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# *Curtobacterium sp.* MA01 generates oxidative stress to inhibit the plant growth

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ABSTRACT

Soil habitat bacteria associate with plants to offer growth benefits or to cause detrimental effects on host-plants. The present study was aimed to know the basis for inhibition of petunia plant growth by *Curtobacterium sp.* MA01. The bacterial isolate MA01 infected petunia plants reduced the growth of roots and shoots without causing any disease. The genomic DNA of MA01 was isolated and the bacterium was identified as *Curtobacterium sp.* MA01 by molecular and phylogenetic studies. The bacterial interaction caused the degradation of protein synthesis in infected plants. However, the high rate of lipid peroxidation was observed in plants associated with bacteria than their control. The significant reduction of antioxidants such as carotenoids, catalase and polyphenol oxidase activities were found in bacterium treated plants. The results of this study revealed that *Curtobacterium sp.* MA01 caused the oxidative stress and declined the antioxidants to suppress the growth of petunia plants.

## 1. Introduction

Microorganisms are major living organisms in soil, which play vital role to decompose several biological and organic materials. The numbers of bacterial and fungal genera were reported as phosphate solubilizers, nitrogen fixers, plant growth hormone producers to enhance the growth of plants (Kang et al., 2015b; Radhakrishnan et al., 2015). On the other hand, those microorganisms secrete some of the toxic metabolites to prevent the growth of plant pathogens (Kang et al., 2015a; Radhakrishnan et al., 2018) and weeds or plants (Radhakrishnan et al., 2017). The plant growth suppressing microbes can be used as bioherbicides to control the weed populations (Boyette and Hoagland, 2015). The growth and yield of crop plants are affected by diversified weed growth in agricultural fields. The application of chemicals to prevent the weed growth has a possibility to develop multiple herbicide resistant weed populations (Culliney, 2005) and affects the food quality due to the deposition of pesticides and herbicides (Kim et al., 2013). The harmless and environmental friendly microorganisms or their products might be useful to prevent the weed growth. Several groups of bacteria and fungi were reported as weed controlling agents, but the mechanism of weed growth suppression is not studied well (Radhakrishnan et al., 2018).

Recently, we reported that the bur cucumber seed extracts and *Enterobacter spp.* I-3 inhibited the weed growth by altering hormones and nutrients uptake (Lee et al., 2015; Radhakrishnan et al., 2017). The understanding of bioherbicidal microbial interaction with plants would be helpful to find out the economic importance of those microorganisms. The previous studies highlighted the bioherbicidal effects of bacteria and fungi on weed seed germination and biomass variations, while few studies were conducted in biochemical changes in weeds (Radhakrishnan et al., 2018). An analysis of stress related parameters including reactive oxygen species (ROS) and antioxidants in plants reveals the detrimental biomolecular changes of infected plants (Sewelam et al., 2016).

Petunia is an ornamental plant which contains economically important secondary metabolites. The growth of plants is affected by several biotic and abiotic environmental factors. Previously, we showed the stunted growth of petunia due to the effect of a bacterial species (Radhakrishnan and Sandhya, 2019) and identified as *Curtobacterium spp.* which are Gram-positive bacilli present in soil and plants (Mariita et al., 2015; Bulgari et al., 2014) and not harmful to human (Agarkova et al., 2012). Some of the *Curtobacterium spp.* promote the plant growth and act as a biocontrol agents against pathogens (Feng et al., 2017). There is no report on bioherbicidal activity of *Curtobacterium spp.* This

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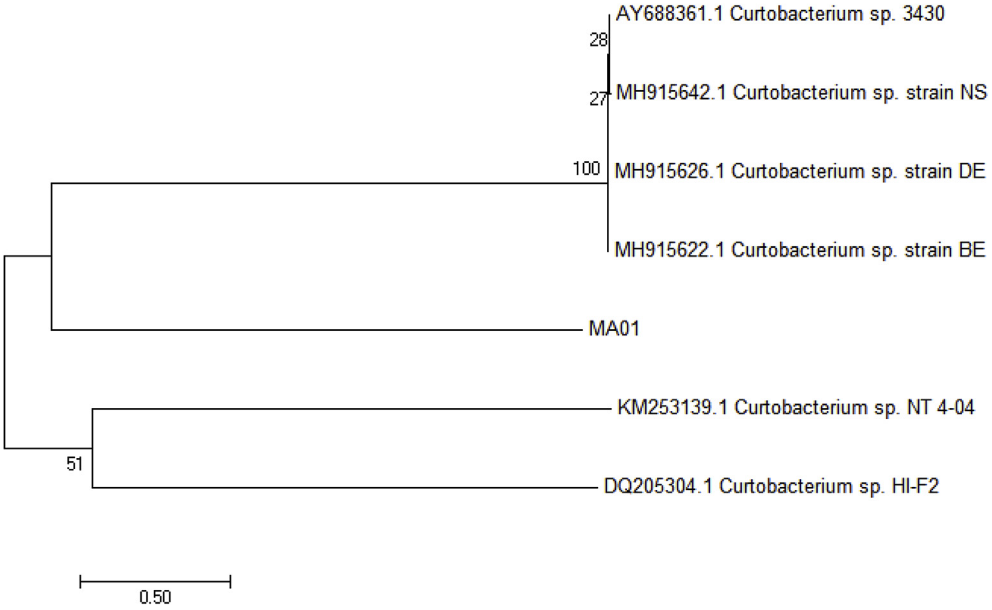

**Fig. 1.** Phylogenetic tree of the bacterial isolate *Curtobacterium sp.* MA01 (*KT456284.1*), based on the 16S rDNA sequences of MA01 and related bacteria.

study was aimed to reveal the growth inhibitory effect of *Curtobacterium spp.* on petunia plants by analyzing oxidative stress and protein metabolisms.

## 2. Materials and methods

### 2.1. Bacterial culture and identification

In our preliminary study, the infected petunia plants grown soil were collected and bacterial strain MA01 was isolated from soil and roots of petunia, and then it reduced shoot and root growth (Radhakrishnan and Sandhya, 2019). For identification, the bacterial strain MA01 was cultured on nutrient broth for 2 days at 30 °C. The genomic DNA of bacterial culture was extracted and the bacterium was identified on the basis of partial 16S ribosomal DNA (rDNA) sequence. The forward primer (518F: CCAGCAGCCGCGGTAATACG) and reverse primer (800R: TACCAGGGTATCTAATCC) were used to sequence the 16S rDNA. The obtained MA01 sequences were submitted to the BLAST search program (http://www.ncbi.nlm.nih.gov/BLAST/) to know the nucleotide sequence homology. The closely identical sequences obtained from BLAST search results were aligned through CLUSTAL W and their phylogenetic relation was detected on neighbor-joining tree constructed by using MEGA software.

### 2.2. Plant proteins, lipid peroxidation and antioxidants analysis

To find out the reason for petunia plant growth suppression by bacterial isolate MA01, the content of proteins, lipid peroxidation, and antioxidants was analysed and compared with control plants. The plant samples were ground with 10 mM phosphate buffer (pH 7.0) and centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatant of the samples were separated and used for biochemical analysis. The content of proteins in petunia plants was determined by using Bradford (1976) method. The lipid peroxidation in the supernatants was determined by quantification of malondialdehyde (MDA) according to the procedure of Ohkawa et al. (1979). Catalase (CAT) activity was assayed using the method described by Aebi (1984). However, plant samples were homogenized with 50 mM Tris–HCl buffer (pH 7.0) containing 3 mM MgCl$_2$, 1 mM EDTA (Ethylenediaminetetraacetic acid), and 1.0% PVP (Polyvinylpyrrolidone) and centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatant was collected to assess CAT activity. Polyphenol oxidase (PPO) activities were estimated using the method described by Kar and Mishra (1976). Plant samples were homogenized in 0.1M phosphate buffer (pH 6.8) and centrifuged at 2 °C for 15 min at 17,000 rpm. The supernatant was used to measure the activity of PPO. Photosynthetic pigments were extracted from the leaves and ground using 80% acetone. The carotenoid contents were estimated according to the method of Lichtenthaler (1987).

### 2.3. Statistical analysis

The data obtained from biochemical studies were expressed as mean ± SE and one-way analysis of variance was done by using Duncan's multiple range test. All statistical analyses were performed at the level of $P$ values $< 0.05$ by using SPSS 10.0 (SPSS Inc. USA).

## 3. Results and discussion

Bioherbicides are ecologically important to control the weed growth and sustainable agriculture. Previously, we observed that less number of roots and leaves and while limited length of roots and leaves in petunia plants affected by a bacterial strain MA01, and it was suggested that it might be useful to reduce the weed population (Radhakrishnan and Sandhya, 2019). In this present study, the bacterial strain MA01 was identified by their ITS region of DNA sequences and phylogenetic analysis. The obtained sequences were analysed by highest sequence homology proportion, lowest E values and query coverage in BLAST programme. Sequence of other genera was used to authentic the candidate bacterium. The phylogenetic results of this study showed that the isolated bacterium was *Curtobacterium spp*. MA01 (Fig. 1). The sequence was submitted to NCBI GenBank and was assigned Acession No. KT456284.1.

The bioherbicidal substances from bacteria and their mode of actions are diversified. For example, *Enterobacter sp.* I-3 significantly suppressed the weeds of *Cyperus microiria* Maxim, *Digitaria sanguinalis* L. and *Alopecurus aequalis* Sobol (Radhakrishnan et al., 2017) by producing indole acetic acid (IAA), which might trigger the ethylene biosynthesis in infected weeds or plants (Park et al., 2015). Similarly, *Curtobacterium spp.* produce T-2 toxin and trichothecenes (Ueno et al., 1983) would be a reason for preventing weed growth.

The toxins or plant growth suppressing substances from bioherbicidal agents are possible to alter the plant metabolisms. The protein is

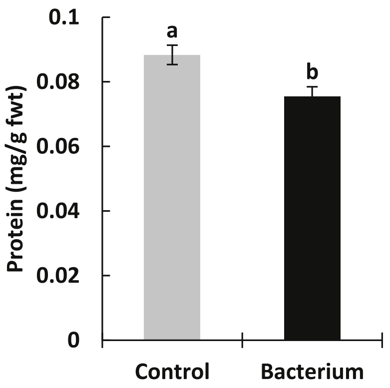

**Fig. 2.** Influence of *Curtobacterium sp.* MA01on protein contents in Petunia plants.

an important biomolecule to regulate cellular functions. The protein content of petunia treated with *Curtobacterium spp.* MA01 decreased as 15% than their controls (Fig. 2). During stress condition, the proteolytic enzymes might act to degrade the proteins (Radhakrishnan and Lee, 2013a).

Moreover, an accumulation of MDA reveals that plants are under oxidative stress (Radhakrishnan and Lee, 2013b). Fig. 3 showed that control plants had 36.41 mg of MDA/g fwt, but bacterial interaction elevated the MDA (147.68 mg of MDA/g fwt) level, which reflected that *Curtobacterium spp*. MA01 cause the detrimental effects and create the oxidative stress to suppress the plant growth. Gill and Tuteja (2010) reported the higher accumulation of MDA damages membrane proteins, ion channels, inactivating receptors and enzymes and leads to decrease in membrane fluidity of cells.

Antioxidants are playing vital role to scavenge the ROS production and reduce the oxidative stress. Catalase, peroxidase, superoxide dismutase are major enzymes to reduce the ROS in cells. Unfavorable environmental conditions decrease those antioxidants in plants (Radhakrishnan and Lee, 2013b). Catalase converts $H_2O_2$ into water and oxygen. In current study, the catalase activity was declined as 32.5% in plants infected by bacteria when compared to bacteria free plants. Similarly, Plants treated with *Curtobacterium spp.* MA01 suppressed the expression of PPO (31.0%) in petunia plants (Fig. 3). PPO is a nuclear encoded enzyme to prevent the oxidative stress. The results of this study revealed that bacterium strongly influence the antioxidants enzyme activities to reduce the plant growth. Moreover, non-enzymatic antioxidants are decreased during stress conditions. One of the major plant pigments, carotenoids (non enzymatic antioxidants) act as scavenger of singlet oxygen by quenching process, which is helpful to reduce the oxidative stress and photooxidation (Siefermann-Harms, 1987). The results of our study showed that the content of carotenoids was significantly decreased (34.2%) in bacterium treated plants.

In conclusion, *Curtobacterium spp.* MA01 affected the plant growth by altering protein metabolisms, enzymatic and non-enzymatic antioxidants to suppress the growth of petunia plants. This bacterial isolate would be useful to develop bioherbicide to prevent the weed growth and their populations.

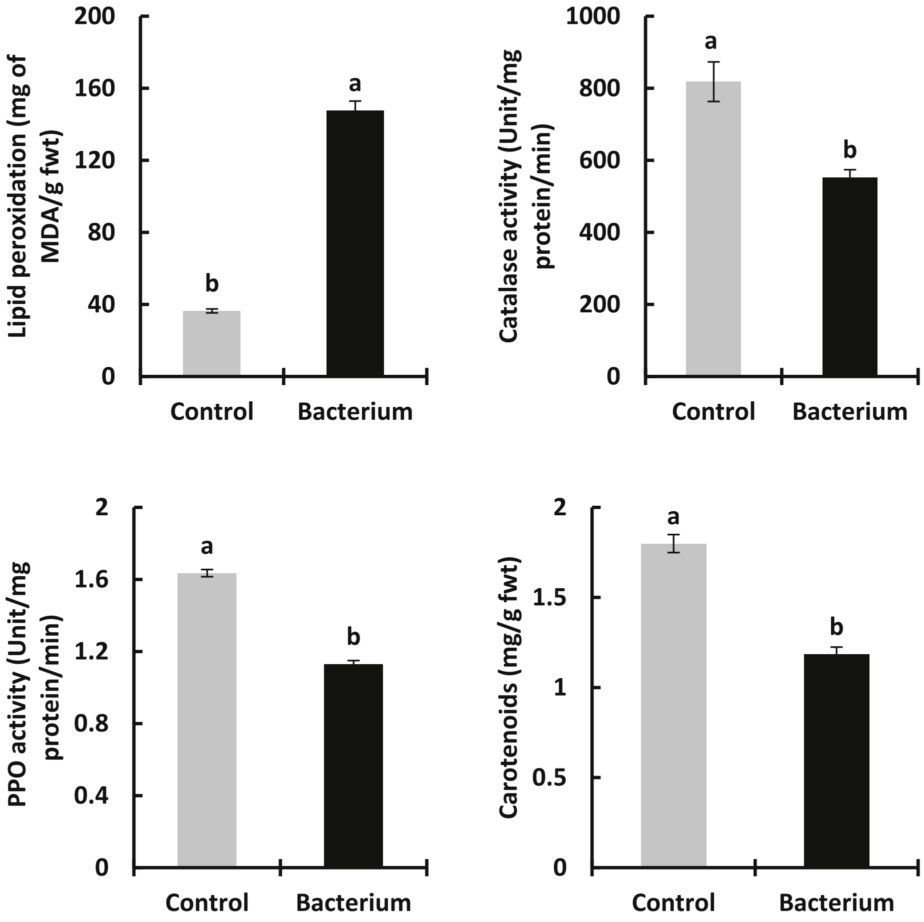

**Fig. 3.** Effect of *Curtobacterium sp.* MA01on lipid peroxidation and antioxidants in Petunia plants.

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