RESEARCH ARTICLE Agricultural Science Digest, Volume 40 Issue 4: 370-375 (December 2020)

Do *Phaseolus vulgaris* Development Stages Influence the Total Rhizospheric Bacterial and the Phytate Utilising Community?

R.T. Maougal[1, 2], M. Kechid[1, 2], K. Baziz[3], A. Djekoun[1]

10.18805/ag.D-286

ABSTRACT

Background: The occupation of soil by plants is known to induce changes in the soil chemical and physical properties by shoot decomposition and root growth and secretion.

Methods: In this study we investigate the degree to which the total and the phytate mineralising rhizospheric communities bacteria are affected by the growth of six Recombinant Inbred Lines (RILs) of common bean (*Phaseolus vulgaris*). The six RILs tested were contrasted on adaptation to P (phosphorus)-deficiency from sensitive to tolerant. Rhizosphere samples were taken three times during the plant developmental stage and the changes in the density and the phytase activity of those communities relative to the P content were studied. Bacterial community was followed by culturing and measuring total community DNA of soil to allow a cultivation-independent analysis, by amplification of the 16S rRNA gene using real time PCR.

Result: Our results showed that successional moves in the rhizosphere bacterial density as plant mature confirmed that plants select their own rhizosphere community. Moves of the bacterial community in the rhizosphere were more pronounced in mature common bean and phytase enzymatic activity confirmed the ability of this plant to mobilise a functional bacterial community when the bean needs high quantities of phosphorus. This study demonstrates that common bean selects a microbial community and its density change in response to plant growth.

Key words: Common bean, Plant growth stage, Population dynamics, Rhizosphere, Soil phytase activity.

INTRODUCTION

Carbonaceous compounds are abundantly released from root into the rhizosphere (da Rocha, 2009). These rhizodeposits influence the root- associated microbial communities (Belimov *et al*., 2009) by stimulating the growth of bacterial and fungal population in the surroundings of the roots comparing to the bulk soil (Subhashini, 2013), phenomenon called rhizosphere effect (Rovira, 1965). Specific bacteria in the plant rhizosphere are beneficial to plant growth by using the available nutriments of N, P and K. They play important roles in the terrestrial ecosystem by participating in almost all biochemical processes (Xu, 2007).

During root growth, local soil characterisations are increasingly affected by the roots (Girme and Sarnoba, 2018) and a number of studies have indicated that the structural and functional diversity of rhizosphere population is affected by the plant species due to differences in root exudation and rhizodeposition in different root zones (Søressen, 1997). Plant roots create selective pressures that influence the local microbial communities, causing effects on their abundance and composition (Gaumont Guay *et al*., 2008).

Phosphorus, one of the major nutrients limiting plant growth, rapidly immobilized after addition to soil as a soluble fertilizer and thus, it becomes less available to plant. Low P availability affects particularly legumes with high phosphorus requirements, especially beans (Graham and Vance, 2003). The sensitivity of legume to their deficiency has been widely documented and is generally attributed to large amount of carbon rotation and P-dependent energy required in the metabolism (Schulze and Drevon, 2005). Furthermore, soil enzyme activities influence the growth of plants (Sun *et al*., 2015). Soil enzymes, such as phosphatase, have also been regarded as biological indicators of soil health by playing key roles in nutrient cycling and agricultural ecosystems (Alkorta *et al*., 2003). Among a diversity of phosphatases enzyme, phytase is the only enzyme that has the specific capacity to degrade phytate, the major constituent form of soil organic P (comprise up to 60%-80% of the soil total P)

[1]Laboratoire de Génétique, Biochimie et Biotechnologies Végétales (GBBV), Département de Biologie et d'Ecologie, Faculté de Biologie, Université Frères Mentouri Constantine 1, Route de Ain El Bey, 25000 Constantine, Algeria.

[2]Département de Biotechnologie, Institut de la Nutrition, de l'Alimentation et des Technologies Agro-Alimentaires (INATAA), Université Frères Mentouri Constantine 1, Route de Ain el Bey, 25000 Constantine, Algeria.

[3]Département de Pharmacie, Faculté de Médecine, Université Hadj Lakhdar. Route de Tazoult, 05000 Batna, Algeria.

Corresponding Author: R.T. Maougal, Laboratoire de Génétique, Biochimie et Biotechnologies Végétales (GBBV), Département de Biologie et d'Ecologie, Faculté de Biologie, Université Frères Mentouri Constantine 1, Route de Ain El Bey, 25000 Constantine, Algeria. Email: rym.maougal@umc.edu.dz

How to cite this article: Maougal, R.T., Kechid, M., Baziz K. and Djekoun, A. (2020). Do *Phaseolus vulgaris* Development Stages Influence the Total Rhizospheric Bacterial and the Phytate Utilising Community? Agricultural Science Digest. 40(4): 370-375.

Submitted: 01-06-2020 **Accepted:** 02-07-2020 **Published:** 10-09-2020

(Bargaz *et al*., 2015). Soil microbes (Thakur *et al*., 2014) can largely affect enzyme activities (Ros *et al*., 2006).

The effect of plant developmental stage on rhizosphere microbial communities has not been so widley investigated (Mougel *et al*., 2006). The change from young plant, flowering plant, to a plant in senescence may influence the microbial community structure. This modification also varies with the plant type and microbial community composition (Watt *et al*., 2006). We have investigated changes in microbial biomass in the rhizosphere of *phaseolus vulgaris* to reveal the relationship among the rhizosphere bacterial community, soil phytase activity and the developmental stage of the plant.

MATERIALS AND METHODS

Site description and growing condition

Six recombinant inbred lines (RILs) of *P. vulgaris* selected from a previous screen of the crossing of two parental RILs (BAT477 and DOR364) (Drevon *et al*., 2011) were used. Three RILs are P deficiency- sensitive: 147, 83, 29 and three RILs are P deficiency resistant: 104, 34 and 115.

These RILs were cultivated in field during 2017 in the agronomic station of CNCC (Centre National de Certification et de Controle des semences) in the region of Constantine, Algeria (36°15′N, 6°41′E, 630 m). This soil contained 357, 301, 309, 28.8, 3.1 and 0.59 g kg^{-1} soil clay, silt, sand, organic-C, organic-N and P, respectively. The pH was alkaline (pH water: 8:2). The RILs were grown under field conditions without fertilizer application.

Sampling of roots and soil

Three sampling dates corresponding to representative developmental stage of the plant were chosen: vegetative stage (April), stem elongation (May) and flowering (June). For each of these stages, the plants were gently shaken and the samples of rhizospheric soil defined as the roots adhering soil were collected for the 6 RILs.

Plant biomass and P uptake

Shoot-root ratio was determined for each development stage. Each plant was carefully harvested (roots and shoots were separated). Plant roots and shoots were then placed in individual foil packets, dried at 65°C for 48 h to determine total plant biomass as dry weight. The P contents in the plant tissues were analysed by the molybdovanadate method as described by Sadzawka *et al*. (2006).

Density of total cultivable bacteria and phytate utilising bacteria

In order to determinate the density of bacteria, 3 g of rhizosphere soils were suspended in 30 ml of sterile Phosphate Buffered Saline (PBS), pH 7.2 on an orbital shaker (120-rev min^{-1}). The density of the total and the phytate utilising cultivable bacteria were estimated using a culture method (Jorquera *et al*., 2008). The same dilutions were also plated on Angle medium as modified by Richardson and Hadobas (1997) to obtain the number of cultivable bacteria with ability to utilize phytate. the bacteria solubilising phytate was indicated by the development of a clearing zone around the colonies. The number of microbial colonies was expressed as log CFU g^{-1} of dry soil.

Soil phytase activity

The phytase activity was determined according to Araujo *et al*. (2008) by measuring the determination of Pi released from the phytate.

Soil DNA extraction and purification

DNA extraction from the rhizosphere soil material was performed using the modified Mannitol-Based Methods (Fatima *et al*., 2014) for soil DNA extraction. The final DNA extracts was quantified using the NanoDrop 2000 (Thermo Scientific) and stored at -20°C before use.

Quantification of the 16S rRNA gene by real time PCR

The Amplification of the 16S rRNA gene by a real time PCR was undertaken from soil DNA and was performed using a SYBERGreenH detection system. DNA was amplified in a total reaction volume of 20 ml. The reaction mixtures contained 1 mM of each primer 314F (5′ CCTACGGAGG CAGCAGCAG 3′) and 515R (5′ATTCCGCGGCTGCTGGCA 3′) (López-Gutiérrez *et al*., 2004) and 1 ng of DNA. The real time PCR conditions were as described by Plassart *et al*. (2012). Purity of amplified products was checked by the observation of a single melting peak and the presence of a unique band of the expected size in a 3% agarose gel stained with ethidium bromide.

Statistical analysis

The assay was carried out in a complete randomised design and the data was analysed statistically with the variance analysis, using the statsoft Inc. (2011) Satistica (Data Analysis Software), version 7.1. Values of bacterial densities were means of 3 replicates per soil. Values of growth and nodulation were means of 10 replicates per RIL.

RESULTS AND DISCUSSION

Density of total cultivable bacteria and phytate utilising bacteria

The density of the total culturable bacteria was significantly greater in P-sensitive RILs rhizosphere than in the P-sufficient RILs rhizosphere (Fig 1a). The density of total culturable bacteria was low at the beginning of the season (vegetative stage) (10^2 CFU g^{-1} dry soil) but increased over time in both P- deficiency sensitive and P-deficient RILs soil rhizosphere to reach the maximum value at flowering stage (10^7 CFU g^{-1} dry soil for the P- deficiency sensitive RILs and 10^4 CFU g^{-1} dry soil for the P- deficiency resistant RILs). The major difference can be attributed to plant development stage, this is consistent with Smalla *et al*. (2001). This evolution is probably due to difference in quantity and nature of rhizodeposition during the plant development as well as climate change (Cregut *et al*., 2009).

Overall, 3% of the total rhizosphere bacterial isolates were able to grow and to produce a clearing zone on angle medium, suggesting a bacterial phytate mineralization which is in agreement with previous studies (Maougal *et al*., 2014a) this was considered as the cultivable functional community structure. For this phytate mineralising bacteria a slight but significant difference has been observed between the RILS in the first stage and then the bacterial densities increase to the flowering stage. Our findings suggest that plants affect the dynamic of P in the soil by directing the composition of the rhizosphere microbial community. The results are consistent with previous works reporting that rhizosphere microorganisms directly and indirectly influence the composition and biomass of natural plant communities (Mendes *et al*., 2013).

Bacterial abundance was affected by the RILs also Our data suggests that the P deficiency sensitive RILs has an influence on the microbial community colonizing its roots by adapting their microbial community or mobilizing more bacteria on their rhizosphere. This capacity could be an efficient strategy to mobilize and increase the inorganic P (Hinsinger *et al*., 2011, Maougal *et al*., 2014a).

Estimate the size of the total bacterial community

The Fig 2 showed that the detection of the total 16S rRNA gene copy number per g of soil in the rhizosphere of common bean varied from RILs to another along the phenology. Globally, it is noted that a significant difference exists between the P-deficiency resistant and the P-deficiency sensitive lines, the density of the total bacteria was significantly greater in the P-deficiency sensitive RILs rhizosphere (until 7.10^{10} copy of gene) than in the P-sufficient RILs rhizosphere. (4.10^{10} copy of gene). The density of total bacteria was low at the beginning of the season (vegetative stage) and increased over time in both P- deficiency resistant and P deficiency sensitive RILs soil rhizosphere to reach the maximum value at flowering stage (7.10^{10} copy of gene).The application of real time-PCR approaches demonstrates that the measurement of total soil biomass (extracted DNA) changes during the plant growth. We detected a rhizosphere effect; specifically an increased relative abundance of bacterial population in the neighbourhood of the root and this increase or evolution was dependent to plant phonological stage.

Plant biomass and P use efficiency of plants

Both P-deficiency sensitive and P- deficiency resistant lines of common bean seems to grow identically during the first two phonological stages studies (Fig 3), but at the flowering stage it is noted that there is a significant difference between the P- deficiency sensitive's lines (147, 83, 29) and the P-deficiency sufficient lines (104, 34, 115) with the highest value for the line 104. This variability may be due to the reduction in N_2 fixation induced by a P-deficiency in the soil (Drevon *et al*., 2015) because plants need a high energy for the reduction of atmospheric N_2 by the nitrogenase (Roratu

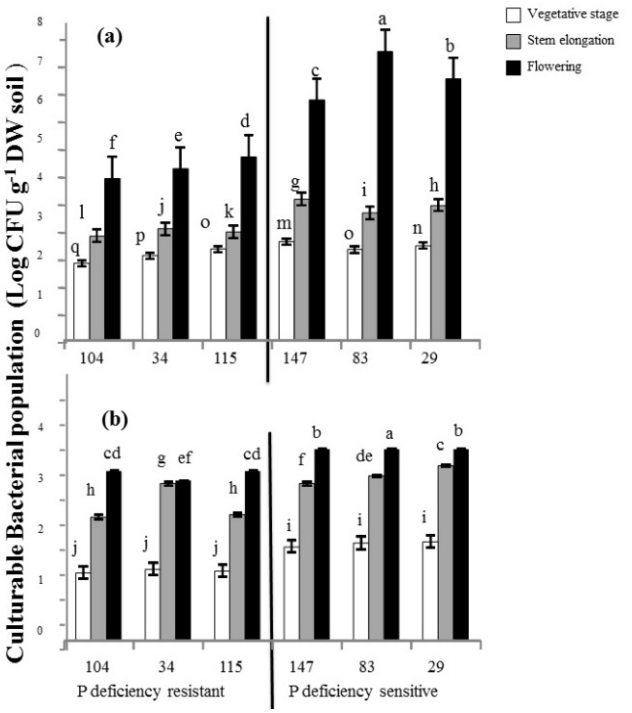


Fig 1: Enumeration of bacterial CFU per gram dry weight (DW) from rhizosphere soil of 6 RILs (104, 34, 115, 147, 83 and 29) of *Phaseolus vulgaris* on LB medium (a) and angle medium (b). Soils were sampled at vegetative stage (white bars), stem elongation (grey bars) and flowering (black bars). Bars are means ± SD (n = 3) and for each P level, different letters denote significant differences between RILs (one-way ANOVA, $p < 0.05$).

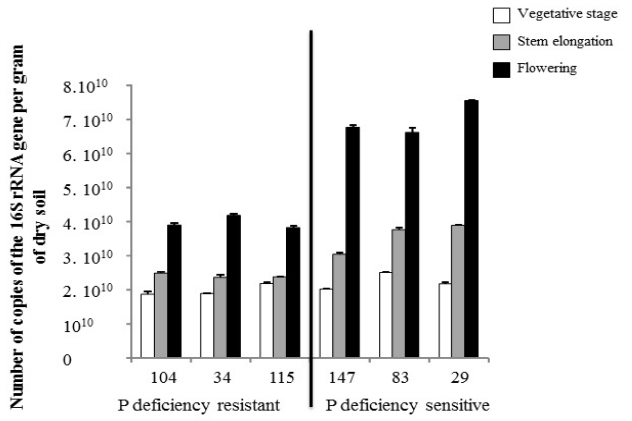


Fig 2: Quantification of the 16S rRNA gene of bacterial per gram dry weight (DW) from rhizosphere soil of 6 RILs (104, 34, 115, 147, 83 and 29) of *Phaseolus vulgaris* on LB medium (a) and angle medium (b). Soils were sampled at vegetative stage (white bars), stem elongation (grey bars) and flowering (black bars). Bars are means ± SD (n = 3) and for each P level.

et al., 2009). This suggests that the rhizospheric bacteria have stimulated the mechanisms associated with roots for transformation of soil organic P into soluble Pi as the only source of P that the root can absorb.

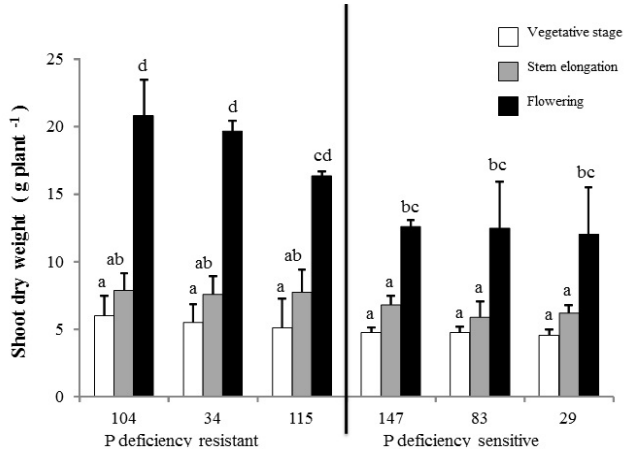


Fig 3: Biomass of shoot of the common bean RILs 104, 34, 115, 147, 83 and 29 in vegetative stage (white bars), stem elongation (grey bars) and flowering (dark bar). Data are mean ± SD of ten replicates, different letters denote significant differences between RILs (one-way ANOVA, $p < 0.05$).

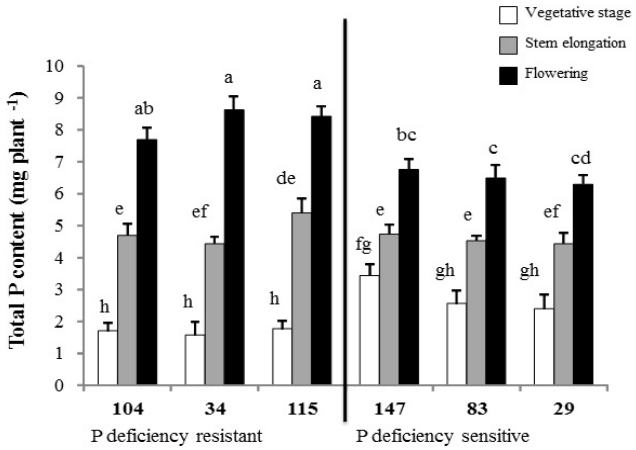


Fig 4: P content in shoot of the common bean RILs 104, 34, 115, 147, 83 and 29 in vegetative stage (white bars), stem elongation (grey bars) and flowering (dark bar). Data are mean ± SD of ten replicates, different letters denote significant differences between RILs (one-way ANOVA, $p < 0.05$).

The P use efficiency of plants measured (Fig 4) presented a significant ($p < 0.01$) increase of the shoot P concentration observed from a phonologic stage to another. The content of the shoot parts P in P- deficiency sufficient plants studied (104, 34 and 115) are significantly higher than in P- deficiency sensitive's this may be due to the nodulation of *P. vulgaris* under P deficiency (Maougal *et al*., 2014a). This would optimize the efficiency in use of the rhizobial symbiosis by producing more biomass per unit of P consumed (Vadez and Drevon, 2001).

Soil phytase activity

An important finding of this work is that a rich soil phytase activity was observed in the rhizosphere of the P deficiency sensitive RILs (Fig 5). This could be a consequence of the

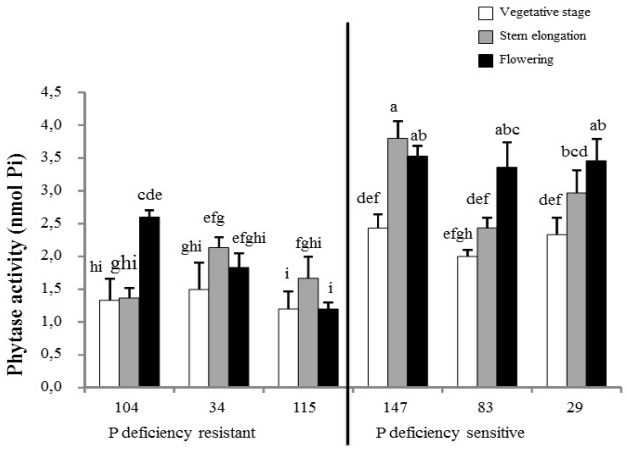


Fig 5: Phytase activities of rhizosphere soils of the six bean genotypes (104, 34, 115, 147, 83 and 29) harvested at vegetative stage (white bars), stem elongation (grey bars) and flowering (dark bar). Data are mean ± SD of ten replicates; different letters denote significant differences between RILs (one-way ANOVA, $p < 0.05$).

mobilisation of the bacteria. When the legume growth is limited by low P availability, the rhizospheric bacteria could contribute to a normal growth (Maougal *et al*., 2014 a, b). Additionally, the symbiotic nitrogen fixation in common bean is affected by P-deficiency more than other legumes (Drevon *et al*., 2015). Overall the mean values of the phytase activity were significantly greater in the rhizosphere of the P-deficiency sensitive lines (from 1.20 nmol Pi to 3.53 nmol Pi) than in the P- deficiency resistant RILs. As expected the RILs can affect the phytase activity depending of their P demand (Araùjo *et al*., 2008, Lazali *et al*., 2013). This suggests that the size of cultivable phytate mineralising bacterial community was combined to the phytate activity in the rhizosphere. It has been reported that the phytase activity is synthetized during inorganic phosphorus limitation (Li *et al*., 1997).

During plant growth, the phytase activity increases significantly in the rhizosphere. The lowest values were obtained during vegetative stage; the highest value was noted during stem elongation for the P-deficiency sensitive RIL 147, but as a whole, the highest values were noted during flowering stage for both P-deficiency sensitive and P- deficiency resistant lines. As common bean needs high quantities of P, the limitation of this element in the rhizosphere environment could induce more phytase activity in the active microbial community (Lim *et al*., 2007, Maougal *et al*., 2014a). The highest density observed of phytase at the flowering stage of common bean development would be due to stimulation brought by larger contribution of rhizodeposits.

CONCLUSION

To conclude our results, it is suggested that the distribution of the bacterial population in the rhizosphere varied temporally and was depended on plant stage and P

demanding which appears to stimulate numbers and activities of bacteria performing phytase from organic soil. Our results highlight a strong interdependence of phytate mineralising bacterial with soil phytase activity, suggesting that plant affects dynamic of P in the soil by directing the composition of the rhizosphere microbial community according to their physiological needs. Further investigations should be extended to determine the structure of the rhizospheric community.

ACKNOWLEDGEMENT

The authors are very grateful to M. El Haddef El Okki for the help on statistical analysis. We also wish to acknowledge N. and W. from the CNCC for their help and Yasmine for English revision.

REFERENCES

Alkorta, I., Aizpurua, A., Riga, P., Albizu, I., Amezaga, I., Garbisu, C. (2003). Soil enzyme activities as biological indicators of soil health. Reviews Environmental Health 18: 65-73.

Araújo, A.P., Plassard, C., Drevon J.J. (2008). Phosphatase and phytase activities in nodules of common bean genotypes at different levels of phosphorus supply. Plant and Soil. 312: 129-138.

Bargaz, A., Zaman-Allah, M., Farissi, M., Lazali, M., Drevon, J.J., Maougal, R.T., Carlsson G. (2015). Physiological and molecular aspects of tolerance to environmental constraints in grain and forage legumes. International Journal of Molecular Sciences. 16: 18976-19008.

Belimov, A.A., Safronova, V.I., Sergeyeva, T.A., Egorova, T.N., Matveyeva, V.A., Tsyganov, V.E., Borisov, A.Y., Tikhonovich, I.A., Kluge, C., Preisfeld, A., Dietz, K. J. Stepanok, V.V., (2001). Characterisation of plant growth-promoting rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane-1-carboxylate deaminase. Canadian Journal of Microbiology. 47: 642-652.

Cregut, M., Piutti, S., Vong, P.C., Slezack-Deschaumes, S., Crovisier, I., Benizri, E. (2009). Density, structure and diversity of the cultivable arylsulfatase-producing bacterial community in the rhizosphere of field grown rape and barley. Soil Biology and Biochemistry. 41(4): 704-10.

da Rocha, U.N., van Overbeek, L., van Elsas, J.D. (2009). Exploration of hitherto uncultured bacteria from the rhizosphere. FEMS Microbiol Ecol. 69: 313-328.

Drevon, J.J., Alkama, N., Araujo, A.P., Beebe, M., Blair, M., Attar, H., *et al.* (2011). Nodular diagnosis for ecological engineering of the symbiotic nitrogen fixation with legumes. Proceedings of Environmental Sciences. 9: 40-46.

Drevon, J.J., Abadie, J., Alkama, N. andriamananjara, A., Amenc, L., Bargaz, A., Carlsson, G., Lazali, M., Ghoulam, C., Ounane, S.M. (2015). Phosphorus use efficiency for N_2 fixation in the rhizobial symbiosis with legumes, In: Biological Nitrogen Fixation, [F.J. de Bruijn (eds.)], Wiley, Sons, Inc., p. 455-464.

Fatima, F., Pathak, N., Rastogi Verma, S. (2014). An improved method for soil DNA extraction to study the microbial assortment within rhizospheric region. Molecular Biology International. 2014: 518960.

Gaumont-Guay, D., Black, T.A., Barr, A.G., Jassal, R.S. Nesic, Z. (2008). Biophysical controls on rhizospheric and heterotrophic components of soil respiration in a boreal black spruce stand. Tree Physiology. 28: 161-171.

Girme, M.S. and Sarnoba, D.H. (2018). Effect of nutrient supply system on biometry and rhizosphere microflora of soybean, pigeon pea and sorghum. Agricultural Science Digest. 38: 22-26.

Graham, P.H., Vance, C.P. (2003). Legumes: Importance and constraints to greater use. Plant Physiology. 3: 872–877.

Hartmann, A. Rothballer, M., Schmid, M. (2008). Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. Plant and Soil. 312(1-2): p. 7.

Hinsinger, P, Betencourt, E., Bernard, L., Brauman, A., Plassard, C., Shen, J., Tang, X., Zhang, F. (2011). P for two, sharing a Scarce Resource: Soil Phosphorus Acquisition in the Rhizosphere of Intercropped Species. Plant Physiology. 156: 1078-1086.

Jorquera, M.A, Hernández, M.T., Rengel, Z., Marschne, P., Mora, M.L. (2008). Isolation of culturable phosphobacteria with both phytate-mineralization and phosphatesolubilization activity from the rhizosphere of plants grown in a volcanic soil. Biology and Fertility of Soils. 44: 1025-1034.

Lazali, M., Zaman-Allah, M., Amenc, L., Ounane, G., Abadie, J., Drevon, J.J., (2013). A phytase gene is over-expressed in root nodules cortex of *Phaseolus vulgaris* - rhizobia symbiosis under phosphorus deficiency. Planta. 238: 317-324.

Li, J., Hegeman, C.E., Hanlon, R.W., Lacy, G.H., Denbow, D.M., Grabau, E.A. (1997). Secretion of active recombinant phytase from soybean cell-suspension cultures. Plant Physiolology. 114: 1103-1111.

Lim, B.L., Yeung, P., Cheng, C., Hill, J.E. (2007). Distribution and diversity of phytatemineralizing bacteria. ISME Journal. 1: 321-330.

López-Gutiérrez, J.C., Henry, S., Hallet, S., Martin-Laurent, F., Catroux, G., Philippot, L. (2004). Quantification of a novel group of nitrate-reducing bacteria in the environment by real-time PCR. Journal of Microbiology Methods. 57: 399-407.

Maougal, R.T., Brauman, A., Plassard, C., Abadie, J., Djekoun, A., Drevon, J.J. (2014a). Bacterial capacities to mineralize phytate increase in the rhizosphere of nodulated common bean (*Phaseolus vulgaris*) under P deficiency. European Journal of Soil Biology. 62: 8-14.

Maougal, R.T., Bargaz, A., Sahel, C., Amenc, L., Djekoun, A., Plassard, C., Drevon, J.J. (2014b). Localization of the Bacillus subtilis beta-propeller phytase transcripts in nodulated roots of *Phaseolus vulgaris* supplied with phytate. Planta. 239: 901-908.

Mendes, R., Garbeva, P., Raaijmakers, J.M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic and human pathogenic microorganisms. FEMS Microbiology Reviews. 37: 634-663.

Mougel, C., Offre, P., Ranjard, L., Corberand, T., Gamalero, E., Robin, C., Lemanceau, P. (2006). Dynamic of the genetic structure of bacterial and fungal communities at different development stages of *Medicago truncatula* Jemalong J5. New Phytologist. 170: 165-175.

Plassart, P., Terrat, S., Thomson, B., Griffiths, R., Dequiedt, S., Lelievre, M., Regnier, T.,Nowak ,V., Bailey, M., Lemanceau, P., Bispo. A., Chabbi, A., Maron, P.A., Mougel, C., Ranjard, L. Philippe Lemanceau. (2012). Evaluation of the ISO Standard 11063 DNA Extraction Procedure for Assessing Soil Microbial Abundance and Community Structure. PLoS ONE. 7(9): e44279. doi:10.1371/journal.pone. 0044279.

Richardson, A.E. Hadobas P.A. (1997). Soil isolates of *Pseudomonas* spp. that utilize inositol phosphates. Canadian Journal of Microbiology. 43: 509-516.

Ros, M., Pascual, J.A., Garcia, C., Hernandez, M.T., Insam, H. (2006). Hydrolase activities, microbial biomass and bacterial community in a soil after long-term amendment with different composts. Soil Biology and Biochemistry. 38: 3443-3452.

Rotaru, V. Sinclair, T.R. (2009). Interactive influence of phosphorus and iron on nitrogen fixation by soybean. Environmental and Experimental Botany. 66: 94-99.

Rovira, A.D. (1965). Interactions between plant roots and soil micro--organisms. Annual Review of Microbiology. 19: 241-266.

Sadzawka, A., Carrasco, M. A., Grez, R., Mora, M. L., Flores, H., Neaman, A. (2006). Recommended methods of analysis for soils in Chile. (In Spanish.) Serie de Actas INIA no. 34. Santiago, Chile: Instituto de Investigaciones Agro--pecuarias.

Schulze, J., Drevon, J.J. (2005). P-deficiency increases the O_2 uptake per N_2 reduced in alfalfa. Journal of Experimental Botany. 56: 1779-1784.

Smalla, G., Wieland, A., Buchner, A., Zock, J., Parzy, S., Kaiser, N., Roskot, H., Heuer, Berg. G. (2001). Bulk and Rhizosphere Soil Bacterial Communities Studied by Denaturing Gradient Gel Electrophoresis: Plant-dependent Enrichment and Seasonal Shifts Revealed. Applied and Environmental Microbiology. 4742-4751.

Sørensen, J. (1997). The rhizosphere as a habitat for soil micro--organisms. In Modern Soil Microbiology. [Eds. J.D. van Elsas, J.T Trevors and E.M.H.] Wellington. Marcel Dekker, New York. pp 21-45.

Subhashini, D.V. (2013). Isolation of rhizospheric bacteria and their effect on germination of tobacco seed and growth of seedlings. Agricultural Science Digest. 33(2): 127-130.

Sun, K., Liu, J., Gao, Y., Jin, L., Gu, Y., Wang. W. (2015). Isolation, plant colonization potential and phenanthrene degradation performance of the endophytic bacterium *Pseudomonas sp*. Ph6-gfp. Scientific Reports 4: 5462.

Thakur, D., Kaushal, R., Shyam, V. (2015). Phosphate solubilising microorganisms: role in phosphorus nutrition of crop plants- a review. Agricultural Reviews. 35:159-171.

Vadez, V., Drevon, J.J. (2001). Genotypic variability in phosphorus use efficiency for symbiotic N_2 fixation in common bean (*Phaseolus vulgaris*). Agronomie, EDP Sciences. 21(67): 691-699.

Watt, M., Kirkegaard, J.A., Passioura, J.P. (2006). Rhizosphere biology and crop productivity. Australian Journal of Soil Research. 44(4): 299-317.

Xu, H., Biswas, D.K., Li, W.D., Chen, S.B., Zhang, S.B. Jiang, G.M., Li, Y.G. (2007). Photosynthesis and yield responses of ozone-polluted winter wheat to drought. Photosynthetica. 45: 582-588.