

REGULAR ARTICLE

Effect of *Rhodopseudomonas palustris* G5 on seedling growth and some physiological and biochemical characteristics of cucumber under cadmium stress

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ABSTRACT

At present, we investigated whether *Rhodopseudomonas palustris* G5 strain treatments diminished the damage on cucumber seedlings caused by cadmium (Cd) stress. Growth and physiological parameters of cucumber seedlings were determined at laboratory after co-treating seedlings with *Rhodopseudomonas palustris* G5 strain and different concentrations of Cd (50, 100, 150, 200, 300 mg L⁻¹). The results indicated that Cd stress significantly reduced shoot height, root length, total chlorophyll content, root activity, but increased malondialdehyde (MDA) content. In addition, lower concentrations of Cd stress increased soluble sugar and soluble protein contents, and improved the activities of superoxide dismutase (SOD; EC: 1.15.1.1) and peroxidase (POD; EC: 1.11.1.7), but higher concentrations of Cd decreased the soluble sugar and soluble protein contents, and inhibited SOD and POD activities. G5 strain application markedly reduced the toxic effects of Cd on cucumber seedlings. In sum, G5 strain treatment significantly alleviated damage on cucumber seedlings caused by Cd, and increased shoot height, root length, total chlorophyll content, root activity, the contents of soluble sugar and soluble protein, as well as the activities of SOD and POD, but decreased MDA content compared with non-treating plants.

Keywords: *Rhodopseudomonas palustris*; Cadmium; Cucumber seedlings; Physiological parameters; Antioxidant enzymes

INTRODUCTION

Cadmium is one of environmentally toxic contaminants. Cadmium is taken up from the soil by plants, which means that it can potentially threaten human health via the food chain (Fargasova, 2001; Gill et al., 2011). Some studies have shown that Cd can adversely affect plant growth, photosynthesis, and some physiological parameters (El-Beltagi et al., 2010; Mishra et al., 2014; Xu et al., 2008). For example, Mishra et al. (2014) suggested that supplemental exposure to Cd could affect the plant metabolic status at higher doses, stunt growth, and cause chlorosis and necrosis in shoot cultures of ashwagandha. El-Beltagi et al. (2010) reported that Cd inhibited antioxidant enzyme activity, decreased water potential in radish. Cadmium stress promoted the generation of reactive oxygen species (ROS). Excessive accumulation of ROS induced lipid peroxidation, and then damage DNA, RNA, proteins, and membranes (Weckx et al., 1996). Under environmental stress, in order to resist ROS damage, plants formed protective mechanisms

including enzymatic and non-enzymatic mechanisms (Mishra et al., 2014). However, when the stress level exceeded the tolerance threshold of the plant, ROS cannot be eliminated, leading to decreasing of antioxidant enzyme activities and growth inhibition (Li et al., 2008). Therefore, the development of strategies to mitigate damage caused by Cd has received considerable attention. Popova et al. (2009) reported that salicylic acid treatment attenuated cadmium damage on pea seedlings. Li et al. (2013) showed that applying silicon and cerium to soils enhanced lettuce resistance to Cd stress.

Photosynthetic bacteria, which are common microorganisms in the natural environment, can produce 5-aminolevulinic acid (ALA), indole acetic acid (IAA), porphyrin compounds, and antiviral factors (Nunkaew et al., 2014; Sasaki et al., 2005). These substances can enhance the activity of plant cell, which improves photosynthesis efficiency and promotes the production of antioxidant enzymes that enhance plant resistance to stress. Some studies have

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Received: 22 February 2016; Revised: 20 October 2017; Accepted: 23 October 2017; Published Online: 28 October 2017

shown that photosynthetic bacteria producing ALA can regulate plant metabolism and prevent the oxidative damage in plant cells (Nunkaew et al., 2014; Xu et al., 2016). Nevertheless, whether G5 strain can lessen the toxicity of Cd is still elusive. Therefore, we determined the alleviation effect of *Rhodospseudomonas palustris* G5 strain on Cd damage in cucumber and analyzed mechanisms underlying detoxification of G5 strain by measuring the growth and physiological indexes of cucumber seedlings under the stress of Cd. Thus, this study will contribute to isolate the G5 strain and further apply it in practice.

MATERIALS AND METHODS

Microbial inoculants

Rhodospseudomonas palustris G5 strain, which could produce ALA, was isolated by our laboratory. Under anaerobic conditions, G5 strain was cultured in improved RCVBN medium for 7 days at 28°C, with a PPF of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light (Xu et al., 2016). The cells of G5 strain were collected by centrifugation at 5,000 r min^{-1} for 15 min. In sterile water, the pellets were resuspended to reach a final concentration of 10^9 CFU mL^{-1} .

Experimental design

The cucumber seeds were cleansed with 70% ethanol for surface sterilization for 2 min, then further sterilized with 0.05% sodium hypochlorite for 10 min. Thirdly, the seeds were washed in sterile water. Finally, the seeds allowed to germinate on water-soaked sterile paper in Petri dishes at 28°C in the dark for 4 days.

$\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ was dissolved in Hoagland's nutrient solution to obtain Cd concentrations of 0, 50, 100, 150, 200 and 300 mg L^{-1} . After that, Hoagland's nutrient solution that contained different concentrations of Cd was placed into plastic trays of 5 cm high, 24 cm wide and 37 cm long, respectively. The seedlings were randomly divided into 2 groups, A and B, and every group was divided into five subgroups. Group A seedlings were treated in Hoagland's nutrient solution that contained different concentrations of Cd. Group B seedlings were treated in a similar way, except that 10 mL of *Rhodospseudomonas palustris* G5 strain was added. The nutrient solution was renewed two times weekly. Each treatment had 3 repeated samples and each one contained 10 plastic trays. Cucumber seedlings without Cd and G5 strain treatments were used as controls. The seedlings were put into climate chamber which was operated with an illumination time of 12 h/day and relative humidity of 70% at diurnal temperature (day/night) 30/23°C. After 13 days of treatment, the physiological and biochemical indexes of the leaves were determined with leaves at the same position on each stem.

Measurement of growth parameters

At 13 days after G5 strain and Cd treatment, the shoot heights and root lengths were measured for G5 strain and every Cd treatment according to Lutts et al. (1996).

Measurement of physiological and biochemical indexes of cucumber seedlings

The activities of SOD and POD were measured according to the methods of Wu and Von Tiedemann (2002). The contents of chlorophyll and malondialdehyde (MDA) was assayed as described by Hegedüs et al. (2001). Soluble sugar content was analyzed according to the methods of Zarco-Tejada et al. (2005). Soluble protein content was determined using the methods of Bradford (1976). Root activity was determined according to the methods of Kerley (2000).

Data analysis

All data presented here were means of three replicates. The mean and the standard deviation (\pm SD) were calculated using Microsoft Excel 2007. The data were analyzed at significant levels of $P < 0.05$ using one way ANOVA.

RESULTS AND DISCUSSION

The effects of G5 strain and Cd treatment on shoot height and root length of cucumber seedlings

At present, Cd stress significantly inhibited the growth of cucumber ($P < 0.05$) (Table 1). And 300 mg L^{-1} Cd resulted in the most obvious reduction in shoot height and root length (decreased by 29.24%, and 14.01% compared to the control, respectively). All G5 strain treatment diminished inhibition on the shoot heights and root lengths of cucumber seedlings caused by Cd stress ($P < 0.05$). The greatest significant improvement in shoot height occurred when cucumber seedlings were treated with G5 and 200 mg L^{-1} Cd (increased by 19.00% compared to that of the seedlings under 200 mg L^{-1} Cd stress) ($P < 0.05$) (Table 1), whereas the largest increase of 14.51% in root length was observed at the seedlings treated with G5 strain and 300 mg L^{-1} Cd treatment ($P < 0.05$). These results showed that G5 strain had the ability to enhance cucumber resistance to Cd stress. Current results were in agreement with the findings of Xu et al. (2016), finding that *Rhodospseudomonas palustris* could increase plant biomass under abiotic stress.

The effects of G5 strain and Cd treatment on total chlorophyll content and root activity in cucumber seedlings

Chlorophyll is a key indicator of damage inflicted on plant by heavy metals (Wang et al., 2006). Heavy metals stresses often decreased the total chlorophyll content. Current studies showed that 50 to 300 mg L^{-1} Cd significantly decreased the total chlorophyll content in cucumber leaves ($P < 0.05$) (Table 2). The largest fall in total chlorophyll

Table 1: Effects of G5 strain and Cd treatment on shoot height and root length of cucumber seedlings

Concentration (mg L ⁻¹ Cd)	Shoot height (cm)		Root length (cm)	
	-G5	+G5	-G5	+G5
0	13.92±0.23 ^a	15.26±0.22 ^a	5.21±0.03 ^a	5.94±0.03 ^a
50	12.37±0.36 ^b	13.89±0.28 ^b	5.07±0.09 ^b	5.76±0.07 ^a
100	11.86±0.21 ^c	13.27±0.17 ^c	4.94±0.08 ^c	5.33±0.05 ^b
150	10.77±0.15 ^d	12.51±0.25 ^d	4.82±0.01 ^d	5.43±0.02 ^b
200	10.21±0.20 ^e	12.15±0.16 ^d	4.67±0.07 ^e	5.21±0.06 ^{bc}
300	9.85±0.13 ^e	11.52±0.19 ^e	4.48±0.05 ^f	5.13±0.06 ^c

Data are means±SD, different lowercase letters in the same column indicate significant differences at $P < 0.05$

Table 2: Effects of G5 strain and Cd treatment on total chlorophyll content and root activity in cucumber seedling

Concentration (mg L ⁻¹ Cd)	Chlorophyll content (mg g ⁻¹ FW)		Root activity (µg g ⁻¹ h ⁻¹)	
	-G5	+G5	-G5	+G5
0	3.65±0.70 ^a	3.9±0.26 ^a	351.43±18.84 ^a	393.82±11.82 ^a
50	2.15±0.2 ^b	2.93±0.13 ^b	295.27±15.58 ^b	368.54±14.62 ^b
100	1.69±0.21 ^{bc}	2.23±0.19 ^c	256.62±11.83 ^c	328.17±13.53 ^c
150	1.35±0.30 ^c	1.94±0.36 ^{cd}	217.90±12.27 ^d	312.68±11.58 ^{cd}
200	1.21±0.13 ^c	1.62±0.18 ^d	156.31±9.75 ^e	291.65±10.90 ^{de}
300	1.02±0.4 ^c	1.49±0.13 ^d	126.84±11.47 ^f	270.19±15.56 ^e

Data are means±SD, different lowercase letters in the same column indicate significant differences at $P < 0.05$

content occurred under 300 mg L⁻¹ Cd treatment (decreased by 72.05% compared to the control). The decrease of total chlorophyll content could be caused by the disturbance of light-harvesting complex formation, inhibition of chlorophyll biosynthesis and photooxidation breakdown of free chlorophyll (Pružinská et al., 2003). G5 strain treatment significantly increased total chlorophyll content of cucumber seedlings exposed to different concentrations of Cd ($P < 0.05$). Current results agree with findings of Nunkaew et al. (2014), reporting that photosynthetic bacteria producing ALA could increase the chlorophyll content of plant under NaCl stress. In addition, Akram et al. (2013) demonstrated that ALA was a potential plant growth regulator and was considered as a precursor for chlorophyll biosynthesis.

Root activity indicates the metabolic status of whole root system, and affects physiological state of above-ground plant traits (Parys et al., 1998). In order to analyze the effect of G5 strain on decreasing the damage on cucumber seedlings caused by Cd stress, the root activities of cucumber seedlings were measured after G5 strain treatment. The result showed that Cd stress reduced root activity of cucumber seedlings compared to the control plant ($P < 0.05$) (Table 2). Root activity showed a decreasing trend as the Cd concentrations rose and the largest reduction was observed after cucumber seedlings had been treated with 300 mg L⁻¹ Cd (63.91% reduction compared to the control). After treated with G5 strain, the root activities of the seedlings under Cd stress significantly improved ($P < 0.05$), but by different amounts. G5 strain had the greatest influence on the root activities of cucumber seedlings treated with 300 mg L⁻¹ Cd, which

increased by 113.02% compared to seedlings treated only with 300 mg L⁻¹ Cd ($P < 0.05$). These results showed that G5 strain treatment increased root activity of cucumber, and further promoted plants for the uptake of nutrients and water.

The effects of G5 strain and Cd treatment on the soluble sugar and soluble protein contents in cucumber seedlings

Carbohydrates regulate plant growth and enhance plant resistance to stress (Gill et al., 2003). Soluble proteins induce the activities of metabolic enzymes, detoxification enzymes and antioxidant enzymes (Naeem et al., 2011). Under adverse environmental stress, plants often accumulate soluble sugar and soluble protein for reducing their freezing point and osmotic potential, and to activate metabolic enzymes, detoxification enzymes and antioxidant enzymes to help to adapt to the environment (Naeem et al., 2011; Cui et al., 2015). However, when the stress level was overloaded, the soluble sugar and soluble protein contents fell, which impaired plant stress resistance. 50 mg L⁻¹ Cd stress significantly increased the contents of soluble sugar and soluble protein compared to the control plants ($P < 0.05$) (Table 3). However, the contents of soluble sugar and soluble protein reached the lowest level after 300 mg L⁻¹ Cd stress ($P < 0.05$) (Table 3). The treatment of G5 strain increased the soluble sugar and soluble protein contents of cucumber seedlings exposed to Cd. G5 strain had the greatest influence on cucumber seedlings treated with 200 mg L⁻¹ Cd in terms of the soluble sugar contents, and the contents were increased by 57.34%, while G5 strain most improved soluble protein contents of cucumber seedling by 83.73% when treated with 300 mg L⁻¹ Cd

Table 3: Effects of G5 strain and Cd treatment on the contents of soluble sugar and soluble protein in cucumber seedling

Concentration (mg L ⁻¹ Cd)	Soluble sugar content (mg g ⁻¹ FW)		Soluble protein content (mg g ⁻¹ FW)	
	-G5	+G5	-G5	+G5
0	6.13±0.01 ^b	8.25±0.06 ^b	7.25±0.08 ^b	9.24±0.11 ^b
50	7.28±0.06 ^a	8.86±0.03 ^a	8.73±0.10 ^a	9.82±0.29 ^a
100	5.83±0.16 ^c	8.10±0.26 ^b	6.75±0.04 ^b	8.87±0.10 ^c
150	5.21±0.05 ^d	7.84±0.05 ^c	5.93±0.16 ^c	7.73±0.23 ^d
200	4.29±0.45 ^{cd}	6.75±0.04 ^d	4.54±0.45 ^d	6.18±0.15 ^e
300	3.83±0.7 ^e	5.29±0.01 ^e	2.95±0.70 ^e	5.42±0.24 ^f

Data are means±SD, different lowercase letters in the same column indicate significant differences at $P < 0.05$

Table 4: Effects of G5 strain and Cd treatment on antioxidant enzymes and MDA contents in cucumber seedling

Concentration (mg L ⁻¹ Cd)	SOD (U mg ⁻¹ Pro)		POD (U mg ⁻¹ Pro)		MDA (μmol g ⁻¹ Pro)	
	-G5	+G5	-G5	+G5	-G5	+G5
0	183.58±16.47 ^b	226.59±21.53 ^b	69.58±1.78 ^b	75.30±1.62 ^c	39.24±2.21 ^e	26.39±0.90 ^e
50	226.49±30.78 ^a	282.74±25.64 ^a	90.87±3.37 ^a	96.7±2.88 ^a	46.95±1.79 ^d	31.32±5.40 ^d
100	157.64±18.68 ^{bc}	191.28±18.27 ^c	71.36±1.06 ^b	88.16±5.71 ^b	49.73±1.27 ^{cd}	35.96±4.21 ^{cd}
150	136.90±12.83 ^{cd}	166.08±11.80 ^{cd}	64.35±3.31 ^c	71.64±2.70 ^c	53.46±1.04 ^c	40.64±3.21 ^{bc}
200	111.24±10.90 ^d	145.59±9.86 ^d	56.26±1.29 ^d	72.75±1.06 ^c	57.53±3.72 ^b	42.75±1.68 ^b
300	68.26±6.41 ^e	98.62±7.06 ^e	43.35±1.53 ^e	63.38±1.78 ^d	64.71±1.58 ^a	51.64±1.15 ^a

Data are means±SD, different lowercase letters in the same column indicate significant differences at $P < 0.05$

($P < 0.05$). After G5 strain treatment, the contents of soluble protein and soluble sugar in the cucumber seedlings increased significantly, suggesting G5 strain treatment improved plant resistance to Cd stress by increasing the contents of soluble protein and soluble sugar.

The effects of G5 strain and Cd treatment on antioxidant enzyme activities and MDA contents in cucumber seedlings

SOD and POD are the main antioxidant enzyme to scavenge reactive oxygen species (ROS) induced by pollutants and protect cells from damage (Cui et al. 2015). Under biotic and abiotic stress conditions, ROS were produced and then damaged the plant cell. In order to diminish the damage caused by excessive ROS, plants improve stress resistance by enhancing the activities of SOD and POD (Cui et al. 2015). However, when stress exceeded the tolerance threshold of the plant, ROS cannot be eliminated, which results in the decrease in the activities of SOD and POD (Li et al. 2008). Current results showed that increasing in the Cd concentrations led to improving the SOD and POD activities in the cucumber seedling at first. But these were then followed by decreases in the activities (Table 4). When the Cd concentration was 50 mg L⁻¹ Cd, the activities of SOD and POD in the cucumber seedlings reached to a maximum of 23.37% and 30.60%, respectively, compared to the control ($P < 0.05$). The G5 strain treatment increased SOD and POD activities of cucumber seedlings under Cd stress. G5 strain most improved the SOD and POD activities of the cucumber seedlings when treated with 300 mg L⁻¹ Cd, and the activities increased by 44.48% and 46.21%, respectively. This suggested that G5 strain could increase the plant capacity

to adapt to stress and alleviate the damage caused by Cd stress. This findings could be attributed to the fact that G5 strain decreased heavy metal bioavailability in the soil, and produced physiologically active substances, which increase the activities of antioxidant enzyme of plants under stress, enhance plant stress responses, and relieve stress damage to plants (Nunkaew et al., 2014; Xu et al., 2016).

MDA is produced mainly from membrane lipid peroxidation, which means it can be used to assess the degree of membrane lipid peroxidation of plant. Many studies showed that heavy metal stress led to the increasing of ROS, and then caused lipid peroxidation (Mishra et al., 2014; Xu et al., 2008). Current results found that MDA content of cucumber seedling increased with the improving of Cd concentration ($P < 0.05$) (Table 4). The highest MDA content was observed in seedling treated with 300 mg L⁻¹ Cd stress, which showed a 64.91% increase compared to the control plants. The increasing of MDA content indicated that the Cd treatments had led to oxidative stress. However, after treatment with G5 strain, the MDA content of cucumber seedlings among all the treatment fell significantly ($P < 0.05$). G5 strain produced the largest reduction in the MDA content of cucumber seedlings treated with 50 mg L⁻¹ Cd, which decreased by 33.29% ($P < 0.05$). In sum, the application of G5 strain alleviated the toxic effects of Cd, and inhibited lipid peroxidation in cucumber seedlings.

CONCLUSION

The present study demonstrated that G5 strain alleviated the damage of Cd stress on cucumber seedling, showing

that G5 strain promoted plant growth, and improved content of soluble protein, soluble sugar and chlorophyll, as well as root activities and activation of antioxidant enzymes, and further led to the diminishing of MDA content in cucumber seedlings exposed to Cd stress. The detoxification mechanism caused by G5 strain under Cd stress is closely connected to the activation of antioxidant enzymes and the accumulation of soluble protein and soluble sugar, which minimizes the oxidative damage induced by Cd stress. Owing to the complexity of the three-way interactions among G5 strain, plant and Cd. Therefore, further studies need to investigate the mechanisms underlying these interactions.

ACKNOWLEDGMENTS

This study was supported by the Science and Technology Planning Project founded by Science and Technology Commission in Henan Province, China (No. 162102310588) and by the School-based Program of Zhoukou Normal University (No. ZKNUB215210, ZKNUB2201703).

Contributions of authors

Honglian Ge designed the study. Honglian Ge, Zhonghua Liu and Fuli Zhang performed the experiments and analyzed the data, as well as wrote the paper.

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