

## The Effects of Temperature, pH, and Bactericides on the Growth of *Erwinia pyrifoliae* and *Erwinia amylovora*

Rosemary Shrestha<sup>1</sup>, Seon Hwa Lee<sup>2</sup>, Jang Hyun Hur<sup>1</sup> and Chun Keun Lim<sup>1\*</sup>

<sup>1</sup>Division of Bio-Resources Technology and <sup>2</sup>Division of Biological Environment, Kangwon National University, Chuncheon 200-701, Korea

(Received on February 1, 2005; Accepted on February 19, 2005)

In this comparative study, the effects of temperature, pH, and bactericides on the growth of *Erwinia pyrifoliae* and *Erwinia amylovora* were investigated. The maximum temperature for the growth of both *Erwinia* species was estimated to be 36°C. The maximum specific growth rates of *E. pyrifoliae* and *E. amylovora* were observed at 27°C and 28°C, respectively, and no significant growth differences were shown at their optimum temperatures. However, at lower temperatures ranging from 12-21°C, *E. pyrifoliae* showed higher growth rates with doubling times shorter than those of *E. amylovora*. Distinct growth rates at these temperatures revealed that *E. pyrifoliae* is more cold-tolerant than *E. amylovora*. The optimum pH for the growth of both pathogens was 7.5 and growth was not seen at pH ≤ 5.0 and ≥ 10.0. These results showed that the effect of pH on the growth of *E. pyrifoliae* and *E. amylovora* was similar. Minimum inhibitory concentrations (MICs) of copper sulfate, oxolinic acid, streptomycin, and tetracycline, which inhibited growth of *E. pyrifoliae* and *E. amylovora*, were determined. The strains of both pathogens were able to grow at 0.08-0.32 mM copper sulfate, but not at higher concentrations. However, none of the tested strains grew in the presence of oxolinic acid (0.001 mM), streptomycin (0.1 mM), and tetracycline (0.01 mM) concentrations. These results suggested that all strains of both *Erwinia* species were sensitive to tested bactericides and indicated no occurrence of resistant strains of *E. pyrifoliae* in Korea.

**Keywords** : doubling time, *hrpN<sub>ep</sub>*, minimum inhibitory growth, *Pyrus pyrifolia*, shoot blight

*Erwinia pyrifoliae*, a causal pathogen of shoot blight disease of the Asian pear (*Pyrus pyrifolia* cv. Singo), was isolated in Chuncheon, Korea (Rhim et al., 1999; Shrestha et al., 2003). The disease symptoms of shoot blight are similar to those of fire blight and bacterial shoot blight of pear (BSBP), which are caused by *Erwinia amylovora* and

a Japanese *Erwinia* species, respectively (Beer et al., 1996; Kim et al., 2001; Mizuno et al., 2000). The morphological, cultural, and biochemical characteristics of *E. pyrifoliae* were also similar to those of *Erwinia amylovora* and a Japanese *Erwinia* sp. (Kim et al., 1999; Shrestha et al., 2003). However, molecular studies, including total DNA-DNA relatedness, sequence analysis of the 16S-23S rRNA intergenic transcribed spacer (ITS) region, and plasmid profiles, revealed that these three *Erwinia* species are genetically dissimilar (Jock et al. 2003; Maxson-Stein et al. 2003).

The effects of temperature and pH on growth have been studied in several phytopathogenic bacteria, including *E. amylovora* (Ark, 1937; Billing et al., 1961; Billing, 1974), *Pseudomonas syringae*, and *Xanthomonas pruni* (Young and Luketina, 1977). These effects had not yet been examined in *E. pyrifoliae*. To date, *E. pyrifoliae* has not been reported in other parts of the world, which suggests that this pathogen is endemic and unique to Korea. *E. amylovora*, however, exists worldwide, in Europe, the eastern Mediterranean, Pacific Asia, and North America (Bonn and Zwet, 2000). Different spatial distributions of these two *Erwinia* species may be attributed to differences in their physiological properties. Growth, a chief determinant of the physiological properties of the pathogens, is affected by a number of factors such as temperature, hydrogen ion concentration (pH), salt concentration, nutrition, etc. Therefore, the growth of a location-specific pathogen, *E. pyrifoliae*, was investigated at various temperatures and pH concentrations and which was subsequently compared with that of *E. amylovora*, a more cosmopolitan pathogen.

In Korea, *E. pyrifoliae*, the causal agent of shoot blight of the Asian pear, is controlled by eradication of diseased host plants. This practice prevents multiplication and dissemination of *E. pyrifoliae* in major pear orchards. No alternative methods are currently used to control this pathogen. In the case of *E. amylovora*, chemical control combined with sanitation, pruning, eradication, and insect control have been used as methods for controlling and eliminating this pathogen. Van der Zwet and Keil (1979) summarized

\*Corresponding author.

Phone) +82-33-250-6437, FAX) +82-33-256-8254  
E-mail) chunkeun@kangwon.ac.kr

data collected on the chemical control of fire blight on apples and pears which had been published from 1920-1979 and found that copper compounds and antibiotics acted as effective bactericides against *E. amylovora*. Bactericides, which may be essential to the establishment of an integrated control system for reducing shoot blight incidence, have not yet been examined for their effect on the growth of *E. pyrifoliae*. In this investigation, we evaluated the sensitivity of *E. pyrifoliae* to copper sulfate, oxolinic acid, streptomycin, and tetracycline for the selection of effective bactericides against this pathogen.

## Materials and Methods

**Bacterial strains and culture media.** The bacterial strains

used in this study are listed in Table 1. The strains of *E. pyrifoliae* and *E. amylovora* were routinely grown on Mannitol Glutamic Yeast (MGY) agar plates at 28°C and maintained in Nutrient Broth (NA) containing 20% glycerol at -70°C (Shrestha et al., 2003). Pathogenicity and HR properties of the strains used in this study are also listed in Table 1. Pathogenicity tests on immature pear fruits, *P. pyrifolia* cv. Singo, were performed using approximately  $2 \times 10^7$  colony forming unit (cfu)/ml of each strain as described by Shrestha et al. (2003). For HR assay,  $2 \times 10^7$  colony forming unit (cfu)/ml of each strain were also used and infiltrated into tobacco leaves (*Nicotiana tabacum* cv. Burley), as described by Shrestha et al. (2005). After infiltration, the plants were kept at room temperature and the HR was scored within 24 h.

**Table 1.** List of bacterial strains used in the present study

Strains <sup>a</sup>	Host	Location, collection date	Characteristics		References
			Pathogenicity	HR	
<i>Erwinia pyrifoliae</i>					
WT3 (KCCM 10283)	<i>Pyrus pyrifolia</i>	Jichonri, Chuncheon, Korea, 1995	+	+	Shrestha et al. (2003)
WT4 (KNUCPB 964)	<i>P. pyrifolia</i>	Jichonri, Chuncheon, Korea, 1996	+	+	This study
WT5 (KNUCPB 955)	<i>P. pyrifolia</i>	Jichonri, Chuncheon, Korea, 1995	+	+	This study
WT6 (KNUCPB 966)	<i>P. pyrifolia</i>	Jichonri, Chuncheon, Korea, 1996	+	+	This study
WT7 (KNUCPB 967)	<i>P. pyrifolia</i>	Duck Duwonri, Chuncheon, Korea, 1996	+	+	This study
WT8 (KNUCPB 968)	<i>P. pyrifolia</i>	Jichonri, Chuncheon, Korea, 1996	+	+	This study
WT9 (KNUCPB 989)	<i>P. pyrifolia</i>	Owolri, Chuncheon, Korea, 1998	+	+	This study
WT10 (KNUCPB 9610)	<i>P. pyrifolia</i>	Jichonri, Chuncheon, Korea, 1996	+	+	This study
WT11 (KNUCPB 9811)	<i>P. pyrifolia</i>	Owolri, Chuncheon, Korea, 1998	+	+	This study
WT15 (KNUCPB 9515)	<i>P. pyrifolia</i>	Jichonri, Chuncheon, Korea, 1995	+	+	This study
WT18 (KNUCPB 9718)	<i>P. pyrifolia</i>	Duck Duwonri, Chuncheon, Korea, 1997	+	+	This study
WT20 (KNUCPB 9520)	<i>P. pyrifolia</i>	Jichonri, Chuncheon, Korea, 1995	+	+	This study
WT24 (KNUCPB 9724)	<i>P. pyrifolia</i>	Owolri, Chuncheon, Korea, 1997	+	+	This study
WT25 (KNUCPB 9525)	<i>P. pyrifolia</i>	Duck Duwonri, Chuncheon, Korea, 1995	+	+	This study
WT30 (KNUCPB 9630)	<i>P. pyrifolia</i>	Jichonri, Chuncheon, Korea, 1996	+	+	This study
WT36 (KNUCPB 9736)	<i>P. pyrifolia</i>	Owolri, Chuncheon, Korea, 1997	+	+	This study
WT43 (KNUCPB 9743)	<i>P. pyrifolia</i>	Duck Duwonri, Chuncheon, Korea, 1997	+	+	This study
WT59 (KNUCPB 9759)	<i>P. pyrifolia</i>	Owolri, Chuncheon, Korea, 1997	+	+	This study
Ep1 (DSM 12162)	<i>P. pyrifolia</i>	Chuncheon, Korea, 1996	+	+	Kim et al. (1999)
Ep4 (DSM 12394)	<i>P. pyrifolia</i>	Chuncheon, Korea, 1997	+	+	Kim et al. (1999)
Ep8 (DSM 12393)	<i>P. pyrifolia</i>	Chuncheon, Korea, 1995	+	+	Kim et al. (1999)
Ep16 (DSM 12163)	<i>P. pyrifolia</i>	Chuncheon, Korea, 1996	+	+	Kim et al. (1999)
<i>E. amylovora</i>					
ATCC 15580 <sup>†</sup>	<i>P. communis</i>	U.K.	+	+	Dye D. W.
LMG 1877	<i>Cydonia oblonga</i>	Denmark	+	+	Hockenhuil J.
LMG 1946	<i>P. communis</i>	Belgium	+	+	Vantomme R.
LMG 2068	<i>Rubus idaeus</i> ,	U.S.A.	+	+	Hayward A.
Ea246	Blackberry	U.S.A.	+	+	Reis A.

<sup>a</sup>Type strain; ATCC, American Type Culture Centre; DSM, Deutsche Sammlung von Mikroorganismen; KCCM, Korean Culture Center of Microorganisms; KNUCPB, Kangwon National University Collection of Phytopathogenic Bacteria; LMG, Laboratorium voor Microbiologie; +, positive to pathogenicity on immature pear fruit and hypersensitive response (HR) on tobacco leaves.

**Measurement of growth at different temperatures and pH.** Two strains of *E. pyrifoliae* (WT3 and Ep16) and one strain of *E. amylovora* (ATCC 15580) were used to evaluate the effects of temperature and pH on growth. Growth at different temperatures and pH concentrations were automatically monitored *in vitro* using the turbidimetric system BioscreenC (Labsystems, Finland). To study the effects of temperature, a fresh culture of each strain was inoculated into 5 ml MGY broth and cultured for 16 h at 28°C in a shaking incubator at 160 rpm. Optical density was adjusted to 0.5 at 600 nm absorbance and 300 µL of each strain was inoculated into honeycomb plates containing 100 cuvettes, as mentioned by Begot et al. (1996). Growth was measured every ten minutes at 3 degree intervals, ranging from 12-39°C. As the growth of both *Erwinia* species was found to be the most favorable in the range of 27-30°C, each species was again grown at 1 degree intervals at temperatures within this range.

To study the effects of acidity and alkalinity on the growth of these pathogens, MGY broth with pH ranging from 2.0-9.0 was prepared at intervals of 0.5 units. The growth of both *Erwinia* species at different pH concentrations was then measured using the same procedure as described for the temperature experiment. Five successive cuvettes of the same column were used for each strain in both parameters. Two cuvettes of each column were filled with the same volume of non-inoculated medium. The data were stored in the desktop computer which was interfaced with the analyzer. The individual growth curve of each inoculated cuvette was further analyzed by Excel Software (Microsoft Windows 2003) and an average of five cuvettes was taken for each strain in each parameter.

#### Calculation of doubling time and specific growth rates.

The optimum temperature and pH for growth were estimated to be the values at which maximum specific growth rates of the strains were observed. The specific growth rates and doubling times were calculated from the exponential phase of growth using following formulae, as given by Pirt (1975) and Ingraham et al. (1983):

$$M_1 = 2nM_0$$

$$\log M_1 = n \log 2 + \log M_0$$

$$n = \frac{\log M_1 - \log M_0}{\log 2}$$

$$k = \frac{n}{T_1 - T_0}$$

$$g = \frac{1}{k} = \frac{T_1 - T_0}{n}$$

where,  $M_0$  = initial O.D. of each strain,  $M_1$  = final O.D. of each strain,  $T_0$  = initial time,  $T_1$  = final time,  $n$  = no. of

generation,  $k$  = growth rate constant and  $g$  = doubling or generation time.

Plots for the specific growth rate versus temperature and pH were developed to analyze the linear relationship with the growth of *E. pyrifoliae* and *E. amylovora*.

#### Determination of Minimum Inhibitory Concentrations (MICs).

Twenty-two strains of *E. pyrifoliae* and five strains of *E. amylovora* were used to evaluate sensitivity to four bactericides: copper sulfate, oxolinic acid, streptomycin, and tetracycline (Table 1). MICs were determined to be the concentrations at which tested bactericides inhibited *in vitro* growth of these pathogens. The MGY agar was prepared, cooled to 50°C, and amended with filter-sterilized copper sulfate to final concentrations of 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, and 1.2 mM; oxolinic acid to 0.001, 0.002, 0.004, 0.008, and 0.016 mM; streptomycin to 0.1, 0.2, 0.4, 0.8, 1.60, and 3.20 mM; and tetracycline to 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, and 0.64 mM. All strains were cultured overnight and O.D. was adjusted to 0.6 at 600 nm absorbance. 5 µL of each O.D.-adjusted strain was spotted on the MGY agar plates amended with different concentrations of selected bactericides, which were then incubated at 28°C, as described by McManus and Jones (1994). Triplicate agar plates were used for each concentration and the experiment was repeated three times. Growth was evaluated for 48 h at 12 h time intervals.

## Results and Discussion

#### The effects of temperature and pH on growth rate.

Specific growth rates and doubling times calculated for the strains of *E. pyrifoliae* and *E. amylovora* at monitored temperatures are given in Table 2. None of the strains grew at 39°C. The temperature maximum for growth was estimated at 36°C for both *Erwinia* species, since, when standard physiological and biochemical tests based on Bergey's manual were performed during their identification (Shrestha et al., 2003), the strains of these pathogens did not grow at 37°C. At maximum temperature, doubling times were calculated to be  $2.69 \pm 0.10$  h,  $2.88 \pm 0.18$  h, and  $2.72 \pm 0.06$  h for *E. pyrifoliae* WT3, *E. pyrifoliae* Ep16, and *E. amylovora* ATCC15580, respectively (Table 2). Low growth rates with long doubling times were observed at 33-36°C (Fig. 1). The optimum temperatures for growth were observed at 27°C for *E. pyrifoliae* and 28°C for *E. amylovora*. At optimum temperature, doubling times were calculated to be  $1.53 \pm 0.02$  h,  $1.50 \pm 0.04$  h, and  $1.44 \pm 0.02$  h for *E. pyrifoliae* WT3, *E. pyrifoliae* Ep16, and *E. amylovora* ATCC15580, respectively; no significant differences were observed in their growth rates (Table 2). High growth rates with short lag phases were observed for both

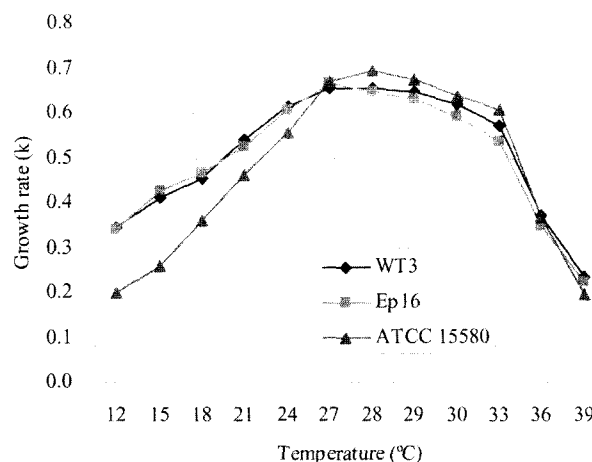
**Table 2.** Doubling times estimated for *E. pyrifoliae* and *E. amylovora* at different temperatures

Temperature (°C)	Doubling time (h)		
	<i>E. pyrifoliae</i>		<i>E. amylovora</i>
	WT3	Ep16	ATCC15580
12	2.89±0.18	2.92±0.12	4.97±0.08
15	2.43±0.15	2.35±0.12	3.84±0.12
18	2.20±0.07	2.15±0.03	2.76±0.05
21	1.85±0.06	1.90±0.03	2.16±0.05
24	1.62±0.04	1.64±0.03	1.80±0.03
27	1.53±0.02	1.50±0.04	1.49±0.03
28	1.53±0.04	1.55±0.02	1.44±0.03
29	1.55±0.03	1.58±0.05	1.48±0.02
30	1.61±0.03	1.69±0.08	1.56±0.03
33	1.75±0.05	1.86±0.14	1.65±0.04
36	2.69±0.10	2.88±0.18	2.72±0.06
39	4.27±0.26	4.51±0.28	5.11±0.25

<sup>a</sup>Mean doubling time of five replicates of each strain ± S.D.

pathogens at temperatures between 27 and 30°C. At temperatures ≤ 21°C, both pathogens grew slower with lower growth rates, longer doubling times, and more prolonged lag phases than those at ≥ 24°C (Fig. 1). However, distinct growth rates were observed at temperatures ≤ 21°C with doubling times of 1.85 ± 0.06 h, 1.90 ± 0.03 h, and 2.16 ± 0.05 h for *E. pyrifoliae* WT3, *E. pyrifoliae* Ep16, and *E. amylovora* ATCC15580, respectively. Growth rates were more distinctive at 12°C and showed doubling times of 2.89 ± 0.18 h, 2.92 ± 0.12 h, and 4.97 ± 0.08 h for *E. pyrifoliae* WT3, *E. pyrifoliae* Ep16, and *E. amylovora* ATCC15580, respectively. These results showed that *E. pyrifoliae* had higher growth rates and shorter doubling times with a prolonged lag phase at ≤ 21°C when compared to those of *E. amylovora* (Fig. 1). Based on these results, it was concluded that *E. pyrifoliae* is more cold-tolerant than *E. amylovora*. Regarding the epidemic survey of shoot blight disease, *E. pyrifoliae* has so far been found only in the northern parts of South Korea, including Chuncheon which are much colder in winter than are the southern parts, where major pear cultivation areas are located. Although this result does not explain the reason that *E. pyrifoliae* is endemic to Korea, it can be speculated that its cold-tolerant character is one of the factors that has enabled this pathogen to survive better in the northern parts of the country than in the southern parts during the winter season.

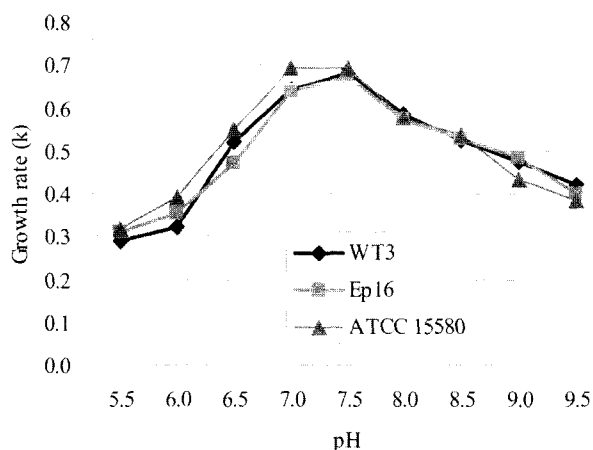
Specific growth rates and doubling times calculated for the strains of both *E. pyrifoliae* and *E. amylovora* at different pH concentrations are given in Table 3. Neither of the pathogens was able to grow at pH ≤ 5.0 and ≥ 10.0. Low growth rates and long doubling times were observed in both pathogens at concentrations of moderately acidic

**Fig. 1.** Plot of the growth rates of *Erwinia pyrifoliae* and *E. amylovora* determined at different temperatures. WT3 and Ep16 represent strains of *E. pyrifoliae*, whereas ATCC 15580 represents *E. amylovora*.**Table 3.** Doubling times estimated for *E. pyrifoliae* and *E. amylovora* at different pH

pH	Doubling time (h) <sup>a</sup>		
	<i>E. pyrifoliae</i>		<i>E. amylovora</i>
	WT3	Ep16	ATCC15580
5.5	3.45±0.35	3.22±0.13	3.15±0.12
6.0	2.62±0.04	2.82±0.11	2.55±0.13
6.5	1.92±0.02	2.12±0.03	1.82±0.05
7.0	1.55±0.02	1.56±0.02	1.44±0.08
7.5	1.47±0.03	1.49±0.08	1.44±0.07
8.0	1.71±0.04	1.75±0.03	1.73±0.07
8.5	1.91±0.04	1.89±0.08	1.86±0.08
9.0	2.10±0.23	2.07±0.18	2.32±0.05
9.5	2.37±0.10	2.51±0.10	2.62±0.04

<sup>a</sup>Mean doubling time of five replicates of each strain ± S.D.

pH (5.5-6.5) and alkaline pH (8.5-10). At pH 5.5, doubling times were calculated to be 3.45 ± 0.35 h, 3.22 ± 0.13 h, and 3.15 ± 0.12 h for *E. pyrifoliae* WT3, *E. pyrifoliae* Ep16, and *E. amylovora* ATCC15580, respectively (Table 3). Similarly, doubling times of 2.37 ± 0.10 h, 2.51 ± 0.10 h, and 2.62 ± 0.04 h for *E. pyrifoliae* WT3, *E. pyrifoliae* Ep16, and *E. amylovora* ATCC15580, respectively, were calculated at pH 10.0. The most favorable pH was found at 7.5 for both pathogens, which showed optimum growth with short doubling times of 1.47 ± 0.03 h, 1.49 ± 0.08 h, and 1.44 ± 0.07 h in *E. pyrifoliae* WT3, *E. pyrifoliae* Ep16, and *E. amylovora* ATCC15580, respectively (Table 3, Fig. 2). These results showed similar effects of pH on the growth of these two pathogens, although some variation was observed between acidic (pH 5.5-6.0) and alkaline conditions (9.0-9.5) (Table 3).



**Fig. 2.** Plot of the growth rates of *Erwinia pyrifoliae* and *E. amylovora* determined at different pH. WT3 and Ep16 represent strains of *E. pyrifoliae*, whereas ATCC 15580 represents *E. amylovora*.

**Copper sulfate, oxolinic acid, streptomycin, and tetracycline sensitivity.** MICs of copper sulfate, oxolinic acid, streptomycin, and tetracycline were estimated for the strains of both *E. pyrifoliae* and *E. amylovora*. All strains of both pathogens were capable of growing in the presence of  $\leq 0.08$  mM copper sulfate. Eight strains (WT5, WT6, WT7, WT15, WT20, WT36, WT47, and WT59) of *E. pyrifoliae* and two strains (LMG 1877 and Ea246) of *E. amylovora* further grew in concentrations of up to 0.32 mM copper sulfate, which indicates variation in their sensitivity to this bactericide. However, none of the strains of *E. pyrifoliae* and *E. amylovora* were capable of growing in the presence of oxolinic acid, streptomycin, or tetracycline. MICs of 0.001 mM, 0.1 mM, and 0.01 mM were determined for oxolinic acid, streptomycin, and tetracycline, respectively, showing their sensitivity to all tested bactericides. These findings suggest that tested bactericides can be used as a part of integrated pest management to control *E. pyrifoliae*, along with eradication of diseased plants, the only method used currently in Korea for control of *E. pyrifoliae*.

## References

- Ark, P. A. 1937. Variability in the fire blight organism, *Erwinia amylovora*. *Phytopathology* 27:1-28.
- Beer, S. V., Kim, J. H., Zumoff, C. H., Bogdanove, A. J., Laby, R. J., Gustafson, H. L., Momol, T., Aldwinckle, H. S., Tani, A. and Tamura, O. 1996. Characterization of bacteria that cause "bacterial shoot blight of pear" in Japan. *Acta Hort.* 411:179-181.
- Begot, C., Desnier, I., Daudin, J. D., Labadie, J. C. and Lebert, A. 1996. Recommendations for calculating growth parameters by optical density measurements. *J. Microbiol. Methods* 25:225-232.
- Billing, E. 1974. The effect of temperature on the growth of the fire blight pathogen, *Erwinia amylovora*. *J. Appl. Bacteriol.* 37:643-648.
- Billing, E., Baker, L. A. E., Crosse, J. E. and Garrett, C. M. E. 1961. Characteristics of English isolates of *Erwinia amylovora*. *J. Appl. Bacteriol.* 24:195-211.
- Bonn, W. G. and Van der Zwet, T. 2000. Distribution and economic importance of fire blight. In Vanneste J. L. (ed), *Fire blight: The disease and its causative agent, Erwinia amylovora*. CABI publishing, UK, pp 37-53.
- Ingraham, J. L., Maloe, O. and Neidhardt, F. C. 1983. *Growth of bacterial cell*. Sinauer Associates Inc. Massachusetts, USA.
- Jock, S., Jacob, T., Kim, W.-S., Hilgebrand, M., Vosberg, H. P. and Geider, K. 2003. Instability of short-sequence DNA repeats of pear pathogenic *Erwinia* strains from Japan and *Erwinia amylovora* fruit tree and raspberry strains. *Mol. Genet. Genomics* 268:739-749.
- Kim, W.-S., Gardan, L., Rhim, S. L. and Geider, K. 1999. *Erwinia pyrifoliae* sp. nov., a novel pathogen affecting Asian pear trees (*Pyrus pyrifoliae* Nakai). *Intl. J. Sys. Bacteriol.* 49:899-906.
- Kim, W.-S., Jock, S., Paulin, J. P., Rhim, S.-L. and Geider, K. 2001. Molecular detection and differentiation of *Erwinia pyrifoliae* and Host range analysis of the Asian pear pathogen. *Plant Dis.* 85:1183-1188.
- Maxson-Stein, K., McGhee, G. C., Smith, J. J., Jones, A. L. and Sudin, G. W. 2003. Genetic analysis of a pathogenic *Erwinia* sp. isolated from pear in Japan. *Phytopathology* 93:1393-1399.
- Mc Manus, P. S., and Jones, A. L. 1994. Epidemiology and genetic analysis of streptomycin-resistant *Erwinia amylovora* from Michigan and evaluation of oxytetracycline for control. *Phytopathology* 84:627-633.
- Mizuno, A., Sato, S., Kaeai, A. and Nishiyama, K. 2000. Taxonomic position of the causal pathogen of bacterial shoot blight of pear. *J. Gen. Plant. Pathol.* 66:48-58.
- Pirt, S. J. 1975. Principles of microbe and cell cultivation. Blackwell, Oxford, UK.
- Rhim, S.-L., Voelkisch, B., Gardan, L., Paulin, J. P., Langlotz, C., Kim, W.-S. and Geider, K. 1999. An *Erwinia* species, different from *E. amylovora* cause a necrotic disease of Asian pear trees. *Plant Pathol.* 48:514-520.
- Shrestha, R., Koo, J. H., Park, D. H., Hwang, I., Hur, J. H. and Lim, C. K. 2003. *Erwinia pyrifoliae*, a causal endemic pathogen of shoot blight of Asian pear tree in Korea. *Plant Pathol. J.* 19:294-300.
- Shrestha, R., Tsuchiya, K., Baek, S. J., Bae, H. N., Hwang, I., Hur, J. H. and Lim, C. K. 2005. Identification of *dspEF*, *hrpW*, and *hrpN* loci and characterization of the *hrpN<sub>ep</sub>* gene in *Erwinia pyrifoliae*. *J. Gen. Plant Pathol.* 71 (in press).
- Young, J. M., Leuketina, R. C. and Marshal, A. M. 1977. The effects on temperature on growth *in vitro* of *Pseudomonas syringae* and *Xanthomonas pruni*. *J. Appl. Bacteriol.* 42:345-354.
- Van der Zwet, T. and Keil, H. L. 1979. Fire blight - A bacterial disease of rosaceous plants. United States Department of Agriculture Handbook 510, Washington DC, USA.