Fungi, P-Solubilization, and Plant Nutrition

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The application of plant beneficial microorganisms is widely accepted as an efficient alternative to chemical fertilizers and pesticides. It was shown that annually, mycorrhizal fungi and nitrogen-fixing bacteria are responsible for 5 to 80% of all nitrogen, and up to 75% of P plant acquisition.

Keywords: sustainable agriculture ; fungi ; P-solubilization ; alternative P-sources

1. Introduction

The continuously growing human population determines the increased global demand for high agricultural productivity, which, at the same time, should follow the principles of sustainability and circular economy. In the last 15 years, several biotechnological approaches were developed to enhance plant growth and health with safe and environmentally mild alternatives, including those based on microorganisms. The practical realization of the proposed strategies will significantly reduce the indiscriminate use of chemical fertilizers and pesticides. Despite the environmentally harsh conditions of surviving, soil contains many individual microbial taxa, including different members of the three domains of life. Although researchers still sub-estimate (for methodological reasons) microbial diversity [1], microorganisms are one of the key components of both natural and cultivated soils thus affecting the soil quality and plant productivity [2]. It should be noted that long-term chemical fertilization and application of pesticides decreased both the soil microbial species richness and microbial–plant beneficial interactions as a part of the plant holobiont [3]. Therefore, rebuilding soil productivity by applying bioeffectors (biostimulants or biofertilizers) is a priority and one of the most studied biotechnological alternatives to chemical fertilizers.

The term biofertilizer has different definitions but in general, it includes plant beneficial microorganisms and their derivates (metabolites), excluding biocontrol agents [4][5][6][7]. Among beneficial microorganisms, bacteria and fungi are considered the most important in helping plant nutrient acquisition and improving plant health. In general, the co-existence of fungi and bacteria is reported in microbiomes with different profile characteristics (animal, soil, and food microbiomes) [8]. Particularly in soils, they are reported as the most abundant microorganisms with 10^2–10^4 times more biomass than protists, archaea and viruses [1]. Amongst various functions that bacteria and fungi perform in soil ecosystems, particularly important is their contribution to plant growth and development, and plant diversity. It was demonstrated that fungi play an important role in the utilization of easily available and more complex litter-derived C than bacteria, thus actively taking part in soil formation [9]. However, both bacteria and fungi are present in the soil microbial hotspots where the soil organic carbon decomposition is much higher than in the bulk soil [10].

It was shown that annually, mycorrhizal fungi and nitrogen-fixing bacteria are responsible for 5 to 80% of all nitrogen, and up to 75% of P plant acquisition [2]. However, while bacteria are the most studied soil microorganisms and most frequently reported in the scientific literature, the role of fungi is relatively understudied [11], although they are the primary organic matter decomposers and govern soil carbon and other elements cycling [12]. It is also well established that fungi, particularly in the zone with an abundant presence of fungal hyphae or roots and hyphae (mycosphere or mycorrhizosphere, respectively), greatly affect bacterial growth in soil and, consequently, their interactions with plants [13]. The potential rapid growth and distribution of a given functional bacterium within the community are often linked to fungi as mediators of ecological processes which also impact the diversity of bacterial communities [14]. On the other hand, the cooperation between fungi and bacteria is a selective process depending on the soil, although this phenomenon should be further studied in soil and other systems as well [13][14].

Fungi are known as a diverse and multifunctional group of soil microorganisms, which demonstrate a high capacity to adapt to various adverse abiotic conditions such as salinity, drought, heavy metals, and extreme pH [15][16][17]. It is also important to mention that fungi manifest significant tolerance to low water activity (a_w) values and high osmotic pressure as proved when growing on solid substrates [18], preserving at the same time high metabolic activity. In soil, 1.5 million fungal species can be found free-living in the bulk soil or as endophytes occupying plant tissues, the mycorrhizae being the most studied beneficial fungus–plant association [19].

Many fungi are able to solubilize insoluble phosphates or facilitate P-acquisition by plants and, therefore, form an important part of the commercial microbial products, with *Aspergillus*, *Penicillium* and *Trichoderma* being the most efficient.

2. P-Bearing Sources

2.1. Conventional P-Sources

The amount of P that is available to plants in cultivable soils is frequently low [20]. To satisfy the need of the plants, the P is added to soil in the form of phosphate fertilizers, but the overall P use efficiency is low because, although plants utilize a fraction of soluble P, the rest rapidly forms insoluble complexes with soil constituents [21]. Therefore, frequent application of soluble forms of inorganic P is normally above what would be necessary under ideal conditions. Even under adequate P-fertilization, only a fraction of the applied P is acquired by the first year's plant growth [22]. A part of the chemical P-fertilizer can be converted into sparingly soluble calcium (alkaline soils), aluminum, and iron (acidic soils) salts of P or be fixed to soil minerals. It was estimated that in the middle of this century about 14 million tons of phosphate fertilizers will be applied, seven million of which will remain in the soil [22].

The basic raw material to produce phosphate fertilizers is rock phosphate composed mainly of the phosphate mineral apatite [$Ca_5(PO_4)_3(Cl/F/OH)$]. The rock phosphate is processed to remove the bulk of the contained impurities, resulting in a concentrate with a content of P_2O_5 ranging from 26% to 34% and up to as much as 42%. It should be noted that lower P-concentrations in rock phosphate and lower quality deposits generate more waste materials, and, on the other hand, more energy and chemicals are required per ton of useful phosphate produced [23]. It is also important to mention the great risk of contamination with different metals present in varying concentrations in rock phosphate and its fertilizer derivates [24][25]. Three facts should be considered when assessing the current situation in this field. One of the main reasons for concern in both phosphate rock-mining and P-fertilizer industries is that inexpensive, high-grade rock phosphate reserves could be exhausted in the next 60 to 80 years as phosphate-bearing ore is a finite non-renewable resource [21][23][26]. P-fertilizer use has increased by around 2.5% per year, 4- to 5-fold during the last 50 years and is projected to increase in the first three decades of the 21st century by 20 Tg per year [27]. Second, there are no substitutes for phosphorus in agriculture, but, on the other hand, phosphorus is recycled by using animal manure and sewage sludge (although there are serious concerns about these alternatives). Finally, it is important to note that the cultivation soil available per capita on a global level is lowering while the world population is increasing by 250,000 people every day (approximately 80 million per year). This situation will result in the enhanced need for food and, consequently, phosphate demand will increase at a rate of 1.5–2% [27]. Therefore, there is an urgent need to find novel phosphate sources and/or novel P-solubilization techniques for ensuring phosphate availability.

2.2. Alternative P-Sources

2.2.1. Struvite

Here, the emphasis will be on potential P-bearing sources such as struvite and bone char. Struvite is a P recovery product, and it should be mentioned that scientific attention to the recovery of P is increasing because of its agricultural and industrial importance and bearing in mind the rapid depletion of natural P-resources. Struvite ($NH_4MgPO_4{\cdot}6H_2O$) is relatively abundant in soils and lakes and can be found naturally formed through a variety of reactions with sources such as bird droppings and fish bones, but also in water treatment plants in form of crystals in wastewater pipes [28][29]. Controlled struvite formation can be carried out in reactors during the sludge digestion process thus forming a magnesium ammonium phosphate fertilizer for its use in agriculture [30]. It is also important to note that the NH_4^+ of the struvite composition can be readily replaced by potassium, thus forming $MgKPO_4{\cdot}6H_2O$ (potassium struvite) [31]. This form of struvite could serve as a source of different important nutrients, which enhance its fertilizing value. As with all other P-bearing natural sources, struvite can be added to soils without any previous treatment, but its fertilizing effect is not well pronounced. When assessing the agronomic value of struvite, it should be also mentioned that the solubility of different sources of struvite depends on their chemical and physical composition and soil characteristics such as pH [32]. Application of soluble P during early plant growth is crucial to determine high growth and plant development with optimal yield. However, struvite added to soil–plant systems is not able to satisfy the need of P during this phase of the plant growth as it is only slightly soluble in water (1–5 %) [33]. Even when applied to soil in combination with soluble chemical P-fertilizers, dissolution of struvite is not registered and therefore its presence is not beneficial to early plant growth [34]. In general, struvite solubility is very low in water, gradually decreasing from pH 7.0 to 8.5 in calcareous soils with a high pH

[35]. Therefore, it is important to study how to increase the struvite solubility to meet the plant P demand, particularly in the early stage of plant growth during the establishment of the root system, always bearing in mind that this slow-release fertilizer has a P-concentration similar to different superphosphates [36]. Application of *Aspergillus niger* is an option to increase the solubility of struvite, releasing soluble P [37]. A general scheme for struvite solubility and functionality depending on the type of soil, soil conditions (pH, presence of organic acids), characteristics of the struvite related to its nature and formation mode could serve when assessing the nutritional need of the respective crop to optimize its yield.

2.2.2. Biochar and Bone Char

Two main types of biochar could be distinguished, depending on the origin of the material before the treatment: biochar and bone char. Biochar is a biologically derived material produced after a thermal degradation of organic materials such as agricultural wastes. The production of biochar in the absence or very little presence of oxygen is now a very used process mainly due to the energy potential of the respective organic sources [38]. Particularly interesting and well-studied is the pyrolysis of agricultural crop residues producing biochar, which is further applied as a soil amendment [39]. In soil, biochar amendment is accepted as a sustainable approach with multifaceted benefits starting with the management of agricultural wastes, bioenergy production, carbon sequestration during biochar production, improving soil biological and physical characteristics, improving resistance to diseases, and promoting the growth of plants [40]. It was reported that biochar application positively affects the stress tolerance of plants to different abiotic factors derived from industrial activities or climate change such as salinity, drought, metal toxicity and high temperature [41][42]. Another important benefit of applying biochar is its P-content. It was suggested that recycling agricultural residues through biochar may improve sustainable P-recycling bearing in mind the enormous number of agricultural wastes and particularly manures, which have four times higher P-content compared to some solid wastes [43]. Independently of the form of P in the wastes, biochar is much richer in P, which is always insoluble. During the thermal treatment of biochar, the P-content ends in insoluble complexes with Al, Fe, Ca, Mg, and other metal cations [44].

Bone char is another potential alternative source of P and particularly in low-income countries is considered a very attractive slow-release P-bearing source [45]. Like in the case of biochar, bone char is produced via the pyrolysis process, in which bone-containing material is treated in absence of oxygen and at temperatures ranging from 200 to 700 °C. Until recently, residual materials of meat production were efficiently used as high-protein components of animal feed. However, because of the bovine spongiform encephalopathy crisis in the European meat industry, this use of animal wastes is now strictly controlled. The main treatment options are incineration and pyrolysis, which sterilize the product, preventing the transmission of diseases associated with raw animal products [46], and increase the concentration of desirable nutrients such as P and calcium (up to 47%) producing bioapatite and huge amounts of ash, the valorization of which is a major concern. Therefore, apart from using bone residues for their energy content (~17,000 kJ kg^{-1}), these wastes can be applied to soil as part of the sustainability strategies of the fertilizer industry and agriculture [47]. An important advantage of this P-bearing source is its purity compared to rock phosphate, as it is almost totally free of heavy metals and radionuclides. For example, the cadmium concentrations in bone char range up to only 3.03 mg kg^{-1} in cattle and pig bone but up to 556 mg kg^{-1} in rock phosphate [48].

Both bio and bone char are sources of an inorganic, insoluble form of P. Here, P-solubilizing microorganisms could be tested and used as a tool for high and rapid solubilization in fermentation systems or in soil [49][50]. To facilitate the microbial P-solubilizing function and due to their highly porous structure, bone char and biochar were used as soil amendments and simultaneously as cell/spore carriers [49][51]. Biochar derived from agricultural wastes, including bones, can be considered a potential carrier for the formulation of microbial inoculants and might replace other cheap and widely used commercial materials such as peat. It should be noted that, when assessing the role of biochars as potential P-sources and soil P-improvers, researchers should also distinguish between different types of these products formed at different temperatures, 500–700 °C being the most appropriate [49][52].

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