ショ糖-酒石酸カリウム平衡密度勾配遠心法によるpotyvirus管状封入体の純化

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
著者	野田, 千代一 前田, 孚憲 井上, 成信
巻/号	54巻3号
掲載ページ	p. 319-322
発行年月	1988年7月

日植病報 54: 319-322 (1988)
Ann. Phytopath. Soc. Japan 54: 319-322 (1988)

短　報
Phytopathological Note

Isopycnic Separation of Potyviral Cylindrical Inclusions by Sucrose-Potassium Tartrate Density Gradient Centrifugation

Chiyoichi NODA*, Takanori MAEDA* and Narinobu INOUYE*

Key words: potyvirus, turnip mosaic virus, potato virus Y, bean yellow mosaic virus, purification of cylindrical inclusion.

Cytoplasmic cylindrical inclusions are one of the main characteristics of potyvirus group[1,2] and are controlled by portions of the virus genomes[1-5]. The study of cylindrical inclusions is very important to gain some understanding of their functions in host cells[6,7]. It also has been pointed out that antisera to inclusion proteins are potentially useful in diagnosing virus infections[5] and studying the relationships among potyviruses[6]. The purification of many cylindrical inclusions has been reported already[6-11]. Most experiments conducted so far on their purification have employed sucrose density gradient centrifugation as the final step in this process. However, it is difficult to separate cylindrical inclusions as a single band by this method since they are not uniform in size[12].

This paper describes an improved method for purification of cylindrical inclusions, induced by turnip mosaic virus (TuMV) obtained from *Statice* sp.[13], bean yellow mosaic virus (BYMV, P 25M strain)[14] and potato virus Y (PVY) from potato[15], reducing the amount of organic solvents needed for clarification by treating inclusion preparations with them at a later step in the process of purification, and using isopycnic centrifugation in a sucrose-potassium tartrate density gradient.

TuMV[13] was propagated in *Brassica rapa* var. *komatsuna*. Infected plants, 2–5 weeks after inoculation, were placed in the dark for 48 hr to reduce starch contamination prior to harvesting of leaves. Systemically infected leaf tissue was homogenized in a blender with a cold solution containing 3 ml of 500 mM potassium phosphate buffer (PB) (pH 7.5) and 5 mg sodium sulfite per gram of fresh tissue and then filtered through two-layers of gauze. Triton X-100 was added to the homogenate to a final concentration of 5% and after stirring for 1 hr at 4 C, the mixture

* Research Institute for Bioresources, Okayama University, Kurashiki 710, Japan　岡山大学資源生物科学研究所

1) Edwardson, J. R. (1966). Amer. J. Bot. 53: 359–364. 2) Matthews, R. E. F. (1982). Intervirology 17: 9–199. 3) Dougherty, W. G. and Hiebert, E. (1980). Virology 104: 174–182. 4) Edwardson, J. R. (1974). Fla. Agric. Exp. Stn. Monograph No. 4. 5) Purcifull, D. E., Hiebert, E. and McDonald, R. G. (1973). Virology 55: 275–279. 6) Hiebert, E., Purcifull, D. E., Christie, R. G. and Christie, S. R. (1971). *Ibid.* 43: 638–646. 7) Edwardson, J. R. and Christie, R. G. (1978). Annu. Rev. Phytopathol. 16: 31–55. 8) Hiebert, E. and McDonald, J. G. (1973). Virology 56: 349–361. 9) Zettler, F. W., Abo El-Nil, M. M. and Hartman, R. D. (1978). CMI/AAB Descriptions of Plant Viruses, No. 191. 10) Lima, J. A. A., Purcifull, D. E. and Hiebert, E. (1979). Phytopathology 69: 1252–1258. 11) Nagel, J., Zettler, F. W. and Hiebert, E. (1983). *Ibid.* 73: 449–454. 12) Hiebert, E., Purcifull, D. E. and Christie, R. G. (1984). Methods in Virology 8: 225–280. 13) Iino, N., Inouye, N. and Maeda, T. (1987). Nogaku Kenkyu 61: 171–184. 14) Inouye, T. (1973). *Ibid.* 54: 155–171. 15) Maeda, T., Hidaka, S. and Hidaka, Z. (1975). Ann. Phytopath. Soc. Japan 41: 284 (Abstr.).

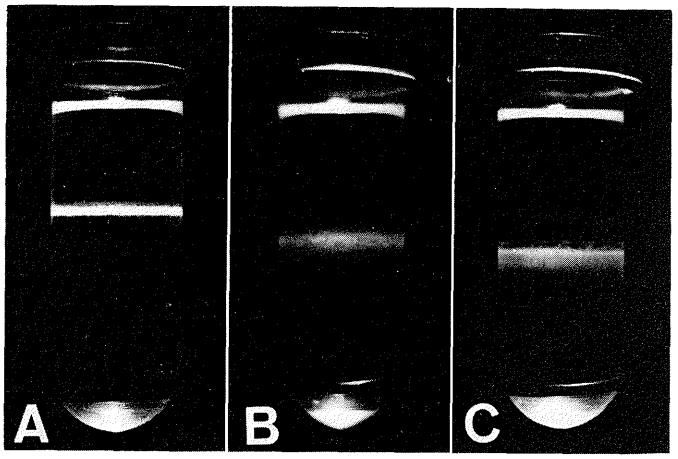

Fig. 1. Isopycnic bandings of three potyviral cylindrical inclusions after centrifugation in sucrose-tartrate density gradients. A: turnip mosaic virus, B: bean yellow mosaic virus, and C: potato virus Y.

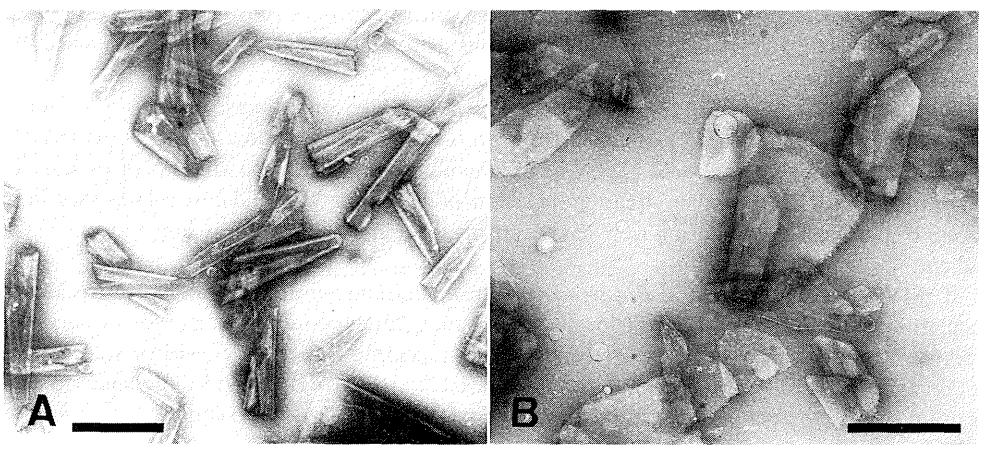

Fig. 2. Electron micrographs of purified turnip mosaic virus inclusions (A) and bean yellow mosaic virus inclusions (B), negatively stained with 1% ammonium molybdate. Scale represents 1,000 nm.

was centrifuged at 7,000×*g* for 20 min. The pellet was resuspended, with the aid of a Teflon homogenizer, in 2 ml of 50 mM PB (pH 8.2) containing 0.1% 2-mercaptoethanol (2-ME) per gram of the initial tissue and stirred again with 5% Triton X-100 for 1 hr at 4 C. After centrifugation at 7,000×*g* for 20 min, the pellet was resuspended in 0.3 ml of 20 mM PB (pH 8.2) containing 0.1% 2-ME per gram of the initial tissue. The resuspended material was shaken for 30 sec with 0.15 ml chloroform and 0.15 ml carbon tetrachloride per gram of the initial tissue. After centrifugation at 600×*g* for 5 min, the supernatant was collected. The pellet containing organic solvents was reextracted in the same way and centrifuged. The two supernatants were combined and centrifuged at 7,000×*g* for 20 min and the pellet was resuspended in 20 mM PB (pH 8.2) containing 0.1% 2-ME. The resuspended material was layered (3 ml per centrifuge tube) onto a linear density gradient made of 50–100% sucrose-potassium tartrate in water (w/v, a 1 : 1 mixture of them) and centrifuged in a Hitachi RPS25A rotor at 23,000 rpm for 1 hr. A sharp

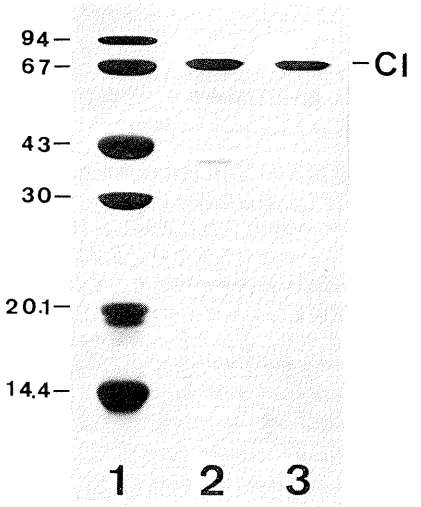

Fig. 3. SDS-PAGE analysis of the protein subunits of purified TuMV inclusions (lane 3) and the preparation before density gradient centrifugation (lane 2). Inclusion protein (CI) is indicated. Lane 1, MW marker proteins: phosphorylase b 94,000 (94), bovine serum albumin 67,000 (67), ovalbumin 43,000 (43), carbonic anhydrase 30,000 (30), soybean trypsin inhibitor 20,100 (20.1), and α-lactalbumin 14,400 (14.4).

band of inclusions was detected (Fig. 1). While no band appeared when the preparation extracted from healthy tissue was centrifuged in the same density gradient. The inclusion zone collected with a pipette was diluted 4-fold with 20 mM PB (pH 8.2) and centrifuged at 7,000×*g* for 20 min. The pellet was resuspended in a small volume of 20 mM Tris-HCl (pH 8.2). The distance of the band from the meniscus did not change during prolonged centrifugation for more than 1 hr. This may indicate that a period of 1 hr for centrifugation is sufficiently long for the isopycnic banding of inclusions. The yield of inclusions, as determined by ultraviolet spectrophotometry[12], was *ca.* 5 mg/100 g tissue. Purified inclusions examined in electron microscope contained striated inclusions similar to those in the purified preparations reported for TuMV[8,16] (Fig. 2).

A major band with MW 70,000 was detected in the SDS-PAGE analysis[17] of purified inclusions, while two minor components, MW 35,000 and 56,000, in addition to inclusion protein were found in the sample prior to gradient centrifugation (Fig. 3). The major band with MW 70,000 well corresponds to that reported for dissociated TuMV-cylindrical inclusion protein[8]. Electron microscopic observation and SDS-PAGE analysis of the purified preparations revealed the high purity of the purified TuMV-inclusions.

Furthermore, to evaluate the suitability of this method for separating other potyviral inclusions, BYMV[14]- and PVY[15]-inclusions were purified from the infected *Nicotiana clevelandii* and *Vicia faba*, respectively. Figure 1 shows that each of their inclusions was separated as a single band in the density gradient. In a comparative experiment of TuMV-, PVY- and BYMV-inclusions, the three inclusions banded at different positions in the gradient, thus indicating that each inclusion has its own particular density.

The advantages of this method are that it requires less amount of organic solvents needed for clarification and cylindrical inclusion is separated as a single band in a density gradient.

16) McDonald, J. G. and Hiebert, E. (1975). Virology 63: 295–303. 17) Laemmli, U. K. (1970). Nature 227: 680–685.

和 文 摘 要

野田千代一・前田孚憲・井上成信：ショ糖-酒石酸カリウム平衡密度勾配遠心法による potyvirus 管状封入体の純化

カブモザイクウイルス (TuMV) の管状封入体の純化法に改良を加えた。TuMV 感染コマツナ葉をリン酸緩衝液で磨砕後，Triton X-100 処理と遠心分離とを繰り返した後，有機溶媒処理を行った。この部分純化試料を 50～100% のショ糖-酒石酸カリウムの密度勾配に重層し，日立 RPS25A ローターで 23,000 rpm, 1 時間遠心分離した。その結果，1 本のシャープな封入体のバンドが形成された。純化試料の電顕観察および SDS-PAGE 分析の結果，封入体の構造はよく保持されており，また宿主成分の混入もほとんどなかった。収量は約 5 mg/100 g 生葉であった。同様にしてジャガイモ Y ウイルス (PVY) とインゲンマメ黄斑モザイクウイルス (BYMV) の管状封入体を純化することができた。本法は有機溶媒の量が少量ですむこと，サイズの不均一な封入体を単一なバンドとして得ることができるという利点がある。

(Received February 3, 1988)