ENZYMES OF RAMIE WHITE ROOT-ROT FUNGUS *Rosellinia necatrix* (HART.) BERL.

by

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The experimental methods used were the same described by Garren (1) or Lanphere (2). The qualitative tests were made *in vitro* in which the existence of enzyme was detected by the reaction of end-products which yield within a certain periods of incubation after adding various substrates to the enzymic extracts of the mycelial mats.

Preparation of Enzymic Extract

Mycelium of the fungus grown on the potato-juice medium (2% sucrose added) for six months, filtered by Büchner funnel, washed thoroughly with distilled water, and dried in the air on the filter paper.

A certain weight of the material was ground in the mill with sterilized sand and was allowed to extract enzyme for 24 hours after adding 4 volumes of distilled water and a few drops of toluol. The enzymes were precipitated by adding 3 volumes of 95% alcohol to the filtered extracts, and the precipitated enzymes on filter paper were dried at the room temperature and then the enzymic extract was made up to 1 per cent solution with distilled water. The temperature during the incubation periods throughout this experiment was held at 30°C. Toluol was used as antiseptic. Tests were made in the usual methods and the summary is as follows.

Test of Enzymic Activity

For the determination of amylase, invertase, inulase, cellulase and pectinase, the reducing sugars were tested by Fehling's test after the indicated periods of incubation. For lipase determination, methyl acetate was alkalized with sodium hydroxide, and methyl red was used as the indicator. If the enzyme present, the mixed solution would become red as the results of acid formation. Maltase and lactase were tested with Barfoed's reagent.

Tests of urease and asparaginase were made with Nessler's solution in the presence of ammonia. In the test for tannase, reactive solution was precipitated with alubumin and the filtrate was neutralized with sodium hydroxide, and then Barfoed's reagent was used to test the presence of sugar.

In the test for laccase, hydroquinon and phloroglucin were used as the substrates. The brownish red or brown color occurred in the mixed solution will indicate the presence of the enzyme. Tyrosin and carbolic acid were used as the reactive substrates for tyrosinase test. After a certain periods of incubation, the presence of enzyme was indicated by the change of the soultion's color. One per cent glucose was added to the enzymic soultion to test the presence of zymase. The occurrence of CO_3 was noted for the presence of the enzyme. Rennet was detected by the coagulation of fresh milk after several hours of incubation. Oxidase, peroxidase and catalase were detected by adding guaiac gum and H_2O_2.

Experimental Results

Table 1.—Results of enzyme tests of the mycelial mats of ramie white root-rot fungus.

Enzymes	Substrates	Periods of incubation	Reaction
Amylase	Starch	4 hours	+
Invertase	Sucrose	2 days	+
Inulase	Inulin	3 days	+
Maltase	Maltose	10 days	?
Lactase	Lactose	10 days	+
Cellulase	Filter paper	12 days	+
Pectinase	Pectin	10 days	+
Lipase	Methyl acetate	5 days	+
Zymase	Glucose	2 days	+
Rennet	Milk	8 hours	?
Urease	Urea	2 days	+
Asparaginase	Asparagin	2 days	+
Tannase	Tannic acid	5 days	+
Laccase	Hydroquinon Phloroglucin	24 hours	+
Oxidase	Guaiac gum	2 hours	+
Peroxidase	Guaiac gum+H_2O_2	½ hours	+
Catalase	H_2O_2		+
Tyrosinase	Tyrosin Carbolic acid Thymol	10 days	?

As shown in *Table 1*, 15 enzymes in the mycelium of *Rosellinia necatrix* were tested with positive reaction, while the tests for maltase, rennet and tyrosinase showed negative results.

1. Garren, R. H.: Phytopath. 28 (1938), 839～845.
2. Lanphere, W. M.: Phytopath. 24 (1934), 1244～1249.

苧麻白紋羽病菌の酵素

馬鈴薯煎汁培養基上に生育せる本病菌菌絲の酵素について定性試験を行つた処，amylase, invertase, inulase, lactase, cellulase, pectinase, lipase, zymase, urease, asparaginase, tannase, laccase, oxidase, peroxidase, catalase の 15 酵素を檢出し得たが，maltase, rennet 及び tyrosinase の 3 酵素は檢出することが出来なかつた．