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Control of Soybean Sprout Rot Caused by *Pythium deliense* in Recirculated Production System

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A soybean-sprout rot epidemic occurred in a mass production soybean sprout factory in 2000 and 2001 in Korea, which caused up to 20% production loss. Among the causal pathogenic bacteria and fungi, *Pythium deliense* was found to be the dominant pathogen of severe root and hypocotyls rot, particularly in recirculating water system. An average of 90% of the isolated fungi from the rotted sprout on potato dextrose agar were *Pythium* sp. The fungal density of *Pythium* in the sampled water was monitored in the recycled water system for 1 year using a selective medium (corn meal agar with Pimaricin, 10 mg; Rifampicin, 10 mg; and Ampicillin, 100 mg per 1 liter). The drained water from the soybean-sprout cultivation always had a certain amount of fungus in it. The removal of *Pythium* from the recycling water system must be thorough, safe, and environment friendly. However, the pathogen in the water was easily found even after ozone and chlorine treatments, which were devised on the recycling system for the removal of microorganisms. 5-μm pore size filter was applied and was able to successfully control the disease. As the sprout industry increasingly shifts into mass production, the demand for water will increase continuously. Recycling water for sprout production is eco-friendly. However, a process must be devised to be able to first decompose organic matters before *Pythium* zoospores are filtered.

Keywords **:** *Pythium deliense*, recycled water, rot, soybean sprout.

Soybean sprout has been a traditional food for over 1,000 years now in Korea. As the cultivation of soybean sprout shifts to mass production of commercial products, the demand for enormous amount of water has likewise increased. For example, 1,000-1,500 tons of water is needed to produce 20-25 tons of sprout products daily. Watering in sprout cultivation is effective in lowering the temperature, eliminating organic matter, and supplying oxygen inside the container (Park and Kim, 1998). However, it is difficult to obtain 1,000 tons or more of fresh water from the underground. In terms of environment-friendly water use, recycled water system needed to be introduced in mass production factories.

Yun and Kim (2003a) reported that *Pythium deliense* is the causal organism of severe root and hypocotyls rot of soybean sprout. High temperature, humidity and CO_2 concentration in the middle of commercial containers are much more conducive to rotting due to their high densities. Rotting usually becomes an epidemic during summer especially in mass production systems using recycled water. However, the problem is hardly found in small factories using only fresh water. Although only up to 20% of rot was detected, economic loss reached up to 30-35%. Sprout rot has caused unstable yield and low quality of the products, and selection has become time and labor consuming.

Epidemiological study has become necessary to evaluate *Pythium* rot against several bacteria and fungi of soybean rot. Several fungi, such as *Fusarium moniliforme, F. oxysporum, F. solani* (Oh and Park, 1996), and two bacteria, *Pseudomonas putida* (Park et al., 1997a) and *Erwinia carotovora* (Park et al., 1997b) were reported as the causal pathogens of soybean sprout rot. In addition, *Rhizoctonia solani, Macrophomia phascoli, Colletotricum* sp., and *Pseudomonas fluorescence* Biotype II have been reported in Japan (Takao et al., 1986). There was a need to evaluate *Pythium* rot to determine whether or not it was the major epidemic fungus in a particular epidemic incidence that occurred in recycled system. Five or more genus of pathogenic bacteria of soybean sprout rot were found in the same factory, but those were all endemic (Yun and Kim, 2003b).

After finding the epidemic microorganism responsible for severe soybean sprout rot, the disease should be controlled reasonably and in an eco-friendly way. Because of the consumers' demand for organic soybean sprout, chemical control is not appropriate.

This paper reports that the epidemic soybean sprout was caused by the pathogen *Pythium* based on the dynamics of *Pythium* occurrence on the diseased sprout and in water. The use of small pore-sized filter was an effective and eco-friendly way in controlling *Pythium* in recycled water. Daily yield was used to evaluate the control method.

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Materials and Methods

Isolation of pathogenic *Pythium* sp. Diseased soybean sprouts were sampled from a commercial factory (Umsung, Chungbuk) just before they were packed. Fungal isolation was conducted 15 times throughout the year. About 40 plates of potato dextrose agar (PDA) were used every isolation. The symptoms of rotted sprouts were mainly water-soaked brownish hypercotyl and cotyledon. Surface-sterilization of rotted soybean sprout was done partly with 70% ethyl alcohol for 1 min. Two or three parts of the diseased sprout were placed on each PDA plate. After 48 h culturing at 25°C, mycelia were checked whether or not these were that of *Pythium* sp. The fungus grew fast and had white fungal mycelium which covered the plate within 2 days.

Pathogenic tests were conducted on the germinated soybean seed in the test tube with 2% agar. Three grains of surface-sterilized soybeans with 0.5% NaOCl were placed on each pure-cultured *Pythium*-like agar block. Pathogenesis of each fungus was decided after 4 days of culture under darkness at 25°C.

Recycled water system and water sampling. Commercial soybean sprout factories make use of recycled water systems, which have a daily capacity of 1,500 tons. The recycled system clears the used water of sprout cultivation by means of ozone, sodium hypochloride, and several filters to reduce organic matters and microorganisms (Fig. 1). After treatments, the recycled system gathers water on 40-ton tanks. In order to measure the density of *Pythium* sp., sterilized 1-liter bags were used. Water samples were collected from: 1) the drain of container in the cultivation room; 2) fresh underground water; 3) right after the recycling treatments; and 4) 40-ton tank, which was ready to supply the culture room. Sampling was done 17 times from July 29, 2000 to May 2, 2001.

Culturing the *Pythium* on the selective medium. The density of the *Pythium* sp. in the water was measured by a selective medium (corn meal agar with Pimaricin, 10 mg; Rifampicin, 10 mg; and Ampicillin, 100 mg per 1 liter). 10 ml of sampled water was poured on each selective agar plate for 24 h at 25°C, then the sampled water in the medium was discarded and the plate incubated for one more day at 25°C. White and fast-growing mycelium colony on the agar was typical of *Pythium* (Jee et al., 2000). Seed health test was conducted 500 domestic and imported seeds on the selective medium. Without surface sterilization, 10 seeds were tested on each 9-cm Petri plate.

Application of 5-μm pore filter and daily yield check. The recycled water system originally made use of the 10-μm pore filter. However, organic matter in the water from the soybean sprout culture was easily clogging the filter. Hence, on August 15, 2001, the filter system was changed to 5-μm pore size to block the zoospore of *Pythium* sp. Yield was calculated as the product of soybean sprout per the amount of soybean seeds. Domestic and imported seeds from China were managed differently.

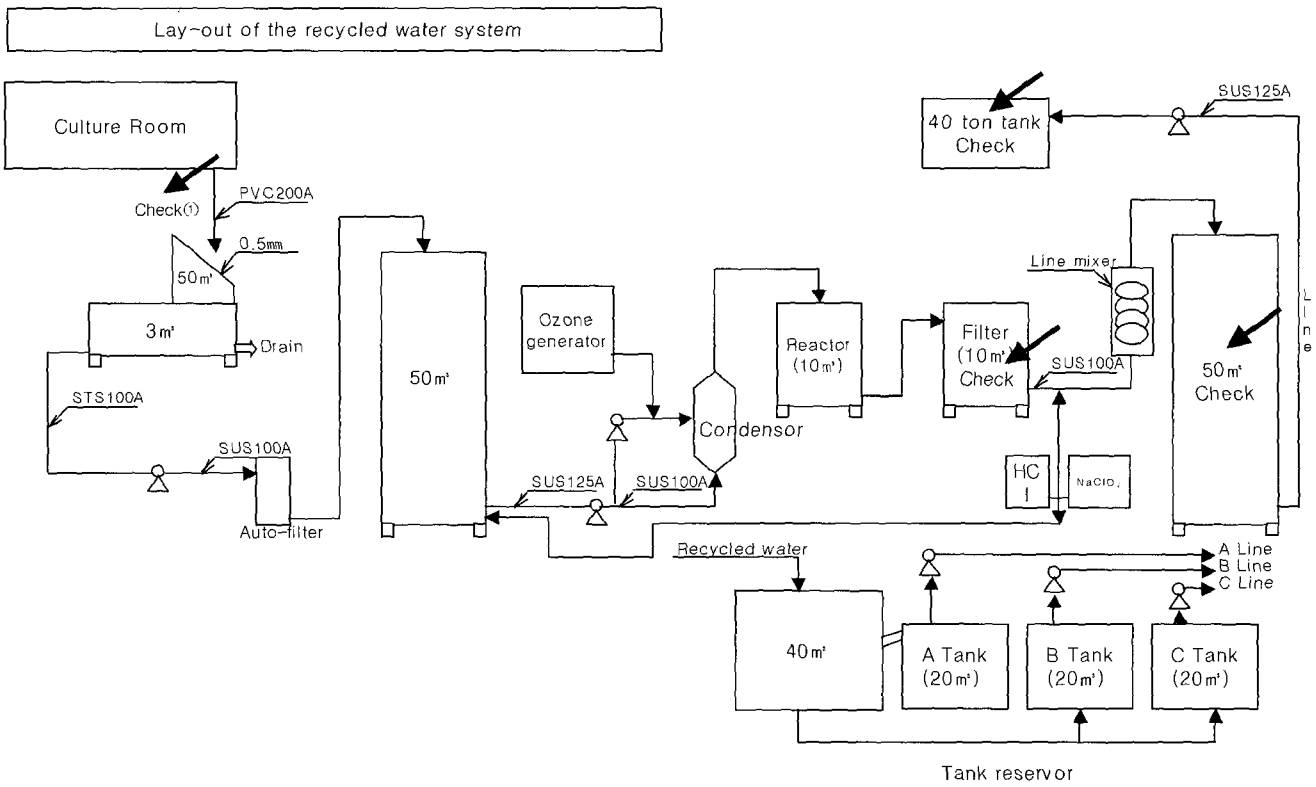

Fig. 1. Layout of the recycled water system for soybean sprout culture. Control of microorganisms and removal of organic matter were done by means of ozone, chloride, and micro filter system. The sampling sites were: 1) culture room; 2) 50 m^3 tank; 3) 40-ton tank; and 4) the ground water. Sample sites are marked by thick arrows.

Results and Discussion

Fungal isolation from the rotted soybean sprout was conducted 15 times, and the major fungal isolate was found to be *Pythium* sp. at 100%-32% (Table 1). A total of 404 plates contained *Pythium* sp., which grew fast and covered the plates within 2 days. Pathogenecity tests were conducted in the test tubes (pictures not shown) with the mycelial agar block. The fast-growing white mycelia on the tested seeds were covered, and most of the seeds failed to germinate. Among the isolates, 40 were identified as *Pythium deliense* (Yun and Kim, 2003a). The frequency (72%-100%) of *Pythium* sp. isolates was relatively high in the summer of 2000, and dropped to 35%-50% in the winter. Since soybean sprout rot at the factory was an epidemic in the summer, the seasonal pattern of *Pythium* occurrence was highly correlated. Moreover, this fungus has a high optimum temperature, which is 30°C or more (Kim and Park, 1997). Lowering the temperature of the container in the summer was deemed very important. It is possible to shorten the showering period if the temperature is low enough. Whereas, the seasonal differences of the soybean sprout bacteria was not apparent (Yun and Kim, 2003b). In addition, the bacterial profile on the recycled water system for sprout production was quite diverse, but the fungal profile on the same system was quite simple and *Pythium* sp. was the dominant species among the pathogenic fungi. The symptom after a 4-day growth in the tube was too severe to match the original symptoms isolated. The original symptom was detected in the pathogenecity test of sprout culturing using the container (Yun and Kim, 2003a).

Pythium sp. was not detected in the underground water (data not shown). In addition, the fungus was not found from the 1,000 soybean seeds during the seed-health test. Because this study failed to find the original source of *Pythium* in this particular system, it was not able to confirm whether the fungus goes around the recycled water system (Fig. 2) and causes severe epidemic rot. The drained water after showering of the sprout culture always had the fungus. *Pythium* sp. was found four times in the 40-ton tank, which was to be used to shower the sprouts. The fungus was also found at least three times in the water right after the

Table 1. Frequency of *Pythium* sp. from the rotted soybean sprout[a]

Sampling	Date	No. of sample plates	Plates with *Pythium*	Frequency (%)
1	22-Jul-2000	30	28	93.3
2	29-Jul-2000	33	24	72.7
3	03-Aug-2000	44	35	79.6
4	10-Aug-2000	44	41	93.2
5	22-Aug-2000	43	37	86.1
6	06-Sep-2000	40	35	87.5
7	27-Sep-2000	44	44	100.0
8	19-Oct-2000	40	30	75.0
9	29-Nov-2001	40	14	35.0
10	15-Dec-2001	40	25	62.5
11	05-Feb-2001	40	23	57.5
12	12-Mar-2001	40	25	62.5
13	19-Apr-2001	40	14	35.0
14	08-May-2001	40	16	40.0
15	16-May-2001	40	13	32.5

[a] *Pythium* plates were determined as showing white and fast-growing mycelial colony. Each isolate was tested for pathogenecity using 2% agar test tube.

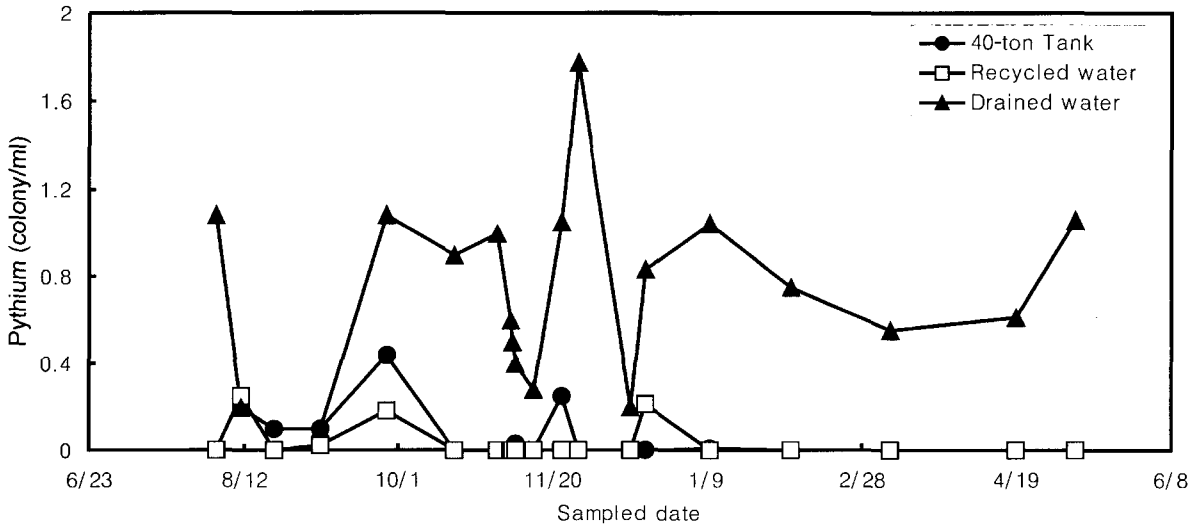

Fig. 2. The density of *Pythium* (colony/ml) in each sampled water before the use of 5-μm size pore filter. Drained water was sampled from the culture container drain. Recycled water was taken right after all the recycling treatments. The 40-ton tank water was ready to supply the cultivation. Although the density of the underground water was not shown, it did not have any fungus.

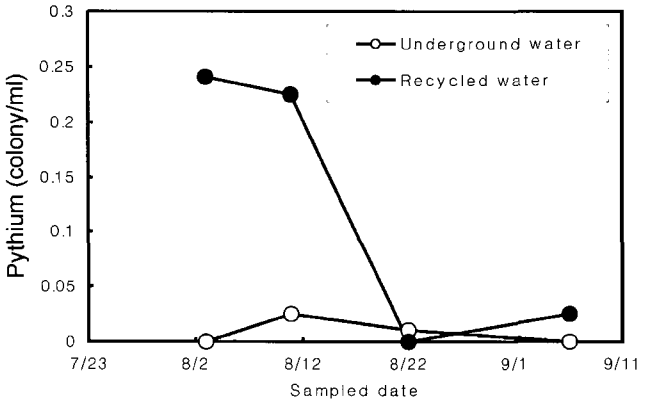

Fig. 3. The density of *Pythium* (colony/ml) in each sampled water after the use of 5-μm size pore filter. Drained water was sampled from the culture container drain. Recycled water was taken right after all the recycling treatments. The 40-ton tank water was ready to supply the cultivation.

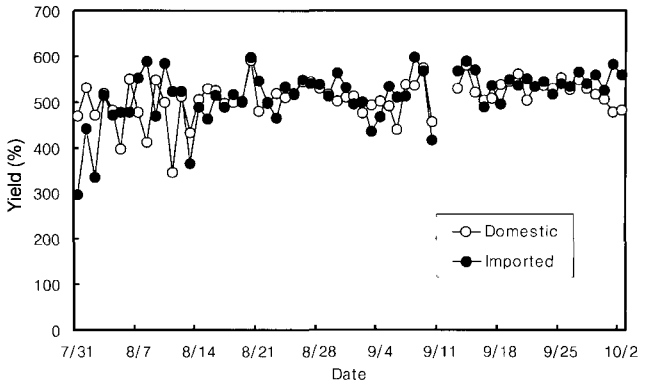

Fig. 4. Daily yield of soybean sprout production of domestic and imported soybean. Filter treatment means that the 5-μm filter size was applied to the recycled water system at that time.

recycling. This means that the fungal pathogen in the water after the treatments, such as ozone and chlorine, was not completely removed by the existing recycling facility and, hence, was not effective in eradicating the fungus.

Some chemicals were effective in controlling *Pythium* in water, such as 100 ppm of H_3PO_3 and 20 ppm of NaOCl, in the laboratory. However, with the consumers demand for organic soybean sprout, chemical control against the disease is not acceptable. Since the zoospore diameter of this fungus is up to 10-μm, 5-μm pore size filter was used. Organic matters from soybean germination always clogged the recycled filter system when 10-μm pore size filter was used. Pre-treatment of the recycled water needed to be done first in order to decompose the organic matter to filter the fungal zoospore effectively.

Figure 3 shows the density of the fungus in the recycled and underground water. Compared with Figure 2, the scale of the fungal density was 1/10. The fungal density was controlled more precisely due to the small pore size filter. However, severe clogging may occur and may stop the whole sprout production system. This problem must be solved to be able to come up with eco-friendly means to control the disease. Daily yields were monitored for 2 months including before and after the use of 5-μm pore size filter. After the adoption of this filter in mid-August, the yield became more stable than before (Fig. 4). Yields were around 500-550% of either domestic or imported soybeans. Stability was preferred over unstable high yield to be able to predict the product. After the filtering treatment, the fungus was not found (Fig. 3).

In Korea, there are more than 3,000 soybean sprout factories. Most of these factories are using home-made style containers which are small and shallow in depth. As the consumers demand for more reliable and fresh sprout increases, factories must produce them more massively under well-controlled environment. Based on these industrial changes, research on the cultivation of soybean sprout under well-controlled watering and bigger and deeper container conditions must be pursued (Yun and Kim, 2003b). Results of this study showed that in the mass production of soybean sprouts, filtering is an effective and eco-friendly means to control the disease. Without the removal of organic materials before filtering, successful *Pythium* control by filter would not be possible.

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