SHORT COMMUNICATION

Partial Purification of the Virus Associated with Enset Chlorotic Leaf Disease

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Abstract

Purification procedures developed for other members of the bacilliform DNA (BADNA) virus genus did not work for the virus associated with enset (*Ensete ventricosum*) chlorotic leaf streak disease whereas continuous check-ups of the same diseased enset leaf samples by dot blot ELISA gave positive reaction to banana streak virus antiserum. Therefore, incubation of the diseased leaf samples under -70°C for different periods of time was thought to rupture the mucilaginous tissue for facilitating the purification. Based on this approach, a minimum incubation period of 4 weeks under -70°C was found effective in liberating the BADNA particles from the enset leaf. The only problem encountered was keeping the virus particle at its correct density in cesium sulphate gradient. The bullet shaped virus particles (bacilliform DNA virus) associated with enset chlorotic leaf streak were found to have a length of 100-175 nm with an average of 137.5 nm and a width of 25-32 nm, averaging 28.5 nm, which is in accordance with the other BADNA genus members.

Introduction

Enset (*Ensete ventricosum*) is the major staple food crop for the populace of the Southern Peoples Regional State of Ethiopia. Enset chlorotic leaf streak diseasefig is one of the major limiting factors for the production of the crop and is caused by a BADNA virus (Mesfin et al. 1996). Purification protocols developed for other BADNA members which include cacao swollen shoot (Adomako et al. 1983), brown spot of yam (Harrison et al. 1973, James et al. 1973), *Colocasia* bacilliform (Gumpf et al. 1981), rubus yellow net (Jones & Roberts 1976), rice tungro bacilliform (Omura et al. 1983, Singh et al. 1984), sugar cane mild mosaic (Lockhart et al. 1992) and banana streak (Lockhart 1986) viruses did not work for ECLSV (Mesfin *et al.*, unpublished). On the other hand, repeated check-ups of diseased leaves using dot-blot immunoassays (Mesfin et al. 1996) and molecular techniques revealed the presence of a BADNA virus. A renewed purification procedure was therefore developed to liberate the bacilliform virus particles from enset leaves and thereby elucidate its morphology and dimensions.

Materials and Methods

Virus Source

Diseased enset corms were collected from Hagerselam, Sidamo, Ethiopia, and grown in the greenhouse of the department of virology, Wageningen Agricultural University (WAU), in The Netherlands. Leaf samples were cut from such plants for the purpose of this activity.

Incubation

The deep freeze (-70°C) in the department of virology, WAU, was used to keep the leaf samples of 50 g each in a plastic bag for periods of 1, 2, 3, 4, and 5 weeks. Enset leaf samples which were leftovers in deep freeze (-70°C) from the hybridization work were used in this purification and liberated the virus particles

associated with the enset chlorotic leaf streak disease which were observed under the electron microscope. Since the same purification protocol had failed to liberate the virus in repeated trials, incubation period was considered as treatment to know the cause for the success of purification.

Virus Purification

The enset BADNA virus was partially purified from diseased enset leaves collected from the greenhouse at the department of virology, WAU. Purification procedures were monitored by electron microscopic examination of aqueous extracts and high speed pellets at each stage of the process. Except the leaf incubation under -70°C, purification protocols are modifications adopted from methods used for other BADNA virus members (Lockhart 1986, Lockhart et al. 1992, Omura et al. 1992). Details are as follows. Immediately after taking out the incubated leaves (50 g) from the freezer, the leaves were then extracted using 1:3 (w/v) in a blender in cold 0.1 M Tris-HCl, pH 6, containing 0.5% (w/v) Na_2SO_3, 0.5% (v/v) 2-mercaptoethanol, 0.25% (w/v) maceroenzyme and 0.25% (w/v) cellulase. The extracted suspension was incubated for 4 hr at room temperature. The pH of the extract was adjusted to 7.4 and stirred for 30 min. After that, the suspension was put on low speed centrifugation (LSC), 10,000 rpm for 10 minutes at 4°C using a Sorval GSA rotor. The supernatant was collected and the pellet was resuspended in 100 ml Tris-HCl buffer, pH 7.4. It was again blended and put on LSC. After pooling the supernatant, Triton X-100 (2% v/v) was added and the suspension was stirred for 10 minutes. Then after, the suspension was layered over a 6 ml cushion of 20% (w/v) sucrose in 0.01 M Tris-HCl, pH 7.4 and put on high speed centrifugation, 35,000 rpm for 2 hr at 4°C using Ti 45 rotor. The high speed pellets were resuspended for overnight in 0.01 M Tris-HCl, pH 7.4. This suspension was shaken briefly with 0.5 volume chloroform and put on LSC at 10,000 rpm, 10 minutes at 4°C. The aqueous phase was re-extracted with chloroform as above on LSC. The final supernatant was loaded on a preformed 0-40% Cs_2SO_4 gradient in Phosphate Buffered Saline concentration-Tween containing 20% (w/v) sucrose (Harrison & Roberts 1973) and centrifuged in a SW 28.1 rotor for 5 hr at 140000 g at 4°C. The virus suspected band was removed with a Pasteur pipette and dialysed against 0.01 M phosphate buffer, pH 7.2, to remove the cesium salts. At the same time, all layers were checked for the presence of the virus particles by electron microscopy.

Electron Microscopy

Leaf dip and partially purified preparations were negatively stained with 2% sodium phosphotungstate, pH 6.8 (PTA). For immunosorbent electron microscopy (ISEM) tests, carbon coated grids were floated on drops of 20 μl Banana Streak Virus (BSV) antiserum which was diluted 1:1000 times with 0.05 M phosphate buffer, pH 7 and incubated at 37°C for 15 min. After rinsing in the same phosphate buffer, the antibody-coated grids were dried and floated on drops of partially purified ECLSV suspensions and incubated for 20 hr at 4°C while no evaporation could occur. After rinsing with phosphate buffer and staining with PTA, examination of the grids was made on Zeiss electron microscope. Virus particle measurements were made from electron micrographs of partially purified virus stained with PTA. The lattice spacing of crystalline catalase (Wrigley 1968) was used as an internal calibration standard.

Results and Discussion

Symptomatology

Corms of diseased enset plants from Hagereselam did not develop typical chlorotic streaks at Wageningen, The Netherlands. Symptoms were more pronounced during the winter than the summer period.

Incubation

Diseased enset leaf samples kept under -70°C for 1-3 weeks did not yield the bacilliform virus particles on purification. However, the same leaf samples kept for periods of 4 weeks and longer revealed the BADNA particles in sufficient amount on purification (Fig. 1). The freezing effect might have contributed in breaking apart the fibrous tissues of the leaves

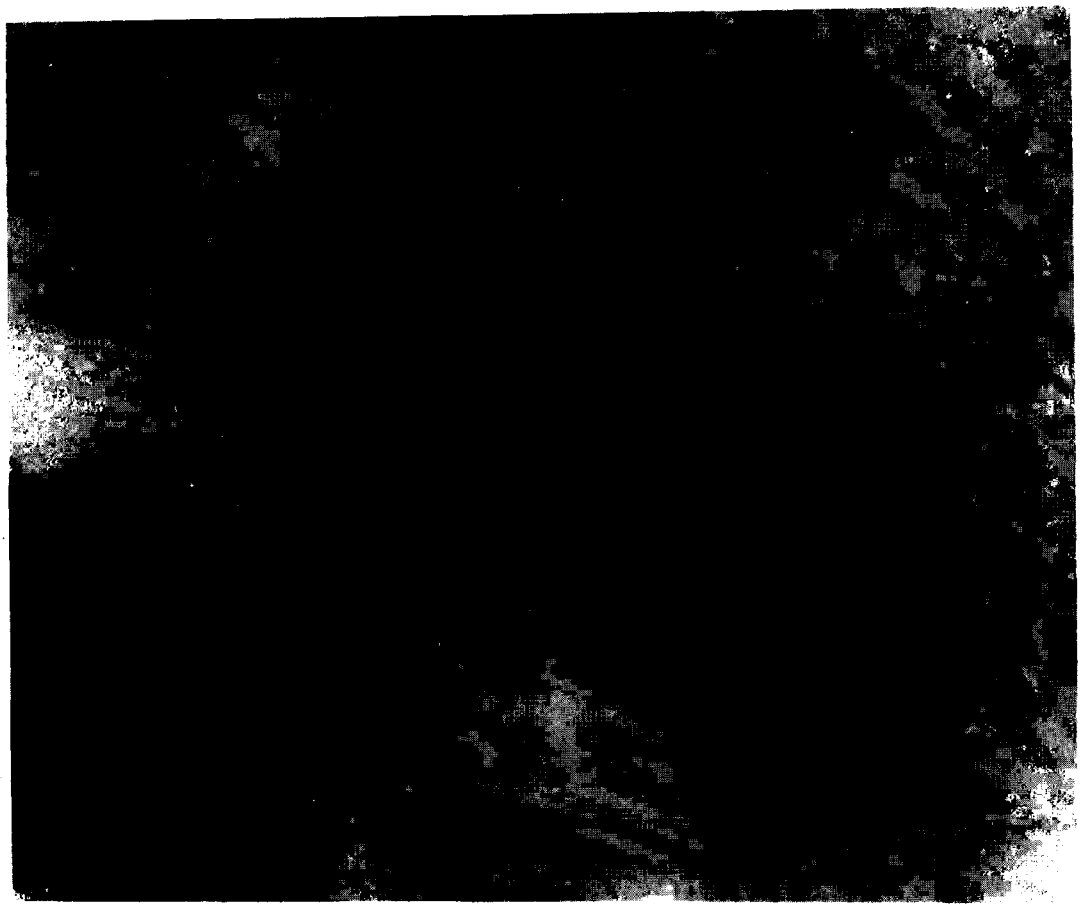

Fig. 1. BADNA virus particles trapped on BSV-antiserum coated grids from partially purified extract of viral diseased leaf of enset

and thereby resulted in easy liberation of the BADNA particles during the extraction process.

Virus Purification

The age of enset leaves did not contribute positively towards the virus purification effort as leaves tried in previous purifications from the heart leaf, first expanded leaf and the oldest leaves did not yield the enset BADNA virus particles even in the presence of the cellulotic enzymes. Rather the incubation of the leaves at least for 1 month period and the addition of cellulotic enzymes like maceroenzymes and cellulase (Singh et al. 1984) in the extraction buffer were instrumental in liberating the BADNA particles from the fibrous tissue of enset leaves. The problem encountered was the distribution of the BADNA particles in all layers of Cs_2SO_4 gradient. Therefore, manipulation of the concentration of cesium salt in sucrose gradient should be tried to bring the virus particles in their right density.

Electron Microscopy

The bacilliform particles associated with enset chlorotic leaf streak disease were not observed in the leaf dip preparations. It was only from the partially purified suspension of 4 weeks and longer incubated leaf samples that the BADNA particles were clearly seen for the first time

after a year's painstaking purification effort. This coincidence was a mere chance since it was accidentally purified from leaf samples left after completion of hybridization. Critical analysis led the reason on incubation period and this was proved by the treatments evaluated. BADNA virus particles trapped from the partially purified preparation (top part) on the antibody-coated and incubated grids were clearly seen in Fig. 1. Measurement of 56 particles from electron micrographs of a partially purified preparation for enset leaves with typical chlorotic leaf streaks and stained with PTA gave mean particle dimensions of 118-125 x 29.5-30 nm. This is in harmony with the measurement reported for the BADNA genus group (Brunt et al. 1990).

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