

Chitin Component in Haustorial Wall of Powdery Mildew Fungus of Barley

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Introduction

According to a microscopic observation on the infection process of powdery mildew fungus on barley, the formation of appressorium, halo and haustorium occurred within 6 hr, 9 hr and 12 hr, respectively. Success or failure of the infection has been considered to be determined at early stage of haustorial formation¹⁾.

The process of haustorial formation of barley powdery mildew was reported in detail by Bushnell, and it is generally believed that haustoria probably occupy a key position in the interchange of substances between fungi and their hosts²⁾, although no direct evidence has been shown for this concept. Concerning chemical components of haustoria and haustorial walls, nothing has been known except the fact that haustorial wall is continuous to the external mycelial wall³⁾.

In this paper the authors report results of histochemical experiment on the chitin component in the haustorial wall.

Material and Method

Observation on the Fungal Walls in Infection Process : Primary leaves of 8 day-old seedling of *Hordeum vulgare* L, cultivar Russian 74 were inoculated with synchronized conidia of either compatible or incompatible race of *Erysiphe graminis hordei*, Hr74 or Hh4. The epidermis of the inoculated leaves were stripped at intervals of time, and fixed with a mixture of formalin—acetic acid—50% ethanol (5/5/90 v/v/v) for 1 hr, and were washed twice with 95 % ethanol. For observation of fungal walls a modified chitosan reaction was applied to the epidermal strips to avoid severe shrinkage occurred in the standard reagent, 16 M KOH, of van Wisselingh's method⁴⁾. Namely epidermal strips were dipped into 4 M KOH and heated gently on flame at the temperature below 140°C, washed with 95 % ethanol, water, and stained with I₂-KI solution (I₂-1 g, KI- 3 g, H₂O- 300 ml) and 5 % sulfuric acid.

Observation on Isolated Haustorial Wall : Surface mycelia of race Hr 74 on Russian 74 were removed 10-day after inoculation. Epidermis of the infected leaves were detached and chopped with a razor blade in a small amount of 0.02 M phosphate buffer (pH 6.5) containing 0.3 M mannitol. The chopped juice was filtrated with 1 layer of gauze and centrifuged at 500 × g for 15 min. The precipitate was collected as crude haustoria fraction, and subjected to the chitosan reaction described above.

Result and Discussion

To know a general figure of chitosan reaction in powdery mildew fungus, the epidermis 10-day after inoculation was tested. The conidia, conidiophores, hyphae, septa and haustoria showed positive chitosan reaction, purple to reddish purple (Plate 1. a,

b, c). Results of chitosan reaction on host and fungal organs were shown in Table 1. On the basis of these results, heat treatment was performed for 2 hr.

Table 1. Effect of heat treatment for 1 or 3 hr periods on the color development in chitosan reaction.

Organ	Coloration	
	Heat Treatment (hr)	
	1	3
Host Epidermis	Blue	Colorless
Conidium	Orange purple	Purple
Septum	Reddish purple	Purple
Appressorium	Reddish purple	Purple
Haustorium	Reddish purple	Purple
Hypha	Reddish purple	Purple
Halo	Yellow	Colorless

In the compatible combination, Russian 74-Hr 74, formation of secondary hyphae and lobes was observed at 24 hr after inoculation (Plate 1. d). At 19 hr lobes differentiated and were stained positively (Plate 1. f). At 16 hr haustoria were still immature, but showed positive reaction (Plate 1. g), and at 12hr primordial haustoria were already formed and showed the remarkable positive reaction (Plate 1. h). However, in the incompatible combination, Russian 74-Hh4, haustorial formation did not occur even at 24 hr after inoculation and halo was stained darker yellow (Plate 1. e).

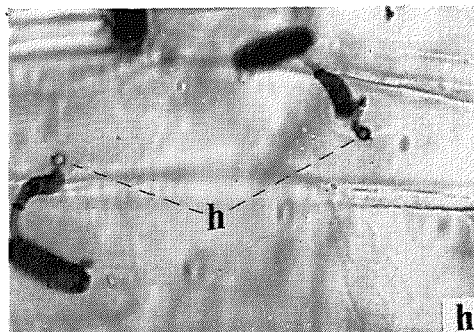
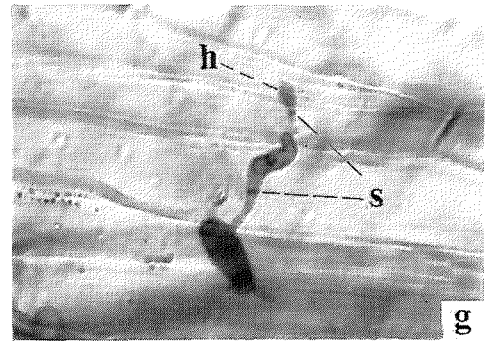
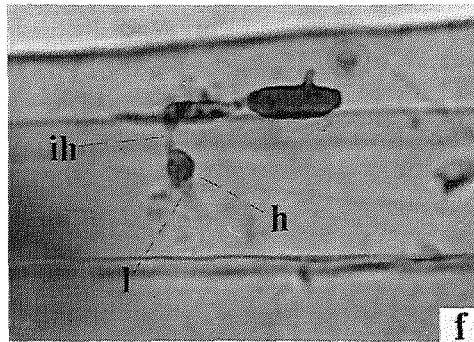
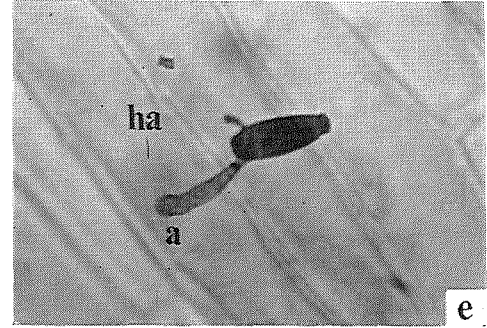
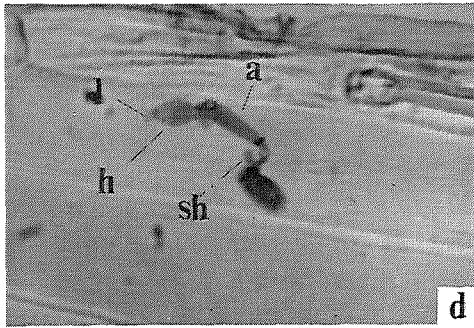
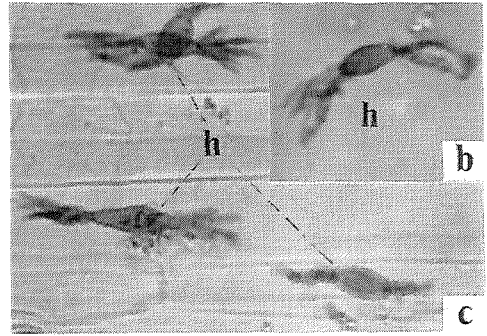
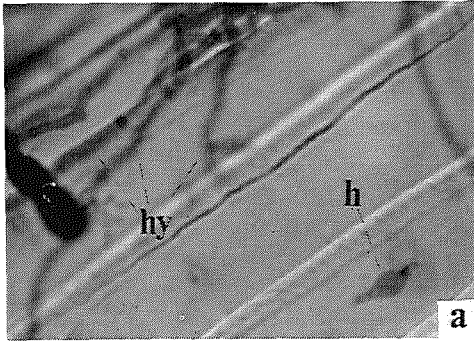
These results clearly show that haustorial walls, even if primordial, contain chitin as other hyphal and conidial walls do. It is thus unlikely that haustorial function as nutrient absorbing organ is due to a specific wall composition. Characterization of chitins and other wall components is essential for further interpretation of the specific function of haustoria, hence is under way.

Acknowledgement

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- hy : hypha
- h : haustorium
- a : appressorium
- l : lobe
- sh : secondary hypha
- ha : halo
- s : septum
- ih : infection hypha

Plate 1.

Chitosan reaction in the infection process of *E. graminis hordei* race Hr 74 (compatible) and race Hh 4 (incompatible) on *H. vulgare* L. cultivar Russian 74.

- a : Hyphae and haustorium of the fungus as observed 10-day after inoculation : Pretreatment with alkaline causes detachment of most of the mycelia from leaf surface. Note the haustorium in the cell detached from hypha. Hyphae and haustorium were stained reddish purple indicating presence of chitin in the wall.
- b : Haustorium of Hr 74 isolated from leaves of Russian 74, a compatible host.
- c : Mature haustoria of Hr 74 observed 10-day after inoculation.
- d : Infection structure of Hr 74 observed 24 hr after inoculation. Conidial and appressorial walls are stained darker comparing with those of young haustorium and primordial secondary hypha.
- e : Appressorial formation of an incompatible race Hh 4 on Russian 74. Halo was stained yellow while conidium and appressorium were stained reddish purple.
- f : Infection structure of Hr 74 on Russian 74 (compatible host), as observed 19 hr after inoculation. Haustorium was still immature and lobes have just started to form. Note positive reaction at infection hypha and lobes.
- g : Infection structure as observed 16 hr after inoculation in the same combination as above. Note dark stain at septa.
- h : Infection structure as observed 12 hr after inoculation in the same combination as above. Note the positive reaction of the primordial haustorium.

オオムギうどんこ病菌吸器細胞壁におけるキチン成分について

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純寄生性病害の感染成立に中心的役割を演ずると考えられている吸器の性質を明らかにするために、オオムギうどんこ病菌の吸器形成過程における吸器壁のキチン質の生成に関して組織化学的に検討した。その結果、吸器壁はキトーサン反応陽性で、菌糸壁と同様キチン質がその骨格をなしているものと考えられる。さらに、経時的な検討の結果、感染初期に形成される吸器原基において、すでにキチン反応が陽性であり、キチン合成系の活性化は、吸器機能発現のための一つの重要な過程であると推定される。