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Mitigative Effect of *Bacillus subtilis* QM3 on Root Morphology and Resistance Enzyme Activity of Wheat Root under Lead Stress

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Abstract

Lead (Pb) is an environmental pollutant extremely toxic to plants and other living organisms including humans. In order to research the relieve effect of *Bacillus subtilis* QM3 on wheat roots (*Triticum aestivum* L.), after wheat seeds germination for two days, wheat root caused, the experimental materials were divided into four large groups and each large group was placed in 6 petri dishes as six small groups, and then four large groups respectively cultivated with sterile water (CK), 10^8 CFU/ml *B. subtilis* QM3 (B1), 10^7 CFU/ml *B. subtilis* QM3 (B2) and 10^6 CFU/ml *B. subtilis* QM3 (B3) for 2 days, after that stressed with lead nitrate, Pb $(NO)_2$, Pb^{2+} concentration calculation at five concentrations (50, 250, 500, 1000, 2000 mg/L), sterile water and different Pb^{2+} concentration liquid respectively cultivated the 6 small groups in each large group measuring root morpholog and assaying changes of antioxidant enzyme activity. The results showed that: with the increase of the Pb^{2+} concentration, root morphology index and the activity of antioxidant enzyme increased first and then decreased. Root morphology index reached the maximum in 50 mg/L Pb^{2+} concentration. *B. subtilis* QM3 clearly promoted the growth of the root and the antioxidant enzyme activity ($p < 0.05$). Without Pb stress, *B. subtilis* QM3 had the best improving effect on root morphology. When Pb^{2+} concentration was 50 mg/L, superoxide dismutase (SOD) and ascorbate peroxidase (APX) reached the maximum. SOD activity, compared with CK, B1, B2 and B3 respectively, increased by 8.05%, 27.41% and 9.79%. APX activity, compared with CK, B1, B2 and B3 respectively, increased by 52.70%, 111.15% and 14.16%. Catalase (CAT) and peroxidase (POD) reached the maximum at the Pb^{2+} concentration was 500 mg/L. CAT activity, compared with CK, B1, B2 and B3 respectively, increased by 59.93%, 83.46% and 70.59%. POD activity, compared with CK, B1, B2 and B3 respectively, increased by 2.88%, 10.11% and 7.67%. Result suggested that *B. subtilis* QM3 could improve root growth and antioxidant enzyme activity of the wheat root under lead stress.

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Keywords

***B. subtilis* QM3, Resistance Enzyme Activity, Root Morphology, Lead Stress**

1. Introduction

With the rapid expansion of the city and the rapid development of the modern industry and agriculture, the pollution of heavy metals in natural environment is becoming more and more serious. Lead (Pb), one of the oldest known metals, is a pervasive and persistent environmental occupational toxic metal, and Pb poisoning remains a health threat [1]. The release of Pb represents a serious problem for human life by entering the food chain. A variety of environmental stresses like soil salinity, drought, extreme of temperature and heavy metals are known to cause oxidative damage to plants either directly or indirectly by triggering an increased level of production of reactive oxygen species (ROS) [2]-[7]. Lead toxicity to plants is mainly because of heavy metal destroyed the normal physiological function in plant, heavy metal toxicity may also be exerted by the fact that heavy metals induce secondary oxidative stress by importing the formation of harmful reactive oxygen species (ROS) [8]. These ROS include superoxide $\left(O_2^-\right)$, hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2) that are produced as by products during membrane linked electron transport activities as well as by a number of metabolic pathways [4] and in turn cause damage to the biomolecules such as membrane lipids, proteins, chloroplast pigments, enzymes, and nucleic acids [5]. When lead enters the plant cells, like various heavy metals, it induces an oxidative stress in growing plant parts due to enhanced production of reactive oxygen species (ROS), and cell damages result in a reduction of plant productivity [9] [10].

To combat the oxidative damage plants have the antioxidant defense system comprising of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX).

Wheat is one of the major grain crops in the world. The global annual demands have increased year by year, but due to the deterioration of the environment and other unfavorable factors severely restrict the increase of wheat yield. Study on the growth of lead early effects on crops, has been a focus of the field of ecological environment in recent years. The root is the most important part of the combination of heavy metals in plants, and is also the most vulnerable part to heavy metal toxicity. It is the underground vegetative organs of plants, playing a very important role in crop production. Therefore, the study on effects of heavy metal in soil on plant root system is of important significance.

A few studies on alleviation of heavy metal stress-induced inhibition of activity of antioxidant enzyme have been published. However, in relation to bacteria, the studies on the alleviation effect of bacteria on plant growth under heavy metals stress are limited. To understand the biology of plants and microbial ecology, many studies performed with bacteria have focused on evaluating the colonization pattern of vegetative tissues, as well as the effects of bacteria on plant growth [11]. *B. subtilis* QM3 is a strain of antagonistic strains which have biological control function. It was isolated from dung of QingHai yak. Careful study is necessary to determine whether *B. subtilis* QM3 can alleviate inhibition of wheat root growth which is induced by heavy metal stress. Previous study showed that *B. subtilis* QM3 had an auxo-action on wheat seed germination and had a promotion effect on the growth of tomato.

This experiment is intended to investigate the alleviation effect of *B. subtilis* QM3 on growth and enzyme activities of wheat roots that under different concentration of lead stress; it will be helpful to identify toxic critical values of Pb in soils due to wheat's response, and lay a theoretical foundation for the alleviation of heavy metal pollution and the original innovation of microbial inocula.

2. Materials and Methods

2.1. Plant Material and Bacterial Suspension Preparation

Wheat seeds from the Research Institute of wheat in Shanxi Province, China. *B. subtilis* strain QM3 (College of life science, Shanxi Normal University, China) was grown at 37˚C for 3d in nutrient broth (NB) medium, under vigorous shaking (150 rpm). Cultures were centrifuged once at 3000 g for 5 min at 20˚C to collect the bacteria and then rinsed three times with sterile water, and diluted in sterile water solution in order to reach an OD_{600nm}

of 0.8 (10^8 CFU/ml *B. subtilis* QM3). At the same time diluted the bacterial suspension liquid 10 times (10^7 CFU/ml *B. subtilis* QM3) and 100 times (10^6 CFU/ml *B. subtilis* QM3) to reserve.

2.2. Plant Treatment and Lead Stress Conditions

Wheat seeds were surface-sterilized with 0.1% Mercuric chloride solution for 10 min and then rinsed three times with sterile water. Then soaked in sterile water for 24 h and then transferred to Petri dishes for germination. After two days' germination, root caused, then transferred the uniform growth of wheat seeds to other Petri dishes, seedlings were divided into four large groups, each group was placed in 6 petri dishes as six small group, four large groups respectively cultivated with sterile water (CK), 10^8 CFU/ml *B. subtilis* QM3 (B1), 10^7 CFU/ml *B. subtilis* QM3 (B2) and 10^6 CFU/ml *B. subtilis* QM3 (B3) for 2 days. Pb $(NO_3)_2$ treatment performed on the fifth day, Pb^{2+} concentration calculation at five concentrations (50, 250, 500, 1000, 2000 mg/L), sterile water and different Pb^{2+} concentration liquid respectively cultivated the 6 small groups in each large group for 4 days, after that root morphology index and resistance enzyme activity were evaluated. The seedlings were grown in a constant temperature light incubator (25˚C day/20˚C night; 12 h/12 h, light/dark period; and 55% relative humidity).

2.3. Root Growth Analysis

After 9 days' growth, three uniform seedlings were selected from each Petri dish for the determination of root growth by using Root scanner. All measurements were performed in three replicates.

2.4. SOD Assay

SOD activity was measured through the photo-reduction of nitro blue tetrazolium chloride (NBT) [12]. About 500 mg fresh tissues were homogenized in 5 ml of 100 mM Na-phosphate buffer (pH 7.8) containing 0.1 mM EDTA, 0.1% (v/v) Triton X-100 and 1% (w/v) polyvinyl pyrrolidone (PVP). The extract was centrifuged at 8000 × g for 30 min at 4˚C. The reaction mixture contained 50 µL of 33 mM NBT, 100 µL of 10 mM L-methionine, 50 µL of 0.0033 mM riboflavin in 250 µL of 50 mM sodium phosphate buffer. The reaction mixture was placed under lamp below 15 W for 25 min before the reaction was stopped by switching off the light. Non-illuminated and illuminated reactions without supernatant served as control. The absorbance was measured at 560 nm. One unit of SOD activity was defined as the quantity of SOD required to produce a 50% inhibition of NBT.

2.5. CAT Assay

The activity of CAT was assayed according to Beers and Sizer [13]. Fresh samples (500 mg) were homogenized in 5 ml of 50 mM Tris/NaOH buffer (pH 8.0) containing 0.5 mM EDTA, 1% (w/v) PVP and 0.5% (v/v) Triton X-100. The homogenate was centrifuged at 8000 × g for 30 min at 4˚C and after dialysis supernatant was used for enzyme assay. Assay mixture in a total volume of 3.0 ml contained 2000 µl of 100 mM NaH_2PO_4 buffer (pH 7.0), 800 µl of 200 mM H_2O_2 and 200 µl enzyme. The decomposition of H_2O_2 was followed at 240 nm (extinction coefficient of 0.036 $mM^{-1} \cdot cm^{-1}$) by decrease in absorbance. Enzyme specific activity is expressed as mmol of H_2O_2 oxidized min^{-1} $(mg\ protein)^{-1}$.

2.6. APX Assay

About 500 mg root sample were homogenized in 5 ml of 50 mM Na-phosphate buffer (pH 7.8) containing 1% PVP, 1 mM ascorbic acid and 1 mM PMSF as described by Moran *et al.* [14]. After centrifugation at 8000 × g for 30 min at 4˚C, the supernatant was dialyzed against the same extraction buffer and it served as enzyme. APX was assayed according to Nakano and Asada [15]. Reaction mixture in a total volume of 1 ml contained 50 mM K-phosphate buffer (pH 7.0), 0.2 mM ascorbic acid, 0.2 mM EDTA, 20 mM H_2O_2 and enzyme. H_2O_2 was the last component to be added and the decrease in absorbance was recorded at 290 nm (extinction coefficient of 2.8 $mM^{-1}cm^{-1}$) using a UV-Vis spectrophotometer at 30 s intervals up to 7 min. Correction was made for the low, non enzymic oxidation of ascorbic acid by H_2O_2. The specific activity of enzyme is expressed as mmol ascorbate oxidized min^{-1} $(mg\ protein)^{-1}$.

2.7. POD Assay

Samples (500 mg) were ground in potassium-phosphate buffer, pH 6.1 (1 ml 100 mg^{-1} fresh weight) with PVP

(1.0 $g \cdot g^{-1}$ of fresh weight) at 4˚C and centrifuged for 30 min at 8000 rpm. Supernatant was used as the crude enzyme extract. Peroxidase activity was assayed spectrophotometrically at 25˚C following the oxidation of guaiacol. The reaction mixture consisted in 100 µl 20 mM guaiacol and 100 µl 10 mM H_2O_2 in 700 µl potassium-phosphate buffer pH 6.1. One hundred microlitres of each crude enzyme extract was added, and the increase in absorbance at 470 nm was followed. Enzyme activity was expressed as absorbance per minute per mg protein (ΔAbs $min^{-1} \cdot mg^{-1}$ protein). Protein concentration was determined by the Bradford method [16].

2.8. Statistical Analysis

One way analysis of variance (ANOVA) and Duncan's multiple range tests were carried out to determine significant differences ($p < 0.05$) between the means by Data Processing System (DPS, version 7.05) and EXCEL program.

3. Results

3.1. Mitigative Effect of *B. subtilis* QM3 on the Growth of Root

Lead nitrate treatment results in the variation of root morphology (**Table 1**). As shown in **Table 1**, when without

Table 1. Effects of *B. subtilis* QM3 on the growth of root under lead stress.

Pb^{2+} concentration (mg/L)	Treatment	Len (cm)	SA (cm^2)	Vol (cm^3)	PA (cm^2)
0	CK1	5.35 ± 0.01c	28.86 ± 1.50d	12.33 ± 1.03c	9.12 ± 0.98d
	B1	9.46 ± 0.06b	45.19 ± 0.12c	36.14 ± 0.10b	14.43 ± 0.14c
	B2	10.26 ± 0.10a	104.27 ± 3.62a	90.57 ± 0.57a	34.41 ± 0.50a
	B3	9.51 ± 0.04b	58.68 ± 0.02b	36.61 ± 0.04b	18.69 ± 0.02b
50	CK2	12.41 ± 0.03b	91.49 ± 1.14b	54.45 ± 0.48c	29.27 ± 1.04b
	B1	13.09 ± 0.03a	50.54 ± 0.69d	49.07 ± 0.07d	15.78 ± 0.34d
	B2	7.52 ± 0.37c	68.40 ± 0.35c	72.48 ± 0.67a	21.49 ± 0.29c
	B3	6.97 ± 0.38d	102.27 ± 4.66a	70.39 ± 0.95b	34.46 ± 0.58a
250	CK3	4.25 ± 0.49b	38.70 ± 0.53c	28.68 ± 0.57c	12.26 ± 0.87b
	B1	6.06 ± 0.65a	41.14 ± 0.16b	21.56 ± 0.10d	13.47 ± 0.37b
	B2	5.86 ± 0.72a	46.35 ± 1.79a	51.92 ± 1.25a	20.73 ± 1.26a
	B3	3.76 ± 0.08b	42.39 ± 0.35b	32.04 ± 0.07b	13.68 ± 0.38b
500	CK4	3.62 ± 0.95c	29.46 ± 0.89b	18.80 ± 0.47b	9.47 ± 0.20b
	B1	4.58 ± 0.10bab	23.73 ± 0.81c	13.07 ± 0.08c	5.81 ± 0.02d
	B2	5.19 ± 0.14a	32.81 ± 1.60a	28.24 ± 0.39a	22.43 ± 0.61a
	B3	4.08 ± 0.09bc	23.06 ± 0.07c	8.36 ± 0.24d	8.08 ± 0.84c
1000	CK5	3.33 ± 0.01c	27.45 ± 0.41a	7.56 ± 0.50a	8.76 ± 0.21a
	B1	3.99 ± 0.46b	18.25 ± 0.02b	7.18 ± 0.05a	7.41 ± 0.01b
	B2	4.93 ± 0.03a	16.47 ± 0.49c	4.20 ± 0.07b	5.17 ± 0.03d
	B3	3.29 ± 0.01c	18.87 ± 1.03b	7.36 ± 0.12a	6.08 ± 0.22c
2000	CK6	1.86 ± 0.048c	6.41 ± 0.31d	1.62 ± 0.08d	1.99 ± 0.03c
	B1	3.26 ± 0.01a	8.91 ± 0.03b	3.56 ± 0.19b	2.82 ± 0.02b
	B2	2.40 ± 0.07b	11.16 ± 0.05a	4.04 ± 0.20a	3.57 ± 0.03a
	B3	1.85 ± 0.04c	7.53 ± 0.47c	2.42 ± 0.01c	2.81 ± 0.02b

CK, B1, B2 and B3 respectively represent the control group (treated with sterile water), 10^8 CFU/ml *B. subtilis* QM3 (B1), 10^7 CFU/ml *B. subtilis* QM3 (B2) and 10^6 CFU/ml *B. subtilis* QM3 (B3). Len, SA, Vol and PA in the table respectively represent the root length, the root surface area, the root volume and the root projection area. Values in the table are given as mean ± SD for 3 replicates. Different letters in the same column mean significant difference among treatments at 0.05 ($p < 0.05$).

Pb stress, *B. subtilis* QM3 had an obvious promotion effect on root growth, root length (Len), compared with CK1, B1, B2 and B3 respectively increased by 76.82%, 91.78% and 77.76%; surface area (SA) compared with CK1, B1, B2 and B3 respectively increased by 56.58%, 261.30% and 103.33%; volume (Vol) compared with CK1, B1, B2 and B3 respectively increased by 193.11%, 634.55% and 196.92%; projection area (PA) compared with CK1, B1, B2 and B3 respectively increased by 58.22%, 277.30% and 104.93%. Without *B. subtilis* QM3 treatment, even a low Pb^{2+} concentration did have effect on root growth, under 50 mg/L Pb^{2+} concentration, root morphology index reached the maximum, Len was obviously higher than those without Pb concentration, increased by 131.96%, under 250, 500, 1000, 2000 mg/L Pb^{2+} concentration, Len was significantly inhibited; under 50, 250 and 500 mg/L Pb^{2+} concentration, SA was obviously higher than those without Pb concentration, under 50 and 250 mg/L Pb^{2+} concentration, Vol and PA were obviously higher than those without Pb concentration, this result indicated that the root length was the most affected by lead stress, but under 1000 and 2000 mg/L Pb^{2+} concentration, Len, SA, Vol and PA were all inhibited. Appearing above phenomenon may due to the dual role of Pb in plant, low Pb^{2+}concentration can promote the growth of plants. When Pb^{2+} was 500 mg/L, Len compared with CK1, CK4, B1, B2 and B3 respectively reduced by 32.34%, 14.39%, 2.99% and 27.74%, B1, B2 and B3 all had alleviation effect on root under lead stress; When Pb^{2+} was 1000 mg/L, Len compared with CK1, CK5, B1, B2 and B3 respectively reduced by 37.76%, 25.42%, 7.85% and 38.50%, B1 and B2 had a mitigation effect, B3 is the opposite. When Pb^{2+} concentration were 50, 250, 500, 1000, 2000 mg/L, not all concentrations of the *B. subtilis* QM3 bacteria liquid have a role in remission, and between different Pb^{2+}concentration stress, the effect of the same concentration of *B. subtilis* QM3 is different. There is no fixed promotion relationship between different concentration of *B. subtilis* QM3 bacteria liquid and root morphology under lead stress, but in a certain degree, *B. subtilis* QM3 had an alleviation effect on the growth of the wheat root under lead stress.

3.2. Mitigative Effect of *B. subtilis* QM3 on SOD Activity

$Pb(NO_3)_2$ treatment caused an induction in the activity of SOD (**Figure 1**). With the increase of lead concentration, SOD activity of B1, B2 and B3 treatments for wheat root and the CK showed a tendency that SOD activity were increased and then decreased. Basically under the Pb concentration of 50 mg/L SOD activity reached the maximum, compared with CK, B1, B2 and B3 respectively increased by 8.05%, 27.41% and 9.79%. Under lead concentrations were 0 mg/L, 50 mg/L, 250 mg/L, 500 mg/L, SOD activity of wheat root under the treatment of B1, B2 and B3 were higher than that of CK, while under the lead concentrations were 1000 mg/L and 2000 mg/L, SOD activity had no regular variation. When Pb concentration was 1000 mg/L, there's no difference between CK, B1 and B3, while the activity of B2 treatment was the lowest. When Pb concentration was 2000

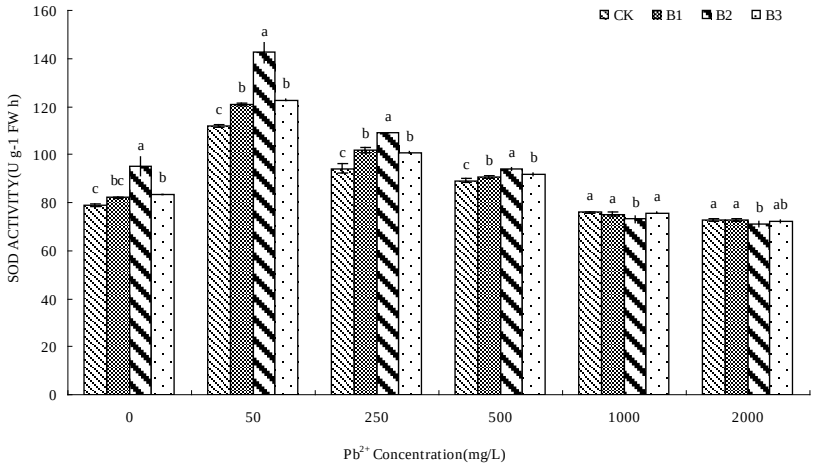

Figure 1. Effects of *B. subtilis* QM3 on SOD activity of wheat root under different lead stress. CK, B1, B2 and B3 respectively represent the control group (treated with sterile water), 10^8 CFU/ml *B. subtilis* QM3 (B1), 10^7 CFU/ml *B. subtilis* QM3 (B2) and 10^6 CFU/ml *B. subtilis* QM3 (B3). Values in the chart are given as mean ± SD for 3 replicates. Different letters in the same Pb^{2+} concentration column mean significant difference among treatments ($p < 0.05$).

mg/L, CK and B1 treatment had a slightly higher activity than B2 and B3. In the Pb solution concentrations were 0 mg/L, 50 mg/L, 250 mg/L, 500 mg/L, SOD activity of *B. subtilis* QM3 treatment was enhanced obviously than CK. While the lead concentration were 1000 mg/L and 2000 mg/L, among different treatments there's a little change in the activity of SOD.

3.3. Mitigative Effect of *B. subtilis* QM3 on CAT Activity

With the increasing of lead concentration, the result showed a tendency that CAT activity were increased and then decreased (**Figure 2**). Under the Pb concentration of 500 mg/L CAT activity reached the maximum, compared with CK, B1, B2 and B3 respectively increased by 59.93%, 83.46% and 70.59%. On the whole, CAT activity of wheat root under the treatment of B1, B2 and B3 were higher than that of CK, and B2 treatment was enhanced obviously than any other treatments. When the Pb^{2+} concentration were 2000 mg/L, there 's no change between CK and B3, B1 treatment had the highest activity, however, B2 treatment had the lowest activity. Under the lead stress (0, 50, 250, 500, 1000 mg/L), *B. Subtilis* QM3 could improve CAT activity.

3.4. Mitigative Effect of *B. subtilis* QM3 on APX Activity

Pb $(NO_3)_2$ treatment caused APX activity of B1, B2 and B3 treatments for wheat root and the CK showed a tendency that APX activity were concomitant increased in roots at the range of low Pb treatment levels and then decreased under high levels of Pb treatment (**Figure 3**). Similar to CAT activity, APX activity of wheat root under the treatment of B1, B2 and B3 were higher than those in CK. Under the Pb concentration of 50 mg/L APX activity reached the maximum, compared with CK, B1, B2 and B3 respectively increased by 52.70%, 111.15% and 96.28%, B2 treatment was enhanced obviously than any other treatments.

3.5. Mitigative Effect of *B. subtilis* QM3 on POD Activity

With the increasing of lead concentration, POD activity of B1, B2 and B3 treatments for wheat root and CK showed a tendency that POD activity were increased in low Pb treatment levels and then decreased in high Pb treatment levels (**Figure 4**). At the Pb concentration was 500 mg/L, POD activity reached the maximum, compared with CK, B1, B2 and B3 respectively increased by 2.88%, 10.11% and 7.67%. Overall, under the high level lead stress (1000 mg/L), POD activity had no significantly difference between CK, B1 and B3, B2 had a slightly higher POD activity. Under the lead concentrations were 0 mg/L, 50 mg/L, 250 mg/L, 500 mg/L and 2000 mg/L, POD activity of B1, B2 and B3 treatments for wheat root were higher than that of the CK.

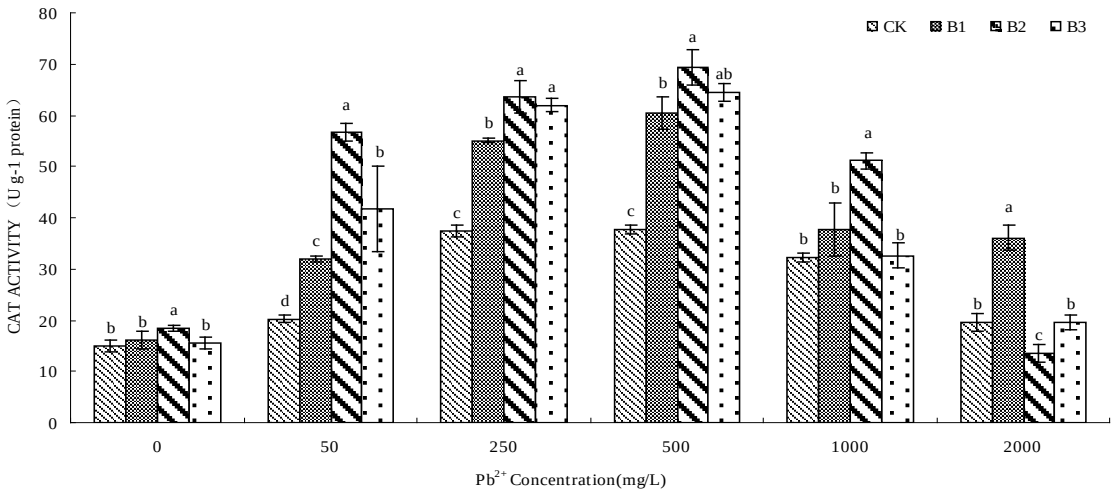

Figure 2. Effects of *B. Subtilis* QM3 on CAT activity of wheat root under different lead stress. CK, B1, B2 and B3 respectively represent the control group (treated with sterile water), 10^8 CFU/ml *B. subtilis* QM3 (B1), 10^7 CFU/ml *B. subtilis* QM3 (B2) and 10^6 CFU/ml *B. subtilis* QM3 (B3). Values in the chart are given as mean ± *SD* for 3 replicates. Different letters in the same Pb^{2+} concentration column mean significant difference among treatments ($p < 0.05$).

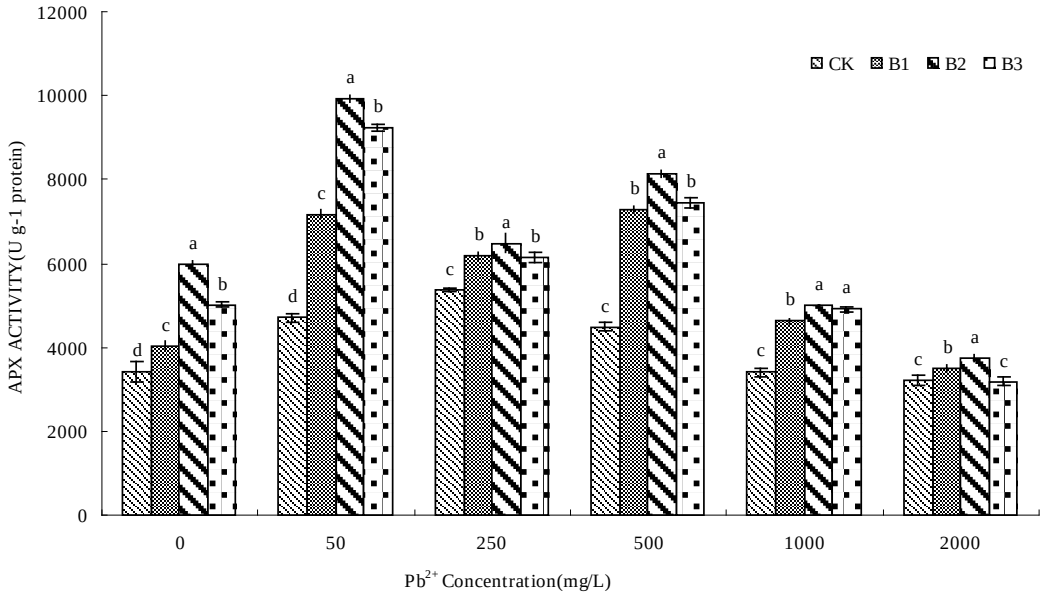

Figure 3. Effects of *B. subtilis* QM3 on APX activity of wheat root under different lead stress. CK, B1, B2 and B3 respectively represent the control group (treated with sterile water), 10^8 CFU/ml *B. subtilis* QM3 (B1), 10^7 CFU/ml *B. subtilis* QM3 (B2) and 10^6 CFU/ml *B. subtilis* QM3 (B3). Values in the chart are given as mean ± *SD* for 3 replicates. Different letters in the same Pb^{2+} concentration column mean significant difference among treatments ($p < 0.05$).

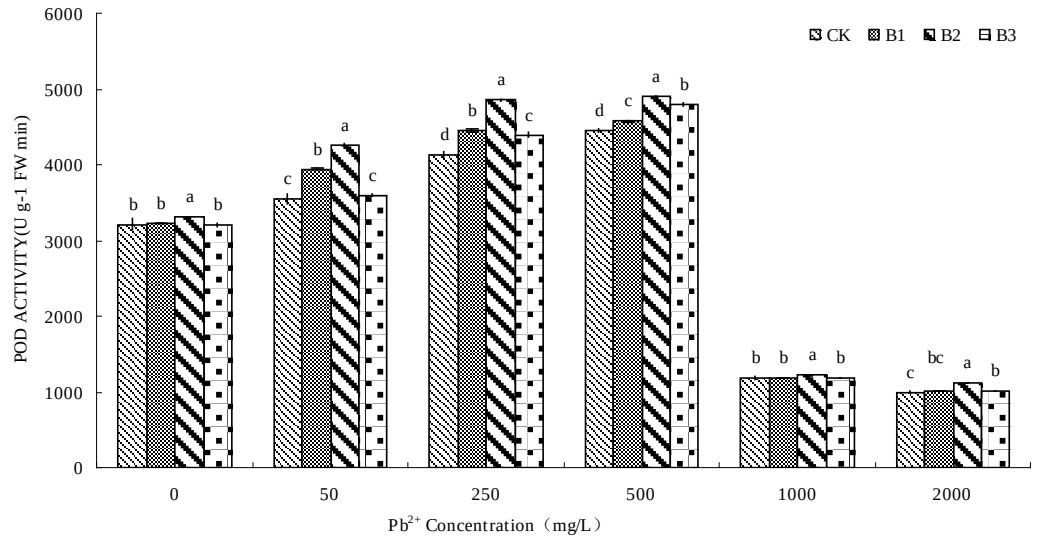

Figure 4. Effects of *Bacillus subtilis* QM3 on POD activity of wheat root under different lead stress. Values in the chart are given as mean ± *SD* for 3 replicates. CK, B1, B2 and B3 respectively represent the control group (treated with sterile water), 10^8 CFU/ml *B. subtilis* QM3 (B1), 10^7 CFU/ml *B. subtilis* QM3 (B2) and 10^6 CFU/ml *B. subtilis* QM3 (B3). Different letters in the same Pb^{2+} concentration column mean significant difference among treatments ($p < 0.05$).

4. Discussion

Lead is one of the most abundant heavy metals polluting the soil environment [17]-[20]. It is readily absorbed by plants mainly through the root system and thereafter exerts its toxicity symptoms. The effects of Pb phytotoxicity include stunted growth, chlorosis, blackening of the root systems [18], alteration in water and nutritional status of plants [15] as well as various plant processes [18] [20].

A number of reports have showed the inhibitory and toxic effect of different heavy metal on the germination of seeds and the growth of plant seedling [21] [22]. It is well known that root growth is more sensitive than seed germination to metal toxicity [23] [24]. In addition, because plant roots are the first point of contact for heavy metal toxic factors, the reduction in root length was more prominent in plants when exposed to different of Pb and Cd treatments in comparison with the growth of shoots [25] [26]. Some researches [27]-[29] found that low concentrations of Pb in the nutrient solution stimulated seed germination, while high concentrations resulted in the inhibitory effect, suggesting the dual role of Pb in plant. Our result showed that lead stress caused a significant inhibition on wheat root growth, including the root length, root surface area, root volume and root projection area. However, under 50 mg/L Pb^{2+} concentration, the wheat root growth was promoted, it may due to the dual role of Pb in plant. From other Pb^{2+} concentrations stress, in a certain degree, *B. subtilis* QM3 had an alleviation effect on the growth of the wheat root under lead stress.

In many plant species heavy metals have been reported to cause oxidative damage due to production of ROS [4] [7] [30]-[32]. To resist oxidative damage, the antioxidant enzymes and certain metabolites present in plants play an important role leading to adaptation and ultimate survival of plants during periods of stress [16] [32]. Induction in the activities of antioxidative enzymes is a general strategy adopted by plants to overcome oxidative stress due to the imposition of environmental stresses [7] [33].

The current results show an increase of SOD activity in wheat seedlings growing in the presence of lead. SOD considered as a first defense against ROS as it acts on superoxide radicals, which are produced in different compartments of the cell and are precursors of the other ROS [34]. Increase in SOD activity is attributed to the increase in superoxide radical concentrations.

CAT activity increases under lead phytotoxicity, and this increase can be also explained by a substrate induction, in order to maintain low levels of H_2O_2 as an adaptive mechanism [35].

POD is located in cytosol, cell walls, vacuoles and extracellular spaces. It is considered as stress marker enzyme having a broad specificity for phenolic substrates and a higher affinity for H_2O_2 than CAT. POD consumes H_2O_2 to generate phenoxy compounds that are polymerized to produce cell wall components such as lignans [35]. Increase in POD is correlated with lead stress suggesting it to be an intrinsic defense tool [10].

Our results show that resistance enzyme activity increased in low Pb^{2+} concentration level, the results of our experiments are same as the results of the previous studies, plants can resist the heavy metal stress by improving the activity of the resistance enzymes. Whereas resistance enzyme activity decreased in high Pb^{2+} concentration level (1000, 2000 mg/L). In the same Pb^{2+} concentration treatment, enzyme activity of wheat root under the treatment of B1, B2 and B3 were higher than that of CK, and B2 treatment was enhanced obviously than any other treatments. While under high concentration of Pb^{2+} (1000, 2000 mg/L), each index had little change.

The results suggest that under low Pb^{2+} concentration, wheat roots can show a strong ability of antioxidant by improving the resistant enzyme activities; In the low Pb^{2+} concentration, compared with the control group, the treatment of *B. subtilis* QM3 contributed to enzymes activity of the wheat root improved to a certain degree, so as to restrain the excessive accumulation of reactive oxygen caused by lead stress, reduce lipid peroxidation of cell membrane and maintain the relative integrity and orderliness, at last, relieved the inhibition to the root system growth. By comparison, 10^7 CFU/ml *B. subtilis* QM3 bacteria liquid treatment was enhanced obviously than any other treatments. 10^8 CFU/ml *B. subtilis* QM3 has a higher concentration, and 10^6 CFU/ml *B. subtilis* QM3 has a lower concentration, these two reasons may lead to their alleviate effect are not as good as 10^7 CFU/ml *B. subtilis* QM3 solution. However, plant self-protection ability is limited, When the Pb^{2+}concentration over 1000 mg/L, membrane lipid system of wheat roots would be thoroughly damaged by active oxygen, the activities of the resistant enzyme are reduced, at the same time the treatment of *B. subtilis* QM3 solution to wheat root that under high level of Pb^{2+} (>1000 mg/L) had no obvious relieve effect on SOD and POD activity. Lead concentration was too high to cause plant excessive injury, lead to alleviate effect not obvious.

5. Conclusion

Plant-associated bacteria can have beneficial effects on the growth of their host. Nevertheless, the role of plant growth promoting bacteria, in terms of plant metal stress tolerance, has not been investigated in depth. When the plants exposed to Pb, the bacterium depressed Pb induced oxidative stress by improving antioxidant enzymes (SOD, CAT, APX, POD), promoted relative plant growth, resulting in increased plant tolerance to Pb. The result indicated that *B. subtilis* QM3 promoted better growth in plants cultivated in the presence of Pb. This phenome-

non appears to be attributed to a mechanism that decreases Pb concentrations in the root via a beneficial interaction between the bacteria and the plant roots.

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