STUDIES ON SCLEROTIUM-FORMING FUNGI

I. *Sclerotium cepivorum* Berk and *Sclerotium tuliparum* Klebahn

Part 3. Pectinase Activity and Preparation

By R. P. Asthana, M.Sc., D.I.C., Ph.D. (London), F.A.Sc.
(*Mycologist to Government, C. P. & Berar, Nagpur*)

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There are certain factors which condition invasion of particular hosts by some fungi and not by others. The chief relevant references are given below.

Walker, Lindegren and Bachmann[6] report the presence of toxic substances in the juice extracted from succulent onion scales. They remark that these toxins are of two general types, one which is neither removed nor broken down readily by heat and one which is volatile and passes off from the extracted juice at room temperature within a few hours. There is a gradual decline during storage of onion bulbs in the amount of volatile toxin, a decline which is hastened by increase in temperature. The fungal spores generally become more sensitive with age to the volatile toxin. When comparing onion pathogens and non-pathogens they found no strict negative correlation between pathogenicity to onion and sensitiveness to the toxins, which only indicates that other factors enter into the determination of the parasitic relation. Considering the onion parasites as between themselves there was evident a negative correlation between aggressiveness of parasitic attack and sensitiveness to the dissolved and volatile toxins. The presumption, however, is that as the parasites invade the tissue the host toxins, though attenuated by fungus enzymes, may possibly exert some retarding effect upon the invader. If this be the case, it is suggested that the host toxins may be one of the numerous factors which determine the degree of parasitism attained by a given parasite.

Vasudeva[5] gave an analysis of the factors responsible for the failure of *Monilia fructigena* to attack onion and *Botrytis Allii* to attack apple. He observed that the chief feature shown by spores of *Monilia*, when placed in wounds on onion, is their failure to germinate. This is due to the presence of a thermolabile substance which can be extracted with ether or chloroform. On the other hand, the failure of *B. Allii* to attack apple tissue is not due to any inhibitory or retarding action of apple juice. It could be made

to parasitise by adding to the inoculum a certain concentration of a nitrogenous substance. The effect of a nitrogenous compound in stimulating attack by *B. Allii* was found to run parallel with its effect in stimulating the secretion of the pectinase enzyme. He also found that by artificially ripening the apples, they become susceptible to *B. Allii* attack.

Chona[2] studied the enzymic behaviour of certain apple-attacking fungi (*Botrytis cinerea, Fusarium fructigenum*) with that of parasites on potato (*Pythium* sp., *Phytophthora erythroseptica*). Ordinarily the apple-attacking fungi did not attack potato and *vice versa* but he found that with the supply of an additional nitrogenous food a certain amount of such cross infection could be brought about. The pectinase activity could be retarded by pH concentration and by the action of certain plant extracts and chemicals, the retardation depending on the medium into which the enzyme was secreted.

Menon[3] has shown that the behaviour of the pectinase is modified by the nutrient medium in which the fungus is growing. According to him the nature of the nutrient medium modifies the capacity of a fungus to secrete pectinase. He assumes that certain substances are adsorbed from the nutrient medium and this adsorption modifies the properties of the pectinase.

Thornberry[4] has dealt with the pectinase activity of eight strains of *Fusarium* sp. from tobacco stems, two of *Sclerotium bataticola* (*Macrophomina phaseoli*), *Sclerotinia sclerotiorum, S. trifoliorum, Rhizoctonia* sp. from tobacco, three strains of *Thielaviopsis brasicola, Phytomonas* (*Bacterium*) *mori, P. tabaca* (*Bact. tabacum*), and *P. angulata* (*Bact. angulatum*). The determination of the pectinase activity was according to the method of Neuberg and Ostendorf (*Biochem. Z.*, ccxxix, p. 464, 1930). According to them extracts from pectase-active plant tissues hydrolyse the ester linkage of the half calcium salt of monomethyl tartaric acid. The ester being water-soluble and hydrolysable by pectase into soluble methyl alcohol and insoluble half calcium salt of tartaric acid, this method of determining pectase activity offers promise of utility for quantitative measurements based upon the precipitate formed. Thornberry by working with the above method observed that freshly isolated cultures of *Fusarium* sp. gave moderate hydrolysis, whereas little or no activity was shown by those that had undergone repeated subculturing since removal from their host. *S. sclerotiorum* and *S. trifoliorum* were only slightly active but considerable hydrolysis took place in the tubes inoculated with *M. phaseoli.* The tobacco *Rhizoctonia* gave negative results, while those obtained with *T. basicola* were variable.

The pectinase enzyme of the two fungi, *Sclerotium cepivorum* and *S. tuliparum*, was prepared from cultures on plugs as well as on flasks,

Only the enzyme excreted by the fungi was taken into account. The enzyme by the plug method was prepared by placing blocks of potato, turnip, etc., in boiling tubes having absorbent cotton wool, soaked with water, at the bottom. Such tubes were autoclaved, inoculated and incubated at 20° C. for different periods. The decayed portion as well as the fungal growth was removed and the juice squeezed, centrifuged and tested for pectinase. To obtain pectinase from flask cultures, 40 c.c. of medium were inoculated in 500 c.c. conical flasks and incubated at 20° C. for 10 to 20 days. The liquid was then filtered off and tested for pectinase. The test of activity was the usual one of the disintegration of potato discs (50 μ thick) as described by Brown.[1] For each experiment, as far as possible, potato discs were taken from the same potato so as to avoid tissue variation.

Preparations of the external enzyme were made in a standard manner from 15 days old plug cultures of potato, carrot, turnip, tulip and onion. These, when tested on standard potato discs, gave the activities shown diagrammatically in Fig. 1.

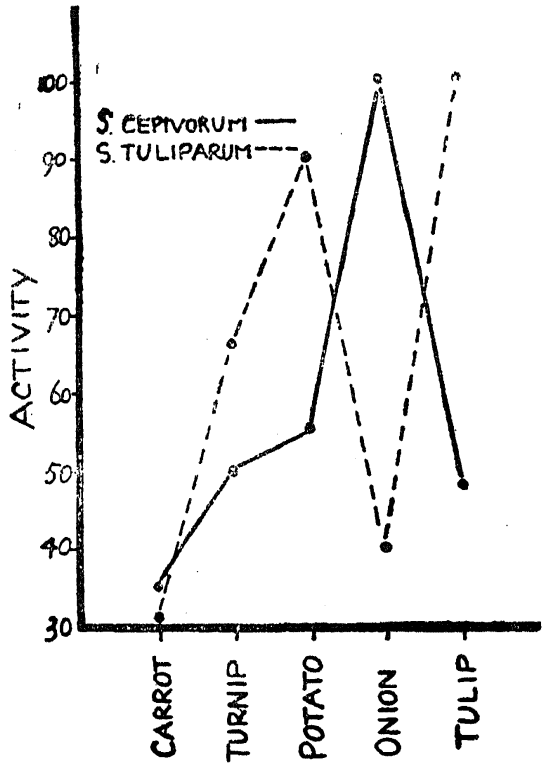

FIG. 1. Pectinase activity of *S. cepivorum* and *S. tuliparum* on different plug cultures.

From the figure it is clear that on media other than onion the activity of *S. cepivorum* is less than that of *S. tuliparum*. This applies also to tulip as a medium. On the other hand, the enzyme prepared from *S. cepivorum* on onion plugs is much stronger than that of *S. tuliparum* on this medium.

The corresponding diagram for the enzymatic extracts prepared from 15 days' old flask cultures is given in Fig. 2.

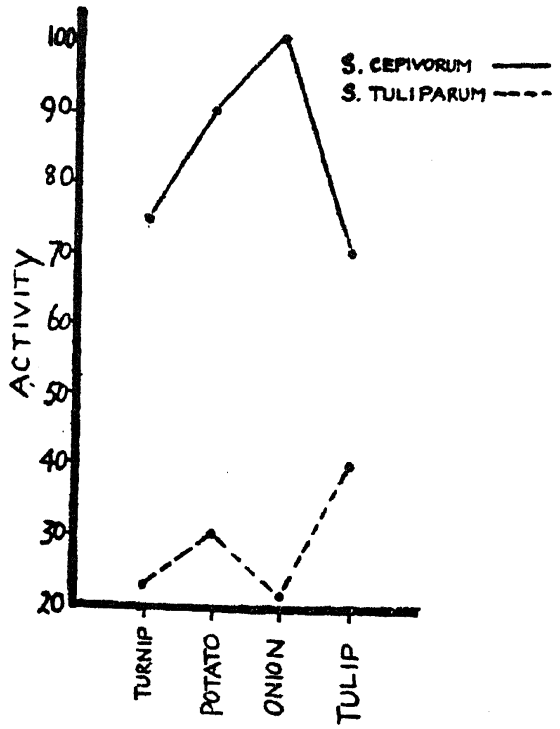

FIG. 2. Pectinase activity of *S. cepivorum*, *S. tuliparum* on flask cultures.

It will be noticed that here in all cases the pectinase activity of *S. cepivorum* is much stronger than that of *S. tuliparum*, but the tendency of the curves is the same as in the previous figure.

The enzyme from both the fungi was also obtained from 15 days' old flask cultures of synthetic and 20 per cent. tulip and onion extracts. The pectinase activity on different cultures from the two fungi are given in Table I.

TABLE I

Fungus	Pectinase activity			
	Richard's solution	Brown's starch	20% onion	20% tulip
S. cepivorum ..	100	71	83	80
S. tuliparum ..	100	83	71	86

The activities recorded in Figs. 1 and 2 have reference to potato discs as test material. A comparative study of potato, tulip and onion discs gave the data shown in Table II in which the times required for disintegration are recorded.

A comparison of columns 2 and 5 of this table shows that *S. cepivorum* produces a more active enzyme than *S. tuliparum* when onion plugs are used as media and that the converse applies when tulip plugs are used.

TABLE II

Fungus	Pectinase from onion plug			Pectinase from tulip plugs		
	Potato discs	Tulip discs	Onion discs	Potato discs	Tulip discs	Onion discs
S. cepivorum ..	40 min.	3 hrs. and 30 min.	1 hr. and 50 min.	60 min.	2 hrs. and 55 min.	2 hrs. and 10 min.
S. tuliparum ..	..	3 hrs.	3 hrs. and 40 min.	40 min.	2 hrs. and 25 min,	3 hrs. and 20 min.

Comparison of columns 2 and 3 shows that though the enzyme of *S. tuliparum* is only about half as active as that of *S. cepivorum* when tested on potato discs, it is fully more active when tested on tulip material. In other words tulip material is specifically more sensitive to the enzyme of *S. tuliparum.*

Similarly a comparison of columns 5 and 7 shows that the enzyme of *S. cepivorum* is specifically more active on onion material than is the enzyme of *S. tuliparum.*

Effect of various factors on Pectinase activity.—The data as regards the *effect of H-ion concentration* on the pectinase activity are given in Fig. 3.

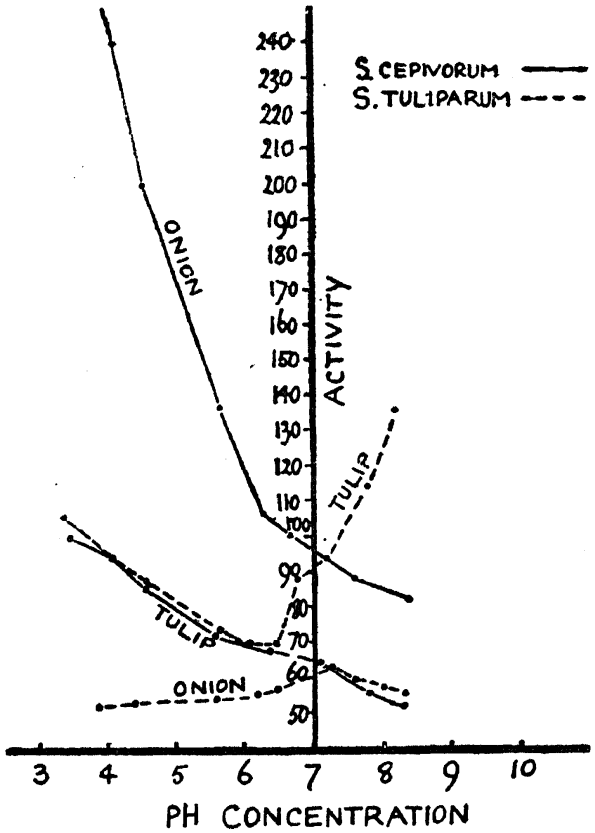

FIG. 3. Relation of H-ion concentration to the pectinase activity of *S. cepivorum* and *S. tuliparum.*

To obtain a range of pH, different amounts of N/20 HCl or NaOH were added. As a control to this experiment another range of pH was set up without the extracts which were tested for pectinase activity. Here it was found that within ten hours time there was no disintegration in the potato discs within 3·5–8·6 pH range. Therefore any effect shown within this range was due to the pectinase present. The pectinase activity of the extract without any added acid or alkali was taken to be 100.

A study of the above figure shows that there is a marked liking of *S. cepivorum* enzyme for acidity, especially the one from onion plugs. The curves for the preparation of *S. tuliparum* do not slope continuously to the right but either show no definite response to pH concentration at all or show a minimum of activity near the neutral point and a rather steep upward gradient on the alkaline side.

It has already been shown experimentally that the growth of *S. cepivorum* is fairly constant over a wide range (2·2 – 8·2) of H-ion concentration. A higher pH than 8·2 causes the growth rate to fall considerably. On the other hand the growth of *S. tuliparum* shows a well-defined optimum near the neutral point. The range of *S. tuliparum* in the alkali side is greater than that of *S. cepivorum* and conversely for the acid side.

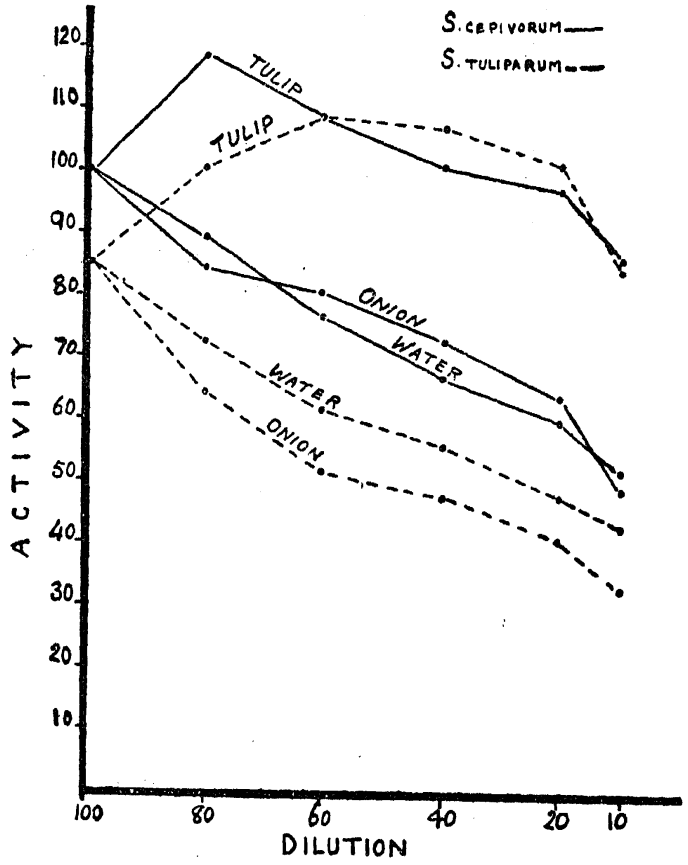

FIG. 4. The relative effect of dilution by water, onion and tulip juice on the pectinase activity from onion plugs.

It is interesting to recall in this connection that *S. cepivorum* is favoured in its growth, as stated above, by an acid reaction, so that to that extent the reactions of the fungus and of its enzyme are similar.

The relative retarding effects of various diluting substances (water, tulip or onion juice) are shown in Figs. 4 and 5. In the former the medium used was onion and in the latter tulip plugs. The cultures in both cases were three weeks old.

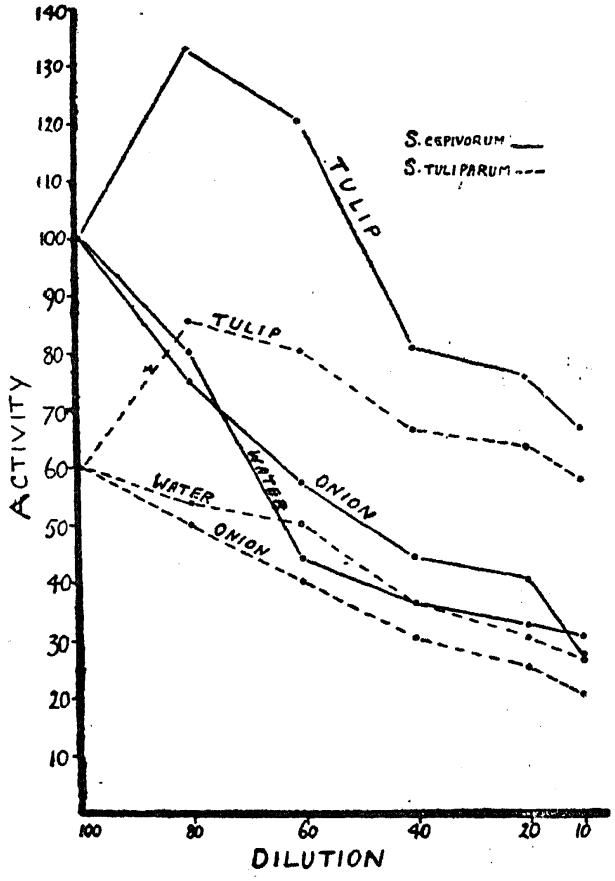

FIG. 5. The relative effect of dilution by water, onion and tulip juice on the pectinase activity from tulip plugs.

From both of these figures it is seen that certain dilutions by tulip juice instead of reducing considerably increase the activity while the presence of onion juice retards. The extracts of both fungi behaved similarly in this respect.

SUMMARY

1. *Sclerotium cepivorum* and *Sclerotium tuliparum* both were found to excrete pectinase enzyme on a variety of media.

2. *Sclerotium cepivorum* gave more active preparations of this enzyme when grown on onion than on tulip tissue, and the converse was true for *S. tuliparum*.

3. There was evidence that tulip tissue was specifically more sensitive to the enzyme of *S. tuliparum* than to that of *S. cepivorum* and conversely.

4. The enzyme prepared from *S. cepivorum* was more tolerant of acidity than that of *S. tuliparum*.

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