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Isolation of Rhizobacteria in Jeju Island Showing Anti-Fungal Effect against Fungal Plant Pathogens

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To select active bacterial strains to control plant diseases, 57 bacterial strains were isolated from the rhizosphere of the plants growing in various areas such as coast, middle and top of Halla Mountain in Jeju Island. Anti-fungal effect of isolated bactrial strains was tested *in vitro* by incubating in potato dextrose agar with isolates of four fungal plant pathogens *Rhizoctonia solani, Fusarium oxysporum, Colletotrichum gloeosporioides* and *C. orbiculare*, respectively. Thirty-four bacterial strains inhibited the hyphal growth of the plant pathogens, from which 17 strains inhibited one of the tested fungi, 10 strains two fungi, six strains three and a strain TRL2-3 inhibited all of the tested fungi. Some bacterial strains could inhibit weakly the hyphal growth of the plant pathogens, whereas some did very strongly with apparent inhibition zone between the plant pathogens and bacterial strains indicating the unfavorable condition for hyphal growth. Although there was no apparent inhibition zone, some bacterial strains showed a strong suppression of hyphal growth of plant pathogens. Especially, the inhibition by TRL2-3 was remarkably strong in all cases of the tested plant pathogens in this study that could be a possible candidate for biological control of various plant diseases.

KEYWORDS: Antagonistic bacteria, Anti-fungal activity, Biological control, Plant disease

Plant protection using antagonistic microorganisms has been tried in many crops (Handelsman and Stabb, 1996; van Lenteren, 1995). Recently, this type of disease control has been search-lighted because of their beneficent characteristics such as environment favorable, no selection of resistance pathogen, etc. (Albajes *et al.*, 1999). In many cases, however, the application of the antagonist to the field could not control plant diseases contrary to its expectation even if the antagonists has showed an high level of anti-fungal activity in the greenhouse (Rovira and Davey, 1974). It seems that the efficacy of the antagonists is changed in the rhizosphere by some unfavorable environments such as humidity, temperature, other competitive microorganism, etc. (Kloepper and Mariano, 2000). Therefore, the efficacy of the antagonists could be maintained and protect plant disease if the environment may be kept in the favorable condition for the antagonists.

Recently, hydroponic cultivation has been rapidly increased in Korea including Jeju Island (Nam *et al.*, 2001). There are two systems in hydroponic cultivation, artificial soil medium culture system and water culture system (Nam *et al.*, 2001). It is likely that the biological control could be useful control method in hydroponic cultivation in which the rhizopheral environment could be well controlled. Moreover, for satisfaction of the demand of consumers for more fresh and safe products, the control should be substitute biological control by pesticide.

Until now, numerous bacterial strains showing anti-fungal activity have been reported, however, very rarely in Jeju Island. This study was carried out to isolate the antagonistic bacteria from the rhizosphere of plant in various areas in Jeju Island and the anti-fungal activities of the isolated bacterial strains were tested against several plant pathogens.

Twenty-two plants included with roots were collected from various areas of coast and mountain Halla in Jeju. One g fresh weight of the roots which attached soils were removed by tap water was thoroughly homogenized with 1 *ml* of sterilized water in a mortar by using pestle. After filtering with three sheets of cheesecloth, the filtrate was diluted 10 times with sterile water. Three hundred *μl* of each dilute was taken three times and unfolded on tryptic soy agar medium (TSA). The TSA plates were incubated at 28°C for 2 days and the plates containing 1 to 10 colonies were selected. The colonies were classified by visual criteria. By the streaking method every colony was further isolated on TSA. After incubation at 28°C for 2 days the well-developed cells were transferred to TSA. Each bacterial strains were mixed with glycerol 1:1 (v/v) in an ependorftube and stored at −80°C until be used.

The isolated bacteria were spotted on the middle of half side of PDA and then a mycelial disc (5 mm in diameter)

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of the plant pathogen from the margin of growing culture was placed at the center of the opposite side. The antifungal activity was surveyed against 4 plant pathogenic fungi; *Rhizoctonia solani*, *Fusarium oxysporum*, *Colletotrichum gloeosporioides* and *C. orbiculare*. After incubation for 7 days at 28°C, inhibition of mycelial growth of each fungus was rated as following: +, weakly inhibited; ++, strongly inhibited; and +++, very strongly inhibited.

Percent inhibition of hyphal growth by a strain TRL2-3 showing anti-fungal activity to all of the tested plant pathogens was evaluated by following: inhibition rate (%) = [1 − (length of fungal colony near the isolate/length of fungal colony opposite of the isolate)] × 100.

Fifty-seven bacterial strains were isolated from the rhizospheres of the plant roots. Among them 34 bacterial strains inhibited the hyphal growth of at least one plant pathogens tested, in which 17 strains inhibited one fungus, 10 isolates did two fungi, 6 isolates were effective to three fungi simultaneously and one strain TRL2-3 inhibited all of the tested fungi (Table 1). It was more than 50% of the total bacteria isolated and high frequency compare to those of found from other area in Korea (Jeun *et al.*, 2001). Among 34 bacterial strains expressing anti-fungal activity, seven strains were isolated from the coast area, 13 isolates from middle of the mountain and 14 isolates from top of the mountain (Table 1). Although there was no statistical data, more bacteria showing anti-fungal effect were isolated from the rhizospheres of plant roots grown in the mountain than those isolated from the coast areas. There was no explain about that but one can sug-

Table 1. Inhibition of hyphal growth of plant pathogens *Rhizoctonia solani*, *Fusarium oxysporum*, *Colletotrichum gloeosporioides* and *C. orbiculare* by various bacterial strains isolated from the rhizosphere of the plant in Jeju

Bacterial isolate[a]	Inhibition of hyphal growth of plant pathogens by bacterial isolates in PDA			
	Plant pathogen			
	R. solani	*F. oxysporum*	*C. gloeosporioides*	*C. orbiculare*
CRJ1-2	−	−	−	+
CRJ2-2	−	−	−	+
CRJ3-1	+	+	−	−
CRJ3-3	−	++	−	+
CRL1-3	++	−	++	+
CRL4-3	−	+	−	−
CRL5-1	−	−	−	+
MRJ1-1	++	−	−	−
MRJ1-3	−	−	−	+
MRJ2-2	−	−	+	+
MRJ2-3	−	+	−	+
MRJ3-1	−	+	−	−
MRJ3-2	+	−	−	−
MRJ3-3	−	+	−	−
MRL1-2	−	−	+	−
MRL2-1	−	−	+	−
MRL2-2	−	+	−	++
MRL2-3	++	−	+++	++
MRL2-4	−	−	+	+
MRL3-1	−	+	++	++
TRJ1-1	−	++	+	−
TRJ2-1	−	−	+	−
TRJ2-2	−	−	+	+
TRJ3-2	−	−	−	++
TRJ4-1	−	−	−	++
TRJ4-3	−	−	−	++
TRL2-1	−	+	+	−
TRL2-2	−	+	+	+
TRL2-3	++	+++	+++	+++
TRL3-1	−	++	+	+
TRK1-2	−	−	−	++
TRK2-2	+	−	++	+++
TRK3-1	−	−	−	++
TRK3-2	−	−	+	+

[a]C means strains isolated from coast areas of the Mt. Halla; M, from middle areas; T, from top areas, respectively.
[b]−, non inhibited; +, weakly inhibited; ++, strongly inhibited; +++, very strongly inhibited.

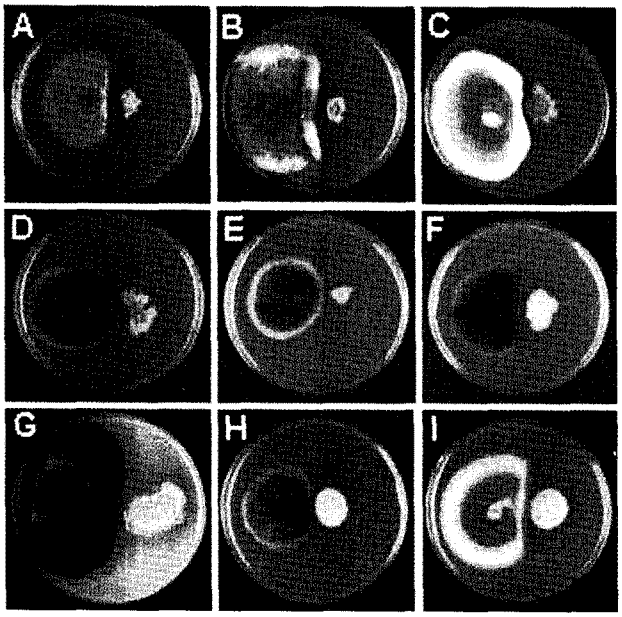

Fig. 1. Inhibition of hyphal growth of plant pathogens *Rhizoctonia solani*, *Fusarium oxysporum*, *Colletotrichum gloeosporioides* and *C. orbiculare* by bacterial strain TRL2-3 (A, B, C, and D), *C. orbiculare* by TRK2-2, TRK1-2 and MRL2-3 (F, G and H), *C. gloeosporioides* by MRL2-3 (I) *in vitro*. Strain BRL2-1 showed no inhibition of hyphal growth of *C. orbiculare* (E).

gest that many effective bacteria prefer relative low temperature in their environment. However, to establish such suggestion, more experiment should be carried out.

The anti-fungal activity was evaluated by the inhibition of hyphal growth of the plant pathogens adjacent to the bacterial strains (Fig. 1). Many bacterial strains had no anti-fungal effects (Fig. 1E). The inhibition of hyphal growth by the bacterial strains was various. Some bacterial strains could inhibit weakly the hyphal growth of the plant pathogens (Figs. 1G and 1H). Several bacterial strains inhibited the fungal growth strongly in which an inhibition zone was observed between the plant pathogens and bacterial strains, indicating the unfavorable condition for hyphal growth (Figs. 1B and 1I). Although there was no apparent inhibition zone, some bacterial strains showed a strong suppression of the hyphal growth of the plant pathogens (Figs. 1A, 1C, 1D and 1F). The bacterial strains showed different anti-fungal effects individually either in quantitatively or qualitatively (Table 1). Half of the effective bacteria showed anti-fungal only one plant pathogen tested. Most of them weakly or strongly inhibited the mycelial growth (Table 1). On the other hand, the bacteria showing anti-fungal effects very strongly were effective to at least three different plant pathogens (Table 1). Based on these results, it is suggested that there may be an effective substance such as lipopoly-saccharides secreted from the bacteria. It was known that the lipopolysaccharides showed antifunal activity (Dow *et al.*, 2000).

The strain TRL2-3 showed the antifungal activity to all tested plant pathogens (Table 1) of which hyphal growths were very strongly inhibited (Table 2). To illustrate the effective anti-fungal activity of the strain TRL2-3, the *in vitro* tests were further replicated resulting in the significant inhibition of plant pathogens by TRL2-3 (Table 2). All of the hyphal growths of the tested plant pathogens were almost inhibited over 50% and *C. orbiculare* was inhibited to 70% by TRL2-3 (Table 2). This bacterial strain may be expected to control plant disease not only through directly anti-fungal activity but also through inducing systemic resistance in plants. Such rhizosphere bacteria have been reported as a growth promoter as well as a resistance inducer (van Loon *et al.*, 1998). They were defined as plant growth promoting rhizobacteria (PGPR; Kloepper *et al.*, 1980). To find effective bacteria for plant disease control, both direct anti-fungal and resistance-inducing efficacy should be tested. Therefore, more additional experiments, such as evidence of resistance expression in planta, should be carried out to select effective bacteria for plant disease control.

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Table 2. Inhibition rates of hyphal growth of plant pathogens *Rhizoctonia solani*, *Fusarium oxysporum*, *Colletotrichum gloeosporioides* and *C. orbiculare* by the selected bacterial strain TRL2-3

Bacteiral strain	Inhibition rate (%) of hyphal growth of plant pathogens (%)[a]			
	R. solani	*F. oxysporum*	*C. gloeosporioides*	*C. orbiculare*
TRL2-3	67.2±9.6[b]	55.0±13.2	58.6±7.4	70.0±11.4

[a]Inhibition rate (%) = [1 − (length of fungal colony near the strain/length of fungal colony opposite of the strain)] × 100.
[b]Values represent means±standard deviation of three separated experiments, each containing six plates per treatment.

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