Minireview

Strategies of organic phosphorus recycling by soil bacteria: acquisition, metabolism, and regulation

Yonsoo Park,1,2# Mina Solhtalab,1,2# Wiriya Thongsomboon1,3# and Ludmilla Aristilde 1,2*
1Department of Civil and Environmental Engineering, McCormick School of Engineering and Applied Science, Northwestern University, Evanston, IL, 60208.
2Department of Biological and Environmental Engineering, Cornell University, Riley-Robb Hall, Ithaca, NY, 14853.
3Department of Chemistry, Faculty of Science, Mahasarakham University, Mahasarakham, 44150, Thailand.

Summary

Critical to meeting cellular phosphorus (P) demand, soil bacteria deploy a number of strategies to overcome limitation in inorganic P (Pi) in soils. As a significant contributor to P recycling, soil bacteria secrete extracellular enzymes to degrade organic P (Po) in soils into the readily bioavailable Pi. In addition, several Po compounds can be transported directly via specific transporters and subsequently enter intracellular metabolic pathways. In this review, we highlight the strategies that soil bacteria employ to recycle Po from the soil environment. We discuss the diversity of extracellular phosphatases in soils, the selectivity of these enzymes towards various Po biomolecules and the influence of the soil environmental conditions on the enzyme’s activities. Moreover, we outline the intracellular metabolic pathways for Po biosynthesis and transporter-assisted Po and Pi uptake at different Pi availabilities. We further highlight the regulatory mechanisms that govern the production of phosphatases, the expression of Po transporters and the key metabolic changes in P metabolism in response to environmental Pi availability. Due to the depletion of natural resources for Pi, we propose future studies needed to leverage bacteria-mediated P recycling from the large pools of Po in soils or organic wastes to benefit agricultural productivity.

Introduction

Phosphorus (P), an essential element for all living organisms, is required for cell membranes, genetic materials, energy carrier molecules, carbon metabolism and metabolic signalling (Westheimer, 1987; Büinemann et al., 2010). Due to limited bioavailable P in soils, soil microorganisms often live under P starvation conditions and thus rely on various strategies to access bioavailable P, by improving P assimilation and optimizing intracellular and extracellular P metabolism (Miller et al., 2010; Richardson and Simpson, 2011; Yang and Post, 2011; Grafe et al., 2018). The concentration of bioavailable P as inorganic P (Pi) is typically low in soil solution (<0.01–1 mg L−1 in highly fertile soils). Of the total P in surface soils, which generally ranges from 20 to 1000 mg P kg−1, organic P (Po) forms comprise 20%–80% (Anderson, 1980; Büinemann et al., 2010; Yang and Post, 2011). Low-molecular-weight phosphomonoesters, such as phytic acid, sugar phosphates and ribonucleotides, and phosphoester polymers, such as nucleic acids and phospholipids, are the predominant forms of Po in soils that originate from microbial cell death and plant debris (Turner et al., 2003; Vestergren et al., 2012; Zhang et al., 2012; Dodd and Sharples, 2015; Hou et al., 2015; Siros and Buckley, 2019). Synthetic phosphonoesters (also called organophosphates) in soils are derived from anthropogenic sources such as herbicides, insecticides, flame retardants and plasticizers (Gao et al., 2016; Yadav et al., 2018; Wang et al., 2019). Beyond natural and synthetic phosphoester compounds with P-O bonds, phosphonates with P-C bonds found in cell membrane lipids, exopolysaccharides and glycoproteins are also present in soils (Table 1) (Metcalf and Van Der Donk, 2009). Besides their natural abundance (Clark et al., 1999; Yu et al., 2013), these compounds have been widely used in agriculture and medicine.

Received 2 March, 2021; accepted 14 December, 2021. *For correspondence. E-mail: ludmilla.aristilde@northwestern.edu; Tel. 847-491-2999; Fax: 847-491-4011. #These authors are co-first authors.

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| Category name                        | Type of bond | Substrate structure (Neutral) | EC No.  | Product                      | Examples of enzymes                                                                 |
|--------------------------------------|--------------|-------------------------------|---------|------------------------------|-------------------------------------------------------------------------------------|
| Phosphoric monoester hydrolase       | P-O          |                               | 3.1.3.- | P$_i$                        | Alkaline phosphatase (ALP)                                                          |
| (Phosphomonoesterase)                |              |                               |         |                              | Acid phosphatase (AP)                                                              |
|                                      |              |                               |         |                              | Nucleotidase                                                                       |
|                                      |              |                               |         |                              | Phytase                                                                             |
|                                      |              |                               |         |                              | Sugar phosphatase                                                                  |
|                                      |              |                               |         |                              | Polynucleotide - phosphatase                                                       |
|                                      |              |                               |         |                              | phosphoserine phosphatase                                                          |
|                                      |              |                               |         |                              | ALP:                                                                               |
|                                      |              |                               |         |                              | γ-proteobacteria (e.g. Pseudomonas)                                                 |
|                                      |              |                               |         |                              | α-proteobacteria (e.g. Rhizobia)                                                   |
|                                      |              |                               |         |                              | Actinobacteria                                                                      |
|                                      |              |                               |         |                              | Firmicutes (e.g. Bacillus)                                                         |
|                                      |              |                               |         |                              | Cyanobacteria (e.g. Gloeobacter)                                                    |
|                                      |              |                               |         |                              | AP:                                                                                |
|                                      |              |                               |         |                              | γ-proteobacteria (e.g. Pseudomonas, Stenotrophomonas, Xanthomonas, Enterobacter, Erwinia, Lysobacter, Serratia) |
|                                      |              |                               |         |                              | α-proteobacteria (e.g. Caulobacter, Methylobacterium, Sphingomonas, Rhodobacter)    |
|                                      |              |                               |         |                              | Firmicutes (Bacillus, Clostridium)                                                  |
|                                      |              |                               |         |                              | Bacteroidetes (Pedobacter)                                                          |
|                                      |              |                               |         |                              | Phytase:                                                                           |
|                                      |              |                               |         |                              | γ-proteobacteria                                                                   |
|                                      |              |                               |         |                              | δ-proteobacteria                                                                   |
|                                      |              |                               |         |                              | γ-proteobacteria (e.g. Pseudomonas)                                                 |
|                                      |              |                               |         |                              | Bacteroidetes, Cyanoacteria, Actinobacteria                                         |
|                                      |              |                               |         |                              | Firmicutes (Bacillus)                                                              |
|                                      |              |                               |         |                              | γ-proteobacteria                                                                   |
|                                      |              |                               |         |                              | Actinobacteria                                                                      |
| Phosphoric diester hydrolase         | P-O          |                               | 3.1.4.- | P-R                         | Exonuclease                                                                         |
| (Phosphodiesterase)                  |              |                               |         |                              | Phospholipase                                                                      |
| Phosphoric triester hydrolase        | P-O          |                               | 3.1.8.- | R-P-R                       | Paraoxonase                                                                        |
|                                      |              |                               |         |                              | γ-proteobacteria (e.g. Pseudomonas, Firmicutes (e.g. Bacillus))                     |
| Triphosphoric acid monoester hydrolase| P-O          |                               | 3.1.5.- | P$_{--}$P$_{--}$           | dGTPase                                                                            |
|                                      |              |                               |         |                              | Common in all bacteria                                                              |
| Phospho-anhydrides hydrolase         | P-O          |                               | 3.6.1.- | P$_i$                        | Adenosine-triphosphatase                                                           |
|                                      |              |                               |         |                              | Inorganic diphosphatase                                                           |
|                                      |              |                               |         |                              | Nucleoside diphosphate-phosphatase                                                 |
|                                      |              |                               |         |                              | Common in all bacteria                                                              |
contemporarily (Galezowska and Gumienna-Kontecka, 2012; Martinez et al., 2018). There is increased recognition of the importance of soil microorganisms in playing an integral role in recycling P from the array of P\textsubscript{o} compounds found in soils (Oberson and Joner, 2005; Bünemann et al., 2010; Zhou et al., 2018). Here we present a review of the strategies employed by soil bacteria to synthesize different P\textsubscript{o} compounds as well as obtaining bioavailable P for their cellular metabolism (Fig. 1): (i) biosynthesis of P\textsubscript{o} compounds, (ii) diversity and selectivity of phosphatase enzymes, (iii) acquisition of P\textsubscript{o} compounds, (iv) regulation of phosphatases, P\textsubscript{o} acquisition and P\textsubscript{o} metabolism and (v) future research directions to be considered towards harnessing these bacterial capabilities for sustainable P management and engineered P recycling.

**Biosynthesis of common P\textsubscript{o} compounds**

Cellular P\textsubscript{i} is required for phosphorylation reactions within the central carbon metabolism and the eventual generation of precursors for the biosynthesis of P\textsubscript{o} biopolymers, namely, nucleic acids and phospholipids, as illustrated in Fig. 2. Here we follow the incorporation of P\textsubscript{i} starting with the initial catabolism of glucose, a common carbohydrate in soils and the derivative of cellulose and starch, two common biopolymers in plant matter. Glucose is phosphorylated to produce glucose-6-phosphate (G6P) by a hexokinase or via the phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS). Hexokinases transfer the phosphoryl group of adenosine triphosphate (ATP) to glucose or other hexose sugars, such as mannose, fructose, or galactose (Cohen, 2014; Prakasham and Kumar, 2019). In the PTS system, PEP is the phosphoryl donor for substrate phosphorylation (Deutscher et al., 2014; Jeckelmann and Erni, 2020). Following the phosphorylation of the sugar, the phosphorylated metabolite, such as G6P, subsequently enters the pentose phosphate pathway or proceeds to glycolysis. Alternatively, glucose can also enter the gluconate pathway through which glucose is oxidated to gluconate and subsequently phosphorylated to 6-phosphogluconate. 6-phosphogluconate can then enter either the pentose phosphate pathway directly or glycolysis through the Entner–Doudoroff pathway (Conway, 1992; Nikel et al., 2015).

Within the pentose phosphate pathway, following oxidation and decarboxylation reactions, G6P generates ribose-5-phosphate (RSP). After phosphorylation with pyrophosphate derived from ATP, RSP becomes 5-phospho-\(\text{d-ribose}-\alpha\)-1-diphosphate (PRPP), which is an important precursor to the biosynthesis of purine and pyrimidine nucleotides, the amino acids histidine and tryptophan, and the cofactors nicotinamide adenine

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dinucleotide (NAD) and NAD phosphate (NADP) (Cohen, 2014; Hove-Jensen et al., 2017). Both purine and pyrimidine nucleotides are essential to biomass growth as they serve as building blocks for RNA, DNA and the energy carrier molecules (ATP, NAD and NADP).

Purine nucleotides, such as adenosine-5'-monophosphate (AMP) and guanosine-5'-monophosphate, can be synthesized either via de novo or salvage pathways. Inosine monophosphate (IMP) is an intermediate of the de novo purine nucleotide biosynthesis and is synthesized by gradually assembling the purine ring on the C-1 of the R5P moiety in PRPP (Kilstrup et al., 2005; Hove-Jensen et al., 2017). In the salvage pathway, several enzymes are involved in adding the phosphoribose R5P to recycled nucleobases to produce monoribonucleotides (e.g. synthesis of AMP by phosphoribosylating adenine) (Hove-Jensen et al., 2017). Further phosphorylation steps on the monoribonucleotide would then produce triphosphorylated ribonucleotides such as ATP, which serves as a phosphoryl or nucleotidyl donor within the cell, or guanosine triphosphate, which also acts as a phosphoryl donor (Walsh et al., 2018).

Pyrimidine nucleotides, such as uridine-5'-triphosphate (UTP) and cytidine-5'-triphosphate (CTP), are also synthesized via de novo and salvage pathways. De novo synthesis of pyrimidine begins with carbamoyl phosphate, a metabolite generated by phosphorylating and aminating dissolved CO₂. Follow-up reactions involve amino acid addition to and subsequent cyclization of carbamoyl phosphate to eventually generate the pyrimidine ring dihydroorotate (Cohen, 2014). Orotate, which is generated from dihydroorotate, reacts with PRPP to produce orotidine 5'-monophosphate and, after decarboxylation, uridine-5'-monophosphate (UMP) (Cohen, 2014). After two phosphorylation steps, UMP yields UTP, which is the precursor to the synthesis of CTP and subsequent synthesis of pyrimidine compounds (Kilstrup et al., 2005). The pyrimidine salvage pathway, unlike the purine salvage pathway, involves a single phosphoribosyltransferase, uracil phosphoribosyltransferase, which utilizes uracil from nucleic acid catabolism or supplied from the environment (Hove-Jensen et al., 2017).

The biosynthesis of phospholipids relies on the combination of CTP, a pyrimidine ribonucleotide, with glyceraldehyde-3-phosphate (GAP), a glycolytic metabolite. The transfer of the acyl group from GAP to the sn-1 and sn-2 positions of glycerol-3-phosphate (G3P) by the G3P acyltransferase yields 1,2-diacylglycerol-sn-G3P (Sohlenkamp and Geiger, 2015). This latter metabolite further reacts with CTP to form cytidine diphosphate-diacylglycerol, which is a common precursor to various phospholipids with an assortment of different headgroups that compose the cell membrane (Parsons and Rock, 2013; Sohlenkamp and Geiger, 2015). In the following section, we discuss the diversity and selectivity of phosphatase enzymes employed by soil bacteria to

Fig 1. Schematic overview of P₃ recycling processes employed by soil bacteria. Decomposition of plant and microbial biomass results in an accumulation of organic matter, including P₃ compounds, in soil. In this review, we explain the strategies of soil bacteria in recycling of P from P₃ coming from soil organic assemblages. As shown in the schematic, these recycling strategies include the degradation of P₃ compounds by intracellular and extracellular phosphatases, the upregulation of genes encoding transporters for the uptake of extracellular P and the incorporation of the scavenged P into the P₃ biosynthesis pathways.

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Fig 2. Overview of the different metabolic pathways involved in P$_o$ biosynthesis in bacterial cells. The metabolic pathways for the biosynthesis of precursors to essential Po biopolymers, nucleotides and phospholipids, starting from glucose are shown. Abbreviations: G6P, glucose-6-phosphate; R5P, ribose-5-phosphate; PRPP, 5-phospho-D-ribose-α-1-diphosphate; IMP/UMP, inosine/uridine monophosphate; CTP, cytidine triphosphate; GAP, glyceraldehyde-3-phosphate; G3P, glyceraldehyde-3-phosphate; 1,2-diacyl-G3P, 1,2-diacylphosphoglycerate; CDP-DAG, cytidine-1,2-diacylphosphosphate.

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hydrolyze different P<sub>0</sub> compounds to achieve P<sub>i</sub> acquisition.

### Diversity and selectivity of phosphatases for P<sub>0</sub> hydrolysis

During P<sub>i</sub> deficiency, soil bacteria produce phosphatase enzymes to recycle P<sub>i</sub> from heterogenous P<sub>0</sub> mixtures for their own usage and to help the plant growth (Fitriatin et al., 1998; Monds et al., 2006). Phosphatases catalyse the hydrolytic cleavage of a bond between P and another atom (Table 1). The majority of phosphatases are involved in hydrolyzing the P-O bonds present in most P<sub>0</sub> compounds found in soils. Phosphomonoesterases (Enzyme Commission number, EC 3.1.3.-) represents the most abundant class of extracellular phosphatases released by soil bacteria to release P<sub>i</sub> from phosphomonoesters (Sharma et al., 2013; Margalef et al., 2017). Alkaline phosphatases, i.e. ALP, (EC 3.1.3.1), acid phosphatases, i.e. AP, (EC 3.1.3.2), and phytases (EC 3.1.3.8 and 3.1.3.26) are the predominant forms of extracellular phosphatases in soils (Eivazi and Tabatabai, 1977; Hui et al., 2013; Sharma et al., 2013). ALPs, the most well-studied group, are encoded by phoA, phoD and phoX genes (Neal et al., 2018). The PhoD and PhoX ALPs were reported to be more abundant than PhoA in marine and terrestrial ecosystems (Luo et al., 2009; Sebastian and Ammerman, 2011; Ragot et al., 2015), with PhoDs having the highest abundance in soils (Luo et al., 2009; Tan et al., 2013; Ragot et al., 2015). In addition to their phosphomonoesterase activities, most PhoDs exhibit phosphodiesterase activity, e.g. PhoD<sub>AP</sub> from *Aphanothec halophytyca* (Kageyama et al., 2011). Based on the signal peptide attached to the protein sequences, it was concluded that PhoD and PhoX ALPs are translocated to the cell membranes (Pop et al., 2002; Monds et al., 2006), whereas the PhoAs are secreted extracellularly or to the periplasm (Kim and Wyckoff, 1991; Monds et al., 2006). A metagenomics study (Luo et al., 2009) reported a 40% higher abundance of cytoplasmic ALPs than extracellular ones in marine bacterial communities. It remains to be determined how the cellular localization of ALPs varies in soil ecosystems.

A soil metagenome analysis for bacterial phosphatase genes showed a higher abundance of ALPs compared to APs in soil (including rhizosphere and bulk soil) (Lidbury et al., 2017a), whereas when considering only the rhizosphere region, the number of gene copies for APs was found to be 10- to 100-fold higher than ALP genes (Fraser et al., 2017). Bacterial APs, known as non-specific acid phosphatases (NSAPs), are active towards a broad range of substrates harbouring different structures (Rossolini et al., 1998). These APs are categorized into three classes A, B and C; the abundance of classes A and C APs was shown to be several times higher than class B APs in different soil types (Neal et al., 2018).

While class A and B APs are mostly periplasmic enzymes, class C APs are associated with the bacterial outer membrane, providing higher accessibility to the P<sub>0</sub> present in the soil and thus making class C APs more advantageous for soil bacteria than the other classes (Neal et al., 2018).

Phytases are phosphatases capable of degrading phytic acid, the primary P<sub>i</sub> storage in plants and grains (Table 1) (Mullaney and Ullah, 2003; Lei et al., 2013). Phytases can also hydrolyze other P<sub>0</sub> compounds including ribonucleotides and sugars phosphates (Sohtalab et al., 2020, Sariyska et al., 2005, Casey and Walsh, 2003, George et al., 2007, Greiner et al., 2009). Based on their mechanism in the sequential hydrolysis of phytic acid, phytases are grouped into 3-phytases (EC 3.1.3.8) and 6-phytases (EC 3.1.3.26). Only a few bacterial phytases are grouped as 6-phytases, including the periplasmic phytase of *Escherichia coli*, one of the most studied bacterial phytases (Lim et al., 2000; Menezes-Blackburn et al., 2013). Most bacterial phytases are classified as 3-phytases, a group of enzymes whose gene abundance was shown to be 4.5-fold higher in P<sub>i</sub>-deficient soil relative to P<sub>i</sub>-rich soil (Yao et al., 2018). Considering their structural differences, bacterial phytases can be classified into four subcategories, namely, histidine acid phosphatases, β-propeller phytases (BPPs), protein-tyrosine phosphatase-like phytases (cysteine phosphatase) and purple acid phosphatases (PAP) (Lei et al., 2013). Among those, BPPs are reported to be the predominant bacterial phytases secreted in the soil near the rhizosphere (Lim et al., 2007; Jorquera et al., 2013). To obtain an accurate evaluation of the bacterial phytase diversity in soil ecosystems, new metagenomic studies of the diversity of phytases based on the updated genomic databases of soil bacteria are still warranted (Lim et al., 2007).

In addition to the three main bacterial phosphomonoesterases mentioned above, PAP-like sequences were also found in genomes of 43 bacteria and four cyanobacteria species (Yeung et al., 2009; Bhadouria and Giri, 2021). Based on homology models, bacterial PAPs are similar structurally to mammalian PAPs in the vicinity of their active sites (Schenk et al., 2000). However, the structures of bacterial PAPs have not been determined yet, and only a few have been characterized for their physiological roles and biochemical properties (Yeung et al., 2009; Zhu et al., 2019; Forrellad et al., 2020). Interestingly, bacterial PAPs from *Burkholderia pyrrocinia* (PAP9), *B. cenocepacia* (BcPAP) and *Mycobacterium tuberculosis* (Rv2577) have alkaline pH optima unlike previously characterized PAPs from plants and
mammals, which are optimally active at the pH range of 4–7 (Yeung et al., 2009; Zhu et al., 2019; Forrellad et al., 2020).

The hydrolysis of phosphodiesters requires the activity of a group of enzymes called phosphodiesterase (EC 3.1.4.-), such as nuclease and phospholipases (Table 1). Phosphomonoesters are the product of these reactions and will subsequently be hydrolyzed to Pᵢ by phosphomonoesterase activities mentioned earlier (Table 1) (Turner and Haygarth, 2005; Bünemann, 2008). Similar to phosphomonoesterases, elevated gene abundance of phosphodiesterases such as exoribonuclease and sphingomyelin phosphodiesterase is found in Pᵢ-deficient soils (Yao et al., 2018). In response to the presence of the toxic phosphotriesters (such as in paraoxon, a metabolite of the insecticide parathion), soil bacteria have evolved enzymes, called phosphotriesterase or organophosphate hydrolase (encoded by opd gene) (Table 1) (Ali et al., 2012; Khalid et al., 2016), to detoxify these compounds by breaking the P-O bond (Theriot and Grunden, 2011). Other P-O hydrolyses are intracellular phosphatases, which are responsible for catalysing the dephosphorylation processes related to regulations, signalling pathways and motility (Zuo and Deutscher, 2001; Garcia-Caballero et al., 2017). The C-P lyases were historically known as the only enzymes that could break down the P-C bonds in phosphonates, but other hydrolyase enzymes, such as phosphonopyruvate hydrolyse and phosphonoacetate hydrolyse (Agarwal et al., 2011), can also target the stable P-C bonds (Quinn et al., 2007; Agarwal et al., 2011). The P-N bonds, which are mostly found in histidine- or arginine-phosphorylated proteins, can be lysed by intracellular phosphatases primarily involved in protein dephosphorylation (Chou et al., 2005), thus not likely relevant to the Pᵢ acquisition of soil bacteria from the extracellular region. The diverse categories of extracellular, periplasmic and membrane-associated phosphatases mentioned here are produced by various soil bacterial species present to facilitate the Pᵢ recycling from Pᵢ-containing natural organic assemblages.

In relation to the presence of high microbial activity in the rhizosphere, phosphatase activities are reported to be higher in the rhizosphere than the bulk soil (Spohn and Kuzyakov, 2013). There is a higher abundance of AP genes in rhizosphere bacteria than in bulk soil (Spohn and Kuzyakov, 2013; Fraser et al., 2017). Among bacterial NSAPs, only class A and C are present in the environment, whereas class B enzymes are from bacteria associated with eukaryotic hosts (Neal et al., 2018; Udaondo et al., 2020). While class A NSAPs are mainly produced by Gram-negative bacteria (including Pseudomonas, Caulobacter, Stenotrophomonas, Methylobacterium, Sphingomonas and Xanthomonas species (Neal et al., 2018), class C NSAPs are found in a broad range of soil bacteria including Bacillus, Clostridium, Enterobacter, Erwinia, Lysobacter, Pedobacter, Pseudomonas, Rhodobacter and Serratia species (Neal et al., 2018). Unlike APs, ALP genes are mostly found in bacterial communities in the bulk soil instead of the rhizosphere (Spohn and Kuzyakov, 2013). Among the diverse range of phyla producing ALPs (Sebastian and Ammerman, 2009; Ragot et al., 2017), Proteobacteria and Actinobacteria were the most abundant communities harbouring both phoD and phoX genes, followed by Firmicutes for phoD and Planctomycetes for phoX genes (Ragot et al., 2017; Liburdy et al., 2021). Particularly, phoD (the most relevant ALP genes in soil bacteria) was found in the orders Actinomycetales, Bacillales, Gloeobacterales, Rhizobiales and Pseudomonadales (Tan et al., 2013; Ragot et al., 2015; Wan et al., 2020).

A diverse range of bacteria including α-proteobacteria, δ-proteobacteria, γ-proteobacteria, Bacteroidetes, Cyanobacteria and Actinobacteria were found to possess the phytase-like genes (Jorquera et al., 2008). As the most abundant bacterial phytase, BPP genes were detected in the strains of Pseudomonas and Bacillus from plant roots (Jorquera et al., 2012), including phytases from Bacillus amylobiliquefaciens (Shim and Oh, 2012; Boukhris et al., 2015), B. laevolacticus (Gulati et al., 2007), B. subtilis (Lim et al., 2007) and Pseudomonas sp. FB15 (Jang et al., 2018). In addition to BPPs, the genes and the phytase activity related to histidine acid phosphatases and PAPs were found in Acinetobacter and Buttiauxella sp. of soil samples (Rix et al., 2020).

Although there is little information about phosphodiesterase enzymes related to soil bacteria, a community proteogenomics study found that archaea and γ-proteobacteria are the major producers of phosphodiesterases particularly in response to Pᵢ deficiency (Yao et al., 2018). Moreover, it was shown that a considerable number of soil bacteria mainly from Actinobacteria and proteobacteria phyla harbour the genes for phospholipase enzymes. For instance, P. fluorescens can catalyse all the degradation steps of glycerophosphodiesterases extracellularly using their glycerolphosphodiesterases, GipQII (Liburdy et al., 2017b). Due to their relevance to biotechnological applications, phosphotriesterases were characterized from several soil bacteria, such as P. diminuta, OPH (Gorla et al., 2009), P. putida (Multry and Karns, 1989; Khalid et al., 2016), Agrobacterium, OpDA (Home et al., 2002), Flavobacterium, OPH (Multry and Karns, 1989; Home et al., 2002) and Burkholderia, OphB (Taesung et al., 2007). With respect to phosphonatase enzymes for breaking the P-C bonds, Pseudomonas species such as P. fluorescens are the notable soil bacterial species shown to produce phosphonatases, PhnA (Panas et al., 2006).

Soil physicochemical properties play an important role in influencing both the bacterial communities producing...
phosphatases (Marschner et al., 2004; Margalef et al., 2017; Ragot et al., 2017) and influencing the stability and activity of phosphatases (Fig. 3) (Allison, 2006; Schimel et al., 2017). For instance, expectedly, ALP abundance was higher in grassland soils with alkaline pH, than in forest soils with acidic pH (Ragot et al., 2017). Based on a soil metagenome analysis (Lidbury et al., 2017a), the number of bacterial AP genes remained the same in both acidic and alkaline pH values, but the number of ALP genes decreased significantly at low pH. Similar to APs, the number of phytase and phosphodiesterase genes has been shown to remain constant in response to high and low pH (Lidbury et al., 2017a). In addition to pH, the type of crops is one of the main factors determining the type of phosphatases and their bacterial producers in agricultural soils. For example, α-proteobacteria Sphingopyxis and Asticcacaulis and the β-proteobacteria Ralstonia and Cupriavidus that harbour phoD genes were more associated with sorghum rhizosphere, whereas α-proteobacterium Bosea and β-proteobacterium Achromobacter with NSAP genes were found more in maize rhizosphere (Neal et al., 2021). Moreover, it was shown that bioavailability of enzyme cofactors can determine the abundance of phosphatase genes in soil. For instance, presence of Ca²⁺ can facilitate high abundance of ALP (phoD and phoX) and BPP genes, whereas the absence of Ca²⁺ is correlated with the abundance of NSAPs in soil (Neal and Glendining, 2019). Interestingly, while the relative abundance of P₀ compounds was negatively correlated with the abundance of phoX-harbouring Actinobacteria, it had no effect on proteobacteria (Ragot et al., 2017) and was correlated positively with the abundance of all phoD-harbouring communities (Ragot et al., 2017; Luo et al., 2019). Furthermore, it was shown that introducing high P₀ and organic matter via long-term manure supplementation enhanced the gene abundance of ALPs, phytases, exopolyphosphatases and pyrophosphatases in soil more than inorganic fertilization (Ma et al., 2020). Among ALP genes, phoX genes were found in fewer ecosystems than phoD genes (Ragot et al., 2015). Moreover, the bacterial communities producing PhoD are different in different types of soil. For instance, in vertisol and planosol (both rich in clay minerals), proteobacteria are the dominant phoD-associated bacteria, whereas the Deinococcus-Thermus and Firmicutes were predominant in leptosol (extremely gravelly and/or stony soil) (Ragot et al., 2017). Therefore, depending on the type of soil in the rhizosphere of a specific crop, a combination of different environmental factors such as land use, pH and nutrient concentrations (P₀, Pᵢ, total carbon and nitrogen) can determine the type of bacterial communities harbouring phosphatase genes in the rhizosphere (Margalef et al., 2017; Ragot et al., 2017; Sun et al., 2020).

Kinetic parameters of phosphatases vary depending on the type of phosphatase (Hui et al., 2013), the origin of the enzyme (Hui et al., 2013), soil type (Giaveno et al., 2010), soil depth (Stone and Plante, 2014) and the extent of P amendments (Zhang et al., 2018). While similar half-saturation constants (Kᵢ) were found for ALPs and APs across different types of soil, higher maximum initial rates
activity of AP remained unaffected (Olsson et al., 2013). In deep soils compared to surface soil, kinetic parameters of soil-associated phosphatases \((V_{\text{max}}\text{ and } K_m)\) decreased. However, while phosphatase catalytic efficiency was maintained in the first meter of soil profiles, it decreased by 50% at 140 cm soil depth (Stone and Plante, 2014).

The catalytic efficiency of soil-associated phosphatases is dependent on their structural stability, which is influenced by soil environmental factors such as pH, temperature, adsorption to minerals and organic matter. The optimal activity of APs was found at pH 5.2 and ALPs at pH 9.2 (Hui et al., 2013). Among different minerals, montmorillonite was shown to be destructive to the catalytic activity of ALPs (Zhu et al., 2016). Binding to goethite (an iron-oxyhydroxide mineral) also led to a decrease in the activity of ALP (Zhu et al., 2016), but the catalytic activity of AP remained unaffected (Olsson et al., 2012). Phytase was also adsorbed greatly to different minerals surfaces (Giaveno et al., 2010). Among those minerals, kaolinite and montmorillonite caused the greatest activity loss upon adsorbing phytases. However, the clays with heterogeneous surfaces, such as oxisol clays, could enhance the phytase stability in soil (Giaveno et al., 2010). Consistent with the positive effect of heterogeneous mixtures, it was shown that the addition of organic matter, such as biochar amendments, also increased ALP activity up to fourfold (Khadem and Raiesi, 2019). Furthermore, ALP immobilization on montmorillonite and goethite was shown to protect the enzyme against heavy metal inhibition (Wang et al., 2017; Tan et al., 2018).

In addiction to gene expressions in the bacterial community, the abundance of different forms of P in the soil can affect the catalytic activity of the phosphatases (Zhang et al., 2018). The addition of \(P_i\) inputs to the \(P_i\)-deficient forest soil led to a decrease in soil phosphatase catalytic efficiency (i.e. \(V_{\text{max}}/K_m\)) due to inhibition of enzymes by \(P_i\). However, in \(P_i\)-rich soils where the initial catalytic efficiency of phosphatases was higher, the extra addition of \(P_i\) inputs to the soil further increased the phosphatase efficiency (Zhang et al., 2018). The strategies evolved by bacteria for the uptake of the released \(P_i\) from \(P_o\) after extracellular phosphatase activity as well as the strategies developed for the direct uptake of \(P_o\) compounds will be discussed in the following section.

**Acquisition of \(P_i\) and \(P_o\) compounds by soil bacteria**

Upon hydrolysis of \(P_o\) compounds by the activity of phosphatases discussed in the previous section, the resulting \(P_i\) is assimilated by soil bacteria primarily via phosphate inorganic transporters (Pit) and the phosphate-specific transport (Pst) system (Wanner, 1996). Primarily studied in *E. coli*, the low-affinity Pit transporters assimilate \(P_i\) under \(P_i\)-replete conditions and the high-affinity Pst system is induced under \(P_i\)-depleting conditions (Hoffer et al., 2001a; Hsieh and Wanner, 2010; McCleary, 2017). Similar trends of transitioning to the Pst system with reduced \(P_i\) availability were observed in other soil bacteria such as *Bacillus* species, *Burkholderia cenocepacia*, *Pseudomonas aeruginosa*, *Sinothizobium melilotii*, *Streptomyces coelicolor* and *Staphylococcus aureus* (Qi et al., 1997; Voegele et al., 1997; Hoshino, 1998; Antelmann et al., 2000; Saier Jr et al., 2002; Hoi et al., 2006; Voigt et al., 2006; Martín et al., 2011; Kelliher et al., 2018; Barreiro and Martínez-Castro, 2019; Shropshire et al., 2021). Though \(P_i\) is the most favourable form of P to bacteria, soil bacteria are also capable of assimilating \(P_o\) compounds such as organophosphates and phosphonates (Table 1).

The organophosphate-phosphate antiporters (OPA) family allows the transport of various phosphorylated metabolites, such as G3P, G6P, 2- or 3-phosphoglycerate, or PEP (Elvin et al., 1985; Lemieux et al., 2004a; Albermann et al., 2014). These \(P_i\) metabolites represent intermediates in the glycolytic pathway in cellular carbon metabolism. The G3P transporter (GlpT) and hexose-6-phosphate transporter:antiporter (UhpT), both of which are members of the OPA family, are well-studied in *E. coli* (Winkler, 1966; Ambudkar et al., 1990; Brzoska et al., 1994; Fann and Maloney, 1998; Auer et al., 2001; Huang et al., 2003; Lemieux et al., 2004a). Homologues to the *glpT* gene have been identified in *B. subtilis* and *P. aeruginosa* as well (Castañeda-Garcia et al., 2009). In *Salmonella typhimurium*, the phosphoglycerate transporter protein (PgtP), another member of the OPA family, catalyses the transport of 2PGA, 3PGA and PEP (Varadchachary and Maloney, 1991). In addition, *E. coli* has the Ugp (uptake of glycerol phosphate) system, an alternative transport system for glycerophosphoryl diesters, encoded by the *ugpBAECQ* operon (Schweizer et al., 1982; Brzoska and Boos, 1988). *Corynebacterium glutamicum* lacks genes homologous to those encoding GlpT, UhpT and PgtP but contains homologues of the genes encoding the Ugp system (Ishige et al., 2003). Reports of *C. glutamicum* utilizing G3P as a sole P source led to the conclusion that G3P is taken up by the Ugp system encoded by its *ugpAECB* operon (Eggeling and Bott, 2005; Wendisch and Bott, 2005; Lindner et al., 2012). In addition to the aforementioned transporters in the OPA family, other novel bacterial transporters that may be involved in \(P_o\) utilization have been reported. For instance, a recent study that performed a comparative genomics analysis on eight *Flavobacterium* strains detected the expression of six
distinct phosphate utilization system transporter complexes (PusCD) and proposed that these complexes may target phosphodiester in soil, most likely lipid headgroups and nucleic acids (Liburdy et al., 2021).

Phosphonates (with C-P bonds), found in soil from both biogenic and anthropogenic (e.g. herbicides) sources, can be a potential P source to soil bacteria subjected to P deficiency (Hilderbrand and Henderson, 1983; McGrath et al., 2013; Hove-Jensen et al., 2014). The C-P lyase pathway involving the phosphonate transporter (Phn) system is considered a major phosphonate catabolism route in bacteria and is well-studied in E. coli (McGrath et al., 2013; Hove-Jensen et al., 2014). The 14-gene operon, phnzCDEFGHIKLNMOP, in E. coli encodes the proteins required for the uptake and assimilation of phosphonates via the C-P lyase pathway with the first three genes (phnCDE) encoding an ABC-type phosphonate transport system (Metcalf and Wanner, 1993; White and Metcalf, 2004; Stasi et al., 2019). Via the C-P lyase pathway, alkylphosphonates are converted to the corresponding alkanes and P (McGrath et al., 2013). A survey of bacterial genes revealed that the genes encoding the phosphonate transport polypeptides are found as either phnCDE or phnCDEE, in which there are two distinct transmembrane polypeptides (Hove-Jensen et al., 2014). The survey revealed that E. coli, Burkholderia pseudomallei and P. stutzeri have phnCDE for the Phn system, whereas S. melloti, Ochrobactrum anthropic, Agrobacterium radiobacter, Mesorhizobium loti and Bradyrhizobium japonicum have phnCDEE (Hove-Jensen et al., 2014). Moreover, P. stutzeri has an additional operon relevant to the C-P lyase pathway, htxABCDEFGHIJKLMN, where htxBCD encodes the ABC transport system for phosphonate (White and Metcalf, 2004; Hove-Jensen et al., 2014). Orthologous genes to phnCDE were identified in C. glutamicum and S. aureus (Ishige et al., 2003). Unlike the Phn system activated under P limitation, it has been reported that P\textsubscript{i}-insensitive 2-aminoethylphosphonic acid (2EAP) degradation in P. putida NG2 and BIRD-1 (Teman and Quinn, 1998; Murphy et al., 2021). A recent study identified the proteins relevant to 2EAP assimilation in P. putida BIRD-1, whereby 2-aminoethylphosphonate XVW (Aep XVW) was the primary P\textsubscript{i}-sensitive 2EAP transporter with AepSTU serving an auxiliary role and 2EAP permease (AepP) was essential for P\textsubscript{i}-insensitive growth on 2EAP (Murphy et al., 2021).

A metagenomics study on the soil microbial community grown with legume and grass found that microorganisms involved in P\textsubscript{o} mineralization were more abundant in soil grown with legume, whereas microorganisms involved in P\textsubscript{i} solubilization (from inorganic P minerals) were more abundant in the soil grown with grass (Zhou et al., 2018). This study identified the AI and P contents as well as P\textsubscript{o} composition in the soil as the major factors of the reported differences (Zhou et al., 2018). Therefore, in addition to P availability, this study suggests that different environmental factors may influence the activation of P acquisition strategies in soil bacteria. In the following section, we discuss the regulation involved in P\textsubscript{o} acquisition and metabolism depending on the P availability.

Phosphatases, P\textsubscript{o} acquisition and P\textsubscript{o} metabolism

As discussed above, bacteria can scavenge P\textsubscript{i} from P\textsubscript{o} compounds in their environment through enzymatic action using phosphatases, including ALPs, APs, phytases, phospholipases and nucleases. We also highlighted that, in addition to P\textsubscript{i} uptake, many bacteria can directly transport P\textsubscript{o} compounds such as organophosphates and phosphonates as alternative P sources. When the environmental P\textsubscript{i} level is limited, bacteria increase the expression of P\textsubscript{i}-scavenging enzymes and several P transporter systems to increase P\textsubscript{o} acquisition (Wanner, 1996; Santos-beneit, 2015). In addition, soil bacteria can modify their metabolic network related to the biosynthesis of P\textsubscript{o} compounds such as phospholipids, nucleotides and nucleic acids. In this section, we discuss the mechanisms employed by soil bacteria to regulate the following processes: enzyme production for P\textsubscript{i}-scavenging from P\textsubscript{o}, expression of P\textsubscript{o} transporters and metabolic changes in P\textsubscript{o} biosynthesis under P\textsubscript{i}-limiting conditions. The overview schematic of bacterial regulation of these processes is illustrated in Fig. 4.

Many of the genes involved in the production of P\textsubscript{i}-scavenging enzymes and P\textsubscript{o} transporters are controlled by a global regulatory mechanism called the phosphate (pho) regulon (Santos-beneit, 2015; Martin and Liras, 2021).

The pho regulon is inactive under replete P\textsubscript{i} condition but, during P\textsubscript{i} scarcity, the pho regulon is activated to regulate a set of genes towards maintaining an adequate supply of P\textsubscript{i} (Wanner, 1990; Santos-beneit, 2015; Morimoto et al., 2016; Martin and Liras, 2021). Mediated by the pho regulon, P\textsubscript{i} is transported primarily through the Pho system upon depletion in P\textsubscript{i}, whereas the Pit system is the primary P\textsubscript{i} transporter when P\textsubscript{i} is replete (Rosenberg et al., 1977; Willsky and Malamy, 1980; Qi et al., 2016; Grafe et al., 2018). Specifically, the pho regulon upregulates pstSCAB, the operon that encodes
the proteins of the Pst transporter system (Wanner, 1990; Vuppada et al., 2018). Importantly, the regulation of the 
pho regulon is critical to the subsequent regulation of enzymes involved in P₇ acquisition from P₈ compounds.

In bacteria, the extracellular P₇ level is the responsible signal that regulates the pho regulon (Rao et al., 1993; Vuppada et al., 2018) which is mediated by a two-component regulatory system composed of an inner-membrane sensor protein and a transcriptional response regulator (Wanner, 1990; Santos-beneit, 2015). First characterized in E. coli, this regulatory system of the pho regulon consists of two proteins: PhoR for the sensor protein and PhoB for the response regulator protein (Tommassen et al., 1982). These proteins are called by different names in other bacteria, for instance, the corresponding proteins in B. subtilis are PhoR and PhoP (Hulett et al., 1994; Novák et al., 1999; Kocan et al., 2006; Glover et al., 2007). When environmental P₇ is abundant, PhoR remains in its repressor form, which functions as a phosphatase that keeps PhoB inactive (Wanner, 1990; Carmany et al., 2003). Under P₇ limitation, PhoR undergoes autophosphorylation to be converted to the activator form, which functions as a kinase that activates PhoB via phosphorylation (Wanner, 1990; Gao and Stock, 2013). The active PhoB (i.e. phosphorylated PhoB) subsequently binds to promoter regions of the genes controlled by the pho regulon to upregulate or suppress targeted gene transcription by interacting with the RNA polymerase sigma factor RpoD in the promoter regions of those genes (Makino et al., 1993;
Blanco et al., 2002). The specific DNA sequences, which are targeted by phosphorylated PhoB, are two 11-base pair direct-repeat sequences known as Pho boxes (Blanco et al., 2002). The consensus DNA sequences in the Pho boxes differ in different bacteria (Blanco et al., 2002; Yoshida et al., 2012; Sola-Landa et al., 2013). Beyond PhoP, assimilation, the pho regulon may additionally control several genes involved in nitrogen, carbon and iron metabolism (Santos-beneit, 2015; Millan-oropeza et al., 2020). Bioinformatic genome analysis together with transcriptional studies on the response to PhoP depletion has been used to identify novel members of the pho regulon (Baek and Lee, 2006; Lidbury et al., 2016; Ordóñez-Robles et al., 2017; Martinez-Castro et al., 2018). The heterogeneity in the occurrence of pho regulon members was observed at the species-specific level within the same genus. (Lidbury et al., 2016; Ordóñez-Robles et al., 2017; Lidbury et al., 2021).

In most cases, the following genes encoding phosphatase-type enzymes (responsible for the hydrolytic release of P from P-O containing P compounds) are upregulated in a pho regulon-dependent manner during PhoP starvation: phoA, phoX and phoD for ALPs; phoN for AP (in S. typhimurium); phoD for phospholipase; appA and phyC for phytases; glpQ and upgQ for glycero phosphodiester phosphodiesterases, and ushA for S’nucleotidase (Wanner, 1990; Kasahara et al., 1991; Russell D. Monsd et al., 2006; Santos-beneit, 2015; Lidbury et al., 2016; Martín et al., 2017; Grafe et al., 2018; Lidbury et al., 2017b). Exceptions include Streptomyces tsukubaensis whose phoA and phoD genes are not controlled by the pho regulon (Martínez-Castro et al., 2018). The pho regulon also regulates the phnCDEFHIJKLMNOP operon, which encodes several C-P lyase enzymes that catalyse the release of PhoP, by breaking C-P bonds (Stasi et al., 2019). In addition to phosphatase regulation by the pho regulon, a few non-pho regulated phosphatases have been reported. For instance, the phoC gene, which encodes a class A NSAP in Morganella morganii (Thaller et al., 1994), and at least three C-P lyases [phosphonoacetalddehyde (encoded by phnX), phosphonoacetaldehyde hydrolase (encoded by phnA), and phosphonopyruvate hydrolase (encoded by palA)] are pho regulon-independent (Quinn et al., 2007). The regulation of the phoC gene of M. morganii has not yet been elucidated. The phnX, phnA and palA genes are regulated by distinct transcriptional regulators encoded by lysR, phnR and palR genes respectively (Quinn et al., 2007).

Several PhoP transporter genes that are regulated positively by the pho regulon include the Phn system for phosphonate uptake (phnCDE) and the Ugp system for the uptake of glycero phosphodiesteras and G3P (ugpBAECQ) (Wanner, 1990; Santos-beneit, 2015). Unlike the Phn system, the recently identified aminoethylphosphate transporter AepP is PhoP-insensitive (Murphy et al., 2021) and thus is not regulated by the pho regulon. Other PhoP transporters such as GlpT, UhpT and PgtP systems, all of which belong to the OPA family by transporting their respective organophosphate via anion exchange with internal PhoP, are generally not regulated by the pho regulon (Elvin et al., 1985; Wanner, 1996; Lemieux et al., 2004b). Despite the similar transport mechanism of these OPA-type transporters, each is regulated by a distinct regulatory system.

In E. coli, the GlpT system, which is a G3P transporter encoded by the glpT gene is not controlled by the pho regulon (Larson et al., 1982; Wanner, 1996; Law et al., 2009). The glpT gene is a member of the glp regulon, which regulates the catabolism of glyceroL, G3P and glycero phosphodiesteras (Larson et al., 1992). The transcription of glpT and other genes in the glp regulon are regulated by the G3P regulon repressor (GlpR). The transcription of glpT and other genes in the glp regulon are regulated by the G3P regulon repressor (GlpR), encoded by the glpEGR operon in E. coli (Yang and Larson, 1998; Lemieux et al., 2004a; Escapa et al., 2013). GlpR repressor decreases the expression of the genes in the glp regulon by binding to the operators which are close to or overlapping with their promoter regions (Yang and Larson, 1998). The expression of GlpT is induced by extracellular G3P, which binds to the GlpR repressor to lower its binding affinity, alleviate the GlpR repression of the glpT gene and consequently increase the GlpT expression (Cozzarelli et al., 1968; Law et al., 2009). The expression of GlpT requires the presence of cyclic adenosine monophosphate (cAMP), which forms a complex with the cAMP receptor protein (CRP) and binds to the promoter region to activate the transcription of the glpT gene (Castañeda-García et al., 2013). The glpT gene as well as other genes in the glp regulon are additionally governed by catabolite repression through products of the glycero lipid biosynthetic pathway (Lemieux et al., 2004a). Under PhoP starvation conditions, G3P is mainly transported by the pho regulon-dependent Ugp system, leading to the increase in internal PhoP concentration (Xavier et al., 1995). A high level of internal PhoP will stimulate the uptake of G3P by the GlpT system, which takes up G3P by exchanging out PhoP, thus balancing the intracellular PhoP level (Xavier et al., 1995). Unlike in E. coli, the GlpT system in B. subtilis is encoded by the glpQT operon which is induced by PhoP depletion as well as glyceral (Antelmann et al., 2000; Lidbury et al., 2017b).

The UhpT transporter, which is encoded by the uhpT gene, mediates the uptake of hexose-6-phosphate (Sonna et al., 1988; Cattoir et al., 2020). The synthesis of UhpT transporter is tightly regulated by the products of
the uhpcABC regulatory genes: a response regulator UhpA, a histidine kinase UhpB and a receptor/transporter protein UhpC (Islandt and Kadner, 1993; Schwöppe et al., 2002; Västermark and Saier, 2014; Cattoir et al., 2020). The UhpB protein functions as an activator of UhpA, which regulates uhpt transcription (Weston and Kadner, 1988; Wright and Kadner, 2001). The UhpC protein has a high sequence similarity to the Uhpt transporter (Islandt and Kadner, 1993) and can serve as a G6P-specific transporter with a relatively low transport rate compared to UhpT (Västermark and Saier, 2014). The UhpC protein functions as a high-affinity G6P receptor that signals UhpB to undergo autophosphorylation and activate the UhpA regulator in the presence of external G6P (Wright and Kadner, 2001; Västermark and Saier, 2014; Cattoir et al., 2020). Thus, the expression of the Uhpt transporter is induced by extracellular G6P (Dietz and Heppel, 1971; Hoffer et al., 2001b; Castañeda-García et al., 2013, Cattoir et al., 2020). Similar to the GlpT system, the expression of the Uhpt transporter requires the cAMP-CRP complex to bind to the promoter site of the uhpt gene (Castañeda-García et al., 2013; Cattoir et al., 2020). The Uhpt transporter has been suggested to mediate the uptake of G6P as a carbon source, not a phosphate source (Hoffer et al., 2001b). Under Pi limitation, the pho regulon upregulates the production of phosphatases which can degrade G6P in the periplasm. Consequently, much higher concentrations of the extracellular G6P are required to induce the expression of Uhpt (Hoffer et al., 2001b).

In addition to transporting P_o, both GlpT and UhpT have also received considerable attention due to their ability to transport fosfomycin, an antibiotic commonly used to treat urinary tract and gastrointestinal infections (Santoro et al., 2011; Kurabayashi et al., 2015; Ballestero-Téllez et al., 2017; Hirakawa et al., 2018; Aghamali et al., 2019; Cattoir et al., 2020). Studies on fosfomycin susceptibility in enterohaemorrhagic E. coli found that the expression of glpT and uhpt genes are positively controlled by both CRP-cAMP and a global transcriptional regulator called FNR, which is activated under anoxic conditions (Kurabayashi et al., 2015; Kurabayashi et al., 2017).

In S. typhimurium, the Pgtp protein, which is encoded by the pgtp gene, is a specific transporter for PEP, 2PGA and 3PGA (Saier et al., 1975). The two proteins PgtB and PgtC are involved in the induction of the pgtp transcription by modulating the activity of PgtA in response to inducer binding (Jiang et al., 1988; Yang et al., 1988). The PgtA regulator is thought to function as a derepressor which becomes active under the inducing condition and subsequently facilitates the transcription of the pgtp gene (Jiang et al., 1988). Recently, Jiang et al. (2021) reported a newly identified protein named VrpA as a potential positive regulator of pgtp. In response to low glucose availability, the cAMP-CRP induces the transcription of vrpA and activates the expression of PgtP to upregulate 3PGA uptake (Jiang et al., 2021). Besides regulation of P_i or P_o acquisition, bacteria alter several intracellular processes to manage P allocation in response to different environmental P availability. Several soil bacteria remodel their lipid membranes to reduce the proportion of P-containing lipids by replacing phospholipid with glycolipid (Geiger et al., 1999; Lidbury et al., 2016). For instance, S. meliloti was reported to replace phospholipids with phosphate-free lipids (Geiger et al., 1999). Using proteomics analysis of soil Pseudomonas species, Lidbury et al. (2016) found that proteins involved in lipid remodelling (PlicP, DagK, OlsA, OlsB, Cfa and TauD) in the pho regulon had a higher expression when these species (P. putida, P. fluorescens and P. stutzeri) were grown under P_i limitation (Lidbury et al., 2016). In B. subtilis, P_i starvation initiates the synthesis of teichuronic acid, which is a phosphocontaining anionic polymer that replaces teichoic acid, a phosphate-rich anionic polymer in the cell wall (Allenby et al., 2005; Botella et al., 2011; Fritz and Mascher, 2014; Devine, 2018). Under this condition, the active PhoP represses the genes involved in teichoic acid synthesis such as tagAB and tagDEF but upregulates the genes involved in teichuronic acid (tauA-H operon) (Devine, 2018). The biosynthesis of nucleotides and nucleic acids is also affected by P availability. Phosphorus starvation leads to the accumulation of guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp), collectively called (p)ppGpp (Spira and Yagi, 1998; Sivapragasam et al., 2017). The (p)ppGpp molecules are the key signalling molecules modulating general metabolism and mediating the stringent response, the bacterial metabolic response to nutrient starvation and other stress (Hauryliuk et al., 2015). When P_i is depleted, the level of (p)ppGpp increases and triggers the stringent response to inhibit primary bacterial metabolism, including amino acid metabolism, carbohydrate metabolism, ion transport systems, and the synthesis of ribosomal RNA, and DNA replication, transcription and translation (Hauryliuk et al., 2015; Wu et al., 2020). In B. subtilis, the (p)ppGpp molecules directly inhibit...
DNA replication by inhibiting primase, an essential enzyme in the replication process (Wang et al., 2007). While the stringent response generally downregulates DNA and nucleotide synthesis (Traxler et al., 2008), it has been shown to promote the purine salvage pathway, which recycles purines, in S. coelicolor (Sivapragasam and Grove, 2016) and Agrobacterium fabrum (Sivapragasam et al., 2017). The functions of (p)ppGpp and the molecular mechanisms of (p)ppGpp-mediated regulation are complex and beyond the scope of this article. These topics are presented in detail in the review article by Hauryliuk et al. (2015).

**Future research directions**

In the previous sections, we have presented what is known regarding Pₐ cycling by soil-associated or relevant bacteria, from the biosynthesis of Pₐ compounds to the acquisition of P from Pₐ either via the activity of phosphatase enzymes in releasing Pₐ or by direct Pₐ assimilation with specific transporter systems, and the regulation involved in these processes depending on the Pₐ availability (Fig. 1). Despite the important role of bacteria in soil P cycling, there is limited understanding on species-specific diversity of phosphatases, Pₐ transporter systems and their regulation. For instance, bacterial orders such as Solibacterales, Acidobacterales and Actinomycetales, which showed significant roles in P cycling, remain poorly characterized (Bergkemper et al., 2016). Such lack of species-specific information on soil bacteria stems from the well-established fact that less than 1% of soil microorganisms are considered cultivable by standard cultivation methods (Rappé and Giovannoni, 2003; Vartoukian et al., 2010; Giagnoni et al., 2018). Thus, to this date, research on soil bacteria often relies on referencing model organisms such as E. coli and B. subtilis. However, some metabolic processes and regulatory mechanisms differ from species to species. For instance, the details of how the pho regulon senses Pₐ availability and regulates responses vary among different organisms (Santos-benet, 2015). The PgtP transporters, which are found in S. typhimurium are absent in the commonly studied E. coli K12 (Goldrick et al., 1988; Albermann et al., 2014). The regulation of phosphatase genes in S. tsukubaensis is considerably different from the model organism S. coelicolor (Martinez-Castro et al., 2018). Furthermore, few studies have addressed diesterase enzymes (Yao et al., 2018), despite the importance of these enzymes in the initial degradation of P polymers such as nucleic acids. The diversity of diesterases, the bacterial communities involved in their secretion and the soil conditions that can affect their production or reactivity remain to be elucidated. To establish a detailed understanding of bacterial processes in soil P cycling, more species-specific studies beyond the model organisms are needed. Metagenomics represents an important approach employed to gain information on uncultivated bacteria (Vartoukian et al., 2010). However, due to the small size of genomic libraries related to the genomes of the soil bacteria, there is still limited information regarding the species-dependent diversity of APs and phytases in soil bacterial communities. Therefore, new metagenomics studies on the updated genes in the database can be useful in acquiring information about the P-scavenging capabilities of the microbial communities present in soil environments, albeit resolving any insights on regulatory mechanisms would still be challenging with this approach. We identified the following research topics, which are imminent to improve our understanding of the role of soil bacteria in cycling Pₐ and provide insights into the potential biotechnological applications of these bacteria in sustainable agriculture:

- Study of Pₐ metabolism and regulations of P metabolism and P acquisition in representative pure cultures of soil bacteria to obtain information beyond those provided by the studies with E. coli and B. subtilis.
- Genomic, proteomic and exoproteomic studies of single and mixed cultures of soil bacteria under different P availability and P sources.
- Metagenomics studies on soil bacteria and Pₐ utilization under different soil environments, i.e. different pH, climate, land usage, P amendments, and so on.
- Studies on Pₐ metabolism and regulation by soil bacterial communities.
- Determination of stability and lifespan of different classes of phosphatases in response to different soil characteristics, such as different minerals and organic matter.
- Determination of kinetic parameters of soil-associated phosphatases and the influencing factors (metal cations, mineral surface chemistry, pH).
- Establishing curated databases for kinetics and stability parameters of soil phosphatases to establish accurate predictive models.
- Molecular modelling and atomic-scale studies on the interactions and reactivity of phosphatases with different mineral surfaces and organic matter complexes.
- Studies on the possible mechanistic role of bacterial chemotaxis towards P/Pₐ resources in the soil.

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