trunks. Enzymatically each strain behaves nearly the same also, with both having a high α -D-galactosidase activity and low β -D-glucosidase and acid phosphatase activities. It is noted, however, that the activities of each enzyme per milligram of protein is higher in the cultures than in the fresh basidiocarps.

An attempt to isolate cultures of strains P-67-1 and P-67-2 proved to be unsuccessful. Although strain P-67-1 did produce basidiocarps in the test tube culture after the primary isolation (Plate VII), the secondary isolation did not fruit in time to be included in this study. Strain P-67-2 (Plate VII) never produced any fructifications in the test tube culture.



P-67-1

P-67-2

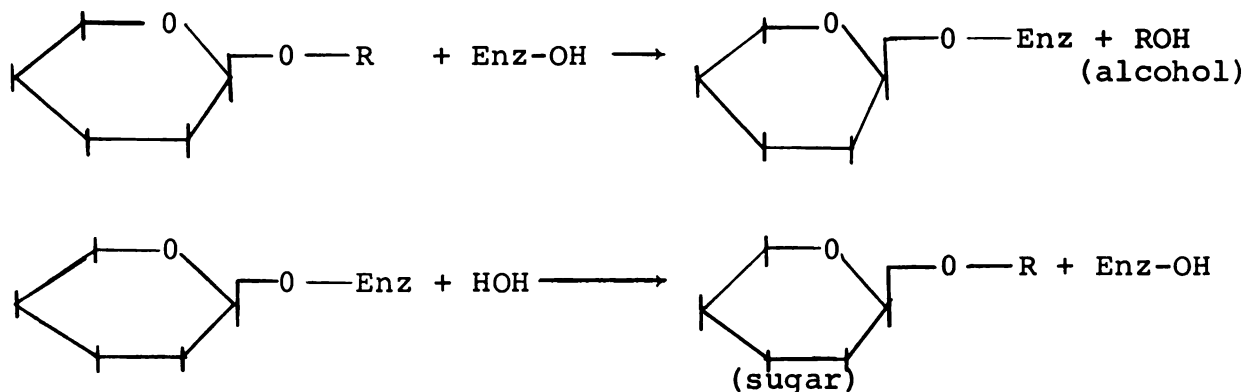
Plate VII

Primary isolates of naturally growing Pleurotus ostreatus, strains P-67-1 and P-67-2, on 2.5% malt extract agar slants showing basidiocarp formation in strain P-67-1.

V. DISCUSSION

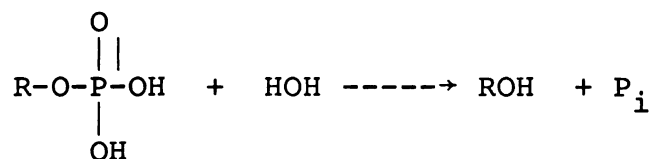
It is not surprising that Pleurotus ostreatus proved to be an excellent source of α -D-galactosidase, β -D-glucosidase and acid phosphatase, however, the specific role of these enzymes in the metabolism of this organism is obscure.

Pigman (1944) and Gottschalk (1950) proposed a mechanism for the action of the glucosidases in which a sugar and an alcohol are released following enzymatic cleavage:



It is known that fungal cell walls consist of chitin and cellulose. Cook (1962) has recently detected the presence of galactose in the cell wall of filamentous fungi. Therefore, α -D-galactosidase and β -D-glucosidase could provide galactose and glucose units as well as various alcohols for the metabolic scheme.

Phosphatase catalyzes the following reaction:



and thus provides for inorganic phosphate (White, Handler and Smith, 1964).

This study was conducted with the objective of detecting some relationship between enzymatic activity and basidiocarp development. However, certain factors involved tend to raise more questions than to provide definite answers.

Due to the difficulty in obtaining naturally growing Pleurotus ostreatus in any stage of development other than the mature basidiocarp stage, another means of obtaining various growth stages was necessary. Since this organism fruits in culture, this provided a means for the study of the stages of development of the basidiocarp. However, the cultural growth conditions are not the same as natural conditions for basidiocarp development and the results may vary accordingly.

Bulmer and Li (1966) were able to demonstrate a very high specific activity for phosphatase in the meiotic tissue of Calvatia cyathiformis in comparison with the pre-meiotic and post meiotic tissues which lead them to believe that phosphatase may be directly related to the meiotic process.

The high phosphatase activity during meiosis may be due to an increased metabolic activity in the puffball as it produces its vast number of basidiospores. Under cultural conditions for Pleurotus ostreatus it was difficult to obtain sufficient quantities of meiotic stages and the material studied was limited to the pre-meiotic, young basidiocarp and post meiotic, mature basidiocarp stages. This difficulty of obtaining meiotic stages may be due to the extended period of time over which the basidiocarps are produced in Pleurotus ostreatus in contrast to those of Calvatia cyathiformis and also to a difference in structure.

Even if the high phosphatase activity was due to the metabolic activity of basidiospore production, it is doubtful that Pleurotus ostreatus, consisting of about 20-30% meiotic tissue, would compare to the puffball which contains about 80-90% meiotic tissue.

Data on the enzymatic behavior in culture show that the enzymes tested performed best in the pH range of 4.0-5.0 and in a temperature range of 40-50°C. These conditions do not exactly correspond to the natural optimums of Pleurotus ostreatus which are about 25-30°C and 5.2-6.8 pH. However, in all three of the above instances, each enzyme behaved similarly to α -D-galactosidase, β -D-glucosidase and acid phosphatase found in Calvatia sp. The actual results obtained were similar to those of Bulmer and Li

(1966) and Li and Shetlar (1964) in their work on the puffball.

Results on the specific activities of the enzymes during the three stages of development tested showed a threefold increase in α -D-Galactosidase activity when comparing the mycelium to the mature basidiocarps (13.6 to 44.3). β -D-glucosidase activity ranged from 9.72 to 12.06 to 19.04 as development proceeded, while acid phosphatase activity tapered off slightly as the basidiocarp reached maturity.

In comparing the activities of the cultured basidiocarps to those of P-67-1, a naturally growing strain of Pleurotus ostreatus of similar origin, fallen aspen logs, it was noted that although the fructifications produced in culture differed in shape and size from the fresh basidiocarps, they both produced the same type of basidiospores (Plates IV and V), and they both reacted similarly with respect to enzymatic activity. The cultured basidiocarps showed a high α -D-galactosidase (44.3) and low β -D-glucosidase (19.04) and acid phosphatase (2.12) activities. Naturally growing fructifications had a similar pattern with α -D-galactosidase activity at 9.56 and β -D-glucosidase and acid phosphatase activities at 5.41 and 1.15 respectively.

Two different strains of naturally growing Pleurotus ostreatus did not behave the same when compared enzymatically. Strain P-67-1 and strain P-67-2 were not only

different in appearance and texture but also enzymatically. Specific activities of strain P-67-1 were 9.56, 5.41 and 1.15 for α -D-galactosidase, β -D-glucosidase and acid phosphatase while P-67-2 had values of 10.21, 4.02 and 14.89 for the same enzymes. This large increase in acid phosphatase activity (1.15 to 14.89) between strains P-67-1 and P-67-2 could be significant in that enzymatic differences might exist in other strains as well. Many more strains would have to be tested, however, to come to any definite conclusions.

Although a basidiocarp of P-67-1 was produced in culture (Plate VII) an attempt to grow it on a larger scale did not produce fructifications until this research had been completed. Strain P-67-2 did not fruit in culture (Plate VII) indicating that this strain may possibly be heterothallic and unable to produce a fruiting body from the germination of a single spore.

The main problem in working with cultures of P. ostreatus under laboratory conditions is that of obtaining synchronous growth on agar media for the collection of the basidiocarps. Development of the basidiocarp varies from culture to culture with respect to time making the growth of this organism very unpredictable. Studies on changes in environmental conditions, light effects and aeration might lead to the development of better fruiting bodies.

The sampling of more material from developing basidiocarps as well as of meiotically active stages in addition to the sampling of more strains or species would provide for a more thorough study of enzymatic activity. The results could perhaps lead to some definite answers as to the correlation between α -D-galactosidase, β -D-glucosidase and acid phosphatase activities and fruitification.

VI. SUMMARY

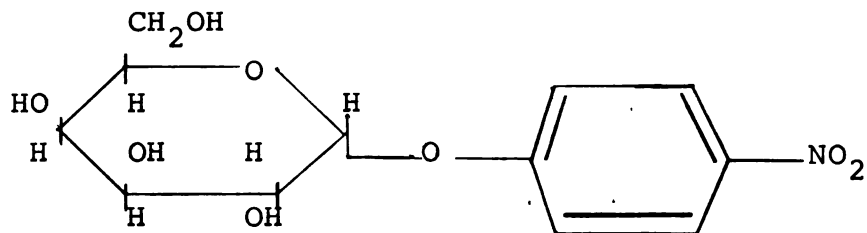
1. Pleurotus ostreatus (Fr) Kummer, commonly known as the oyster mushroom, proved to be an excellent source for the enzymes, α -D-galactosidase, β -D-glucosidase and acid phosphatase.
2. Since this organism fruits in culture, a study of the enzymatic activities was possible during different stages of basidiocarp development.
3. Results on the enzymatic behavior of the enzymes α -D-galactosidase, β -D-glucosidase and acid phosphates show optimum performance at a pH of 4.0-5.0 and a temperature of 40-50°C.
4. α -D-galactosidase activity reached a peak as the basidiocarp developed to maturity. β -D-glucosidase activity showed only a slight increase while acid phosphatase activity decreased slightly after maturity.
5. Strain variations among P. ostreatus seem to differ not only in appearance but also in enzymatic activity.
6. Fresh basidiocarps of P. ostreatus found growing on dead elm showed almost a fifteenfold increase in acid phosphatase activity over the strain obtained from fallen aspen trees.

APPENDIX

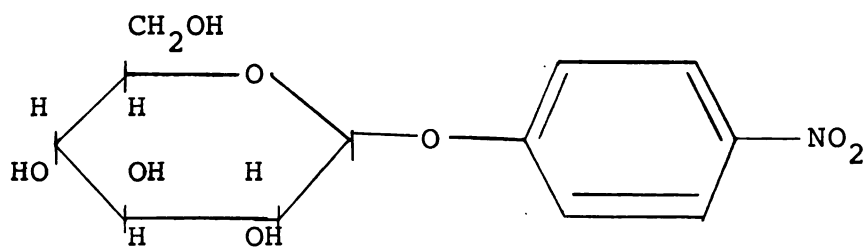
Components of Difco malt extract: (Difco Laboratories Inc., Detroit, Michigan)

A dehydrated barley infusion containing diastase, dextrin, dextrose, protein and barley salts.

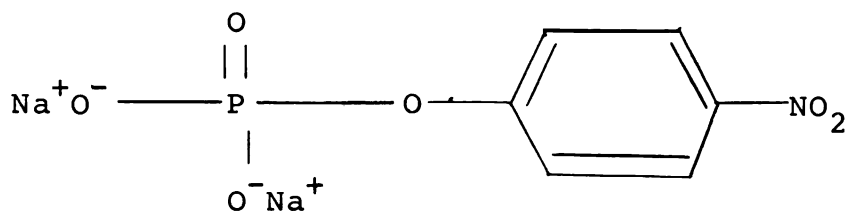
Structural formulas of the Chromogenic Substrates:



p-nitrophenyl - α-D-galactopyranoside



p-nitrophenyl - β-D-glucopyranoside



p-nitrophenyl phosphate (di Na⁺)

Information on the Collection of the Strains of Pleurotus
ostreatus:

<u>Strain</u>	<u>Collection Date</u>	<u>Information</u>
P-64-10	5/16/64	Collected near Rose City, Michigan on fallen aspen, <u>Populus tremuloides</u> Mich.
P-67-1	6/11/67	Collected in Rose Lake Rec- reation Area near Lansing, Michigan from fallen aspen.
P-67-2	6/12/67	Collected in Meridian Town- ship, Michigan on a dead elm, <u>Ulmus americana</u> L.

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