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Screening of suitable carriers for *Bacillus amyloliquefaciens* strain QL-18 to enhance the biocontrol of tomato bacterial wilt

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ABSTRACT

Soilborne plant diseases seriously limit agricultural production. Due to environmental concerns, sustainable agricultural practices must involve the use of environmentally friendly alternatives, such as biocontrol microbes (BMs), to suppress various plant diseases. However, the success of BM application largely depends on the carrier formulation. An ideal carrier that can support the survival of a BM while discouraging the growth of the target pathogen is expected to enhance the performance of the BM in plant disease control. In this study, various commercial composts and peats were evaluated as carriers for *Bacillus amyloliquefaciens* QL-18, a BM used to control tomato bacterial wilt (TBW) caused by the soilborne bacterial pathogen *Ralstonia solanacearum*. Among the evaluated carriers, rapeseed cake compost (RCC) was found to be the best carrier, acting as both a substrate and food source for *B. amyloliquefaciens* QL-18. RCC facilitated the growth and survival of strain QL-18 by supplying adequate levels of dissolved carbon and nitrogen. Moreover, aqueous extracts of RCC alone inhibited *R. solanacearum* growth, suggesting that certain RCC components are toxic to the pathogen. Greenhouse and field experiments demonstrated that RCC improved the ability of *B. amyloliquefaciens* QL-18 to reduce the incidence of TBW and the population of the pathogen in the tomato rhizosphere. Although other carriers maintained large populations of *B. amyloliquefaciens* QL-18, similar to those obtained with RCC, they were not deemed suitable carriers because they constituted a poor nutrient supply for *B. amyloliquefaciens* QL-18 growth; thus, they had less of an impact on disease control. The principle of BM carrier selection should be adopted in the future when developing new bio-products for the effective control of soilborne plant diseases.

1. Introduction

By 2050, the human population will most likely increase by 2–4 billion people (Cohen, 2003), necessitating an increase in food production. However, plant diseases continue to play a major limiting role in agricultural production. As the control of plant diseases using classical pesticides raises serious concerns about food safety, environmental effects and pesticide resistance, sustainable agricultural practices must involve the use of environmentally friendly alternatives for effective plant disease control. In addition to balanced fertilization (Abro et al., 2013; Dordas, 2008), soil biosolarization (Domínguez et al., 2014), and other agroecological practices (Wezel et al., 2014), biocontrol microbes (BMs) have increasingly been used to suppress phytopathogens for sustainable agriculture (Camprubí et al., 2007; García-Seco et al., 2013; Khaled et al., 2008; Ongena and Jacques, 2008; Weller, 2007). Although the inoculation of plants with BMs is now well established, the formulation of inocula with reliable and consistent effects under field conditions remains a bottleneck that limits industrial-scale applications of BMs. In addition, the choice of an appropriate carrier for the formulation is critical for successful BM implementation (Malusa et al., 2012).

An appropriate carrier for BMs must exhibit the following properties: a high water-holding capacity, chemical and physical uniformity, a near-neutral or readily adjustable pH, a reasonable cost (Ferreira and Castro, 2005; Stephens and Rask, 2000), no BM toxicity and environmental safety (Benrebah et al., 2007). One important selection strategy is based on the multiplication and survival of beneficial microbes during storage (Malusa et al., 2012). Although previous screening strategies have been successfully

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applied (Accinelli et al., 2009; Albareda et al., 2008; Riveracruz et al., 2008; Singh et al., 2007), they do not consider the effect of carrier application on the survival of pathogens in soil. Indeed, carriers of BMs applied to soil might facilitate the survival of pathogens in the absence of a host, potentially causing low stability and decreased efficacy of biocontrol products under field conditions. Therefore, the ideal carrier screening strategy should consider both the BM and the pathogen.

Ralstonia solanacearum is a soilborne bacterial phytopathogen that causes wilt on a wide host range, including tomato, potato, tobacco and other important crops (Elphinstone, 2005). Soil fumigation, short rotation, and resistant cultivars have been suggested as integrated control strategies for bacterial wilt (French, 1994); however, efficient control measures for this pathogen in the field are still unavailable due to its long-term survival in soil and water, large genetic diversity, and broad geographical distribution (Elphinstone, 2005). *Bacillus amyloliquefaciens* strain QL-18 has been recently reported as a potential BM that can efficiently reduce the disease incidence of tomato bacterial wilt (TBW) under greenhouse conditions (Wei et al., 2011). Nonetheless, unstable *B. amyloliquefaciens* QL-18 performance has been observed in the field when applied with a mixture (1:1 in weight) of pig manure compost (PMC) and rapeseed cake compost (RCC), which has been used as a model carrier for different BMs targeted against various soilborne diseases (Wu et al., 2009; Zhao et al., 2011; Zhang et al., 2013). In this study, *B. amyloliquefaciens* QL-18, *R. solanacearum*, and tomato plants (c. v. Hezuo 903) were employed as a model system to establish carrier screening principles for the development of a biocontrol formulation. Peat and compost, two common carriers of BMs (Albareda et al., 2008; Riveracruz et al., 2008), were selected as potential candidate carriers for *B. amyloliquefaciens* QL-18.

It is hypothesized that the ideal commercial carrier should both prolong the shelf life of *B. amyloliquefaciens* QL-18 and promote its ability to suppress TBW without facilitating the growth of *R. solanacearum* in soil. To select a suitable carrier for *B. amyloliquefaciens* QL-18, a series of *in vitro*, greenhouse and field experiments were carried out to evaluate (1) the shelf life of *B. amyloliquefaciens* QL-18 in different carrier (compost or peat) formulations; (2) the ability of compost or peat to serve as the sole nutrient source for *B. amyloliquefaciens* QL-18 and *R. solanacearum* survival; (3) the effects of carriers on the survival of *B. amyloliquefaciens* QL-18 and *R. solanacearum* in soil; and (4) the effects of carriers on the ability of *B. amyloliquefaciens* QL-18 to reduce the disease incidence of TBW under greenhouse and field conditions.

2. Materials and methods

2.1. Bacterial strains and culture conditions

The bacterial strains *R. solanacearum* QL-Rs1115 (Race 1, Biovar 3) and *B. amyloliquefaciens* QL-18 were stored in 20% glycerol at −80 °C, and were recovered on CPG (French et al., 1995) and LB agar plates, respectively. A single colony of each bacterium was routinely cultured for further studies. *R. solanacearum* QL-Rs1115 was cultured using CPG medium; *B. amyloliquefaciens* QL-18 was cultured using LB medium. Both bacterial strains were cultured at 28 °C with shaking at 170 rpm for 48 h on a rotary shaker.

2.2. Carrier characteristics

Five composts and two peats (peat1 and peat2, as reference carriers) purchased from different Chinese commercial enterprises were used as formulation carriers for *B. amyloliquefaciens* QL-18 (Table 1). The selected properties of these carrier candidates are listed in Table 1. The carriers were dried to a moisture content of 5% in an oven (Memmert, Germany) at 80 °C for 24 h, finely ground in a hammer mill to pass through a 1-mm screen, and stored at room temperature for further studies.

2.3. Evaluation of carriers as a substrate for B. amyloliquefaciens QL-18

The effect of the carrier as a substrate on the survival of *B. amyloliquefaciens* QL-18 was evaluated using plastic bottles equipped with a 0.22-μm filter membrane in the cap to allow air exchange (Fig. S1). The bacterial culture of *B. amyloliquefaciens* QL-18 was suspended in 500 mL of 0.01 mol L^{-1} phosphate buffer, and 20 mL of a suspension of *B. amyloliquefaciens* QL-18 (5.0×10^9 CFU mL^{-1}, as determined by the plate count method with LB medium) was mixed with 100 g of each carrier. Carriers treated with 20 mL of 0.01 mol L^{-1} phosphate buffer were used as controls. Each treatment included three replications (three bottles). In addition, these plastic bottles were covered with black plastic bags to avoid the influence of light on the survival of *B. amyloliquefaciens* QL-18. The bottles were stored at 25 °C and periodically sampled at 0, 10, 20, 30, 60, 90, 120 and 180 days post-inoculation. The population of *B. amyloliquefaciens* QL-18 in each carrier at each sampling time point was determined by the plate count method using *Bacillus*-semiselective medium salt-V8 (Tumer and Backman, 1991).

2.4. Evaluation of carriers as a food source for B. amyloliquefaciens QL-18 and R. solanacearum QL-Rs1115

Different carrier media serving as the sole nutrient source were evaluated for their effects on the growth of *B. amyloliquefaciens* QL-18 and *R. solanacearum* QL-Rs1115. Ten grams of each carrier was added to 50 mL of distilled water in a 250-mL conical flask, followed by sterilization at 115 °C for 30 min. Seed suspensions of *B. amyloliquefaciens* QL-18 (5.0×10^8 CFU mL^{-1} in 0.01 mol L^{-1} phosphate buffer, as determined by the plate count method with LB medium) and *R. solanacearum* QL-Rs1115 (5.0×10^9 CFU mL^{-1} in 0.01 mol L^{-1} phosphate buffer, as determined by the plate count method with CPG medium) were inoculated separately into each culture medium at a ratio of 1:1000 and grown at 28 °C with shaking at 170 rpm for 3 days on a rotary shaker. The populations of *B. amyloliquefaciens* QL-18 and *R. solanacearum* QL-Rs1115 were determined at 0, 1, 2, and 3 days after inoculation by the plate count method with CPG and LB media, respectively. Control treatments (LB culture medium for *B. amyloliquefaciens* QL-18 and CPG culture medium for *R. solanacearum* QL-Rs1115) without a carrier were included. Each treatment included three replications (three flasks). The resulting suspension was transferred to a 50-mL centrifuge tube and centrifuged at 8000 × *g* for 10 min at 4 °C. The contents of dissolved organic carbon (DOC) and total soluble nitrogen (Nts) in the supernatant were determined using a Liqui TOC (Elementar, Germany).

2.5. Evaluation of the toxicity of aqueous carrier extracts toward R. solanacearum

Extracts of the carriers were collected in a series of weight-to-water volume ratios (1:25, 1:10, 1:5, and 1:2) in 250-mL flasks after shaking in a rotator at 200 rpm for 1 h at room temperature. The extracts were filtered through two layers of cheesecloth, and the resulting filtrate was centrifuged at 10,000 × *g* for 10 min. The supernatant was then filtered through a 0.22-μm filter and stored at 4 °C until further use. Next, the inhibitory effect of the cell-free

Table 1
Properties of the carriers used in this study.

Carrier	pH	EC (ms/cm)	OM (%)	TN (%)	K_2O (%)	P_2O_5 (%)	Source
RCC	6.75	9.00	71.2	8.64	0.56	1.15	Xintiandi Amino Acid Fertilizer, Ltd. (Yixing, Jiangsu)
PMC	7.28	6.32	22.2	1.79	0.6	2.19	Tianniang Agricultural Technology, Ltd. (Changshu, Jiangsu)
CMC	8.41	4.99	53.1	1.51	0.84	2.00	Apollo Bio-product, Ltd. (Jintian, Jiangsu)
CDC	7.08	7.35	32.1	1.38	1.57	0.45	Lianye Bio-technology, Ltd. (Jiangyin, Jiangsu)
HRC	7.64	3.97	47.7	2.3	0.78	0.37	Mingzhu Fertilizer, Ltd. (Nanjing, Jiangsu)
Peat1	4.45	0.43	53.6	1.9	0.06	0.04	Biyuan Peat Development, Ltd.
Peat2	5.11	0.49	34.9	1.33	0.69	0.17	Ziran Horticulture, Ltd. (Shanghai)

*RCC: rapeseed cake compost; PMC: pig manure compost; CMC: chicken manure compost; CDC: cow dung compost; HRC: herb residue compost; EC: electrolytic conductivity; OM: organic matter; TN: total nitrogen.

carrier extract on the growth of *R. solanacearum* QL-Rs1115 was evaluated. After spreading 200 μL of a suspension (10^8 CFU mL^{-1} in 0.01 mol L^{-1} phosphate buffer, as determined by the plate count method with CPG medium) of *R. solanacearum* QL-Rs1115 onto CPG medium plates, 100 μL of cell-free carrier extract was added using the Oxford cup method (Fig. S2). There were three replications for each extract. The plates were then kept at 30 °C, and the zones of inhibition were evaluated after 48 h. This experiment was repeated twice.

2.6. Effects of carriers on the survival of *B. amyloliquefaciens* QL-18 and *R. solanacearum* QL-Rs1115 in soil

This experiment was designed to evaluate the effects of two carriers (RCC and peat2), either alone or in combination with *B. amyloliquefaciens* QL-18, on the survival of *R. solanacearum* QL-Rs1115 in soil. Plastic bottles were used as vessels, and the soil used in the experiment was collected from a tomato field in the town of Qilin. The soil was air-dried and sieved through a 1-mm silver sieve. Six treatments were included: (1) in CK, the soil was not amended with a carrier or *B. amyloliquefaciens* QL-18; (2) in RCC, the soil was amended with RCC but not *B. amyloliquefaciens* QL-18; (3) in peat2, the soil was amended with peat2 without *B. amyloliquefaciens* QL-18; (4) in QL-18, the soil was inoculated with a *B. amyloliquefaciens* QL-18 suspension without a carrier amendment; (5) in RCC + QL-18, the soil was amended with both RCC and the *B. amyloliquefaciens* QL-18 suspension; and (6) in peat + QL-18, the soil was amended with both peat2 and the *B. amyloliquefaciens* QL-18 suspension. For each treatment, the carrier was mixed to homogeneity with 300 g of soil at a ratio of 1:100 (w/w). Ten milliliters of a *B. amyloliquefaciens* QL-18 suspension (3.0×10^9 CFU mL^{-1} in 0.01 mol L^{-1} phosphate buffer, as determined by the plate count method with LB medium) was mixed with the soil in each microcosm according to the experimental design. All treatments were inoculated with 10 mL of a QL-Rs1115 suspension (4.2×10^9 CFU mL^{-1} in 0.01 mol L^{-1} phosphate buffer, as determined by the plate count method with CPG medium). The water content of the soil was adjusted to 35% with sterilized water. The microcosms were maintained at 25 °C in a dark chamber. Each treatment consisted of three replicates (three bottles), and the soil was periodically sampled from each bottle. The number of viable cells of *R. solanacearum* QL-Rs1115 and *B. amyloliquefaciens* QL-18 in the soil was estimated by plating 10-fold serial dilutions on a modified semi-selective medium, South Africa (SMSA-E) (French et al., 1995) and salt-V8 plates, respectively.

2.7. Biocontrol of TBW under greenhouse and field conditions

A greenhouse experiment was first designed to evaluate the effects of two carriers (RCC and peat2), either alone or in combination with *B. amyloliquefaciens* QL-18, on the incidence of TBW. The experimental design and soil amendments were the same as those described in Section 2.6. Tomato cv. Hezuo 903 seeds were planted in plastic pots (8 cm in diameter) containing 300 g of steam-sterilized soil. The soil used in this study was collected from a tomato field in Qilin, a vegetable production base for the nearby urban population in Nanjing City, China, where *B. amyloliquefaciens* QL-18 and *R. solanacearum* QL-Rs1115 were isolated; bacterial wilt disease is a constant problem at this location. The seedlings were grown in a greenhouse at temperatures ranging from 25 to 35 °C and watered daily with sterilized water. Seedlings at the three-leaf stage were transplanted into ceramic pots containing 5 kg of air-dried paddy soil (pH 6.2 with 2.67% organic matter) for further study. The soil was treated with 100 mL *B. amyloliquefaciens* QL-18 suspension (5.0×10^8 CFU mL^{-1}) according to the experimental design. One week later, all the treatments were inoculated with 100 mL of an *R. solanacearum* QL-Rs1115 suspension (5.0×10^6 CFU mL^{-1}). Each treatment included 3 blocks with 15 pots (three seedlings were planted in each pot). Therefore, a total of 45 seedlings were used for each treatment. The pots were randomly arranged in a glass greenhouse with a temperature ranging from 25 to 35 °C and a relative humidity of approximately 70–90%. The disease incidence was recorded daily for two months according to the method described by Wei et al. (2011). Three tomato plants were randomly selected and carefully uprooted from each treatment every 10 days after inoculation with the pathogen. The *R. solanacearum* population in the rhizosphere of the tomato plants was determined by plating 10-fold serial dilutions on SMSA-E medium.

A subsequent experiment was carried out to evaluate the effects of *B. amyloliquefaciens* QL-18 formulations (referred to as RCC18 and Peat18) on the suppression of TBW in a field in the town of Qilin. A suspension of *B. amyloliquefaciens* QL-18 was mixed with RCC and peat2 separately in a 1:5 (v/w) ratio, and the resulting mixtures were designated as RCC18 and Peat18. The final density of *B. amyloliquefaciens* QL-18 was approximately 5.0×10^8 CFU g^{-1} of formulation prior to field application. Tomato seedlings (cv. Hezuo 903) were grown in seedling trays with a nursery substrate (commercially available from Huaian Agricultural Technological Development Ltd., Huanyin, Jiangsu, China), which was evenly mixed with 1% (w/w) RCC18 or Peat18; no amendment (CK) was also used as a control. For transplanting, 20 g of RCC18 or Peat18 was applied to the soil for each plant. Overall, three treatment conditions were set up: RCC18 + RCC18, Peat18 + Peat18, and CK + CK. Each plant was treated with the same formulation at both seedling and transplant time points. Each treatment was replicated five times (five plots of ~1.5 m × 8.0 m), and the plots were arranged in a completely randomized design. Chemical fertilizers (Stanley fertilizer stock Co., LTD, Shandong, China) were applied in addition to the treatment amendments to maintain sufficient soil nutrient levels. Standard agronomic practices were used to grow the tomato plants without any pesticides. The disease incidence (proportion of wilted plants to total number of plants) was

determined at the time of harvest by counting the number of wilted plants in each plot.

2.8. Statistics

The microbial count data were log10 transformed prior to performing the statistical analyses. The data were statistically evaluated at the 5% level using an ANOVA procedure (Holm–Sidak method) with SigmaPlot (Systat Software, San Jose, CA, USA). Linear regression was employed to analyze the relationship between the bacterial population and the contents of dissolved organic carbon and total soluble nitrogen.

3. Results and discussion

3.1. Shelf life of B. amyloliquefaciens QL-18 in different carriers

A long and stable shelf life is one of the most important commercial characteristics of biocontrol products. Therefore, the population dynamics (an index of shelf life) of *B. amyloliquefaciens* QL-18 using different candidate carriers was determined using semi-selective salt-V8 medium. The total numbers of colonies formed on salt-V8 plates ranged from 10^3–10^6 CFU g^{-1} dry carrier for the control treatments (containing no *B. amyloliquefaciens* QL-18 cells, Fig. 1A); for the *B. amyloliquefaciens* QL-18-treated carriers, the number of colonies formed on salt-V8 plates exceeded 10^8 CFU g^{-1} dry carrier during storage at 25 °C for 180 days (Fig. 1B). This result indicated that the colonies from the treated carriers were composed of *B. amyloliquefaciens* QL-18 cells.

The abilities of the carriers to support adequate *B. amyloliquefaciens* QL-18 survival differed, with RCC and peat2 maintaining the largest populations of *B. amyloliquefaciens* QL-18. At the end of the storage period, the viable numbers of *B. amyloliquefaciens* QL-18 in RCC and peat2 were greater than 8.7 and 8.5 log CFU g^{-1} dry carrier, respectively. A high viable cell count capable of suppressing soilborne plant pathogens is the most important factor in obtaining a high-quality inoculant, and the acceptable standard for the number of BMs in the carrier material varies from one country to another. However, in many countries, formulations containing 10^7 CFU g^{-1} or more are considered standard (Accinelli et al., 2009; Rebah et al., 2002). The Chinese bio-organic fertilizer production standard stipulates that the functional microorganism content should be greater than 2.0×10^7 CFU g^{-1} dry formulation after storage for 6 months at room temperature. The high viable cell counts of *B. amyloliquefaciens* QL-18 observed in all carriers may be largely attributed to spore formation, which supports the survival of the strain in stressful environments (Emmert and Handelsman, 1999).

3.2. Carriers act as a food source for B. amyloliquefaciens QL-18 and R. solanacearum QL-Rs1115

In addition to serving as a substrate, a carrier should supply nutrients for the biocontrol agent while ideally discouraging the growth of the pathogen. Compared to the other carriers, RCC appeared to be the best food source for *B. amyloliquefaciens* QL-18, as clearly demonstrated in Fig. 2A, C and E. The plate counts of *B. amyloliquefaciens* QL-18 in the RCC medium were significantly ($P < 0.001$) higher than those in the other media (Fig. 2A). Furthermore, the larger *B. amyloliquefaciens* QL-18 population observed in the RCC medium was closely related to the higher contents of dissolved organic carbon (Fig. 2C) and total soluble nitrogen (Fig. 2E). The contents of dissolved organic carbon and total soluble nitrogen were significantly and positively related to the *B. amyloliquefaciens* QL-18 populations on days 1 ($P < 0.0184$ and $P < 0.0060$, respectively), 2 ($P < 0.0117$ and $P < 0.0120$, respectively), and 3 ($P < 0.00632$ and $P < 0.0151$, respectively) of cultivation. After 3 days of *B. amyloliquefaciens* QL-18 cultivation, the dissolved organic carbon and total soluble nitrogen contents were significantly higher in the RCC medium than in the other media, with increases of 146.2% and 190.3%, respectively, indicating that more nutrients were released from RCC. The other carriers also had the potential to serve as a substrate for *B. amyloliquefaciens* QL-18 (Fig. 1). However, they were not good nutrient sources, as *B. amyloliquefaciens* QL-18 proliferated only slowly or stopped growing in the media based on these carriers (Fig. 2A). Taken together, these results suggest that RCC can serve as a nutrient source for *B. amyloliquefaciens* QL-18.

Unlike what was observed with the *B. amyloliquefaciens* QL-18 inoculum, no significant ($P > 0.223$) linear relationship was observed between the dissolved organic carbon and the total soluble nitrogen contents for the *R. solanacearum* populations. The

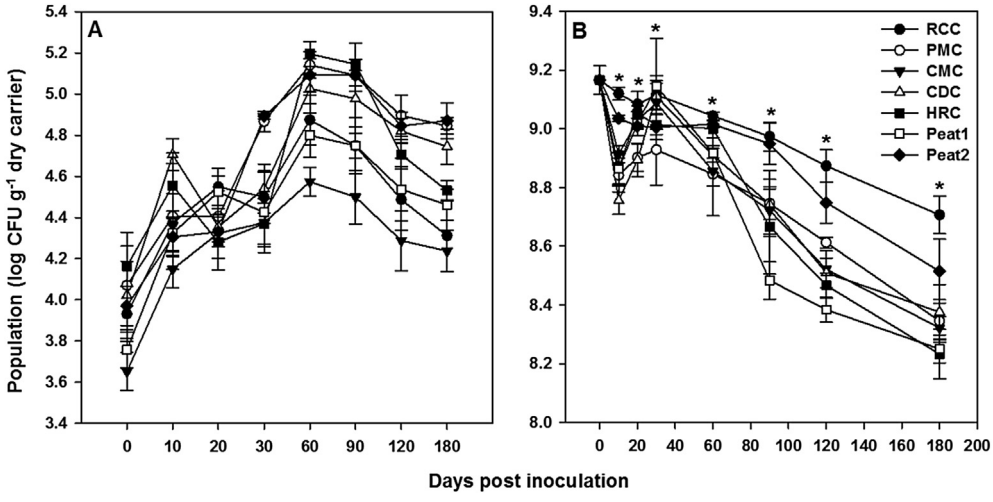

Fig. 1. Shelf life of *B. amyloliquefaciens* QL-18 in different carrier formulations. Plate counts on V8-salt medium from rapeseed cake compost (RCC), pig manure compost (PMC), chicken manure compost (CMC), cow dung compost (CDC), herb residue compost (HRC), peat1, and peat2 carriers inoculated with 0.01 mol L^{-1} phosphate buffer (A) or *B. amyloliquefaciens* QL-18 (B) were determined on different days post-inoculation. An asterisk on the top of the bar represents a significant difference ($P < 0.05$, Holm–Sidak method) in the inoculum populations between the carriers at the same time point.

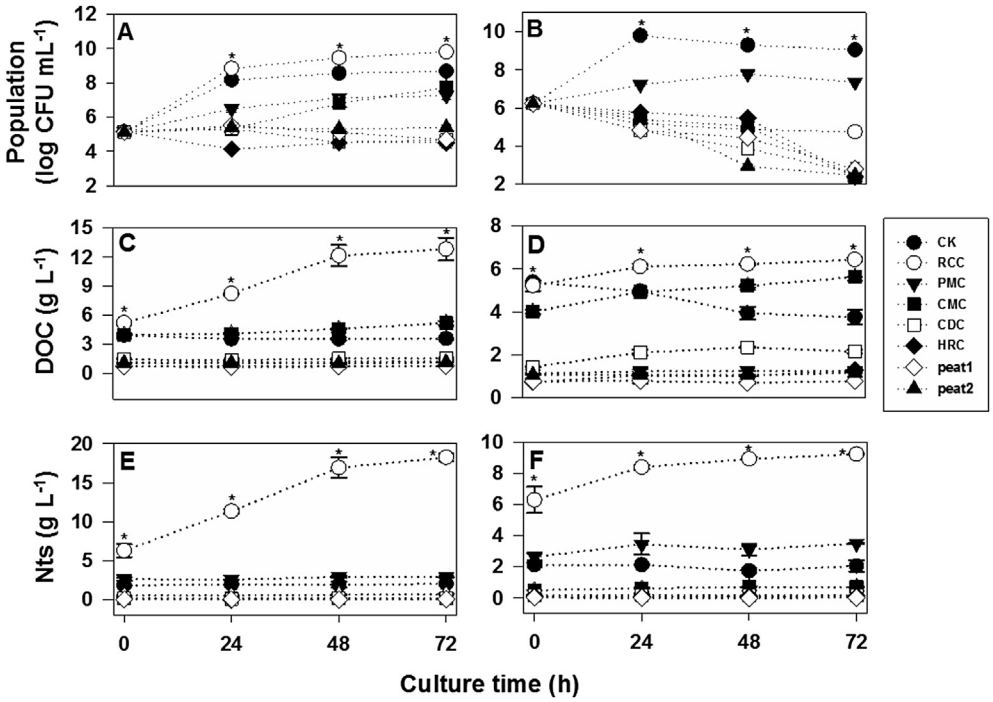

Fig. 2. Changes in inoculum populations (A and B), dissolved organic carbon (C and D), and total soluble nitrogen (E and F) in the RCC (rapeseed cake compost), PMC (pig manure compost), CMC (chicken manure compost), CDC (cow dung compost), HRC (herb residue compost), peat1, and peat2 carriers at 0–3 days after inoculation of *B. amyloliquefaciens* QL-18 (A, C, and E) or *R. solanacearum* QL-Rs1115 (B, D, and F). LB and CPG media served as controls (CK) for the incubation of *B. amyloliquefaciens* QL-18 and *R. solanacearum* QL-Rs1115, respectively.

growth of *R. solanacearum* QL-Rs1115 was discouraged in RCC medium (Fig. 2B), even though this medium contained sufficient nutrients for the growth this pathogen (Fig. 2D and F). Further results suggested that RCC might contain components that are toxic to *R. solanacearum*, as indicated by the presence of clear halos in the 1:2 and 1:5 extract treatments (Fig. 3); this finding potentially explains the failure of *R. solanacearum* to proliferate in the nutrient-rich RCC medium (Fig. 2B). Rapeseed (*Brassica napus*) contains glucosinolates, which are hydrolyzed by myrosinase to isothiocyanates, and previous studies showed that *Brassica* cultivars with high isothiocyanate and glucosinolate contents could effectively reduce *R. solanacearum* (Arthy et al., 2005) and *Rhizoctonia solani* populations Handiseni et al. (2013). In addition, an increase in the *R. solanacearum* population observed in PMC (Fig. 2B) indicated that this compost could not be used as a carrier for controlling bacterial wilt disease because it might facilitate *R. solanacearum* growth when applied to soil.

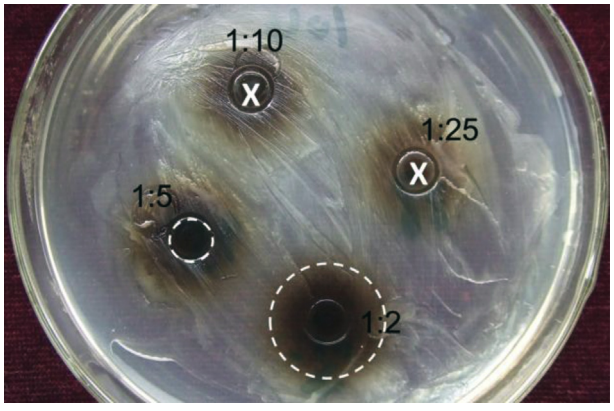

Fig. 3. Inhibition of the growth of *R. solanacearum* QL-Rs1115 by cell-free RCC extracts collected in a series of weight-to-water volume ratios (1:25, 1:10, 1:5, and 1:2). After 200 μL of an *R. solanacearum* QL-Rs1115 suspension (10^8 CFU mL^{-1}) was spread onto plates containing CPG medium, four test ratios (100 μL) of a cell-free carrier extract were added using the Oxford cup method. The plates were then cultivated at 30 °C for 48 h to evaluate the resulting inhibition zones (without *R. solanacearum* cells). Clear inhibition zones indicated by white circles were only observed in treatments with 1:5 and 1:2 ratios.

3.3. Effects of carriers on the survival of *B. amyloliquefaciens* QL-18 and *R. solanacearum* QL-Rs1115 in soil

The effects of peat2 and RCC on the survival of *B. amyloliquefaciens* QL-18 and *R. solanacearum* QL-Rs1115 in soil were evaluated for a period of 36 days (Fig. 4A and B). The tested carrier amendments ($P < 0.001$, $F = 103.351$) and treatment times ($P < 0.001$, $F = 255.228$) were found to significantly affect the survival of strain QL-18 in soil. Although the populations of *B. amyloliquefaciens* QL-18 decreased slowly over time in all treatments, the carrier amendments did facilitate its survival compared with the control, with the populations in the RCC + QL-18 treatment being significantly larger than those in the peat + QL-18 and control treatments at 8 ($P < 0.001$ and $P = 0.005$, respectively), 16 ($P < 0.001$ and $P < 0.001$, respectively), and 36 days post-inoculation ($P < 0.001$ and $P < 0.001$, respectively).

The survival of *R. solanacearum* QL-Rs1115 in soil was significantly decreased by the applied carrier amendment ($F = 224.442$, $P < 0.001$), the presence of *B. amyloliquefaciens* QL-18 ($F = 692.270$, $P < 0.001$), and the treatment time ($F = 2132.102$, $P < 0.001$), and there was a statistically significant interaction between these three factors ($P < 0.001$). The *R. solanacearum* QL-Rs1115 population decreased rapidly in all the treatments except in peat2. Compared with RCC, the peat2 amendment facilitated the survival of *R. solanacearum* QL-Rs1115 in soil, with significantly larger populations in the peat2 treatment ($P < 0.001$) compared with the RCC

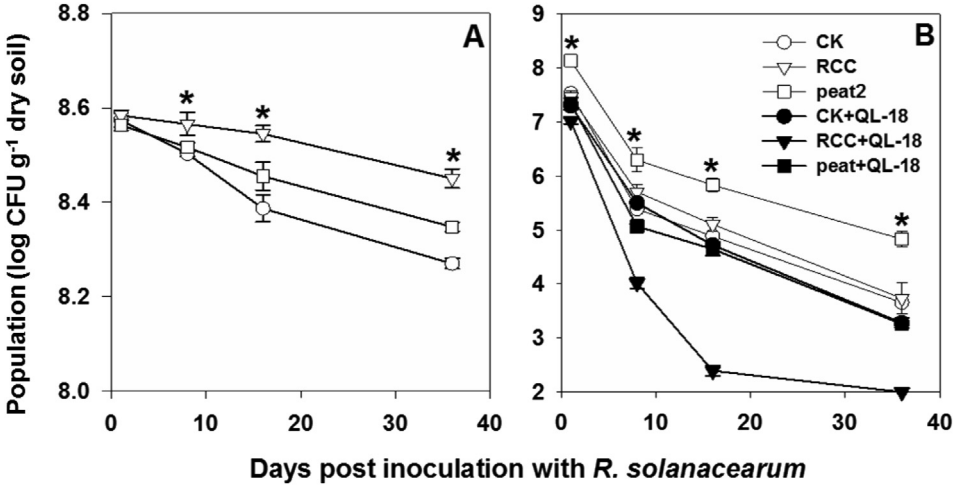

Fig. 4. *B. amyloliquefaciens* QL-18 (A) and *R. solanacearum* QL-Rs1115 (B) population dynamics in soil treated with RCC, peat2, QL-18, RCC + QL-18, peat + QL-18 or no amendment (CK) on different days post-inoculation. Asterisks indicate the presence of significant differences (Holm–Sidak method; $P < 0.05$) among treatments for a particular day post-inoculation with *R. solanacearum*.

and control treatments. Although the RCC extract had an inhibitory effect on the growth of *R. solanacearum*, the effect of the RCC amendment on the survival of *R. solanacearum* QL-Rs1115 in soil was not significant ($P = 0.076$). This loss of a suppressive effect might be due to the low dose (1%) of RCC applied to the soil. In fact, one study employed the same nutrient source and demonstrated that the minimum dose resulting in toxic effects was 41.7 g kg^{-1} soil (Elberson et al., 1996). The dose applied in the present study was 10 g kg^{-1}; thus, the amended soil was not toxic to *R. solanacearum* (Fig. 4B).

However, the combination of RCC with *B. amyloliquefaciens* QL-18 did significantly reduce the *R. solanacearum* QL-Rs1115 population ($P < 0.001$): the populations were significantly smaller in the RCC + QL-18 treatment than in the other treatments at 8 ($P < 0.001$), 16 ($P < 0.001$), and 36 ($P < 0.001$) days post-inoculation. Although *R. solanacearum* QL-Rs1115 cells were not detected in the RCC + QL-18 treatment at 36 days post inoculation, the populations in the other treatments ranged from 3.26 to 4.83 log CFU g^{-1} dry soil.

3.4. Application of *B. amyloliquefaciens* QL-18 with RCC efficiently suppressed TBW

The incidence of TBW was significantly affected by the applied carrier amendments ($F = 21.248$, $P < 0.001$) and the presence of *B. amyloliquefaciens* QL-18 ($F = 319.469$, $P < 0.001$) (Fig. 5A and B). Independent of the presence of a carrier, strain QL-18 significantly reduced the disease incidence by 40–75%. Additionally, the RCC amendment significantly ($P < 0.001$) enhanced the performance of *B. amyloliquefaciens* QL-18. In the absence of strain QL-18, the carrier amendment had no significant effect on disease suppression ($P > 0.241$). Inefficient disease control by a biocontrol agent may be largely due to failure of its establishment in a soil in which nutrient availability is limited. RCC not only supports the proliferation of *B. amyloliquefaciens* QL-18 but also might increase the abundance of bacteria and actinomycetes in rhizosphere soils (Zhang et al., 2013), which might help to reduce *R. solanacearum* populations through nutrient competition and antagonism.

The peat2 amendment had no positive effect on performance ($P = 0.873$), and the lower biocontrol efficacy of *B. amyloliquefaciens* QL-18 observed using peat2 as a carrier clearly demonstrated the drawbacks of previously applied carrier selection principles. The consistently high cell density of *B. amyloliquefaciens* QL-18 found in peat2 demonstrated that peat is a good carrier based on these previous principles (Fig. 1). However, the peat2 amendment also prolonged the survival of *R. solanacearum* in the soil compared with RCC and the non-amended treatments (Figs. 4B and 5B). Because it is not toxic to *R. solanacearum*, peat2 could serve as a substrate that facilitates the saprophytic growth of the pathogen. Therefore, peat2 might be a good carrier for other applications, though it is not a suitable choice for the suppression of *R. solanacearum*.

The *R. solanacearum* populations in the rhizosphere soil increased significantly ($P < 0.001$, $F = 1986.737$) with the number of days post-inoculation for all treatments. However, the soil inoculated with *B. amyloliquefaciens* QL-18 showed significant reductions in *R. solanacearum* populations ($P < 0.001$, $F = 1120.375$) compared with the treatments without strain QL-18. The ability of *B. amyloliquefaciens* QL-18 to suppress *R. solanacearum* was also affected by the carrier amendment ($P < 0.001$, $F = 12.491$): the *R. solanacearum* populations in the rhizosphere soil of the RCC + QL-18 treatment were significantly lower than those in the other 5 treatments at 20 ($P < 0.001$, $F = 22.166$), 30 ($P < 0.001$, $F = 44.007$), 40 ($P < 0.001$, $F = 181.118$), 50 ($P < 0.001$, $F = 145.301$), and 60 ($P < 0.001$, $F = 74.119$) days post-inoculation.

The results from the field experiment confirmed the ability of the RCC18 formulation to suppress TBW (Fig. 6). *B. amyloliquefaciens* QL-18 together with RCC successfully reduced the disease incidence by 69.5%, whereas *B. amyloliquefaciens* QL-18 with peat2 only reduced the disease incidence by 20.2%. Such types of formulations have been designated as bioorganic fertilizers (BOF), and they can be applied to soil to control soilborne diseases. It is suggested that plant seedlings should be treated with BOFs twice: at the seedling and transplanting stages. At the seedling stage, the amount of BOF applied ranges from 0.5% to 2.0% of the total seedling substrate; for transplantation, the dosage ranges from 15 to 2000 g depending on the crop (Wei et al., 2011; Wu et al., 2009; Zhang et al., 2014).

4. Conclusion

The results of this study reveal that RCC is one of the best carriers for *B. amyloliquefaciens* QL-18 to control bacterial wilt disease of tomato. When combined with RCC, *B. amyloliquefaciens* QL-18 can more effectively suppress *R. solanacearum* invasion than

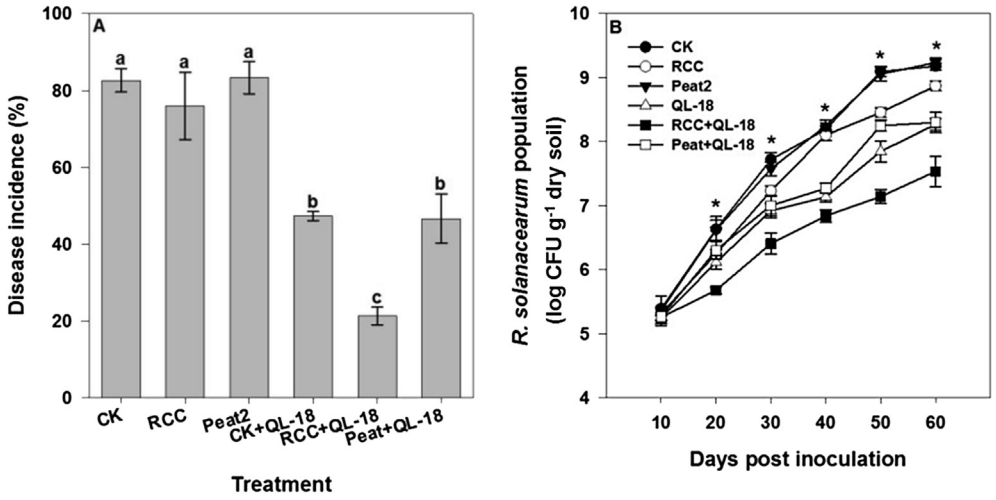

Fig. 5. The disease incidence (A) and population dynamics (B) of *R. solanacearum* in soil treated with rapeseed cake compost (RCC), peat2, QL-18, RCC + QL-18, peat + QL-18 or no amendment (CK) on different days post-inoculation. Different letters and asterisks indicate the presence of significant differences (Holm–Sidak method; $P < 0.05$) among the treatments for a particular day post-inoculation with *R. solanacearum*.

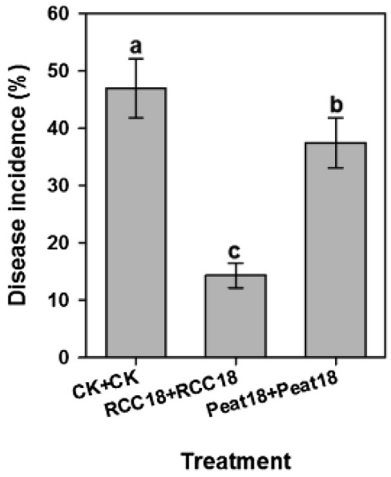

Fig. 6. The disease incidence in field plots treated with two *B. amyloliquefaciens* QL-18 formulations (RCC18 and peat18). RCC18 and peat18 refer to the mixtures of *B. amyloliquefaciens* QL-18 with RCC and peat2, respectively. The disease incidence was evaluated at the time of harvest. Tomato plants treated with RCC18 at both the seedling and transplanting stages (RCC18 + RCC18 treatment) showed the lowest disease incidence ($P < 0.001$). Different letters indicate significant differences (Holm–Sidak method; $P < 0.05$) among the treatments.

when combined with other carriers or alone. The sufficient levels of nutrients supplied by RCC prolong the survival of strain QL-18 in soil. In addition, certain components in RCC that are toxic to *R. solanacearum* might allow *B. amyloliquefaciens* QL-18 to compete with the pathogen for nutrients, further contributing to disease control. Carriers such as peat2 might prolong the shelf life of BMs; however, they cannot supply nutrients to BMs for long-term pathogen suppression.

Though RCC was demonstrated to be a good carrier for *B. amyloliquefaciens* QL-18 in the present study, it is not clear whether RCC could be used as a universal carrier for various beneficial microorganisms targeting different soilborne plant diseases; further investigations are needed to make such a general conclusion. Remarkably, the results presented here offer a very important perspective for carrier selection, as soilborne plant diseases are becoming increasingly serious (Butler, 2013; Wei et al., 2011). Future studies involving carrier screening should consider target pathogens to avoid supplying pathogens with nutrients and a substrate.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cropro.2015.05.010.

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