농약과학회지 제13권 제1호 (2009)
The Korean Journal of Pesticide Science Vol. 13, No. 1, pp. 45~53

ORIGINAL ARTICLE/CONTROL

Antagonistic Effect of *Lactobacillus* sp. Strain KLF01 Against Plant Pathogenic Bacteria *Ralstonia solanacearum*

Anupama Shrestha[1], Kyu-up Choi[1], Chun Keun Lim[1], Jang Hyun Hur[2] and Saeyoull Cho[1]*

[1]Division of Applied Biology, College of Agriculture and Life Science, Kangwon National University, Chuncheon 200-701, Korea, [2]Division Of Biological Environment, College of Agricultural and Life Sciences, Kangwon National University, Chuncheon 200-701, Korea

(Received February 5, 2009; accepted February 23, 2009)

Abstract

An antagonistic bacterial strain KLF01 was isolated from rhizosphere of tomato and identified to be *Lactobacillus* sp. by biochemical and genetic analysis. This strain showed antagonism against the used plant pathogenic bacteria like *Ralstonia solanacearum*, (bacterial wilt), *Xanthomonas axonopodis* pv. *citri*, (Citrus canker), *Xanthomonas campestris* pv. *vesicatoria* (Bacterial spot), *Eriwinia pyrifoliae* (Shoot-blight) and *Eriwinia carotovora* subsp. *carotovora* group (Potato scab) through agar well diffusion method. *In planta* test done by drench application of strain KLF01 (4×10^8 cfu/ml) into the experimental plot containing tomato (*Solanum lycopersicum* L.) cultivar 'Lokkusanmaru' and red pepper (*Capsicum annuum* L.) cultivar 'Buja' plants, in pot test post-inoculated with the plant pathogenic bacteria, *R. solanacearum* significantly reduced the disease severity, compared to the non-treated plants.

Key words Bacterial wilt, Biocontrol, *Lactobacillus* sp., KLF01, *Ralstonia solanacearum*

Introduction

Lactic acid bacteria (LAB) are chemo-organotrophic, phylogenetically diverse group of bacteria that are gram-positive, non-sporulating, coccus or rod shaped with less than 50 mol% G+C in their DNA (Hammes *et al.*, 1991). LAB use fermentable carbohydrates as energy source. Hexoses are degraded mainly to lactate (homofermentatives) or formate (heterofermentatives) and additional products such as acetate, ethanol, CO_2 are also formed. They include various genera as *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Leuconostoc*, *Carnobacterium*, *Propionibacterium* and *Bifdobacterium* (Wood and Holzapfel 1955). Isolation of various LAB from different products like milk, fermented foods and plants, has been frequently reported previously but its study on isolation from soil is rare, however some spore-forming LAB was isolated from the soil (Suzuki and Yamasato 1994).These isolated strains from soil showed antibacterial activities against some gram-positive bacteria (Chene *et al.*, 2005). Moreover, atypical streptobacteria and betabacteria, as well as *Lactobacillus* species from plants: *L. plantaruim*, *L. fermentum* and small numbers of *L. brevis*, *L. casei*, *L. viridescens*, *L. cellobiosus* and *L. saflivarias* were also isolated and reported (Mundt and Hammer 1968; Sharpe 1981; Stirling and Whittenbury 1963).

The inhibitory factors resulting from the metabolism of oxygen in LAB and the cellular catabolism products of these bacteria have been described by Piard *et al.*, in 1991. A number of authors have reported the capacity of *L. diacetylactis* and *L. citrovorum* to inhibit undesirable microorganisms, notably *Pseudomonas* sp. Although these

*연락저자 : Tel. +82-33-250-6431, Fax. +82-33-256-8254
E-mail: saeyoullcho@kangwon.ac.kr

studies establish the role of organic acids in the observed inhibitions, it was suggested that there must be the involvement of other antibacterial factors too (Piard and Desmazeaud 1991). The antibacterial effects of LAB have been extensively studied (Stiles *et al.*, 1996). The microbistatic and microbicidic action of LAB is based on both competition for nutrients and production of various antimicrobial compounds such as organic acids, hydrogen peroxide, bacteriocins and low molecular weight antimicrobial agents (Ouwehand 1998).

LAB has a long history of showing antagonistic effect against food spoilage microorganisms and human pathogens. However, its study against plant pathogens is limited. Conventional use of chemical pesticides or soil fumigants like methyl bromide has been seriously questioned because of environmental and human health hazard. Therefore, the biological control of plant pathogens is of paramount importance nowadays (Hayward *et al.*, 1991 & Bernal *et al.*, 2002). *Ralstonia solanacearum*, a serious soil-borne pathogen which causes bacterial wilt, is a major constraint in the production of tomato, pepper, potato, tobacco, banana, egg-plant and many other economically important plants in tropical, subtropical and warm temperate regions of the world (Hayward *et al.*, 1991). It can survive for more than five years without a host, making its control quite challenging. The efficiency of conventional practices to control this disease is also limited (Sung *et al.*, 2005). In previous report, biological control agents like avirulent mutants of *R. solanacearum* (Dong *et al.*, 1999), genetically engineered antagonistic bacteria (Kang *et al.*, 1995), some rhizobacteria like, *Streptomyces* spp. (El Albyad *et al.*, 1996), *Bacillus* spp. (Sung *et al.*, 2005) and *Paenibacillus* sp. strain KPB3 (Jungi *et al.*, 2007) are used to control bacterial wilt of tomato (*Solanum lycopersicum* L.). Likewise, the antibacterial activity of some LAB has been reported against bacteria (Ronel *et al.*, 1986) and *Fusarium* spp. (Laitila *et al.*, 2002). However, the use of LAB against *R. solanacearum* is yet unknown. Antibacterial activity *Lactobacillus* sp. strain KLF01 has been observed both *in vitro* and *in planta* tests in this study. Therefore, objective of this study was to investigate *in vitro* and *in planta* effect of antagonistic strain KLF01 and its implementation as a biological control against *R. solanacearum*.

Materials and methods

Bacterial isolation and identification

Strain KLF01 was isolated from rhizosphere of tomato on field located in Chuncheon, Gangwon province, Republic of Korea. The collected soil was serially diluted in sterile water, and plated onto MRS agar (De Man, Rogosa and Sharpe) plates, and incubated overnight at 37℃. About 250 colonies isolated were subjected to *in vitro* antibacterial assay against *R. solanacearum* for bioassay. Only one isolates among them showed clear inhibitory zone against *X. axonopodis* pv. citri, *X. campestris* pv. *vesicatoria* and *R. solanacearum*. This strain was selected and named as KLF01. This strain was stored by freeze-drying in 10% skimmed milk for its long-term preservation, as described by Perry *et al.*, 1995. LAB were isolated and maintained in MRS broth. MRS media was solely used for cultivation of KLF01 in all the experiments.

Physiological and biochemical test and 16S rRNA analysis

Strain KLF01 was subjected to physiological and biochemical tests, based on Bergey's manual of systemic bacteriology. Biochemical characterization of this strain was done using API50 CHB/ API20E Kits (BioMerieux, France) according to manufacturers' instructions. Total genomic DNA was isolated using a lysozyme dodecyl sulfate lysis procedure described previously (Sambrook and Russell, 2001). 16S rRNA gene was amplified using fD1 (5'-AGAGTTTGATCATGGCTCAG3') and rP2 (5'ACGGTTACCTTGTTACGACTT-3') primers (Weisburg *et al.*, 1991). PCR amplification was carried out in 25 μl reaction volumes containing 20 mole of each primer, 20 μl concentration of dNTPs (dATP, dGTP, dCTP and dTTP) (Promega Co. USA), 5 units of taq polymerase (Biotools Co.) with 10 ng of DNA. PCR analysis was performed with a DNA thermal cycler (Perkin-Elmer Applied Biosystems, Foster City, CA, USA). The PCR product was analyzed by electrophoretic separation in 1% (w/v) QA-agarose (Qbiogene, Irvine, CA), containing 0.5 μg/L ethidium

bromide. The PCR products were excised from the gel and purified using a QIAquick gel extraction kit (Qiagen Inc., Hilden, Germany). Purified DNA was ligated into the pGEM-T easy vector (Promega Co.). Plasmid containing the 16S rRNA region was then directly sequenced using an ALFred autocycle sequencing kit, with M13 forward and reverses primers. DNA homology searches were carried out with the NCBI databases, using the BLAST network service (Altschul *et al.*, 1990).

Extraction of antimicrobial substance

The cell-free supernatant was collected and filtered through a sterile 0.45 μm syringe filter (Sartorius, Gottingen, Germany) into sterile screw cap tubes (Han *et al.*, 2007). The cell suspension and supernatant of KLF01 were used to investigate antibacterial activity.

In vitro antibacterial assay against different bacteria

Strain KLF01 isolated from the soil, strains *Lactococcus* sp. strain KLC02 (Reference strain) and *Pediococcus* sp. strain KPD03 (Reference strain) were subjected to *in vitro* antibacterial assay against different plant pathogenic bacteria. Agar well diffusion method as described by Benkerroum and Sandine in 1988 with some modifications was carried out. MRS agar plates were overlaid with 7ml Soft MGY agar (containing 0.75% agar) inoculated with 100 μl of the overnight growth culture of the indicator strain and incubated for 3 hrs. After incubation for 3 hrs, wells were punched out of the agar and 10 μl of cell suspension and cell free suspension of the test organism was poured into each well separately. The antibacterial activity was assayed by observing inhibitory zones in the background of each indicator organisms after 18-24 hrs of incubation. Strain KLF01 and the other two reference strains, fractionated into bacterial suspension and bacterial free culture supernatant were subjected to antibacterial assay under different conditions. Each assay was performed in triplicate.

In planta antibacterial assay at green house

Three weeks old red pepper and tomato seedlings were used to perform this experiment at green house. The experimental design was a randomized complete block design with five treatments and six plants in each treatment. *Lactobacillus* sp. strain KLF01, *Lactococcus* sp. strain KLC02 and *Pediococcus* sp. strain KPD03 culture (1×10^8 cfu /ml) were prepared in 50 ml of MRS broth and *Paenibacillus* sp. strain KPB3 was prepared in 50 ml of M5 broth.5 ml of strain *Lactobacillus* sp. strain KLF01, *Lactococcus* sp. strain KLC02, *Pediococcus* sp. strain KPD03 and *Paenibacillus* sp. strain KPB3 were drenched into each pot under treatment separately. This method was based on as reported by Molina *et al.*, (1998). The control plants were treated with 5 ml tap-water. One week after drench treatment, 10 ml of *R. solanacearum* suspension (1×10^8 cfu/ml) was applied to roots of all plants and transplanted to new pot. Disease severity was observed after ten days of pathogen treatment. The experiment was conducted three times with completely randomized design with 5 plants per replication.

Statistical analysis

The data were subjected to analysis of variance using SAS version 8 (SAS institute, Cary, NC). Mean values among treatments were compared by the Tukey's test at α = 0.05 level of significance. Disease development on each plant was rated using the following scale: 5 = plant dead; 4 = 76 to 100% leaves with symptoms; 3 = 51 to 75% of leaves with symptoms; 2 = 26 to 50% of leaves with symptoms; 1 = < 25% of leaves with symptoms; and 0 = no symptoms. The disease index was calculated from the disease ratings by the following formula: Disease Index = [$\sum$ (rating no. × no. of plants in the rating) / (total no. of plants × highest rating)] × 100%. Control effect % was calculated by the following formula. Control effect index = [D.S of control - D.S /D.S of the control of the sample] × 1

Results

Physiological characteristics and 16S rRNA analysis of isolate KLF01. The phenotypic characteristics of the isolate KLF01 was found to be facultative anaerobic and catalase negative. The optimal temperature for their growth was

approximately 37℃. The isolates and reference strains grew optimally at pH 6.0-7.0. Various *Lactobacillus* sp. shows the positive carbohydrate fermentation pattern for fructose, D (+) glucose, lactose, maltose, galactose and mannose. The strain KLF01 showed positive results for above mentioned carbohydrates while it showed negative results for arbutin and variable results for amygdalin and gentiobiose (Table 1). Thus, the patterns of carbohydrate fermentation for strain KLF01 was similar to those of *Lactobacillus* sp. Phylogenetic analysis of the strain KLF01 was done by nucleotide sequence analysis of its 16S rRNA gene. The phylogenetic tree based on the 16s rRNA gene sequences with different lactic acid bacteria by neighbor-joining method revealed its relatedness toward the *Lactobacillus fermentum*, *Pediococcus acidilactici*, *Lactococcus lactis* and *Leuconostoc* sp (Fig. 1). Moreover, 16S rRNA sequence alignment of the strain KLF01 with those of *Lactobacillus delbrueckii* AY773950, *Lactobacillus lactis* M58823, *Lactobacillus fermentum* AB362626, *Lactobacillus fermentum* Eu559594, *Pediococcus acidilactici* Eu147311, *Lactobacillus lactis* M58823, *Lactococcus lactis* Eu074844,

Table 1. Biochemical characteristics of strain KLF01

No.	Substrate	Strain KLF01.
1	RIBose	+
2	GALactose	+
3	GLUcose	+
4	FRUctose	+
5	MaNnosE	+
6	AMYgdalin	V
7	ARButin	-
8	ESCulin	+
9	MALtose	+
10	LACtose	+
11	MELibiose	+
12	RAFfinose	+
13	GENtiobiose	V
14	D TAGatose	+
15	2-Keto-Gluconate	+
16	2-Keto-Gluconate	+
17	5-Keto-Gluconate	+

Isolate KLF01 showed different carbohydrate fermentation patterns. -, negative ; +, positive; v, variable.

Table 2. Antibacterial activity of strain KLF01 against different bacteria

Indicator strains	Agar well diffusion method[a)]
Salmonella sp. (Positive control)	+++
Water (Negative control)	-
Ralstonia solanacearum	++
Xanthomonas pv. *vesicatoria*	++
X. axonopodis pv. *citri*	+++
X. pv. *pruni*	-
Eriwinia pyrifoliae	++
E. carotovora subsp. *carotovora*	++
E. amlyovora	-
Bacillus. subtilis	+++
B. lichenifirmis	++

[a)] Determined by measuring the average diameter of clear zone of inhibition -, no inhibition < 1 mm ; + weak inhibition (< 5 mm); ++mild (= 5 mm); +++, strong inhibition (> 10 mm).

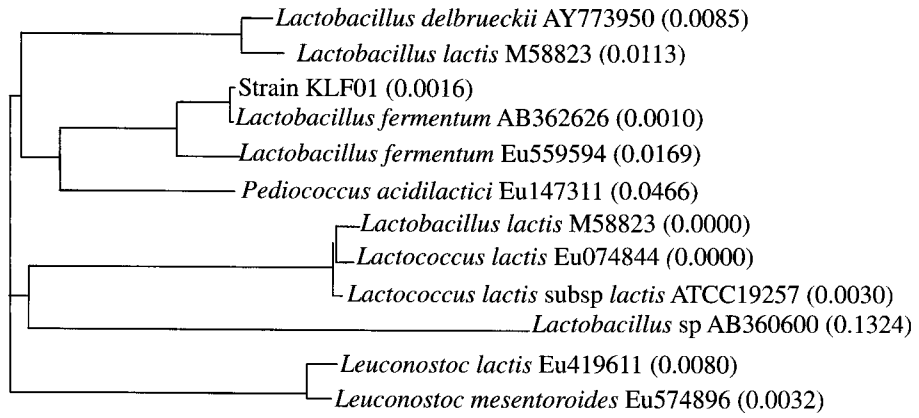

Fig. 1. Phylogenetic tree based on the 16S rRNA gene sequences of different members of lactic acid bacteria. The branching pattern was generated by the neighbor-joining method.

Lactococcus lactis subsp lactis ATCC19257, *Lactobacillus* sp AB360600, *Leuconostoc lactis* Eu419611 and *Leuconostoc mesentoroides* Eu57489 revealed 98% homology with *Lactobacillus fermentum*. Therefore, strain KLF01 was identified as *Lactobacillus* sp.

In vitro antibacterial effect of KLF01

The antibacterial substances produced from the reference strains *Lactococcus* sp. strain KLC02 and *Pediococcus* sp. strain KPD03 and strain KLF01 showed antagonism against different plant pathogenic bacteria like *R. solanacearum*, (a serious soilborne disease which causes bacterial wilt), *X. axonopodis* pv. *citri*, (Citrus canker), *X. campestris* pv. *vesicatoria* (Bacterial spot), *E. pyrifoliae* (Shoot blight) and *E. carotovora* subsp. *carotovora* group (Potato scab). *Salmonella* sp. was taken as positive control whereas water as negative control. Strain KLF01 was able to show various degree of inhibition against different bacteria. The degree of antagonism was ranged from mild (++) i.e. (=5 mm) to strong (>10 mm) (+++) inhibition zone. The zone of inhibition observed was clear and consistent. The degree of antagonism shown by strain KLF01 against *X. axonopodis* pv. *citri* was the greatest followed by *X. campestris* pv. *vesicatoria*, *R. solanacearum* and *E. carotovora* subsp. *carotovora* respectively. *Salmonella* sp. was used as positive control and water as negative control to compare the antagonistic results (Fig. 2). The other two antagonistic strains also showed similar effects against different bacteria with little or more variation (data not shown).

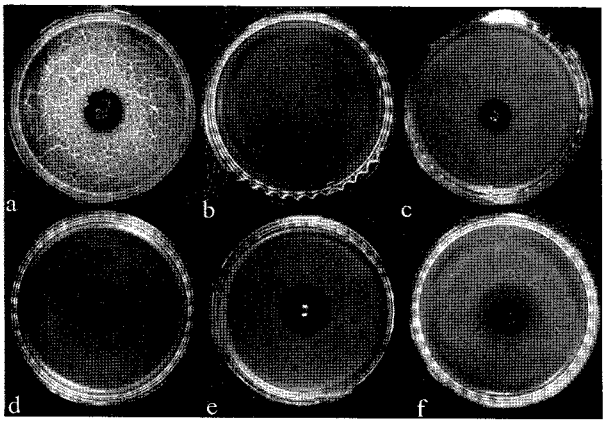

Fig. 2. Antibacterial activity of strain KLF01 against different bacteria using agar well diffusion method where (a) *Salmonella* sp., (b) water, (c) *R. solanacearum*, (d) *X.* pv. *vesicatoria*, (e) *X. axonopodis* pv. *citri*, (f) *E. carotovora* subsp. *carotovora*.

In planta antagonistic activity

Bio control efficacy of the strain KLF01 along with *Paenibacillus* sp. strain KPB3, strain KLC02 and strain KPD03 for the control of bacterial wilt caused by *R.*

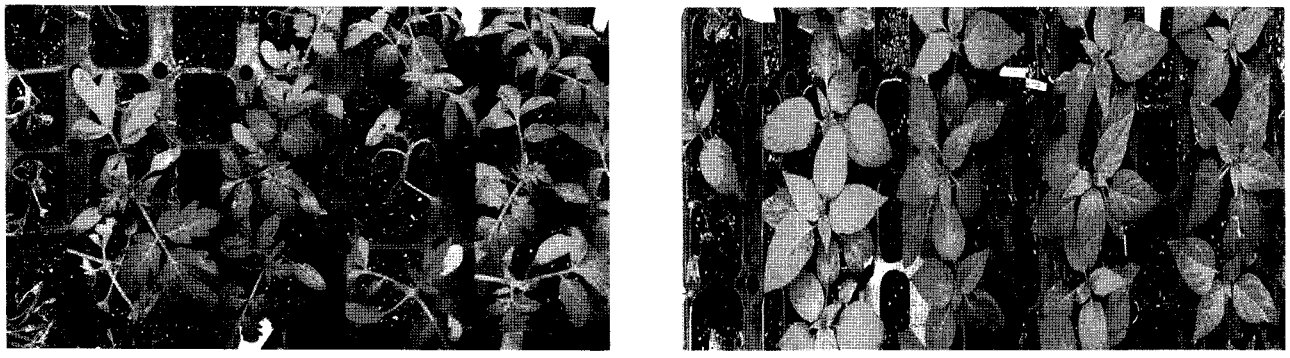

Fig. 3. Symptoms of bacterial wilt on tomato cultivar Lokkusanmaru and red pepper cultivar Buja, treated with (a) Water, (b) *Paenibacillus* sp. strain KPB3, (c) *Lactobacillus* sp. strain KLF01, (d) *Lactococcus* sp. strain KLC02, (e) *Pediococcus* sp. strain after inoculated by *R. solanacearum*.

Five ml of bacterial suspension (1×10^8 cfu/ml) was drenched and while control plants were not treated with the bacterial suspension. After 1 week the treated plants and control plants were artificially post- inoculated with *R. solanacearum* (1×10^8 cfu/ml) under greenhouse condition at 28℃. Disease severity was visually assessed on 10^{th} day after the application of bacterial wilt pathogen. Untreated set of plants showed wilting symptoms. All the other treated plants showed resistance to wilting.

Table 3. Mean value of disease severity and bio control efficacy of the bio control (BCAs) against the bacterial wilt of tomato and red pepper caused by *R. solanacearum*

Treatment[X)]	Meana[b)]			
	Disease severity[y)]		Biocontrol efficacy[w)]	
	Tomato	Red pepper	Tomato	Red pepper
Lactobacillus sp. strain KLF01	0.291[b]	0.235[b]	0.636[a]	0.718[a]
Lactococcus sp. strain KLC02	0.408[b]	0.233[b]	0.482[ab]	0.718[a]
Pediococcus sp. strain KPD03	0.264[b]	0.236[b]	0.664[a]	0.719[a]
Paenibacillus sp. strain KPB3	0.522[ab]	0.343[b]	0.344[b]	0.577[b]
Control	0.786[a]	0.833[a]		

[y)] Disease severity was visually assessed on 10[th] day after the application of bacterial wilt pathogen, *R. solanacearum*, using = [Σ (rating no. × no. of plants in the rating)/ total no. of plants × highest rating)] × 100.[w)] Control efficacy % = D.S of control-D.S of the sample/ D.S of control x 100

[ab)] Means with different superscripts differs significantly at $P < 0.05$, average of disease index in three experiments. (Tukey HSD[a)]). Values with the same letter are not significantly different according to Tukey's test ($\alpha = 0.0$).

solanacearum on tomato and red pepper was evaluated under green house conditions. The control (plant treated with water) showed disease symptoms and ultimately collapsed after 14 days after inoculation of pathogen while plants pretreated with *Lactococcus* sp. strain KLC02, *Pediococcus* sp. strain KPD03 and strain KLF01 (10^8 cfu/ml) significantly reduced the disease severity and their bio-control efficacy was higher than the control (Fig. 3c). Strain KLF01 reduced disease severity up to 63% against bacterial wilt on tomato and up to 71% bacterial wilt on red pepper. In addition, all other reference strains were also able to reduce disease severity of bacterial wilt symptoms caused by *R. solanacearum* on tomato and red pepper. Strain KLF01, KLC02, KPD03 and KPB3 reduced disease severity up to 63%, 48%, 66% and 34% respectively against bacterial wilt on tomato and 71%, 71%, 71.9% and 57% against bacterial wilt on red pepper respectively under green house conditions. *In vitro* results of strain KLF01 against *R. solanacearum* were not significant in comparison with those of other plant pathogenic bacterial strains, however biocontrol efficacy observed under green house conditions was significant (Fig. 3).

Discussion

R. solanacearum is considered to be one of the most important plant pathogenic bacteria which cause great economic losses world-wide (Hayward *et al.*, 1991). Since none of the chemical agents satisfactorily control *R. solanacearum*, biological control using antagonistic micro-organisms is a subject of increasing interest. Various control strategies, including host-plant resistance (Dalal *et al.*, 1999), cropping systems (Dalal *et al.*, 1999), soil amendments (Vincent *et al.*, 1998), integrated control (Katayama *et al.*, 1987) and biological agents, *Paenibacillus* sp. strain KPB3 (Jungi *et al.*, 2007) were reported. Various isolates of *Bacillus* sp. (Bernal, G *et al.*, 2002; Lemessa and Zellar 2007) and *Paenibacillus* sp. strain KPB3 (Jungi *et al.*, 2007) were previously reported to control bacterial wilt caused by *R. solanacearum*.

The use of microorganisms for biological purposes has become an effective alternative to control plant pathogenic bacteria. The production of various antimicrobial substances as lactic acid, acetic acid, hydrogen peroxide and bacteriocins produced by LAB has been reported. Moreover, antibacterial substances of LAB like bacteriocins have been reported to show antibacterial activity only against gram positive food spoilage bacteria (Seuk *et al.*, 2000) and human pathogens *Salmonella typhimurium*, *E. coli* and *Staphylococcus aureus* (Wen *et al.*, 2006), unlike their activity against plant pathogenic bacteria. LAB was also reported to show antagonistic effect (Ronel *et al.*, 1986) and antifungal activity (Laitila *et al.*, 2002) against some phytopathogens. Therefore, to obtain a more effective

control of bacterial wilt, these antagonistic strains were selected and tested for their antibacterial effect under greenhouse conditions too. Under greenhouse conditions, plant inoculated with the antagonistic strains reduced disease severity.

Analysis of the data from three replicates of each experiment with tomato and red pepper indicated that diseased plants were significantly reduced in compared to the control after post-inoculation of antagonistic strains. The number of dead plants over a period of time was generally less in all experiments in tomato and red pepper plants treated with antagonistic strains than control (water treatment). In addition, growth of these treated plant were healthy in compare to the untreated plant. However, some leaves were observed to be dried and little faded away in some plants (Fig. 3). The biocontrol efficacy of *Lactobacillus* sp. strain KLF01 was more significant than other two strains on tomato while there was no such significant difference on red pepper among all these strains treated plants. The biocontrol efficacy of *Lactobacillus* sp. strain KLF01, *Lactococcus* sp. strain KLC02 and *Pediococcus* sp. strain KPD03 were significantly higher than *Paenibacillus* sp. strain KPB3 (Jungi *et al.*, 2007). Though, *in vitro* test of strain KLF01 showed mild zone of inhibition against *R. solanacearum* under green house conditions the antagonistic effect in planta was significantly greater than *in vitro* conditions. These kinds of variability on *in vitro* and *in planta* test have been frequently reported. Thus, these antagonistic strains could significantly reduce the disease severity in case of tomato and red pepper plants infected by *R. solanacearum* under green house conditions.

The results of the present study indicate that the use of these antagonistic LAB strains may offer a potential alternative as a natural, biocontrol agent against *R. solanacearum*. Since the above mentioned bacterial pathogen is critical in the initiation and progression of wide variety of plant diseases, the present findings suggest that the implementation of *Lactobacillus* sp. strain KLF01 may offer a novel approach in the biological control of this disease. However, biocontrol of soil borne diseases is complex because these diseases occurs in the dynamic environment at the interface of the rhizosphere therefore, effect of the antagonistic *Lactobacillus* sp. strain KLF01 against *R. solanacearum* and other plant pathogenic bacteria is yet to be observed in the field conditions.

Acknowledgements

This study was supported by a grant from the Rural Development Administration fund (20080601-036-026-001-03-00), Republic of Korea

Literature Cited

Altschul, S.F., W. Gish, W.E. Miller, W. Myers and D. J. Lipman (1990) Basic local alignment search tool. *J. Mol. Biol.* 215:403~410.

Benkerroum, N. and W. E Sandine (1988) Inhibitory action of nisin against *Listeria monocytogenes. J. Dairy Sci.* 71:3237~3245.

Bernal, G., A. Illanes and L. Ciampi (2002) Isolation and partial purification of a metabolite from a mutant strain of *Bacillus* sp. with antibiotic activity against plant pathogenic agents. *E.J.B.* ISSN:0717~3458.

Chen, Y.-S., F. Yanagida and T. Shinohara (2005) Isolation and identification of lactic acid bacteria from soil using an enrichment procedure. *Lett in Appl Microbiol.* 40:195~200.

Dalal, N. R., S. R. Dalal, V. G. Golliwar and R. I. Khobragade (1999) Studies on grading and pre-packaging of some bacterial wilt resistant brinjal (*Solanum melongena* L.) varieties. *J. Soils Crops.* 9:223~226.

Dong, C., X. Zeng and Q. Liu (1999) Biological control of tomato bacterial wilt with avirulent bacteriocinogenic strain of *Ralstonia solanacearum. J. S. China Agric Univ.* 20:1~4.

El Abdyal, M. S., M.A. el Sayed and A. R. el Shanshourie (1996) Effects of culture conditions on the antimicrobial activities of UV-mutants of *Streptomyces corchorusii* and *S. spiroverticillatus* against bean and banana wilt pathogens. *Microbiol. Res.* 151:201~211.

Hamad S.H., M.C. Dieng, M.A. Ehrmann and R.F. Vogel (1997) Characterization of the bacterial flora of sudanese sorghum flour and sorghum sourdough. *J. Appl. Microbiol.* 83:764~770.

Hammes, W.P., N. Weiss, and W.P. Holzapfel (1991) The genera *Lactobacillus* and *Carnobcterium.* In the prokaryotes. A handbook on the biology of bacteria: ecoophysiology, isolation, identification, applications (eds Balwos, A., Truper, H. G., Dworkin, M., Harder, W. and Schleifer, K.H. Springer,

New york, USA, pp. 1535~1594.

Han K.S., Y. Kim, S.H. Kim and S. OH (2007) Characterization and purification of acidocin 1B, a bacteriocin produced by *Lactobacillus acidophilus* GP1B. *J. Microbiol. Biotechnol.* 5:774~83.

Hayward, A. C. (1991) Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum. Annu. Rev. Phytopathol.* 29:65~87.

Jungi. Ki Suk, Nagesh. S. Ipper, Seon Hwa Lee, Anupama Shrestha, Hye Jin You, Joon Hee Han, Jun Mo Cho and Chun Keun Lim (2007) Effects of a soil-born *Paenibacillus* sp. strain KPB3 on suppression of bacterial wilt disease caused by *Ralstonia solanacearum. The Korean J. of Pesticide Science.* 10:313~319.

Kang, Y., G. Mao, C. Lu and L. He (1995) Biological control of bacterial wilt of tomato by extracellular protein defective mutants of *Pseudomonas solanacearum. Acta Phytophylacica Sin.* 22:287~288.

Katayama. K and Kimura. S (1987) Ecology and protection of bacterial wilt of potato 2. Some control methods and their integration. Bull. *Nagasaki Agric. For. Exp. Stn.* 15:29~57.

Lemessa, F and W. Zellar (2007) Screening rhizobacteria for biological control of *Ralstonia solanacearum* in Ethiopia. *Bio control.* 42:336~344.

Laitila, A., H.L. Alakomi, L. Raaska, T. Mattila-Sandholm and A. Haikara (2002) Antifungal activities of two *Lactobacillus plantarum* strains against *fusarium* moulds in vitro and in malting of barley. *J. Appl. Microbiol.* 93:556~576.

Molina, L., C. Ramos, M.C. Roncheil, S. Molin and J.L. Ramos (1998) Construction assays. *Appl. Environ. Microbiol.* 64: 2072~2078.

Mundt, J. O., and J. L. Hammer. (1968) *Lactobacilli* on plants. *Appl. Microbiol.* 16:1326~1330.

Ouwehand, A.C. (1998) Antimicrobial components from lactic acid bacteria. in lactic acid bacteria microbiology and functional aspects ed. Salminen, S. and von Wright, A. pp. *New York:Marcel Dekker.* pp. 139~159.

Perry, S.F. (1995) Freeze drying and cryopreservation of bacteria. In: methods in molecular biology. J. G. Day and M. R. McLellan (eds.) *Humana Press Inc.* 21~30.

Piard J.C. and M. Lait Desmazeaud (1991) Inhibiting factors produced by lactic acid bacteria: oxygen metabolites and end-products from catabolism. 71:525~541.

Ronel Visser, H. Wilhelm Holzapfel, J. Johannes, T. Bezuidenhout, and M Kotze Johann es (1986) Antagonism of lactic acid bacteria against phytopathogenic bacteria. *Appl. Environ.* 52:552~555.

Sambrook, J and D.W. Rusell (2001) Molecular Cloning: A laboratory manual. Cold harbour laboratory press, Cold Spring Harbor, New York, USA.

Seuk Hyun Ko and Ahn Cheol (2000) Bacteriocin production by Lactococcus lactis KCA2386 isolated from white kimchi. *The Korean Journal of Food Science and Technology.* 9:263~269.

Sharpe, M. E. (1981) The genus *Lactobacillus*. p. In M. P. Starr. H. Stolp. H. G. Trulper. A. Barlows. and H. G. Schlegel. *The prokaryotes. Springer-Verlag, Berlin.* pp. 1653~ 1679.

Stiles, E.M. (1996) Biopreservation by lactic acid bacteria. *AntonieLeeuwenhoek.* 70:331~345.

Stirling, A. C., and R. Whittenbury (1963) Sources of the lactic acid bacteria occurring in silage. *J. Appl. Bacteriol.* 26:86~90.

Sung, P. J., J. K. Shin, H. B. Cho, and S. D. Kim (2005) Isolation, identification and biological control activity of SKU-78 strain against *Ralstonia solanacearum. J. Korean Soc. Appi. Biol. Chem.* 48:48~52.

Suzuki, T. and K. Yamasato (1994) Phylogeny of spore-forming LAB based on 16S rRNA gene sequences. *FEMS Microbiol Lett.* 115:13~17.

Vincent, V. M. and T. W. Mew (1998) Effect of a soil amendment on the survival of *Ralstonia solanacearum* in different soils. *Phytopathology.* 88:300~305.

Weisburg, W. G., S. M. Barns, D. A. Pelletier and D. J. Lane (1991) 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* 173:697~703.

Wen-Hsin Lin., Hwang Chin-Fa, Chen Li-Wei, Tsen Hau-Yang (2006) Viable counts, characteristic evaluation for commercial lactic acid bacteria products. *Food Microbiol.* 23:74~81.

Wood, B. J. B. and W.H. eds. Holzapfel (1955) The genera of lactic acid bacteria. Blackie Academic & Professional, UK.

세균성 시들음병에 대한 식물성 유산균(*Lactobacillus* sp.)의 저해효과

아누파마 슈레스타[1] · 최규업[1] · 임춘근[1] · 허장현[2] · 조세열[1]*

[1]강원대학교 생물자원공학부 응용생물전공, [2]강원대학교 자원생물환경학과

요 약 KLF01으로 명명된 세균이 토마토의 뿌리에서 분리 되었고 생화학적, 유전학적 검증을 통해 Lactobacillus sp.로 동종 되었다. *In vitro* 실험에서 식물성 세균인 *Ralstonia solanacearum*를 비롯하여, *Xanthomonas axonopodis pv. citri, Xanthomonas campestris pv. vesicatoria, Eriwinia pyrifoliae, Eriwinia carotovora subsp. carotovora group*에게도 저해 효과를 나타내는 것으로 확인 되었다. 고추와 토마토를 이용한 *in vivo* 실험에서는 특히 *R. solanacearum*에 대해 대조구와 비교 시들음병의 진행을 저해하는 것으로 관찰 되었다.

색인어 세균성 시들음병, 생물학적방제, 식물성유산균, KLF01, *Ralstonia solanacearum*