Virus-like Particles from Abnormal Growing Oyster Mushrooms, *Pleurotus florida* and *P. ostreatus*

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菌絲生長이 不進한 사철느타리 및 느타리버섯으로부터 Virus粒子의 分離

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ABSTRACT: This study aimed to investigate possible cause of slow and abnormal growth of oyster mushrooms, *Pleurotus florida* and *P. ostreatus* collected from bad crop farms. Spherical virus particles of 30 nm in diameter from *P. florida*, 23 nm particles from *P. ostreatus*, and both 23 and 30 nm particles were also found from interspecies mated culture between *P. florida* and *P. osreatus*. The virus particles might be associated with the bad crop of *Pleurotus* species.

KEYWORDS: virus, oyster mushroom, *Pleurotus* spp.

Introduction

Oyster mushrooms (*Pleurotus* spp.) have been very popular with Korean peoples as one of special foods which possess the unique flavor, taste and nutritive value (Bano, *et al.* 1963; Pyo, *et al.* 1975: Go, *et al.* 1984). Furthermore, the mushrooms attract our attention with potential effect reducing cancer tumor cells in mice (Kim, *et al.* 1972).

The spcies of *Pleurotus, P. ostreatus, P. florida* and *P. sajor-caju,* have been cultivated as important cash crops with the success of rice-straw culture since 1970 in Korea (Park *et al.* 1975, 1977a, 1977b). The cultivation areas of oyster mushrooms have been greatly increased year by year since begining of rice straw cultures. In 1988, 29, 137 tons of oyster mushrooms were produced on the rice straw culture beds of 2,749, 742 m^2 throughout the whole contry (Ministary of A. F. F. Statistics, 1989).

Some of diseases and insect-pests of oyster mushrooms have been reported with the enlargement of cultivation area and continous cropping in a limited area. The cultural procedures such as preparation of the culture substractes, spawning, regulation of cultural environments and harvest usually bring mutural contamination of the harmful microorganisms and insect-pests.

In 1987, one grower of the oyster mushrooms near city of Suweon unfortunately experienced bad crop resulting in unknown causal agent on the cultural substrates. The growing oyster mushrooms on the cultural beds were almost completely destroyed by the various molds considered to be non-pathogenic. In a preliminary study to investigate the causal agent of the bad crop, the authors found out an interesting fact that isolate obtained from the above noted disordered oyster mushroom showed significant slow growth of the mycelia in comparison with the normal isolates. So, it was concluded the isolate itself might be responsible for the slow growth of the mycelia by contamination of pathogenic virus. In this experiment, the causal agent was investigated.

Materials and Methods

Fungal culture: Abnormal oyster mushrromcultures (*Pleurotus* spp.) were obtained from farms where many competition fungal pathogens and yellow bloth disease caused by *Pseudomonas* spp. were severe. The mycelia of *Pleurotus* spp. were isolated from the centre of caps of abnormal mushroom. They were placed on potato dextrose agar (PDA) medium (Booth, 1971). The mycelia were grown at 24℃ and repeatedly transferred to new media. These cultures were stored at 4℃ until use.

Purification of virus: The procedure to purify virus particles was followed by methods of Harmsen, *et al.* (1991) with some modifications. Diseased *Pleurotus* was inoculated on to mushroom complete agar medium (Raper, *et al.* 1972) and incubated at 25℃ for 7 days. The mycelia were shaking cultured by 120 rpm at 25℃ for 4 days. After incubation the cells were collected by filtration with nylon cloth. The cells were then broken by grinding in a Nihon-Seiki homogenizer for 5 to 10 mim on an ice bath in two volumes of 0.01 M sodium phophate buffer (pH 7.5) contaning 0.5% mercapto ethanol. The cell debris was removed by centrifugation at 7500 rpm for 60 min in a Hithachi RPRS-2-1213 rotor. The supernatant solution was collected and 10% polyethylenglycol M.W 6000 and 0.6 M NaCl was added to precipitate the virus particles at 4℃ for over night or 2 hours in a stirring condition. The precipitate was collected by centrifugation at 7000 rpm for 10 min and resuspended in 0.1 M sodium phosphate buffer (pH 7.5). The suspension was stirred slowly for over night or 2 hours at 4℃ and clarified by centrifugation at 9000 rpm for 15 min in a Hitachi RPR 20-2-2885 rotor. The supernatunt was collected and stored at 4℃ as partally purified viruses.

Sucrose density gradient centrifugation: Partially purified virus was centrifused in sucrose density gradient (10-40% W/V) in 0.1 M sodium phosphate buffer (pH 7.0) at 25000 rpm for 150 min in Hitachi SRP 28SA swing rotor. The viruses were collected by centrifugation at 40,000 rpm for 60 min at 4℃ in a Hitachi RP50T-2-269 rotor, otherwise, sucrose polyethylenglycol gradient in 0.1 M sodium phosphate buffer (pH 7.5) was centrifuged. The purified virus solution was observed its spectrum under UV light.

Electron microscopy observation: The purified viruses were dropped on a 150-200 mesh grid which covered by formvar film and negatively stained with 2% phosphotungstic acid at pH 7.0 after removing excessive water. The virus particles were observed with Hitachi H-800 electron microscope.

Ultrastructure of inter-cellular tissue: Abnormal growing mycelium cultured on a cellophane membrane paper which placed on MCM was used for examination of infection tissue. Infected tissues were prefixed for 30 min with 3% glutaaldehyde in Millonings phosphate buffer (pH 7.3) containg 2.26% sodiumphosphate monobasic, 2.25% sodium hydroside and 5.4% glucose then washed 2 times as described by Hetta, *et al.* (1971). Prefixed tissue was postfixed with 2% osmic acid and dehydrated with acetone after washing with phosphate buffer. Dehydrated tissue was embedded with Epon 812 through 3 successive transfers (15 min for each step) under vacuum condition. Embedded tissue was hardened for 1 hour at 70℃ and then 2 hours at 100℃ with 15% DMP-30 for accelerating. Thin section of the tissue was made with ultramicrotome (Sourvall MT 6000). The thin sectioned tissue was stained with 2% uranyl acetate for 2 min and for 5 min in Reynolds lead citrate before examination under the eletron microscope.

Results and Discussion

Partially purified virus-like particles from abnormal growing cultures were further purified through either sucrose density gradient 10-40% or sucrose PEG density gradient.

Two light scattering bands located 2.4-2.6 cm and 4.5-5.4 cm below the meniscus were observed following sucrose density gradient centrifugation of partially purified virus (Fig. 1).

The upper band formed 2.4-2.6 cm below the

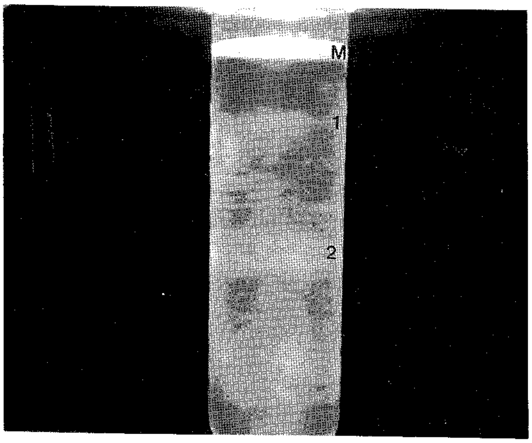

Fig. 1. Specific light scattering band produced by virus preparation of *P. florida* in sucrose density gradient centrifugation. M; Meniscus, 1 and 2; Viral light scatter

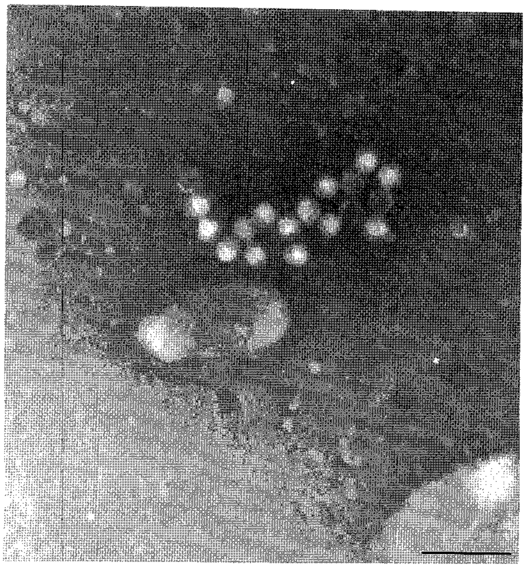

Fig. 2. Cell free virus particles from abnormal growing culture of *P. florida* (Bar equels 100 nm).

meniscus was a mixture of RNA empty virus particles and some of host cell debris, but the lower band formed 4.5-5.4 cm blow the meniscus contained spherical virus particles.

Prurified virus-like particles of *P. florida* were spherical type of about 30 nm in diameter (Fig. 2). But the particles from grey type isolate of *P. ostreatus* were a little smaller than those of *P. florida*. The particles were 23 nm in diameter as shown

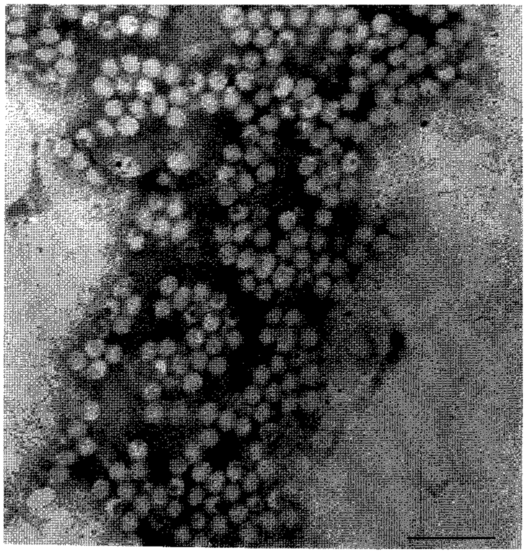

Fig. 3. Cell free virus particles from abnormal growing culture of *P. ostreatus* (Bar equels 100 nm).

Fig. 3. As shown Fig. 2 and Fig. 3 generally one kind of virus was observed in each species, respectively. However, in case of interspecies mating culture between *P. florida* and *P. ostreatus* showed both kinds of particles, 23 and 30 nm (Fig. 4). There were some empty particles among virus-like particles (arrow in Fig. 2). The empty particle was a little smaller than that of normal one. Other form of rod shape or filamentous particles was not observed.

It was the first time to find out the presence of virus particles from *P. florida*. It was very typical that virus particle in oyster mushroom was one type unlike other higher fungi. Common mushroom *Agaricus bisporus* contaminated several kinds of isomatric particles such as 25 nm and 34 nm in diameter and bacillifrom particles of 19 nm wide and 50 nm long (Atkey, P. T. 1985; Dieleman-van Zaayen, A. 1969; Dieleman-van Zaayen, *et al.* 1981). Oak mushroom, *Lentinus edodes* also has 25, 29, and 30 nm virus particles including flexible rod type of 15-17×1500 nm in maximum length, and rigid rod type of 25-28×280-300 nm (Mori, *et al.* 1974; Ushiyama, 1975; 1983; 1984). However, in case of inter-species mating culture between *P. florida* and *P. ostreatus* there were two types of virus, 23 nm and 30 nm particles. It

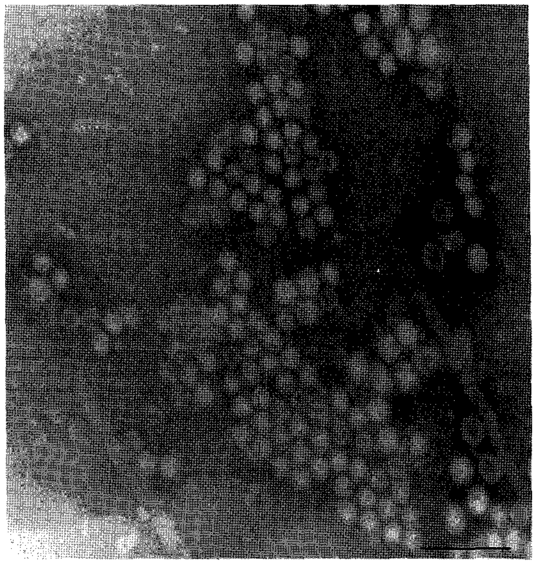

Fig. 4. Cell free virus particles from abnormal growing isolate of inter species mated culture between *P. florida* and *P. ostreatus* (Bar equels 100 nm).

means that there is a possibility to contaminate other kinds of virus in oyster mushroom through mating or protoplast fusion.

The virus particles were present throughout the cytoplasm in tissue of *P. florida*. The particles appeared dispersly and aggregated loosely as shown Fig. 5. The virus particles in ultra-thin section were a little smaller than those of cell free virus particles. The diameter of the particles in ultrathin section was averaged to 26 nm in *P. florida* instead 30 nm of cell free particles. The particles were shown in the cytoplasm but not in cell organells such as mitochondria and nucleus. but, often the particles were found in small vacuoles.

Purified 30 nm virus preparation was subjected to ultraviolet (U.V.) light analysis. The optimal spectrum absorbance of the virus was at 259.5 nm but minimum was at 242 nm as shown in Fig. 6.

The A260 : A280 (nm) obsorption ratio was about 1.59. As shown the UV spectrum, the virus preparation was consisted of nucleic acid. Usually around 260 nm of UV spectrum known that nucleic acid was contained in it.

As the above results, virus or virus-like particles were observed on the abnormal growing culture of *P. florida* and *P. ostreatus*. The particles were spherical type of 30 nm from the *P. florida* and 23 nm from the *P. ostreatus* in diameter, respectively. The virus particles might be associated with the bad crop of *Pleurotus* species in Korea.

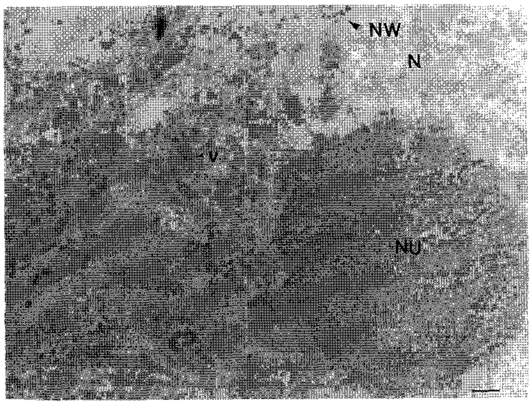

Fig. 5. Ultrathin sections of virus infected vegetative mycelium of *P. florida* (Bar equels 100 nm) NW; nuceli wall, N; nucleus, NU; nucleolus, V; virus.

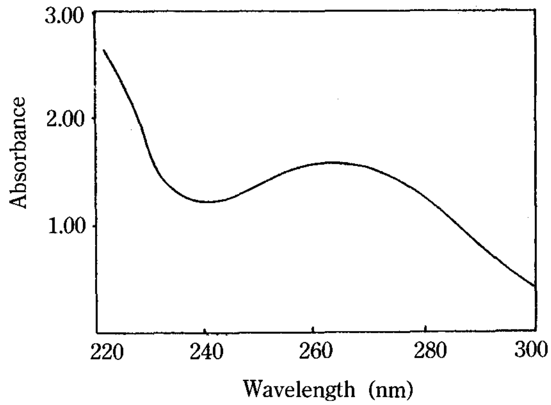

Fig. 6. Ultraviolet absorption spectrum of purified 30 nm virus particles from *P. florida*.

摘 要

菌絲生長이 不進한 사철느타리버섯 (*P. florida*), 느타리버섯 (*P. ostreatus*) 및 이들 두 種의 交配菌株로부터 30 nm 및 23 nm의 求刑 virus粒子를 單一 혹은 混合形態로 分離하였다. 사철 느타리버섯으로부터 分離한 virus의 最適 spectrum은 259.5 nm였다.

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