Plant Pathol. J. 17(3) : 141-148 (2001)

The Plant Pathology Journal

Occurrence and Biological Control of Postharvest Decay in Onion Caused by Fungi

Joon Taek Lee, Dong Won Bae[1], Seun Hee Park, Chang Ki Shim, Youn Sig Kwak[2] and Hee Kyu Kim*

Department of Agricultural Biology, Genetic Engineering Institute, Gyeongsang National University, Chinju 660-701, Korea

[1]Central Laboratory, Gyeongsang National University, Chinju 660-701, Korea

[2]Kumho Life and Environmental Science Laboratory, Kwangju 500-712, Korea

(Received on November 20, 2000)

Postharvest decay of onion bulbs was examined by inspecting the commercial packages in the market or in storage. Bulb rot incidence was unexpectedly high, and onion bulbs with 1st quality grade were rotten most severely by 51%, followed by 32% for 2nd and 21% for 3rd grades. This indicates that larger bulbs had higher incidences of bulb rots. Major pathogens associated with basal and neck rots were *Fusarium oxysporum* and *Aspergillus* sp. or *Botrytis allii*, respectively, of which basal rot was most prevalent and damaging during storage. Among the epiphytic microorganisms from onion plants, several *Bacillus* and *Paenibacillus* spp. and previously selected *Pseudomonas putida* and *Trichoderma harzianum* had inhibitory efficacy against bulb rot pathogens. Among these *B. amyloliquefaciens* BL-3, *Paenibacillus polymyxa* BL-4, and *P. putida* Cha 94 were highly inhibitory to conidial germination of *F. oxysporum* and *B. allii*. *P. putida* Cha 94, *B. amyloliquefaciens* BL-3, *P. polymyxa* BL-4, and *T. harzianum* TM were applied in the rhizoplane of onion at transplanting. Initially antagonist populations decreased rapidly during the first one month. However, among these antagonists, rhizoplane population densities of BL-3, Cha 94, and TM were consistently high thereafter, maintaining about 10^4-10^5 cells or spores per gram of onion root up to harvest time. The other bacterial antagonist BL-4 survived only for two months. TM was the most effective biocontrol agent against basal rot, with the number of rotten bulbs recorded at 4%, while that of the control was 16%. Cha 94 was effective for the first 20 days, but basal rot increased thereafter and had about the same control efficacy as that of BL-3 and BL-4. When the antagonists were applied to the topping areas of onion bulbs at harvest, TM was the most effective in protecting the stored onion bulbs from neck rotting. The second effective antagonist was BL-3. TM and BL-3 completely suppressed the neck rot in another test, suggesting that biocontrol of postharvest decay of onion using these microorganisms either at the time of transplanting or at harvesting may be promising.

*Corresponding author.
Phone) +82-55-751-5443, FAX) +82-55-751-5199
E-mail) heekkim@nongae.gsnu.ac.kr

Keywords : *Allium cepa* L., biological control, postharvest decay.

The current status of postharvest diseases of onion (*Allium cepa* L.) in Korea is little understood, although losses caused by these diseases are remarkable (Lee, 1988; Chung, 1982). In storage conditions, a number of fungi, including *Fusarium* spp., *Botrytis* spp., and *Aspergillus niger*, were found on diseased onion bulbs (Schwartz and Mohan, 1995). Also, some opportunistic bacteria are associated as endophytic microflora with naturally infected onion bulbs from farms and packing sheds. These include *Pseudomonas aeruginosa, Serratia marcescens, Bacillus cereus, Erwinia, Klebsiella, Enterobacter*, and *Escherichia* spp. (Presly, 1985).

Soilborne pathogens invade roots by direct penetration and/or through wounds. Invasion of the stem plate is achieved either by growth of the pathogen from infected roots or through natural wounds in the lower portion of the stem plate area. Initial invasion by neck- rotting pathogens was reported to begin at the neck of onion at the time when some plant leaves are dead by wilting and breakdown, or after the death of the entire top at maturity, and thereafter they penetrate slowly into the inside tissue of the bulb (Machacek, 1929; Abawi and Lorbeer, 1971). However, Maude and Presly (1977) reported the main source of neck rot in stored bulbs in relation to the etiology of *Botrytis allii*. When infected seeds germinate, the cotyledon tips are invaded by the fungal mycelium, contaminating the seed coat that remains attached to cotyledons when seedlings emerge from the soil. The fungal mycelium then grows downward into the tissues and invades the neck of the onion bulb internally.

The germination of *A. niger* spores was stimulated by onion bulb sap. No spore germinated at 13°C while minimum temperature for mycelial growth on onion scales was 17°C (Bertolini and Tian, 1997). Stow (1975) observed that rotting of large bulbs was more severe than that of small ones under the optimal conditions of 30°C and RH 50-80%.

The storage disease was substantially reduced, if topped onions were dried at 30°C under sufficient airflow. The treatment was most effective if the crop was removed from the field for drying within 48 h of topping, thus, avoiding severe infection of the damaged green neck tissue (Maude et al., 1984). Bulb rot (*Fusarium oxysporum* f. sp *cepae)* of onions in the field and its subsequent development during storage was reduced by fertilizer treatments containing P, whereas, yields responded to N applications (Ashour et al., 1973). Fungicide treatment had also been attempted (Chung, 1992; Maude and Presly, 1977; Ashour et al., 1973; Moity et al., 1982), but this approach is not desirable for fresh products which are released shortly to the market.

Biological control should is one of the most reliable approaches to protect bulbs from either storage pathogens or other opportunistic microorganisms, which are associated with postharvest losses. A few biocontrol have been tried against postharvest diseases (Moity et al., 1982; Jayswal et al., 1990). However, in Korea no attempts have been made so far to determine the biotic factors involved in postharvest losses of onion, and thus to develop biological control strategies. The objectives of this study were to assess current postharvest losses due to storage diseases of onion, to isolate and identify pathogens associated with storage disease, and to select and determine the feasibility of antagonistic microorganisms for their biocontrol potentials.

Materials and Methods

Occurrence of bulb rot in commercial onion packages. Onion bulbs (*Allium cepa* L.) in commercial packages (20 kg/package) with three different quality grades (1st, 2nd, and 3rd grades) were provided by or purchased from the Namhae Branch of the National Agricultural Cooperative Federation (NACF), Korea. Rotten or decayed bulbs were inspected visually from November 1995 to April 1996.

Isolation and identification of microbes associated with bulb rot in storage. Rotten onion bulbs in storage or in the market were collected from Changryeong, Namhae, Hamyang, and Euiryeong of Gyeongnam province in Korea from August 1996 to February 1997. Onion bulb tissues were surface-sterilized with 1% sodium hypochlorite for 1 min, rinsed in sterile distilled water, and placed on water agar in an incubator at 28°C. Fungal mycelium outgrowing from the tissues were transferred to potato-dextrose agar (PDA), and maintained on PDA with half concentration (hereafter designated as HPDA) at 28°C. Isolated fungi were identified by their physiological and morphological characteristics according to the previous descriptions (Everts and Schwartz, 1985; Jayaswal et al., 1990; Schwartz and Mohan, 1995).

Pathogenicity of fungal isolates was estimated by the frequency of major fungi colonizing onion bulb rots during storage (at 4°C for 45 days) of the onion bulbs which were purchased from the Namhae Branch of NACF. During storage, neck rot and basal rot were examined at intervals of 20 days.

Selection of antagonists against bulb rot pathogens. Epiphytic microorganisms were isolated from onion growing in fields to select antagonists against bulb rot fungi. Onion leaves were sampled from four locations, Hamyang, Changryeong, Namhae, and Euiryeong in Korea at 10-day intervals from February 1996 to March 1996. The collected samples were immersed in 9 ml of sterile 0.1 M $MgSO_4$ and shaken for 30 min at 200 rpm in a rotary shaker (KMC, Korea). The sample suspensions were serially diluted, and aliquots of 0.1 ml were plated on nutrient agar (NA) or HPDA and incubated at 28°C for 2 days. Bacterial colonies with variable colony shapes were chosen randomly, and cultured on HPDA to test their antagonistic activities by the dual culture method. A total of 100 isolates including *Pseudomonas putida* Cha 94 and a fungal antagonist *Trichoderma harzianum* TM which had been previously used in the laboratory were selected and tested for antagonistic activity in the first screening experiment.

For antagonistic activity test, each antagonist was inoculated at five places, 3 cm apart from the center with equal spacing on the periphery of a HPDA plate and incubated at 28°C for 1 day, and a mycelial disc 9 mm in diameter of the pathogen grown on PDA at 28°C for 1 week was inoculated in the center of the plate. The plate was placed at 28°C. After 1 week of incubation, inhibition of the mycelial growth adjacent to the antagonists was examined.

Effect of antagonistic bacteria on the conidial germination of *Fusarium oxysporum* and *Botrytis allii.* In the first screening experiment, three good bacterial strains *P. putida* Cha 94, *Bacillus amyloliquefaciens* BL-3, and *Paenibacillus polymyxa* BL-4 were further tested for biocontrol activity against *F. oxysporum* and *B. allii* isolated from decayed onion bulbs. Each antagonist was cultured in PD broth at 28°C for 24 h and 48 h, respectively, and the bacterial suspension was mixed with conidia (5×10^5/ml) of *F. oxysporum* and *B. allii,* then pipetted in a hole slide glass placed at 28°C and 20°C, respectively. Conidial germination was examined every 30 min.

Preharvest application of selected antagonists for the control of basal rot caused by *F. oxysporum.* *Pseudomonas putida* Cha 94, *B. amyloliquefaciens* BL-3, *P. polymyxa* BL-4, and *T. harzianum* TM were used for studies of biocontrol effect by field application. For selecting rifampicin-resistant mutants of the antagonistic bacteria for population monitoring in rhizoplane of onion after treatment, 100 µl of PD broth culture of Cha 94, BL-3, and BL-4 was evenly spread on HPDA amended with 50 µg/ml of rifampicin (Sigma Chemical Co., St. Louis, USA) ($HPDA_{rif50}$). After incubation at 28°C for 4 days, colonies occurring spontaneously on the media were transferred to fresh HPDArif50. Selected rifampicin-resistant mutants were stored in 30% aqueous glycerol at -70°C.

Rifampicin-resistant mutants of BL-3, BL-4, and Cha 94 were grown on PD broth for 48 h at 28°C and harvested by centrifugation at 5000 rpm for 10 min, then rinsed three times with 0.1 M $MgSO_4$. The number of viable cells in suspension was adjusted approximately to 1×10^7-10^8/ml. Also, conidia of TM were collected from the PDA cultures grown at 28°C for one week,

adjusted to the concentration of 10^8 spores/ml.

Onion cv. Changryeong-Daego (Seoul Seed Co., Korea) was used for the biocontrol experiment in a field in Hamyang, Korea, which has a cropping history of onion for 20 years with year-by-year, onion-rice rotation during the last 10 years. Onion seedling roots were dipped for 1 h in the suspensions of the antagonistic bacteria or fungal spores. Then, the treated plants were planted in plots in the field (plot size: 2 × 2.2 m) on November 4, 1996 by the randomized complete block design with three replications.

To examine the bacterial and fungal populations on the onion rhizoplane, root samples (1 g from 0.5 cm below the stem plate) were transferred to 9 ml of sterile distilled water and shaken on a rotary shaker at 200 rpm for 30 min at 28°C. For the bacterial pathogens, the suspensions were serially diluted and plated on HPDA amended with 50 mg/ml rifampicin ($HPDA_{rif50}$). For *T. harzianum* TM, Trichoderma-seletive medium (NH4NO3, 1 g; glucose, 3 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; K_2HPO_4, 0.9 g; KCl, 0.15 g; agar, 24 g; PCNB, 0.2 g; rose bengal, 0.15 g; and chloramphenicol, 0.25 g per liter of distilled water) was used instead of $HPDA_{rif50}$. Populations of the antogonists on the onion rhizoplane were examined monthly.

As the onion plants were treated on roots, the basal rot of onion was examined during the storage to examine the biological control effect of the antagonist treatments. At harvest (July 23, 1997) roots and tops of onion were clipped, and onion bulbs were packed in mesh bags (50 bulbs in a bag), and stored at ambient temperature (32-38°C at daytime; 21°C at night time) for 1 month. Then, the onion bulbs were stored at 4°C for 1/2 month. Basal rot symptom development was examined every 20 days after storage.

Biocontrol effect of antagonist treatments at topping on the control of onion neck rot. Onion neck rot is presumably caused by infection of *B. allii* or *Aspergillus* sp. through harvesting practice i.e. neck topping. Therefore, antagonistic bacterial and fungal suspensions prepared as above were sprayed on the topping areas of onion bulbs immediately after removing shoots (topping) at harvest (July 1, 1997). Onion roots were also clipped, but not treated with the antogonists. The treated onion bulbs were packed in mesh bags (50 bulbs per bag) stored at ambient temperature (32-38°C and 21°C for day and night) until August 19, 1997 before storing at 4°C for one and a half months from August 23 to October 6. During storage, the bulbs treated were inspected every 20 days for neck rot symptom development. Each treatment had three replicates of three mesh bags. Incidences of neck rot and severity were examined.

Onion bulb decay index was used for describing severity of neck rot, which was based on the degree of symptoms developed: 0, no neck rot; 1, slight internal neck rot; 2, moderate internal neck rot; 3, moderate neck rot and bulb decay; 4, severe neck rot and bulb decay.

Results

Occurrence of bulb rot in commercial onion packages. Onion bulb rot occurred more severely than expected. When the commercial onion packages in storage and in the market were examined, about 37% of the total onion bulbs examined were rotten with the highest incidence (51%) in the 1st grade onion packages (Fig. 1). Onion bulb rot was less severe in the 2nd and 3rd grades with bulb rot of 32% and 28%, respectively. One of the most important criteria for grading onion bulbs is the size, which means that larger bulbs had more bulb rot than smaller ones.

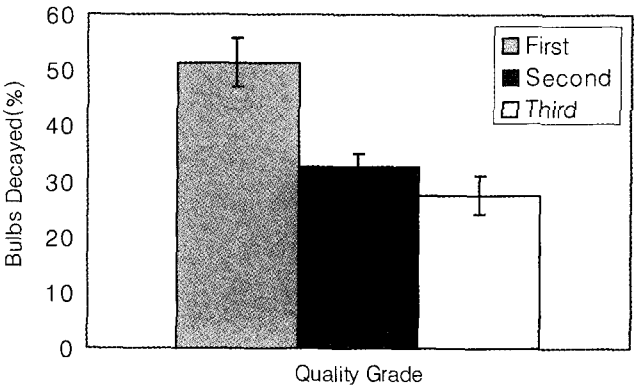

Fig. 1. Postharvest decay of onion bulbs in commercial packages with 1st, 2nd, and 3rd quality grades. Onion bulbs sampled from commercial packages in storage and in the market were inspected from November 1995 to April 1996.

Isolation and identification of microorganisms associated with onion bulb rot. Various fungi and bacteria were isolated from rotten bulbs. Among them, major pathogens related to bulb rots were identified as *F. oxysporum*, *Aspergillus* sp., and *B. allii* (data not shown). Specially, *F. oxysporum* was related with basal rot, while *Aspergillus* sp. and *B. allii* were related to neck rot. When the causal influence of the pathogens in bulb rot was expressed by the frequency of their colonization on decayed bulbs, *F. oxysporum* had a value of approximately 21-30%, and the other two 11-20% (data not shown), indicating that *F. oxysporum* may be the most frequent and damaging. However, no basal rot symptoms caused by this pathogen were observed in the experimental field in this study.

Inhibitory effects of antagonists against bulb rot pathogens. Among the epiphytic microorganisms from onion plants during the growing season of 1996, several *Bacillus* and *Paenibacillus* spp. and previously selected *P. putida* and *T. harzianum* had inhibitory efficacies against bulb rot pathogens (Table 1). All of antagonistic bacteria, except *B. pantothenticus* BD-1, showed inhibitory effect against the three storage fungi with somewhat variations in the degree of mycelial growth inhibition. In particular, *B. amyloliquefaciens* BL-3 and BL-5, *B. macerans* DD-8, *P. putida* Cha 94, and *T. harzianum* TM were highly inhibitory to *Aspergillus* sp., most of which strongly inhibited the mycelial growth of *B. allii*. *Paenibacillus polymyxa* BL-4 and BE-2 and *T. harzianum* TM showed the highest inhibitory effect against *F. oxysporum*.

Table 1. *In vitro* antagonism of selected microorganisms against onion bulb decay fungi during storage

Antagonist		*Apergillus* sp.	*Botrytis allii*	*Fusarium oxysporum*
Species	Strain			
Bacillus amyloliquefaciens	BL3	+++[a]	++++	++
	BL5	+++	++++	+++
	BL6	++	+++	+++
B. circulans	BJ-8	++	+++	++
B. macerans	DD-8	+++	++++	+++
B. pantothenticus	BD-1	–	–	+++
B. pumilus	BB-7	+	++	+++
B. pulvifaciens	BH-7	+	++	+++
B. subtilis	BC-21	++	++++	+++
Paenibacillus polymyxa	BL-4	++	++++	++++
	BE-2	++	++++	+++++
Pseudomonas putida	Cha94[b]	+++	+++	++
Trichoderma harzianum	TM[b]	+++	++++	++++

[a] Inhibition of fungal mycelial growth by dual culture of an antagonist and a pathogen on potato-dextrose agar with half concentration at 28°C for 5 days: +++++, > 6 mm; ++++, 5-6 mm; +++, 4-5 mm; ++, 3-4 mm; +, 2-3 mm; –, no inhibition.
[b] Selected strains from the previous study.

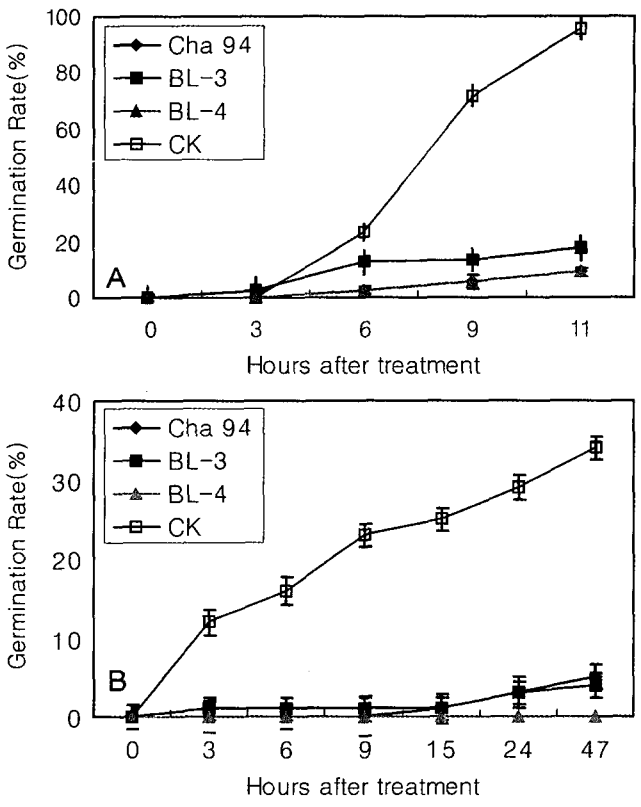

Fig. 2. Effects of antagonistic bacteria, *Bacillus amyloliquefaciens* BL-3, *Paenibacillus polymyxa* BL-4, and *Pseudomonas putida* Cha 94, on suppression of conidial germination of *Fusarium oxysporum*, a basal rot pathogen of onion bulbs (**A**), and *Botrytis allii*, a neck rot pathogen of onion bulbs (**B**). Antagonists were cultured in PD broth mixed with conidia of *F. oxysporum* or *B. allii*, and incubated at 28°C or 20°C.

Conidial germination of *Fusarium oxysporum* was suppressed to below 15% 11 h after treatment of antagonists, compared to 95% under the antagonist-free conditions at 28°C (Fig. 2A). BL-4 and Cha 94 were more efficient in inhibiting conidial germination than BL-3. For *B. allii*, a low temperature fungi, conidial germination rate was also suppressed to 0-5% by the antagonistic treatments as compared with 33% of the control when incubated at 20°C for 47 h (Fig. 2B). No spore was germinated in the spore suspension treated with BL-4.

Preharvest application of selected antagonists for the control of basal rot caused by *F. oxysporum*. *Pseudomonas putida* Cha 94, *B. amyloliquefaciens* BL-3, *P. polymyxa* BL-4, and *T. harzianum* TM were applied in the rhizoplane of onion. Initially antagonist populations decreased rapidly during the first 1 month. However, among these antagonists, rhizoplane population densities of BL-3, Cha 94, and TM were consistently high thereafter, mainintaining about 10^4-10^5 cells or spores per gram of onion root up to harvest time (Fig. 3). The other bacterial antagonist BL-4 survived only for 2 months.

Effect of preplanting treatments of antagonists on the control of postharvest decay (basal rot) of onion was examined. Basal rot incidence was low at harvest time in *T. harzianum* TM and *P. putida* Cha 94, compared with the control and other antagonists. *T. harzianum* TM was the most effective biocontrol agent against basal rot, with the number of rotten bulbs recorded at less than 4%, while that of the control was 16% (Fig. 4). *P. putida* Cha 94 was effective for the first 20 days, but basal rot increased thereafter and had about the same control efficacy as that of *B. amy-*

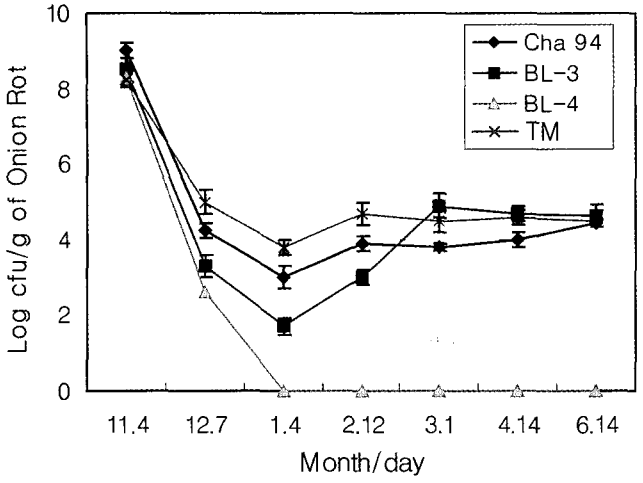

Fig. 3. Population changes of antagonists *Bacillus amyloliquefaciens* BL-3, *Paenibacillus polymyxa* BL-4, *Pseudomonas putida* Cha 94, and *Trichoderma harzianum* TM on onion rhizoplane during the cultivation period after preplanting treatment. Roots of onion seedlings were dipped in the suspensions of the antagonists for 1 hr, and transplanted on November 4 in a field at Hamyang. Data, averages, and standard deviations of three replications are shown in the graph.

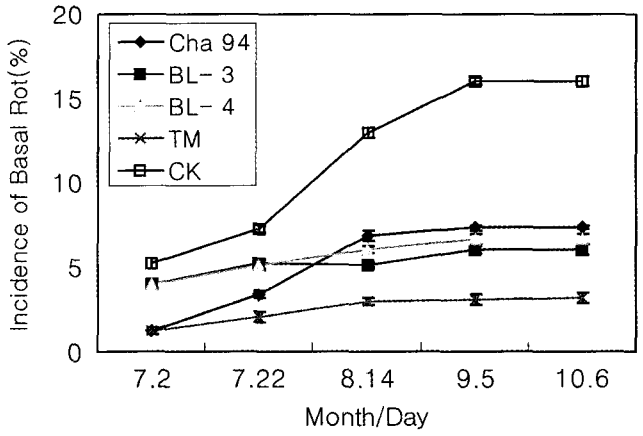

Fig. 4. Incidence of basal rot on onion bulbs during storage as influenced by preplanting treatment of antagonists *Bacillus amyloliquefaciens* BL-3, *Paenibacillus polymyxa* BL-4, *Pseudomonas putida* Cha 94, and *Trichoderma harzianum* TM. After harvest (July 1, 1997), onion bulbs were packed in mesh bags (50 bulbs in a bag), and stored at ambient temperature (32-38°C at daytime; 21°C at night time) until August 19, and stored at 4°C for the rest of the time. Data are averages and standard deviations of three replicates.

loliquefaciens BL-3 and *P. polymyxa* BL-4.

Biocontrol effect of antagonist treatments at topping on the control of onion neck rot. Onion neck rot during storage was more prevalent than basal rot. When the antagonists were applied to the topping areas of onion bulbs at harvest, the disease was generally suppressed with variations among the antagonists. As in basal rot, *T. harzianum*

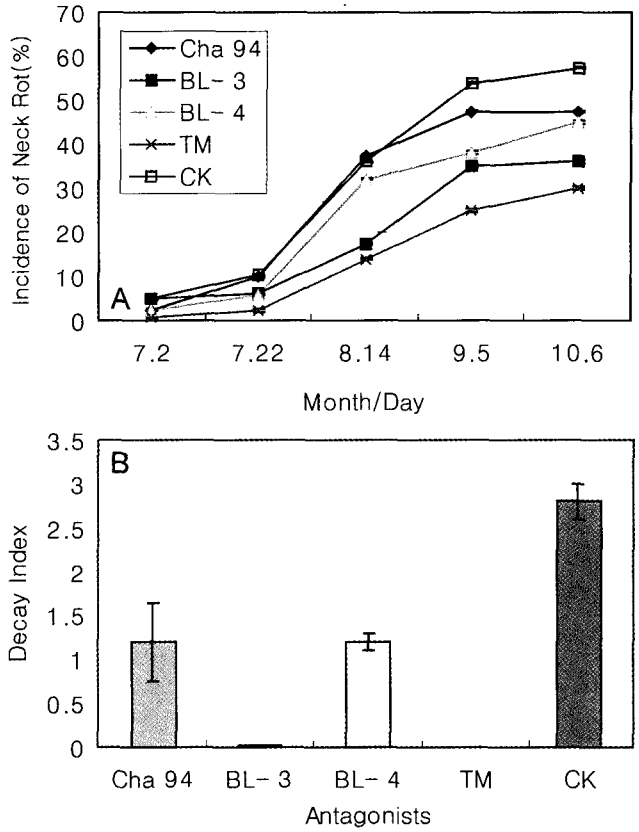

Fig. 5. Incidence (**A**) and severity (decay index) (**B**) of neck rot of onion bulbs during storage as influenced by antagonistic treatments (*Bacillus amyloliquefaciens* BL-3, *Paenibacillus polymyxa* BL-4, *Pseudomonas putida* Cha 94, and *Trichoderma harzianum* TM) at harvest. After harvest (July 1, 1997), onion bulbs were packed in mesh bags (50 bulbs in a bag), and stored at ambient temperature (32-38°C at daytime; 21°C at night time) until August 19, and stored at 4°C for the rest of the time. Data are averages and standard deviations of three replicates. Decay index: 0, no neck rot; 1, slight internal neck rot; 2, moderate internal neck rot; 3, moderate neck rot and bulb decay; 4, severe neck rot and bulb decay.

TM was the most effective in protecting the stored onion bulbs from neck rotting (Fig. 5A). The second most effective antagonist was BL-3. However, when another set of samples was inspected based on disease severity (disease index), BL-3 and TM completely suppressed neck rot, while the other antagonists had a control efficacy of about 50% relative to the control (Fig. 5B).

Discussion

During storage of agricultural products, there are two types of losses; rotting and desiccation losses which generally occur in high and low humidity conditions, respectively. Storage condition of 50-80% humidity was optimum to minimize 'rotting and sprouting' at 30°C (Stow, 1975). In

this study, the current status of postharvest losses of onion bulbs was examined from November 1995 to April 1996. Unexpectedly, onion bulb rot was found very severe during storage. This high losses can be attributed to the wet weather condition during harvesting at the time of the study. Onion bulbs from the commercial packages of the 1st grade were most severely decayed with 51% bulb rots, followed by the 2nd and the 3rd grades with 32% and 28% bulb rots, respectively. Similar results were also reported by Stow (1975). The reason why onion bulbs from the 1st grade packages were most severely decayed was presumably due to the higher moisture content of the bigger onion bulbs than the small ones. The present criteria for quality grading are primarily based on the size of bulbs, and large bulbs may contain more moisture and loose tissues than small ones.

The importance of timely harvest, rapid handling and marketing to minimize postharvest losses of short-day onions grown in warm environments had been emphasized when cold storage is not used (Wall and Corgan, 1994). In Korea, most harvesting is done from late spring to early summer. At this time of the year, intermittent rain shower is frequent, and accordingly harvested bulbs was improperly air-dried. To promote air drying of onion bulbs, packages are often piled to about 1.2 m high outdoors, which may provide optimum environmental conditions for high-temperature fungi like *Aspergillus* sp. to invade the green neck tissue of onion. Optimum harvest time was at 80% maturity, according to Wall and Corgan (1994).

In storage conditions, many fungi, including *Fusarium* spp., *Botrytis* spp., and *Aspergillus niger*, were recorded on diseased onion (Schwartz and Mohan, 1995). Among the fungi, the most frequent and damaging was *B. allii* (Presly, 1985), which was reported to infect bulbs through wounds produced during harvesting, leaf removal and marketing (Smalley and Hansen, 1962). However, Maude and Presly (1977) contended that the seed-borne fungus, *B. allii*, invaded deeply within the neck tissues of maturing bulbs. Chung (1982) reported that postharvest losses of onions due to *Botrytis* spp. and *Fusarium* spp. were greater with delayed harvesting. He further observed that neck rot was predominant for late-harvested bulbs. Harvesting of onion crops with mechanical removal of the foliage (topping), followed by postharvest drying at ambient temperatures (ca. 18°C) resulted in an increase in neck rot incidence (Maude et al., 1984). The disease was substantially reduced if topped onions were dried at 30°C with an hourly airflow of 425 m^3. It was suggested that initial invasion by a weak parasite such as *Aspergillus niger* began at the neck of the onion at breaking over, or after the death of the entire top at maturity (Machacek, 1929). Conidial germination was stimulated by onion bulb sap from such wounds (Tanaka, 1981).

Cother et al. (1976) identified *P. aeruginosa* from internal brown spot rot of onion, which develops lesions only in the leaf bases near the center of the bulb, but neither in immature bulbs nor on wound-inoculated leaves. Cother and Dowling (1986) also postulated that *Pseudomonas*, including *P. aeruginosa, Erwinia, Klebsiella, Enterobacter*, and *Escherichia* spp., *Serratia marcescens*, and *Bacillus cereus* on onion bulbs may become opportunistic pathogens when changes in host physiology are triggered by high temperature at bulb maturity.

Contrary to the reports in many other countries, this study showed that the most damaging pathogen for onion in Korea was *F. oxysporum*. However, no basal rot was observed during the growth in fields. This suggests that the fungal pathogen may survive and remain quiescent with steady and slow growth only enough to maintain the biomass of the pathogen at rhizosphere environments in the fields, but not enough to proliferate beyond the threshold population to be epidemic during the cultivation period. It may be during storage when the pathogen activates and causes rots due to various conditions including environments and plant physiology favorable to the pathogen. On the other hand, neck rot was severe during the ambient temperature storage, probably caused by high-temperature fungi such as *Aspergillus* sp.

No cultivars were determined to be resistant to the pathogens or opportunistic bacteria (Cother et al., 1976). Also, the postharvest decay is influenced profoundly by environmental factors which have yet to be well understood. Moreover, chemical treatment for storage products is not desirable at all because of the harmful effect of chemical residue. Therefore, biological control using microorganisms is one of the most promising ways to reduce storage diseases. One possibility for substantially increasing plant yields without imposing environmental threats is to make use of certain microorganisms that occur in all agricultural soils that protect plants from pathogenic or opportunistic bacteria. Fluorescent Pseudomonads, particularly *Pseudomonas putida* and *Pseudomonas fluorescens*, which are commonly isolated from the plant rhizosphere, have been shown to protect plants from fungal infection (Joyaswal et al., 1990). Pusey et al. (1984) attempted postharvest biocontrol of stone fruit brown rot by *Bacillus subtilis*. Promising microbes from ecological niche for biocontrol of cucumber wilt fungus *F. oxysporum* f. sp *cucumerinum* were *T. harzianum, Gliocladium virens*, and *P. putida* (Kim and Jee, 1987). Moity et al. (1982) reported that the combination of iprodione with *T. harzianum* (ipro-25M), an iprodione-tolerant, showed control against white rot. However, no attempts on biocontrol of onion storage decay have been published so far.

Some antagonistic microbes in this study seem to be promising as biocontrol agents. Various antagonistic bacteria were from the phylloplane of onion crops. Among them, *B. amyloliquefaciens* BL-3 and *P. polymyxa* BL-4 were effective in reducing pathogen growth and spore germination. Also, previously isolated antagonists *P. putida* Cha94 and *T. harzianum* TM had similar activities. However, the BL-4 population on onion rhizoplane rapidly decreased and gave less control effect on basal rot. Among the bacterial antagonists, BL-3 was most effective in controlling both basal and neck rots. The fungal antagonist TM has better or similar control efficacy than BL-3. *B. amyloliquefaciens* strain BL-3, producing heat-stable antifungal protein (Bae, 1999), reduced decay regardless of the inoculum level or the isolate of pathogenic fungi tested, and was effective at temperatures of 10-30°C.

Treatments of the antagonists applied at seedling transplanting time or sprayed immediately after topping reduced basal rot and neck rot incidences. Especially for neck rot, a scoring method of bulb decay based on the degree of rot symptoms was developed, which may facilitate the evaluation of neck rot. After antagonisitc microoganisms, Cha 94, BL-3, BL-4, and TM were treated, an evaluation of the biocontrol potentials of applied strains was done by inspecting samples for decay based on the disease index of onion bulb. BL-3 and TM were the most effective. Onion bulbs with as high as disease index 1 maintain the integrity and firmness, and were not distinguishable from completely healthy ones. This may explain the contradictory results that slight incidence of neck rot in BL-3 had a disease index 0 (Fig. 5, 6), although each result was derived from different samples.

The control of postharvest decay of onion can be further improved by combining chemical and biological treatments to seed or seedlings at the time of transplanting. Also, integrated approaches after harvesting such as improved storage methods should be attempted to facilitate drying and to eliminate the chance of neck rot infection. A new method, alternative to the conventional way of stocking onion packages, to prevent the physical damage of bulbs during storage should also be established to minimize the postharvest decay of onion bulbs.

Acknowledgment

This research was funded by the SGRP/HTDP (Special Grants Research Program/High-Technology Development Project) of the Ministry of Agriculture and Forestry (MAF) of Korea.

References

Abawi. G. S. and Lorbeer, J. W. 1971. Pathological histology of four onion cultivars infected by *Fusarium oxysporum* f. sp. *cepae*. *Phytopathology* 61:1164-1169.

Abawi, G. S. and Lorbeer, J. W. 1971. Populations of *Fusarium oxysporum* f. sp. *cepae* in organic soils in New York. *Phytopathology* 61:1042-1048.

Abd-El Moity, T. H., Papavizas, G. C. and Shatla, M. N. 1982. Induction of new isolates of *Trichoderma harzianum* tolerant to fungicides and their experimental use for control of white rot of onion. *Phytopathology* 72:396-400.

Ames, A. 1915. The temperature relations of some fungi causing storage rots. *Phytopathology* 5:11-19.

Ashour, W. A., Ali, M. D. H., Morsy, A. A. and Diab, M. M. M. 1973. Effects of some cultural practices and fungicides on basal bulb rot of onion. *Agriculture Research Review* 51(3): 153-162.

Bae, D. W. Purification and characterizaton of an antifungal protein from *Paenibacillus macerans* PM-1 antagonistic to rice blast fungus, *Pyricularia oryzae* Cavara. Ph. D Thesis. Gyeongsang Natl. Univ., Chinju. 85 p.

Bertolini, P. and Tian, S. P. 1997. Effect of temperature on production of *Botrytis allii* conidia on their pathogenicity to harvested white onion bulbs. *Plant Pathology* 46:432-438.

Chung, H. D. 1982. Control of onion bulb rot during low temperature storage by postharvest fungicide treatment. *J. of the Korean Society for Horticultural Science* 23(2):109-121.

Cother, E. J. and Dowling, V. 1986. Bacteria associated with internal breakdown of onions bulbs and their possible role in disease expression. *Plant Pathology* 35(3):329-336.

Cother, E. J., Darbyshire, B. and Brewer, J. 1976. *Pseudomonas aeruginosa* cause of internal brown spot rot of onion. *Phytopathology* 66(7):828-834.

Eastburn, D. M. and Butler, E. E. 1991. Effect of soil moisture and temperature on the saprophytic ability of *Trichoderma harzianum*. *Mycologia* 83(3):257-263.

Jayaswal, R. K., Fernandez, M. A. and Schroeder III, R. G. 1990. Isolation and characterization of a *Pseudomonas* strain that restricts growth of various phytopathogenic fungi. *Applied and Environmental Microbiology* 56(4):1053-1058.

Kim, H. K. and Jee, H. J. 1988. Influence of rhizosphere antagonists on suppression of cucumber wilt, increased cucumber growth and density fluctuation of *Fusarium oxysporum* f. sp. *cucumerinum* Owen. *Korean J. Plant Pathol.* 4(1):10-18.

Lee, J. T. 1998. Fungi associated with storage diseases of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) and biological control of postharvest decay. M.S Thesis. Gyeongsang Natl. Univ., Chinju. 47-56 pp.

Machacek, J. E. 1929. The black mold of onions, caused by *Aspergillus niger* Tiegh. *Phytopathology* 19:733-739.

Maude, R. B., Shipway, M. R., Presly, A. H. and O'Connor, D. 1984. The effects of direct harvesting and drying systems on the incidence and control of neck rot (*Botrytis allii*) in onions. *Plant Pathology* 33:263-268.

Maude, R. B. and Presly, A. H. 1977. Infection of onions by *Botrytis allii*. *Ann. of Appl. Biol.* 85(1):165.

Presly, A. H. 1985. Studies on *Botrytis* spp. occurring on onions (*Allium cepa* L.) and leeks (*Allium porrum L.*). *Plant Pathol-*

ogy 34:422-427.

Pusey, P. L. and Wilson, C. L. 1984. Postharvest biological control of stone fruit brown rot by *Bacillus subtilis*. *Plant Disease* 68:753-756.

Schwartz, H. F. and Mohan, S. K. 1995. Compendium of onion and garlic diseases. APS Press. 54 p.

Stow, J. R. 1975. Effects of humidity on losses of bulb onion (*Allium cepa*) stored at high temperature. *Expl. Agric* 11:81-87.

Wall, M. M. and Corgan, J. N. 1994. Postharvest losses from delayed harvest and during common storage of short-day onions. *HortScience* 29(7):802-804.