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The influence of phosphate solubilizing microorganisms on symbiotic nitrogen fixation: Perspectives for sustainable agriculture

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Abstract

Phosphorus is one of the most major macro-element after nitrogen (N) for both plants and microorganisms. However, the availability of P seems to be widely limited in soils due to its chelation with other insoluble compounds. The use of chemical fertilizers based phosphorus is an unsuccessful strategy, because it induces soil and water pollution. Moreover, chemical fertilizers could react with other elements such as iron (Fe), manganese (Mg) and calcium (Ca) and precipitate on soil surfaces which reduce the availability of soluble forms of phosphorus. The presence of phosphate solubilizing microorganisms in soil represents a major pathway for solubilizing and releasing inorganic phosphates into soluble forms which could be directly used by plants. Indeed, a large number of microorganisms that are able to fix atmospheric nitrogen could be involved in P solubilization mechanisms. Here, we emphasize the influence of phosphate solubilizing microorganisms (PSM) on symbiotic nitrogen fixation, and the exploitation of the most performant strains to reduce land degradation, pollution and to improve sustainable agriculture.

1. Introduction

Ensuring food consumption will become the most challenging concept in the upcoming years due to the world human's expansion. In order to meet this challenge, the study of soil biological systems and agro-systems is needed for a better understanding of different processes and interactions leading to stability in agro-systems. Conventional agriculture was ultimately based on the use of chemical fertilizers, e.g: manufactured water-soluble phosphatic (WSP) fertilizers (superphosphates) which had improved the green revolution especially in correcting phosphorus deficiencies. However, chemical fertilizers could trigger water pollution and land degradation which is a negative way to sustain production bases and develop crop productivity. Hence, the use of microbial inoculants (biofertilizers) including phosphate solubilizing microorganisms (PSM) in agriculture represents an efficient approach to optimize phosphorus (P) and nitrogen (N) uptake for plants. Indeed, microbial inoculants are cost effective, eco-friendly and renewable source of plants nutrients [1]. In this context, Jain et al (2005) reported on the use of rhizobium and phosphate solubilizing bacteria (PSB) to enhance some legume crop productivity due to their vital role in N_2-fixation and P-solubilization [2]. Moreover, the introduction of efficient strains of P-solubilizing species *of Bacillus megaterium* biovar *phosphaticum*, *Bacillus polymyxa*, *Pseudomonas striata*, *Aspergillus awarmoni* and *Penicillium digitatum* in the rhizosphere of crops and soils has widely been reported in regard of increasing phosphorus availability in soils [3]. However, dating research on P-mobilizing soil microorganisms need more understanding of how PSM and other microorganisms interact within rhizosphere. Molecular techniques provide new opportunities in terms of detecting the presence and the abundance of specific microorganisms or quantifying the expression of target genes directly in soil or in rhizosphere with high levels of sensitivity. For instance, specific primers based on conserved regions have been described for various microorganisms associated with P solubilization. In addition, phylogenetic analysis of bacterial diversity based on 16S-RNA genes sequences, could help in further applications for assessment of diversity focusing particular traits. Throughout this current review, we aimed to show the influence of PSM on the symbiotic nitrogen fixation based on various researches on this area of study in order to update the importance of microbial inoculants to protect environment as well as food security in the earth. Thus, we reported on the latest advances by giving new insights about PSM and nitrogen fixing symbiosis which represent a great impact on improving sustainable agriculture in the upcoming years.

2. Phosphate solubilizing Microorganisms

Microorganisms in soil represent a major component in different processes and activities which maintain the quality of agro-ecological conditions in the earth. Indeed, numerous and beneficial activities could be performed by microorganisms in soil including decomposition, nutrient mobilization, mineralization, and storage or release of nutrient and water. Moreover, certain microorganisms play a major role in the biological concept of nitrogen fixation as well as denitrification. Beyond all these contributions, some microorganisms have the ability of phosphate solublization which facilitates the conversion of insoluble phosphatidic forms to soluble compounds that are directly used by plants [4,5].

The most important genera of phosphate solubilizing microorganisms are: *Bacillus* and *Pseudomonas* [6], followed by *Aspergillus* and *Penicillium* from the important fungal genera [7] and finally a nematofungal genra which include one species known as *Arthrobotrys oligospora* for its ability to solubilize different rock phosphate [8]. However, studies on phosphate solubilizing microorganisms (PSM) that can induce nodule formation on legumes are limited. Few reports have been focused on the concept of *Rhizobium*-legumes symbiosis and their ability to solubilize inorganic phosphates [9-12]. It has been reported that the ability of phosphate solubilization is mainly related to the nature of nitrogen source in the media. Besides, a high level of solubilization by PSM could be widely enhanced by the presence of ammonium salts rather than nitrogen source. This could be explained by the extrusion of protons in order to compensate the ammonium uptake, leading to a decreased extra-cellular pH [12,1]. Overall, the most performant PSM strains should therefore be exploited for studying the physiological, biochemical and molecular mechanisms of phosphate solubilization under stressed ecosystems, and especially in the dry areas.

3. Evaluation of phosphate solubilizing microorganisms *in vitro* cultures

Investigations aiming the isolation and screening of PSM are mainly based on serial dilutions or enrichment techniques on different mediums based phosphorus e.g: Pikovskaya medium, Sperber's basal medium and the National Botanical Research Institute's Phosphate (NBRIP) medium (Figure 1) [14-16]. Such evaluations of PSM require pure liquid culture medium to assess the different pathways used during phosphate solubilization process [17]. The most important mechanism which is usually involved in phosphate solubilization is production of organic acids. Indeed, among organic acids that have been found in liquid culture filtrates are: oxalic acid, citric acid and lactic acid. The identification of these compounds was performed by paper chromatography on thin layer chromatography or by high performance liquid chromatography and certain enzymatic methods to allow more accurate identification [18]. It was reported that organic acids can act differently during solubilization process: they can either directly dissolve the mineral phosphate as a result of anion exchange of PO_4^{2-}, or chelate both iron and aluminum ions associated with phosphate [19]. Moreover, it was noticed that in certain cases phosphate solubilization could be induced by starvation process [20]. Studies showed that there is no significant correlation between the acids produced by PSM and the released phosphorus in the medium [21]. Thus, the role of organic acids produced is widely expressed by the decrease of pH, chelation of cations and by competing with phosphate for adsorption sites in soil [22].

Besides, some inorganic acids could be involved in the solubilization process, e.g: hydrochloric acid which have the ability to solubilize inorganic phosphate but less effectively compared with organic acids at the same pH [23].

Numerous studies have scrutinized that acidification is not the most adapted pathway during solubilization process and the decrease of pH is not correlated with the ability to solubilize mineral phosphate [24]. Hence, chelation seems to be important in P solubilization since the presence of 0.05M EDTA in the medium has the same solubilizing effect when *Penicillium bilaii* was added [25]. It was showed that among nodulation bacteria, e.g: *Rhizobium* and *Bradyrhizobium*, the phosphate solubilizing activity of theses microorganisms was related to their ability to decrease the pH of the medium [26].

Some studies were focused on the evaluation of PSM in different culture mediums, in order to provide more accurate solubilization that could help in a better understanding of biochemical pathways of P solubilization process (Figure 3)[27, 28]. Indeed, a study was performed to test the phosphate solubilization ability of certains microorganisms isolated from Iranian calcareous soils which are known with a predominance of calcareous and a low level of tricalcium phosphate $Ca_3(PO_4)_2$.. The screening of 446 was performed on Sperber's basal medium agar plate in the presence of two different source of P (Tricalcium phosphate and inositol hexa-phosphate).

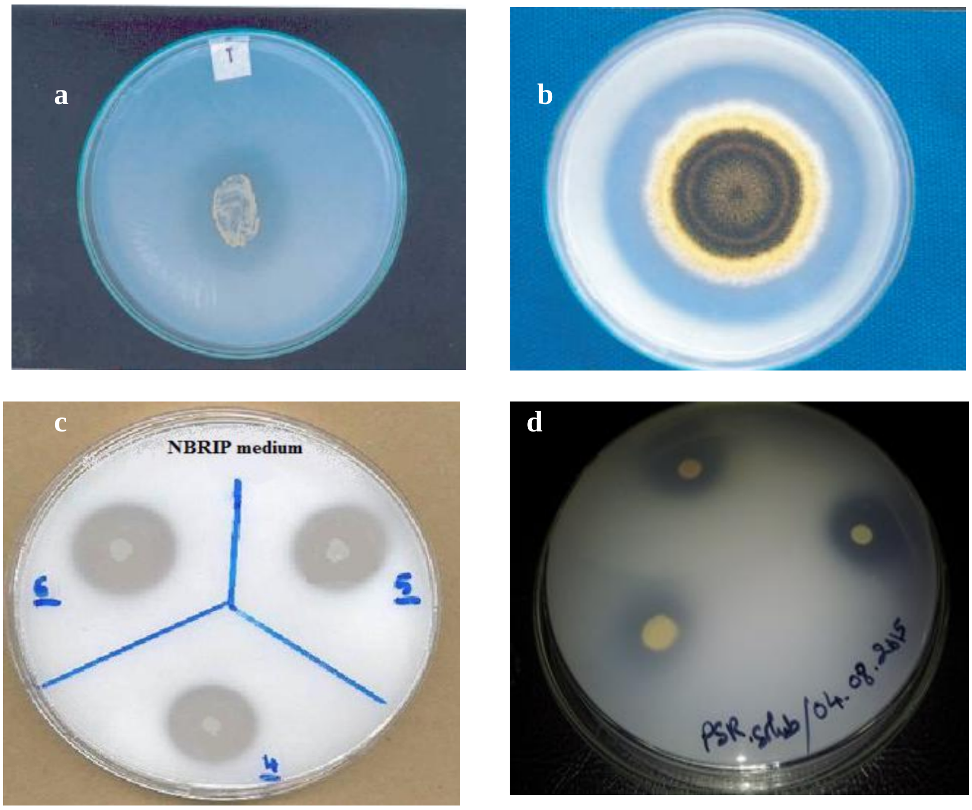

Figure 1: Evaluation of phosphate solubilizing microorganisms (PSM) in different mediums. Appearance of phosphate solubilizing bacteria (a) and fungi on Pikovskaya medium (b) [30]; (c) Solubilization of three bacterial isolates originated from a phosphate rock deposit of a Moroccan mine, on solid medium NBRIP [31]; (d) Phosphate solubilizing rhizobia (PSR) and nodulating faba bean (*vicia faba L.*) in Morocco performed on Sperber's basal medium [32].

The authors suggested a second evaluation on Sperber's basal medium liquid culture of *Rhizobium leguminosarum bv.viciae* isolates along with *Bacillus sp.* and *Pseudomonas fluorescens* used as positive controls. It was observed that *Rhizobium leguminosarum bv.viciae* mobilized a significant amount of TCP in liquid medium ($P < 0.05$; 197.1 mg/l in 360 hours) comparing to the other tested rhizobia. Moreover, the authors noted that the release of soluble P was significantly correlated with the final pH found in the filtrates which could be explained by the great importance of acid production during the mobilization process of phosphates [27].

The evaluation of phosphate soubilization ability by microorganisms should be performed by using different techniques and medium cultures. Hence, Liu et al [28] demonstrated that phosphate solubilization ability of PSB performed differently on three different techniques: NBRIP plate culture, NBRIP liquid culture and in sandy soil incubation. A poor correlation was noted in the first technique between sizes of halo zone and water soluble phosphorus (WS-P). The second case showed a decrease in pH (pH<4.3) and a visible release of WS-P reaching an amount of 523.62 mg/l with 3 days of incubation. Finally, all PSB strains showed a low level of solubilization when were they incubated with a sandy soil. Through this investigation, the authors emphasized the importance of performing phosphate solubilization in different ways in order to provide a significant quantification of soluble phosphate in the medium [29].

It was pointed out that the use of agar plate method in PSM screening could reduce the quality of identifying other PSM strains that showed a low phosphate solubilization in solid medium. Therefore, most studies recommended the need of testing PSM strains in liquid culture as well as soil medium to assess P dissolution simultaneously [27].

The advanced biochemical and molecular mechanisms of phosphate solubilization need more investigations, especially of some symbiotic nodule bacteria that still ignored.

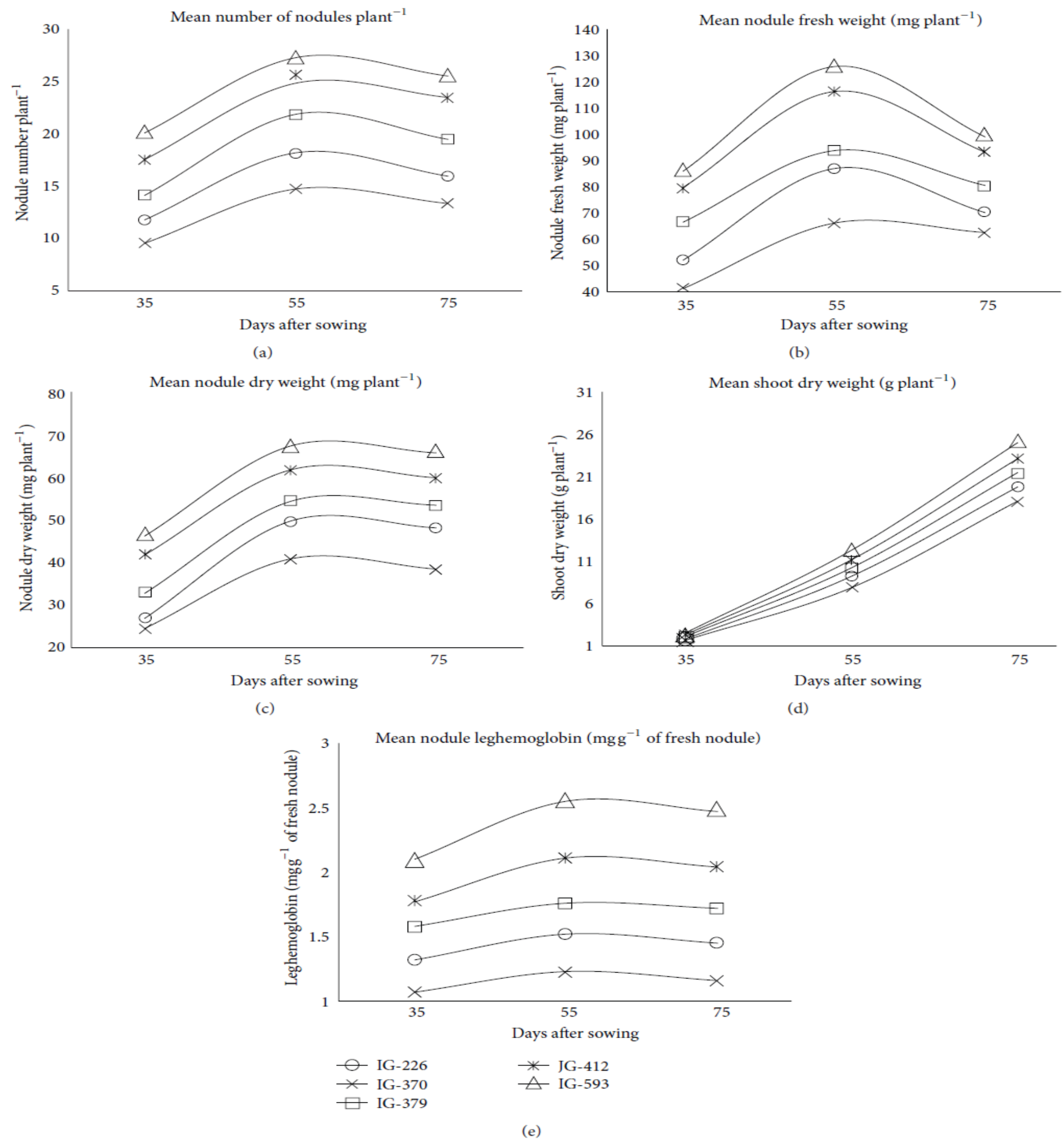

Figure 2. Coinoculation of chickpea by *Rhizobium* and PSM and its effect on symbiotic parameters (a-d): nodule number, fresh and dry weight and leghemoglobin content (e) in nodular tissues of different chickpea genotypes (IG-226; IG-370; IG-379; JG-412; IG-593). The experiment was performed comparing to inoculation with *Rhizobium* and PSM alone [12].

4. Phosphate solubilizing microorganisms and their effect on symbiotic traits

The importance of phosphorus and nitrogen in plants nutrition has been clarified in several investigations. It is well known that a combined inoculation between nitrogen fixers and PSM contribute to a high yield production rather than applying a single inoculation [33]. Indeed, when legumes are inoculated by nitrogen fixers and PSM, their roots are entirely colonized by these microorganisms which enhance the growth by providing plants by nitrogen and phosphorus [34]. It was reported that the application of phosphate-solubilizing bacteria (PSB) (*Pseudomonas striata*) and *Rhizobium* induce a significant agronomic yield [35, 36], compared with the mono-inoculation based *Rhizobium*. In another study which was carried out along 10 years through a long term trials, using PSB and nitrogen fixing symbiosis, the seed production was significantly higher and the nitrogen fixation was found to be more effective better than superphosphate implementation [37]. In this context, a similar study showed that single or combined inoculation with PSM and *Rhizobium* genera contribute to a positive effect on the yield as well as nutrient uptake for legumes crops [38, 39, 16].

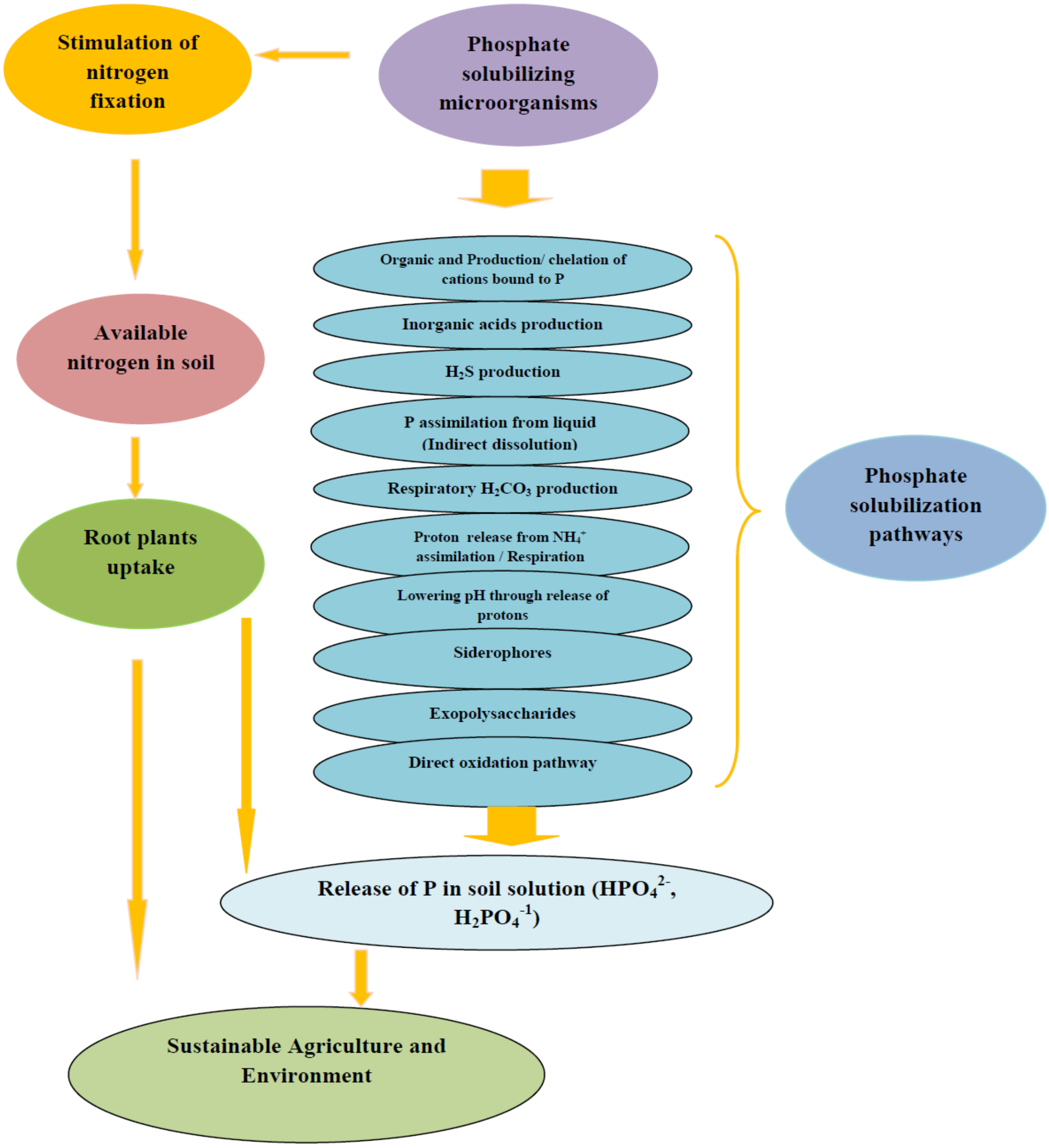

Figure 3: A conceptual theme exhibiting the main mechanisms and contribution of PSM to sustainable agriculture by increasing symbiotic nitrogen fixation and Phosphorus uptake by plants.

Several field experiments examined the influence of the bio-inoculation by PSB and rhizobium by evaluating different symbiotic traits such as nodule leghemoglobin [12]. Indeed, it was reported that, through an evaluation of rhizobium associated with PSB inoculants of chickpea, the yield was higher in terms of nodule number (27.66 nodules. Plant^{-1}), nodule fresh weight (144.9 mg.Plant^{-1}), nodule dry weight (74.30 mg.plant^{-1}), shoot dry weight (11.76 g.plant^{-1}) and leghemoglobin content (2.29 mg.g^{-1} of fresh nodule). Moreover this bio-inoculation showed a significant enhancement in terms of grain and straw yield (Figure 2) [12].
A study was carried out in the semi arid Pampas of Argentina to assess the influence of *Pseudomonas putida* on leguminous-rhizobia symbiosis [40]. This investigation was performed in this region since the distribution of phosphorus is not suitable for plant nutrition. It was showed that *Sinorhizobium meliloti* 3DOh13 and *Bradyrhizobium japonicum* TIIIB strains demonstrated a significant rate of solubilization for both alfalfa and soybean plants. Moreover, two strains of *Pseudomonas putida* performed a high plant growth promotion. Therefore, the authors suggested the use of mixed inoculants containing both *Pseudomonas* and Rhizobia in

agricultural practices in order to increase symbiotic parameters and nutrient uptake for legumes. A similar investigation pointed out the significant influence of PSM on crop yield and fertility in different sites exposed to various climatic regions in India [41]. The study aimed to demonstrate the effect of phosphate solubilizing bacterial strains *Pantoea cypripedii* (PSB-3) and *Pseudomonas plecoglossicida* (PSB-5) on maize and wheat grain production as well as fertility at different agroclimatic regions. Thus, a significant increase was widely noted especially in grain yield, phosphorus (P) uptake, soil organic carbon, available P, enzyme activities and P-solubilizing microorganisms in all trials at different sites. Overall, as kaur et al [41] suggested, it would be important to implement soils with the tested strains *P. cypripedii* (PSB-3) and *P. plecoglossicida* (PSB-5) with rock phosphate (RP) to increase plant growth promotion and improvement of fertility according to various agro climatic conditions in different sites in India.

A recent study examined phosphate solubilizing rhizobia nodulating faba bean (*Vicia faba L.*) in Morocco [32]. The phosphate solubilization ability was examined *in vitro* and under greenhouse condition in the presence of two reference strains of *Rhizobium leguminosarum* (PSRA and PSRB). All strains were tested for their resistance to environmental constrains (e.g: pH, salinity and temperature variation. It was noted that PSR19, PSR26 and PSR29 performed a significant increase in all symbiotic parameters ($p < 0.01$). The highest number of nodules ($34.333\pm1.527.plant^{-1}$), nodule dry weight ($53.077\pm3.434$ $mg.plant^{-1}$), of shoot dry weight (1.039 ± 0.051 $g.plant^{-1}$) and the highest symbiotic effectiveness were registered by PSR29. Overall, the study highlighted the importance of introducing the most performant strains in terms of their ability to solubilize inorganic phosphate and to increase symbiotic traits of nitrogen fixation, in defected soils in Morocco. Bio-inoculants are usually synthesized as carrier-based on effective strains [42, 43]. Indeed, biological systems are therefore utilized not only for their economical and ecofriendly input, but also for their great interest in improving soil quality and ecosystems [44]. As it is indicated above, P availability was studied either in pot experiments or under field condition, by introducing screened PSM [45,46]. However, inconsistent performance at different site could reduce the feasibility of bio-inoculation. Otherwise, the size of PSM population for instance could influence the symbiotic traits in soil. Therefore, multiple trials are recommended for a consistent performance instead of a single experiment [47].

5. Perspectives for sustainable agriculture

The study of phosphate solubilizing microorganisms (PSM) and their influence on symbiotic nitrogen fixation to sustain agriculture depend mainly on their ability to colonize a particular rhizosphere habitat (Figure 3). Indeed the investigation of PSM provides useful information to prospect the deficiency of phosphorus in marginal soils. Conventional methods such as plate counting and most probable number techniques are limited to extend the characterization of these microorganisms. Moreover, less than 1% of PSM in the environment can usually be cultured by standard techniques and do not perform a significant result on artificial media. Hence, the identification of high diversity of PSM in the rhizosphere is performed now by using strain specific DNA profiles which provide a considerable number of heterogenous groups of microorganisms [48, 49]. Most molecular biological techniques are not based on cultivation methods which lead to microbiologist to screen some habitats of new natural microbial communities [50, 51]. Indeed, the most important techniques are cloning and sequencing which lead to determine PSM genera and species that are present in the community, however , they are time lasting. It was reported that hybridization and profiling are faster in terms of targeting sequences but they require more skills and knowledge [52]. Besides, ribosomal DNA restriction analysis (ARDRA) or ribosomal intergenic spacer analysis (RISA) could be used for further investigation of PSM colonization as well as their community structure.

The genetic basis of organic acids produced by PSM could help in cloning the encoded genes of mineral phosphate solubilizing (mps) ability to different bacteria, especially Rhizobia which can colonize a particular rhizosphere and induce nodule formation. In fact, the activity of microorganisms present in the rhizosphere is a major point of defining the result of bio-inoculation. Indeed, the rhizosohere is a major source of carbon which is used by microbial communities in soil to produce different kinds of organic acids [53, 54, 16].

Some genes are involved in different oxidative metabolism which induces gluconic acid for instance. It was showed that the enzyme assessing this reaction, dehydrogenase (GDH), has pyrroloquinoline as a cofocateur (PQQ). For that reason, the gene involved in the biosynthesis and transport of PQQ could be cloned from beneficial bacteria and introduced to others [55, 56]. In addition, it would be challenging to transfer the genes involved in PQQ to Rhizobium in the aim of enhancing their symbiotic effectiveness and their phosphate solubilization activity. Thus, the modeling rhizobium strains will acquire a double activity of phosphate solubilizing (PS) activity as well as the biological nitrogen fixation.

Conclusion

Phosphate solubilizing microorganisms (PSM) have a great impact on symbiotic nitrogen fixation through bioinoculation trials. This approach represents an important advantage toward soil fertility, sustainability and food security in the earth. However, researches still limited in terms of screening performant strains of PSM. For that reason, further investigations of phosphate solubilization pathways are needed by integrating molecular and biochemical advanced techniques, in order to enhance P solubilization ability along with symbiotic nitrogen fixation process.

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