Plant Pathol. J. 18(4) : 199-203 (2002)

The Plant Pathology Journal

Soft Rot of Onion Bulbs Caused by *Pseudomonas marginalis* Under Low Temperature Storage

Yong-Ki Kim*, Seung-Don Lee, Chung-Sik Choi, Sang-Bum Lee and Sang-Yeob Lee

Plant Pathology Division, National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon 441-707, Korea

(Received on April 2, 2002)

Soft rot occurred severely in onion bulbs stored under low temperature (5°C) in storage houses at Changyoung, Kyungnam province, Korea in early 2000. Water-soaking and yellowish-brown lesions initially appeared on the outside scales of diseased onion bulbs, gradually progressing into the inside scales. Among the bacterial isolates obtained from the lesions, K-2 isolate was found to be responsible for the disease, which grew at a temperature range of from 0° to 36°C with optimum temperature of 30°-33°C. However, it showed strong pathogenicity to onion bulbs at 25°and 5°C at 3 days and 2 months, respectively. The bacterium also caused soft rot on potato and showed hypersensitive reactions to tobacco and potato. The causal bacterium of onion soft rot was identified as *Pseudomonas marginalis* based on morphological, biochemical, and physiological characteristics including LOPAT. Soft rot in onion under low temperature storage caused by *P. marginalis* has not been previously reported.

Keywords : low temperature, onion, *Pseudomonas marginalis*, soft rot.

Onion (*Allium cepa* L.) has been widely cultivated in Korea as an important vegetable crop. Since most onions are harvested intensively from mid May to late June, these are stored under low temperature condition of about 2°-5°C for a long period. However, onion harvesting often coincides with the rainy season in Korea, thus, adequate ventilation to maintain low humidity in the low temperature storehouse is not readily achieved. In addition, onion bulbs are easily wounded and spoiled by pathogenic and even by saprophytic fungi inhabiting the surface of the bulbs because of their soft and weak tissues (Kasmire and Cantwell, 1992).

Onions stored under low temperature condition have been known to be attacked mainly by psychrophilic fungi such as *Botrytis allii*, *Penicillium* sp., and *Fusarium* sp. in Korea (Kim et al., 2001). However, a soft rot of onion bulbs caused by bacteria under low temperature storehouse occurred at Changyoung in early 2000. Incidence of the disease reached up to 15% in severely damaged warehouse. The causal bacterial agent was identified and the pathogenicity of the isolate to onion bulbs under low temperature condition was proven in this study.

Materials and Methods

Isolation of causal agents. Onion bulbs showing water-soaking or yellowish-brown rot under low temperature storage condition were used for isolation. These were washed with tap water and cut longitudinally. The diseased scale tissues were cut into 5 mm cubes by using sterilized surgical blade. Three pieces of onion scale were ground in 1 ml of distilled water using a mortar and pestle. The suspension was streaked onto nutrient broth yeast (NBY) extract medium (NBY, 8 g; yeast extract, 2 g; K_2HPO_4, 2 g; KH_2PO_4, 0.5 g; glucose, 2.5 g; agar, 15 g; and distilled water, 1 l). Plates were incubated at 28°C for 48 h. Discrete colonies were re-streaked onto NBY plates for pure isolation.

Characterization of the causal agents. Three isolates were used for identification. Electron micrographs of the isolates were taken with EM (H-800, Hitachi Co.) as previously reported by Lee et al. (1999). Bacteriological characteristics of the isolates were examined by using the methods described by Lelliott and Stead (1987) and Palleroni (1984) (Bergy's Manual). Gram stain, colony color, LOPAT test (levan type colonies), oxidase reaction, potato rot or pectate liquefaction, arginine dihydrolase, tobacco hypersensitivity, egg yolk reaction, fluorescent pigment on King's medium B (proteose peptone#3, 20 g; $K_2HPO4{\cdot}3H_2O$, 2.5 g; $MgSO_4{\cdot}7H_2O$, 6 g; glycerol, 15 ml; agar, 15 g; and distilled water, 1 l), potato rot and hypersensitivity reaction, and pectate degradation test were conducted according to the method described by Lelliott and Stead (1987). Biochemical characteristics of the isolates such as levan production, oxidation reaction, arginine hydrolysis activity, and nitrate reduction were tested by using the methods described by Goto and Takikawa (1984a and 1984b). Utilization of carbohydrates was also investigated by using the Biolog system.

Pathogenicity test. Healthy onion bulbs were used for the pathogenicity test. With the use of a toothpick, wounds were made on an onion bulb to inoculate the causal agent. The bacterial suspension obtained from 48-h old cultures on NBY agar was

*Corresponding author.
Phone) +82-31-290-0446, FAX) +82-31-290-0453
E-mail) yongki@rda.go.kr

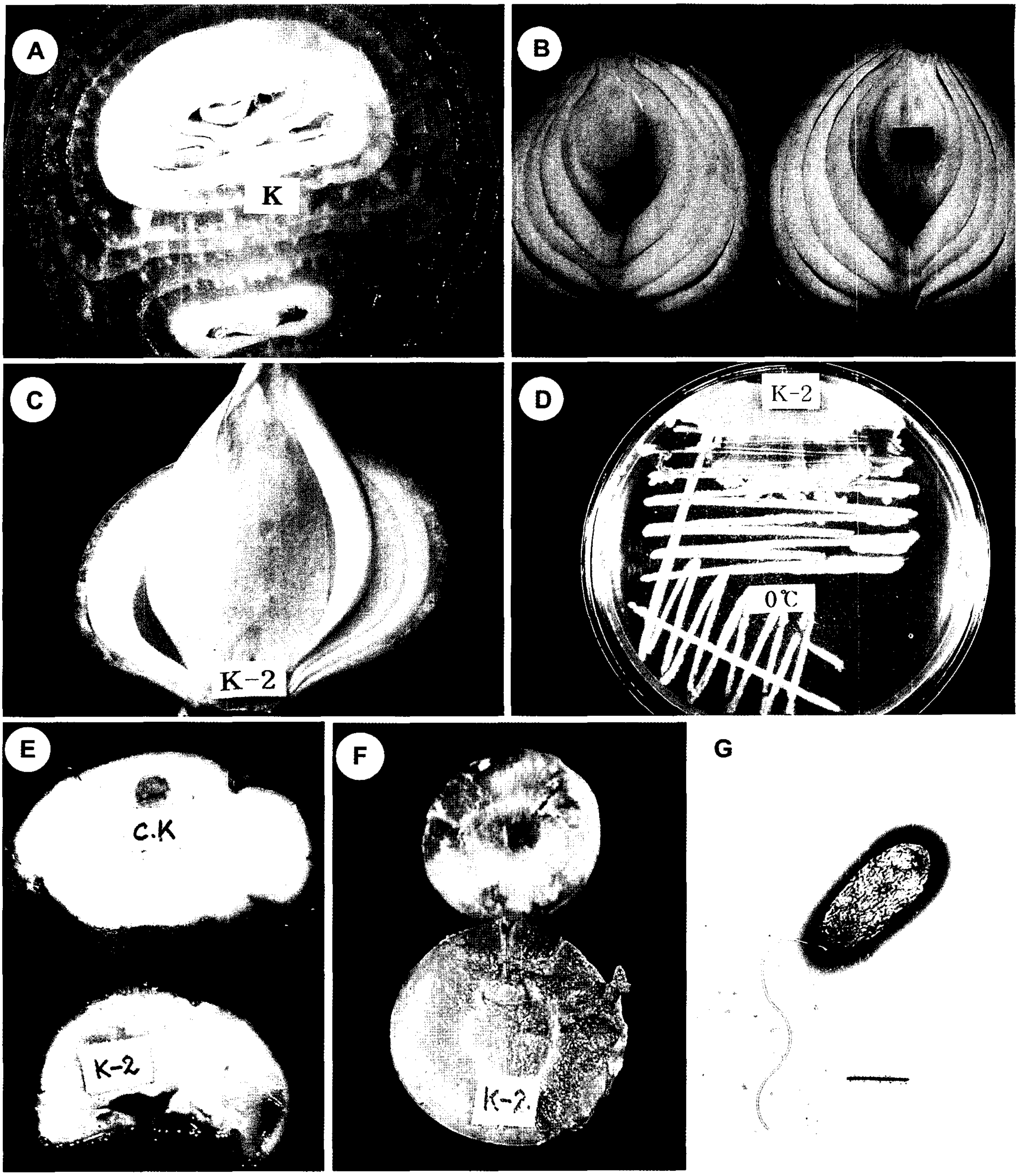

Fig. 1. Symptoms of soft rot on naturally infected onion bulb (**A**). Artificially inoculated onion bulb with *Pseudomonas marginalis* K-2 at 25℃ (**B**) and 5℃ (**C**). Symptoms of soft rot formed on onion bulb 3 days and 2 months after inoculation with *Pseudomonas marginalis* K-2 at 25℃ and 5℃, respectively. Growth of K-2 isolate cultured at 0℃ on nutrient broth yeast extract agar for 7 days (**D**). Pathogenicity bioassay showing tissue degradation after 2 days caused by application of cultured *P. marginalis* K-2: (**E**) potato; (**F**) carrot. Electron micrograph of the isolate (**G**). Bar represents 1 μm.

collected in distilled water and adjusted to 1.0×10^7 cfu/ml. The causal agent was inoculated longitudinally from the neck part and transversely from the outer to the inner part of the onion bulb at the level of 100 μl (10^7 cfu/ml) on the wound. Onion bulbs inoculated were incubated at 25°C in a moist chamber for 3 days. The inoculated bulbs were examined by making a longitudinal section of the bulbs from top to bottom. In order to confirm pathogenicity of the casual agent under low temperature condition, onion bulbs inoculated with the bacterial suspension as described above were incubated at 5°C for 2 months. Meanwhile, pathogenicity test against carrot and potato was similarly conducted using sliced samples.

Growth test of the pathogen at different temperature regimes. To investigate growth pattern of K-2 isolate which showed the strongest pathogenicity among three strains, growth rates of the pathogens at different temperatures (5°, 13°, 17°, 20°, 23°, 26°, 30°, 36°, 40°, and 45°C) were investigated by measuring turbidity on temperature ingredient apparatus (Temperature gradient incubator, Bio-photo recorder, Advantec Toyo Kaisha, LTD). In addition, growth patterns under low temperature condition were investigated by culturing the strain at 5°, 0°, -2°, and -5°C on NBY agar.

Results

Disease symptoms. Soft rot of onion bulbs stored under low temperature condition (5°C) occurred in storage houses at Changyoung in early 2000. The disease incidence reached up to 15% in severely damaged storage house. Infected onion scales showed water-soaking and yellowish-brown rot (Fig. 1A). Symptoms developed along the outer scale of onions at the early growth stage and gradually to inner parts.

Isolation and identification of causal agents. Three types of creamy white colonies on NBY agar were isolated from diseased onion bulbs. The bacterium was rod shaped and gram negative (Fig. 1G). The bacterium was positive for levan production, oxidase activity, arginine dihydrolase test, casein hydrolysis, and nitrate reduction. Hypersensitive response on the leaves of tobacco and tomato was positive. The bacterium grew well at 36°C, but did not grow at over 40°C (Fig. 2). The bacterium produced fluorescent pigments on King's medium B agar. It also showed positive utili-

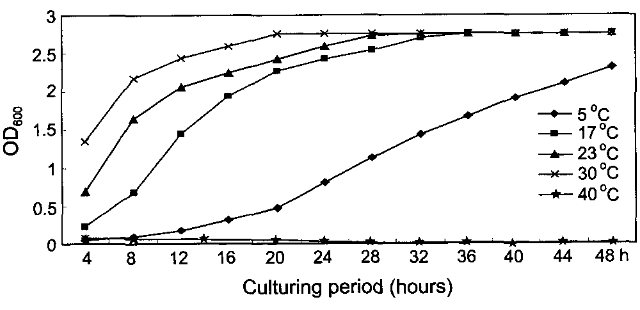

Fig. 2. Growth of *Pseudomonas marginalis* K-2 isolate in the LB broth at different temperature regimes.

Table 1. Isolation of pathogenic bacteria from infected onion bulbs stored under low temperature

Symptoms	Infection site	No. of isolates (isolation ratio)	Pathogenicity[a]	
			25°C	5°C
Soft rot	Inner scale	4 (2.3)	+	–
Water-soaked lesions	Outer scale	7 (4.0)	+(++)[b]	–
Water-soaked lesions	All scale	15 (8.7)	+(+++)[c]	+
Healthy	None	147 (85.0)	–	–

[a]+, ++ and +++ means little, moderate, and strong pathogenicity, respectively.
[b]two isolates (L-1, G) among seven isolates showed moderate pathogenicity.
[c]10 isolates among 15 isolates caused severe symptom within 3 days at 25°C, while one isolate (K-2) caused moderate symptom in 2 months at 5°C.

Table 2. Comparison of characteristics of the bacterial isolates *Pseudomonas marginalis* as reported by Ohuchi et al. (1983) and *P. marginalis* type strain causing soft rot in onion

Characteristics	Present isolate			*Pseudomonas marginalis*[a]	
	K-2	G	L-1	Japan isolate	Type strain
No. of flagella	1-3	1-6	1-6	1-5	1-5
Gram reaction	–[b]	–	–	–	–
Fluorescence on KB	+	+	+	+	+
Growth at 41°C	–	–	–	–	–
Levan production	+	–	–	–	+
Oxidase	+	+	+	+	+
Arginine dihydrolase	+	+	+	+	+
Casein hydrolysis	+	+	+	+	+
Nitrate reduction	+	+	+	+	+
Hypersensitive response	+	–	–	–	+
Utilization of carbohydrates	+	+	+	+	+
D-alanine	+	+	+	+	+
L-arabinose	+	+	+	+	+
Cellobiose	+	–	–	+	+
Glucose	+	+	+	+	+
Meso-inositol	+	+	+	+	+
Mannitol	+	+	+	+	+
L-rhamnose	–	–	–	–	–
Sorbitol	+	+	+	+	+
Sucrose	+	–	–	–	+
D-Galactose	+	+	+	–	+
Butylate	+	+	+	+	+
2-ketogluconate	+	+	+	+	+
Propionate	+	+	+	+	+
Saccharate	+	+	+	+	+
Trehalose	+	+	+	+	+
Gluconate	+	+	+	+	+
Potato soft rot	+	–	–	+	+

[a]*Pseudomonas marginalis* reported by Ohuchi et al. (1983) and type strain of *P. marginalis* NIAS-1330, respectively.
[b]– = negative; + = positive response.

Table 3. Comparison of LOPAT and confirmation characters of the present K-2 isolate with *Pseudomonas marginalis* type strain reported by Lelliott & Stead (1987)

Characters	Present isolate	*Pseudomonas marginalis* type strain
LOPAT characters		
Levan type	+[a]	+
Oxidase reaction	+	+
Potato rot	+	+
Arginine dihydrolase	+	+
Tobacco hypersensitivity	+	+
Confirmation characters		
2-keto gluconase production	+	+
Egg yolk reaction	+	+
Nitrate reduction	+	+
Acid from sucrose	–	+

[a]– = negative; + = positive response.

zation of D-alanine, L-arabinose, glucose, meso-inositol, sorbitol, sucrose, D-galactose, butylate, 2 ketogluconate, propionate, saccharate, trehalose and gluconate, but negative utilization of cellobiose and L-rhammose (Table 2). This showed pectolytic activity in the pectate degradation test. The bacterium K-2 was different from *Pseudomonas marginalis* reported by Ochuchi et al. (1983) in terms of utilization of sucrose and galactose, levan production, and hypersensitive response on tobacco plants. However, its characteristics were similar with those of *Pseudomonas marginalis* type strain NIAS-1330 (Table 2). In addition, the K-2 isolate was consistent with *Pseudomonas marginalis* type strain reported by Lelliott and Stead (1987) in terms of LOPAT and confirmation characters (Table 3). The isolates obtained from diseased onion bulbs in this study were identified as *Pseudomonas marginalis* based on the description in Bergey's manual.

Pathogenicity tests. When the onion bulbs were wound-inoculated with the cell suspension of *Pseudomonas marginalis* K-2 at 25°C, water-soaked and yellowish-brown lesions appeared on the scales of the inoculated bulbs (Fig. 1B). Rot symptoms developed gradually into the inside scales of the onion bulbs. The K-2 isolate caused soft rot when the onion bulbs wound-inoculated with the bacterial suspension were incubated for 2 months at 5°C (Fig. 1C). In the pathogenicity test against carrot and potato, the slices were severely rotten with light-yellowish (for potato) and dark-red (for carrot) lesions 2 days after inoculation (Figs. 1E and 1F).

Growth of the pathogen at different temperatures. The pathogen grew well at 0°C in nutrient broth yeast extract agar (Fig. 1D), but it hardly grew at -2°C. In the growth pattern at different cultural temperatures, *Pseudomonas marginalis* K-2 grew best at 30°C but did not grow at over 40°C in the LB broth (Fig. 2).

Discussion

The bacterial isolate obtained from the rotten onion bulbs stored under low temperature condition was identified as *Pseudomonas marginalis* based on morphological and biological tests. Characteristics of the isolate examined in this study were identical to the bacterium described in Bergey's manual. This bacterium caused similar symptoms on stored onion bulbs artificially inoculated at 25° and 5°C at 3 days and 2 months, respectively.

A similar disease on onion bulbs stored at low temperature condition caused by *Burkholderia cepacia* was previously reported by Yi and Park (1999). However, *P. marginalis* was readily distinguished from *B. cepacia* in terms of various biological characteristics such as arginine dihydrogenase, nitrate reduction, and production of fluorescent pigments on King's medium B agar. *P. marginalis* is a known opportunistic pathogen causing soft rot on a wide range of hosts including onion, lettuce, and Chinese cabbage (Lelliot and Stead, 1987).

Choi and Han (1990c) reported that *P. marginalis*, *P. syringae*, and *P. cepacia* caused onion bulb rot in fields and market places. However, soft rot of onion bulbs caused by *P. marginalis* stored under low temperature has not been reported previously. When onions are harvested from the field, they are undercut and hand pulled. Sometimes the tops are trimmed and the bulbs are then allowed to dry for 2-7 days under field condition. Leaf wounds caused by topping during harvesting are considered as important entrance sites for the pathogen.

It is assumed that the disease occurs severely in low-temperature warehouses because *P. marginalis* contaminates onion bulbs during harvest by moving in between onion scales with rainwater through wounds caused by topping, finally causing soft rot. The temperature in a storage facility normally should be kept at 0°-5°C, the desired temperature for long-term storage of the commodity. However, the casual agent, *Pseudomonas marginalis,* can grow well at above 0°, and can cause soft rot of onion at 5°C. Therefore, if onions were infected in the field and carried into the warehouse, severe damage of onion bulbs may occur. This study confirmed that *P. marginalis* causes soft rot in potato and carrot, as well as in onion. Since *P. marginalis* causes soft rot in various hosts such as Chinese cabbage (Choi and Han, 1989), lettuce (Chioi and Kim, 1989), garlic (Choi and Han, 1990a), ginger (Choi and Han, 1990b), broccoli (Canaday et al., 1991), and rice (Cottyn et al., 1996), much attention must be observed so as not to contaminate the produce by *Pseudomonas marginalis* in the field.

Acknowledgment

The authors wish to thank Mr. Jun-Sung Lee of the Virology Laboratory for his technical assistance in electron microscopy, and Mr. Hee-Dae Kim of the Onion Experimental Station for his assistance in the conduct of this study.

References

Canaday, C. H., Wyatt, J. E. and Mullins, J. A. 1991. Resistance in broccoli to bacterial soft rot caused by *Pseudomonas marginalis* and fluorescent *Pseudomonas* species. *Plant Dis.* 75:715-720.

Choi, J. E. and Han, K. S. 1989. Bacterial rot of Chinese cabbage caused by *Pseudomonas marginalis* pv. *marginalis*. *Korean J. Plant Pathol.* 5:328-330.

Choi, J. E. and Han, K. S. 1990a. Studies on the bacterial soft rot disease of lilliaceae crops. 3. Rot of garlic caused by *Pseudomonas* spp. *Korean J. Plant Pathol.* 6:86-90.

Choi, J. E. and Han, K. S. 1990b. Bacterial soft rot and rhizome rot caused by *Erwinia carotovora, Pseudomonas marginalis* and *P. solanacearum. Korean J. Plant Pathol.* 6:363-368.

Choi, J. E. and Han, K. S. 1990c. Studies on the bacterial soft rot disease of lilliaceae crops in Korea. 4. Bacterial bulb rot of onion caused by *Pseudomonas* spp. *Korean J. Plant Pathol.* 6:358-362.

Choi, J. E., Han, K. S. and Yu, S. J. 1989. Identification of bacteria causing soft rot disease of carrot. *Korean J. Plant Pathol.* 5: 349-353.

Choi, J. E. and Kim, H. Y. 1989. Identification of bacteria causing rot disease of vegetables in the fields and post-harvest period in Korea. IV. Bacterial rot of disease of lettuce. *Korean J. Plant Pathol.* 5:65-71.

Cottyn, B., Van Outryve, M. F., Cerez, M. T., De Cleene, M., Swing, J. and Mew, T. W. 1996. Bacterial disease of rice. II. Characterization of pathogenic bacteria associated with sheath rot complex and grain discoloration of rice in the Philippines. *Plant Dis.* 80:438-445.

Goto, M. and Takikawa, Y. 1984a. Methods for identification of plant pathogenic bacteria (3). *Crop Protect.* 38:432-437.

Goto, M. and Takikawa, Y. 1984b. Methods for identification of plant pathogenic bacteria (4). *Crop Protect.* 38:479-484.

Kasmire, R. F. and Cantwell, M. 1992. Postharvest handling systems: Underground vegetables (roots, tubers and bulbs). pp. 271-275 In: Kader A. A. (ed). Postharvest technology of horticultural crops. The Regents of the University of California, Division of Agriculture and Natural Resources, University of California.

Kim, Y. K., Lee, S. B., Lee, S. Y., Lee, Y. H., Kim, H. D., Yun, T. and Park, S. W. 2001. Ecology and control of postharvest disease of garlics and onions. pp. 56-89 In: Chung, M. N. (ed). Crop Protection Research 2000. National Institute of Agricultural Science and Technology, Suwon, Korea.

Lee, S. D., Yoon, C. M., Lee, Y. K., Choi, Y. C. and Cho, Y. S. 1999. Occurrence and distribution of bacterial canker of red pepper caused by *Clavibacter michiganensis* subsp. *michiganesis*. Res. Plant Dis. 5:105-110.

Lelliott, R. A. and Stead, D. E. 1987. Methods for diagnosis of bacterial diseases of plants. Blackwell Scientific Publications, Oxford. 216 p.

Ohuchi, A., Ahsawa, T. and Nishimura, J. 1983. Two pathogenic bacteria, *Erwinia rhapontici* (Millard 1924) Burkholder 1948 and *Pseudomonas marginalis* pv. *marginalis* (Brown 1918) Stevens 1925, causing a soft rot of onion. *Ann. Phytopath. Soc. Japan* 49:619-626.

Palleroni, N. J. 1984. Genus I. *Pseudomonas*. In: Bergey's manual of systematic bacteriology. Vol. I, Ed. by N. R. Krieg and J. G. Hort, P. 141-219. Williams and Wilkins, Baltmore. 964 p.

Schwartz, H. F. and Mohan S. K., 1995. Compendium of onion and garlic diseases. The APS Press, St. Paul, Minnesota, USA

Yi, Y. K. and Park, Y. M. 1999. Soft rot of onion stored under low temperature condition caused by *Erwinia rhapontici* and *Burkholderia cepacia.* J. Agricul. Sci. Tech. (Andong Univ.) 6:33-40.