



## Original Research Article

# Effect of phosphorus and potassium content of plant and soil inoculated with *Paenibacillus kribensis* CX-7 strain antioxidant and antitumor activity of *Phyllanthus emblica* in colon cancer cell lines

Zhang Ai-min<sup>1,2</sup>, Zhao Gang-yong<sup>2</sup>, Zhang Shuang-feng<sup>2</sup>,  
Zhang Rui-ying<sup>3</sup> and Zhu Bao-cheng<sup>1</sup>

<sup>1</sup>College of Life Science, Agricultural University of Hebei, China

<sup>2</sup>Biological Technology Center of Hebei University, China

<sup>3</sup>The Academy of Life and Sciences of Hebei University, Baoding 071001, China

\*Corresponding author e-mail: [zhu2222@163.com](mailto:zhu2222@163.com), [zhangam2008@yahoo.com.cn](mailto:zhangam2008@yahoo.com.cn)

## ABSTRACT

### Keywords

*Paenibacillus kribensis*;  
Phosphate and potassium solubilization;  
Soil phosphorus and potassium content; Plant phosphorus and potassium content.

*Paenibacillus kribensis* CX-7 strain with the ability of solubilizing phosphate and potassium was chosen from wheat field sample of Chang'an, Shanxi. The activities of strain CX-7 as biological fertilizer to release P and K nutrient for plants and soil of field were determined through field experiments. We found that the application of culture fluid of strain CX-7 was able to significantly increase the content of phosphorus and potassium in plants and that in the soil. The content of potassium in the soil increased by more than 10 mg/kg, the content of phosphorus increased by 3~11 mg/kg. The average content of phosphorus in the plant increased by 15.59% and the content of potassium increased by 30.05%. By biological statistics standards, the differences between the inoculated and the control had reached significant or very significant levels. Apart from this, the yield of maize, wheat, sweet potato, rice, tobacco and cotton inoculated with CX-7 can be increased by 12.3%, 8.2%, 13.6%, 9.5%, 15.2% and 8.5%. Our results implied that *Paenibacillus kribensis* CX-7 strain had potential for usage as a biological fertilizer.

## Introduction

Microbiological fertilizer has the ability of improving the structure of soil and increasing the quality of agriculture products, thus its research and development has been emphasized (Lin and Wang *et al.*, 2001, Ma, 2007). There are many kinds of microbiological fertilizer. It can be classified as rhizobium fertilizer, nitrogen-fixation microorganism fertilizer, phosphate solubilizing bacteria

antibiotic microorganism fertilizer and compound microbiological fertilizer (Chen, 1985). The phosphate and potassium solubilizing microorganism can not only activate the insoluble phosphate and potassium mineral, but also change that into available phosphorus and potassium. Thus the quality of crop can be improved, the effect of environment can be decreased, the physicochemical

property can be made better and the cost of production can be reduced (Xue, 2005).

Silicate bacteria has the ability to decompose soil potassium minerals, activate potassium for the absorption and utilization of plant, and to increase crop yields (Sun and Zhang, 2006; Tang and Zhang, 2008, Xu et al., 2007, Sheng et al., 2001). It was used widely and the majority of Chinese scholars generally believe that the silicate bacteria is specifically *Bacillus mucilaginosus* (Zhang and Zuo, 2000). *Bacillus mucilaginosus* grows well on potassium feldspar supplemented, nitrogen-free medium, which indicates that it has the ability of nitrogen fixation and decomposition of the mineral potassium (Jing and Xu, 1997). However, we separated a new *Paenibacillus kribensis* CX-7 strain, which has the ability of solubilizing phosphate and potassium mineral (Zhang et al., 2013).

Soil is the basis for crop production. It can provide mechanical support, water and oxygen, and provide the necessary nutritional elements for the crop. Soil microorganisms play an important role in the decomposition of soil organic matter and nutrient release (Han et al., 2011). Silicate bacteria as the effect of potassium solubilization, phosphate-solubilization and nitrogen-fixing, it can increase crop yields, improve quality of agricultural products in production (Wang et al., 2009). This study was designed using *Paenibacillus kribensis* CX-7 strain separated from the soil sample of Chang'an, Shanxi. The field experiment has been done to verify the real impact on crop nutrients and soil nutrients, and thus providing a theoretical basis for the large-scale application of this strain.

## Materials and Methods

### Strain

*Paenibacillus kribensis* CX-7 strain—separated from the soil sample of Chan'an, Shanxi, China.

### Crop

Maize, wheat, sweet potato, rice, tobacco and cotton—the main crops in the north of China.

### Inoculation experiment

Cotton, corn, wheat, tobacco, sweet potatoes and rice were inoculated with *Paenibacillus kribensis* CX-7 strain fermentation broth at a dosage of 7500 mL/hac.. The inoculate method is basal-applying. The *Paenibacillus kribensis* CX-7 strain fermentation broth was watered in the crop root. The bacteria content is  $4.5 \times 10^8$  cfu/mL. The plant yield, phosphorus and potassium contents of the treated and the control were measured. At the time, the biostatistics can be calculated between the control and treatment.

### Measuring of soil phosphorus and potassium content

The soil phosphorus and potassium contents of the inoculated and the control were measured. Available phosphorus content was measured according to GB12297-87. (Gasser et al., 2011). Available potassium content was determined with reference to the GB7859-87 (Petrus Ahmed et al., 2010.).

The Olsen and Sommers method (1982) was used to measure the available phosphorus of the plant. Plant potassium

content determination with reference to the tetraphenylboron sodium turbidimetry (Zhang and Zuo, 2000).

## Results and Discussion

### The effect of *Paenibacillus kribensis* CX-7 strain on soil phosphorus and potassium nutrients

Phosphorus and potassium are essential elements for plant growth and development, and they are the supplementary elements of conventional fertilizer in agricultural production and application. When inoculated with *Paenibacillus kribensis* CX-7 strain, available soil phosphorus and potassium content increased significantly. According to soil sample test results of Wuji, Jixian, Gaocheng Gaoyang County, the available soil (cotton or corn) potassium is 113.3 ~ 117.6 mg/kg when inoculated with CX-7 strain. It is 23~27 mg/kg higher than the uninoculated soil and 11.9~16.2 mg/kg higher than the soil treated with 7.5kg K<sub>2</sub>SO<sub>4</sub>. The wheat soil available potassium is 103~110 mg/kg and the available phosphorus is 10~18 mg/kg when inoculated with CX-7 strain.

The content of available potassium is 10~18 mg/kg than the uninoculated soil and the content of available phosphorus is more 3.0~11.0 mg/kg higher than the uninoculated soil. The soil (cotton or corn) available potassium is 109 mg/kg when inoculated with the *Paenibacillus kribensis* CX-7 strain. It is 27 mg/kg higher than the uninoculated one. Thus, the soil available phosphorus and potassium content can be increased significantly by applying CX-7 strain and the soil fertility can be improved effectively.

### The effect of *Paenibacillus kribensis* CX-7 strain application on crop yield

Soil phosphorus and potassium not only improve the level of phosphorus and potassium nutrition of the plant body, but also improve the quality of the crop; the increase of plant phosphorus can increase the crude protein content and the essential amino acids content. The improvement of potassium nutrient can promote the changes of a series of enzyme activity. So the metabolism of carbohydrates can be improved and the degeneration-resistant behaviour can be enhanced.

From table 1, we can see that the yield of cotton, corn, tobacco, wheat, potato and rice can be increased to a certain degree when inoculated with the *Paenibacillus kribensis* CX-7 strain compared to that of the control. The increasing rate of cotton is 8.5% in Hejian city, that of tobacco is 15.2% in Lingshou county, that of corn is 12.3% in Lingshou county, that of rice is 9.5% in Lingshou county, that of potato is 13.6% in Gaoyang county, that of wheat is 8.2% in Gaoyang county and that of potato is 11.8% in Guan county.

From table 2 we can see that the plant phosphorus content of cotton, corn, tobacco, wheat, potato and rice can be increased when inoculated with *Paenibacillus kribensis* CX-7 strain when compared to that of the control. The average increasing rate is 15.59%. The significant difference of the inoculated and the control is supported by statistical analysis ( $t=4.02 > t_{p=0.01}=3.707$ ). The largest increase was seen in cotton at a rate of 32.68%. The next one is wheat and corn and the increasing rate is more than 20%. Thus, the plant phosphorus content can be increased by inoculation with *Paenibacillus kribensis* CX-7 strain.

**Table.1** Increasing effect of *Paenibacillus kribensis* CX-7 strain on the yield of plants

Plant	Site	Groups	Yield (Kg/hm <sup>2</sup> )	Increasing (Kg)	Increasing rate (%)
Cotton	Hejian	Treatment	3910.5	307.5	8.5
		Control	3603.0		
Tobacco	Lingshou	Treatment	2667.0	352.5	15.2
		Control	2314.5		
Maize	Lingshou	Treatment	10179.0	74.4	12.3
		Control	9063.0		
Rice	Lingshou	Treatment	8556.0	744.0	9.5
		Control	7812.0		
Potato	Gaoyang	Treatment	32199.0	3846.0	13.6
		Control	28353.0		
Maize	Dingzhou	Treatment	8679.0	1140.0	15.1
		Control	7539.0		
Wheat	Gaoyang	Treatment	8727.0	664.5	8.2
		Control	8062.5		
Potato	Guan	Treatment	28162.5	2967.0	11.8
		Control	25159.5		

**Table.2** Effect of CX-7 strain on crop phosphorus content of different plants

Plant	Site	Groups	P content (mg/Kg)	Plant growth period	Increase rate(%)	Increasing rate(%)
Cotton	Hejian	Treatment	0.5514	Bud period	0.1358	32.68
		Control	0.4156			
Tobacco	Lingshou	Treatment	0.3016	Florescence	0.032	11.87
		Control	0.2696			
Maize	Lingshou	Treatment	0.6901	Jointing period	0.1215	21.37
		Control	0.5686			
Rice	Lingshou	Treatment	0.4034	Jointing period	0.0302	8.09
		Control	0.3732			
Potato	Gaoyang	Treatment	0.7422	Expand period	0.0378	5.37
		Control	0.7044			
Wheat	Gaoyang	Treatment	0.6086	Double ridge stage	0.139	29.60
		Control	0.4696			
Potato	Guan	Treatment	0.7784	Harvest period	0.047	6.43
		Control	0.7314			

n=6 t=4.02 P=0.05 t=2.447 P=0.01 t=3.707

The cause of the plant phosphorus increasing perhaps is because of *Paenibacillus kribensis* CX-7 strain has the ability of solubilizing phosphate and it increase the plant absorb the phosphorus. The phosphorus level increasing results in the yield increasing of many plant.

wheat, potato and rice can be increased when inoculated with *Paenibacillus kribensis* CX-7 strain when compared to that of the control. The average increasing rate is 15.59%. The significant difference of the inoculated and the control is supported by statistical analysis ( $t=4.02 > t_{P=0.01}=3.707$ ).

From table 3 we can see that the plant potassium content of cotton, corn, tobacco,

**Table.3** Effect of *Paenibacillus kribensis* CX-7 strain on potassium content of plants

Plant	Site	Groups	K content (mg/kg)	Plant growth period	K increment (mg/kg)	Rate of increase%
Cotton	Hejian	Treatment	2.4514	Bud period	0.4456	22.22
		Control	2.0058			
Tobacco	Lingshou	Treatment	3.8738	Florescence	1.5108	63.94
		Control	2.3630			
Maize	Lingshou	Treatment	4.5320	Jointing period	0.687	17.87
		Control	3.8450			
Rice	Lingshou	Treatment	3.8795	Jointing period	1.1959	44.56
		Control	2.6836			
Potato	Gaoyang	Treatment	3.3537	Expand period	1.3583	68.07
		Control	1.9954			
Maize	Dingzhou	Treatment	0.5905	Aging period	0.0315	5.64
		Control	0.5590			
Maize	Gaoyang	Treatment	3.9114	Double ridge stage	0.5381	15.95
		Control	3.3733			
Potato	Guan	Treatment	9.8319	Harvest period	0.2078	2.16
		Control	9.6241			

n=7 t=4.19 P=0.05 t=2.365 P=0.01 t=3.499

From the previously mentioned experiments, we can deduce that the phosphorus and potassium content of different parts of the cotton, maize, tobacco, potato and rice can be increased compared to that of the control when inoculated with *Paenibacillus kribensis* CX-7 strain. Potassium especially can be increased significantly and the result is supported by biostatistics.

Potassium increasing can enhance many enzyme activity in the plant. So the plant yield can be increased.

Through field experiments, the ability of *Paenibacillus kribensis* CX-7 strain to increase phosphorus and potassium content for plants was determined and its use as a biological fertilizer was investigated. The phosphorus and potassium content can be increased significantly. According to soil sample test results, the cotton soil available potassium is 113.3 ~ 117.6 mg /kg when inoculated with *Paenibacillus kribensis* CX-7 strain. It is 23~27 mg/kg higher than the uninoculated soil and it is 11.9~16.2 mg/kg higher than the soil treated with 7.5kg K<sub>2</sub>SO<sub>4</sub>. The content of available potassium of wheat is 10~18 mg/kg higher than the uninoculated soil and the content of available phosphorus is 3.0~11.0 mg/kg higher than the uninoculated soil. The corn soil available potassium is 27 mg/kg higher than the uninoculated one.

When treated with *Paenibacillus kribensis* CX-7 strain, not only did the soil P and K increase, but the phosphorus and potassium content in different plant parts also saw an increase. The average phosphorus content can be increased by 15.59%, and the potassium content can be increased by 30.5%. Through biological

statistic analysis, the difference between the treated and the control was found to be significant or very significant.

Not only the phosphorus and potassium content can be increased, but the yield of maize, wheat, sweet potato, rice, tobacco and cotton inoculated with CX-7 can be increased by 12.3%, 8.2%, 13.6%, 9.5%, 15.2% and 8.5%.

The plant and soil potassium and phosphorus content can be increased obviously when inoculated with *Paenibacillus kribensis* CX-7 strain. The mechanism needs to be further researched, but it has potential for using as biological fertilizer.

## Acknowledgments

In the field experiment, we will thank the workers in the Agriculture Bureau of many county. We also thanks the students in Hebei agriculture university.

## References

- Chem. 56:670-675. Chen, H.K., 1985. Soil microbiology. Shanghai: Shanghai Science and Technology Press.
- Gasser, M.O., M.H. Chantigny et al., 2012. Plant-available and water-soluble phosphorus in soils amended with separated manure solids. J. Environ. Qual. 41(4):1290-1300.
- Han, L., D. Wang and Lin, R.F, 2011. Isolation and screening of antagonistic silicate bacterium. Chinese. J. Soil Sci. 42(1):77-80.
- Jing, C.L., and Xu, L.H. 1997. Microbial Resource. Beijing: Science Press.pp. 151.
- Lin, Q.M., H. Wang and Zhao, X.R. 2001. Capacity of some bacteria and fungi in

- dissolving phosphate rock. J. Microbiol. 28(2):26-29.
- Ma, C.H., 2007. Application and research progress of phosphate-solubilizing microorganisms. Anhui. Agricul. Sci.Bull. 13(4): 34-36.
- Olsen, S.R., and Sommers, L.E. 1982 Phosphorus. In: Page AL, editor. Methods of Soil Analysis: Chemical and Microbiological Properties. ASA and SSSA; Madison, WI, USA: pp. 403-430.
- Petrus, A.C., O.H. Ahmed et al., 2010. Effect of K-N-humates on dry matter production and nutrient use efficiency of maize in Sarawak, Malaysia. Scienti. World.J. 10(6): 1282-1292.
- Sun, D.S., and Zhang, Q. 2006 . Screening of silicate bacteria and bio-leaching silicon from silicate ores, J. Xi'an University.Sci.Technol. 26(2):235-239.
- Tang, L., and Zhang, J.Z. 2008. Isolation, purification and identification of silicate-dissolving bacterial strains and studies of their biological characteristics. Shandong. Agricul. Sci. 1:71-73.
- Sheng, X.F., W.Y. Huang and Cao, X.Y. 2001. Dissolution of feldspar and potassium uptake by the strain NBT of silicate bacterium. Plant Nutrit. Fertili. 7(4):459-466.
- Wang, W., J. Li, J.S. Liu and Zhu, B.C. 2009. Isolation of the Silicate Bacteria Strain and Determination of the Activity of Releasing Silicon and Potassium. J. Anhui Agricul. Sci.37(17):7889-7891.
- Xue, B.G., 2005. Screening and identification of the Bacterium which have efficiency on resolving phosphorus and potassium and in nitrogen fixation. J. Huazhong. Agricul. University. 24(1):43-48.
- Xu, D.Y., F. Li and He, X.L. 2007. Isolation of silicate bacteria and their capability of releasing potassium. J. Huaibei Coal Industry. Teachers. College. 270: 42-44.
- Zhang, A.M., G.Y. Zhao *et al.*, 2013. Solubilization of insoluble potassium and phosphate by *Paenibacillus kribensis* CX-7: A soil microorganism with biological control potential. African J. Microbiol.Res.7(1): 41-47.
- Zhang, D.L., and Zuo, J.L. 2000. Contrast analysis on determination of inorganic element K with flare photometer and sodium tetraphenylboron. Jingxi Forestry Sci. Technol. 5:26-28