horticulturae

MDPI

Article

Isolation and Identification of *Lysinibacillus* sp. and Its Effects on Solid Waste as a Phytate-Mineralizing Bacterium in an Aquaponics System

Edna Rocio Riaño-Castillo [1], Juan Carlos Rodríguez-Ortiz [1], Hye-Ji Kim [2], María de la Luz Guerrero González [1], María Fernanda Quintero-Castellanos [1] and Pablo Delgado-Sánchez [1,*]

[1] Faculty of Agronomy and Veterinary, Universidad Autónoma de San Luis Potosí, Soledad de Graciano Sánchez 78321, SLP, Mexico; ednarociorc@gmail.com (E.R.R.-C.); juancarlos.rodriguez@uaslp.mx (J.C.R.-O.); luz.guerrero@uaslp.mx (M.d.l.L.G.G.); maria.quintero@uaslp.mx (M.F.Q.-C.)

[2] Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 47907, USA; hyejikim@purdue.edu

* Correspondence: pablo.delgado@uaslp.mx; Tel.: +52-(444)-8261314 (ext. 1141)

Abstract: Sedimentable solids generated in aquaponic systems are mainly composed of organic waste, presenting molecules such as phytate, which can be a potential source of inorganic nutrients through mineralization. This work aimed to isolate and identify phytase-producing bacteria and evaluate the inoculation effects of pure strains on mineralization and nutrient release from solid waste generated in aquaponic systems at different oxygen and temperature conditions. The bacteria were isolated from the settleable solids of a commercial aquaponic system and molecularly identified by amplifying the 16S rRNA gene. Subsequently, two tests were carried out: 1. Test for the biochemical identification of phytase-producing bacteria; 2. In vitro mineralization test, where the ability to mineralize phytate and release nutrients under different oxygen conditions [0 rpm (2.1 mg L^{-1}) and 200 rpm (7.8 mg L^{-1})] and temperatures (24 and 37 °C) were evaluated. Our findings show that two pure strains of *Lysinibacillus mangiferihumi* can mineralize phytate under conditions of 200 rpm and 24 °C, mainly increasing the mineralization of PO4- and Ca, a property that has not yet been reported for this species. On the other hand, at 0 rpm and 24 °C, an increase in K was observed (control conditions), while the conditions of 200 rpm and 24 °C, regardless of bacterial inoculation, favored a rise in S, Mg, and Fe. The *Lysinibacillus* strains obtained in this investigation are of great importance due to their application in agriculture and the optimization of mineralization in aquaponic systems. A proper combination of oxygen and temperature will lead to a greater availability of nutrients for the growth and development of vegetables.

Keywords: phytate; plant nutrition; plant development; phosphate; calcium

Citation: Riaño-Castillo, E.R.; Rodríguez-Ortiz, J.C.; Kim, H.-J.; Guerrero González, M.d.l.L.; Quintero-Castellanos, M.F.; Delgado-Sánchez, P. Isolation and Identification of *Lysinibacillus* sp. and Its Effects on Solid Waste as a Phytate-Mineralizing Bacterium in an Aquaponics System. *Horticulturae* **2024**, *10*, 497. https://doi.org/10.3390/horticulturae10050497

Academic Editor: Nikolaos Katsoulas

Received: 11 April 2024
Revised: 7 May 2024
Accepted: 8 May 2024
Published: 11 May 2024

1. Introduction

Aquaponics is an integrated production of fish and plants in which, in symbiosis with microorganisms, effluent and fish wastes are used as a source of nutrients for plant growth [1,2]. In aquaculture systems, most fish metabolize only 20 to 30% of the food supplied, and 70 to 80% is released into the systems as solid waste (dissolved, suspended, and sedimentable), composed mainly of excreted feces and uneaten food [3]. The solid waste generated in these systems is usually collected in mechanical filters (hydrocyclones and settlers) before being pumped to a hydroponic unit. Part of the dissolved nutrients in water can be assimilated by plants (i.e., inorganic forms), while suspended and sedimentable solid waste, composed mainly of organic waste (non-assimilable forms), can be a potential source of inorganic nutrients for plants to be mineralized [4,5].

The major component of fish diets is protein, which is mainly derived from vegetable products such as soy, wheat, and corn, and which stores 70 to 80% of total phosphorus in phytic acid or phytate [6]. It is estimated that 67% of phosphorus in fish feed pellets is phytic acid [7].

Phytic acid is a molecule that is negatively charged. It has a high affinity to some metal ions, forming a chelate with Ca^{2+}, Fe^{2+}, Zn^{2+}, Cu^{2+}, and Mn^{2+}, which reduces the absorption of these minerals in monogastric animals, including fish [6]. Since the digestive tracts of fish do not produce the enzyme phytase [6,8], the ingested phytate is excreted into feces, remaining deposited in the sediments [8]. Thus, part of the phosphorus is not fully available as a nutrient for plants in aquaponics since it is not found in its ionic form as orthophosphate (i.e., $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}).

Phytase is an enzyme that catalyzes the hydrolysis of the phosphodiester bonds in phytate, liberating orthophosphate groups (PO_4^-) [9]. Some studies have found that bacteria with the ability to produce phytases can also promote plant growth through the production of indole acetic acid (IAA) and siderophores [10]. The identification of phytase-producing bacteria is critical since they may play an essential role in mineralizing the phytate present in solid waste in systems, increasing the availability of phosphorus and possibly also some elements that can be chelated with the metal ions. In addition, these bacteria may contribute to reducing the impact caused in traditional aquaculture and closed recirculating aquaculture systems (CRASs), where the solid waste containing high N and P contents generated in the systems is released to the environment, contributing to the eutrophication of receiving water bodies [11].

Several bacteria such as *Bacillus*, *Klebsiella terrigenous*, *Escherichia coli*, *Pseudomonas* sp., *Roultella* sp., *Citrobacter braakii*, and *Enterobacter* have been identified as phytate mineralizers in previous studies, which have reported that the activity of the phytase enzyme could be affected by different conditions, such as pH, Ca^{2+} requirement, nitrogen and carbon sources, growth phases (stationary phase), and anaerobic conditions [12–14]. However, limited studies have been carried out in aquatic habitats, and whether there are other microorganisms with the same capability to mineralize and degrade phytate in solid waste from aquaculture and recirculating aquaponic systems (RASs) is still under investigation [4,8,15,16].

In aquaponics, investigating the biologically induced mineralization and degradation of the phytate present in solids will allow for the establishment of macro- and micronutrients that can be released into the system to optimize plant nutrition and decrease the use of fertilizers [17]. This work aimed to isolate and identify phytase-producing bacteria from a commercial aquaponic system and evaluate their effect on phytate mineralization and nutrient release from solid waste under different oxygen and temperature conditions.

2. Materials and Methods

2.1. Isolation and Identification of Phytate-Mineralizing Bacteria

Samples of solid waste were collected (March 2018) from the hydrocyclone and plant bed of a commercial aquaponic system with tilapia (*Oreochromis niloticus*) and various vegetables (lettuce, basil, and mint) located in the aquaponic facilities at Acuaponia Bofish, Guadalajara, JAL, México. The samples were transported and stored at 4 °C in the Biotechnology Lab at the Faculty of Agronomy and Veterinary—Universidad Autónoma de San Luis Potosí (UASLP), México. One gram of each sample was homogenized in nine mL of liquid medium Luria–Bertani (LB) broth (IBI Scientific®, Dubuque, IA, USA), and serial dilutions were performed to obtain 10^6 dilutions. The samples were incubated at 37 °C for 24 h and 72 h, and then 0.1 mL of each dilution was pour-plated on LB solid agar medium and incubated at 37 °C for 24 to 48 h. These primary cultures were used to carry out a second round of isolations and thus obtain pure cultures, which were conserved in 15% glycerol and stored at −80 °C. Macroscopic tests to identify the morphological characteristics of the colonies (color, shape, elevation, margins, diameter, surface, and texture), Gram staining, and tests of pathogenicity or hemolytic capacity in blood agar (to discard strains with hemolysis capacity) were carried out, identifying sixty pure strains.

2.2. Taxonomic Classification of Bacteria at the Molecular Level

The molecular identification of seven pure strains was performed by amplifying and sequencing the 16S rRNA gene. For that, genomic DNA was extracted using the CTAB method described by Wilson (1997) [18]. PCR amplification from 16S rRNA was performed using the primers QUGP-F1 5′-AGTTTGATCCTGGCTCTCAG-3′ and QUGP-R1b 5′-TACCTTGTTACGACTTC-3′ [19], with an initial denaturalization at 94 °C for 3 min followed by 35 cycles of 94 °C for 1 min; 60.7 °C for 35 s; 72 °C for 1 min; and a final extension step of 72 °C for 5 min. The PCR products were separated by electrophoresis in 1.5% agarose. The PCR products were purified with Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) and then sequenced and analyzed for their taxonomic classification at the genus level in the GenBank database (MW035708, MW 035604, MW029858, MW029733) using BLAST (http://www.dna.affrc.go.jp accessed on 24 September 2020).

2.3. Test for the Biochemical Identification of Phytase-Producing Bacteria

Seven strains of *Lysinibacillus* sp. were individually evaluated by triplicate in phytase screening medium (PSM: glucose 10 g L^{-1}, sodium phytate 4 g L^{-1}, $CaCl_2$ 2 g L^{-1}, NH_4NO_3 5 g L^{-1}, KCl 0.5 g L^{-1}, $MgSO_4$ H_2O 0.5 g L^{-1}, $FeSO_4$ $7H_2O$ 0.01 g L^{-1}, $MnSO_4$ H_2O 0.01 g L^{-1}, and agar 15 g L^{-1}; IBI Scientific®, Dubuque, IA, USA) described by Kerovuo et al. (2000) [20]. The strains were incubated at 37 °C for 4 days, and colonies showing halo formation were selected as a positive indication of extracellular phytase production. The diameter of each halo was measured, and the solubilization index of mineralization (IM) was calculated using the formula: IM = (Colony diameter + diameter in halo zone)/Colony diameter. In addition, the growth kinetics of these positive strains were assessed by measuring the optical density of the PSM medium every twelve hours for 120 h at pH 7.0 and 37 °C with shaking at 200 revolutions per minute (rpm).

2.4. In Vitro Mineralization Test on Solid Waste Generated from Aquaponic Systems

The effects of inoculation with two pure strains of phytase-producing bacteria on the ability to hydrolyze phytate (obtained in the biochemical identification test) in the mineralization of solid waste were evaluated under different oxygen and temperature conditions. Solid waste was extracted from an *Oreochromis niloticus* culture in an experimental aquaponic system at the university; fish were fed with commercial fish feed pellets AquaNu-3® (Santa Ana Pacueco, GTO, México) containing 40% crude protein.

A three-level factorial design (2 × 2 × 3) was employed to evaluate temperature, 24 and 37 °C; oxygen level, anaerobic (0 rpm; 2.1 mg L^{-1}) and aerobic (200 rpm; 7.8 mg L^{-1}) conditions; and inoculation, without (control) or with Sp-5 or Sp-6 strain. Therefore, there were 12 evaluation treatments, each consisting of three replicates, totaling 36 experimental units. Oxygen conditions and temperature (37 °C) were maintained with magnetic stirring at 200 rpm and temperature-controlled hot plates. The temperature conditions were chosen to evaluate conditions close to the ambient temperature (24 °C), which werereported as conducive to good microorganism growth and development (37 °C) [21].

Each experimental unit consisted of a 250 mL flask, with 200 mL of solid waste obtained from the aquaponic systems. For treatments with an inoculation of bacteria, Sp-5 and Sp-6 strains were pre-cultivated in LB (Luria–Bertani) medium for 48 h at 37 °C and 200 rpm. After each experimental unit was inoculated at 4% with an optical density OD600 = 0.3 and with colony forming units of 2.7 × 107 CFU mL^{-1}. The inoculum concentration of 4% was chosen because it was optimum for phytase production in a previous report on *Bacillus* strains [22].

2.5. Determination of the Phytate and Nutrients Present in the Solid Waste

At the beginning (day 0) and end of the experiment (day 12), phytate analysis (mg/100 g of sample) was performed by using the method described by Wheeler and Ferrel (1971) [23]; total nitrogen (%) was analyzed using the Kjeldahl method at a 1:1:1.5

ratio of the sample, catalyst (potassium sulfate and mercury oxide), and concentrated sulfuric acid (H_2SO_4), with four replicates. The percentage of organic matter was analyzed using the calcination method, following the Paneque et al. (2010) [5] manual for organic matter analysis. These data were utilized to determine the relative content of each variable. Additionally, at the end of the experiment, samples of solid waste were collected, dried at 70 °C for 72 h, and digested with 3.2 N of nitric acid (HNO_3) for the analysis of phosphorus (P), potassium (K), calcium (Ca), sulfur (S), magnesium (Mg), iron (Fe), copper (Cu), manganese (Mn), molybdenum (Mo), and zinc (Zn) using optical emission spectrophotometry with inductively coupled plasma (iCAPTM 6000 Plus of Thermo Fisher Scientific™, Waltham, MA, USA).

2.6. Determination of the Nutrient Release during the Mineralization of the Solid Waste

At the beginning and every two days of the experiment (over 12 days), the following variables were analyzed in liquid solution: pH and electrical conductivity (μS cm^{-1}), determined with a multiparameter Thermo Fisher Scientific™ Orion Star™ A211 with an accuracy of ±0.002 (Thermo Spectronic, Waltham, MA, USA), and phosphate (PO_4^- in mg L^{-1}) by using UV-vis spectrophotometry Genesys 20 (Thermo Spectronic, Waltham, MA, USA) at a wavelength of 470 nm, following the yellow vanadate molybdate method described by Rodríguez and Rodríguez (2002) [24]. On the other hand, 10 mL samples of each experimental unit (200 mL) were collected at the beginning and every other day, and they were stored at −20 °C. At the end of the experiment, the initial samples at day 0 and the samples at day 10 were selected for the analysis of K, Ca, Mg, S, Fe, Mn, Mo, Cu, and Zn by using optical emission spectrophotometry with inductively coupled plasma (iCAPTM 6000 Plus of Thermo Fisher Scientific™, Waltham, MA, USA).

2.7. Statistical Analysis

The results obtained were presented as the average ± SD and statistically analyzed with STATISTICA version 7 software (StatSoft Inc., Tulsa, OK, USA) using ANOVA and Tukey's honestly significant difference (HSD) test at a 5% level of significance ($p < 0.05$). A 2 × 2 × 3 factorial analysis with their respective interactions was also performed using the same program.

3. Results

3.1. Isolation and Identification of Phytate-Mineralizing Bacteria

Sixty bacterial strains were isolated from the sedimentable solid samples obtained from the commercial aquaponic farm. It was found that six strains had the capacity to cause partial hemolysis (alpha) and total hemolysis (beta) of red blood cells (discarded strains), forty-seven strains presented the microscopic characteristics of Gram-negative and -positive bacteria in the form of coccus and micrococcus (Figure S1), and seven strains presented the microscopic characteristics of Gram-positive, rod-shaped bacteria arranged in pairs or chains with a rounded shape (Figure S2; Table S1). These seven strains (referred to as Sp-1 to Sp-7) were selected for molecular identification because their characteristics presented those of the *Bacillaceae* family, which is a group known for its phytate-mineralizing and plant growth-promoting activities, antifungal metabolites production, and biological control agent [22,25,26].

Based on the BLAST analysis of the 16S rRNA sequence (Figure S3), the Sp-1 to Sp-7 strains were classified as belonging to the genus *Lysinibacillus* (Table S1). These strains were evaluated with the biochemical phytate mineralization test using phytase screening medium (PSM) to identify phytase-producing bacteria. Only the Sp-5 and Sp-6 strains, which shared 90% identity with *L. mangiferihumi* according to the molecular identification, showed the formation of a halo during 36 to 48 h of cultivation, thus indicating degradation of the phytate molecule (Figures 1 and 2). The Sp-6 bacterium presented a higher mineralization index than Sp-5, with a value of 2.1 ± 0.3 cm compared to the Sp-5 strain's lower

index value of 1.1 ± 0.2 cm at pH 7.0. The growth kinetics in these two strains showed similar growth, reaching OD = 0.516 (Sp-6) and 0.432 (Sp-5) at 120 h.

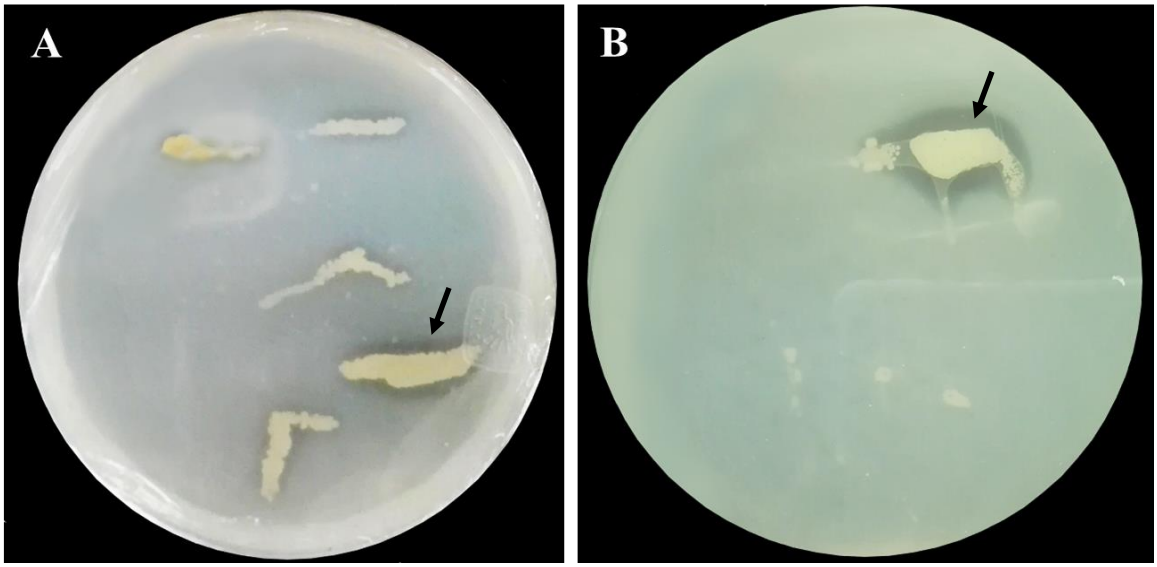

Figure 1. Phytate-mineralizing bacteria. Arrows indicated the halo zone formation by mineralization of insoluble phosphate with bacterial isolates of (**A**) strain Sp-5 and (**B**) strain Sp-6 on a phytase screening medium (PSM). (*n* = 3).

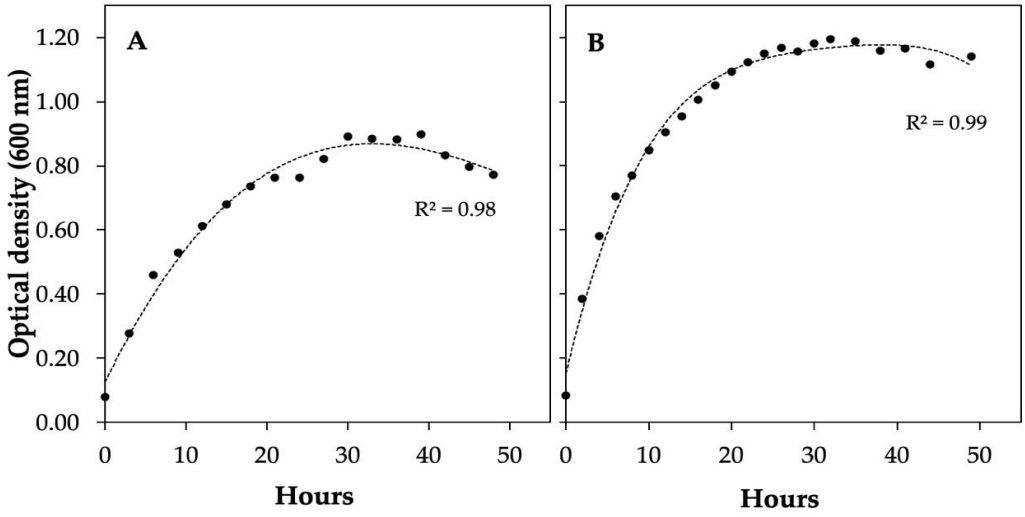

Figure 2. Growth kinetics of pure strains of *Lysinibacillus* phytate mineralizers (*n* = 5): (**A**) strain Sp-5; (**B**) strain Sp-6.

3.2. Phytate Mineralization and Phosphate Release from Sedimentary Solid Waste

According to the results obtained in the biochemical phytate mineralization, the data on the Sp-5 and Sp-6 strain inoculations, phytate mineralization, and phosphate release from solid waste collected from an experimental aquaponic system were evaluated. Regardless of the inoculation, oxygen, and temperature conditions, the relative contents of organic matter (OM) and total nitrogen (N) measured from the solid waste on day 12 were significantly lower than the initial content (day 0), ranging from 64% to 85% and 48% to 84%, respectively (Figure 3A,B). At the beginning, the relative contents corresponded to 65 ± 5% OM and 3 ± 0.2% N, while on day 12, it was 42 ± 0.02% at 55 ± 0.7% OM and 1.4 ± 0.03% at 2.5 ± 0.04% N. Regarding the aerobic conditions, a significant change in the phytate content was observed regardless of bacterial inoculation and temperature,

reaching values of 17 to 40% compared to the initial 100% (2.87 ± 0.17 mg/100 g of sample) (Figure 3C).

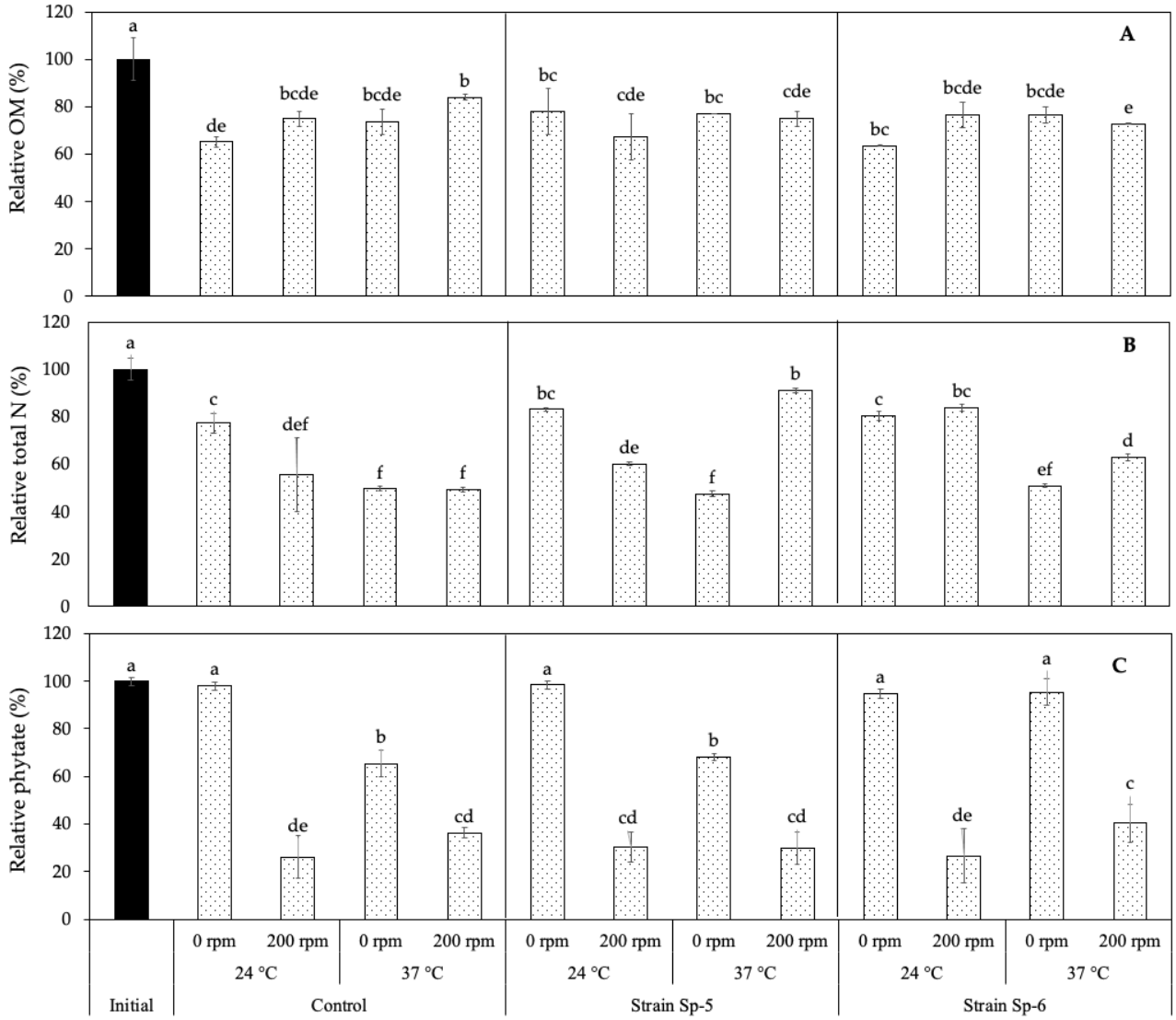

Figure 3. The changes in relative concentrations (%) of (**A**) organic matter, (**B**) total nitrogen, and (**C**) phytate relative to the initial concentration and after inoculation without/with *Lysinibacillus* strains collected in aquaponic systems for 12 days. The data represent the average ± SD. (n = 4). Different letters represent significant differences between treatment and time of $p \leq 0.05$.

On the other hand, the effects of the inoculations with the pure strains of *L. mangiferihumi* were evaluated under different oxygen and temperature conditions. Thus, in determining if the phytase activity of the strains was affected under these growth conditions, our results show that PO_4^- was significantly increased from day 2 in the treatments inoculated with pure strains of Sp-6 and Sp-5 compared to the control (Figure 4A). There was no temperature effect on the release of PO_4^- between the control and Sp-5 and Sp-6 treatments under the conditions evaluated (Figure 4B,C). In contrast, aerobic conditions (200 rpm) positively affected phytate mineralization from day 6, independently of the control and bacterial strain, with higher phosphate concentrations in the treatment inoculated with the Sp-5 and Sp-6 strains; these differences were not significant (Figure 4D,E).

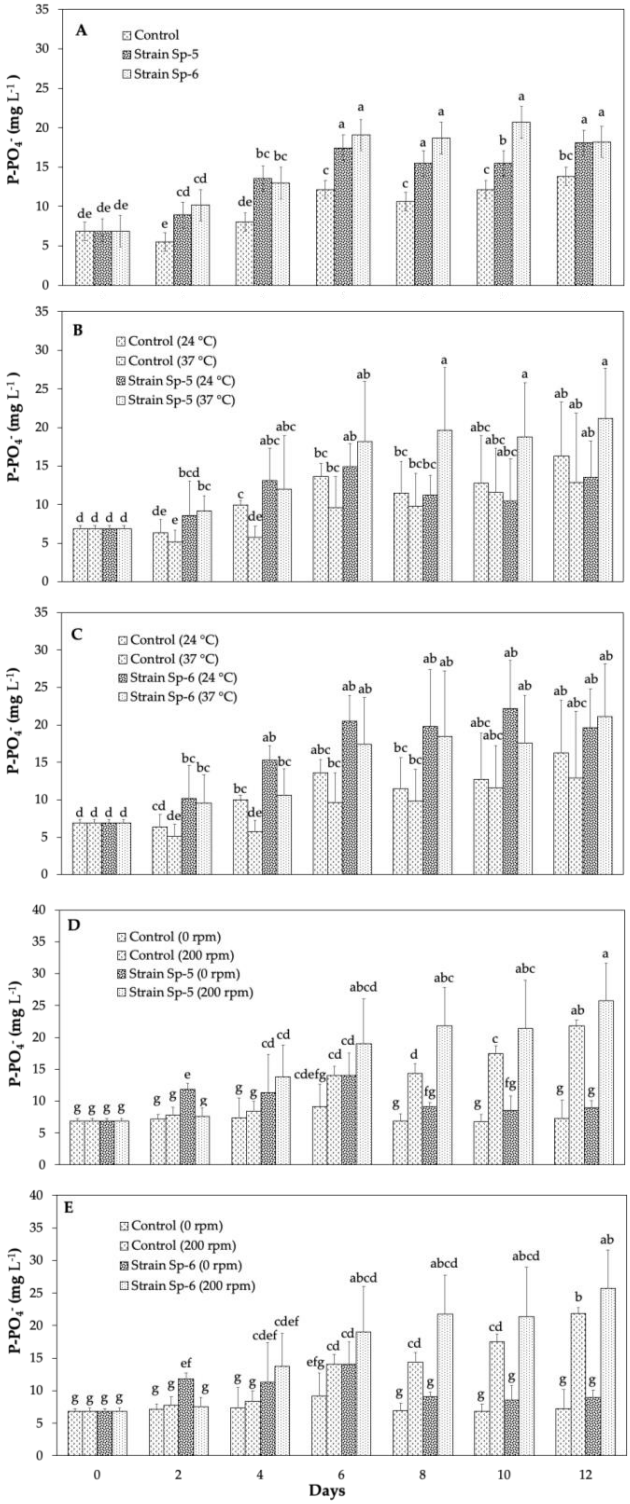

Figure 4. Effect of the inoculation of pure strains of phytate-mineralizing *Lysinibacillus* on the availability of inorganic P in the solution of the solids generated in aquaponic systems. (**A**) Effect of strains of *Lysinibacillus* phytate mineralizers on the mineralization of solids in the system. (**B**) Effect of *Lysinibacillus* (Sp-5) phytate mineralizers under different temperature conditions (24 and 37 °C). (**C**) Effect of *Lysinibacillus* (Sp-6) phytate mineralizers under different temperature conditions (24 and 37 °C). (**D**) Effect of *Lysinibacillus* (Sp-5) phytate mineralizers in different oxygen conditions (anaerobic and aerobic conditions). (**E**) Effect of *Lysinibacillus* (Sp-6) phytate mineralizers in different oxygen conditions (anaerobic and aerobic conditions). The data represent the mean ± SD. (n = 3). Different letters represent significant differences between treatments and time (days) at $p \leq 0.05$.

This increase in PO_4^- coincided with the decrease in the relative phytate content at day 12 relative to the initial phytate content at day 0, wherein a lower relative content was observed in the treatments with aerobic conditions, presenting significant differences at $p < 0.05$ (Figure 3C). Likewise, the correlation analysis confirmed the importance of aerobic conditions for the processes of phytate mineralization and degradation, as, compared to anaerobic conditions, a high correlation was observed with an R^2 of 0.74 (24 °C) and 0.81 (37 °C); additionally, negative correlation coefficients of −0.86 and −0.90 were observed, indicating that under aerobic conditions, the concentration of phytate decreases and the concentration of phosphate increases (Figure 5).

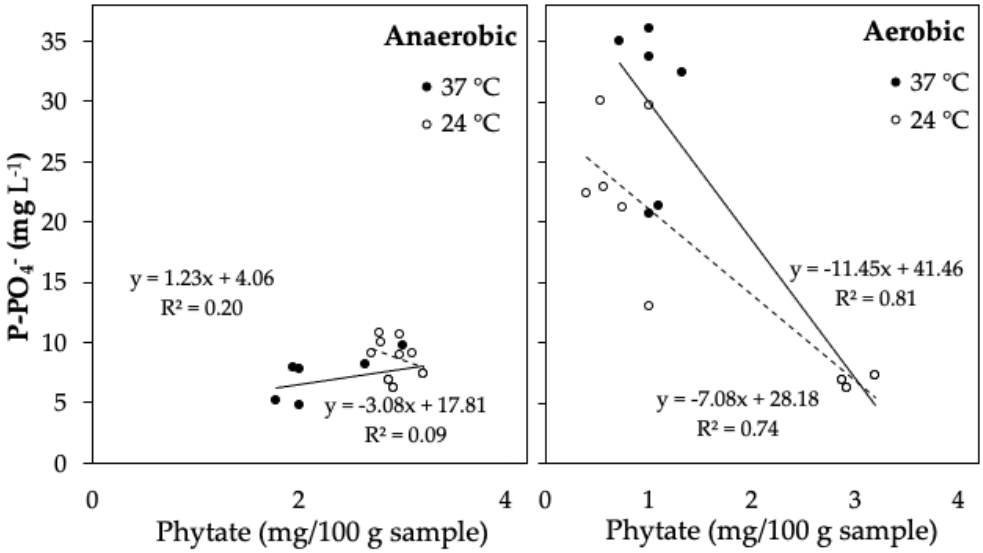

Figure 5. Correlation analysis between the phosphate and phytate variables in anaerobic and aerobic conditions.

3.3. Effect of L. mangiferihumi on the Mineralization and Release of Macro- and Micronutrients

The concentrations of macro- and micronutrients at day 0 were as follows (mg L^{-1}): K, 56.8 ± 13; S, 36.02 ± 6.2; Ca, 54.2 ± 5.7; total P, 28.3 ± 7.8; PO_4^-, 6.85 ± 0.45; Mg, 16.7 ± 1.5; Fe, 0.14 ± 0.14; Zn, 0.1 ± 0.01; Cu, 0.04 ± 0.01; Mn, 0.04 ± 0.02; and Mo, 0.8 ± 0.12. The data obtained at the end of the experiment show a significant increase in each element (Tables 1 and 2). The factorial analysis revealed significant differences at $p \leq 0.0001$ in the release of PO_4^- and total P, indicating that the triple interaction of the inoculation of the Sp-6 strain under aerobic conditions (200 rpm) at 24 °C had a positive effect on the increase in the concentrations (33.4 ± 0.96 and 1193.9 ± 70.7 mg L^{-1}, respectively). Also, significant differences in Ca, Mg, and S ($p \leq 0.01$ and $p \leq 0.05$) were obtained in the triple interactions, where the combination of aerobic conditions, temperature at 24 °C, and inoculation with the Sp-5 and Sp-6 bacteria increased Ca availability. In contrast, aerobic conditions and 24 °C, regardless of bacterial inoculation, favored the release of Mg and S. Meanwhile, there were double interactions ($p \leq 0.05$) between anaerobic conditions and bacterial inoculation for K, where K availability increased without bacterial inoculation (Table 1). Fe presented significant differences among the treatments, and the factorial analysis showed a significant double interaction between aerobic conditions and bacterial inoculation (Table 2). Still, there were significant differences among the treatments in Zn, Cu, Mn, and Mo availabilities.

Table 1. Effect of *Lysinibacillus* on the release and availability of macronutrients during the mineralization of solid waste generated in aquaponic systems.

Inoculation Strain	Temp. °C	Oxygen rpm	K	S	Ca	Total P	P (PO_4^-)	Mg
			mg L^{-1}					
Control	24	0	189.5 ± 52.1 ^{a}	306.2 ± 20.7 ^{de}	90.9 ± 6.9 ^{e}	262.8 ± 3.5 ^{cd}	7.3 ± 1.4 ^{fg}	25.9 ± 0.9 ^{c}
		200	114.6 ± 42.2 ^{e}	470.4 ± 9.4 ^{a}	200.0 ± 3.4 ^{d}	73.6 ± 32.2 ^{f}	18.3 ± 1.4 ^{c}	30.9 ± 0.9 ^{a}
	37	0	139.7 ± 52.7 ^{b}	359.7 ± 17.6 ^{bc}	55.0 ± 3.6 ^{f}	82.9 ± 9.8 ^{f}	6.4 ± 0.6 ^{g}	22.5 ± 0.1 ^{d}
		200	86.6 ± 30.2 ^{e}	329.1 ± 18.2 ^{cde}	55.0 ± 3.2 ^{f}	72.2 ± 10.3 ^{f}	16.8 ± 0.4 ^{cd}	22.3 ± 1.8 ^{d}
Sp-5	24	0	147.1 ± 41.7 ^{bcd}	296.6 ± 3.7 ^{cde}	41.7 ± 3.8 ^{fg}	320.9 ± 71.1 ^{cd}	7.3 ± 2.5 ^{g}	22.7 ± 2.0 ^{d}
		200	122.3 ± 3.79 ^{bcd}	465.9 ± 8.1 ^{a}	301.9 ± 8.1 ^{a}	534.1 ± 6.0 ^{de}	15.0 ± 4.8 ^{d}	31.7 ± 1.1 ^{a}
	37	0	149.9 ± 31.2 ^{bc}	329.2 ± 11.7 ^{ef}	39.5 ± 11.7 ^{gh}	149.3 ± 29.5 ^{ef}	9.8 ± 1.3 ^{ef}	19.5 ± 0.6 ^{e}
		200	102.4 ± 5.5 ^{cde}	364.6 ± 4.8 ^{bc}	277.7 ± 4.8 ^{b}	872.9 ± 114.5 ^{b}	27.8 ± 2.7 ^{b}	28.7 ± 0.1 ^{b}
Sp-6	24	0	125.2 ± 2.4 ^{bcd}	269.0 ± 5.8 ^{q}	33.5 ± 5.8 ^{gh}	356.3 ± 39.2 ^{cd}	10.9 ± 1.3 ^{e}	23 ± 1.0 ^{d}
		200	124.9 ± 6.4 ^{bcd}	473.0 ± 4.5 ^{a}	302.8 ± 4.5 ^{a}	1193.9 ± 70.7 ^{a}	33.4 ± 1.6 ^{a}	31.9 ± 0.8 ^{a}
	37	0	141.5 ± 4.1 ^{b}	303.0 ± 1.2 ^{f}	31.3 ± 1.2 ^{h}	143.9 ± 19.9 ^{ef}	8.1 ± 1.0 ^{g}	19.0 ± 1.0 ^{e}
		200	92.1 ± 14.6 ^{de}	323.0 ± 34.8 ^{bcd}	238.3 ± 34.8 ^{c}	638.9 ± 1.0 ^{b}	27.01 ± 1.0 ^{b}	26.1 ± 2.8 ^{b}
ANOVA			***	***	***	***	***	***
Temp × Oxygen			NS	*	NS	NS	NS	NS
Temp × Inoculation			NS	NS	*	***	***	*
Oxygen × Inoculation			*	NS	***	***	***	***
Temp × Oxygen × Inoculation			NS	*	**	***	***	**

Values represent means, and bars indicate ± SD (n = 3). Different letters indicate significant differences between treatments. NS, *, **, *** mean no significant or significant at $p < 0.05$, 0.01, or 0.001, respectively.

Table 2. Effect of *Lysinibacillus* on the release and availability of micronutrients during the mineralization of solid waste generated in aquaponic systems.

Inoculation Strain	Temp. °C	Oxygen rpm	Fe	Zn	Cu	Mn	Mo
					mg L^{-1}		
Control	24	0	0.9 ± 0.3^{a}	0.7 ± 0.1	ND	ND	0.1 ± 0.9
		200	0.7 ± 0.1^{abc}	1.0 ± 0.2	0.03 ± 0.01	ND	0.2 ± 0.2
	37	0	0.3 ± 0.1^{de}	0.8 ± 0.01	ND	ND	0.3 ± 0.03
		200	0.1 ± 0.1^{e}	0.6 ± 0.02	0.02 ± 0.03	ND	0.5 ± 0.2
Sp-5	24	0	0.4 ± 0.06^{cde}	0.6 ± 0.01	ND	ND	ND
		200	1.0 ± 0.05^{a}	0.8 ± 0.3	0.04 ± 0.02	0.3 ± 0.2	0.6 ± 0.4
	37	0	0.9 ± 0.08^{ab}	0.3 ± 0.2	0.06 ± 0.02	ND	0.2 ± 0.1
		200	1.0 ± 0.1^{a}	0.9 ± 0.4	0.02 ± 0.05	0.4 ± 0.02	0.3 ± 0.2
Sp-6	24	0	0.4 ± 0.07^{bcde}	0.7 ± 0.09	0.05 ± 0.09	ND	0.2 ± 0.3
		200	0.9 ± 0.2^{a}	0.5 ± 0.05	0.04 ± 0.07	0.4 ± 0.06	0.2 ± 0.3
	37	0	0.3 ± 0.1^{de}	0.8 ± 0.3	0.06 ± 0.03	ND	0.2 ± 0.02
		200	0.6 ± 0.03^{abcd}	0.5 ± 0.1	0.02 ± 0.08	0.3 ± 0.1	ND
ANOVA			*	NS	NS	NS	NS
Temp × Oxygen			NS	NS	NS	NS	NS
Temp × Inoculation			**	NS	NS	NS	NS
Oxygen × Inoculation			*	NS	NS	NS	NS
Temp × Oxygen × Inoculation			NS	NS	NS	NS	NS

Values represent means and bars indicate ± SD (n = 3). Different letters indicate significant differences between treatments. Detection limit: Cu < 0.05; Mn < 0.01; Mo < 0.01. ND means not detected. NS, *, **, mean no significant or significant at $p \leq 0.05$, or 0.01, respectively.

4. Discussion

4.1. Isolation and Identification of Phytate-Mineralizing Bacteria

Limited studies have been carried out on characterizing the bacterial communities present in solids from aquaponic systems and their application for organic matter mineralization and plant growth promotion [8,27]. To our knowledge, this is the first study on the isolation and identification of pure strains from the solids accumulated in aquaponic systems and their application as inoculum for optimizing the degradation of organic matter in solids.

In this study, through microscopic and molecular analyses, we isolated and identified seven bacteria strains belonging to the genus *Lysinibacillus* with 87 to 92% identity or similarity (Table S1 and Figures S2 and S3). This genus belongs to the Gram-positive mesophilic bacteria from the Bacillale order and is phylogenetically related to the *Bacillus* genus [12]. A study of metagenomic analyses carried out on the taxonomic characterization of bacterial communities present in aquaponic systems up to the phylum level found that 93.2% of the molecular readings corresponded to bacteria of the *Firmicutes* phylum. This group is divided into several classes, mainly Bacilli, Clostridia, and Mollicutes [27]. This supports the findings in the present study as *Lysinibacillus* was possibly present as part of the Bacilli class, Bacillale order.

The results from the biochemical mineralization test at pH 7.0 show a greater phytate degradation by the Sp-6 (2.1 $\pm$ 0.3 cm) than the Sp-5 (1.1 $\pm$ 0.2 cm) strain at 36 and 48 h of bacterial growth (Figure 1). The growth kinetics of these strains in phytate screening medium at pH 7.0 and 200 rpm present an exponential growth from approximately 12 to 60 h, not reaching a deceleration phase (Figure 2). To our knowledge, there are no studies on phytate activity in *Lysinibacillus.* However, data reported on different species of *Bacillus* spp. show that experiments performed under similar conditions (PSM medium, pH 6.5–7.5, 37–40 °C, and 180–200 rpm) have presented growth kinetics different to our study, reaching an OD600 of 1.2 and presenting an exponential phase of 12 to 36 h; a stationary phase of 36 to 48 h with a higher peak of phytase production; and followed by a deceleration phase [22,28–34]. The above shows that *Lysinibacillus* presents a capacity to mineralize phytate, with a slower growth (OD_{600} of 0.43 in Sp-5 and OD_{600} of 0.52 in Sp-6) concerning *Bacillus.* Nonetheless, further enzymatic studies are still required to determine and confirm its highest peak in the production of the phytase enzyme.

Through molecular identification, these two strains (Sp-5 and Sp-6) showed 90% identity or similarity with *Lysinibacillus mangiferihumi* (Table S1). Notably, this bacterium has not been implicated as a pathogen for fish and plants. *Lysinibacillus* sp. can produce toxins and bacteriocins that aid in disease control [21].

The Sp-1, Sp-3, and Sp-7 strains also showed high identity with *L. mangiferihumi*; however, these did not show phytate-mineralizing activity. This difference may be due to genetic variation among the different populations within the same species, thereby influencing the enzyme activity for phytate degradation somewhat [35]. Therefore, this is a hypothesis that must be studied.

4.2. Phytate Mineralization in Sedimentary Solid Waste from Aquaponic Systems and Their Effect on Phosphorus Solubilization

Some studies have reported that a large portion of the nutrients from fish feed are present in an organic and insoluble form in the uneaten feed and fish feces (solid waste) generated in aquaponic systems, and so are not readily available to plants [36]. A few recent studies on mineralization in aquaponics show differences between aerobic and anaerobic conditions, but which conditions are better still needs to be clarified [4,17,37]. Therefore, we have evaluated the effects of inoculation with pure strains of *L. mangiferihumi* (Sp-5 and Sp-6) on phytate mineralization and nutrient release under different temperature (24 °C and 37 °C) and oxygen (anaerobic and aerobic) conditions.

The decrease in the content of OM and total N after 12 days of mineralization (Figure 3A,B) could be caused by different biochemical processes mediated by the het-

erotrophic microorganisms present in solid waste. Part of the nutrients, mainly carbon and nitrogen, generated during the decomposition process can be used by microorganisms to perform their metabolic functions, and other parts of the nutrients may be available for plants [4,38].

The results obtained on the release of phosphorus in the PO_4^- form show a significant increase from day 2 of the mineralization process in the treatments inoculated with pure *L. mangiferihumi* strains (Sp-6 and Sp-5) compared to the control (Figure 4A). Contrastingly, there were no differences between the 24 and 37 °C temperature conditions (Figure 4B,C). In aerobic conditions, a significant effect was observed, where PO_4^- concentrations increased on day 6 with anaerobic conditions in both the control and Sp5 and Sp-6 treatments (Figure 4D,E).

This release of PO_4^- coincides with the decrease in phytate during the experiment. The relative phytate content showed no significant differences between the phytate concentrations in the control treatment and the treatments inoculated with the pure strains of *L. mangiferihumi* in anaerobic conditions (Figure 3C). Meanwhile, in the treatments with aerobic conditions, regardless of bacterial inoculation (control; Sp-5 and Sp-6) and temperature, a significant decrease was observed on day 12 compared to the initial phytate content at day 0 (Figure 3C). The above shows that the presence of *L. mangiferihumi* favors an increase in PO_4^-, and that aerobic conditions are an important factor that favors both bacterial activity in phytate mineralization and an increase in PO_4^-. The correlation study supported the importance of oxygen in bacterial activity, where high negative correlation coefficients of −0.86 and −0.90 at 24 °C and 37 °C, respectively, were obtained (Figure 5).

On the other hand, the decrease in phytate, both in the control and inoculated treatments under aerobic conditions at 24 and 37 °C, did not show any differences (Figure 3C). This does not match with the PO4 increase, since the concentration of this element's release was statistically higher in the inoculated treatments than in the control (Figure 4A). These results could be due to two reasons: (1) the aerobic conditions were ideal to allowing the phytate-degrading ability of the microbial populations to enact enzymatic activity on the contents naturally present in the solids (control); and (2) the inoculated bacteria (Sp-5 and Sp-6) helped to degrade the phytate present in the solids, possibly solubilizing other forms of non-assimilable phosphorus such as hydroxyapatite, monocalcium phosphate, dicalcium phosphate, and tricalcium phosphate, which can also be found in fish feed and, therefore, in the solids accumulated in the systems [39,40].

These findings on Sp-5 and Sp-6 strains are potentially important for their application in agriculture and traditional aquaculture, as well as for the optimization of mineralization in aquaponics, since, recently, the application of P as a fertilizer in agricultural production is still a subject of discussion due to concerns exhausting reserves of phosphoric rock (non-renewable resource) and the little availability of this element in soil [41]. Likewise, in traditional aquaculture and CRASs, these solids are released into the environment, causing environmental pollution and eutrophication, wherein a large portion of phosphorus is found in forms that cannot be assimilated to plants [11].

4.3. Effect of L. mangiferihumi on the Release of Macro- and Micronutrients during the Mineralization Process

Lysinibacillus is a genus that has not yet been studied much; however, it has found important roles in agricultural and environmental applications, such as growth-promoting capacity in plants, biocontrol activity against phytopathogenic fungi, and aid in the uptake of heavy metals [42–45]. Nevertheless, this study found that *L. mangiferihumi* acts as a phytate-mineralizing species that helps to release and increase mainly PO_4^- and Ca in aquaponic solutions.

A factorial analysis of the macro- and micronutrients was carried out on day 10 of the mineralization process, when the concentrations of PO_4^- were the highest in the treatment inoculated with the Sp-6 strain compared to the control and the treatment inoculated with the Sp-5 strain (Figure 3A). This increase in nutrients at day 10 of mineralization,

compared to the initial time (Tables 1 and 2), shows an important effect of the evaluated conditions on the release of nutrients and is supported by studies that report that the nutrient release during the organic matter degradation is influenced by different factors such as pH, temperature, oxygen, biological activity, and C:N ratio [38].

The significant increases in Ca and Mg (Table 1) coincided with the treatments that showed the highest degradation of phytate and release of P and PO_4^-, which might be explained by the biological activity of *L. mangiferihumi* in phytate degradation. On the other hand, the significant increase in S could be explained by the degradation of the protein present in the solids, since this element is present in the structure of some amino acids, mainly methionine, which is one of the amino acids essential and required for the initiation of protein synthesis and which is supplemented as an additive in fish feed because the organism cannot synthesize it by itself [46].

Contrarily, the anaerobic conditions without bacterial inoculation favored a significant increase in K. Therefore, this could be due to the biological activity occurring in these conditions or possibly due to the use of this element for the metabolic processes of the microbial population in aerobic conditions, as K is necessary for the activation of some enzymes and its concentration in the cells of Gram-positive bacteria is influenced by the concentration of teichoic acids in the cell wall [35].

In a hydroponic solution, it is reported that the ideal concentrations of macronutrients for the growth of vegetables are from 30 to 60 mg L^{-1} of P in the form of PO_4^-; 240 to 350 mg L^{-1} of K; 140 to 240 mg L^{-1} of Ca; and 45 to 55 mg L^{-1} Mg [47]. Compared to the above, the concentrations obtained in the present study were within the suggested range for PO_4^- and 60 mg L^{-1} higher for Ca, but 15 mg L^{-1} and 50 to 160 mg L^{-1} lower for Mg and K, respectively, than those suggested in hydroponics. Some researchers have reported that K may be mandatory as a supplement in aquaponic systems since fish feed presents a low composition of this element. Therefore, the K concentration in the solids is lower, which indicates that mineralization is insufficient to reach the concentrations suggested in hydroponics [9]. However, in the present study, the final concentration of K in the solids at day 12 day of mineralization was 5.7 to 7.2 mg g^{-1} (Table S2), which is higher than the 0.2 mg g^{-1} reported by Delaide (2017) [48] with and lower than the 7.31% reported by Raffie and Roos (2007) [49]. Therefore, studies that show the low availability of K in the solids generated in these systems and evaluate biological processes that optimize the degradation of solids in anaerobic conditions need to be conducted.

There are no significant differences between treatments and interactions in the releases of Zn, Cu, Mn, and Mo, which indicates that the factors evaluated did not significantly influence their release or mineralization (Table 2). Unlike these elements, Fe significantly increased, changing in concentration from 0.9 to 1.0 mg L^{-1}. The factor analysis showed a significant interaction, in which its increase was favored in three interactions: (1) under anaerobic conditions at 24 °C without bacterial inoculation (control); (2) under aerobic conditions at 37 °C with inoculation *of L. mangiferihumi*; and (3) under aerobic conditions at 24 °C, regardless of bacterial inoculation (Table 2). These data show that the availability of Fe could be affected by the different conditions evaluated in the experiment. Therefore, we cannot clearly define which factors caused this increase. The above could have occurred because Fe is one of the elements that oxidizes and is easily affected by pH and the presence of other elements, causing changes in its chemical composition, or generating its precipitation in the solution [3]. For this reason, it is necessary to carry out more studies that define what conditions significantly increase the levels of the form available and assimilable to the plant.

Micronutrients are essential for plant growth in smaller quantities; their function is vital for plant development since they serve as activators of enzymatic reactions and are involved in different physiological processes in plants [50]. The suggested or recommended concentrations are 1 to 2 mg L^{-1} Fe, 0.3 mg L^{-1} Zn, 0.05 mg L^{-1} Cu, 0.5 to 1.0 mg L^{-1} Mn, and 0.05 mg L^{-1} Mo [38]. Based on the above concentrations, our results show that the Fe, Cu, and Mn concentrations at day 10 of mineralization do not reach the concentrations suggested for the plant, while remaining in the organic solids at concentrations of 2.9 to

5.0 mg g^{-1} for Fe, 0.2 mg g^{-1} for Cu, and 0.5 to 0.7 mg g^{-1} for Mn (Table S2). Therefore, this indicates that more studies are still needed to evaluate other factors that may influence the release of these micronutrients and optimize the mineralization of these elements in these systems.

The results reported above coincide the relative increase in electrical conductivity, which indicate the salt concentration in the solution. In anaerobic conditions, the electrical conductivity (EC) showed few increases in its value, as the control ranged between 2.07 ± 0.04 (day 0) to 2.17 ± 0.13 µS cm^{-1} (day 12) with a relative increase of 4.83 ± 5.66%; the treatment with *L. mangiferihumi* presented 2.1 ± 0.05 µS cm^{-1} at day 0, reaching an EC of 2.31 ± 0.13 µS cm^{-1} (Sp-5) and 2.30 ± 0.06 µS cm^{-1} (Sp-6) at day 12, with relative average increases of 11.34 ± 4.53% and 11.37 ± 1.58%. The aerobic conditions presented high EC with respect to the anaerobic conditions, and a higher concentration in the treatments with bacterial inoculation was seen compared to the control. The treatment inoculated with Sp-5 ranged from between 2.07 ± 0.05 µS cm^{-1} (day 0) to 2.45 ± 0.04 µS cm^{-1}, and the treatment with Sp-6 ranged from between 2.1 ± 0.03 µS cm^{-1} (day 0) to 2.44 ± 0.04 µS cm^{-1} (day 12), with relative average increases of 18.34 ± 2.64% and 16.49 ± 1.09%, respectively. The control presented an EC ranging from 2.08 ± 0.04 µS cm^{-1} (day 0) to 2.28 ± 0.08 µS cm^{-1} (day 12), with a relative average increase of 9.61 ± 3.50%.

Additionally, during the mineralization process under anaerobic conditions, the pH increased from 7.6 ± 0.07 to 8.3 ± 0.14 and 8.5 ± 0.14, whereas under aerobic conditions, the pH decreased from 7.6 ± 0.13 to 6.5 ± 0.08 (control) and 5.9 ± 0.08 (Sp-5 and Sp-6 treatments). The differences in pH behavior may be due to the different physical, chemical, and biological processes that can be carried out in anaerobic and aerobic conditions during the degradation of organic matter [51,52].

In aquaponics, aspects such as pathogen control, nutrient availability, proliferation of microalgae, and diseases in plants and fish still need to be solved and improved for aquaponic farmers [1]. Recent studies have focused on evaluating the efficiency of these systems through the application or inoculation of beneficial microorganisms that enhance nitrification processes [53], growth [54–56], and pathogen control [57].

Li et al. (2019) [53] report an increase in the transformation of TAN to nitrate through the implementation of a biofilter composed of porous polymer particles suspended or enriched with nitrifying bacteria, showing removal efficiencies of 71.8% for TAN and 58.5% for nitrite. Piñero et al. 2023 [55] report that using plant growth-promoting bacteria such as *Azotobacter* and *Azospirillum* improves mineral absorption, the efficiency of nitrogen utilization, and plant development. Sirakov et al. (2016) [57] and other researchers [58–60] report on the control of pathogens by using nutrient competition and the production of secondary metabolites such as antibiotics through the inoculation of *Bacillus* spp., *Saccharomyces*, and *Lactobacillus* spp. The present study provides other exciting findings to optimize the mineralization or release of nutrients by applying *L. mangiferihumi*.

5. Conclusions

Our results show that the activity of the identified bacteria, such as phytate mineralizers isolated from the hydrocyclone of an aquaponic system, requires oxygen to carry out its function. The pure strain of *L. mangiferihumi* (Sp-6) favored the degradation of phytate under oxygen conditions and at a temperature of 24 °C, leading to a significant increase in the release of phosphate and Ca during the mineralization process in comparison to the Sp-5 strain and the control. This information is helpful for potential application in agriculture and the optimization of mineralization in aquaponic systems.

The increase in nutrients during the mineralization may not be caused solely by the bacterial activity of *L. mangiferihumi*. It could also be due to the biological activity of other microorganisms and/or chemical reactions during the mineralization under different oxygen and temperature conditions. It was found that anaerobic conditions favor an increase in K. In contrast, the combination of aerobic conditions and temperature at 24 °C increased the availability of S, Mg, and Fe independently of the inoculation with *L. mangiferihumi.*

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae10050497/s1; Figure S1: General microscopic identification of strains isolated from solid waste; Figure S2: Microscopic identification of *Lysinibacillus* strains; Figure S3: Electrophoresis of 1.2% agarose gel; Table S1: Morphological characterization and molecular identification of the strains isolated of solid waste; Table S2: Final concentration of minerals (macro- and micronutrients) in the solid waste.

Author Contributions: Conceptualization, E.R.R.-C., H.-J.K. and P.D.-S.; methodology, E.R.R.-C., J.C.R.-O., M.d.l.L.G.G. and P.D.-S.; investigation, E.R.R.-C.; resources, M.d.l.L.G.G.and J.C.R.-O.; writing—original draft preparation, E.R.R.-C.; writing—review and editing, P.D.-S., M.d.l.L.G.G., M.F.Q.-C. and H.-J.K.; funding acquisition, P.D.-S. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Consejo Nacional de Ciencia y Tecnología CONACYT-Fondo FORDECYT (No. 297525 to Pablo Delgado Sánchez) and MSc. student grant (No. 634137 to Edna Roccio Riaño Castillo).

Data Availability Statement: Data are contained within the article and Supplementary Materials.

Acknowledgments: We are grateful to Francisco Javier Pérez Vázquez from UASLP for his technical assistance in analyzing the phytate in the samples.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Goddek, S.; Joyce, A.; Kotzen, B.; Burnell, G. *Aquaponics Food Production Systems*; Springer Open: London, UK, 2019. [CrossRef]
2. Masabni, J.; Niu, G. Chapter 10: Aquaponics. In *Plant Factory Basics, Applications and Advances*, 1st ed.; Kozai, T., Niu, G., Masabni, J., Eds.; Acadamic Press: Cambridge, MA, USA, 2022. [CrossRef]
3. Goddek, S.; Delaide, B.; Mankasingh, U.; Ragnarsdottir, K.V.; Jijakli, H.; Thorarinsdottir, R. Challenges of Sustainable and Commercial Aquaponics. *Sustainability* **2015**, *7*, 4199–4224. [CrossRef]
4. Goddek, S.; Delaide, B.P.; Joyce, A.; Wuertz, S.; Jijakli, M.H.; Gross, A.; Eding, E.H.; Bläser, I.; Reuter, M.; Keizer, L.P.; et al. Nutrient mineralization and organic matter reduction performance of RAS-based sludge in sequential UASB-EGSB reactors. *Aquac. Eng.* **2018**, *83*, 10–19. [CrossRef]
5. Paneque, V.; Calaña, J.; Calderón, M.; Borges, Y.; Hernández, T.; Caruncho, M. *Manual de Técnicas Analíticas para Análisis de Suelo, Foliar, Abonos Orgánicos y Fertilizantes Químicos*, 1st ed.; Instituto Nacional de Ciencias Agrícolas, Departamento de Biofertilizantes y Nutrición de las Plantas, INCA: La Habana, Cuba, 2010.
6. Cao, L.; Wang, W.; Yang, C.; Yang, Y.; Diana, J.; Yakupitiyage, A.; Luo, Z.; Li, D. Application of microbial phytase in fish feed. *Enzym. Microb. Technol.* **2007**, *40*, 497–507. [CrossRef]
7. Tyson, R.V.; Simonne, E.H.; Treadwell, D.D.; White, J.M.; Simonne, A. Reconciling pH for Ammonia Biofiltration and Cucumber Yield in a Recirculating Aquaponic System with Perlite Biofilters. *HortScience* **2007**, *43*, 719–724. [CrossRef]
8. da Silva Cerozi, B.; Fitzsimmons, K. Use of *Bacillus* spp. to enhance phosphorus availability and serve as a plant growth promoter in aquaponics systems. *Sci. Hortic.* **2016**, *211*, 277–282. [CrossRef]
9. Makkar, H.P.S.; Perumal, S.; Becker, C. Plant Secondary Metabolites. In *Methods in Molecular Biology*, 1st ed.; Humana Press: Totowa, NJ, USA, 2007; ISBN 978-1588299932.
10. Singh, P.; Kumar, V.; Agrawal, S. Evaluation of Phytase Producing Bacteria for Their Plant Growth Promoting Activities. *Int. J. Microbiol.* **2014**, *2014*, 426483. [CrossRef] [PubMed]
11. Lazzari, R.; Baldisserotto, B. Nitrogen and Phosphorus Waste in Fish Farming. *Bol. Do Inst. De Pesca* **2008**, *34*, 591–600.
12. Dunlap, C. *Lysinibacillus mangiferihumi*, *Lysinibacillus tebacifolii* and *Lysinibacillus* Variants are Later Heterotypic Synonyms of *Lysinibacillus sphaericus*. *Int. J. Syst. Evol. Microbiol.* **2019**, *69*, 2958–2962. [CrossRef] [PubMed]
13. Jorquera, M.A.; Hernández, M.T.; Rengel, Z.; Marschner, P.; Mora, M.d.l.L. Isolation of culturable phosphobacteria with both phytate-mineralization and phosphate-solubilization activity from the rhizosphere of plants grown in a volcanic soil. *Biol. Fertil. Soils* **2008**, *44*, 1025–1034. [CrossRef]
14. Mittal, A.; Singh, G.; Goyal, V.; Yadav, A.; Kumar, N. Production of phytase by acid-thermophilic strain of *Klebsiella* sp. DB-3FJ711774 using orange peel flour under submerged fermentation. *Innov. Rom. Food Biotechnol.* **2012**, *10*, 18–27.
15. Maitra, N.; Whitman, W.; Ayyampalayam, S.; Samanta, S.; Sarkar, K.; Bandopadhyay, C.; Aftabuddin, M.; Sharma, A.; Manna, S. Draft genome sequence of the aquatic phosphorus solubilizing and mineralizing Bacterium *Bacillus* sp. Strain CPSM8. *Genome Announc.* **2014**, *2*, e01265-13. [CrossRef] [PubMed]
16. Maitra, N.; Manna, S.K.; Samanta, S.; Sarkar, K.; Debnath, D.; Bandopadhyay, C.; Sahu, S.K.; Sharma, A.P. Ecological significance and phosphorus release potential of phosphate solubilizing bacteria in freshwater ecosystems. *Hydrobiologia* **2015**, *745*, 69–83. [CrossRef]

17. Goddek, S.; Schmautz, Z.; Scott, B.; Delaide, B.; Keesman, K.J.; Wuertz, S.; Junge, R. The Effect of Anaerobic and Aerobic Fish Sludge Supernatant on Hydroponic Lettuce. *Agronomy* **2016**, *6*, 37. [CrossRef]
18. Wilson, I.G. Inhibition and facilitation of nucleic acid amplification. *Appl. Environ. Microbiol.* **1997**, *63*, 3741–3751. [CrossRef]
19. Barghouthi, S.A. A universal method for the identification of bacteria based on general PCR primers. *Indian J. Microbiol.* **2011**, *51*, 430–444. [CrossRef]
20. Kerovuo, J.A. Novel Phytase from *Bacillus* Characterization and Production of the Enzyme. Master's Thesis, University of Helsinki, Helsinki, Finland, 2000.
21. Jamal, Q.M.S.; Ahmad, V. *Lysinibacilli*: A Biological Factories Intended for Bio-Insecticidal, Bio-Control, and Bioremediation Activities. *J. Fungi* **2022**, *8*, 1288. [CrossRef] [PubMed]
22. Demirkan, E.; Baygin, E.; Usta, A. Screening of Phytate Hydrolysis *Bacillus* sp. Isolated from Soil and Optimization of the Certain Nutritional and Physical Parameters on the Production of Phytase. *Turk. J. Biochem.* **2014**, *39*, 206–214. [CrossRef]
23. Wheeler, E.L.; and Ferrel, R.E. A method for phytic acid determination in wheat and wheat fractions. *Cereal Chem.* **1997**, *48*, 312–320.
24. Rodríguez, H.; Rodríguez, J. *Métodos de Análisis de Suelos y Plantas*, 1st ed.; Trillas, S.A., Ed.; de CV: Mexico City, Mexico, 2013; ISBN 978-607-17-0593-8.
25. Balaban, N.P.; Suleimanova, A.D.; Valeeva, L.R.; Chastukhina, I.B.; Rudakova, N.L.; Sharipova, M.R.; Shakirov, E.V. Microbial Phytases and Phytate: Exploring Opportunities for Sustainable Phosphorus Management in Agriculture. *Am. J. Mol. Biol.* **2017**, *7*, 11–29. [CrossRef]
26. Slepecky, R.A.; Ernest, H. The Genus *Bacillus*-Nonmedical. In *The Prokaryotes*; Edward, F., Delong, S., Erko, S., Thompson, F., Eds.; Springer: New York, NY, USA, 2006. [CrossRef]
27. Schmautz, Z.; Graber, A.; Jaenicke, S.; Goesmann, A.; Junge, R.; Smits, T.H.M. Microbial diversity in different compartments of an aquaponics system. *Arch. Microbiol.* **2017**, *199*, 613–620. [CrossRef]
28. Anis Shobirin, M.H.; Farouk, A.; Greiner, R. Potential Phytate-degrading Enzyme Producing Bacteria Isolated from Malaysian Maize Plantation. *J. Biotechnol.* **2009**, *8*, 3540–3546.
29. Gulati, H.K.; Chadha, B.S.; Saini, H.S. Production and characterization of thermostable alkaline phytase from *Bacillus laevolacticus* isolated from rhizosphere soil. *J. Ind. Microbiol. Biotechnol.* **2007**, *34*, 91–98. [CrossRef] [PubMed]
30. Jain, U.; Chauhan, N. *Bacillus cereus* Phytase-Detection, Purification, Characterization and Physiological Role. *Int. J. Sci. Res. Dev.* **2014**, *2*, 14–19.
31. Joseph, I.; Raj, R.P. Isolation and Characterization of Phytase Producing *Bacillus* Strains from Mangrove Ecosystem. *J. Mar. Biol. Assoc. India* **2007**, *49*, 177–182.
32. Oh, B.-C.; Choi, W.-C.; Park, S.; Kim, Y.-O.; Oh, T.-K. Biochemical properties and substrate specificities of alkaline and histidine acid phytases. *Appl. Microbiol. Biotechnol.* **2004**, *63*, 362–372. [CrossRef] [PubMed]
33. Olajuyigbe, F.M. Optimization of extracellular phytase production from *Bacillus amyloliquefaciens* PFB-02 Grown on Selected Agricultural Waste. *Appl. Trop. Agric.* **2016**, *5*, 92–97.
34. Ramani, V. Organic Phosphate Mineralization by *Bacillus sphaericus* and *Pseudomonas cepacia*. *Int. J. Agric. Technol.* **2014**, *10*, 915–922.
35. Madigan, M.; Martinko, J.; Parker, J. *Biología de los Microorganismos*, 14th ed.; Pearson: Madrid, Spain, 2010; ISBN 9788490352793.
36. Schneider, O.; Sereti, V.; Eding, E.; Verreth, J. Analysis of nutrient flows in integrated intensive aquaculture systems. *Aquac. Eng.* **2005**, *32*, 379–401. [CrossRef]
37. Monsees, H.; Keitel, J.; Paul, M.; Kloas, W.; Wuertz, S. Potential of aquacultural sludge treatment for aquaponics: Evaluation of nutrient mobilization under aerobic and anaerobic conditions. *Aquac. Environ. Interact.* **2017**, *9*, 9–18. [CrossRef]
38. Beristain, B. *Organic Matter Decomposition in Simulated Aquaculture Ponds*; Wageningen Universiteit: Wageningen, The Netherlands, 2005.
39. Henao, C.B.; Rincón, S.C. Aislamiento y Producción de Bacterias Fosfato Solubilizadoras a Partir de Compost. Bachelor's Thesis, Universidad Javeriana, Bogotá, Colombia, 2008.
40. Hua, K.; Bureau, D.P. Modeling Digestible Phosphorus Content of Salmonid Fish Feeds. *Aquaculture* **2006**, *254*, 455–465. [CrossRef]
41. Dillow, R.K. International Institute for Sustainable Development (IISD). In *Encyclopedia of Global Warming and Climate Change*; Philander, S., Ed.; Sage Publications: Sauzendaux, CA, USA, 2008. [CrossRef]
42. Balogun, S.; Gbadebo, A.; Sharaf, O.; Hareem, A.S. Cadmium Uptake by Biomass of *Lysinibacillus sphaericus* KY203810 and *Enterobacter cloacae* KY203811 from Steel Company Dumpsite. *Pac. J. Sci. Technol.* **2017**, *18*, 265–276.
43. Naureen, Z.; Rehman, N.; Hussain, H.; Hussain, J.; Gilani, S.; Housni, S.; Mabood, F.; Khan, A.; Farooq, S.; Abbas, G.; et al. Exploring the Potentials of *Lysinibacillus sphaericus* ZA9 for Plant Growth Promotion and Biocontrol Activities Against Phytopathogenic Fungi. *Front. Microbiol.* **2017**, *8*, 1477. [CrossRef] [PubMed]
44. Rafique, M.; Sultan, T.; Ortas, I.; Chaudhary, H.J. Enhancement of maize plant growth with inoculation of phosphate-solubilizing bacteria and biochar amendment in soil. *Soil Sci. Plant Nutr.* **2017**, *63*, 460–469. [CrossRef]
45. Yang, L.-L.; Huang, Y.; Liu, J.; Ma, L.; Mo, M.-H.; Li, W.-J.; Yang, F.-X. *Lysinibacillus mangiferahumi* sp. nov., a new bacterium producing nematicidal volatiles. *Antonie Van Leeuwenhoek* **2012**, *102*, 53–59. [CrossRef] [PubMed]
46. Vásquez, W. *Principios de Nutrición Aplicada al Cultivo de Peces*, 1st ed.; Editorial Juan XVIII Ltda: Los Llanos, Colombia, 2004.
47. Castellanos, J. (Ed.) *Manual de Producción de Tomate en Invernadero*, 1st ed.; Intagri S.C: Celaya, México, 2009; p. 456.

48. Delaide, B. *A Study on the Mineral Elements Available in Aquaponics, Their Impact on Lettuce Productivity and the Potential Improvement of Their Availability*; Université de Liège-Gembloux Agrio Biotech: Gembloux, Belgium, 2017.
49. Rafiee, G.; Saad, C.R. Nutrient cycle and sludge production during different stages of red tilapia (*Oreochromis* sp.) growth in a recirculating aquaculture system. *Aquaculture* **2005**, *244*, 109–118. [CrossRef]
50. Barker, A.; Pillbeam, D. *Handbook of Plant Nutrition*; Taylor & Francis: London, UK, 2006.
51. Krachler, R.F.; Krachler, R.; Stojanovic, A.; Wielander, B.; Herzig, A. Effects of pH on Aquatic Biodegradation Processes. *Biogeosciences Discuss.* **2009**, *6*, 491–514. [CrossRef]
52. Mc Cauley, A.; Clain, J.; Olson-Rutz, K. Soil pH and Organic Matter. *J. Mont. State Univ.* **2017**, *8*, 1–12.
53. Li, C.; Zhang, B.; Luo, P.; Shi, H.; Li, L.; Gao, Y.; Lee, C.T.; Zhang, Z.; Wu, W.-M. Performance of a pilot-scale aquaponics system using hydroponics and immobilized biofilm treatment for water quality control. *J. Clean. Prod.* **2019**, *208*, 274–284. [CrossRef]
54. Bartelme, R.P.; Oyserman, B.O.; Bloom, J.E.; Sepulveda-Villet, O.J.; Newton, R.J. Stripping Away the Soil: Plant Growth Promoting Microbiology Opportunities in Aquaponics. *Front. Microbiol.* **2018**, *9*, 8. [CrossRef]
55. Piñero, M.C.; Collado-González, J.; Otálora, G.; López-Marín, J.; del Amor, F.M. Plant Growth Promoting Bacteria for Aquaponics as a New Strategy That Grants Quality and Nutrient Efficiency in Kohlrabi Cultivation. *Horticulturae* **2023**, *9*, 1299. [CrossRef]
56. Mangmang, J.S.; Deaker, R.; Rogers, G. Inoculation effect of *Azospirillum brasilense* on basil grown under aquaponics production system. *Org. Agric.* **2016**, *6*, 65–74. [CrossRef]
57. Sirakov, I.; Lutz, M.; Graber, A.; Mathis, A.; Staykov, Y.; Smits, T.H.M.; Junge, R. Potential for Combined Biocontrol Activity against Fungal Fish and Plant Pathogens by Bacterial Isolates from a Model Aquaponic System. *Water* **2016**, *8*, 518. [CrossRef]
58. Khastini, R.; Idaryanto, F.R.; Alimuddin; Wahyuni, I.; Sari, I.J.; Puspita, N.; Wahyuni, A.F. Microbial consortia effects on the yields of water spinach in milkfish aquaponics system. *IOP Conf. Ser. Earth Environ. Sci.* **2019**, *383*, 012040. [CrossRef]
59. Mohamed, M.S.; Zaki, F.M.; Ahmedn, O.M. Effect of the Probiotic (*Bacillus* spp.) on Water Quality, Production Performance, Microbial Profile, and Flood Safety of the Nile Tilapia and Mint in Recirculating Aquaponic System. *Egypt. J. Aquat. Biol. Fish.* **2022**, *26*, 351–372.
60. Rivas-García, T.; González-Estrada, R.R.; Chiquito-Contreras, R.G.; Reyes-Pérez, J.J.; González-Salas, U.; Hernández-Montiel, L.G.; Murillo-Amador, B. Biocontrol of Phytopathogens under Aquaponics Systems. *Water* **2020**, *12*, 2061. [CrossRef]