On Gelatinized Hyphal Aggregates in Corn Smut Galls

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樋浦 光男*・樋浦 誠* : トウモロコシ黒穂菌腫のゼラチン化した菌糸塊について

Several investigators^{1,2,3)} have reported that the hyphae of the corn smut fungus, *Ustilago maydis*, are gelatinized at an early stage of chlamydospore formation, and appear as short angular or irregular spine-like segments. If a bit of smut gall tissues in an early stage of development be dissected and observed under a microscope, it is rather easy to find out various gelatinized, colorless masses giving rise to sphaerical, angular, sometimes forked or three forked segments of hyphae, scattering among pieces of gall tissues. These masses appear to be soft, flexible bodies not easily broken, and separable from the gall tissue by dissection. The gelatinized masses are not stained with potassium iodide, cotton blue, acetocarmine and other ordinary stains. So, it is difficult to observe in detail the ramification of the hyphae inside of the masses. No information, so far, has been obtained on the nature of the gelatinized substance.

The present note deals chiefly with the elucidation of the chemical nature of the gelatinized substance in question.

Forty g of hyaline portions of corn smut galls in an early stage of development were cut into small pieces, boiled with 40 ml of water for 5 min in an Erlenmeyer flask softly plugged with cotton, and filtered with filter paper. After cooling, the filtrate was centrifuged to obtain a clear solution to which a two-fold quantity of 99 % ethanol was added to get a whitish precipitate. The purification of the precipitate was once more repeated. (No. 1 : 0.1 mg).

On the other hand, the residual pieces treated as above-mentioned, were kept at 60 C for 2 hr with 40 ml of HCl-acetic acid mixture (1 N HCl : 45% acetic acid, 3 : 1 v/v), and filtered, centrifuged to get a clear solution. When the residual pieces macerated in this way were examined under a microscope, various ramifications of hyphae (Figs. 2 & 3) were clearly observed, suggesting that the gelatinized matrix might be dissolved in the course of treatment. Then, the acid filtrate was treated to obtain a precipitate with 99% ethanol, just as above described. The yield of the whitish precipitate was found higher than that of the boiling water extract. (No. 2 ; 0.15 mg). These two kinds of whitish precipitate quite resembled zeagallan^(4,5) previously reported by the senior author.

In order to make this supposition certain, the following experiments were carried out. No. 1, No. 2, and 0.2 mg of zeagallan were dissolved in 10 ml of 0.2 N H₂SO₄,

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1. Christensen, J. J. (1931). *Phytopath. Z.* 4:129-188. 2. Ehrlich, H. G. (1958). 50:622-627.
3. Sleumer, H. O. (1932). *Z. Botan.* 25:209-263. 4. Hiura, M. (1975). *J. Agr. Chem. Soc. Japan* 49:185-187. 5. Hiura, M. (1976). *Ibid.* 50:121-126. 6. Hiura, M. (1974). *Ann. Phytopath. Soc. Japan* 40:452-453.

respectively, and hydrolysed at 120 C for 1 hr by autoclave. After cooling, they were neutralised with barium carbonate, filtered, concentrated at 60 C, and then spotted on Toyo filter paper No. 51 A. As a solvent, n-butanol : acetic acid : water (4 : 1 : 1) was used, and irrigasion was done twice by the ascending method. The chromatogram was sprayed with sodium hydroxide (1% in 80% ethanol) follwed with silver nitrate (1% in 80% actone), fixed with thiosulfate, and washed in running water.

The chromatogram (Fig. 4) indicates that the gelatine-like substance in question may be composed of zeagallan. This further suggests that the gelatine-like substance is produced by the smut fungus, since it was revealed by the senior author⁽⁶⁾ that zeagallan is not only isolated from the smut spore (teliospore or chlamydospore), but also produced in synthetic solutions by the fungus. (Recieved May 25, 1977)

Fig. 1. Many hyaline, hyphal spine-like segments of various types, scattering on the surface of a part of a gelatinized hyphal aggregate.

Figs. 2 & 3. The ramification of hyphal aggregates released from the gelatinized substance.

Fig. 4. A chromatogram of acid hydrolysates of the glucan, zeagallan and two kinds of polysaccharides obtained in the present investigation : Z, spots from hydrolysed zeagallan, from the top, glucose, gentiobiose, probably triose and tetraose (rather obscure).

N, spots from the polysaccharide dissolved in the HCl-acetic acid mixture. A top spot is unknown.

W, spots from the polysaccharide dissolved in boiling water.

GE, a spot of authentic gentiobiose.

G, a spot of authentic glucose.