Effects of Inoculants (*Chlorobium limicola* and *Rhodopseudomonas palustris*) on Cucumber (*Cucumis sativus L.*) Seedlings in Stimulating their Nutrient Uptake and Growth

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Abstract: Rhizobacteria-containing biofertilizers are perfect tools to promote plant growth for the superiority of reducing environmental damages. Two strains of *Chlorobium limicola* and *Rhodopseudomonas palustris* were applied in the experiment as potential inoculants for cucumber seedlings. Significant enhancement in the availability of macronutrient elements N, P and K were observed in soils, and further improvement in their uptake was also obtained in cucumber plants. Accumulation of essential micronutrients Fe and Zn were detected both in the roots and shoots. The two strains increased chlorophyll and carotinoid synthesis, plant height, stem diameter, wet weight and dry weight. Various doses had significant effects on plant growth stimulation, *C. Limicola* with 10^7 cells mL^{-1} and *R. Palustris* with 10^8 cells mL^{-1} seem to be better on the whole.

Keywords: Biofertilizer, macronutrient, micronutrient, photosynthesis, strains.

INTRODUCTION

Utilization of fertilizers is really necessary for plant growth, but excessive and repeated use of chemical fertilizers may spoil the soil, water and even atmosphere. Rhizobacteria containing biofertilizers are perfect tools to improve plant nutrient and production and reduce environmental damages [1]. Nitrogen is the most major limiting factors to plant growth. The most important contribution to biological nitrogen fixation comes from the symbiotic nitrogen fixing bacteria which characteristically infect the roots of plants (e.g., leguminous crops) with a high degree of host specificity. A relatively broad utilization could be achieved by non-symbiotic nitrogen fixing bacteria, various free-living microorganisms which are capable of nitrogen fixation, which may be related to the closeness of the root-microorganism association in that living plant roots release a wide variety of simple organic compounds which may be used as food by free-living soil bacteria (rhizobacteria) [2]. *Chlorobium limicola* and *Rhodopseudomonas palustris* are ones of them. There is sufficient published evidence to propose photosynthetic bacteria can fix N_2 effectively either cultured singly or in mixed culture in symbiotic association with heterotrophic bacteria [3], and has function as a nitrogen fertilizer for recycling and conserving essential nutrients in some crops ecosystem including: maize [4], mangrove [5], tomato [6], association with straw etc.. About one third people in the world suffer from the inadequate intakes of Fe, Zn and vitamin A [7], especially in developing countries [8]. Cucumber cultivation has an important role in agricultural production. So we checked the role of the two strains in macronutrient and micronutrient elements uptake and growth promotion of cucumber.

MATERIALS AND MATHODS

Chlorobium limicola and *Rhodopseudomonas palustris* are two newly isolated strains from soil. Seeds of cucumber (*Cucumis sativus L. cv. Jinchun4*) were surface sterilized with $HgCl_2$ (0.1%) for 10 min, rinsed with deionized water and placed on filter paper moistured by deionized water, and germinated at 28°C in constant temperature incubator in dark. After germination the seedlings were transferred to plastic pot (Φ_{bottom}10cm×16cm×Φ_{top}15cm) containing 1.2 kg autoclaved soil. The cells in cul-tured bacterial broth of both two strains were adjusted to 10^7, 10^8 and 10^9 cells mL^{-1}, and 150 mL of bacterial broth was applied to each pot every two days, and the application of deionized water and chemical fertilizer were using as controls. The experiment of examining cucumber growth was set in a Completely Randomized Block Design with eight treatments each content four replicates and the replicate represented by ten plants under controlled conditions at 26 ± 2°C and a 16 hr photoperiod created by using illumination incubator. The morphological characters such as height of the plants, stem diameter, wet weight and dry weight were estimated at 40 days after treatment are calculated. We used the Minolta SPAD-502 chlorophyll meter to measure the relative chlorophyll contents and

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chromatography for carotinoid contents of the third fully developed leaves.

Shoot and root tissues were separated after harvesting and were air-dried at 70°C for 5 days, and soil samples were collected after the experiment and air-dried for chemical analysis. Plants were digested in H_2SO_4-H_2O_2 for the determination of total nitrogen. Available N in soils was determined by alkaline-proliferation law. P was determined by the Berthelot reaction and molybdenum blue method [9], soil samples were extracted with 0.5 M $NaHCO_3$ (pH 8.5). Plants and soils were digested using 1M NH_4OAC(pH 7) for the determination of K using an atomic absorption spectrometer. Atomic absorption spectrometer was also used for the determination of Fe and Zn [10].

The data was analyzed statistically for standard deviation by using sigma plot software. The mean values were compared, using Duncan's multiple range test at P<0.05.

RESULTS

The treatment inoculation with *C. Limicola* and *R. Palustris* significantly increased N, P and K uptake in cucumber plants. The two strains had stronger effect on N uptake then P and K uptake. The amounts of available N, P and K nutrients in soils were significantly increased as compared to control, which might be attribute to the capability to fix atmospheric nitrogen, solubilize phosphate and potassium by *C. Limicola* and*R. Palustris*. *R. Palustris* with 10^8 cells mL^{-1} and *C. limicola* with 10^7 cells mL^{-1} have even stronger and more stable effect then with other concentrations (Table **1**).

Accumulation of Fe in both plant parts of roots and shoots increased significantly in various treatments of *C. Limicola* and *R. Palustris* as compared to the control of deionized water, and even higher then the control of chemical fertilizer in roots, and reaching to the maximum level in *R. Palustris* with 10^8 cells mL^{-1}. Level of Zn showed similar trend with that of Fe except

Table 1: Effects of *C. Limicola* and *R. Palustris* on Macronutrient Uptake of Cucumber Seedlings and on the Soil N, P, K Concentrations (n=4; ± s.e.)

treatment (cells mL^{-1})	cucumber(mg.g dw^{-1})			soil(mg kg^{-1})		
	N	P	K	N	P	K
deionized water	19.1±0.82c	1.83±0.10b	27.5±2.41b	70.12±4.32c	8.92±0.50d	80.22±7.8c
R. palustris(10^7)	22.3±0.78ab	2.09±0.08a	31.6±2.01a	92.75±5.19a	10.89±0.67ab	98.07±8.5b
R. palustris(10^8)	22.4±0.98a	2.06±0.11a	31.5±1.93a	98.85±2.07a	10.65±0.81ab	110.16±10.5a
R. palustris(10^9)	20.0±1.11bc	2.04±0.08a	30.5±2.21a	88.80±3.55ab	10.31±0.15bc	98.97±8.7b
C. limicola(10^7)	21.8±0.83ab	2.10±0.10a	31.0±1.58a	82.63±4.25ab	11.33±0.38a	112.34±5.6a
C. limicola(10^8)	20.4±1.02bc	2.12±0.14a	30.2±1.33a	90.76±1.77ab	11.50±1.16a	94.61±7.2b
C. limicola(10^9)	21.6±1.30ab	2.08±0.09a	30.6±2.71a	81.74±1.84b	9.62±1.27cd	95.32±5.8b

All data are means of 4 replications ±standard error.
Means with the same letter within a column are not significantly (P >0.05) different according to Fisher's Protected LSD Test.

Table 2: Effect of *C. Limicola* and *R. Palustris* on the Contents of Fe and Zn in Cucumber Seedlings (n=4; ± s.e.) (mg. kg dw^{-1})

treatment (cells mL^{-1})	roots		shoots	
	Fe	Zn	Fe	Zn
deionized water	415.6±18.3e	123.1±3.21d	532.7±19.5c	146.6±4.68c
chemical fertilizer	475.5±17.6d	144.6±4.44c	608.9±20.2abc	176.2±3.11ab
R. palustris(10^7)	601.4±16.4ab	156.8±2.61b	612.8±21.8ab	182.7±4.65a
R. palustris(10^8)	655.4±20.5a	166.2±0.98ab	654.4±16.4a	175.5±4.12ab
R. palustris(10^9)	602.8±17.5ab	167.5±5.21ab	572.7±17.2bc	174.2±2.08ab
C. limicola(10^7)	588.2±21.4bc	174.9±3.89a	594.6±18.5abc	178.4±1.22ab
C. limicola(10^8)	532.3±18.2c	162.8±4.56ab	566.2±18.9bc	175.9±5.97ab
C. limicola(10^9)	560.1±18.6bc	170.8±2.17a	548.3±17.7c	165.8±3.26b

Table 3: Effects of *C. Limicola* and *R. palustris* on the Plant Height, Stem Diameter, Wet Weight, Dry Weight, Relative Chlorophyll Contents and Carotinoid Contents of Cucumber Seedlings (n=4;±s.e.)

treatment (cells mL^{-1})	Height (cm $plant^{-1}$)	stem diameter (cm $plant^{-1}$)	wet weight (g $plant^{-1}$)	dry weight (g $plant^{-1}$)	Chlorophyll (mg g^{-1})	Carotinoid (mg g^{-1})
deionized water	13.8±0.86e	1.37±0.05d	7.37±0.84e	0.72±0.11c	10.0±0.82d	3.25±0.48b
chemical fertilizer	14.1±1.29e	1.40±0.07cd	7.51±0.62de	0.74±0.07c	10.6±0.98c	2.99±0.71c
R. palustris(10^7)	25.6±0.76a	1.43±0.06bcd	9.98±0.91a	0.88±0.02a	11.1±0.89b	3.55±0.27a
R. palustris(10^8)	25.0±0.84a	1.43±0.16bcd	9.98±0.01a	0.88±0.22a	11.8±0.52a	3.64±0.67a
R. palustris(10^9)	15.0±1.28d	1.47±0.14bc	7.96±0.92cd	0.61±0.01d	6.50±0.99e	1.80±0.20d
C. limicola(10^7)	23.1±0.42b	1.50±0.10ab	8.10±0.62c	0.62±0.02d	11.6±1.38ab	1.89±0.28d
C. limicola(10^8)	18.2±1.36c	1.57±0.03a	9.05±0.61b	0.71±0.08c	11.2±1.35b	3.57±0.71a
C. limicola(10^9)	21.5±0.46b	1.57±0.22a	10.0±0.673a	0.80±0.06b	10.1±0.74d	3.26±0.09b

reaching to the maximum level in *C. Limicola* with 10^7 cells mL^{-1} in roots and *R. Palustris* with 10^7 cells mL^{-1} in shoots. It is obviously that the two stains could promote the enrich of Fe and Zn in cucumber, and the effect seems to be stronger in roots than in shoots (Table **2**).

Both strains showed a promoting effect on plant growth, morphological and biochemical parameters repre-senting at Table **3** showed a best response to the *R. Palustris* inoculants with 10^8 cells mL^{-1}, and treatment with 10^7 cells mL^{-1} is following. Morphological parameters showed a good response to *C. limicola* with 10^9 cells mL^{-1}, however, several parameters responsing to *R. Palustris* with 10^8 cells mL^{-1}were lower then control (Table **3**).

DISCUSSION

The experiment demonstrated that *C. Limicola* and *R. Palustris* stimulated the availability of the macronutrient element N, P and K in soils, and make further improvement on the uptake of them in cucumber plants. The two stains could also promote the accumulation of essential micronutrients of Fe and Zn. Fe is the maximum micronutrient for plant growth. Fe and Zn can accelerate chlorophyll synthesis. So we can see the application of the two stains enhanced growth, while the probably physiological basis might be that an increased chlorophyll and carotinoid synthesis makes a stronger photosynthesis, which may provide more energy for nitrogen fixation, allowing for more efficient plant growth [11], therefor wet weight and dry weight enhanced, and a more intensive uptake of nutrients might also cause a promoting growth of cucumber [12]. The increasing N uptake in our experiment might be related to the fact that photosynthetic bacteria which *C. Limicola* and *R. Palustris* falls into is a genus of photosynthetic prokaryotes, which are known to fix atmospheric nitrogen [13, 14] and induce resistance, seem to achieve their promised contribution to plant growth.Plant growth promoting rhizobacteria stimulate the growth of plants by helping to provide nutrient to the host plant, or indirectly by positively influencing root growth and morphology or by aiding other beneficial symbiotic relationships, or helping to control patho-genic organism [15-17]. Whether the increasing uptake of P and K should due to the role of strains by aiding other beneficial symbiotic relationships or their own role needs further reaserch. The perfect behaviour of the two strains appears therefore a priority to really envisage a future for the use of them as a inoculant for cucumber.

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