Korean Jour. Botany
Vol. 21, 39~44 (1978)

Fluorometric Measurement of Acid Phosphatase Activity in the Angiosperm Parasite (*Cuscuta cephalanthi*) and its Host (*Hedera helix*)[1]

Sangtae Lee
(Department of Biology, Jeonbug National University, Jeonju)

寄生被子植物(*Cuscuta cephalanthi*)과 그 宿主(*Hedera helix*)에서 acid phosphatase活動의 螢光分析法에 依한 測定

李 相 泰
(全北大學校 文理科大學 生物學科)

ABSTRACT

Acid phosphatase activities were analyzed in μg tissue samples from an angiosperm parasite (*Cuscuta cephalanthi*) and its host plant (*Hedera helix*) by a fluorometric microtechnique. The apex and the coiling portion of the parasite axis exhibited greater enzyme activies than other portions of the hypha. Acid phosphatase activity in the haustorium was 2—3 times that in the hyphal axis. The vascular bundles of the normal host exhibited the greatest enzyme activity. The acid phosphatase activity in the host infected by the parasite decreased to the activity level of the haustorium.

INTRODUCTION

Investigations of *Cuscuta* and other parasitic angiosperms have shown that the haustroium functions as an efficient absorbing and conducting element. Dorr (1972) observed that haustorial cells develop a conspicuous "wall labyrinth" that enlarges the absorbing surface 6 to 20 times. Ultramicroscopic studies of the main portion of the haustorium have revealed some similarities to the development of sieve elements: these include enucleation, disintegration of tonoplasts, presence of typical plasmatic filaments and production of a smooth endoplasmic reticulum (Dorr, 1972). Jacob and Neumann (1968) reported that after a period of 24 hours, 75~90% of the saccharose was translocated from the saccharose-treated leaf of *Vicia faba* to *Cuscuta*. A unidirectional host-to-parasite transfer of both photosynthetic and inorganic nutrients seems to occur through the plasmodesmata of the haustorium (Kollmann and Dorr, 1971; Dorr, 1969; Allred, 1966).

Since photosynthesis in *Cuscuta* is negligible, intensive absorption of nutrients is obligatory. However, it has remained uncertain how the haustorium penetrates the host tissues. Establishment usually involves both mechanical and chemical destruction of host cells and tissues (Kuijt, 1969). It is known that the initial penetration of the haustorium always involves destruction of parenchyma tissue between adjacent collenchyma strands of the host tissue (Noriel-

1) This work was done in the laboratory of Plastic Surgery Department, Johns Hopkins University School of Medicine, Maryland, U.S.A. The author expresses gratitude to Dr. Michael J.C. Im in the laboratory for his technical assistance and to Dr. Aubrey W. Naylor, Department of Botany, Duke University, Durham, NC, for valuable discussion.

lo, 1967). Previously it has been assumed from histochemical observations that glycerophosphatase (Tripodi, 1971) and acid phosphatase (Onofeghara, 1972; Kuijt, 1965) are involved in haustorial penetration. These enzymes are already known to be associated with the autolysis of cells in fasting animals and in the resorption of the amphibian tail (de Duve, 1963).

Since quantitative biochemical studies of the haustorium have been hampered because of the difficulty in obtaining enough fresh material for analysis, only histochemical techniques have previously been used (Onofeghara, 1972). By employing the microanlytical methods of Lowry (1953) and Jonek, et al (1977), however, which permit assaying for a variety of enzyme activities in μg size samples, the short-comings of histochemical methods can be circumvented. In the present study, Lowry's microanalytical techniques(Lowry and Passoneau, 1964) were adapted to investigate the haustorial region of *Cuscuta*. With this technique it was possible to obtain reproducible quantitative acid phosphatase measurements.

MATERIALS AND METHODS

The studies were carried out on the population of *Hedera helix* L. plants that had been parasited by *Cuscuta cephalanthi* Engelm. For assay purposes, portions of young *Cuscuta* plants were collected in a vegetative condition before there was any evidence of flowering. Campbell and Moss's (1961) technique was used for determining acid phosphatase activity. Acid phosphatase activity was directly proportional to the fresh weight of the stem tissue; the fluorescence of the reaction product was linear during 60 minuets of incubation at 37°C; the optimal concentration of the substrate was in the range from 1.3—10mM; the optimal *p*H of acetate buffer was 5.3; and magnesium ion did not cause activation of the acid phosphatase activity of *C. cephalanthi*.

One centimeter long stem segments were homogenized in distilled water (10% w/v) with a ground-glass homogenizer. Acid phosphatase activity along the axis was obtained by using 1 cm segments from the following portions of the stem: 1) the apex, 2) an uncoiled portion midway between the apex and the coiling portion, 3) 1 cm above a coiling portion, 4) the coiling portion itself, and 5) portions 1 cm and 6) 5 cm below the coiling segment as shown in Fig. 2.

The fluorometric analysis was carried out essentially as described by Campbell and Moss (1961). The assay method is based on the measurement of fluorescence of the reaction product, α-naphthyl phosphate (Sigma, St. Louis), 100 mM acetate buffer (*p*H 5.3), 2 mM magnesium chloride, 0.02% (w/v) homogenate in a total volume of 55 μl. The mixture was incubated for 60 minutes at 37°C. Blank tubes containing reagent mixture only and standard tubes containing 2—8 nmoles of α-naphthol were run simultaneously. After incubation, a 5 μl aliquot was diluted in 1 ml of 0.5 N NaOH. Fluoresence was measured in a fluorometer (Farrand Optical, Mt. Vernon, N.Y.) equipped with Corning glass No. 5860 as a primary and Nos. 3387 and 4308 as a secondary filter.

For quantitative histochemical studies, fresh samples from the host-parasite complex and uninfected host petiole were frozen in liquid nitrogen, sectioned transversely at 25 μ thickness in a cryostat, and dehydrated overnight under a vacuum at −20°C. The freeze-dried samples were stored in vacuum tubes at −20°C until the enzyme activity was assayed. The freeze-dried sections (Fig. 1) show histological integrity without staining. The outer cortex (oc), inner cortex (ic), phloem (ph), xylem (x), and pith (pt) were separated under the stereomicroscope from the infected and uninfected petioles of the host, as were the advancing tip (t), middle (m), and basal (b) portions of the haustorium, and the inner (pi) and outer (po) portions of the parasite cortex which were adjacent to the haustorium. Less than 1 μg tissue

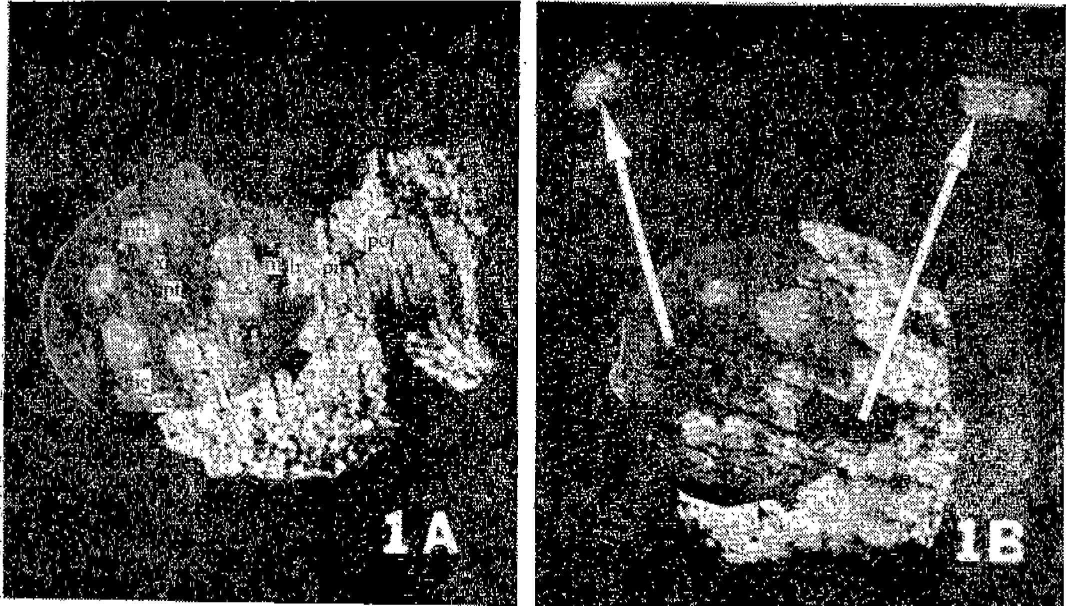

Fig. 1. Dark field micrograph(×20) of the freeze-dried transverse sections of the parasite-host complex. (A) shows the names of the parts from which enzyme activities were analyzed and also the haustorial tip removed is shown by the hollow. (B) demonstrates microdissections of the vascular bundle and a whole haustorium.

samples were weighed on a quartz fiber microbalance (Lowry, 1953) and transferred into the microtest tube, 4×24mm. Details of the instrumentation and techniques have been described by Lowry (1953). The tissues were incubated in 10 μl of reagent mixture for 30 minutes at 37°C. After incubation, 8 μl aliquots were mixed with 1 ml of 0.5 N NaOH. Blank tubes containing reagent mixture only and standard tubes containing 1—2 nmoles of α-naphthol were processed

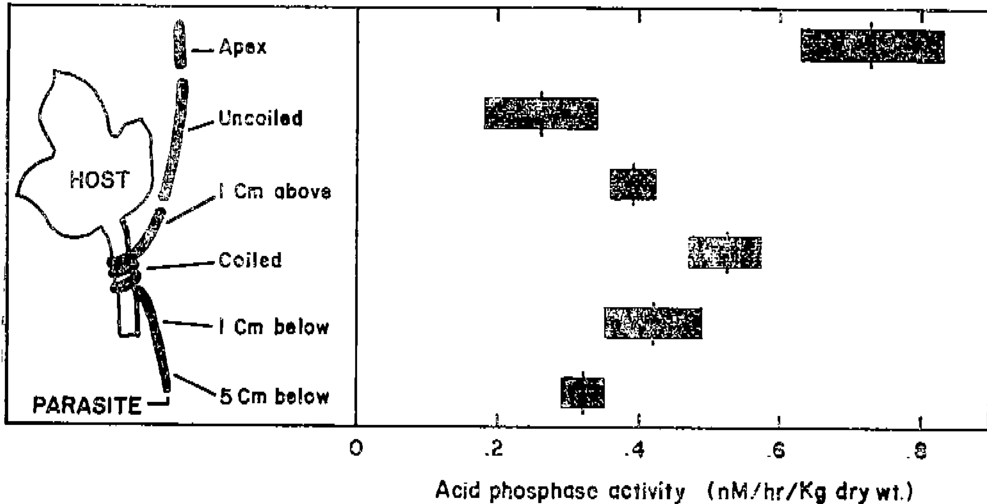

Fig. 2. Acid phosphatase activity along the *Cuscuta* stem. The diagram at the left shows the areas along the parasite stem where acid phosphatase activity was measured. Vertical and horizontal lines on the right represent mean and 95% confidence interval estimate, respectively.

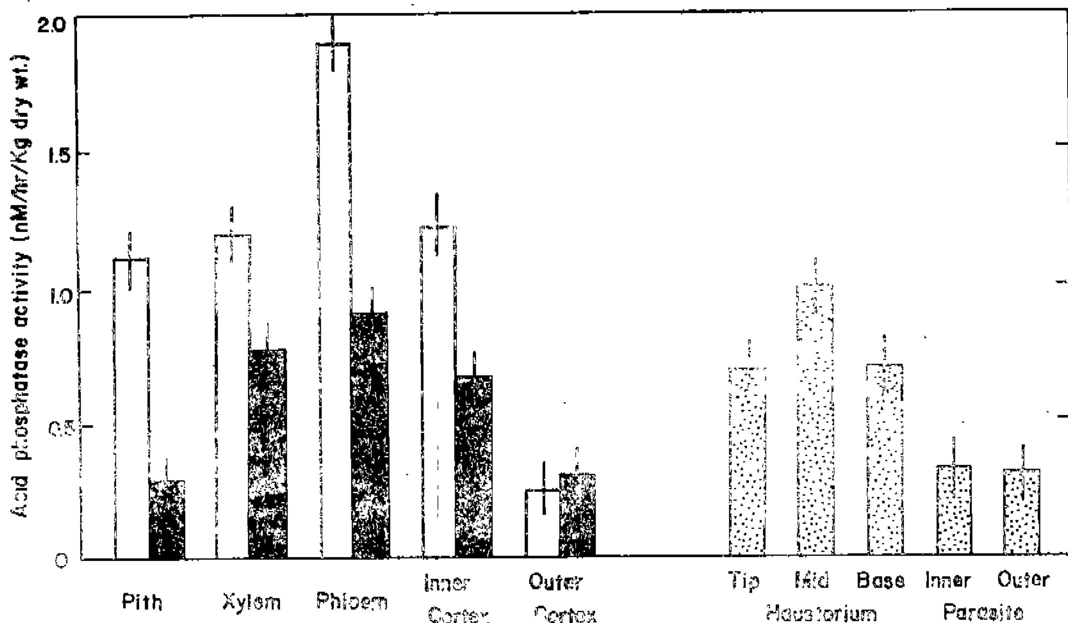

Fig. 3. Mean acid phosphatase activity with standard error (vertical lines) in the normal (open bars) and infected host (solid bars) and the haustorial coil(dotted bars) of the parasite. See Fig. 1A and the text for the sampled portions.

simultaneously. Fluorescence was measured as described above.

RESULTS

Figure 2 shows acid phosphatase measurements along the parasite's axis. The greatest activities were observed in the apex and the coiling portion(0.73 and 0.52 nM/hr/mg. fr. wt., respectively). If the activity of the apex is excluded, a gradual decrease distally from the coiling portion was demonstrated. The uncoiling item stem portions exhibited the lowest activity (see Fig. 2).

Fig. 3 summarizes the distribution of acid phsophatase activity in the uninfected host and in the parasite-host complex. Acid phosphatase activity in the haustorium was 2—3 times that in the adjacent stem of the parasite. There appeared to be no differential distribution of acid phosphatase activity within the haustorium and the parasite axis. The uninfected host plant (open bars, Fig. 3) exhibited highest activity in the phloem and lowest in the outer cortex. In general, enzyme activity in the xylem, phloem and inner cortex of the infected host(solid bars, Fig. 3) decreased to 50~60% of uninvaded tissue. The pith of the parasitized host exhibited only a third of the normal activity. There was no enzymatic alteration in the outer cortex of the host. Enzyme activity in the host tissues was similar to that in the haustorium (dotted bars, Fig. 3).

DISCUSSION

Acid phosphatase participates in intracellular digestion by its nonspecific hydrolytic action of phosphate bonds (de Duve, 1966). The results obtained by measuring portions along the parasite's axis do not effectively support the assumption that acid phosphatase reacts as a hydrolyte involved in penetration of the host, since there was greater acid phosphatase activity in the apex than there was in the haustorial coil

High acid phosphatase activity in meristematic

and differentiating tissues has been shown in many plants; for example, the root tips of *Allium cepa*, *Vicia faba*, and *Pisum sativum* (Jensen, 1956); the outer cells of the pith in *Rauwolfia* (Mia and Pathak, 1968); the trichoblasts of grass roots (Avers and Grimm, 1959); and in the shoot apical meristem in *Picea canadensis* (Van den Born, 1963) and *Pinus lambertiana* (Forsket and Miksche, 1966). High activities in haustoria have been found also *Tipinanthus* (Onofeghara, 1972), *Phithirusa*, and *Antidaphne* (Kuijt, 1965). This study revealed the two-fold activity in the haustorium compared with the axis of the haustorial coil. The distribution pattern of acid phosphatase activity along the stem of *C. cephalanthi* parallels the distribution of active metabolism, i.e., the cellular differentiation and the absorption and translocation of nutrients in the apex and haustorium, respectively.

Acid phosphatase activity in non-meristematic tissues in uninfected host plants was at least four times that in the parasite. Upon penetration of the haustorium the activity drops by 30~50%, a level which is equivalent to that of haustorium activity. Anatomical study by Peirce (1893) showed that *Cuscuta* produced a prehaustorium to invade the host tissues and later pushed a haustorium along the prehaustorial path. During this process the enzyme activity would be expected to be high at the surrounding host tissues, no measurements were made in this study, however. Thomson (1925) discovered that after the completion of intrusive haustorium growth, the superficial cells of the haustorium, especially those near the tip, protruded separate filaments in various directions by dissolving a small opening into the partitions between adjacent parenchyma cells of the host. The present study showed no evidence for acid phosphatase activity. If measurement was made far after accomplishing this process accompanied by the enzyme involvement, the enzyme activity should have returned to the normal activity. There did not appear to be any evidence to support this assumption.

Singh (1972) reported that the activities of phosphorylase and RNase in *Medicago sativa* decreased after *Cuscuta* infection, and the activities returned to the level of the control plants after removing the parasites. According to his results there seemed to be no correlation between the enzymes and haustorial penetration. If or only if the enzymes are involved in haustorial penetration, their activities should be recovered as soon as the formation of haustorium is completed, ignoring the removing of the parasites from the host. No such experiment was made for the present study, but de Duve's(1963) inclusion of these enzymes, as well as acid phosphatase in the lysomal enzymes, makes the role of acid phosphatase in the haustorial penetration doubtful. However, there is no direct evidence that acid phosphatase does not work as a hydrolyte for the haustorial penetration.

I observed that the lower portion of the haustorial coil withered soon after haustorial penetration, whereas the upper portion remained intact until the inflorescence was produced at the coil. Interestingly, the acid phosphatase activity is slightly higher in the lower portion of the coil than in the upper portion (Fig. 2). The comparison gives a clue supporting the autolytic action of the enzyme.

In conclusion, the role of acid phosphatase in haustorial penetration of host tissues by *C. cephalanthi* was not conclusively demonstrated, nevertheless, the techniques used in this study appear to be useful ones in assaying the hydrolytic role of this enzyme in haustorial penetration. In the future, the study of the distribution and activity of acid phosphatase and other hydrolytic enzymes involved in haustorial penetration at different developmental sequences of the haustorium should yield new information that will allow us to gain greater insight into the mechanism through which *Cuscuta* establishes its parasitic relationship with the host.

摘　要

寄生被子植物(*Cuscuta cephalanthi*)과 그 宿主(*Hedera helix*)에서의 acid phosphatase活動을 螢光分析法에 依하여 μg크기의 組織切片으로부터 測定하였다. 寄生植物의 acid phosphatase活動은 主軸의 頂端과 宿主를 감고 있는 部分에서 훨씬 높았으며 haustorium에서의 活動은 宿主를 감고 있는 主軸의 2—3倍나 되었다. 寄生植物의 被害를 받지 않은 正常宿主의 同 酵素活動은 維管束에서 가장 높았고 被害를 받은 後에는 haustorium의 水準으로 떨어졌다.

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(Received September 9, 1978)