THE KOREAN JOURNAL OF MYCOLOGY
Vol. 24, No 4, p246-254 December 1996

Printed in S. KOREA

Xylogone sphaerospora, a New Fungal Pathogen of Cultivated *Ganoderma lucidum*

Jong-Kyu Lee*, Gyung Ja Choi[1], Kwang Yun Cho[1], Se Jong Oh[2], and Jeong Sik Park[2]

**Department of Forest Resources Protection, Kangwon National University, Chunchon 200-701, Korea, [1]Agrochemicals Screening Division, Korea Research Institute of Chemical Technology, Taejeon 305-606, Korea, [2]Division of Applied Microbiology, Agricultural Science and Technology, R.D.A, Suwon 441-707, Korea*

영지의 새로운 병원성진균 *Xylogone sphaerospora*

이종규* · 최경자[1] · 조광연[1] · 오세종[2] · 박정식[2]

강원대학교 삼림자원보호학과,
[1]한국화학연구소 스크리닝연구부,
[2]농업과학기술원 응용미생물과

ABSTRACT: Since the mid of 1980's, cultivation area and production of *Ganoderma lucidum* have been increased annually in Korea. However, the presence of a fungal disease has become a major limiting factor in the cultivation of *Ganoderma lucidum*, causing a serious economic loss. The present study was carried out to isolate and identify the pathogenic fungus to *Ganoderma lucidum*. Several fungi isolated from the wood logs showing typical symptoms were tested whether they are pathogenic to *Ganoderma lucidum* or not by cross-pairing culture method, flask inoculation method, and wood log inoculation method. The pathogenic fungus produced ascomata. Mature ascomata was spherical, dark, thick-walled, 45~95 μm diameter. Asci were thin-walled, evanescent when mature, disintegrate early. Ascospores were spherical, hyaline, glaborous, thick-walled, refractive, 3.6~4.3 μm in size. Conidiophores soon became abundantly septate and broke up into arthrospores, which are cylindrical, 3~6 μm long and 3~4 μm wide. Based on the observations under dissecting microscope, light microscope and scanning electron microscope, teleomorph and anamorph of the pathogenic fungus were identified as *Xylogone sphaerospora* Von Arx & Nilsson and *Sporendonema purpurascens* (Bonordon) Mason & Hughes, respectively. *X. sphaerospora* is first reported as a pathogenic fungus of *Ganoderma lucidum*.

KEYWORDS: *Ganoderma lucidum*, Mushroom Disease, Pathogenic Fungus, Scanning electron Microscopy(SEM), *Sporendonema purpurascens*, *Xylogone sphaerospora*

Concerns on *Ganoderma lucidum* is being increased as a food for health as well as a medicine in Korea. Because of the various pharmacological functions, the market scale of drinks supplemented with the extract from *Ganoderma lucidum* estimated up to 125 million dollars in 1995, and the demand of *Ganoderma lucidum* is prospected to be greatly increased from now on. Since the mid of 1980's, cultivation area and production of *Ganoderma lucidum* have been increased annually in Korea, and the most popular cultivation method is wood log pot cultivation using oak wood log. Cultivation area and the amount of production increased over 3 times and 4 times during last 5~6 years, respectively. Total

*Corresponding author

amounts of production was 1,823M/T in 1993, and 2,901M/T in 1994 (Cho *et al.*, 1995).

Since the late of 1980's, however, a new disease occurred in *Ganoderma lucidum* cultivation house is rapidly spreading over the entire cultivation area including Kangwha, Cheolwon, Shintanjin, and giving severe damages in the production of *Ganoderma lucidum* (Fig. 1)(Lee and Cho, 1995). In addition, mass import of *Ganoderma lucidum* with low quality and price from China is being another limiting factor in domestic cultivation of *Ganoderma lucidum*.

Nevertheless, so far, no research results about the causing agent, disease occurrence, ecological development and control method of the disease were reported. Studies on the diseases of cultivated edible fungi were mostly focused on Button mushroom (*Agaricus bisporus*), Oyster mushroom (*Pleurotus ostreatus* and *P. cystidiosus*), Shiitake mushroom (*Lentinus edodes*), Straw mushroom (*Volvariella volvacea*), Jew's ear mushroom (*Auricularia mesenterica*), and Enoke mushroom (*Flammulina velutipes*)(Eicker *et al.*, 1991; Lamber and Wuest, 1979; Sinden, 1971, 1972; Tu and Liao, 1989), and those on the diseases of *Ganoderma lucidum* are very rare (Cha *et al.*, 1989). Therefore, this study was carried out to isolate and identify the causal agent of the disease to establish effective management strategy for the control of the disease.

Materials and Methods

Observation of disease symptoms

Typical symptoms produced on oak wood log by the infection of the fungal pathogen were observed in the surface, and longitudinally cross-sectioned tissue of infected wood log by naked eye or by using dissecting microscope.

Isolation of the pathogenic fungus

Portions of the wood log showing typical symptoms were cut and chopped. These were soaked in sterilized distilled water for 2 hours, and then filtered through sterilized cheese cloth. Filtrate was diluted at 10^4 concentrations, and 100 μl of the diluted solution was placed on potato dextrose agar(PDA) plate with 200 μg/ml of vancomycin and 100 μg/ml of PCNB (Pentachloronitrobenzene). Added solutions were gently spread on the solidified PDA with a sterilized glass rod and plates were kept at 25°C. Mycelial tips were transferred on the fresh media and cultured.

Pathogenicity test

Isolates producing colony color similar to greenish yellow discoloration appeared on the infected wood log were selected and tested their ability to develop disease symptoms. Tests were carried out by cross-pairing culture method, flask inoculation method, and wood log inoculation method.

Cross-pairing culture method

Agar discs of PDA containing rapidly growing mycelium were cut from the edge of *Ganoderma lucidum* cultures by using 7 mm cork borer and placed, up side down, 2 cm apart from the center on PDA plates (dia. 9 cm), and then kept in the dark at 25°C. After a week, same size of agar disc from the isolated culture was paired 4 cm apart from the position on PDA plate inoculated with *Ganoderma lucidum* culture, and cultured to investigate the effects of isolated culture on the mycelial growth of *Ganoderma lucidum* culture.

Flask inoculation method

For the preparation of *Ganoderma lucidum* culture substrate, oak wood sawdust, mixed wood sawdust, and rice bran were mixed at the ratio of 2:2:1 (v/v/v), and moisture content

was adjusted at 65%. After 100 ml of mixed substrate were put into 250 m*l* volumes of flask, autoclaved and cooled, agar discs of PDA with mycelium of *Ganoderma lucidum* were inoculated and kept at 25~27°C. After a week, agar discs from the isolated culture were inoculated and cultured.

Wood log inoculation method

The isolated culture was inoculated to the oak wood logs (dia. 25~30 cm, height 20 cm) in polypropylene bags, in which mycelium of *Ganoderma lucidum* was already grown and formed white mycelial fan all over the surface of wood log. Inoculum was prepared by culturing the fungus in the mixture of oak wood sawdust, mixed wood sawdust, and rice bran at 25~27°C for 3 weeks. Inoculation was performed by spreading 50 m*l* of culture substrate and mycelium on the top of a log, followed by covering sterilized sand on it at 1 cm thickness. For the development of typical symptoms., relative humidity and temperature were controlled at the range required for the cultivation of *Ganoderma lucidum*.

Identification of the pathogen

Morphological characteristics such as shape and size of spore-bearing structure, spore, and hyphae of the pathogen were observed by using dissecting microscope, light microscope, and scanning electron microscope, and these were used as the identification keys for the pathogenic fungus.

Results and Discussions

Observation of disease symptoms

Typical symptoms appeared on the infected wood log were greenish yellow discoloration and the abundant presence of small, round, dark structures (Fig. 4). When the infected wood log was cross-sectioned, discolored parts were shown to be extended into the inside of wood log and the mass round, dark structures were observed on them (Fig. 3). If the disease was fully progressed, infected wood log was changed into black, and easily disintegrated.

Isolation of the pathogenic fungus

Isolates producing greenish yellow colony on the plate was selected, and cultured under the various environmental conditions in order to observe the production of spherical and dark structures, which can be observed on the naturally infected wood logs. Optimum temperature and pH for the mycelial growth of the pathogen were 25~30°C, and 4~5, respectively. Mass production of small, round and dark structures was obtained by giving alternative light condition of 14:10 hours (light: dark) on the media adjusted at pH 4-5 (Fig. 2).

Pathogenicity test

Cross-pairing culture method.

After inoculation of the isolated culture, mycelial growth rate of *Ganoderma lucidum* was retarded, and the growth was finally stopped. Trials to make survival by transferring the edge of *Ganoderma lucidum* cultures on fresh media were failed. On the other hand, mycelium of *Ganoderma lucidum* was normally grown on the plates untreated with isolated cultures (Fig. 5).

Flask inoculation method

Inoculation of the isolated culture in the flask resulted in the cease of mycelial growth of *Ganoderma lucidum* and produced a typical symptom of greenish yellow discoloration (Fig. 6, 7). Long-term incubation made white mycelium disappear, substrate change into black, and finally produced abundant small, round, dark structures. On the contrast, white mycelium was produced very densely in the un-

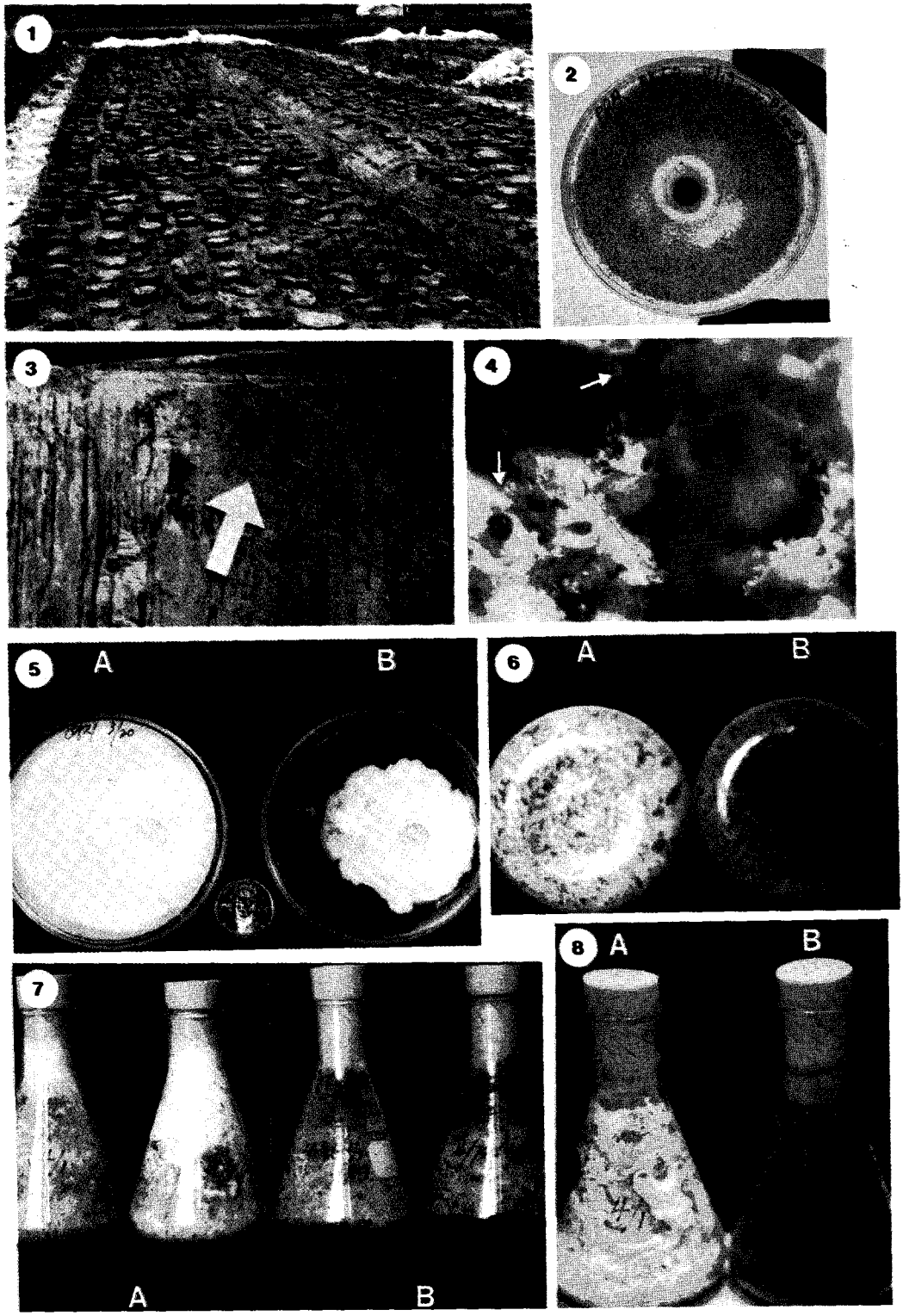
1
2
3
4
5 A B
6 A B
7 A B
8 A B

Fig. 1. A cultivation area of *Ganoderma lucidum* in Kangwha-gun, Kyunggi-Do, Korea, where *Ganoderma lucidum* was once cultivated by wood log sandwich method, but it is no longer cultivated because of the damage by a new pathogenic fungus, *Xylogone sphaerospora*.

Fig. 2. Ascomata formation of *X. sphaerospora* on PDA adjusted at pH 5.0 under the alternating light and darkness conditions(14:10 hours). A lot of tiny and black ascomata of *X. sphaerospora* were formed.

Fig. 3. Ascomata of *X. sphaerospora* formed on the cross-sectioned tissue of oak wood log used for the cultivation of *G. lucidum*. Mycelium of *G. lucidum* are normally grown in white color on the left side, while greenish yellow discoloration, a typical symptom of the infection by *X. sphaerospora*, is shown on the upper right. Arrow indicates very tiny and black ascomata formed on the infected tissue.

Fig. 4. Dissecting microscopy(DM) of ascomata formed in the infected tissues of oak wood log with a pathogenic fungus, *X. sphaerospora*. Arrow indicates spherical and black ascomata in the oak wood chips (×40).

Fig. 5. Pathogenicity of *X. sphaerospora* against *G. lucidum* on PDA plate. Plate A shows normal mycelial growth of *G. lucidum* without the inoculation of *X. sphaerospora*. Plate B shows the cease of mycelial growth of *G. lucidum* by the inoculation of *X. sphaerospora*. Agar disc from *X. sphaerospora* was paired, 4 cm apart from the agar disc of *G. lucidum*, 11 days after inoculation of *G. lucidum*. Cultures were kept in the dark at 25°C, and photo was taken 6 days after pairing of *X. sphaerospora*.

Fig. 6. Pathogenicity test of *X. sphaerospora* against *G. lucidum* in the flask. Agar plugs containing mycelium of *X. sphaerospora* were inoculated 8 days after growth of *G. lucidum* in the flask. Flask A shows fully grown white mycelium of *G. lucidum* to the bottom of flask without the inoculation of *X. sphaerospora*. Flask B shows greenish yellow discoloration at the bottom by the inoculation of *X. sphaerospora*.

Fig. 7. Pathogenicity test of *X. sphaerospora* against *G. lucidum* in the flask. Mycelial growth of *G. lucidum* in flasks A extended up and bottom of the flask, while that in flasks B was retarded and finally stopped when *X. sphaerospora* was inoculated 8 days after inoculation of *G. lucidum*.

Fig. 8. Comparison of two flasks after 4 months incubation with or without the inoculation of *X. sphaerospora*. Flask A shows normally grown white mycelium of *G. lucidum* without the inoculation of *X. sphaerospora*. Flask B shows no white mycelium of *G. lucidum* by the inoculation of *X. sphaerospora*. White mycelium of *G. lucidum* grown before inoculating *X. sphaerospora* was disappeared and changed to dark brown color. Harvested ascomata from the flask B was identical to those of inoculum.

treated flask (Fig. 8). Therefore, isolated culture was proved as a pathogenic fungus to *Ganoderma lucidum* since it first stopped mycelial growth of *Ganoderma lucidum* and produced typical symptoms which can be observed on the naturally infected wood logs. Second, in the inoculated flask, it produced abundant small, round, and dark structures, which are morphologically identical to the cultures used for inoculation.

Wood log inoculation method

Typical symptoms were apppeared on the logs 3 weeks after inoculation of the isolated culture. White mycelium of *Ganoderma lucidum* was changed into brownish, and asexual spores of the pathogenic fungus was observed on the mycelium of *Ganoderma lucidum*. As the disease progresses, the infected wood log showed greenish yellow discoloration, changed gradually into black, and finally decayed (Choi *et al*, 1996).

Identification of the pathogen

The most typical characteristic of the pathogen was the formation of ascomata with ascospores. Ascomata were spherical, astomous, superficial, no appendages. They were first bright, but became brown or black when ripe. Mature ascomata was dark, thick-walled by coiled hyphae, 45~95 μm diameter (Fig. 9, 10). Asci were nearly spherical, thin-walled, evanes-

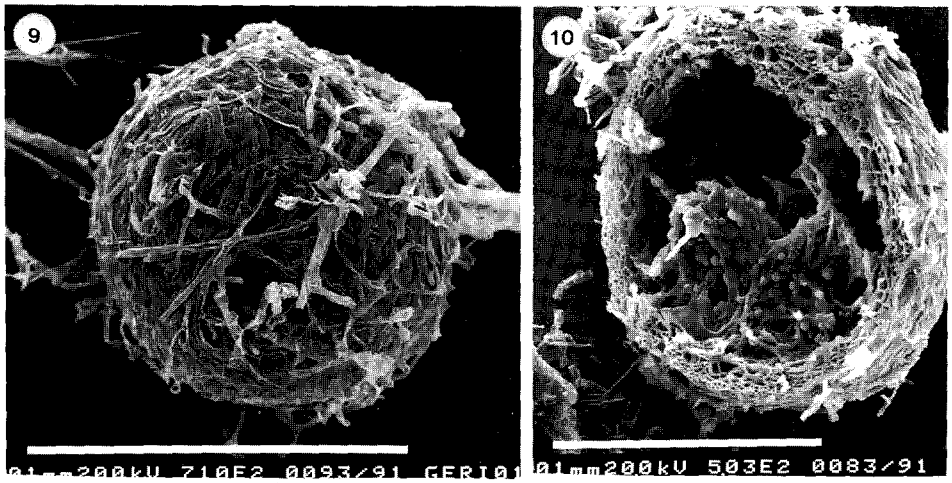

Fig. 9. Scanning electron microscopy(SEM) of ascomata formed by *X. sphaerospora*. Sizes of ascomata were variable and ranged from 45 μm to 95 μm. (bar=0.1 mm)

Fig. 10. Scanning electron microscopy(SEM) of an cross-sectioned ascocarp formed by culture of *X. sphaerospora*. Ascocarp was spherical, astomous, and thick-walled. (bar=0.1 mm)

cent when mature, 8-spored, and 8~12 μm in diameter (Fig. 11). The asci disintegrated early and therefore, it was very difficult to find them (Von Arx, 1981; Von Arx and Nilsson, 1969). Ascospores were spherical, hyaline, glabrous, thick-walled, refractive, 3.6~4.3 μm in size (Fig. 12). Conidiophores was born at the base about 2~2.5 μm broad. They soon became thick-walled, abundantly septate and broke up into arthrospores, which are cylindrical, 3~6 μm long and 3~4 μm broad and had a thick, double wall (Fig. 13, 14).

Based on the morphological characteristics of the isolated culture, the teleomorph stage of the pathogenic fungus was identified as *Xylogone sphaerospora* Von Arx & Nilsson, and the anamorph stage of the fungus was identified as *Sporendonema purpurascens* (Bonordon) Mason & Hughes (Von Arx, 1981). *X. sphaerospora* had been isolated from wood chips from *Pinus sylvestris*, *Picea* sp., and *Betula* sp. in Sweden. Other strains had been isolated from the stored pulp wood chips of different trees in Australia (Von Arx and Nilsson, 1969). So far, it has been known that *Trichoderma* sp. is the only pathogen in the cultivation of *Ganoderma lucidum*. But, *X. sphaerospora* is now reported as a new pathogen of *Ganoderma lucidum* in this paper.

Von Arx and Nilsson (1969) reported that this fast growing fungus is cellulolytic and is able to attack different kinds of hardwoods such as aspen, ash, birch, and beech. The attack starts as a lumenal erosion which ultimately leads to a general dissolution of the cell walls. In a decay experiments, the fungus was cultured on birch wood blocks that had been impregnated with a solution of ammonium nitrate. After 3 months' incubation at 35°C, the loss of wood substance was 11%. By these results, it is conferred that changes of wood log into black and the loss of hardness might be closely related to the decaying processes by the cellulase produced by this pathogenic fungus. Eicker *et al.* (1991) isolated *Sporendonema purpurascens* from mushroom beds and soils, and several isolates are known as pathogenic to mushrooms

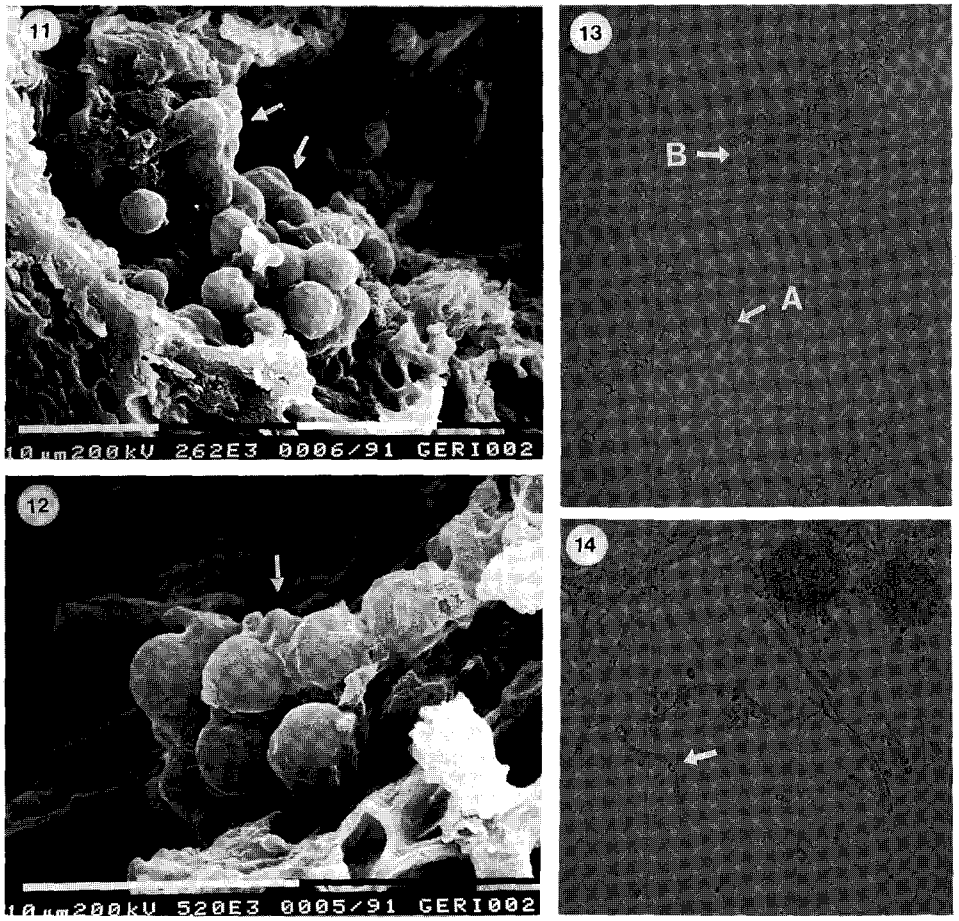

Fig. 11. Scanning electron microscopy(SEM) of asci and ascospores within an ascocarp formed by *X. sphaerospora*. Asci were thin-walled, evanescent when mature, and 8-spored. Arrow indicates the presence of thin-walled asci. (bar=10 μm)

Fig. 12. Scanning electron microscopy(SEM) of magnified asci and ascospores. Ascospores were spherical and 3.6~4.3 μm in size. Arrow indicates the presence of asci. (bar=10 μm)

Fig. 13. Light microscopy(LM) of asexual spores(arthrospores) formed by *Sporendonema purpurascens*. Arthrospores were hyaline, cylindrical, thick-walled, 3~6 μm long and 3~4 μm broad. Arrows indicate arthrospores(A) and initial stage of ascocarp formation(B). Ascocarp was developed from hyphae which become coiled. (×200)

Fig. 14. Arthrospore formation of *S. purpurascens*. Arthrospores formed by septation and breaking up of simple, erect conidiophores. Arrow indicates septation of a conidiophore. (×200)

such as *Agaricus* sp. and *Volvariella* sp.

For the effective control of this pathogenic fungus in the cultivation area of *Ganoderma lucidum*, studies on the physiology and ecology of the fungus, mechanism of disease development against *Ganoderma lucidum*, and screening of effetive control agents are now being conducted.

적 요

1980년대 중반이후로 우리나라에서 영지의 재배

면적과 생산량은 매년 증가해 오고 있지만 1980년대 말기부터 강화, 철원, 신탄지 등지에서 발생하기 시작한 새로운 병원성 진균의 출현에 의해서 영지재배는 막대한 경제적 손실을 입고 있으며 영지재배의 제한적 요인이 되고 있는 실정이다. 이 연구는 영지재배시에 발생하는 새로운 진균성 병해의 효과적인 방제수단을 찾기 위한 노력의 일환으로 병원성 진균을 분리하고 동정하기 위하여 실시되었다. 병원균에 의해 감염된 영지재배 원목에서 분리한 균주가 인공접종시 전형적인 병징을 나타낼 수 있는지 병원성 검정을 실시하였고, 병원성 진균에 의해서 생성되는 병징, 균사, 분절포자, 자낭과, 자낭포자 등을 육안, 해부현미경, 광학현미경, 주사전자현미경 등을 사용하여 관찰하였다. 이 병원균에 의하여 형성되는 자낭과는 성숙시 검은색의 두꺼운 막으로 된 구형으로 직경은 45~95 μm로 크기가 다양하였다. 자낭은 막이 얇고 형성되는 즉시 없어지기 때문에 매우 관찰하기 어려우며 자낭포자는 구형으로 무색이며 비교적 두꺼운 막으로 싸여 반들반들하게 보이고 크기는 3.6~4.3 μm정도로 균일하였다. 분생자병은 즉시 막이 두꺼워지고 격막으로 나누어지면서 분절포자가 형성되었고 분절포자는 원통형으로 길이가 3~6 μm, 폭이 3~4 μm 정도였다. 따라서 이상의 형태학적인 특성에 기초하여 이 병원균의 완전세대는 *Xylogone sphaerospora* Von Arx & Nilsson로, 불완전세대는 *Sporendonema purpurascens* (Bonordon) Mason & Hughes로 동정되었으며, 이 균은 영지의 병원균으로는 처음 보고되는 것이다.

Acknowledgement

We thank Dr. Bae Kyung Sook, Head of Culture Collection Program, Korean Collection For Type Cultures, Genetic Resources Center, Genetic Engineering Research Institute, Taejeon, Korea and Dr. R.A. Samson, Head of Applied Research and Services, Centraalbureau voor Schimmelcultures, The Netherlands, for their discussions on the culture identification. We also thank Mr. Oh, Hyun Woo, Electron Microscopy laboratory, Genetic Engineering Research Institute, Taejeon, Korea, for his contribution to SEM photography. This research was supported by the Agricultural Technology Development Project for UR, Ministry of Science and Technology, Korea.

References

Cha, D.Y., Park, J.S., and Kang, A.S. 1989. Medicinal mushroom(*Ganoderma lucidum*). Pp 382-403. *In*: Cha, D.Y., You, C.H., and Kim, G.P. Eds. Recent technology in edible mushroom cultivation. Nongjinhoe. Suwon, Korea.

Cho, K.Y., Lee, J.K., Kim, B.S., Choi, G.J., and Park, J.S. 1995. Studies on the management of a fungal disease of *Ganoderma lucidum*(I). 79pp. Research Report of the Ministry of Science and Technology, Korea.

Choi, G.J., Cho, K.Y., Lee, J.K., Kim, B.S., and Park, J.S. 1996. Studies on the management of a fungal disease of *Ganoderma lucidum*(II). 81pp. Research Report of the Ministry of Science and Technology, Korea.

Eicker, A., Peng, J.T., and Chen, Z.C. 1991. A survey of pathogenic fungi and weed moulds of cultivated mushrooms in Taiwan. Pp 425-429. *In*: Michael J. Mather. Eds. Mushroom Science XIII. Science and Cultivation of Edible fungi, Vol II. Balkema, Rotterdam, Netherlands.

Lambert, D.H. and Wuest, P.J. 1979. Characteristics of the mushroom pathogen *Verticillium* isolated from four continents and its tolerance to benomyl. Pp 747-757. *In*: Mushroom Science X. Proceedings of 10th International Congress on the Science and Cultivation of Edible fungi, France.

Lee, Jong-Kyu and Cho, Kwang Yun. 1995. *Xylogone sphaerospora*, a new pathogenic fungus of cultivated *Ganoderma lucidum*. *Korean Society of Mycology News Letter*. **7(2)**: 72.

Sinden, J.W. 1971. Ecological control of pathogens and weed moulds in mushroom culture. *Ann. Rev. Phytopathol.* **9**: 411-432.

Sinden, J.W. 1972. Disease problems in technologically advanced mushroom nurseries. *Mushroom Sci.* **8:** 125-130.

Tu, C.C. and Liao, Y.M. 1989. Major diseases of cultivated mushroom and their control in Taiwan. *Mushroom Sci.* **12(2):** 615-626.

Von Arx, J.A. 1981. The genera of fungi sporulating in pure culture. 3rd. ed. 424 pp. A.R. Gantner Verlag K.G., FL-9490 Vaduz, Germany.

Von Arx, J.A., and Nilsson, T. 1969. *Xylogone sphaerospora*, a new ascomycete from stored pulp and chips. *Svensk Botanisk Tidskrift.* **63(3):** 345-348.