



Article

Soil Properties Related to the Occurrence of Rock Phosphate-Solubilizing Bacteria in the Rhizosphere Soil of Faba Bean (*Vicia faba* L.) in Morocco

Abderrazak Rfaki ^{1,2} , Omar Zennouhi ¹, Laila Nassiri ¹ and Jamal Ibijbijen ^{1,*}

¹ Soil and Environment Microbiology Unit, Faculty of Sciences, Moulay Ismail University, 11201 Meknes, Morocco; a.rfaki@cnrst.ma (A.R.); omar.zennouhi@gmail.com (O.Z.); nassiri_layla@yahoo.fr (L.N.)

² Microbiology and Molecular Biology Laboratory (LMBM), National Centre for Scientific Research and Technical Research (CNRST), 10102 Rabat, Morocco

* Correspondence: j.ibijbijen@fs.umi.ac.ma; Tel.: +212-70-13-50-02

Received: 29 March 2018; Accepted: 10 May 2018; Published: 15 May 2018



Abstract: This study focused on revealing the correlations between rock phosphate-solubilizing bacteria (PSB) counts and differing properties of the rhizosphere soil. One hundred and thirty-two samples of rhizosphere soil of faba bean (*Vicia faba* L.) were collected and analyzed from 14 agricultural areas in Meknes, Morocco. The results revealed that all the samples were inhabited with indigenous PSB ranging from 3.55 to 0.002 ($\times 10^5$ CFU/g soil). The correlations between PSB counts and cation exchange capacity, available phosphorus (P), and pH were insignificant; on the contrary, a highly significant correlation ($p \leq 0.01$) was found between the numbers of PSB and total soil bacteria (TB) ($r = 0.80$), total nitrogen (N) ($r = 0.86$), and organic matter ($r = 0.90$). This research enhances our knowledge on PSB population and their interaction with physical, chemical, and biological properties of the rhizosphere soil of faba bean to provide a new index for better use in organic agricultural practices.

Keywords: rock-phosphate-solubilizing bacteria; faba bean; rhizosphere soil; correlation

1. Introduction

In agricultural soils, the solubilization of inorganic phosphate is closely related to soil microorganisms activity [1], such as bacteria [2], fungi [3], and actinomycetes [4]. The rhizosphere of plants is the best ecological niche to isolate plant-growth-promoting rhizobacteria (PGPR), such as rock phosphate-solubilizing bacteria (PSB), which have the ability to promote plant growth and increase plant production. Thus, to provide benefits to plants, PSB thus must be rhizosphere competent through a successful root colonization and persistence against various biotic and abiotic factors. Moreover, rhizosphere was first defined by Hiltner (1904) [5] as the volume of soil influenced by plant roots and their exudates. It is classically distinguished from bulk soil, which corresponds to the area located outside of the rhizosphere, and is therefore non-adhering to roots and not under its influence. The rhizospheric area forms a hot-spot of microbial abundance and activity due to the presence of plant exudates and rhizodeposits [6,7]. Rhizosphere microenvironments are frequently separated into rhizosphere soil (soil–root interface), rhizoplane (root surface), and endosphere (inner root), each possessing distinct features to which microorganisms have to adapt [8]. The rhizosphere has appeared as a versatile and dynamic ecological environment of intense plant–microbe interactions. Otherwise, the composition and structure of phosphate-solubilizing microorganisms (PSM) within rhizospheric soil vary greatly and are influenced largely by the physicochemical characteristics of soil [9]. Furthermore, these microorganisms are generally related to the surface of the soil particles,

especially at the level of the rhizosphere where their metabolic activity is higher [10]. Effectively, in the rhizosphere, root exudates, such as organic acids, are excellent sources of nutrients that can support the growth of microorganisms, which explains their high density at the level of the rhizospheric soil compared to the non-rhizospheric soil [11]. PSM do play a significant role in the biogeochemical cycling of phosphorus (P), increasing the availability of P in the rhizosphere and promoting plant growth. Thus, P is an essential macronutrient for plants and is required for vital functions, such as photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis, cell division, nucleic acid synthesis, and respiration [9]. Also, soils with a calcareous bedrock are especially characterized by their low P content [12]. In the calcareous soils, 88–99% of total inorganic P is bound to calcium, which is primarily responsible for the low P content in the soil solution [13]. Additionally, the addition of soluble P (phosphate fertilizer) to calcareous and alkaline soils may be subject to fixation/precipitation [14]. The P use efficiency (PUE) after application of phosphate fertilizer is low due to the formation of insoluble phosphate complexes through contact with soil colloids [15]. Similarly, only 15–25% synthetic phosphate fertilizers applied to overcome the deficiency of P element remain available to plants, and the rest becomes unavailable [16]. The abundance of PSB at the soil level depends on plant species, soil microbial composition, and soil conditions [17]. However, note that the soil phosphate-solubilizing microorganisms abundance compared with different groups of the microflora vary from soil to soil [18]. In addition, the number of PSB in 13 different sites distributed in some parts of Kenya ranged from 0.38 to 9.1×10^5 CFU g^{-1} of soil [19]. Similarly, Azziz et al. (2012) [20] reported that PSB present in the soils under crop–pasture rotations in a no-tillage regime in Uruguay varied from 0.65 to 62×10^5 CFU g^{-1} of soil. In contrast, Fernández et al. (2015) [21] recorded a low abundance of PSB, located between 0.03 and 0.08×10^5 CFU g^{-1} of soil, in the most productive region of the Argentinean Pampas near Bengolea. The aim of the present research is to study the relationship between density of rock phosphate-solubilizing bacteria population with physical, chemical, and biological properties of the rhizosphere soil of faba bean (*Vicia faba* L.) grown in different regions of Meknes, Morocco.

2. Materials and Methods

2.1. Study Site and Soil Sampling

In the most productive soil in Meknes (Morocco), 14 areas were prospected between April and May 2014 for collecting the samples of rhizospheric soil from *V. faba* (Table 1). Root system samples (5 to 10 cm around the plant and 15 to 20 cm in-depth in the soil) were taken from six randomly chosen plants in the same field. The extracted root system was carefully shaken by hand until the total removal of bulk soil. The remaining roots with rhizospheric soil were separated by brushing and all six subsamples were thoroughly mixed together to obtain a homogeneous composite sample for each study field. The composite soil samples were transferred to polyethylene bags and stored at 4 °C. Afterward, 1 g from each composite soil sample was used for counting the total bacteria (TB) and PSB, while the rest of the soil was air-dried, sieved through a 2-mm sieve, then used for chemical and physical soil properties determination.

2.2. Physicochemical Soil Characteristics

Available P was extracted after the Olsen method [22] and the concentration was determined colorimetrically after [23]. Total nitrogen (N) was estimated by the Kjeldahl digestion method [24]. The organic matter was determined by dosage of organic carbon using the potassium dichromate oxidation method [25]. The cation exchange capacity (CEC) was determined by the Metson method [26] and the pH was measured by a pH meter equipped with a glass electrode with a soil/distilled water ratio of (1/2.5).

2.3. Bacterial Counting

To enumerate TB and PSB in the rhizosphere soil, about 1 g of soil was weighed and transferred into a 250 mL Erlenmeyer flask with 10 mL of a phosphate buffer solution [27]. Subsequently, the solution obtained was stirred at 150 rpm for one hour. After incubation, a series of dilutions of 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-5} was prepared; thereafter, 100 μL aliquots of each dilution were plated on a suitable culture medium. For the enumeration of total bacteria (TB), the Plate Count Agar (PCA) medium was used [28]. The most suitable medium for the enumeration of PSB is NBRIP [29] supplemented with 5 g L^{-1} rock-phosphate powder from the phosphate mine of Khouribga (RPK). The elemental analysis (%) of RPK was described earlier [30] and showed: O, 56.53%; F, 2.42%; Na, 1.81%; Mg, 1.94%; Al, 2.03%; P, 9.37%; S, 0.77%; Sn, 0.12%; Ca, 16.35%; Fe, 0.60%. Before use, the RPK was carefully washed with the extraction solution Mehlich 3 [31] and several times with hot distilled water to remove all traces of available P; then, it was autoclaved and added to the medium of sterile culture as the only source of P. The number of bacteria per gram of soil was determined by the standard method of colony forming unit (CFU) [19] after 3 days of incubation at 28 ± 2 °C. Both media were supplemented with 100 $\mu\text{g}\cdot\text{mL}^{-1}$ of cycloheximide to inhibit fungal growth. Cycloheximide was prepared as a stock solution sterilized by membrane filtration, added to the autoclaved medium, and cooled to 50–55 °C.

2.4. Statistical Analysis

All statistical analyses were performed using the software IBM SPSS Statistics 20.0. The homogeneity of variances was tested for all variables by using the statistic of Levene. To find out if there is a significant correlation between the number of PSB and physical, chemical, and biological properties of our soil samples (TB, pH, organic matter, available P, total N, and CEC), a multiple correlation test was performed. Additionally, the results of all experiments were submitted to analysis of variance (ANOVA). Also, whenever the calculated F Fisher was significant ($p < 0.05$), a test of significant difference with the honesty of Tukey (HSD) was used to compare means.

3. Results

3.1. Physicochemical Soil Properties

The samples of rhizospheric soil from *V. faba* grown in the 14 most productive areas of Meknes were compared for five physical and chemical variables (Table 1).

The pH of rhizosphere soils varied between 6.03 and 8.12 (Table 1). Available P in the studied soils showed that 35.71% had a high concentration, 57.14% an average concentration, and 7.14% of the soils had a low concentration according to the chemical standards of arable soils in the European Union [32]. The highest available P content (41.58 $\text{mg}\cdot\text{kg}^{-1}$) was found at the Seba Ayoun station, while the lowest available P content (14.05 $\text{mg}\cdot\text{kg}^{-1}$) was recorded in the M'haya station (Table 1). The results of the organic matter content in the studied soils showed that 71.43% of the analyzed soils had a high concentration of organic matter, 7.14% had an average concentration, and 21.43% had a low concentration according to the standards of the LUCAS spatial database [33]. Organic matter content varied and ranged between 9.73 and 45.49 $\text{g}\cdot\text{kg}^{-1}$ (Table 1). The cation exchange capacity (CEC) varied between 8.87 and 18.26 $\text{cmol (+)}/\text{kg}$. The analysis of the results of the CEC showed that 35.71% of prospected soils revealed a great capacity for retention of the nutrient cations and 50% of soils showed a very low capacity for nutrient retention (Table 1). On the other hand, the results for the total N content, according to the standards of the LUCAS spatial database [33], showed that 85.71% of the analyzed soils were poor. The highest levels of total N were found at the El-Hajeb station with 0.11 $\text{g}\cdot\text{kg}^{-1}$ of soil, while the lowest total N content was recorded in the EL-Haj Kaddour station with 1.71 $\text{g}\cdot\text{kg}^{-1}$ of soil (Table 1).

Table 1. The physical and chemical characteristics of rhizosphere soil samples of *Vicia faba* collected from 14 sites in the region of Meknes and used for the enumeration of phosphate-solubilizing bacteria (PSB).

No.	Sampling Site	Location	Soil pH	Organic Matter (g·kg ⁻¹)	Available P (mg·kg ⁻¹)	Total N (g·kg ⁻¹)	CEC (cmol (+)/kg)
1	EL-Haj Kaddour (9)	33°49'18 N; 005°25'31 W	6.15 ± 0.08 ^{de}	45.49 ± 0.91 ^a	40.59 ± 0.62 ^a	1.71 ± 0.05 ^a	18.26 ± 1.21 ^{def}
2	El-Hajeb (12)	33°39'45 N; 005°21'21 W	7.22 ± 0.03 ^{abc}	11.70 ± 1.06 ^{ef}	32.84 ± 1.18 ^c	0.11 ± 0.01 ^f	29.33 ± 0.91 ^{cde}
3	Kantina (12)	33°41'23 N; 005°31'37 W	7.94 ± 0.05 ^b	16.48 ± 0.94 ^{de}	40.47 ± 0.65 ^a	0.12 ± 0.01 ^f	29.45 ± 0.62 ^{cde}
4	Bouderbala (12)	33°49'55 N; 005°16'09 W	8.07 ± 0.24 ^b	31.67 ± 0.88 ^b	40.37 ± 0.65 ^a	0.93 ± 0.05 ^{cd}	11.57 ± 0.94 ^f
5	M'haya (9)	33°57'44 N; 005°13'42 W	8.12 ± 0.04 ^a	9.73 ± 0.37 ^f	14.05 ± 0.11 ^f	0.93 ± 0.04 ^{cd}	13.72 ± 0.93 ^{ef}
6	Seba Ayoun (6)	33°54'27 N; 005°26'35 W	7.11 ± 0.09 ^{abcd}	25.43 ± 1.18 ^c	41.58 ± 0.47 ^a	0.78 ± 0.03 ^{de}	8.87 ± 0.11 ^f
7	Ait Hammad (9)	33°52'46 N; 005°09'14 W	6.03 ± 0.18 ^e	22.43 ± 0.91 ^c	27.40 ± 0.58 ^{de}	0.72 ± 0.04 ^e	10.31 ± 0.78 ^f
8	Rass Jerry (12)	33°46'06 N; 005°45'17 W	7.45 ± 0.02 ^{abc}	35.85 ± 1.25 ^b	30.48 ± 0.57 ^{cd}	1.18 ± 0.02 ^b	12.71 ± 0.16 ^f
9	Oued Beht (6)	33°52'15 N; 005°53'46 W	7.62 ± 0.02 ^b	24.52 ± 0.77 ^c	39.96 ± 1.01 ^a	0.78 ± 0.03 ^{de}	42.58 ± 0.95 ^{abc}
10	Agourai (6)	33°37'32 N; 005°38'41 W	7.58 ± 0.04 ^{abc}	13.88 ± 1.29 ^{ef}	29.64 ± 0.93 ^d	0.16 ± 0.03 ^f	44.51 ± 0.50 ^{abc}
11	Ain El Orma (9)	33°54'02 N; 005°46'11 W	7.06 ± 0.06 ^{bcde}	20.58 ± 0.49 ^{cd}	36.62 ± 0.51 ^b	1.17 ± 0.04 ^b	52.62 ± 0.65 ^a
12	Moulay Idriss Zerhoun (12)	34°01'48 N; 005°34'33 W	7.97 ± 0.06 ^b	43.39 ± 0.54 ^a	25.69 ± 0.36 ^e	1.68 ± 0.02 ^a	30.52 ± 0.66 ^{bcd}
13	Ain Jemaa (6)	33°59'03 N; 005°41'39 W	7.53 ± 0.06 ^{abc}	31.06 ± 0.15 ^b	29.90 ± 0.25 ^{cd}	1.09 ± 0.01 ^{bc}	46.50 ± 1.01 ^{ab}
14	Dar Oum Soltane (12)	33°53'56 N; 005°38'50 W	6.03 ± 0.18 ^{cde}	22.43 ± 0.84 ^c	27.06 ± 1.04 ^{de}	0.76 ± 0.01 ^{de}	11.31 ± 0.74 ^{def}

The results represent the mean of (n) repetitions, indicated in brackets behind each sampling site name, and represent the number of fields prospected at the same site ± standard deviation (SD). The different letters in the same column indicate significant differences ($p < 0.05$, Tukey's HSD test). P: Phosphorus; N: Nitrogen; CEC: cation exchange capacity.

3.2. Total Soil Bacteria (TB) and PSB Counting

The number of TB and PSB in the studied soils are presented in Figure 1, and the percentage of the PSB relative to TB is presented in Figure 2.

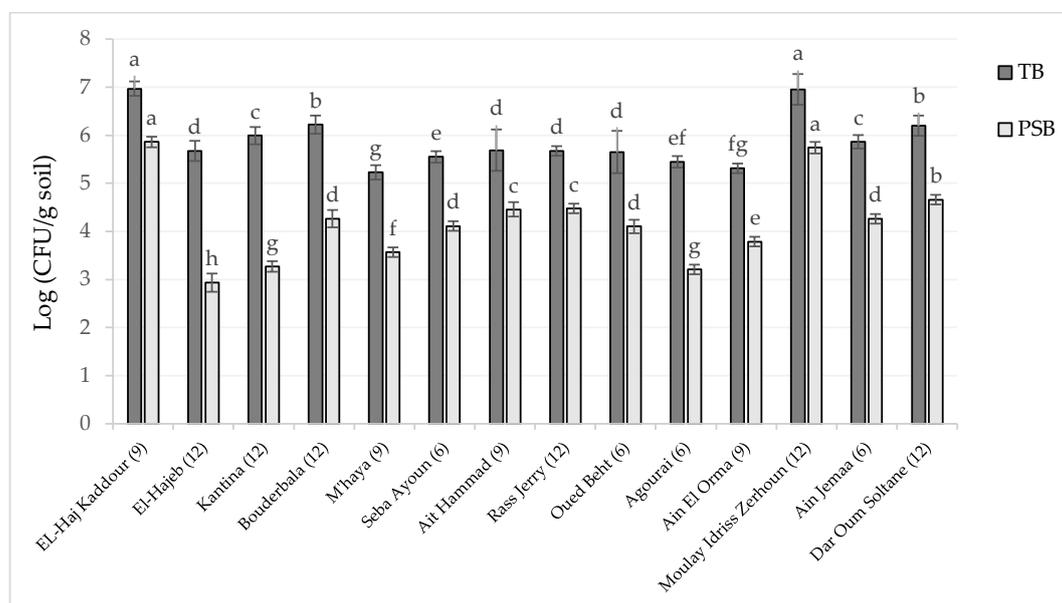


Figure 1. Number of total soil bacteria (TB) and PSB in soil samples. Values are the means of (n) repetitions, indicated in brackets behind each sampling site name, and represent the number of fields prospected at the same site and error bars indicate standard deviation. The different letters in the bars indicate significant differences ($p < 0.05$, Tukey's HSD test). TB: total bacteria; PSB: phosphate solubilizing bacteria.

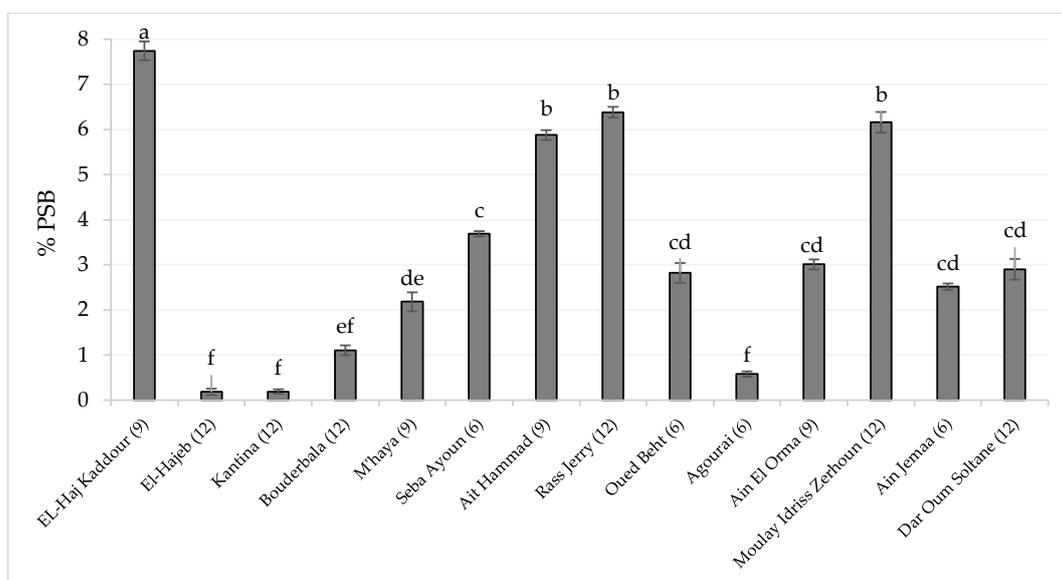


Figure 2. Percentage of PSB (%PSB) compared to TB in soil samples. Values are the means of (n) repetitions, indicated in brackets behind each sampling site name, and represent the number of fields prospected at the same site and error bars indicate standard deviation. The different letters in the bars indicate significant differences ($p < 0.05$, Tukey's HSD test).

The results showed that PSB are present in all prospected soils in this study.

The results indicate that there is an overall difference in the content TB, PSB, and %PSB in different rhizospheric soils of *V. faba* (Table 2). Also, the results of the enumeration of TB ranging between 0.98 (Ain El Orma) and 93.28 (EL-Haj Kaddour) $\times 10^5$ CFU g^{-1} soil. Furthermore, the number of PSB varied between 0.0021 and 7.24 $\times 10^5$ CFU g^{-1} soil, respectively, in Rass Jerry and EL-Haj Kaddour. The %PSB varied between 0.18% and 7.74%, recorded successively, in El-Hajeb and EL-Haj Kaddour.

Table 2. Analysis of variance for the number of TB, PSB, and for the %PSB in the rhizosphere soil of *V. faba*.

		Sum of Squares	ddl	Average Square	F	Signification
TB	Intergroup	11.348	13	0.873	380.847	0.000
	Intragroup	0.064	28	0.002		
	Total	11.412	41			
PSB	Intergroup	28.570	13	2.198	617.152	0.000
	Intragroup	0.100	28	0.004		
	Total	28.669	41			
%PSB	Intergroup	234.418	13	18.032	93.522	0.000
	Intragroup	5.399	28	0.193		
	Total	239.817	41			

TB: total bacteria; PSB: phosphate-solubilizing bacteria; %PSB: percentage of PSB compared to TB.

3.3. The Relationship between Soil Properties and PSB Population

The results of the Pearson correlation coefficient (Table 3) between the soil properties (TB, pH, organic matter, total N, available P, and CEC) and PSB population showed a positive and significant ($p < 0.01$) correlation between number of TB ($r = 0.80$), organic matter ($r = 0.90$), total N ($r = 0.86$), and PSB population. Inversely, pH, available P, and the CEC are not correlated ($p < 0.01$) with PSB population.

Table 3. Multiple correlations between the number of PSB and the physical and chemical characteristics of rhizosphere soil samples of *V. faba*.

	PSB	TB	pH	OM	P	N	CEC
PSB	1						
TB	0.800 **	1					
pH	−0.346	−0.170	1				
OM	0.902 **	0.777 **	−0.148	1			
P	0.031	0.200	−0.080	0.268	1		
N	0.866 **	0.551 **	−0.119	0.822 **	−0.068	1	
CEC	−0.283	−0.195	0.274	−0.149	0.129	−0.101	1

** Indicates a significant correlation at ($p < 0.01$). PSB: phosphate-solubilizing bacteria; TB: total bacteria; pH: pH of soil; OM: organic matter; P: available P; N: total N; CEC: cation exchange capacity.

4. Discussion

The pH of rhizosphere soils in 78.57% of the explored stations revealed the alkalinity of most of the soils studied. This result could be explained by the calcareous rock substrate constituting the plateau of Meknes-Sais. In contrast, 3 stations among the 14 stations surveyed showed an acidic pH. This could be explained by the addition of organic matter (mulch, animal manure) to the soil by some farmers to improve the fertility of their soils, causing a slight soil acidification. However, P is one of the less-mobile nutrients and is less available to plants because of its high reactivity with many soil constituents [34]. In our study, available P was average or low in the case of 64.28% of the studied soils. Similarly, several studies showed that soils with a calcareous bedrock are especially characterized by their low P content due to insoluble phosphate complexes formed through contact with soil colloids (mainly calcium) [12–15]. Moreover, in agricultural soils, the dissolution of inorganic phosphate immobilized by the exchange complexes is closely related to soil microorganisms activity [1].

Maintaining a high level of P has been a major challenge to agricultural scientists, ecologists, and farm managers due to complex bonds formation between available phosphorus and soil cations (Ca^{2+} , Al^{3+} , Fe^{2+} , or Mn^{2+}) depending on soil pH and organic matter [35]. Thus, in contemporary agricultural practices, synthetic phosphatic fertilizer is applied to overcome the P deficiency to plants, which indeed is expensive and poses some serious threats to sustaining the environment. The use of PSM is a sustainable approach for managing P deficiency in agricultural soils [36]. Also, PSB have a great ability to transform insoluble P in the soil into an available form and have great application prospects for eco-agriculture [37].

Heterotrophic bacteria depend on external carbon (C) sources to synthesize energy-rich compounds, such as adenosine triphosphate (ATP). Soil organic matter is a rich source of energy and essential nutrients for microorganisms' growth that needs to be degraded to provide the C sources in the soil. The organic matter content in the studied soils showed that 71.43% of the analyzed soils have a high concentration. This can be explained by plant residues left on the soil surface after each harvest. Also, among the most influential factors on soil quality with respect to soil structure we find especially the aggregate stability and accumulation of organic matter [38]. Moreover, in the present study, we observed that the soils with higher organic matter content showed a higher number of PSB. It may be possible that PSB present in those soils are heterotrophic bacteria. Additionally, we observed that soils with high numbers of TB had more PSB, and it is possible that the organic carbon in the soil supported the growth of both TB as well as PSB as observed in the present study. The P-solubilization performance of PSB depends on the soil organic matter degradation, which gives a rich source of energy and nutrients for P-solubilizers' growth [39]. Otherwise, the CEC, which expresses negative charges per unit mass of soil, is one of the most important characteristics of the soil. Also, only clays and organic matter, by their colloidal properties, can develop significant negative charges on their surface [40]. Furthermore, clays and humic substances most commonly exist in natural soils as clay–humic complexes. Polyvalent cations commonly bridge between the negative charge sites of clay surfaces and negatively charged organic functional groups on humic substances. The main polyvalent cations responsible for the binding of humic and fulvic acids to soil clays are Ca^{2+} , Mg^{2+} , Al^{3+} , Fe^{3+} , K^+ , H^+ , and Na^+ , which will later define the value of the CEC [41]. Our results of the CEC showed that 35.71% of prospected soils revealed a great capacity for retention of cations. This could be mainly attributed to the high content of organic matter in most of the studied soils. For a given agricultural soil, increases in the organic matter content cause an increase of the CEC [42]. In contrast, 50% of soils showed a very low capacity for nutrient cations retention, which is contradictory to the results of the organic matter content. This can be explained by a slow degradation of the organic matter and thus the production of an unstable and unsaturated clay–humic complex, thus causing a decrease of the CEC. Likewise, the total N showed that 85.71% of the analyzed soils were poor, which requires reasonable nitrogen fertilization in this study area to overcome the deficiency. However, these soils generate low crop yields due to a combination of factors that includes their extreme N and P deficiency [43,44].

The number of TB found in all samples analyzed (ranging between 0.98 and 93.28×10^5 CFU g^{-1} soil) is within the theoretical and practical limits indicated in the agricultural soils, which are situated between 10^4 – 10^9 CFU g^{-1} of soil [45]. Moreover, similar results were recently reported—a number of TB between 6 – 14×10^5 CFU g^{-1} soil—from agricultural fields under no-till management in Argentina [21]. Also, a number between 22 and 660×10^5 CFU g^{-1} soil was reported from experimental fields in Uruguay [20]. Furthermore, we observed that the number of PSB in all our samples was still well above 2×10^2 CFU g^{-1} of soil (it varied between 0.0021 and 7.24×10^5 CFU g^{-1} soil). Also, similar results have been reported in Kenyan soils with a number between 0.38 and 9.1×10^5 CFU g^{-1} of soil [19] and in Chinese soils from 6 to 22×10^5 CFU g^{-1} of soil [46]. However, other studies have reported higher values than ours, such as soils under crop–pasture rotations in a no-tillage regime in Uruguay with 0.65 – 62×10^5 CFU g^{-1} soil [20] and 3 – 67×10^5 CFU g^{-1} soil in the rhizosphere of chickpea, mustard, and wheat grown in different regions of Haryana in India [47]. On the contrary, very low numbers have been reported in northern Spain with an abundance of PSB lower than 10^2 CFU g^{-1}

soil [48], in Argentina between 0.03 and 0.08×10^5 CFU g^{-1} of soil [21], and in northern Karnataka in India from 0.01 to 0.18×10^5 CFU g^{-1} of soil [49]. Moreover, these results can be explained by a preferential selection of the microorganisms in rhizospheric soil under the effect of the different root exudates according to the species and the variety of plants [50]. Likewise, it was reported recently that microbial biodiversity in the rhizosphere of different plant species is primarily related to soil conditions and plant genotype [51,52].

Pearson correlation showed a positive and significant ($p < 0.01$) correlation between number of TB ($r = 0.80$), organic matter ($r = 0.90$), total N ($r = 0.86$), and PSB population. Inversely, pH, available P, and the CEC were not correlated ($p < 0.01$) with PSB population. Similarly, Vikram et al. (2007) [49] reported a strong positive correlation between organic carbon content ($r = 0.40$, $p < 0.01$), available N ($r = 0.4$, $p < 0.05$), and PSB population, whereas pH and available P showed no significant correlation with PSB population. Also, Ndung'u-Magiroi et al. (2012) [19] showed a positive and highly significant ($p < 0.001$) correlation between PSB and phosphate-solubilizing microorganisms (PSM) populations ($r = 0.98$), organic C ($r = 0.76$), exchangeable Ca ($r = 0.93$), and exchangeable Mg ($r = 0.92$), while pH and extractable P did not correlate with the PSB population. Thus, no significant correlation between available P and PSB population was recorded in our case, which is consistent with several studies [19,39,49,53,54]. In contrast, other researchers have found that soil P intake increases the size of the PSB population [21,46]. Furthermore, we found that soils rich in organic matter and N contain more PSB, which could be explained by the heterotrophy of PSB requiring exogenous carbon and N sources to ensure vital physiological functions [55–57].

5. Conclusions

The results obtained showed a positive and significant ($p < 0.01$) correlation between PSB population and TB, organic matter, and total N. Contrariwise, pH, CEC, and available P were not correlated with the population of PSB. However, improving the sustainability of agriculture requires the optimal use and management of soil fertility and their physicochemical properties, which are based on the biological processes of soils and their biodiversity.

Author Contributions: I.J. and R.A. conceived and designed the experiments; R.A. and Z.O. performed the experiments; N.L., R.A., and I.J. worked together to analyze the data; R.A. wrote the paper with input from all authors.

Acknowledgments: The authors would like to thank the Dean of the Faculty of Science at Moulay Ismail University for their financial support, and the members of the Regional Center for Agronomic Research of Meknes for their technical support.

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

References

1. Richardson, A.E. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Aust. J. Plant Physiol.* **2001**, *28*, 897–906. [[CrossRef](#)]
2. Zaidi, A.; Khan, M.S.; Ahemad, M.; Oves, M. Plant growth promotion by phosphate solubilizing bacteria. *Acta Microbiol. Immunol. Hung.* **2009**, *56*, 263–284. [[CrossRef](#)] [[PubMed](#)]
3. Khan, M.S.; Zaidi, A.; Ahemad, M.; Oves, M.; Wani, P.A. Plant growth promotion by phosphate solubilizing fungi—Current perspective. *Arch. Agron. Soil Sci.* **2010**, *56*, 73–98. [[CrossRef](#)]
4. Hamdali, H.; Smirnov, A.; Esnault, C.; Ouhdouch, Y.; Virolle, M.J. Physiological studies and comparative analysis of rock phosphate solubilization abilities of actinomycetales originating from Moroccan phosphate mines and of *Streptomyces lividans*. *Appl. Soil Ecol.* **2010**, *44*, 24–31. [[CrossRef](#)]
5. Hiltner, L. Über neuere erfahrungen und probleme auf dem gebiete der bodenbakteriologie unter besonderer berucksichtigung der grundungung und brache. *Arb. Dtsc. Landwirtschaft. Ges.* **1904**, *98*, 59–78.
6. Kamaludeen, S.P.B.; Ramasamy, K. Rhizoremediation of metals: Harnessing microbial communities. *Indian J. Microbiol.* **2008**, *48*, 80–88. [[CrossRef](#)] [[PubMed](#)]

7. Zhuang, X.; Chen, J.; Shim, H.; Bai, Z. New advances in plant growth-promoting rhizobacteria for bioremediation. *Environ. Int.* **2007**, *33*, 406–413. [[CrossRef](#)] [[PubMed](#)]
8. McNear, D., Jr. The rhizosphere—roots, soil and everything in between. *Nat. Educ. Knowl.* **2013**, *4*, 1.
9. Zaidi, A.; Khan, M.S.; Ahmad, E. Microphos: Principles, Production and Application Strategies. In *Phosphate Solubilizing Microorganisms: Principles and Application of Microphos Technology*; Khan, M.S., Zaidi, A., Musarrat, J., Eds.; Springer International Publishing: Cham, Switzerland, 2014; pp. 1–30, ISBN 978-3-319-08215-8, 978-3-319-08216-5.
10. Vazquez, P.; Holguin, G.; Puente, M.; Cortes, A.E.; Bashan, Y. Phosphate solubilizing microorganisms associated with the rhizosphere of mangroves in a semi-arid coastal lagoon. *Biol. Fertil. Soils* **2000**, *30*, 460–468. [[CrossRef](#)]
11. Hinsinger, P. Bioavailability of soil inorganic P in the rhizosphere as affected by root induced chemical changes: A review. *Plant Soil* **2001**, *237*, 173–195. [[CrossRef](#)]
12. Tunesi, S.; Poggi, V.; Gessa, C. Phosphate adsorption and precipitation in calcareous soils: The role of calcium ions in solution and carbonate minerals. *Nut. Cycl. Agroecosyst.* **1999**, *53*, 219–227. [[CrossRef](#)]
13. Tisdale, S.L.; Nelson, W.L.; Beaton, J.D.; Havlin, J.L. *Soil Fertility and Fertilizers*, 5th ed.; Macmillan Publishing Company: New York, NY, USA, 1993; p. 634, ISBN 0-02-420835-3.
14. Baig, K.S.; Arshad, M.; Shaharouna, B.; Khalid, A.; Ahmed, I. Comparative effectiveness of *Bacillus* spp. possessing either dual or single growth-promoting traits for improving phosphorus uptake, growth and yield of wheat (*Triticum aestivum* L.). *Ann. Microbiol.* **2012**, *62*, 1109–1119. [[CrossRef](#)]
15. Vassilev, N.; Vassileva, M. Biotechnological solubilization of rock phosphate on media containing agro-industrial wastes. *Appl. Microbiol. Biotechnol.* **2003**, *61*, 435–440. [[CrossRef](#)] [[PubMed](#)]
16. Shen, J.; Yuan, L.; Zhang, J.; Li, H.; Bai, Z.; Chen, X.; Zhang, W.; Zhang, F. Phosphorus dynamics: From soil to plant. *Plant Physiol.* **2011**, *156*, 997–1005. [[CrossRef](#)] [[PubMed](#)]
17. Katiyar, V.; Goel, R. Solubilization of inorganic phosphate and plant growth promotion by cold tolerant mutants of *Pseudomonas fluorescens*. *Microbiol. Res.* **2003**, *158*, 163–168. [[CrossRef](#)] [[PubMed](#)]
18. Chabot, R.; Antoun, H.; Cescas, M.P. Stimulation de la croissance du maïs et de la laitue romaine par des microorganismes dissolvant le phosphore inorganique. *Can. J. Microbiol.* **1993**, *39*, 941–947. [[CrossRef](#)]
19. Ndung'u-Magiroyi, K.W.; Herrmann, L.; Okalebo, J.R.; Othieno, C.O.; Pypers, P.; Lesueur, D. Occurrence and genetic diversity of phosphate-solubilizing bacteria in soils of differing chemical characteristics in Kenya. *Ann. Microbiol.* **2012**, *62*, 897–904. [[CrossRef](#)]
20. Azziz, G.; Bajsa, N.; Haghjou, T.; Taulé, C.; Valverde, Á.; Igual, J.M.; Arias, A. Abundance, diversity and prospecting of culturable phosphate solubilizing bacteria on soils under crop–pasture rotations in a no-tillage regime in Uruguay. *Appl. Soil Ecol.* **2012**, *61*, 320–326. [[CrossRef](#)]
21. Fernández, L.A.; Agaras, B.; Wall, L.G.; Valverde, C. Abundance and ribotypes of phosphate-solubilizing bacteria in Argentinean agricultural soils under no-till management. *Ann. Microbiol.* **2015**, *65*, 1667–1678. [[CrossRef](#)]
22. Olsen, S.R.; Cole, C.V.; Watanabe, F.S.; Dean, L.A. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. In *USDA Circular*; Government Publication; Printing Office: Washington, DC, USA, 1954; pp. 1–19.
23. Murphy, J.; Riley, J.P. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* **1962**, *27*, 31–36. [[CrossRef](#)]
24. Trivedy, R.K.; Goel, P.K.; Trisal, C.L. *Practical Methods in Ecology and Environmental Media*; Series in Methodology-2; Enviro Media Publication: Karad, India, 1998.
25. Walkley, A.; Black, A. An examination of the Degtjareff method for determining soil organic matter and proposed modification of the chromic acid titration method. *Soil Sci.* **1934**, *37*, 29–37. [[CrossRef](#)]
26. Metson, A.J. *Methods of Chemical Analysis for Soil Survey Samples*; Bulletin 12; Department of Scientific and Industrial Research: Wellington, New Zealand, 1956; p. 207.
27. Araújo, W.L.; Lima, A.O.S.; Azevedo, J.L.; Marcon, J.; Sobral, J.K.; Lakava, P.L. *Manual Isolation of Endophytic Microorganisms*; Department of Genetics—College of Agriculture “Luiz de Queiroz”—Piracicaba ESALQ—USP: São Paulo, Brazil, 2002. (In Portuguese)
28. Atlas, R.M. *Handbook of Microbiological Media*, 4th ed.; Taylor & Francis Group: Boca Raton, FL, USA, 2010; p. 2040.

29. Nautiyal, C.S. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiol. Lett.* **1999**, *170*, 265–270. [[CrossRef](#)] [[PubMed](#)]
30. Hamdali, H.; Hafidi, M.; Virolle, M.J.; Ouhdouch, Y. Rock phosphate solubilizing Actinomycetes: Screening for plant growth promoting activities. *World J. Microbiol. Biotechnol.* **2008**, *24*, 2565–2575. [[CrossRef](#)]
31. Mehlich, A. Mehlich-3 soil test extractant: A modification of Mehlich 2 extractant. *Commun. Soil Sci. Plant Anal.* **1984**, *15*, 1409–1416. [[CrossRef](#)]
32. Tóth, G.; Guicharnaud, R.A.; Tóth, B.; Hermann, T. Phosphorus levels in croplands of the European Union with implications for P fertilizer use. *Eur. J. Agron.* **2014**, *55*, 42–52. [[CrossRef](#)]
33. Tóth, G.; Jones, A.; Montanarella, L. *LUCAS Topsoil Survey. Methodology, Data and Results. JRC Technical Reports; EUR26102—Scientific and Technical Research Series; Publications Office of the European Union: Luxembourg, 2013; p. 141.* [[CrossRef](#)]
34. Giroux, M.; Cantin, J.; Rivest, M.; Tremblay, G. L'évolution des teneurs en phosphore dans les sols selon leur fertilité, leur richesse en phosphore et les types de sol. In *Colloque sur le Phosphore: Une Gestion Éclairée; Ordre des agronomes du Québec: Drummondville, QC, Canada, 2002.*
35. Kuhad, R.C.; Singh, S.; Lata, S.A. Phosphate solubilizing microorganisms. In *Bioaugmentation, Biostimulation and Biocontrol; Singh, A., Parmar, N., Kuhad, R.C., Eds.; Soil Biology Series; Springer: Heidelberg, Germany, 2011; Volume 28, pp. 65–84.* [[CrossRef](#)]
36. Mutai, C.; Njuguna, J.; Ghimire, S. *Brachiaria* Grasses (*Brachiaria* spp.) harbor a diverse bacterial community with multiple attributes beneficial to plant growth and development. *MicrobiologyOpen* **2017**, *6*, e497. [[CrossRef](#)] [[PubMed](#)]
37. Wang, Z.; Xu, G.; Ma, P.; Lin, Y.; Yang, X.; Cao, C. Isolation and Characterization of a Phosphorus-Solubilizing Bacterium from Rhizosphere Soils and Its Colonization of Chinese Cabbage (*Brassica campestris* ssp. *chinensis*). *Front. Microbiol.* **2017**, *8*, 1270. [[CrossRef](#)] [[PubMed](#)]
38. Buscot, F. What are soils? In *Microorganisms in Soils: Roles in Genesis and Functions; Buscot, F., Varma, S., Eds.; Springer: Heidelberg, Germany, 2005; Volume 3, pp. 3–18.* [[CrossRef](#)]
39. Nahas, E. Phosphate solubilizing microorganisms: Effects of carbon, nitrogen and phosphorus. In *First International Meeting on Microbial Phosphate Solubilization: Developments in Plant and Soil Sciences; Velazquez, E., Rodriguez-Barrueco, C., Eds.; Springer SBM: Dordrecht, The Netherlands, 2007; Volume 102, pp. 111–115.* [[CrossRef](#)]
40. Brady, N.C.; Weil, R.R. *The Nature and Properties of Soils*, 13th ed.; Prentice Hall: Upper Saddle River, NJ, USA, 2002; p. 960, ISBN 0130167630, 9780130167637.
41. Rowell, D.L. *Soil Science: Methods and Application; Longman Scientific & Technical Group: New York, NY, USA; London, UK, 1994; p. 350, ISBN 0582087848, 9780582087842.*
42. Mbonigaba Muhinda, J.J.; Nzeyimana, I.; Bucagu, C.; Culot, M. Caractérisation physique, chimique et microbiologique de trois sols acides tropicaux du Rwanda sous jachères naturelles et contraintes à leur productivité. *Biotechnol. Agron. Soc. Environ.* **2009**, *13*, 545–558.
43. He, Z.L.; Yang, X.E.; Baligar, B.C.; Calvert, D.V. Microbiological and biochemical indexing systems for assessing quality of acid soils. *Adv. Agron.* **2003**, *78*, 89–138.
44. Chu, H.; Fujii, T.; Morimoto, S.; Lin, X.; Yagi, K.; Hu, J.; Zhang, J. Soil microbial biomass, dehydrogenase activity, bacterial community structure in response to long-term fertilizer management. *Soil Biol. Biochem.* **2007**, *39*, 2971–2976. [[CrossRef](#)]
45. Kennedy, A.C. Bacterial diversity in agroecosystems. *Agric. Ecosyst. Environ.* **1999**, *74*, 65–76. [[CrossRef](#)]
46. Hu, J.; Lin, X.; Wang, J.; Chu, H.; Yin, R.; Zhang, J. Population size and specific potential of P-mineralizing and—Solubilizing bacteria under long-term P-deficiency fertilization in a sandy loam soil. *Pedobiologia* **2009**, *53*, 49–58. [[CrossRef](#)]
47. Kundu, B.; Nehra, K.; Yadav, R.; Tomar, M. Biodiversity of phosphate solubilizing bacteria in rhizosphere of chickpea, mustard and wheat grown in different regions of Haryana, Indian. *J. Microbiol.* **2009**, *49*, 120–127. [[CrossRef](#)]
48. Peix, A.; Rivas-Boyer, A.A.; Mateos, P.F.; Rodríguez-Barrueco, C.; Martínez-Molina, E.; Velazquez, E. Growth promotion of chickpea and barley by a phosphate solubilizing strain of *Mesorhizobium mediterraneum* under growth chamber conditions. *Soil Biol. Biochem.* **2001**, *33*, 103–110. [[CrossRef](#)]
49. Vikram, A.; Alagawadi, A.R.; Hamzehzarghani, H.; Krishnaraj, P.U. Factors related to the occurrence of phosphate solubilizing bacteria and their isolation in Vertisols. *Int. J. Agric. Res.* **2007**, *2*, 571–580. [[CrossRef](#)]

50. Costa, R.; Götz, M.; Mrotzek, N.; Lottmann, J.; Berg, G.; Smalla, K. Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. *FEMS Microbiol. Ecol.* **2006**, *56*, 236–249. [[CrossRef](#)] [[PubMed](#)]
51. Belimov, A.A.; Puhalsky, I.V.; Safronova, V.I.; Shaposhnikov, A.I.; Vishnyakova, M.A.; Semenova, E.V.; Nadezda, Y.Z.; Natalya, M.M.; Walter, W.; Igor, A.T. Role of plant genotype and soil conditions in symbiotic plant-microbe interactions for adaptation of plants to cadmium-polluted soils. *Water Air Soil Pollut.* **2015**, *226*, 1–15. [[CrossRef](#)]
52. Schreiter, S.; Sandmann, M.; Smalla, K.; Grosch, R. Soil type dependent rhizosphere competence and biocontrol of two bacterial inoculant strains and their effects on the rhizosphere microbial community of field-grown lettuce. *PLoS ONE* **2014**, *9*, e103726. [[CrossRef](#)] [[PubMed](#)]
53. Yahya, A.I.; Al-Azawi, S.K. Occurrence of phosphate-solubilizing bacteria in some Iraqi soils. *Plant Soil* **1989**, *117*, 135–141. [[CrossRef](#)]
54. Kucey, R.M.N. Phosphate solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. *Can. J. Soil Sci.* **1983**, *63*, 671–678. [[CrossRef](#)]
55. Moat, A.G.; Foster, J.W. *Microbial Physiology*, 2nd ed.; Wiley-Interscience: New York, NY, USA, 1988; p. 597.
56. John, L.; Herms, D.; Stinner, B.; Hostink, H. *Mulch Effect on Soil Microbial Activity, Nutrient Cycling, and Plant Growth in Ornamental Landscape*; Ornamental Plant Annual Report and Research Reviews; The Ohio State University-Columbus: Columbus, OH, USA, 2001.
57. Bashan, Y.; Puente, M.E.; Rodriquia, M.N.; Toledo, G.; Holguin, G.; Ferrera-Cerrato, R.; Pedrin, S. Survival of *Azorhizobium brasilense* in the bulk soil and rhizosphere of 23 soil types. *Appl. Environ. Microbiol.* **1995**, *61*, 1938–1945. [[PubMed](#)]



© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).