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Optimizing *Bacillus circulans* Xue-113168 for biofertilizer production and its effects on crops

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In this study, *Bacillus circulans* Xue-113168 biofertilizer was produced through solid state fermentation processes using food waste and feldspar. Results confirmed that solid state fermentation has considerable advantages compared to complex process (solid-state and bio-bleach). The control of pH, temperature, and humidity effectively led to the formation of 2×10^9 cfu/g spore and dissolution of potassium at a rate of 41.53%. Compound microbial fertilizer (CMF), formulated by humic acid and K_2HPO_4 with biofertilizer, has a quick and durable effect. CMF increases the yield of rapeseeds by 75 to 89%, provides higher vitamin C and reduces nitrate in leaf. Yields of selenium-enriched jujube and jujube increased, respectively, in the CMF compared to the matrix control; rates of anthracnose and rust diseases also decreased. Furthermore, our results showed that CMF improved soil properties, such as organic matter, NPK content from 8.83 to 16.16 kg hm^2, and reduced chemical fertilizer from 25 to 11%, respectively. For convenient medium, robust process and good effect, CMF is suitable for potassium deficiency and undeveloped arable land resources.

Key words: compound microbial fertilizer, *Bacillus circulans* Xue-113168, solid-state fermentation (SSF), process, optimize.

INTRODUCTION

Plant biostimulants contain substance(s) and/or micro-organisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality. Biostimulants (Dobbss, 2016; Canellas et al., 2015;Patrick du Jardin, 2015) are ingredient from microbe or others, abiotic stress resistance is promoted, and the effect is not dependent on the nutritional components. Biochemical humic acid are produced from organic by-products and waste residue, waste liquid in organic materials by microbial fermentation and physical and chemical processes (Luciano, 2015). Jujube rust and anthracnose are important diseases that are difficult to control after their occurrence and can cause heavy production loss and low fruit quality. Present research on jujube rust has only dealt with its epidemiology and chemical control. Compound microbial fertilizer (CMF) can palliate Jujube rust and anthracnose, enhance quality attributes of yields, protect and improve soil health by fostering development of beneficial soil microorganisms. Anthracnose and rust disease are soil-borne diseases

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caused by fungi which are difficult to deal with. Zhao et al. (2011) demonstrated how to control *Fusarium* wilt disease in *Cucumis melo*, using bioorganic fertilizer. Plant growth-promoting rhizosphere (PGPR) prevents and controls plant diseases especially soil-borne disease through the followings: competition between the pathogens and space site, the development of antibiotics to inhibit the growth of pathogens and induction of systemic resistance (ISR) of plant (Pacôme et al., 2016). Microbial fertilizers enrich soil fertility (Padmavathiamma et al., 2008), improve fertilizer utilization, inhibit the absorption of nitrate nitrogen, heavy metals, and pesticides by crops, clean up and repair the soil and reduce the occurrence of crop diseases. Biofertilizers could contribute to relieving stresses, that is, energy crisis, scarcity of resources, and environmental pollution (Ho et al., 2010; Basak and Biswas, 2010; Nishanth and Biswas, 2008; Iqbal et al., 2010; Kumar et al., 2010). The application of humic acids, organic wastes and biofertilizers might minimize the pollution caused by chemical fertilizers (Eman, 2008), and the scale of biofertilizers production could be achieved by using a composting mode (Farrell and Jones, 2009).

Optimization of PGPR (Esitken et al., 2010; Kloepper, 2009) by solid state ferment (SSF) in a less-cost and convenient way is urgent. Low water activity in SSF facilitates formation of spore on the solid substrate and the production of large quantities of enzymes, which is different from submerged fermentation (SMF) processes (Barrios-González, 2012). Microbial release of potassium from K-bearing minerals consists of fermentation and bioleach (Lian et al., 2008). This study combines two steps into one.

Furthermore, chitinase and its hydrolysis product have disease resistance effects. Therefore, it is necessary to explore the ability of high dissolving potassium and chitinase activity of strains, its control process, and using it for SSF. In this study, a biological fertilizer, *Bacillus circulans* strain, with a high rate of spore formation and ability to dissolve K and chitinase, was created, making the best use of agricultural and mineral resources (Kloepper, 2009; Zhu et al., 2012). This enhances crop yields, improves the utilization rate of chemical fertilizers and pesticides, and promotes the sustainable development of agricultural industries (Rabish and Keshav, 2013). Thus, the integrated use of PGPR with chitinase and value-added composted organic waste could be highly effective in improving yield.

The present study illustrates the effectiveness of inoculation with PGPR containing chitinase and humic acid in the presence and absence of chemical fertilizer in improving growth, yield and reducing disease under pot and field conditions.

MATERIALS and METHODS

This study screens a large number of available raw materials for a solid substrate through SSF process. The solid substrate does not only supply the nutrients to the anchorage for the cells, but also induces enzymes. The culture medium contains nutrients and the supporting materials. The former are generally flour, while the latter include shrimp shell, spent mushroom substrate, corn bran, potassium feldspar powder, etc. Shrimp shell is rich in chitin for inducing chitinase. K-feldspar is a source of potassium. SMS functions as antibiotics and phytohormone. Flour can be used as nutrients.

K-feldspar was purchased from Lingshou County, Hebei PR, China. The material was ground and then passed through 100 mesh screens. An analysis showed that it contained 10.0% total potassium and insoluble potassium. The shrimp shell was taken down from the South American shrimp. The material was ground and then passed through 16 mesh screens. The analysis showed that it contains 30% protein, 45% ash, and 20% chitin.

SMS of *Pleurotus ostreatus* was obtained from a local market. The material was ground and then passed through 16 mesh screens. The analysis showed that it contained 4.45% organic matter and 1.58% total N. Its C/N was 28/1, water absorption 78 to 80%, and humic acid content 20 to 30%. Corn bran was from the North China Pharmaceutic Corporation, Hebei PR, China. The analysis showed that it contained 11.8% protein, 32% glucose and 11% cellulose.

Potassium dissolving microorganism (KSM)

This K-limited medium is designed to isolate the bacteria that seek to release K from feldspar (Hutchens et al., 2003). *Bacillus circulans* Xue-97316 was isolated from corn rhizosphere soil using K-feldspar as the sole potassium source for the medium (g/L): starch, 5.0; yeast extract, 1.0; $MgSO_4$.$7H_2O$, 0.5; $CaCO_3$, 0.1; $FeCl_3.6H_2O$, 5 mg; pH 7.5). After 1-day incubation at 30°C, *B. circulans* was selected by colony morphology. The single colony looked like a glassy bead. This isolate was originally identified using biochemical and physiological tests. The potassium dissolving rate was determined as = (St-Sc)/It×100%, where St and Sc are the water-soluble potassium in the treatment and matrix, respectively, and It = the total potassium in the treatment.

In order to make the UV light convenient and effective, *B. circulans* strain with a high potassium dissolving rate was bred before using UV mutagenesis and it was screened in a fermentation broth using potassium tetraphenylborate spectrophotometric method. *B. circulans* strains with chitinase activities were previously screened using colloid chitin as a sole C source in a medium. *B. circulans* Xue-113168 was obtained from a series of mutation steps (Figure 1); chitinase and dissolving potassium were deposited as patent strains in the Chinese General Microbiological Culture Collection Center (CGMCC) with the accession number 5155 (Xue, 2013).

Bacillus CGMCC N0.5155 has the ability to dissolve potassium, resist potassium and produce chitinase/chitosanase, etc. The preparation method is as follows: *B. circulans* (potassium bacteria) (*B. circulans* Xue-97316), isolated from maize rhizosphere, in Luancheng, Hebei Province, was put under ultraviolet mutagenesis to obtain 300 single colonies. Then five strains were obtained which have high-throughput screening of 40% potassium solution and 75% sporulation by colony dug block method. After that, under UV mutagenesis, screening with colloidal chitin as sole carbon was done to get three strains whose potassium resistance rate is up to 8%. Afterwards, these strains were respectively placed as follows: in a shake flask of 2.27% K_2O→2.27% K_2O; sterile shake flask→a 4.54 to 5% K_2O; sterile shake flasks→ a 7.5% K_2O; sterile shake flasks → a 10% K_2O; sterile shake flasks→12.5% K_2O; sterile shake flasks→ 15% K_2O containing 12.5 to 15% K_2O tablet isolated from colonies of a single seed, potassium-resistant strains serially passaged 30 times. The mutant bacteria are a high efficient method for bioleaching.

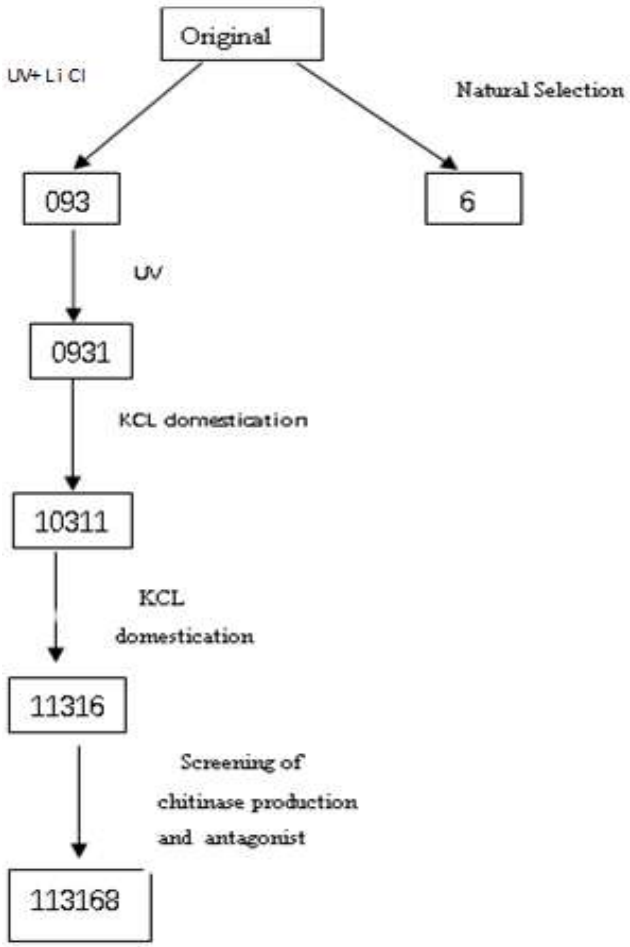

Figure 1. Breeding pedigree of *B. Circulans* Xue 113168.

Preparation of biofertilizer using SSF

The biological fertilizer was made from potassium-dissolving bacteria fermentation; meanwhile, the K releasing course was completed in the process of the preparation. The culture was grown at an initial moisture content of 60 to 65% and 30°C. The SSF was turned over every 48 h for 7 days. The pH was not regulated during the fermentation. Flour and feldspar were used as substrates at 2 and 10% (w/v), respectively, for the SSF with *B. circulans* Xue113168. After 168 h of fermentation, the SSF was autoclaved, maintained overnight and then filtered to detect the soluble potassium in the SSF.

Testing the quality index

Live bacteria counting culture medium are ingrient as follows: (g/L): sucrose (5.0), MgSO4 (1.0), FeCl3 (0.2), yeast extract (0.2), (NH4)2SO4 (0.5), KH2PO4 (1), pH 7.0. The water content was tested using the vacuum oven method. pH was measured using a pH meter in a solid-water ratio (1:1). The CMF was digested with H_2SO_4-H_2O_2. The total N content was determined using the Kjeldahl method; for the total K content, the potassium tetraphenylborate gravimetric method was used and for the total P content, molybdenum antimony anti-colorimetry was used. The organic matter was digested with H_2SO_4-$K_2Cr_2O_7$ and measured using the method of Yeomans and Bremner (1988). To determine humic-C, CMF or SMS was extracted using a mixture of 0.1 M sodium pyrophosphate and. 0.1 M sodium hydroxide at pH 13. The C content of the humic acids was determined using the method of Yeomans and Bremner (1988).

Pot experiment

The experiment was conducted in open air and natural conditions at Hebei to investigate the efficiency of the CMF. The kind of the soil used in the pot experiments is calcareous, with pH 7.8; organic matter, 20.30 g/kg; and total K, 3%. Each pot (20-cm diameter) contained 3 kg of soil. Rapeseed (seven green leaves), obtained from the Hebei Seed Corporation, was used as the inoculant for the experiment in open and natural conditions. The experiments were conducted under six conditions: C1, 65% CMF 3.23 kg/hm^2 + Regular chemical fertilizer 35%; C2, 75% CMF 8.83 kg/hm^2 + Regular chemical fertilizer 25%; C3, 89% CMF 16.16 kg/hm^2 + Regular chemical fertilizer 11%; C4, Sterilized CMF; C5, No fertilizer; C6, 10 kg/hm^2 CH_4N_2O + 20 kg/hm^2, $Ca(H_2PO_4)_2.H_2O$ + 5 kg/hm^2 KCl. Each treatment was performed in quadruplicate.

Field experiments

Field experiments were performed at a Soil and Fertilizer Station in Hebei Province. The experimental layout was a randomized complete block design with four replicates; there was a total of 98 jujube and 0.1332 hm^2 and 0.1385 hm^2 cherry tomato in four different farms in Hebei, China. Three treatments used were: C1, Regular fertilization; C2, Regular fertilization + CMF; C3, Regular fertilization + sterile CMF.

Soil and plant samplings and analyses

Soil samples were dried at room temperature (approximately 25°C) for 2 weeks and then passed through a 2-mm sieve. Soil pH was measured using a pH meter, and 5 g of soil was mixed with 5 ml of distilled water. The available P was extracted with sodium bicarbonate (Oslen et al., 1954), and its concentration was determined using molybdenum antimony anti-colorimetry. The available K in the soil was extracted with 1 M HNO_3, and its concentration was determined using the potassium tetraphenyl-borate gravimetric method.

Soil organic C was determined by oxidizing the organic matter in the soil samples with $K_2Cr_2O_7$ (potassium dichromate) in sulfuric acid (98%) for 30 min and then measuring the concentration of Cr^{3+} formed. Approximately 12 g of a root-free soil sample was individually placed into a 50 ml glass beaker, which were placed into a desiccator (18-dm^3 volume). Forty-five milliliters of ethanol-free $CHCl_3$ was used as a fumigant (22°C, 20 h). The fumigation process was simultaneously started. The non-fumigated soil was shaken (150 rev min^{-1}) for 30 min with 50 ml of 0.5 M K_2SO_4 (mean soil: solution ratio=1:13 (w:v)) and filtered using Whatman paper (No. 42). The fumigated soil was extracted as described earlier after the removal of the $CHCl_3$ from the soil by repeated evacuations (Högberg and Högberg, 2002). The microbial C biomass content was determined using the method of Yeomans and Bremner (1988). Vitamin C content was measured using 2,6- dichloroindophenol titration. The nitrate content was measured using a UV spectrophotometer.

Fungi, Actinomycetes and bacteria in the soil were detected using PDA, Gause No. 1 and Plate Count Agar, respectively.

The total N, total P, and total K contents detected in the strong digested with H_2SO_4-H_2O_2 were determined according to the described methods.

Table 1. The influence of different media on the SSF.

Combination	Corn bran	Spent mushroom substrate	Corn bran + Shrimp shell	Corn bran + Shrimp shell + Spent mushroom substrate
K dissolving rate (%)	2.03±0.21[a]	42.00±1.61[c]	24.09±0.96[b]	43.00±1.72[c]
Living bacteria lg (CFU)	8.02±0.11[a]	10.08±0.13[c]	9.04±0.12[b]	9.16±0.14[b]
Spore formation rate (%)	70.00±3.63[a]	82.62±3.86[b]	80.00±4.02[b]	83.18±3.93[b]

For each column, values not marked with the same letter in superscript are significantly different at $p<0.05$ (Duncan's). For each column, values not marked with the same letter in superscript are significantly different at $p<0.05$ (Duncan's).

Table 2. Results of the orthogonal experiment.

Treatment number	Wheat flour content (%)	$(NH_4)_2SO_4$ content (%)	$MgSO_4$ content (%)	K dissolving rate (%)	Spore concentration lg (CFU)
1	0.5	0.1	0.2	27.58	9.30103
2	0.5	0.5	0.1	22.19	8.30103
3	0.75	0.1	0.1	41.53	9.69897
4	0.75	0.5	0.2	19.85	9.30103

Statistical analyses

The experimental data were subjected to an analysis of variance (ANOVA) using a significance value of $p<0.05$ and Duncan's multiple range test (Duncan's) using SPSS 18.

RESULTS AND DISCUSSION

High dissolving rate of *B. circulans* Xue-113168 and SSF

B. circulans Xue-113168 was isolated from a corn rhizosphere and bred by UV. Its ability to dissolve potassium ores enables *B. circulans* to be applied as a biological fertilizer. Microorganisms that dissolve potassium ores include silicate bacteria, such as *Bacillus mucilaginosus, B. circulans, B. acidophilus*, and fungi (Sheng, 2005; Lian et al., 2008). *B. circulans* Xue-113168 has a high potassium dissolving rate (41%) and also produces chitinase (Xue, 2013).

Potassium dissolving microorganisms (KSM) are able to solubilize 'unavailable' forms of K-bearing minerals, such as micas, illite and k-feldspar, by excreting organic acids that either directly dissolves rock K or chelate silicon ions to bring the K into solution (Sheng, 2005). *B. circulans* Xue-113168 ferments and dissolves K simultaneously. The released soluble K in the fermentation medium was detected. Using potassium feldspar as the substrate of the solid-state fermentation, the detection of spore concentration of 2×10^9 cfu/g, and soluble potassium accounted for 1%. Among the detected isolates, *B. circulans* Xue-113168 displayed the highest K-solubilizing activity, as high as 41.53% after 7days of culture with K-feldspar powder. This cycle is shorter compared to that determined by Lian et al. (2008), who studied SSF and bioleached K-containing minerals for 15 days. One step of SSF adopted in this study has more advantages than the two steps solid-state fermentation combined with bioleaching done by Lian et al. (2008).The mutant bacteria are a high efficient method for bioleaching. Same conclusion was reached by Yingbo et al. (2011).

Utilization of waste as a substrate for SSF of *B. circulans* Xue-113168 for biofertilizer production

The raw materials and formulation were key factors for the SSF and they significantly influenced the overall cost. The formulation of a SMF could be similar to that of SSF according to Barrios-González (2012). However, a SSF medium is typically composed of a solid substrate. As a result, sucrose was replaced with wheat flour for the SSF in this study.

The type and solid state of substance in the SSF medium exert a significant influence on the SSF process, especially the potassium dissolving rate, the amount of live bacteria, and the spore formation rate. The influence of different media on SSF is shown in Table 1.

It can be concluded from Table 2 that the combination of corn bran, shrimp shell, and spent mushroom substrate was the best medium for the SSF. The K-dissolving rate (43.00%) and spore formation rate (83.18%) achieved were the best for all of the groups, and the live bacteria amount 9.16 cfu/g was second only to the spent mushroom substrate group (10.08 cfu/g).

Table 3. Variance analysis of orthogonal experiment.

Resource	III Square sum	df	Mean square	F	Sig.	III Square sum	df	Mean square	F	Sig.
Rectify model	4.03[a]	3	1.34	23.98	0.00	1430.71[a]	3	476.90	2044.95	0.00
Intercept	1319.87	1	1319.87	23542.81	0.00	12813.67	1	12813.67	54944.69	0.00
A	1.28	1	1.28	22.78	0.00	202.28	1	202.28	867.37	0.00
B	2.61	1	2.61	46.52	0.00	878.09	1	878.08	3765.21	0.00
C	0.15	1	0.15	2.64	0.13	350.35	1	350.34	1502.27	0.00
Error	0.67	12	0.06	-	-	2.80	12	0.233	-	-
Total	1324.58	16	-	-	-	14247.182	16	-	-	-
Total corrected	4.71	15	-	-	-	1433.51	15	-	-	-

[a]R -squared = 0.857（Adjusted R-squared = 0.821); [b]R-squared = 0.998 (Adjusted R-squared = 0.998).

Fermentation was formulated to obtain a higher concentration of spores and potassium dissolving capacity for *B. circulans* Xue-113168. It was initially determined through a single factor experiment. The formulation was further optimized through an orthogonal experiment. The design and variance analysis results of the experiment are shown in Tables 2 and 3.

It can be concluded from Table 3 that the range of $(NH_4)_2SO_4$ concentrations was the most important variable. This suggests that in the *B. circulans* Xue-113168 culture, the effects of the three factors on the potassium dissolving ratio were $(NH_4)_2SO_4$ content > $MgSO_4$ content > wheat flour content; all three factors have significant effect on potassium dissolving ratio ($P<0.05$) and that the optimal conditions were wheat flour content, 0.75%; $(NH_4)_2SO_4$ content, 0.1%; and $MgSO_4$ content, 0.2%.

For the spore concentration, it can be concluded that the ranges of $(NH_4)_2SO_4$ and wheat flour concentrations were larger compared to the $MgSO_4$ concentration. Thus, the effects of the three factors on the spore concentration: $(NH_4)_2SO_4$ content > wheat flour content > $MgSO_4$ content, $MgSO_4$ are not significant on spore concentration ($p>0.05$). Optimal conditions were wheat flour content, 0.75%; $(NH_4)_2SO_4$ content, 0.1%; and $MgSO_4$ content, 0.2%. The contents of $(NH_4)_2\ SO_4$ in the media could affect the growth and metabolites of the *B. circulans* Xue-113168 and the mineral bioweathering. This is consistent with Zhi (2012)'s study. The K-dissolving rates are not different between the SMS and Corn bran + Shrimp shell + SMS in the SSF (Table 1), and the humic acids in the SMS also dissolve the K-feldspar, which is complementary to the action of *B. circulans* Xue-113168.

Controlling the SSF process

Spore formation was affected by humidity and temperature. Controlling the moisture content of the SSF process was performed as follows. During the first step, at the end of the sterilization process, the moisture content was adjusted to approximately 60%. During the second step, after the inoculation, the moisture content was adjusted to 60 to 65%. At the end of the fermentation, the moisture content was 50%. The moisture content was controlled using environmental moisture and temperature, which had three phases. During the prophase, the temperature was 33 to 35°C, whereas, at the metaphase, the temperature dropped to 30 to 33°C. However, it increased to 33 to 35°C during the anaphase.

The fermentation time was also an important parameter. By the time that the spores had formed, the fermentation had typically finished. An appropriate temperature and humidity ensured that the spores were formed. When the fermentation lasted for 4 days, the potassium dissolving rate was 24.09%. After the fermentation time was extended to 7 days, the potassium dissolving rate increased to 41.53%. Lian et al. (2008) spent 25 days on the fermentation: 15 days for SSF and 10 days for biological leaching. In this study, the SSF that simultaneously dissolved potassium required 7 days.

The potassium dissolving process was a synthesized multifaceted result. There is no single gene that encodes for a potassium dissolving ability in silicate bacteria, which is similar to the process of dissolving phosphate. It is well known that there are many differences between SSF and SMF in physiology, such as AW and metabolite. The pH range for *B. circulans* Xue-113168 is 5 to 9, and the temperature range is 28 to 37°C. Moreover, the indispensable moisture required for SSF was low. All the characteristics described earlier make *B. circulans* Xue-113168 suitable for SSF. Sheng (2005) demonstrated that the potassium dissolving rate of *Bacillus edaphicus*, which has been proven to have a low potential for commercial use, is low. Tan (1978) reported that 9 to 28% of the potassium that they measured was released by humic acids. It has been suggested that the potassium dissolving rate (41.53%) of *B. circulans* Xue-113168 may include dissolved potassium from K-feldspar through the

Table 4. Results of the quality index determination experiment.

Item	Effective viable cells (billion/g)	NPK ($g.kg^{-1}$)	Organic matter ($g \cdot kg^{-1}$)	N Nitrogen ($g.kg^{-1}$)	P_2O_5 (%)	Available potassium ($g.kg^{-1}$)	Total K_2O ($g.kg^{-1}$)
NY/T798-2015	0.2	80-250	200	-	-	-	-
CMF	2	150	250	12	38	80	100

Table 5. Effect of different soil treatments on the biochemical characteristics of the rapes during the first year (Values are provided as the mean ± SD).

Condition	Dry weight (%)	NO_3 (mg/kg)	Vit C (mg/kg)	Shoot height (cm)	Phosphorus use efficiency (pue)
C1	1.93 ± 0.13^{b}	78.123 ± 1.69^{a}	21.90 ± 0.17^{a}	10.86 ± 0.13^{b}	0.016^{c}
C2	3.53 ± 0.22^{d}	51.38 ± 0.29^{b}	22.82 ± 0.19^{b}	13.11 ± 0.15^{a}	0.15^{e}
C3	2.52 ± 0.12^{c}	53.78 ± 0.29^{c}	22.49 ± 0.1^{c}	13.12 ± 0.12^{a}	0.25^{f}
C4	2.49 ± 0.17^{c}	86.10 ± 1.58^{d}	14.94 ± 0.14^{d}	10.11 ± 0.10^{d}	-0.013a
C5	1.59 ± 0.11^{a}	87.25 ± 1.48^{d}	13.09 ± 0.15^{e}	9.31 ± 0.47^{c}	0.075^{d}
C6	1.71 ± 0.12^{ab}	91.20 ± 1.58^{e}	14.39 ± 0.11^{f}	10.71 ± 0.26^{b}	0^{b}

For each column, values not marked with the same letter in superscript are significantly different at $p<0.05$ (Duncan's). For each column, values not marked with the same letter in superscript are significantly different at $p<0.05$ (Duncan's).

action of humic acids (Friedrich, 1991).

Pot experiment

The results indicate that the rape yield and soil fertility, such as available N, available P, available K and organic matter, have increased. The soil fertility and rape quality and yield were improved by *B. circulan* Xue-113168 and the matrices. In addition, the environment was also improved. Significant increases in dry shoot weights were observed in the rape when the soil was inoculated with *B. circulans* strain Xue-113168 compared to the soil without inoculum, and different treatments affected the soil fertility. The experimental results for the influences of the different treatments on the soil fertility are shown in Table 5. Vitamin C concentration increased by 52.7% in the 75% CMF for the matrix control; however, the nitrate concentration decreased by 77.5% in the 75% CMF for the chemical fertilizer control. The dry weight of the rape increased by 82.9, 40.08, 41.77, 122, and 106.43% in the 75% CMF treatment for the 65% CMF, 89% CMF, substrate, no fertilizer, and chemical fertilizer soils, respectively (Table 4). The alkaline hydrolyzable N and available P contents in the CMF treatments were higher compared to those of the chemical fertilizer treatment, indicating the benefits of a biofertilizer by supplying and enhancing the release of N and P (Table 5). Alkyl hydrolyzable N in the 75% CMF was significantly increased compared to the other groups. The available P and K in the CMF soil for the control were higher. The K content in the rape in the CMF for control was also higher. It can be concluded from Table 5 that the use of a chemical fertilizer can give some nutrients to crops; however, it also decreased soil fertility in the soil by less beneficial microorganism. The use of CMF not only provided indispensable nutrients for crop growth but also increased the organic matter content in the soil, which enriched the soil quality. Remarkably, the available potassium in the soil was increased 1.64 times in the 89% CMF for the substrate group; however, 65 and 75% CMF could not increase the available potassium in the soil compared to the control. Thus, *B. circulans* Xue-113168 was able to dissolve the insoluble potassium in the K-feldspar and other soil K-minerals when enough dose, such as 89% CMF, was utilized. The dose designs were 3.23, 8.83, and 16.16 kg/hm^2. They reduced the amount of chemical fertilizer used (35, 25, and 11%), and improved the phorphase efficiency of the fertilizer used (1.6, 15, and 25% respectively). PUE refers to the increment in soil P status by applying bio-based fertilizers compared to the increment caused by applying chemical fertilizer (Vaneeckhaute et al., 2016). The microbial analyses showed important differences for all treatments (Table 6). The community structure of the microorganisms was altered by the CMF. The numbers of bacteria in the soil increased 1.35 and 1.57 times in the soils amended with CMF 89 and 75%, respectively, compared to the chemical fertilizer soil. Actinomycetes were not different among the various treatments (Table 7). However, the fungal number was lower in the CMF compared to the control group, which might be the reason for the decreased disease occurrence in the CMF treatment. The organic C content in the biofertilizer group was

Table 6. Some chemical properties of the soils in different treatments after the rape harvest.

Condition	Organic matter (%)	Alkyl hydrolysable N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)
C1	2.97±0.06^{d}	93.21±0.67^{b}	1.63±0.10^{ab}	94.83±0.68^{b}
C2	3.05±0.12^{d}	160.33±1.24^{c}	1.64±0.11^{ab}	157.36±1.12^{a}
C3	3.09±0.17^{d}	86.72±0.59^{a}	2.05±0.16^{c}	421.43±2.98^{c}
C4	2.69±0.08^{c}	84.70±0.57^{d}	1.62±0.12^{ab}	159.42±1.08^{a}
C5	2.43±0.08^{ab}	86.24±0.43^{a}	1.82±0.14^{b}	205.91±1.70^{d}
C6	2.5±0.11^{cb}	82.52±0.73^{e}	1.58±0.12^{a}	246.20±1.57^{e}

The data are expressed as the mean ± standard error. Data in a column with a different letter are significantly different at a Duncan's significance level of 0.05.

Table 7. Effect of different treatments on microorganisms (Lg cfu/g dry soil).

Treatment	Silicate bacteria	Bacteria	Fungi	Actinomycetes	Microbial biomass (mg/kg)
Low-dose group	6.19±0.13^{a}	5.42±0.026^{a}	6.93±0.120^{a}	5.19±0.090^{ab}	793±4.6^{a}
Middle-dose group	6.34±0.090^{a}	5.44±0.089^{a}	6.91±0.100^{a}	5.20±0.100^{ab}	835±6.3^{b}
High-dose group	6.54±0.096^{a}	5.41±0.092^{a}	6.92±0.075^{a}	5.32±0.100^{b}	853±6.0^{c}
Matrix control	5.11±0.092^{b}	5.39±0.095^{a}	7.19±0.086^{b}	5.19±0.110^{ab}	511±3.8^{d}
No fertilizer	4.96±0.970^{b}	5.05±0.099^{b}	7.18±0.080^{b}	5.15±0.070^{a}	408±3.3^{e}
Fertilizer control	4.33±0.091^{c}	5.03±0.080^{b}	7.23±0.083^{b}	5.23±0.080^{ab}	382±2.7^{f}

The data are expressed as the mean ± standard error. Data in a column with a different letter are significantly different at a Duncan's significance level of 0.05.

significantly higher compared to the control group, which is in agreement with the microbial biomass C content.

The silicate bacterial counts in the rape rhizosphere increased 1.21, 0.64, 26.12, 113.6, and 160.5 times in the 89% CMF treatment for the 65% CMF, 75% CMF, substrate, no fertilizer, and chemical fertilizer soil, respectively (Table 6). Inoculation of rape with the biofertilizer reduces the recommended fertilizer level by 30%.

Field trials

Yields of selenium-enriched jujube and jujube increased by 6.19 and 8.4%, respectively in the CMF soils compared to the matrix control (Figure 2). Jujube rust significantly reduced as well as the anthrax for the trees. The fruit abscission rate uniformly decreased with the rosy fruit coloration. The average weight of the fruit was approximately 10.9 g for process 2, whereas the average weight for processes 1 and 3 was 9.8 g. The yields of cherry tomato Jinpeng and East St No. 1 increased by 8.41 and 9.29%, respectively, in the CMF soils for the matrix control. Vigorous plant growth, root development, robust stems and dark green leaves were also observed. Late fruit suffered from less plant disease. A developed root system, dark green leaves, and less mosaic disease were all evident compared to the other two treatments. Fruit coloration and less immature fruit were also observed.

Jujube plant number with anthracnose decreased significantly, and rust disease also significantly reduced. The fruit fall off rate reduced, and fruit coloring uniformity was ruddy. Tomato plants thrived well, root system, stem and leaves developed strongly; leaves were invisibly green. Later, the fruits became large and neat and had equitable coloring; and plant diseases also reduced. The mechanisms are synergy and complementary between microorganisms and soil, microbial and nutrient elements (fertilizers), microbes and crops, by means of interaction between microorganisms. CMF in this study helps to address some of the most important challenges such as anthracnose and rust disease facing green agriculture in coming years.

The efficacy components in the CMF are *B. circulans* Xue-113168, its metabolite, silicon and humic acid and chitin (chitosan). Silicon and other elements were released from K feldspar by the *B. circulans* Xue-113168. Data will be reported later. Jayawardana et al. (2016) also verified the reduction of anthracnose disease using rice hull as a silicon source. The chitin in the shrimp shell and spent mushroom substrate can induce *B. circulans* Xue-113168 chitinase. A similar result was reported by Wang et al. (2006) and Chang et al. (2007).

Bioorganic fertilizers and humates are environmentally friendly and inexpensive because they are composed of recycled wastes such as SMS, shrimp shell and corn bran. Humic acids are the adhesives that combine

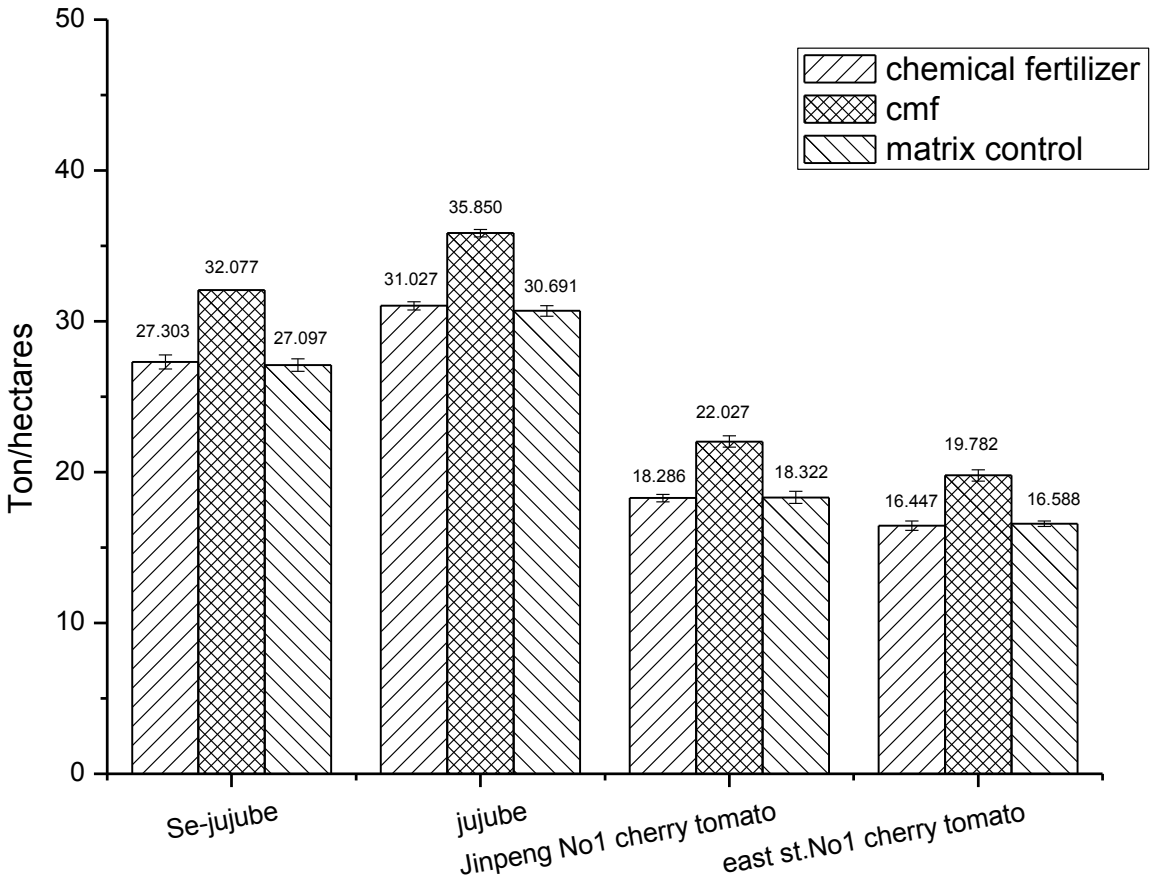

Figure 2. Field test of different treatments on selenium-enriched jujubes, jujubes, and cherry tomatoes. CMF: compound microbial fertilizer matrix control: CMF with *B. circulans* destroyed.

chemical fertilizers with microorganisms. Violante et al. (1999) demonstrated the formation of OH-Al-humate-montmorillonite complexes. However, the interaction among humate, K-feldspar and potassium during SSF or in a compound biofertilizer, which form the K-humate-feldspar complexes, requires further study.

Conclusion

B. circulans Xue-113168 has a 41% potassium dissolving rate and produces Chitinases, induced by chitin, which are in spent mushroom substrate, and shrimp shells. Microorganism combination of Chitinase activity can increase crop yields and lessen the incidence of disease in China's Hebei area. The instance has been reported by Zhao et al. (2011). This study found that the optimal dosage of CMF application was 8.83 kg/hm^2-16.16 kg/hm^2 rapeseed. The study of CMF fertilizer and recycling resource utilization efficiency promote the sustainable development of agriculture industry. The complex formulation and process of CMF is applicable to factories, especially farmers' use on site.

Conflict of interests

The authors have not declared any conflict of interests.

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Abbreviations

CMF, Compound biofertilizer; **SSF,** solid-state fermentation; **PGPR,** plant growth-promoting rhizobacteria.

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