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Straw biochar increases the abundance of inorganic phosphate solubilizing bacterial community for better rape (*Brassica napus*) growth and phosphate uptake

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Abstract

The direct application of inorganic-phosphate-solubilizing bacteria (iPSBs) for improving the efficiency of phosphorus (P) use leads to a low rate of bacterial survival. Biochar is a good inoculum carrier for microbial survival, and diverse feedstocks can have different effects. We generated an iPSB community using seven selected iPSB strains with various phylogenic taxonomies and P-solubilizing abilities. Biochar was then inoculated with the iPSB community and applied to soil in pots seeded with rape (*Brassica napus*). Growth of the rape for four weeks and the effects of biochars produced from six raw feedstocks, rice straw, rice husks, soybean straw, peanut shells, corn cobs and wood, were compared. The synthetic iPSB community had a larger capacity to solubilize inorganic P and exude organic anions than any of the individual strains. The structure of the iPSB community was analyzed by high-throughput sequencing four weeks after inoculation. All seven iPSB strains were detected, dominated by *Arthrobacter defluvii* 06-OD12. The abundance of the iPSB community was significantly correlated with rape biomass, P content and P uptake ($P < 0.05$). The biochar amendments conferred 6.86-24.24% survival of the iPSB community, with the straw biochars conferring the highest survival. The available-P content of the biochar rather than soil pH was the dominant factor for iPSB community structure, suggesting that the biochar material was critical for the survival and functioning of the iPSB community. Our study demonstrates the feasibility of biochar-assisted iPSB improvement of crop growth and P uptake.

Importance

Inorganic phosphate solubilizing bacteria (iPSBs) are commonly proved to be useful as biofertilizer for plant growth and phosphorus (P) uptake. Previous studies dedicated to screen iPSB isolates with high solubilizing capacity. However, the exact limitation would attribute to the low survival rate after direct inoculation, dramatically shortening functioning periods of iPSB strains. A synthetic iPSB community with 7 different generic strains were first-time applied with plant inoculation. By observing the survival condition, including abundance and community structure, the results proved the straw biochar, which compared with other 5 feedstock biochars, was optimal for iPSB community survival.

Keywords: straw biochar; *Brassica napus*; phosphorus; inorganic phosphate solubilizing bacteria; phosphate uptake

Introduction

Phosphorus (P) is an essential macronutrient for plant growth. P, however, is a non-renewable element, so the availability of rock P has been dramatically decreased to satisfy the increased demands for P fertilizers (Elser and Bennett, 2011; Elser et al., 2007; Penuelas et al., 2013). Applied P fertilizers are readily immobilized due to the strong fixation in soil solutions, which renders P unavailable for plant uptake and hinders the movement of P in terrestrial systems (Kochian, 2012). Developing new strategies to increase P-use efficiency are greatly needed.

Microorganisms are active participants in numerous biogeochemical cycles (van der Heijden et al., 2008). Inorganic-phosphate-solubilizing bacteria (iPSB) are major drivers of the biomobilization of soil P, which releases many organic ions or protons to effectively liberate immobilized P and preserve P for being fixed again (Richardson et al., 2009; Richardson and Simpson, 2011). iPSB microbes are also commonly accepted as plant-growth-promoting rhizobacteria (PGPR) and are commonly used as biological inoculants to improve P assimilation and plant productivity (Collavino et al., 2010; Kumar et al., 2012). The low survival rate of the bacteria after being introduced into the soil, however, prevents the sustainability of PGPR inoculation (Bashan et al., 1995; Malusá et al., 2012).

Biochar obtained by the pyrolysis of various raw feedstocks is commonly used as a stable and economic inoculum carrier (Sun et al., 2016). Generally, biochar showed beneficial effects on the abundance, diversity and functional improvement of inoculated microbial community (Lehmann et al., 2011). Biochar with internal

87 porosity provides a protective habitat to prolong PGPR survival, and its strong ability
88 to absorb nutrients can sustainably support the growth of inoculants (Zimmerman et
89 al., 2011), which has been extensively demonstrated (Ahmad et al., 2015; Akhtar et
90 al., 2015). The biochar addition was proved to be of great benefits for regulating
91 phosphate-solubilizing bacteria community (Wei et al., 2018; Wei et al., 2016).
92 Biochars produced from different materials, however, differ in their ability to maintain
93 PGPR due to their different physicochemical properties (Cantrell et al., 2012). A
94 previous study demonstrated the strong influence of biochars with different
95 characteristics on PGPR survival after incorporation into soil (Khan et al., 2014). The
96 selection of biochars is therefore of great importance for PGPR inoculations.
97 We selected biochars pyrolyzed from six raw feedstocks for maintaining the
98 effect of iPSBs on P mobilization to improve plant growth: rice straw (RS), rice husks
99 (RH), soybean straw (SS), peanut shells (PN), corn cobs (CC) and wood (WD). The
100 growth of rape (*Brassica napus*) needs large amounts of P (Belimov et al., 2002) and
101 was thus chosen as the test plant. Rape growth and the P status of the plants and the
102 rhizospheric soil were determined. An iPSB community containing seven microbial
103 strains with varying characteristics was used as a microbial inoculant, and the
104 structures of the communities after application of the six inoculated biochars were
105 analyzed. The relationships between rape growth, biochar addition and iPSB
106 community structure are also discussed.
107

Materials and Methods

Soil sampling and characterization

Soil samples (0-15 cm layer) were collected in May 2015 after crop harvest from the Changshu AgroEcological Experimental Station in Jiangsu Province, China (31°32'N, 120°41'E). The soil contained 0.78% clay, 18.38% silt and 80.84% sand. The samples were air-dried, sieved (0.2 mm) and stored at 4 °C and -20 °C until use and analysis.

Soil pH was determined in a suspension (1:2.5 dry soil:sterilized water (w/v)) using a XL60 pH meter (Fisher Scientific, Asheville, USA). Soil moisture content (%) was calculated as the difference in weight between fresh soil and soil dried in an oven at 300 °C for 24 h. Carbon, nitrogen and sulfur contents were measured with an elemental analyzer (vario MAX CNS, Elementar, Hanau, Germany). P and available-P contents were determined after digestion by strong acid (sulfuric acid) and extraction by sodium bicarbonate using the molybdate-blue method (Murphy and Riley, 1962; Olsen et al., 1954; Parkinson and Allen, 1975), respectively. The acid-digested soils were further used to determine the concentrations of metals (potassium, calcium, sodium, magnesium, iron and aluminum) by inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 7000DV, Perkin Elmer, Waltham, USA). The physical and chemical properties of the Changshu soil are listed in Table S1.

Experimental design and analysis

The diagram of experimental design was presented in Fig.1. In brief, the soil was used for new iPSB strain isolation as well as rape growth with its sterilized version. A common iPSB community is composed of strains from genus *Arthrobacter*, *Streptomyces*, *Bacillus* and *Beta*- and *Gamma-proteobacteria* (Zheng et al., 2017). Based on that, we compared the isolated iPSB strains with a previous isolated iPSB database to select representative iPSB strains based on their morphology, taxonomic diversity and biochemical characterization. Then, 7 representative iPSB strains, including *Arthrobacter defluvii* 06-OD12 (KU647200), *Burkholderia cepacia* 51-Y1415 (KU647244), *Bacillus megaterium* CS22 (MG430229), *Pseudomonas frederiksbergensis* 11-D3 (KU647205), *Rhodanobacter* sp. 25-Y8 (KU647218), *Streptomyces prasinopilosus* 34-Y1 (KU647227) and *Variovorax paradoxus* 19-D4 (KU647212), were chosen to generate iPSB community, which was validated by biocompatibility and P solubilization capacity tests. Besides, six raw feedstocks, including RS, RH, SS, CC, PN and WD, were used to form biochars. The pH, carbon and nitrogen percentages, available P concentration, Brunauer-Emmett-Teller (BET) surface area and pore size of biochar were determined. To see the effect of biochar on iPSB community-assisted crop growth, six iPSB community-adhered biochars (3%) were inoculated into Changshu soil with rape (*Brassica napus*) growth while a soil with iPSB community inoculation but without biochar amendment was treated as control (CK). Before and after 4-week rape growth, the pH, available-P concentration and total P amount of rhizospheric soil, biomass, total P content and P uptake of plant and iPSB community survival rate were measured. The iPSB community structure

was also analyzed by 16S rRNA high-throughput sequencing with iPSB database alignment. Besides, to discriminately quantify the impact of biochar and iPSB community on the plant growth and its P uptake, we conducted another 21-day rape growth with four treatments: rape growth without biochar or iPSB (R-CK), only 3% RS biochar addition (R-B), with only iPSB community inoculation (R-P) and with iPSB community-adhered RS addition (R-BP). The P-related parameters were measured before and after rape growth. The detailed explication could be found in supplementary materials and methods.

Statistical analysis

One-way correlation and variance were determined using SPSS Statistics 21 (IBM, New York, USA). Heatmapping was generated by the PHEATMAP package (http://rpackages.ianhowson.com/cran/pheatmap/) based on R studio (version 3.2.3). Redundancy analysis (RDA), principle component analysis (PCA), variance partition analysis (VPA) and Monte Carlo permutation test were also performed by using R studio (version 3.2.3) with and VEGAN (Oksanen et al., 2007) packages.

Results

Physical and chemical properties of the biochars

All physical and chemical parameters of the six biochars were highly variable between treatments (Table S2). RS had the highest pH (10.30 ± 0.34), and RH had the lowest pH (8.92 ± 0.40) but the highest BET surface area (43.00 ± 3.47 m^2 g^{-1}). Pore size differed significantly among the biochars ($P < 0.05$). C content was significantly higher for CC (74.80 ± 0.87%) and WD (75.93 ± 5.86%) than the other biochars, and N and Olsen-P contents were highest for RS (2.22 ± 0.21% and 685.80 ± 52.14 mg kg^{-1}, respectively).

Selection and characterization of the iPSB strains

We selected seven iPSB strains based mainly on their P-solubilizing ability and phylogenic diversity to artificially simulate an iPSB community. We selected from 133 iPSB strains, 76 from a previous study and 57 from our study. *B. megaterium* CS22 (*Firmicutes*) had the highest hydroxyapatite content (139.35 μg mL^{-1}, Table S3) and ability to solubilize calcium-phosphate (Ca-P) (119.37 ± 2.80 μg mL^{-1}, Table S5) and was thus selected for the community. Six other strains from different classes with good Ca-P-solubilizing ability were also selected: *Rhodanobacter* sp. 25-Y8 (Gammaproteobacteria, 97.34 ± 2.00 μg mL^{-1}), *P. frederiksbergensis* 11-D3 (Gammaproteobacteria, 51.65 ± 16.41 μg mL^{-1}), *V. paradoxus* 19-D4 (Betaproteobacteria, 26.62 ± 4.00 μg mL^{-1}), *S. prasinopilosus* 34-Y1 (Actinobacteria,

17.93 ± 8.98 μg mL^{-1}), *B. cepacia* 51-Y1415 (Betaproteobacteria, 15.14 ± 1.03 μg mL^{-1}) and *A. defluvii* 06-OD12 (Actinobacteria, 13.15 ± 0.17 μg mL^{-1}).

We comprehensively characterized these seven strains before generating the iPSB community. They had various cellular sizes and shapes (0.5-10 μm and spherical or rod-shaped, Fig. 2a). All these strains could survive in the LB, R2A and PVK (with NH_4^+ or NO_3^- as the sole nitrogen source) media but with different enzymatic activities and abilities to hydrolyze carbohydrates and form acids (Table S4). For example, *B. cepacia* 51-Y1415 hydrolyzed 10 of 11 carbohydrates, and *B. megaterium* CS22 hydrolyzed only seven but hydrolyzed potassium gluconate, which is different from the other strains. *B. megaterium* CS22 could also form organic acids from 23 of 49 organic compounds, but *Rhodanobacter* sp. 25-Y8 did not form detectable amounts of any acid. The seven strains formed colonies with different morphologies in LB medium (Fig. 2b). The experiments with bromocresol-purple staining indicated that anion exudation differed substantially between strains that were cultivated in PVK-NH_4^+ and PVK-NO_3 media (Fig. 2c, d). The pHs of the PVK media and ability to secrete organic anions after 72 h of cultivation also varied among the strains (Fig. 2e and Table S6). *B. megaterium* CS22 produced the lowest medium pH (4.73) without secreting lactic, acetic or malic anions but secreted the most succinic anions (196.60 ± 9.98 ppm). *A. defluvii* 06-OD12 produced the highest medium pH (8.04) and secreted abundant organic anions and the most malic anions (107.33 ± 5.71 ppm).

206 *Assessment of iPSB-community generation*

207 The biocompatibility experiment by colony steaking indicated that these seven
208 strains survived well together (data not shown). The abilities of the community
209 generated by mixing the seven strains at the same cell density (10^9 CFU mL^{-1}) to
210 solubilize Ca-P (146.77 μg mL^{-1}) and ferric-phosphate (Fe-P) (29.16 μg mL^{-1}) were
211 significantly higher than those of the individual strains ($P < 0.05$, Table S5). The
212 features of community growth indicated an initial large release of inorganic P (78.28 ±
213 6.89 μg mL^{-1} at 12 h) and then a continuous increase to 146.77 ± 15.81 μg mL^{-1} after
214 144 h of cultivation. All six organic anions were detected from the cultivation of the
215 iPSB community (Table S6). Malic and lactic anions were the two most abundant
216 organic anions (80.48 ± 10.28 and 64.03 ± 5.94 μg mL^{-1} after 144 h, respectively),
217 which increased with the release of inorganic P.

218 *The bacterial, soil and plant responses after four weeks of rape growth*

219 Parameters of the microbes, rhizospheric soil and plants associated with the four
220 weeks of rape growth are presented in Table 1. The bacterial population was largest
221 for RS (2.88 ± 0.45 × 10^8 CFU g^{-1} dry soil), followed by RH and SS (1.89 ± 0.18 and
222 1.52 ± 0.39 × 10^8 CFU g^{-1} dry soil, respectively). iPSB survival after four weeks of
223 rape growth was significantly higher for RH (30.34 ± 1.49%) than CK, followed by
224 RS, SS and PN at 24.24 ± 2.15, 19.58 ± 1.29 and 18.79 ± 2.40%, respectively ($P <$
225 0.05). The pH of the rhizospheric soil was about 6.5-6.9 for all treatments. The total P
226 content was slightly higher for RH, CC and PN than CK. The amount of Olsen P,

however, was significantly higher with biochar addition and was highest for RS (34.93 ± 0.72 mg kg^{-1}, $P < 0.05$). RS and SS had significantly higher plant biomass (6.10 ± 0.57 and 6.14 ± 0.70 g pot^{-1}, respectively), P content (0.66 ± 0.03 and 0.68 ± 0.05 g kg^{-1}, respectively) and P uptake (4.05 ± 0.55 and 4.19 ± 0.36 mg pot^{-1}, respectively) than the other treatments ($P < 0.05$).

Response of the iPSB community after four weeks of rape growth

A total of 61816 reads of the 16S rRNA gene were obtained after filtering for quality and removing chimeric reads. Of these, 15640 sequences with similarities >99% with the iPSB database were accepted as potential iPSB species. The percentage of iPSBs in each biochar treatment ranged from $6.44 \pm 1.55\%$ (CK) to $62.57 \pm 17.27\%$ (RS), with an average of $33.81 \pm 18.58\%$. The abundance of the iPSB community was significantly higher for RS and SS ($50.73 \pm 12.02\%$) than the other biochar-addition treatments (Fig. 3a, $P < 0.05$), and the Shannon diversity index of the community was significantly higher for CK (2.38 ± 0.01) and CC (2.36 ± 0.03) than the other treatments (Fig. 3b, $P < 0.05$).

The pattern of the structures of the iPSB communities is shown in Fig. 4a. Thirty species were aligned, and the seven selected iPSB strains were successfully detected in all treatments. *A. defluvii* 06-OD12 was the dominant species with percentages of the total iPSB populations of 1.04 ± 0.30, 4.61 ± 1.08, 3.99 ± 0.98, 3.13 ± 0.50, 38.28 ± 11.20, 9.65 ± 2.54 and $28.61 \pm 7.77\%$ for CK, WD, PN, CC, RS, RH and SS, respectively. The average abundances of *B. megaterium* CS22, *B. cepacia* 51-Y1415,

P. frederiksbergensis 11-D3, *Rhodanobacter* sp. 25-Y8, *S. prasinopilosus* 34-Y1 and *V. paradoxus* 19-D4 were 4.19 ± 2.23, 2.03 ± 1.83, 0.19 ± 0.25, 1.68 ± 0.65, 4.65 ± 3.14 and 2.09 ± 2.14%, respectively. The PCA of different iPSB strains across different biochar treatments showed that six strains from stimulated iPSB community were well separated from other strains (Figure S1).

The RDA indicated that the first two axes explained 90.72% of the effect of the soil and biochar characteristics on the iPSB community, with RDA1 and RDA2 explaining 85.07 and 5.65%, respectively (Fig. 4b). Biochar available P (AP) and soil AP were the most important factors affecting the iPSB communities, supported by their significant correlations ($P < 0.0001$) in the Monte Carlo permutation tests (Table S7). Other significantly correlated factors were pore size, biochar N content, soil total P content, biochar pH and BET surface area ($P < 0.0001$).

The contribution of each factor and their interactions to the structure of the iPSB communities was quantified, and the percentages of the variance due to one or two variables resolved by the VPA are listed in Table S8. Biochar and soil AP contributed the most, at 0.352 and 0.219%, respectively. The interaction between soil AP and biochar AP explained most of the variance (9.367%) for the iPSB community, followed by the interactions between pore size and pH (3.273%) and between biochar C and N content (3.045%). The analyses of correlations between iPSB community abundance with diversity and the plant parameters are presented in Fig. 5. The abundance of the iPSB community was significantly correlated negatively with its

diversity ($P < 0.001$) but positively with plant biomass, plant P and plant P uptake ($P < 0.001$).

To discriminate the impact of biochar or iPSB community for the rape growth and its P uptake, we conducted another experiment for 21 days. Results (Table S9) showed that solo biochar addition (R-B) or iPSB inoculation (R-P) significantly increased rhizosphere soil and plant P content and increased plant biomass compared with R-CK treatment, while R-P was measured with significantly higher P amounts than R-B in rhizosphere soil ($P < 0.05$). Combined amendment with biochar and iPSB (R-BP) performed significantly higher soil P concentration and plant parameters ($P < 0.05$).

Discussion

Plant biomass and the ability to take up P are thus important indices for assessing the effect of an iPSB community on plant growth. Plant biomass, P content and P uptake in our study were all significantly correlated ($P < 0.001$) with the abundance of the iPSB community (Fig. 5b-d), suggesting that the inoculation of the biochar with the iPSB community positively contributed to rape growth.

Inoculation of microorganism with biochar should perform with improvement effect since the physical and chemical characters of biochar will provide appropriate habitats for microbial community, including its abundance, diversity, nutrient acquirement, mobilization and cycling (Lehmann et al., 2011; Quilliam et al., 2013). All biochars tested in this study were beneficial as inoculum carriers for the survival of the iPSB community, but their efficacy varied, perhaps due to the physical and chemical properties of the individual biochars. RS was the best feedstock as an inoculum carrier; the maintenance of the bacterial population was significantly highest for RS ($2.88 \pm 0.45 \times 10^8$ CFU g^{-1} dry soil, $P < 0.05$; Table 1), perhaps due to its surface area and porosity. BET surface area (35.29 ± 3.02 m^2 g^{-1}) and pore size (27.86 ± 1.22 μm) were significantly higher ($P < 0.05$) for RS than the other biochars except RH (Table S2). The larger pore size would provide a habitat niche for microbes and have positive effects on the capacity to retain nutrients by binding both cations and anions to the surface (Atkinson et al., 2010; Liang et al., 2006). A systematic

study of 32 biochars found that the survival of *Rhizobium tropici* had a quadratic dependence on biochar pore size (Vanek et al., 2016).

The porosity of RS also allowed the retention of significantly ($P < 0.05$) more N (2.22 ± 0.21%) and AP (685.80 ± 52.14 mg kg^{-1}) than the other biochars, which provided a good habitat for the survival of the iPSB community. The correlation analysis (Table S7) found that the structure of the iPSB community depended mostly on biochar AP content ($R^2 = 0.881$), followed by biochar N content ($R^2 = 0.652$), pore size ($R^2 = 0.656$) and BET surface area ($R^2 = 0.352$), suggesting that the survival of the community may dependent on the retention of nutrients by the biochar. Biochar is a good material for the retention of key macronutrients such N and P due to its surface area and availability of anionic and cationic charges (Lehmann, 2007). The nutrient status of biochars, however, depends on the origin of the biochar feedstock and its mode of production. RS was the best feedstock in our study for nutrient storage and bacterial survival. A previous study found that RS biochar could hold more organic C and organic matter for the survival of *Bradyrhizobium* and sulfur-reducing bacteria (Khan et al., 2014). Another study also reported that the benefits of RS biochar to microbes contributed to the significantly higher survival than for other biochars of *Bacillus mucilaginosus*, which had higher carbohydrate contents after four weeks of incubation (Sun et al., 2015).

We used seven strains to generate the iPSB community. All strains were detected by high-throughput sequencing after four weeks of cultivation (Fig. 4a). *A. defluvii*

06-OD12 was the most abundant iPSB strain, followed by *S. prasinopilosus* 34-Y1 and *B. megaterium* CS22. This finding was consistent with previous studies reporting that *Arthrobacter*, *Streptomyces* and *Bacillus* were commonly isolated iPSB genera in rhizospheric soils that promoted plant growth (Aislabie et al., 2006; Gopalakrishnan et al., 2011; Taha et al., 1969; Xuan et al., 2011). *Pseudomonas* has been extensively used as an iPSB genus due to a specific gene for solubilizing inorganic phosphate (Kwak et al., 2015; Miller et al., 2010). *Pseudomonas* in our study, however, was less competitive, perhaps because the biochars provided alkaline environments (pH >8, Table S2), and *Pseudomonas* prefers acidic environments (Garbeva et al., 2004).

The RS and SS biochars that held abundant AP were the best for population growth of the iPSB community (Fig. 4a). The size of the community was significantly correlated with biochar properties and soil AP ($P < 0.05$, Fig. 4b and Table S7). The interaction between soil and biochar AP contributed the most to the structure of the community (Table S8). Soil pH has a strong impact on the abundance of bacterial communities (Shen et al., 2013), and iPSB strains are sensitive to soil pH, with abundance increasing with pH (Zheng et al., 2017). The interaction between soil pH and pore size or N content of biochar would also make greater contribution for iPSB community structuring, which may suggest that the combined effect of soil pH and biochar would provide a better environment for the survival of iPSB community, even for its better functioning. Our results, however, indicated that AP had a larger effect, suggesting that the biochar provided a protective environment for the survival and growth of the iPSB community. The combined effect of biochar and soil AP was

proved to be given the best contribution for the formation and functionality of iPSB community, suggesting that available P was the critical dependent factor for iPSB community. Exogenously amended inoculants may not survive better due to strong competition with native microbes for long periods, so the storage of abundant nutrients by biochars would be critical for increasing community size (Lehmann et al., 2011). Biochars with nearly neutral internal pHs would benefit microbial survival, suggested by the significant correlation between iPSB community abundance and biochar pH rather than soil pH ($P < 0.05$). The biodiversity of the iPSB community indicated by the Shannon index was significantly lower ($P < 0.05$) for RS and SS than the other treatments (Fig. 4b), and the biodiversity of all treatments was significantly negatively correlated ($P < 0.05$) with iPSB community abundance (Fig. 5a), perhaps because the strains of the inoculated iPSB community survived well and became dominant with the assistance of the RS and SS biochars.

The combined usage of RS biochar and iPSB community inoculation was most beneficial for rape growth. In terms of rhizospheric soil (Table S9), RS biochar significantly increased the P contents while the effect of iPSB community was much higher ($P < 0.05$), suggesting that the iPSB community inoculation had the effect of P mobilization and was better functioning on soil P releasing than biochar. As for the impact on plant growth, we could not discriminate significant differences from the effect of solo biochar or iPSB community on the promotion of plant biomass and its P content and uptake; however, the combined application of biochar and iPSB performed significantly higher promotion ($P < 0.05$), which suggested that the RS

365 biochar may provide a better habitat for P releasing by iPSB community to promote
366 rape growth. The biochar was extensively reported with beneficial effects on soil
367 nutrient mobilization and plant nutrient uptake (Atkinson et al., 2010), which was also
368 found in our study (nearly 2 times higher in soil Olsen-P concentration and 2 times of
369 plant P uptake). If we considered the enhancing effect of biochar for P releasing is
370 permanent, the remaining increasing effect observed in R-BP treatment (almost 1.5
371 times higher in available P enhancement and 1.5 times of plant P uptake than R-B
372 treatment) should be attributed to the functioning of iPSB community; however, the
373 actual effect of solo iPSB community inoculation was far less. One reasonable
374 explanation to this phenomenon may be that RS biochar provide a well environment
375 for iPSB survival and strengthen its ability for P mobilization. Although we have
376 found RS biochar was the best for the survival of iPSB community (Table 1), we
377 could not conclude that the biochar was the only reason for iPSB survival since there
378 is not a longer period (more than 3 month) to observe its living status. A similar study
379 of 20 weeks was reported that the biochar addition showed significant impact on the
380 population increase of *Bacillus mucilaginosus* within 4 weeks; however, the survival
381 rate was gradually declined afterwards (Sun et al., 2015). Another study also reported
382 the short-term effect of biochar amendment for the better survival of soil bacteria
383 (Hale et al., 2013). Both of those studies indicate that the biochar was effective but
384 may hardly give a long-term support for bacterial survival. A possibility is that there
385 would be not enough nutrients (such as available N and P) retained in biochar after a

386 long time, and the starving bacteria may enter into a non-culturable state to keep them
387 survive without functioning or even cell division (Sun et al., 2016).

388 The iPSB community benefited crop growth, and the biochar provided suitable
389 conditions for iPSB community survival due to its strong absorption of AP. All
390 biochar feedstocks in this study benefited the iPSB community, but the straw biochars
391 (RS and SS) were best for increasing iPSB community abundance and its functioning
392 in P mobilization.

393

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524 **Figure legends**

525 **Figure 1.** The diagram of experimental design. The soils sampled from Changshu was
526 used in new iPSB strain isolation and its sterilized one were used for rape growth with
527 different treatments. The asterisk (*) indicates that those samples were physically or
528 chemically analyzed. The pound sign (#) suggests that those strains were being
529 biochemically determined. The detail method was explicated in Supplementary
530 Information.

531 **Figure 2**. (a) Morphology of the iPSBs by scanning electron microscopy and colonies
532 after 72 h of cultivation at 30 °C on (b) LB medium, (c) PKV medium with NH_4^+ as the
533 N source and (d) PKV medium with NO_3^- as the N source. *E. coli* without P-solubilizing
534 ability was used as a negative control. (e) Heatmap of the profiles of medium pH and
535 organic-anion exudation by the iPSBs after 144 h of cultivation based on the average
536 of three replicate experiments.

537 **Figure 3.** Abundance (a) and Shannon diversity (b) of the biochars inoculated with the
538 iPSB community. Error bars represent the standard deviation of the replicates (n=4).
539 Different letters indicate significant differences at $P < 0.05$.

540 **Figure 4**. Heatmap and redundancy analysis (RDA) of the iPSB communities with the
541 biochars after four weeks of rape growth based on Bray-Curtis distances. (a) Profile of
542 iPSB community composition and (b) the effect of the physical and chemical properties
543 of the soil and biochar on the iPSB communities. The plotted values are natural-

544 logarithm transformations of relative iPSB community abundance. The columns in (a)
545 are labeled with biochar names and replicate numbers. The seven strains used to
546 generate the iPSB community are identified in red, and the other strains are identified
547 as species or genera.

548 **Figure 5.** Relationships between iPSB community abundance and the Shannon
549 diversity index (a), plant biomass (b), plant P (c) and plant P uptake (d).

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