Isolation of Kenaf leaf peroxisomes to investigate the localization of Hibiscus chlorotic ringspot virus (HCRSV) coat protein (CP)

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ABSTRACT

Coat protein (CP) of Hibiscus chlorotic ringspot virus (HCRSV) had been found to be responsible for the spread of infection. Kenaf (Hibiscus cannabinus) was used as a model organism in this study. As CP is most likely localized in the peroxisomes, there is a need to produce a protocol adapted solely for the isolation of peroxisomes from the leaf of Kenaf as peroxisomal characteristics differ greatly within and amongst plants. At the moment, Sucrose and Percoll density gradient are the two most commonly used method to isolate peroxisomes. Modifications were made to the protocols to optimize the yield of peroxisomes from the leaves of both healthy and virus infected Kenaf. Hydroxypyruvate reductase assay, Western blot analysis and transmission electron microscopy (TEM) were carried out to check the presence and the intactness of the peroxisomes eluded. The presence of CP inside the peroxisomes was also investigated through Western blot after thermolysin treatment to remove outer proteins. The results of this study showed that the protocols yield only low amount of intact peroxisomes. The identification of CP through Western blot also did not yield satisfactory result. As such, this study will serve as a platform for further investigation in the similar direction.

INTRODUCTION

CP of HCRSV was responsible for the spread of infection in the plant it infected. In this study, Kenaf was used as a model organism. As CP was expected to be localized in the peroxisomes, peroxisomes study thus need to be carried out. Firstly, this study will optimize the two protocols used to isolate peroxisomes, namely the sucrose density gradient method and the Percoll density gradient method. The isolated product will then be tested for presence and intactness of peroxisomes. Western blot analysis will also be used to check whether CP is localized in the peroxisomes.

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MATERIALS AND METHODS

Kenaf was grown from seeds and then inoculated with HCRSV. After it reaches the 8-leaf stage, leaves from both healthy and virus infected plants are harvested, dried and ground into a suspension for isolation of peroxisomes using either sucrose density gradient or Percoll density gradient, which has been modified and optimized. The isolated fraction or pellet will then be tested for presence and intactness of peroxisomes using hydroxypyruvate assay, Western blotting and transmission electron microscope. Western blot is also used in an attempt to prove the presence of CP in the peroxisomes. To reach this conclusion, protein on the outside of peroxisomes need to be degraded. This is done by treating the peroxisome with thermolysis, a protease that will digest protein without disrupting peroxisomal membrane.

RESULTS

Comparison between the sucrose density gradient method with Percoll density gradient method
Sucrose density gradient method yield a green peroxisome fraction while the Percoll density gradient method yield a white peroxisome pellet.

Hydroxypyruvate reductase assay
Hydroxypyruvate reductase assay was carried out to check the intactness of the supposed peroxisomes obtained from the two isolation procedure. Two reaction mixture was observed from each sample; one is diluted in buffer containing sugar while the other is diluted in buffer without sucrose. An absorbance at 340nm was then noted for each sample. The assay is done before and after thermolysin treatment. Before thermolysin treatment, the absorbance for the reaction mixture containing sugar was higher as compared to the other. However, after thermolysin treatment, a reverse phenomenon was observed.

Western blotting
Negative result was observed for all samples when sulfite oxidase antibody was used. When CP antibody was used instead, only the samples taken directly from both virus infected and healthy leaves register bands.

Transmission electron microscopy
A putative peroxisome was observed. It is about 106nm in diameter and has a granular matrix. However, no crystalline structure that was characteristic of peroxisomes was observed.
DISCUSSION

The ideal peroxisome isolation protocol

Although sucrose gradient method yields a peroxisomal fraction with much more contaminant as compared to the Percoll density gradient method, sucrose density gradient method is much lower in cost. Moreover, in some experiments where specific targeting to peroxisomes are carried out, contamination might not actually pose too great a problem. As such, both isolation method has its benefits and drawbacks. It is thus up to individuals to choose the best method for their experiment.

Hydroxypyruvate reductase assay

The absorbance result before thermolysin treatment shows clearly the presence of intact peroxisomes as hydroxypyruvate reductase will only be able to oxidize NADH when it is lysed out from peroxisomes.

The result after thermolysin treatment is thus very unexpected. A possible cause might be that thermolysin not only hydrolysis protein, it might also bring about synthesis of certain products, even possibly NADH. Thus, the lysed peroxisomes will provide more raw materials, thus making the absorbance for lysed peroxisomes higher than intact ones.

Western blot analysis

The negative result for all the samples when sulfite oxidase was assayed show a possible error in material and method rather than the problem with the sample. It might be that the primary antibody is denatured or that there is insufficient binding time of antibodies to register bands. On the other hand, the negative result for CP is probably due to the low concentration of protein in the sample.

REFERENCES


