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# Molecular Analysis of double-stranded RNA in Abnormal Growing Oyster-Mushrooms, *Pleurotus florida* and *P. ostreatus* due to Virus Infection

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# Virus 罹病 느타리버섯(*Pleurotus*)으로부터 double-stranded RNA의 分離

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**ABSTRACT: The experiment was performed to find out the possibilities to detect virus infection in oyster mushrooms, *Pleurotus species* by analysis of doublestranded ribonucleic acid (ds RNA). Ds RNA segments were extracted from virus infected isolates which grew abnormally. But virus free isolates didn't show any ds RNA segments. The ds RNA was consisted of one large segment of 8100 base pairs (bp) and 4 smaller segments with 2170, 2120, 1980 and 1984 bp. Whereas, cell free virus particles showed only one larger ds RNA segment. The ds RNA was dissolved by RNase A in low salt, 0.1 M SSC and melted at 85℃. It was possible to use the ds RNA analysis for detecting virus infection directly from the host cells.**

**KEYWORDS: DS RNA. oyster mushroom, virus.**

It has been passed only 40 years since the first experimental evidence of fungal virus came with die-back disease of the cultivated common mushroom, *Agaricus bisporus* by Sinden and Hauser (1950). The virus was found to be associated with a severe disease of the cultivated mushroom.

The major virus or virus-like particles from fungi contain double stranded ribonucleic acid (ds RNA) segments. Evidence for the presence of ds RNA in the fungi led to our attraction with the facters of antiviral activity (Buck, 1979; Suzuki *at al.*, 1974). This antiviral activity was attributed to interferon induction by the viral ds RNA. Therefore, it was to be greatly stimulated research on fungal virus because fungal virus would be good source of ds RNA. Unforfunately ds RNA has toxicity in humen which is comparable to that of baterial toxins (Allexander *at al.*, 1971).

Successful isolation of ds RNA does not depended on the type of tissue, process and method. Therefore, it might be useful for the study of RNA virus replication and detection of virus infection directly from the host tissue (Dodds *at al.*, 1987; Marino *at al.*, 1976; Mori, 1978).

The authors found virus-like particles from abnormal growing oyster mushrooms, *Pleurotus florida* and *P. ostreatus* in a previous research (Go *at al.*, 1992). The particles were spherical with 30 nm in diameter from *P. florida* and 23 nm from *P. ostreatus*, respectively. The virus-like particles might be associated with bad crop of oyster mushrooms. The purified virus preparation showed its U. V. light spectrum of 295.5 nm. It mean that the viral genome would be consisted of nucleic acid. Ds RNA has been recognized as genetic materials of many plants, animals, fungal and bacte-

rial viruses (Libonati, *at al.*, 1980).

In this experiment, ds RNA from the abnormal growing oyster mushrooms due to virus infection was analyzed to find out their genome and possibility to use the ds RNA for detecting virus infection. And the ds RNA was characterized by enzymatic treatment and melting profile, etc.

## Materials and Methods

**Fungal culture:** The fungal cultures used in this experiment were previously described (Go *at al.*, 1992). Some other additional cultures of oyster mushrooms were also collected from bad crop farms throughout the country. A few cultures from abroad were examined for virus infection by ds RNA analysis.

**Ds RNA analysis:** Double-stranded ribonucleic acid (ds RNA) was extracted as described by Harmsen *at al.* (1989). The mushrooms were ground to a fine powder in a mortar jar. In a eppendorf tube, 100 mg of ground materials were mixed with RNA extraction buffer which consist of 250 mM NaCl, 200 mM Tris HCl (pH 7.5), 1 mM EDTA and one volume of phenol/chloroform. The tube was centrifuged at 10,000xg at R. T. for 1 hour. The upper phase was collected and mixed with one volume of 30% ethanol after about 25 mg of Bio-rad cellex N-1 was added. At least 1 hour later, unbounded nuclic acid with the cellex were removed by five successive washing with 1 m*l* STE buffer which consists of 100 mM NaCl, 50 mM Tris HCl (pH 8.0) and 1 mM EDTA in 15% ethanol. The ds RNA was eluted with 250 m*l* STE buffer 3 times successively. The ds RNA was precipitated with one volume of isopropanol for 1 hour. The ds RNA pellet was collected by centrifugation for 30 mins and dried in vacuum condition.

**Agarose gel electrophoresis:** Electrophoresis of ds RNA was done on 1% agarose gel in TAE buffer consist of 40 mM Tris acetate (pH 4.8 or 8.0) and 20 mM sodium acetate in the presence of 5 mg of ethidium bromide per m*l*. The gels were run at 60 mA until satisfactory migration. The RNA bands were observed under UV lamp. ds RNA from virus infected common mushroom, *Agaricus bisporus* as a reference ds RNA.

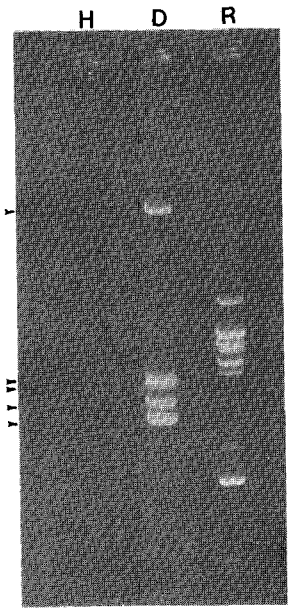


**Fig. 1.** Agarose gel analysis of ds RNA segments extracted from abnormal growing mycelium of *P. florida*. H; Normal growing mycelium, D; Abnormal growing mycelium, R. Reference ds RNA of virus infected common mushroom, *A. bisporus*.

## Results and Discussions

Abnormal growing mycelia of *P. florida* showed 5 segments of double-stranded ribonucleic acid (ds RNA). The largest segment was about 8100 base pairs (bp) and followed by 2170, 2120, 1980, and 1840 bp, respectively. The largest ds RNA band was equivalant to the sum of following 4 ds RNA segments. However, healthy mycelium did not contain any detectable ds RNA (Fig. 1).

The ds RNA contained culture showed virus like particls in previous research (Go, *et al.*, 1992). Ds RNA has been recognized as genetic materials of many plants, animals, fungal and bacterial viruses (Libonati, 1980). Therefore, the ds RNA contained cultures of *Pleurotus* species were associated with virus infection. Also, it is possible to use the ds RNA for the diagnosis of virus infection due to absence of the ds RNA in healthy cultures

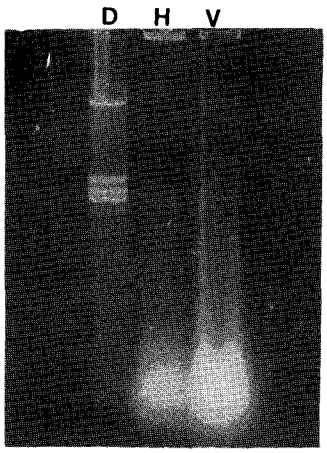


**Fig. 2.** The viral ds RNA segments from virus infected mycelium (D), cell free virus particles (V), and normal growing mycelium (H).

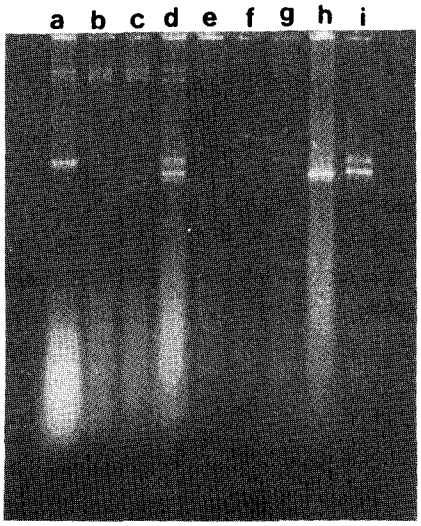


**Fig. 3.** Agarose gel analysis of viral ds RNA extracted from abnormal growing cultures of *Pleurotus* species. *The isolates were collected from following cities. a: Dangjin I, b: Dangjin II, c: Dagjin III, d: Okchun I, e: Okchun II, f: Ansung, g: Suweon, h: Wonju, i: Reference ds RNA from *P. florida*.

of oyster mushrooms like other plants and fungi (Dodds *at al.*, 1987; Morris *at al.*, 1979). The number of ds RNA segments in *Pleurotus* species was different from that of other edible mushrooms. Oak mushroom, *Lentinus edodes* and common mushroom, *A. bisporus* have 10 segments, respectibely (Harmsen *at al.*, 1989; Ushiyama, 1989). This difference was due to the kinds of virus infection. The oyster mushrooms were infected by only one kind of virus in each strain or species usually (Go *at al.*, 1992). But the oak and common mushroom were infected by 3-4 kinds of virus.

The ds RNA segment was extracted from cell free virus-like particles (Fig. 2). But the number of ds RNA segment was only one instead 5 segments of ds RNA from host cells. The cell free virus particles was equal to the largest segment from host mycelium.

As the virus-like particles contained only one ds RNA segment, the virus infected in *P. florida* was single species, and the virus needs only one segment of ds RNA for the multiplication. In the previous research, one kind of virus particles mophologically was observed. Mycoviruses are usually simple virus. In 1982 a proposal was made for the initial classification of ds RNA mycovirus into three families (Mathews, 1982). Among the families, Totiviridae with members that only need one or dicistronic ds RNA for multiplication. But other families need more than one segment. Therefore, the virus from *P. florida* might be belonging to the *Totiviridae* family.

While, in the cell, the number of ds RNA segments were 5. Perhaps the genomic cistron was synthesized from different parts of the whole genome and then encapsidated together in the cell. But this seems premature, because the genomic sequence did not analyzed yet. Ushiyama (1984) also reported similar results from *L. edodes*. The oyster mushroom of abnormal growing and suffering from bacterial yellow bloth disease caused by *Pseudomonas* spp. were analyzed ds RNA. Most of them contained ds RNAs. In case of *P. ostreatus* lane, g, and h in Fig. 3 also contained ds RNA. They showed a little different ds RNA pattern in number and position from the others mainly *P. florida* on the agarose gel.

Although they were belonging to same species, the number of ds RNA segments were different from each other. Isolate of a, b, and c from Dangjin city contained 4 segments in a, 2 in b, and

**Table 1.** Results of ds RNA analysis of leading strain and local isolates in the country and introduced isolates of *Pleurotus* spp. from Jap%an and European countries.

| Isolates | No. of analyzed isolates | No. of ds RNA containing isolates |
|---|---|---|
| Leading strain | 5 | 0 |
| Local isolates | 20 | 0 |
| Japanese isolates | 10 | 2 |
| European isolates | 10 | 3 |

3 in c, respectively. Isolate d from Okchun city contained 5 ds RNA segments but isolate e didn't contain any visible ds RNA segments. However, isolate g contained only one faint segment. The different number of ds RNA segments in host cells might show their severity of viral disease in viral disease of *Pleurotus* species. The severity was increased along with the number of ds RNA segments. But it needs more studies at moment.

To find out the trace of virus disease in Korea whether they were occurred locally or introduced from abroad, domestic and foreign isolates were examined. The extracts from local *P. ostreatus* isolates which distributed naturally throughout the country and leading strains for cultivation now a days in the country didn't show any ds RNA segment (Table 1). However, 2 Japanese and 3 European isolates showed ds RNA segments on the agarose gel. They were ASI2037 and 2038 from Japan and ASI 2142, ASI 2145 and ASI 2150 from Europe. The number is stock cultural number at the Agricultural Sciences Institute (ASI). ASI 2038 was identified to *P. cornucopiae* but the others were *P. ostreatus*. As the above results, the virus disease of oyster mushroom introduced from abroad by individual spawn maker without knowing it and distributed to local farms. The authors have known that a culture called 4401 belong to *P. florida* showing viral symptoms at first was introduced from abroad and distributed to local farmers by a individual spawn producer.

The ds RNA of virus infected oyster mushroom was characterized by enzymatic treatment and melting profile. The ds RNA was resistant to

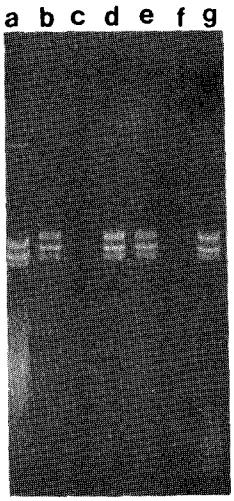


**Fig. 4.** Effects of RNase A and DNase on ds RNA from virus infected *P. florida*. a: Control without enzyme, b: RNase A in 1×SSC, c: RNase A in 0.1×SSC, d: DNase in 1×SSC, e: DNase in 0.1×SSC, f: RNase A in Distilled water (DW), g: DNase in DW.

RNase A in relatively high salt condition (1×SSC) as shown in Fig. 4.

The ds RNA from virus infected *P. florida* showed the maximum UV absorption spectrum at 258 nm and the minimum at 234 nm (Fig. 5). The absorption ratio of A 250/280 was about 1.86. This UV profile was almost same as that of cell free viral preparation in a previous research (Go, *at al.*, 1982). Therefore, the ds RNA from the host cell was the same to that of cell free virus particles.

The UV spectrum was same to that of other edible fungi, *Volvariella volvacea* (Chen, *at al.*, 1988) and *L. edodes* (Ushiyama, 1983) which were the maximum at around 260 nm.

The ds RNA solution in 0.01×SSC was treated at each temperature from 50 to 100℃ by 5℃ interval for 4 min and cooled rapidly on ice then analyzed them with Hitachi UV spectrophotometer 2, 000. The melting profile of the ds RNA increased up to 85℃ in 0.01×SSC solution (Fig. 6). The U. V. melting profile was characteric proerty for duplex nuclic acid molecules.

The thermal denaturation temperature of ds RNA from virus infected *L. edodes* was about 80℃

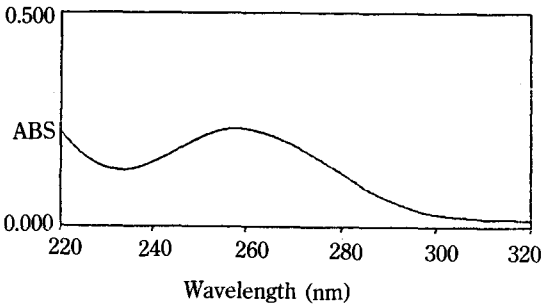


**Fig. 5.** Ultraviolet (U. V.) absorbance spectrum of ds RNA from virus infected *P. florida.*

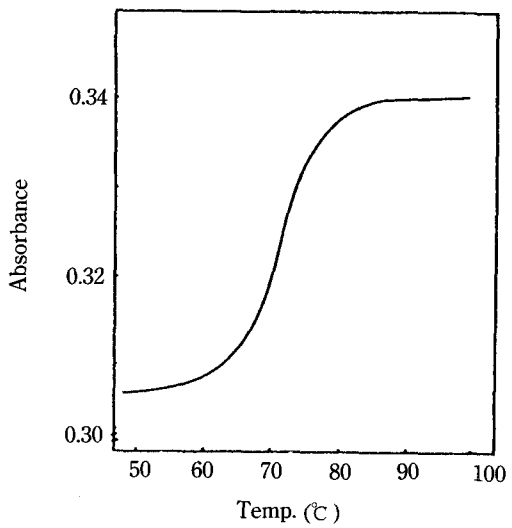


**Fig. 6.** Melting profile of ds RNA from virus infected *P. florida.*

(Ushiyama, 1983). Melting point of ds RNA in *A. bisporus* was at 79.5℃. Therefore, the melting point of ds RNA from *P. florida* was higher than those from *A. bisporus* and *L. edodes.*

## 摘 要

Virus 罹病 느타리버섯(*Pleurotus* spp.)으로부터 二重螺腺 ribo 核酸(ds RNA)을 分離하였다. Ds RNA는 8100 base pairs(bp)의 큰 band와 2170, 2120, 1980 및 1840 bp의 4개 작은 band로 이루어졌다. Ds RNA 分析法으로 느타리버섯의 Virus 罹病與否를 調査한 結果 菌絲生長이 不振하고 細菌性褐色 腐敗病 等에 罹病되고 異常子實體를 形成하는 느타리버섯으로부터 Virus 罹病을 確因하였으나 健全버섯으로부터는 ds RNA를 分離하지 못하였다. 이 病은 海外로부터 傳來한 것으로 보인다. Ds RNA는 底濃度鹽類液(0.1XSSC)에서 RNase A에 鎔解되었으며 85℃에서 特性變化가 發生하였다.

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