

ENZYME ACTION IN ECHINODONTIUM TINCTORIUM  
ELLIS AND EVERHART.

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In a recent paper<sup>1</sup> it was pointed out that information concerning the physiology of the wood-destroying fungi is comparatively meager. The intention to investigate the enzyme action in some of these forms was also expressed there. The present paper is the second of a series dealing with this phase of metabolism of the wood-destroying fungi.

*Echinodontium tinctorium* is perhaps one of the most destructive heart rot diseases in the West. White, Alpine, grand, noble, and Douglas fir, Engelmann's spruce, and western and mountain hemlock have been reported as having been affected. Perhaps by far the greatest damage occurs on white fir and western hemlock. The economic importance of this fungus has been sufficiently discussed by Weir and Hubert<sup>2</sup> and also by Meinecke<sup>3</sup> so that no further discussion of its economic importance or distribution is necessary.

The culture of the fungus used in this study was obtained from a young sporophore by the tissue method. The sporophore was carefully washed with sterile distilled water, dried by means of sterile tissue towelling, and cut open. Small portions of tissue were taken from the interior of the fruiting body and transferred to potato agar slants. After the fungus had made considerable growth, transfers were made from the agar slants to sliced sterile carrots in large Erlen-

<sup>1</sup> Schmitz, H., and Zeller, S. M., Studies in the physiology of the fungi IX. Enzyme action in *Armillaria mellea* Vahl., *Daedalea confragosa* (Bolt.) Fr., and *Polyporus lucidus* (Leys.) Fr., *Ann. Missouri Bot. Garden*, 1919, vi, 193-200.

<sup>2</sup> Weir, J. R., and Hubert, E. E., Forest disease surveys, *U. S. Dept. Agric., Bull.* 658, 1918, 1-23.

<sup>3</sup> Meinecke, E. P., Forest trees common in California and Nevada, *U. S. Dept. Agric., Forest Service*, 1914, 1-67.

meyer flasks, and the cultures incubated for 3 months at a temperature of 32°C. The fungus makes comparatively slow growth both on hard potato agar and on the carrots. While still in an actively growing condition the fungous mats were removed from the flasks, and, when thoroughly dry, were finely ground.

All the methods followed in the present study are similar to those in the former paper.<sup>1</sup> This was done in order to make the results as comparable as possible.

#### *Esterases.*

The esterase activity of *Echinodontium tinctorium* was determined by the action of the ground fungous meal on methyl acetate, ethyl acetate, ethyl butyrate, triacetin, and olive oil emulsion. After 21 days incubation hydrogen ion concentration determinations of the various enzyme cultures and controls were made and compared. Marked esterase activity occurred when methyl acetate and ethyl acetate were used as substrates, a trace of activity when methyl butyrate was employed, but no apparent activity when triacetin and olive oil emulsion were used as substrates.

#### *Carbohydrases.*

Carbohydrase activity is, no doubt, the most important and most interesting phase in the study of the physiology of the wood-destroying fungi with reference to enzyme action. The action of the fungous meal was determined on 1 per cent solutions of maltose, lactose, sucrose, raffinose, potato starch, inulin, white fir, filter paper cellulose, and hemicellulose. After varying periods of incubation the enzyme cultures were filtered and 5 cc. samples of the filtrate treated with 20 cc. of Fehling's solution. In Table I the average results of two titrations are given as the number of cc. of 0.05 N potassium permanganate required to oxidize the dissolved copper oxide. The results indicate evident action on all the substrates.

TABLE I.  
*The Carbohydrase Action of Echinodontium tinctorium.*

Incubation period.	Substrate.	With fungous meal.	With fungous meal auto-claved.	Without fungous meal.
		0.05 N KMnO <sub>4</sub> .		
		cc.	cc.	cc.
14 days	Maltose.....	31.3	19.1	15.1
20 "	Lactose.....	27.5	24.7	21.2
3 hrs.	Sucrose.....	8.2	5.3	0.2
3 days	Raffinose.....	6.1	4.0	0.3
6 hrs.	Potato starch.....	4.4	3.1	0.2
6 days	Inulin.....	7.0	4.5	0.3
30 "	White fir cellulose.....	8.9	3.6	0.2
25 "	Filter paper ".....	7.1	3.2	0.4
30 "	Hemicellulose.....	8.5	4.8	1.5

#### *Tannase.*

Tannase activity was determined by the action of the fungous meal on a 1 per cent solution of tannic acid. After 20 days incubation the cultures were filtered and 5 cc. of the filtrates were titrated against 0.05 N iodine. In all cases negative results were obtained.

#### *Amidase and Urease.*

Acetamide and urea were used as substrates to determine the presence or absence of enzymes which split amino-acids into ammonia and hydroxyl acids. The enzyme cultures were set up in wash bottles with the intakes and outlets sealed by means of rubber tubing and clamps. After 10 days incubation the bottles were connected up with other wash bottles containing distilled water and a few drops of bromothymol blue; air was then drawn through the series by means of a suction pump. The change in color due to the shifting of the hydrogen ion concentration and the length of time necessary to cause this change were noted.

When acetamide was used as a substrate, all tests were negative. With urea as a substrate, however, the color of the indicator changed from yellowish brown (pH 5.6) to bright blue in 3 seconds. In the

tests in the controls a slight change in color was also noted, but in no case going beyond the light blue stage even after air had been drawn through the wash bottles for 3 minutes.

*Rennet.*

The presence of rennet has been variously reported in the wood-destroying fungi. In *Echinodontium tinctorium* fresh milk was coagulated in 2 hours while the controls remained unchanged.

*Catalase.*

When 50 cc. of a 3 per cent solution of hydrogen peroxide were added to 1 gm. of fungous meal, a rapid evolution of oxygen resulted amounting to 42 cc. of the gas in 1 minute.

*Proteases.*

Tryptic and ereptic fermentations were studied by the use of 1 per cent solutions of albumin, peptone, casein, and fibrin in enzyme cultures having neutral, acid, and alkaline reactions. In no case was a positive result obtained with either the biuret or the tryptophane test. In order to check the negative results in the fibrin, this material was stained with Congo red and the color fixed by immersing in boiling water. This stained fibrin was then used as a substrate. In such cultures a liberation of the stain would indicate the digestion of the fibrin. In the enzyme cultures having an alkaline reaction, a slight liberation of the stain was noted, but this also occurred in the alkaline controls. Biuret and tryptophane tests with these cultures also were negative.

SUMMARY.

In *Echinodontium tinctorium* the presence of the following enzymes was demonstrated: esterase, maltase, lactase, sucrase, raffinase, diastase, inulase, cellulase, hemicellulase, urease, rennet, and catalase.