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BIO-HERBICIDE EFFECT OF SALT MARSH TOLERANT *ENTEROBACTER* SP. I-3 ON WEED SEED GERMINATION AND SEEDLING GROWTH

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Abstract

Weeds are major challenges in crop cultivation and cause yield loss. The bacteria based bio-herbicides are emerging against chemical herbicides. This study was aimed to explore the bio-herbicide effect ofsalt marsh tolerant *Enterobacter sp.* I-3 on various weed species. The efficacy of I-3 bacterial isolates against weed growth was compared with I-4-5 bacterial strain. The bacterial strains, I-3 and I-4-5 inhibited the seed germination of *Cyperus microiria* Maxim. *Enterobacter* sp. I-3 showed higher weed control activity than I-4-5. It was confirmed with growth reduction of *C. microiria* Maxim. The seed germination of *Digitaria sanguinalis* L. weed was accelerated during the interaction of I-4-5 and it was drastically declined by I-3 bacterial culture. However, *Alopecurus aequalis* Sobol. seeds treated with either I-3 or I-4-5 bacterial culture showed no significant germination inhibition. The results of this study suggested thatsalt marsh tolerant*Enterobacter* sp. I-3 can be applied as bacterial herbicides to control weeds in agricultural fields.

Key words: Bio-herbicide, *Enterobacter* sp. I-3, Growth inhibition, Weeds.

Introduction

Weeds are strong competitors against crop plants for nutrients uptake, light and space, and inhibit the growth and yield of crops (Arnold *et al*., 1988; Halford *et al*., 2001). The seed germination and growth of weeds are faster than crop plants and also support the insect pest to cause diseases in crops. Although, the tillage and crop rotation practices reduce the weed flora (Marshall *et al*., 2003; Koocheki *et al*., 2009) but those measures cannot provide the estimated result of weed control. To enhance the crop yield against weeds, farmers are using the chemical herbicides including glyphosate, dicamba and 2,4,D, and the deposition of chemicals in soil and water damages the food chain in ecosystems and also harmful to humans (Radosevich *et al*., 1997). Kim *et al*. (2013) reported that 32% of food products in Korea are unappropriate for consumption because of maximum residue limit of pesticides. The frequent use of chemical herbicides induces herbicide-resistant weeds (Batish *et al*., 2007; Aktar *et al*., 2009).

The effective environmental friendly approach is needed to reduce the chemical usage in agricultural fields. The identification of microbial and plant based herbicides would be an alternative to chemical herbicides (Weissmann & Gerhardson, 2001; Omer *et al*., 2010; Lee *et al*., 2015). The allele chemicals such as polyacetylenes, phenollics, flavonoids, steroids, tannins, quinines, terpenoids, fatty acids, organic acids, ketones and other chemicals secreted from plants into their surrounding environment influence the regular growth of nearby plants (Soltys *et al*., 2013) and the decomposition of those plants in soil reduce the seed germination and growth of weeds (Dayan *et al*., 2000). On the other hand, the application of crop beneficial and weed-controlling microbes would be a successful and sustainable management of weeds. Several the studies revealed that microbial association enhance the plant growth and yield by regulating the primary and secondary metabolism of crop plants (Kang *et al*., 2012; Hashem *et al*., 2015; Radhakrishnan *et al*., 2015). However, plant growth promoting bacteria prevent the pathogen infection by their secretion of antibiotics, siderophore and hydrogen cyaninde (Ahmad *et al*., 2008). Very few studies were conducted to determine the bio-herbicide effect of bacteria. Some of the bacterial species such as *Pseudomonas putida*, *Stenotrophomonas maltophilia* and *Enterobacter taylorae* were identified as bioherbicides (Mazzola *et al*., 1995). The usage of deleterious rhizobacteria in weeds controls their growth and is inexpensive than chemical (Arshad & Frankenberger, 1991). Thus, this study was aimed to explore the bio-herbicidal effect of salt marsh tolerant *Enterobacter* sp. I-3 on seed germination and seedling growth of different weed species.

Materials and Methods

In a previous study, we found I-3 and I-4-5 bacterial isolates showed better bio-herbicidal activity than other bacterial species (301) isolated from soil collected from various parts of agricultural fields, Republic of Korea. Two bacterial strains, I-4-5 and I-3 significantly reduced the seedling growth of radish when compared to their controls. (Park *et al*., 2015). In the current study, the bacterial (I-3 and I-4-5) isolates were cultured in Luria Bertani media agar (LB; Merck Co., Germany) for 7 days at 30°C on a shaking incubator at 200 rpm to know their weed control. Weed seeds (*Digitaria sanguinalis* L., *Alopecurus aequalis* Sobol. and *Cyperus microiria*

Maxim.) were surface sterilized with sodium hypochlorite (5 %) for 10 min, and thoroughly rinsed with autoclaved double distilled water. The sterilized seeds were sown in plastic pots containing horticultural soil (13–18% (w/v) peat moss, 7–11% perlite, 63–68% coco-peat, and 6–8% zeolite, ~90 mg/kg NH^{4+}, ~205 mg/kg NO_{3-}, ~350 mg/kg P_2O_5, and ~100 mg/kg K_2O) under controlled green-house conditions (30±2 °C). The distilled water and LB medium were used as control for this experiment. The bacterial cultures (I-3 and I-4-5) were autoclaved and their culture filtrate was used on weed seeds to confirm the metabolites produced from bacteria that inhibit the seed germination. The rate of seed germination was recorded on every day.

I-3 bacterial strain was identified on the basis of 16S rDNA (ribosomal DNA) sequence. The genomic DNA of I-3 bacterial strain was extracted and their 16S rDNA sequence was amplified using the 518F primer (CCAGCAGCCGCGGTAATACG) and 800R primer (TACCAGGGTATCTAATCC) and compared with relative similar sequences. I-3 bacterial isolate was named as *Enterobacter* sp. (Park *et al*., 2015). The different dosages of I-3 culture (25%, 50%, 75% and 100%) were prepared by using sterile LB medium and applied to seeds of *Cyperus microiria* Maxim. The seedling growth was measured after three weeks.

Statistical analysis

The experiments were randomly designed and data obtained from this study were expressed as means±SE. Data were compared with statistical analysis of one way analysis of variance (ANOVA) by Duncan's multiple range test (DMRT) using a SPSS software (V. 13.0, Chicago, USA) and P values <0.05 were regarded as statistically significant.

Results and Discussion

Digitariasanguinalis commonly known as crabgrass belongs to the Poaceae family and it is widely distributed and reproduced seeds by tillage method. The regeneration capacity of this weed from basal node is helped to spread the weed population (Gallart *et al*., 2009). The growth of *D. sanguinalis* affects the crop plant growth and yield including soybean and maize and while the seedling emergence of weeds can be delayed by using spring cover crops, early sowing and increasing crop density (Huarte & Benech-Arnold, 2003; Oreja *et al*., 2017). Some of plant extracts containing allele chemicals prevent *D. sanguinalis* weed germination and growth as a result of membrane damage, photosynthesis reduction and inhibition of cell division (Travaini *et al*., 2016). In the current study, we compared the germination of *D. sanguinalis* seeds extended up to 16 days in bacteria treated and non-treated experiments (Fig. 1). The higher rate of seed germination was observed in bacterial culture of I-4-5 (91%) and their culture filtrate (99%) treatments than their controls (72%). The significant reduction of seed germination was noted in *Enterobacter* sp. I-3 and sterile I-3 culture filtrate (61%).

Some of the bacteria associated with roots causes a detrimental effects on plants, but it might be used as bio-herbicides to inhibit weed growth (Boyette & Hoagland 2015). *Aeromonas, Agrobacterium, Alcaligenes, Bacillus, Burkholderia, Chryseomonas, Enterobacter, Microbacterium, Pseudomonas* and *Xanthomonas* species showed negative effects in plants (Flores-Vergas & O'Hara 2006; Li & Kremer 2006; Kim & Rhee, 2012; Sarwar & Kremer, 1995; Boyette & Hoagland, 2015; Barghouthi & Salman, 2010). The inhibitory effect of bacteria on plants varied with different species or cultivars (Kennedy *et al*. 2001). The current study is the first report to suggest that the use *Enterobacter* sp. I-3 control *D. sanguinalis* seed germination.

However, *A. aequalis* (shortawn foxtail) is a noxious weed in oil seed rape, wheat, barley and other crop cultivation and may cause ~50% of yield loss (Guo *et al*., 2015 and 2016; Iwakami *et al*., 2017). Thifensulfuron-methyl was used to control the weed growth. However, the weed became resistant to herbicides were identified in 2004 (Uchikawa *et al*., 2005). It is suggested that alternative environmental friendly methods are needed to control the weed population. We have attempted to find the affect of bacterial bio-herbicides. The results of the current study showed that the germination of *A. aequalis* seeds was faster than *D. sanguinalis* and it reached maximum (100%) germination within 7 days (Fig. 2). The culture medium (LB), I-3 and I-4-5 bacterial cultures, and their sterile culture filtrates were tested to explore the weed control effects of bacteria, but there was no significant variation between control and bacteria-treated seed germination.

The weed population of *C. microiria* affects the rice growth and yield and plant based bio-herbicide (alfalfa pellet) inhibited the weed growth (Xuan *et al*., 2001). In addition, I-3 and I-4-5 bacterial culture and their culture filtrates significantly suppressed the weed (*C. micoiria*) seed germination (Fig. 3). The results of the present study showed that normal seed germination rate was observed as 76% at 7 days, but it was gradually declined in the treatments of sterile culture filtrate of I-4-5 (60%), I-4-5 bacteria (48%), I-3 bacteria (36%) and their culture filtrates (14%). *Enterobacter* sp. I-3 significantly reduced the seed germination and seedling growth of *C. micoiria* (Fig. 4 and 5). When the bacterial culture was diluted as 25%, 50%, 75% and 100% to find out the efficacy of I-3 bacteria, it showed that 20%, 26%, 32% and 37% of seedling growth reduction, respectively.

The secretion of microbial metabolites can suppress the plant growth (Fredrickson & Elliott 1985). The biosynthesis of auxin from microbes and their interaction with plants were widely reported (Radhakrishnan *et al*. 2013). Previously, we reported that *Enterobacter* sp. I-3 synthesized and secreted high amount of IAA (Park *et al*., 2015) and caused negative effect on weed growth (Sarwar&Frankenberger 1994) due to the stimulation of aminocyclopropane-1-carboxylate (ACC) synthase, which plays vital role in ethylene biosynthesis (Kende, 1993). *Enterobacter* sp. I-3 might suppress the photosynthesis, gibberellins production and several amino acids and while stimulating the abscisic acid accumulation in infected weeds (Radhakrishnan *et al*., 2016).

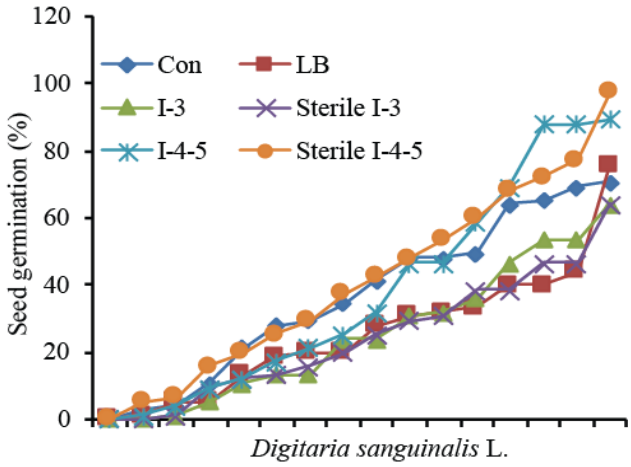

Fig. 1. Effect of bacterial isolates (I-3 and I-4-5) and their culture filtrates on seed germination of *Digitaria sanguinalis* L.

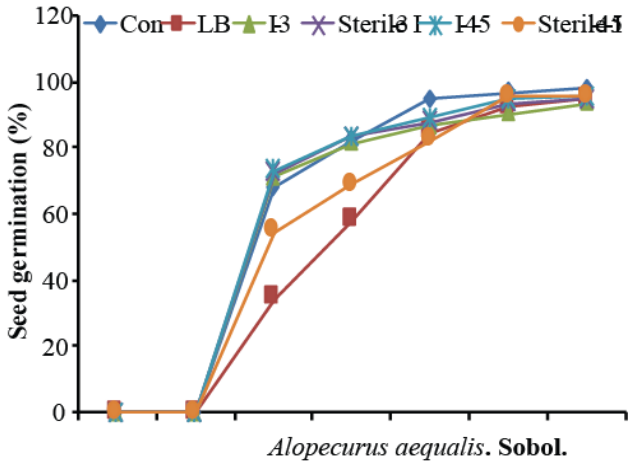

Fig. 2. Influence of bacterial isolates (I-3 and I-4-5) and their culture filtrates on seed germination of *Alopecurus aequalis*. Sobol.

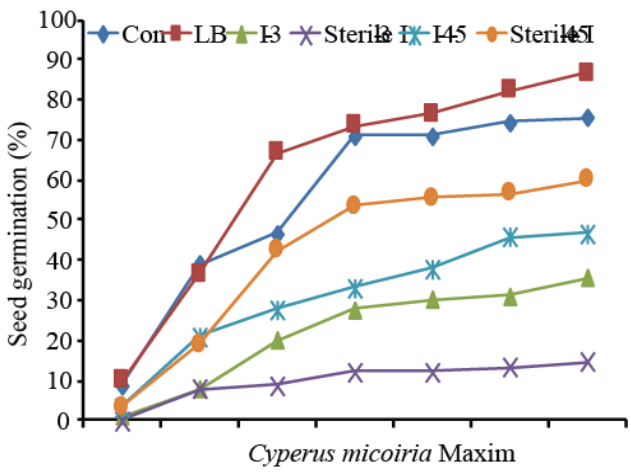

Fig. 3. Effect of bacterial isolates (I-3 and I-4-5) and their culture filtrates on seed germination of *Cyperus micoiria* Maxim.

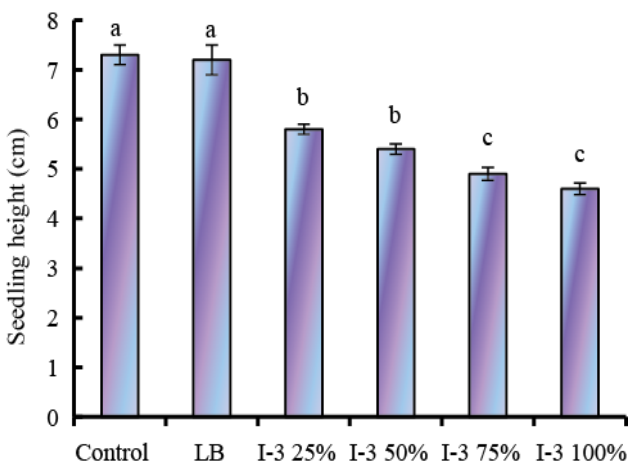

Fig. 4. Effect of bacterial isolate I-3 on seedling growth of *Cyperus micoiria* Maxim.

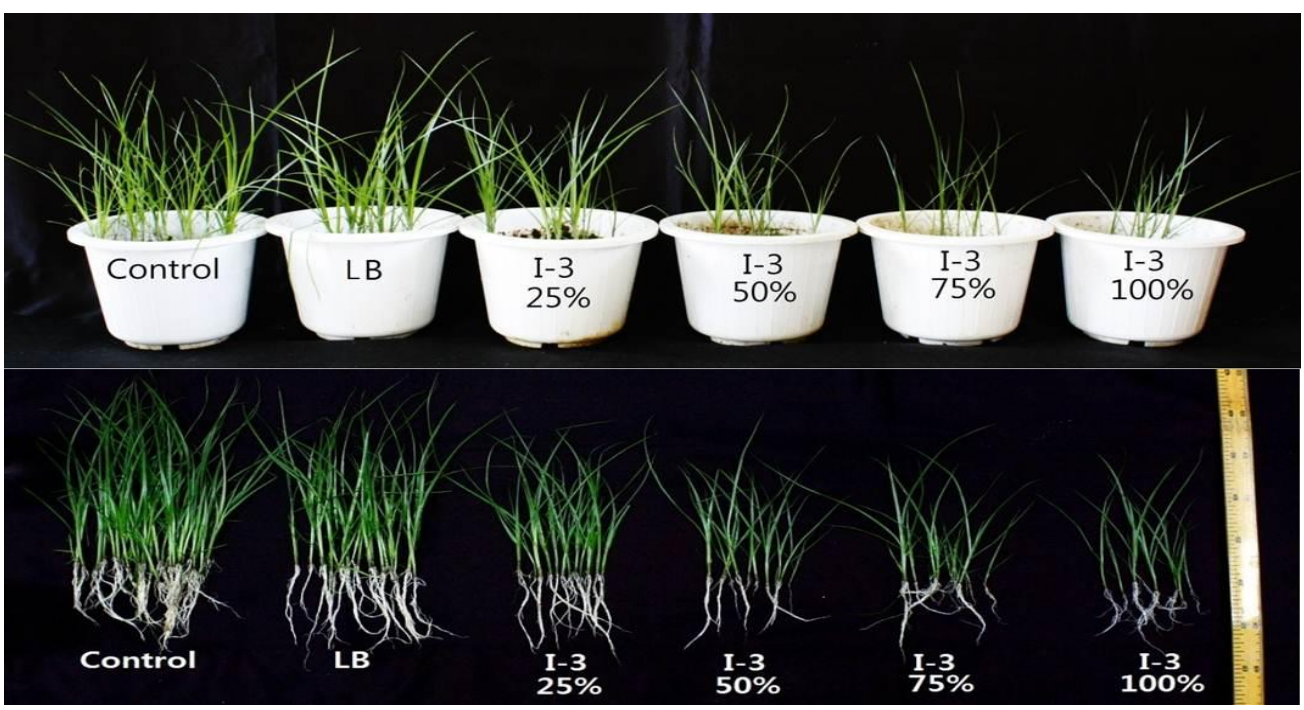

Fig. 5. Influence of bacterial isolate, *Enterobacter* sp. I-3 on seedling growth of *Cyperus C. micoiria* Maxim.

Conclusions

The results of this study concluded that the utilization of *Enterobacter* sp. I-3 would be an effective alternative against chemical herbicides in controlling some of the weed species including *D. sanguinalis* and *C. micoiria*. The enlighten results of current study are giving new idea to develop bacteria based bioherbide to weed management and to protect the soil nature.

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