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Enhancement of Soil Available Nutrients and Crop Growth in Sustainable Agriculture by a Biocontrol Bacterium *Lysobacter enzymogenes* LE16: Preliminary Results in Controlled Conditions

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Abstract: The indiscriminate use of chemical fertilizers has led to adverse environmental impacts and poor crop quality and accelerates the depletion of mineral reserves used for fertilizer production. Microbes are vital in soil nutrient cycling, and some effectively enhance soil nutrient supply and reduce chemical fertilizer usage. Biocontrol bacterium *Lysobacter enzymogenes* LE16 can produce various hydrolases against plant pathogens to mineralize soil organics via enzyme production. Therefore, the enzyme production, soil organic P and N mineralization, and crop agronomic performances induced by *L. enzymogenes* LE16 were investigated by pure culture, soil incubation, and greenhouse pot experiments. *L. enzymogenes* LE16 can hydrolyze lecithin and protein and convert them to inorganic P and NH_4^+-N. Similarly, available P and N increased as this bacterium was inoculated and grown in the tested soil. In the greenhouse pot experiment, phosphomonoesterase and protease produced by *L. enzymogenes* LE16 inoculant effectively mineralized soil organic P and N and enhanced soil available nutrients, thereby improving the nutrient uptake, fertilizer utilization rate, and agronomic efficiency of lettuce and pepper seedlings. Bacterial inoculation increased the lettuce yield by 6.43–11.30% and pepper fruit yield by 43.82–70.32%, even with less chemical fertilizer application. Therefore, *L. enzymogenes* LE16 can hydrolyze lecithin and protein in pure cultures, and mineralize organic P and N in soils, thus improving crop yield and quality and reducing chemical fertilizer application via the production of phosphomonoesterase and protease. *L. enzymogenes* LE16 shows potential for sustainable agriculture beyond plant protection.

Keywords: plant growth promoting bacteria; hydrolytic enzymes; soil organic phosphorus and nitrogen; mineralization; plant growth

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1. Introduction

The increasing population pressure and demand for food have led to an agricultural system dominated by conventional and intensive practices [1–3]. To satisfy the food demand and self-sufficiency of the growing population, farming systems in many nations inevitably require the excessive use of commercial fertilizers to meet the nutrient needs of crops. For example, Chinese farmers have applied up to 600 kg ha^{-1} per year of chemical fertilizers over the last few decades [4] and currently apply approximately 70% more mineral fertilizers to their crops as compared with the rest of the world [5]. The situation is similar in Vietnam, where the number of fertilizers used increased by 7% (nitrogen), 8% (phosphorus), and 10% (potassium) per year from 1995 to 2000 [6]. As a result, the indiscriminate use of chemical fertilizers has led to significant adverse environmental impacts, such as poor soil fertility, air and groundwater pollution, greenhouse gas emissions, and decreased biodiversity [7]. Furthermore, some non-renewable mineral rocks, such as high-grade phosphate reserves used for P fertilizer production, will be depleted within 50 to 100 years, depending on the resource utilization efficiency [8,9]. Therefore,

considering all the adverse impacts of long-term fertilizer use, governmental initiatives are increasingly being developed to reduce the use of chemical fertilizers while ensuring high crop yields and agricultural sustainability. For instance, in 2015, the Ministry of Agriculture in China published the Action Plan of Zero Growth on Chemical Fertilizers by 2020, which emphasizes the need for China to restructure the fertilizer application, improve fertilizer use efficiency, and promote alternative practices to reduce the use of mineral fertilizers in agricultural systems drastically.

Nitrogen (N) and phosphorus (P) are the two most important macronutrients essential for plants. However, the N use efficiency rarely exceeds 40% in crop cultivation due to leaching, volatilization, and denitrification [10]. The utilization efficiency of P fertilizers rarely exceeds 30% because the strong fixation is related to precipitation as Ca salts in calcareous soils or adsorption on Fe and Al (hydro) oxides and/or precipitation as Fe and Al salts in acid soils [11,12]. As a result, surplus N and P fertilizers must be added to soils to sustain high crop yields. Considering the hazardous effects of excessive fertilizer, it is urgent to reduce the application of N and P fertilizers in sustainable agriculture without yield and quality losses.

The mean pool size of soil organic N is approximately 750 g m^{-2}, accounting for over 95% of total N in most agricultural soils [13,14]. Furthermore, the organic P pool accounts for an average of 587 $\pm$ 32 kg ha^{-1} in soils worldwide, which, if available for crop use, will support 117 $\pm$ 6 years of sustainable crop production [15]. Therefore, the efforts to mobilize soil organic N and P may have great potential for reducing N and P fertilizer use in sustainable agriculture. Organic N and P mineralization in soils is a microbe-driven process [16,17]. Microbial transformation of organic N and P compounds into plant-accessible forms depends on proteolytic enzymes and organophosphorus hydrolytic enzymes [16,18]. Therefore, the mineralization of soil organic N and P by microbes is an environmentally friendly and sustainable manner to enhance available nutrients for plant growth and yield.

Lysobacter spp., Gram-negative aerobic bacteria with gliding motility, is an important biocontrol agent for suppressing various pathogenic microbes, including bacteria, fungi, yeasts, algae, and nematodes [19]. *L. enzymogenes* LE16, a new biocontrol bacterium isolated from the rhizosphere of oilseed rape (*Brassica napus* L.) in Yunnan Province, China, significantly inhibits the growth of animal pathogenic bacterium *Staphylococcus aureus*, plant pathogenic bacterium *Pseudomonas syringae* pv. *tabaci*, and several plant pathogenic fungi and oomycetes, including *Colletotrichum gloeosporioides*, *Penicillium italicum*, *Alternaria alternata*, *Rhizoctonia solani*, *Didymella bryoniae*, *Sclerotinia sclerotiorum*, *Phytophthora nicotiana*, and *Phytophthora capsici* [20]. Despite their novel roles in the biocontrol of plant pathogens, serval *Lysobacter* sp. strains have been demonstrated to own the ability to promote plant growth and increase plant yield. For example, applying *L. gummosus* L101 can increase the germination rates and average yield of Styrian oil pumpkins [21]. The 1-aminocyclopropane-1-carboxylate (ACC) deaminase produced by *L. gummosus* OH17 can promote root growth in *Oryza sativa* Nipponbare plants [22]. Therefore, *Lysobacter* sp. strains may have great potential in promoting plant growth. According to our previous research, the production of hydrolytic enzymes (including protease, phosphomonoesterase, and lysozyme) plays essential roles in their plant disease control abilities [20]. Therefore, it is plausible to postulate that the protease and phosphomonoesterase produced by *L. enzymogenes* LE16 may also be involved in mineralizing soil organic N and P and stimulating plant nutrient uptake and growth. Thus, the main objectives of this study were (i) to figure out the abilities of *L. enzymogenes* LE16 in producing protease and phosphomonoesterase and hydrolyzing organic N (protein) and P (lecithin) in culture solutions, (ii) to confirm our hypothesis that *L. enzymogenes* LE16 can mineralize organic N and P and improve available nutrients in soils, (iii) to explore the mechanisms employed by this bacterium in soil organic N and P mineralization, and (iv) to provide some information on the potential use of *L. enzymogenes* LE16 as a biofertilizer in sustainable agriculture to improve soil available N and P nutrients for crops.

2. Materials and Methods

2.1. Preparation of Bacterial Inoculant

L. enzymogenes LE16 maintained on Luria–Bertani agar slants (LB culture medium; 10 g tryptone, 5.0 g yeast extract, 10 g NaCl, 15 g agar, 1.0 L water) at 4 °C were transferred into liquid LB medium (without agar) and incubated in the dark at 28 °C with constant shaking at 109× *g* for 48 h. After that, the broth culture was centrifuged at 7265× *g* for 15 min. The bacterial cells were collected from the bottom of centrifugal tubes and washed twice with 0.85% NaCl solution before being suspended in distilled water as 2 types of inoculants (approximately 1.0×10^3 and 1.0×10^9 cells mL^{-1}, respectively).

2.2. Plate Culture Experiment

1.0 µL of *L. enzymogenes* LE16 inoculum (1.0×10^3 cells mL^{-1}) was inoculated on the center of the test plate with Pikovskaya's agar medium for the detection of lecithin hydrolysis ability [23] and skim milk medium for the detection of protein hydrolysis ability [24]. Plates were incubated at 28 °C for 2 days, and the clear halos produced around colonies were positive results.

2.3. Liquid Culture Experiment

2.3.1. Hydrolysis of Lecithin in Liquid Culture Solutions

A total of 1.0 mL of the inoculant containing 1.0×10^3 cells mL^{-1} was inoculated into 50 mL of liquid-modified Pikovskaya's medium [23] in a 150 mL flask, where lecithin was the sole organic P source, and a total of 30 flasks were included. This bacterial concentration enables us to investigate the processes of bacterial growth and metabolite production in liquid cultures [25]. All flasks were then incubated at 28 °C with constant shaking at 109× *g* for 120 h, after which the bacterial suspension from 1 flask was taken every 24 h and centrifuged at 7265× *g* for 15 min to remove bacterial cells and other solids, with 5 replicate flasks for each measurement. Inorganic P in the cultures was analyzed by molybdenum blue colorimetry [26]. The supernatants from culture solutions were mixed with disodium phenyl phosphate (DPP) buffered at pH 5.0, 7.0, or 9.4, then incubated at 37 °C for 2 h. The released *ρ*-nitrophenol was detected colorimetrically at 570 nm to indicate acid, neutral, and alkaline phosphomonoesterase activities [27]. Each phosphomonoesterase activity (U) unit was expressed as µg *ρ*NP mL^{-1} solution $hour^{-1}$.

2.3.2. Hydrolysis of Protein in Liquid Culture Solutions

The ammoniation ability test medium (ATM) used in this experiment supplied bovine serum albumin (BSA) as the sole N and C source. A total of 1.0 mL of the inoculant containing 1.0×10^3 cells mL^{-1} was inoculated into 50 mL of liquid ATM in a 150 mL flask and incubated at 28 °C with constant shaking at 109× *g* for 120 h. Culture fluids were taken every 24 h and centrifuged at 7265× *g* for 15 min to remove bacterial cells, with 5 replicate flasks for each measurement. NH_4^+-N in the supernatant was analyzed by the indophenol blue spectrophotometry method [26]. The acid, neutral, and alkaline protease activity were measured based on the hydrolysis of the substrate casein buffered at pH 3.0, 7.5, and 10.0, respectively. Free tyrosine reacted with Folin's reagent to produce a blue chromophore, which was spectrophotometrically quantified at 680 nm [28]. Each unit of protease activity (U) was expressed as µg tyrosine mL^{-1} solution $minute^{-1}$.

2.4. Soil incubation Experiment

The cultivated horizon of purplish soil (Regosol, FAO Soil Classification System), which is widespread in Chongqing, Southwest China, was collected and used in this experiment. The soil with a silt loam texture and a pH of 7.1 contains 15.0 g kg^{-1} organic matter, 0.8 g kg^{-1} total N, 19.2 mg kg^{-1} NH_4^+-N, 56.4 mg kg^{-1} of NO_3^--N, 0.6 g kg^{-1} total P, 1.7 mg kg^{-1} water-soluble P, and 6.1 mg kg^{-1} Olsen-P. The air-dried soil was ground to pass through a 1 mm sieve and then placed in 250 mL flasks (50 g soil per flask). The soil in the flasks was moistened with distilled water to reach a maximum field moisture capacity

of 65 ± 2% before being autoclaved at 121 °C for 30 min. Experimental treatments included a blank control (CK, without bacterial inoculant) and the addition of 5.0 mL 1.0×10^9 cells mL^{-1} bacterial inoculant into the soil (BI) [29]. In BI treatment, bacterial inoculants were evenly mixed with soil after sterilization. Each flask was sealed with a porous film to allow air exchange and minimize water evaporation. After that, flasks were incubated at 28 ± 1 °C in the dark for 30 days, and the soil moisture was maintained gravimetrically during the incubation period. A total of 36 replicates for each treatment were included.

Six flasks were randomly chosen every six days, and the soil in two flasks was mixed as one sample for the measurement of soil Olsen P, water-soluble P, 1 N NaOH-hydrolyzable N, and NH_4^+-N by the methods of Pansu and Gautheyrou [26]. Soil phosphomonoesterase and protease activities were detected in the same manner described above, but used 2.0 g of fresh soil samples instead of culture solutions, and used soil buffered at 7.1 instead of culture solution buffered at 7.0 or 7.5. Each unit of soil phosphomonoesterase activity (U) was expressed as μg ρNP g^{-1} soil $hour^{-1}$, and soil protease activity (U) was described as μg tyrosine g^{-1} soil h^{-1}.

2.5. Greenhouse Pot Experiment

Greenhouse pot experiments were conducted with lettuce (*Lactuca sativa* L.) and pepper (*Capsicum annuum* L.) in the greenhouse of Southwest University during their growing seasons in 2019. The same soil used in the *Soil incubation experiment* was formaldehyde sterilized for 12 h to eliminate soil-borne pathogens and placed in pots (2.5 kg soil pot^{-1} for lettuce and 5.0 kg soil pot^{-1} for pepper). Specifically, the soil was placed in a plastic container; formaldehyde was added (25 mg kg^{-1} soil) and evenly mixed. After that, the container was sealed for 12 h to eliminate soil pathogens. The sterilized soil was ventilated for 30 days before being used in the greenhouse pot experiment.

Considering the actual vegetable fertilization in the local areas, the potential use of *L. enzymogenes* LE16 in sustainable agriculture to reduce the application of chemical fertilizers, the results generated from our pre-experiments, and previous studies [30,31], the greenhouse experiment in this study was designed to compare bacterial efficiency in different fertilized soils and to figure out how much chemical fertilizer can be reduced by the application of *L. enzymogenes* LE16 inoculant. Thus, experimental treatments included a blank control without chemical fertilizer or bacterial inoculant (CK treatment), a full chemical fertilizer treatment (CF), a full chemical fertilizer plus bacterial inoculant treatment (CF + BI), a 90% of full chemical fertilizer plus bacterial inoculant treatment (0.9CF + BI), an 80% of full chemical fertilizer plus bacterial inoculant treatment (0.8CF + BI), and a 70% of full chemical fertilizer plus bacterial inoculant treatment (0.7CF + BI). Each pot received 0.375 g N, 0.125 g P_2O_5, and 0.125 g K_2O (lettuce) or 1.50 g N, 0.75 g P_2O_5, and 1.50 g K_2O (pepper) in both the CF and CF + BI treatments. The chemical fertilizers used in this experiment are urea, potassium dihydrogen phosphate, and potassium sulfate, which were applied to pot soils and evenly mixed before transplanting. Compared with CF and CF + BI treatments, chemical fertilizers (including N, P_2O_5, and K_2O) supplied in 0.9CF + BI, 0.8CF + BI, and 0.7CF + BI treatments were decreased by 10%, 20%, and 30%, respectively. The nutrients supplied in CF are close to the number of fertilizers applied in local lettuce and pepper cultivation. After that, one fifteen-day-old lettuce seedling or one twenty-day-old pepper seedling was transplanted in each pot. Bacterial inoculant with approximately 1.0×10^9 cells mL^{-1} was used in this experiment [29]. Furthermore, 250 mL inoculant for lettuce and 500 mL inoculant for pepper were added into the soils around each seedling at the time of transplanting. Each 250 mL liquid inoculant contained 60.71 mg C, 4.86 mg N, and 1.53 mg P, which accounted for 0.16% of the organic matter in the experimental soil; 1.30% of N and 2.80% of P in the fertilizers applied in lettuce seedlings; and 0.65% of N and 0.93% of P in the fertilizers applied in pepper seedlings. Therefore, the effects of C, N, and P in added microorganisms on soil nutrients and plant growth are negligible. The pots were placed in a randomized complete block design, with ten replicates for each treatment. Lettuce and peppers were maintained in the greenhouse

for 40 and 120 days, respectively, with watering every 2 or 3 days. The mature pepper fruits were harvested every three days during the maturing stage.

Plant biomass and the yields of leaves (lettuce) and fruits (pepper) were weighed and recorded. Fresh lettuce leaves and pepper fruits were collected randomly for the determination of vitamin C (Vc) by 2,6-dichloroindophenol titration [32], protein by Coomassie brilliant blue spectrophotometry [33], and soluble sugar by 3,5-dinitro salicylic acid colorimetry [34]. Plant samples were dried and digested with H_2SO_4-H_2O_2. The N in the digestions was analyzed using the Kjeldahl method, P by the molybdenum blue colorimetry method, and K by flame photometry [26]. Plant nutrient uptake (including N, P, and K) was calculated as follows:

$$N \text{ (mg seedling}^{-1}) = DB \times NC$$

where N is the nutrient uptake by plants; DB is the dry biomass of each plant seedling (g); NC is the nutrient (N, P, and K) concentration of dry plant samples (mg g^{-1}).

The soil near the plant roots was collected and divided into two parts. One part of fresh soil was used to determine microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) using the fumigation-extraction method [35] and to determine phosphomonoesterase and protease activity by the methods as described in the *Soil incubation experiment* section. Another part of the soil was air-dried and grounded to pass a 1 mm sieve to measure 1 N NaOH-hydrolyzable N, NH_4^+-N, Olsen P, water-soluble P, and exchangeable K by the methods of Pansu and Gautheyrou [26].

Plant nutrient utilization rate (N, P, and K) was calculated as follows:

$$NUR \text{ (\%)} = (N_F - N_0) \times {F_F}^{-1} \times 100\%$$

where NUR is the nutrient utilization rate of plants; N_F and N_0 are the nutrients uptake (mg seedling^{-1}) with and without fertilizers, respectively; F_F is the number of nutrients (mg seedling^{-1}) in the fertilizers supplied.

The agronomic efficiency of N, P, and K nutrients was calculated as follows [36]:

$$AEN \text{ (g g}^{-1}) = (Y_F - Y_0) \times {F_F}^{-1}$$

where AEN is the agronomic efficiency of nutrients; Y_F and Y_0 are the economic yield (g seedling^{-1}) with and without fertilizers, respectively; F_F is the number of nutrients (g seedling^{-1}) in the fertilizers supplied.

2.6. Data Treatments

Statistical analysis and graphing were conducted using the SPSS 21.0 statistical software package (SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2016 (Microsoft Inc., Redmond, WA, USA). The treatment effects were evaluated by analysis of variance (ANOVA) using the SPSS, and significant differences between treatment means were tested by Fisher's protected least significant difference (LSD) (* $p < 0.05$ or ** $p < 0.01$). Linear correlation coefficients among variables in the soil incubation were calculated by Pearson's correlation analysis ($p < 0.01$). Pearson correlation coefficients among phosphomonoesterase, protease, SQI (soil quality index; the average value of soil 1 N NaOH-hydrolyzable N, NH_4^+-N, Olsen P, water-soluble P, exchangeable K, MBC, MBN, phosphomonoesterase activity, and protease activity), PQI (plant quality index; the average value of plant biomass, economic yield, N uptake, P uptake, K uptake, Vc, protein, and soluble sugar), and PNUI (plant nutrients utilization index; the average value of plant N utilization rate, P utilization rate, K utilization rate, agronomic efficiency of N, agronomic efficiency of P, and agronomic efficiency of K) in the greenhouse pot experiment were determined using all replicates.

3. Results

3.1. Hydrolysis of Lecithin and Protein by L. enzymogenes LE16

Clear halos were produced around the colony on the plate containing Pikovskaya's agar medium (Figure 1A) and skim milk medium (Figure 1B). The result suggested the successful hydrolysis of organic P and N compounds contained in the lecithin and protein by *L. enzymogenes* LE16.

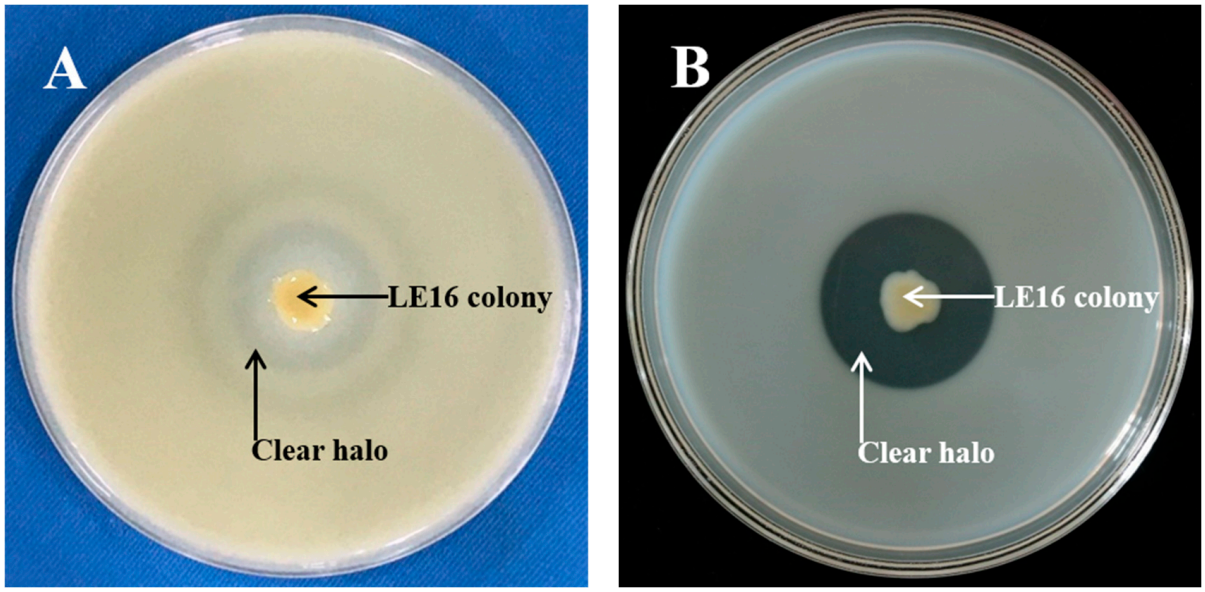

Figure 1. Hydrolysis of lecithin (**A**) and protein (**B**) by *L. enzymogenes* LE16.

3.2. Extracellular Hydrolytic Enzymes Produced by L. enzymogenes LE16

The bacteria can simultaneously produce extracellular acid, neutral, and alkaline phosphomonoesterases in culture solution (Figure 2A). The enzyme activities increased quickly in the early incubation stages and reached the maximum values for the acid enzyme (223 U) at 36 h, the neutral enzyme (115 U) at 24 h, and the alkaline enzyme (136 U) at 48 h after inoculation. After that, the phosphomonoesterase activities slightly decreased during the late incubation period. Inorganic P released from lecithin by *L. enzymogenes* LE16 in culture solution also increased quickly with time in the early incubation stages, but slowed down in the late incubation stages (Figure 2B). During the whole incubation period, inorganic P released from lecithin was positively correlated with phosphomonoesterase activities throughout the entire incubation period (r_{Acid} = 0.797 **, $r_{Neutral}$ = 0.706 **, $r_{Alkaline}$ = 0.668 **, n = 30, $p < 0.01$).

L. enzymogenes LE16 can produce acidic, neutral, and alkaline proteases and hydrolyze BSA in the culture solution (Figure 2C,D). In the early incubation period, the activities of the 3 proteases increased quickly with incubation time and reached 149.8 U (acid), 187.4 U (neutral), and 169.4 U (alkaline) after 48 h of inoculation. After that, the changes in protease activities were minimal. The NH_4^+-N concentration was changed in a similar trend to protease activities and increased from 0 at the initial to 128.9 mg L^{-1} at the end of incubation (Figure 2D). Additionally, the NH_4^+-N released from BSA positively correlated with protease activities (r_{Acid} = 0.899 **, $r_{Neutral}$ = 0.826 **, $r_{Alkaline}$ = 0.858 **, n = 30, $p < 0.01$).

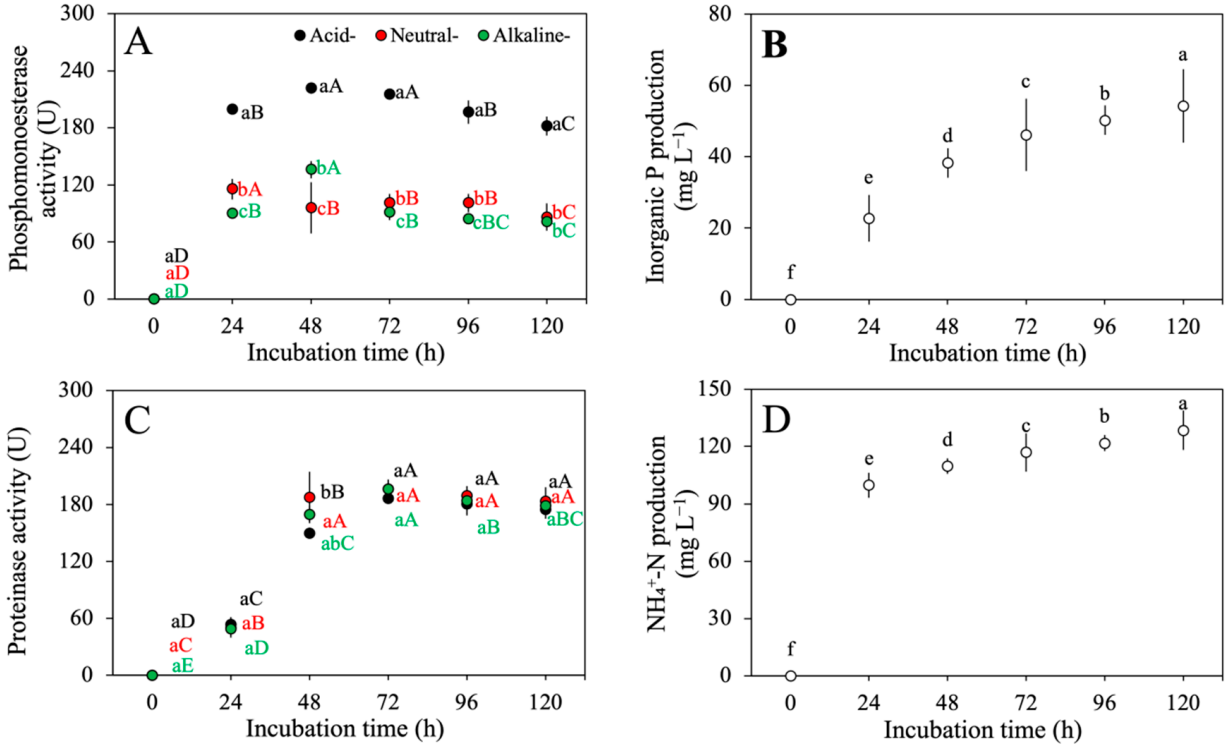

Figure 2. Changes in phosphomonoesterase activity (**A**), inorganic P released from lecithin (**B**), protease activity (**C**), and NH_4^+-N released from BSA (**D**) in culture solutions with *L. enzymogenes* LE16 inoculation. Data are the means of six replicates. Bars represent the SD. Uppercase letters represent significant differences in the same enzyme activity at different times. Lowercase letters indicate differences among the three enzyme activities simultaneously ($p < 0.05$, LSD test).

3.3. Changes in Soil P and N after Inoculation with L. enzymogenes LE16

Phosphomonoesterase activity, protease activity, available P (including Olsen-P and water-soluble P), and available N (including 1 N NaOH-hydrolyzable N and NH_4^+-N) remained almost unchanged in blank control soils (no bacterial inoculation) during the incubation (Figure 3). In contrast, an increasing trend of phosphomonoesterase activities, available P, and available N was observed in the inoculated soils. Compared with the blank control, inoculated soil showed a higher soil phosphomonoesterase activity, which is positively correlated with soil Olsen-P ($r = 0.929$ **, $n = 18$, $p < 0.01$) and water-soluble P ($r = 0.870$ **, $n = 18$, $p < 0.01$) (Figure 4A,B). Similarly, protease activity was positively correlated with 1 N NaOH-hydrolyzable N ($r = 0.811$ **, $n = 18$, $p < 0.01$) and NH_4^+-N ($r = 0.638$ **, $n = 18$, $p < 0.01$) in the soils inoculated with *L. enzymogenes* LE16 (Figure 4C,D).

3.4. Effects of the Test Bacterial Inoculant on Lettuce and Pepper Performances in the Greenhouse Pot Experiment

Compared to applied CF alone, *L. enzymogenes* LE16 inoculation plus CF (including CF + BI, 0.9CF + BI, 0.8CF + BI, and 0.7CF + BI) significantly increased nutrient uptake (including N, P, and K) and the fertilizer utilization rate (including N, P, and K) of both lettuce and pepper seedlings (Table 1). Their biomass were increased by 6.93–11.58% (lettuce) and 19.16–28.04% (pepper), and the economic yield increased by 6.43–11.30% (lettuce) and 43.82–70.32% (pepper) compared with those by CF treatment.

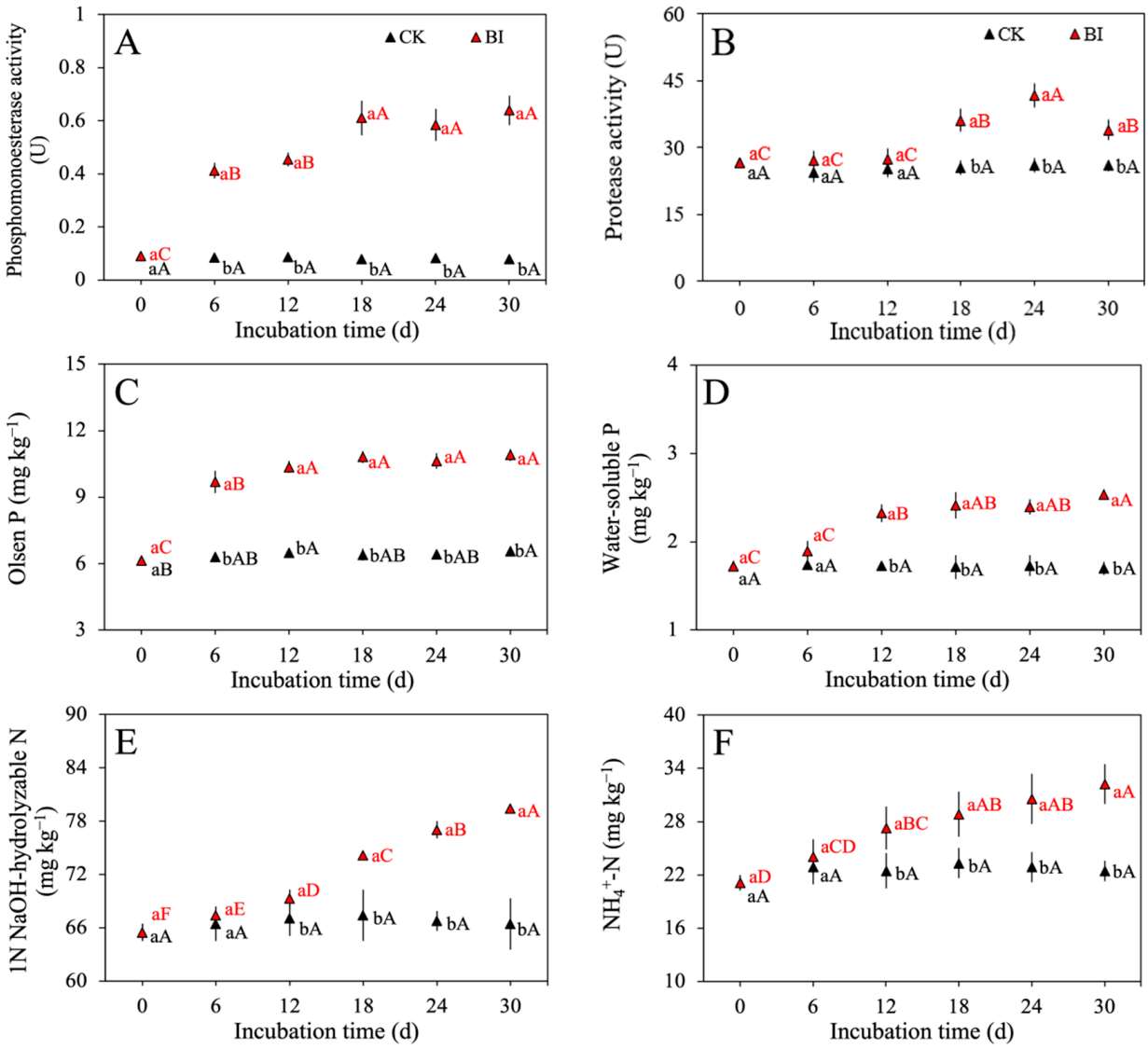

Figure 3. Changes in phosphomonoesterase activity (**A**), protease activity (**B**), Olsen P (**C**), water-soluble P (**D**), 1 N NaOH-hydrolyzable N (**E**), and NH_4^+-N (**F**) in the black control soil (CK) and bacterial inoculated soil (BI). Uppercase letters represent significant differences in the same treatment at different times. Lowercase letters indicate treatment differences simultaneously ($p < 0.05$, LSD test).

Table 1. Effects of *L. enzymogenes* LE16 inoculation on plant biomass, economic yield, nutrient uptake, and nutrient utilization rate.

Crops	Treatments	Biomass Increase Compared to CF (%)	Economic Yield Increase Compared to CF (%)	Nutrient Uptake (mg seedling^{-1})			Nutrient Utilization Rate (%)		
				N	P	K	N	P	K
Lettuce	CK	-	-	59.58 e	13.93 c	140.1 d	-	-	-
	CF	-	-	148.6 d	16.21 b	170.5 c	23.72 e	4.18 b	29.26 c
	CF + BI	11.58 a	11.30 a	154.2 c	18.87 a	205.0 b	25.22 d	9.04 a	62.50 b
	0.9CF + BI	7.44 b c	7.56 b	164.6 a	18.82 a	220.4 a	31.13 c	9.95 a	86.05 a
	0.8CF + BI	6.93 c	6.43 c	168.9 a	18.14 ab	217.0 a	36.45 b	9.64 a	92.62 a
	0.7CF + BI	8.28 b	7.91 b	159.2 b	17.30 ab	207.3 b	37.96 a	8.81 a	92.53 a
Pepper	CK	-	-	444.6 d	125.4 d	861.7 d	-	-	-
	CF	-	-	1468 c	208.9 c	1579 c	68.20 d	25.50 e	57.60 e
	CF + BI	19.67 b	44.55 b	1853 b	266.4 b	1885 b	93.88 c	43.06 d	82.23 d
	0.9CF + BI	19.16 b	43.82 b	1865 b	264.6 b	1923 b	105.22 c	47.24 c	94.74 c
	0.8CF + BI	27.86 a	63.66 a b	2158 a	309.0 a	2097 a	142.77 b	70.07 a	124.0 a
	0.7CF + BI	28.04 a	70.32 a	2212 a	271.4 b	1880 b	168.35 a	63.68 b	116.9 b

In each column, means followed by different lowercase letters indicate significant differences for each plant type at $p < 0.05$.

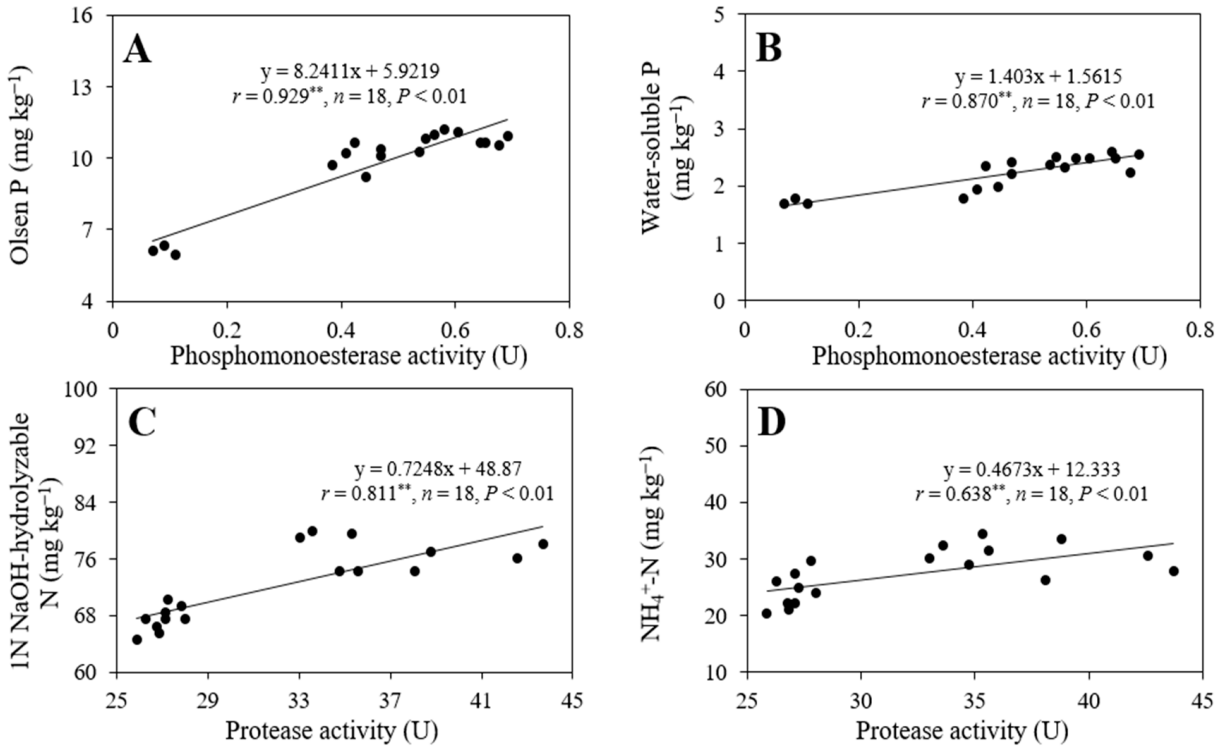

Figure 4. Pearson's correlation analysis between soil phosphomonoesterase activity and Olsen P (**A**) and water-soluble P (**B**), soil protease activity and soil 1N NaOH-hydrolyzable N (**C**) and NH_4^+-N (**D**) after inoculation with *L. enzymogenes* LE16. ** indicates significant result of the correlation analysis.

Lettuce leaves contained the highest protein and soluble sugar in CK, followed by 0.7CF + BI, while CF contained the lowest Vc and protein content (Table 2). Compared with CF alone, *L. enzymogenes* LE16 inoculation plus CF (including CF + BI, 0.9CF + BI, 0.8CF + BI, and 0.7CF + BI) increased lettuce Vc and protein content by about 7.08–30.59% and 27.70–56.32%, respectively. For pepper seedlings, pepper fruit contained the lowest Vc and soluble sugar content in CF. In contrast, *L. enzymogenes* LE16 inoculation plus CF significantly increased the Vc, protein, and soluble sugar content of pepper fruit by 19.98–36.19%, 35.99–54.50%, and 20.42–62.61%, respectively, compared with CF treatment (Table 2).

Table 2. Effect of *L. enzymogenes* LE16 inoculation on the quality of lettuce leaf and pepper fruits.

Treatments	Lettuce			Pepper		
	Vc (mg 100 g^{-1})	Protein (mg g^{-1})	Soluble Sugar (mg g^{-1})	Vc (mg 100 g^{-1})	Protein (mg g^{-1})	Soluble Sugar (mg g^{-1})
CK	23.68 d	10.88 a	14.09 a	72.96 b c	3.36 d	16.64 a
CF	22.59 e	6.57 f	9.34 c	59.21 d	3.89 c	8.96 d
CF + BI	24.19 d	9.09 d	9.57 c	80.64 a	6.01 a	12.58 c
0.9CF + BI	29.50 a	8.39 e	9.28 c	76.16 a b	6.00 a	14.57 b
0.8CF + BI	27.46 c	9.65 c	8.80 c	71.04 c	5.46 b	11.19 c
0.7CF + BI	28.29 b	10.27 b	11.17 b	78.08 a	5.29 b	10.79 c

In each column, means followed by different lowercase letters indicate significant differences for each plant type at $p < 0.05$.

As shown in Figure 5, CF + BI significantly increased the agronomic efficiency of nutrients (AEN, including N, P, and K) for both lettuce and pepper seedlings. AEN is presented in the sequence of 0.7CF + BI > 0.8CF + BI > 0.9CF + BI > CF + BI > CF (no significant difference among 0.8CF + BI, 0.9CF + BI, and CF + BI for lettuce and between

0.9CF + BI and CF + BI for pepper). Compared with CF, AEN in the inoculated soils increased by 40.45–83.67% (lettuce) and 74.13–209.9% (pepper).

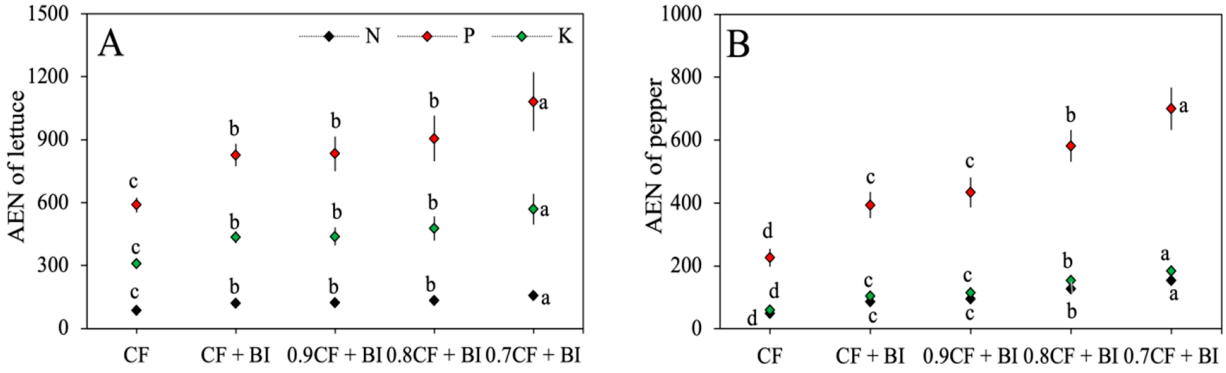

Figure 5. Agronomic efficiency of lettuce (**A**) and pepper (**B**) nutrients. Different lowercase letters indicate significant differences for each nutrient type between different treatments at $p < 0.05$.

3.5. Effects of the Test Bacterial Inoculant on Plant-Grown Soil Properties

As shown in Table 3, soils with bacterial inoculation contained higher available N content (including 1 N NaOH-hydrolyzed N and NH_4^+-N) than those that received CF alone (not significant in the lettuce-grown soil among CF, CF + BI, and 0.9CF + BI and pepper-grown soil between CF and CF + BI). Compared with the blank control, both fertilization and bacterial inoculation increased soil available P content (including Olsen P and water-soluble P), which ranged from 7.74 mg kg^{-1} to 9.73 mg kg^{-1} in the lettuce-grown soil and from 4.80 mg kg^{-1} to 6.06 mg kg^{-1} in the pepper-grown soil. There was no considerable difference in soil available K content, which ranged from 48.42 mg kg^{-1} to 53.51 mg kg^{-1} in lettuce-grown soil and from 23.01 mg kg^{-1} to 29.97 mg kg^{-1} in pepper-grown soil. Protease and phosphomonoesterase activities were highest in inoculated soils, while protease was lowest in soils with CF alone.

Table 3. Effect of *L. enzymogenes* LE16 inoculation on available nutrients and enzyme activity of plant rhizosphere soils.

Plants	Treatments	Available Nutrients (mg kg^{-1})			Protease (U)	Phosphomonoesterase (U)
		N	P	K		
Lettuce	CK	65.89 c	5.26 d	50.96 ab	36.22 b	0.72 b
	CF	65.97 c	7.74 c	50.96 ab	29.06 c	0.74 b
	CF + BI	65.66 c	9.30 a	53.51 a	40.53 ab	1.02 a
	0.9CF + BI	67.02 bc	9.55 a	48.42 b	42.76 ab	1.12 a
	0.8CF + BI	69.09 ab	9.73 a	50.96 ab	44.49 a	1.11 a
	0.7CF + BI	69.78 a	8.70 b	50.12 ab	45.16 a	1.13 a
Pepper	CK	37.35 c	2.91 d	26.76 b	54.89 c	1.21 c
	CF	37.66 bc	5.91 ab	29.976 a	44.06 d	0.99 d
	CF + BI	38.89 b	6.06 a	27.296 b	60.53 bc	1.50 b
	0.9CF + BI	41.74 a	5.65 b	27.83 ab	62.76 ab	1.54 ab
	0.8CF + BI	42.15 a	4.97 c	27.83 ab	65.82 ab	1.61 ab
	0.7CF + BI	42.54 a	4.80 c	23.01 c	68.16 a	1.64 a

Available N = 1 N NaOH-hydrolyzed N + NH_4^+-N; available P = Olsen P + water-soluble P; available K = 1 N ammonium acetate-extracted K. In each column, means followed by different lowercase letters indicate significant differences for each plant type at $p < 0.05$.

The greenhouse pot experiment showed that soil protease and phosphomonoesterase activities were significantly and positively correlated with SQI, PQI, and PUNI for both lettuce and pepper seedlings (r_{SQI} = 0.585 **–0.765 **, n = 60, $p < 0.01$; r_{PQI} = 0.485 *–0.807 **, n = 60, * $p < 0.05$, ** $p < 0.01$; r_{PUNI} = 0.796 **–0.863 **, n = 50, $p < 0.01$) (Table 4).

Table 4. Pearson correlation coefficients between enzyme activities and relevant indexes (SQI, PQI, and PNUI) of the greenhouse pot experiment.

Index	Lettuce				Pepper			
	Protease	Phosphomonoesterase	SQI	PQI	Protease	Phosphomonoesterase	SQI	PQI
Phosphomonoesterase	0.785 **				0.899 **			
SQI	0.585 **	0.765 **			0.627 **	0.757 **		
PQI	0.485 *	0.807 **	0.787 **		0.544 *	0.677 **	0.963 **	
PNUI	0.796 **	0.863 **	0.792 **	0.865 **	0.847 **	0.845 **	0.819 **	0.931 **

SQI = Soil quality index, the average of soil 1 N NaOH-hydrolyzable N, NH4+-N, Olsen P, water-soluble P, exchangeable K, MBC, and MBN; PQI = Plant quality index, the average of plant biomass, economic yield, N uptake, P uptake, K uptake, Vc, protein, and soluble sugar; PNUI = Plant nutrients utilization index, the average of plant N utilization rate, P utilization rate, K utilization rate, agronomic efficiency of N, agronomic efficiency of P, and agronomic efficiency of K. Significant correlations at * $p < 0.05$ and ** $p < 0.01$ (n = 60; except for PNUI, n = 50).

4. Discussion

Organic P can be up to 50% of the total insoluble P in many agricultural soils [37]. The primary forms of organic P in soils are inositol phosphate-like P, monoester phosphate-like P, diester phosphate-like P, and nucleic acid-like P [38,39]. The mobilization of organic P in soils refers to the dephosphorization of organic P compounds to release inorganic P available for plants and the degradation of the rest to small molecular organics. The dephosphorization reactions of P-containing organics are catalyzed by a group of enzymes that may be specific for a single compound or have broad specificity for numerous compounds [40,41]. For example, phytase sequentially hydrolyzes inositol hexaphosphate to various lower-order inositol phosphates and monoester phosphates at a pH of around 7.5. Acid, neutral, and alkaline phosphomonoesterases catalyze hydrolysis reactions of phosphomonoesters, nucleic acids, low-order inositol phosphates, and phosphoproteins at various pH values [40–43]. In the present study, the production of acid, neutral, and alkaline phosphomonoesterases by *L. enzymogenes* LE16 (Figure 2A) implied that this bacterium could produce not only phosphomonoesterase, but also phosphodiesterase (using phosphodiester lecithin) and could potentially hydrolyze phosphomonoesters and simple inositols in soils over a wide pH range. Extracellular phosphomonoesterases produced by the bacterium resulted in the concomitant release of inorganic P from lecithin in culture solutions (Figure 2B). Therefore, the phosphomonoesterase produced could be the mechanism of *L. enzymogenes* LE16 involved in the hydrolysis of organic P, such as many other bacteria reported by Maseko and Dakora [37], Wei et al. [44], and Wu et al. [45]. Moreover, phosphomonoesterase activities slightly decreased after reaching the maximum, while the inorganic P increased slowly in the culture solution (Figure 2A,B). That is, the phosphomonoesterases produced by *L. enzymogenes* LE16 could be suppressed by high inorganic P concentrations, which is consistent with our previous study [46] and the result found by Von Tigerstrom [47]. Plant roots can produce acid phosphomonoesterase, but rarely neutral and alkaline phosphomonoesterase [48–52]. The production of acid, neutral, and alkaline phosphomonoesterases by *L. enzymogenes* LE16 should be important in organic P mobilization and plant P supply in soils at wide pH ranges.

Soil organic N exists mainly in the form of proteins that bind with mineral particles or other organic macromolecular compounds, such as polysaccharides and humus. During N mineralization in soils, proteolysis is considered a rate-limiting step due to the much slower primary phase of protein mineralization than amino acid ammonification [53,54]. As a result, proteases are highly diverse and ubiquitous in soils and provide a large proportion of bioavailable N [55,56]. According to the current experiment, the concentration of NH_4^+-N increased in the liquid mediums during incubation (Figure 2D), which suggested the production and discharge of amino acid dehydrogenases or amino acid oxidases by *L. enzymogenes* LE16, in addition to proteases. Furthermore, protease activities were positively correlated with NH_4^+-N production (r = 0.826 **–0.899 **, n = 30, $p < 0.01$), indicating the effective hydrolysis of organic N by *L. enzymogenes* LE16, such as many other bacteria [57,58].

Plant growth-promoting rhizobacteria (PGPRs) have diverse roles in crop cultivation, including the production of plant growth-promoting substances, solubilization of insoluble nutrients in soils (notably P and K), mineralization of macromolecular organics, and fixation of atmospheric N [59,60]. Applying PGPRs can be considered an alternative to chemical fertilizers for sustainable agriculture [61,62]. In the present study, phosphomonoesterase and protease activities in the inoculated soils increased with time. At the same time, they remained almost unchanged in the soils without bacterial inoculation during the incubation (Figure 3). The soil used for the incubation experiment was sterilized by autoclaving at 121 °C for 30 min before inoculation, suggesting almost all microorganisms were killed. Thus, the increases in enzyme activities in the inoculated soils should be derived from the bacterium inoculated, indicating the successful survival and multiplication of *L. enzymogenes* LE16 in the tested soil. Soil available P and N also increased with time after inoculation (Table 3), which implied that *L. enzymogenes* LE16 releases more available P and N from soil organic compounds than bacterial utilization. If this phenomenon persists, plant available P and N in soils will increase after applying *L. enzymogenes* LE16 inoculant. Furthermore, positive correlations were recorded between phosphomonoesterase activity and available P and between protease activity and available N in the inoculated soils (Figure 4). These results confirmed the involvement and direct contribution of *L. enzymogenes* LE16 to the mineralization of organic P and N in the tested soils by producing phosphomonoesterase and protease.

Generally, genotype (or heredity) and environments (including climate and nutrients) determine crop yield and quality. Nutrient supply is critical to increase yield and improve crop quality under certain climate conditions [31]. Compared with CF alone, *L. enzymogenes* LE16 plus CF significantly increased plant nutrient uptake, utilization rates, and crop yields, even with fewer chemical fertilizers supplied (Table 1). Similar results have been obtained from other identified PGPRs, i.e., *Neosartorya fischeri* [63], *Bacillus megaterium* [64], and *Aspergillus* sp. [65]. Plants absorb nutrients in a balanced way [66]. The improved crop N and P uptake may also promote crop K uptake and the utilization rate in the treatments inoculated with *L. enzymogenes* LE16. Therefore, such as other PGPRs strains, the application of *L. enzymogenes* LE16 can effectively increase the crop nutrient uptake, utilization rate, and yields, which may also be the reason for changes in the quality of lettuce leaf and pepper fruit (Table 2). The AEN is essential for evaluating fertilizer productivity and utilization efficiency [67]. In the present study, the AEN of lettuce and pepper increased as the proportion of CF decreased in fertilizer treatments (Figure 5), resulting in the highest AEN in 0.7CF + BI treatments, which suggests that plants accumulated more nutrients in the CF plus *L. enzymogens* LE16 inoculation treatment than in the treatment of CF alone. Therefore, it is reasonable to postulate that N losses and P fixation in the fertilized soils receiving the bacterial inoculant are lower than in those not receiving it, which supports the conclusion that high agronomic nutrient efficiency often implies high crop yields, but low fertilizer losses [68].

The available soil nutrients (including N, P, and K) measured at crop harvest could be considered the consequent balance among soil fixation, plant absorption, and nutrient mobilization throughout plant growth. In the present study, soil available nutrients increased or remained unchanged in the treatments receiving bacterial inoculation compared with those in the CF treatment (Table 3). Considering the increased nutrient uptake by lettuce and pepper plants and higher soil available nutrients in the harvested soils, it is reasonable to suggest that *L. enzymogenes* LE16 inoculation released more available P and N from soil organic compounds for plant uptake. These results indicated again the inoculated bacterium survives and reproduces in plant-grown soils, and the increased enzyme activities could reflect the active transformation of soil organic compounds into plant available P and N [69]. The significant positive correlations between these two enzyme activities (phosphomonoesterase and protease) and SQI, PQI, and PUNI for both lettuce and pepper seedlings (Table 4) further demonstrated the application of *L. enzymogenes* LE16 in sustainable agriculture to improve crop yield and quality and reduce chemical

fertilizer application. Furthermore, in the present greenhouse pot experiment, plants were well-managed and not infected with pathogens throughout the growth period. Thus, the increase in growth promotion and yields and quality improvement of lettuce and pepper plants could be attributed mainly to the soil organic P and N mineralization by *L. enzymogenes* LE16 via the production of phosphomonoesterase and protease.

5. Conclusions

Lysobacter spp. has long been explored as a biocontrol agent for plant diseases. In the present study, *L. enzymogenes* LE16 hydrolyzed organic N and P compounds in the liquid culture medium and increased soil available N and P via the production of phosphomonoesterase and protease. Lettuce and pepper nutrient uptake, nutrient utilization rate, economic yield, and plant quality were notably enhanced after *L. enzymogenes* LE16 inoculation, and these results were positively correlated with the phosphomonoesterase and protease produced by *L. enzymogenes* LE16. Applying this bacterium under fertilization conditions can reduce the application of chemical fertilizers without sacrificing yield and quality. Our study persuasively demonstrates the potential use of *L. enzymogenes* LE16 as a biofertilizer in sustainable agriculture beyond plant protection. Our future work may focus on evaluating the plant growth promotion effect of *L. enzymogenes* LE16 inoculated into unsterilized soils and field conditions.

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