Phosphate-solubilizing fungi: Isolation, characterization, and impact on soil as potential biofertilizers (Postprint)

Authors: Rim WERHENI AMMERI, Yasmine OCHI, Maroua OUESLETI, Hassen ABDENNACEUR, Najla SADFI ZOUAOUI, Rim WERHENI AMMERI

Date: 2026-03-10T11:06:13+00:00

Abstract

The escalating global demand for sustainable agriculture necessitates the development of effective biological alternatives to conventional chemical fertilizers, particularly those addressing phosphorus (P) use efficiency. This study focused on the isolation and detailed characterization of phosphate-solubilizing fungi from soil or compost to evaluate their impact and potential for use as biofertilizers. Fungal isolation was performed using serial dilution from various sources, followed by molecular and morphological characterization to identify promising strains. Four strains were ultimately selected and identified using morphological, biochemical, and molecular techniques: Aspergillus flavus (CM1), Penicillium crustosum (C3), Penicillium fellutanum (C4), and Metarhizium robertsii (J1). The most active strain was initially tested in liquid and solid media supplemented with synthetic P (Ca3(PO4)2) and was evaluated by measuring fungal biomass and P titration. This strain demonstrated good growth and activity, supporting an optimal temperature of 25°C, a pH of 3, an ammonium concentration of 1.5 g/L, and a glucose addition of 25.0 g/L. The biofertilization potential of the selected strains was then comprehensively evaluated through controlled experiments, including the optimization of growing conditions, quanti fication of soluble P under hermetic storage in soil, and measurement of soil fungal populations to assess their impact. P transformation experiments conducted in hermetic jars showed that CM1 had the highest CO2 release (approximately 7115.30 mg CO2/100 g soil) and the highest soluble P levels at the final sampling time (78.85 mg/L), thus outperforming the other strains. Furthermore, in soil hermetic jars, CM1 (reaching up to 26$×$104 CFU (colony forming units)/g soil) and C4 significantly enhanced soil microbial activity and P bioavailability. These results clearly highlight the potential of the selected fungal strains as

biofertilizers to improve P availability and boost crop productivity in P-deficient soils.

Full Text

Preamble

J Arid Land (2026) 18(2): 339–352 doi: 10.1016/j.jaridl.2026.02.007; CSTR: 32276.14.JAL.20250361 Phosphate-solubilizing fungi: Isolation, characterization, and impact on soil as potential biofertilizers Rim WERHENI AMMERI1,2*, Yasmine OCHI1, Maroua OUESLETI1, Hassen ABDENNACEUR2, Najla SADFI ZOUAOUI1 1 Laboratory of Mycology, Pathologies and Biomarkers (LR16ES05), Faculty of Sciences of Tunis, University Tunis El Manar, Tunis 2092, Tunisia; 2 Laboratory of Treatment and Valorization of Water Rejects, Water Researches and Technologies Center, Borj-Cedria Technopark, University of Carthage, Soliman 8020, Tunisia Abstract: The escalating global demand for sustainable agriculture necessitates the development of effective biological alternatives to conventional chemical fertilizers, particularly those addressing phosphorus (P) use efficiency. This study focused on the isolation and detailed characterization of phosphate-solubilizing fungi from soil or compost to evaluate their impact and potential for use as biofertilizers. Fungal isolation was performed using serial dilution from various sources, followed by molecular and morphological characterization to identify promising strains. Four strains were ultimately selected and identified using morphological, biochemical, and molecular techniques: Aspergillus flavus (CM1), Penicillium crustosum (C3), Penicillium fellutanum (C4), and Metarhizium robertsii (J1). The most active strain was initially tested in liquid and solid media supplemented with synthetic P (Ca3(PO4)2) and was evaluated by measuring fungal biomass and P titration. This strain demonstrated good growth and activity, supporting an optimal temperature of 25°C, a pH of 3, an ammonium concentration of 1.5 g/L, and a glucose addition of 25.0 g/L. The biofertilization potential of the selected strains was then comprehensively evaluated through controlled experiments, including the optimization of growing conditions, quanti fication of soluble P under hermetic storage in soil, and measurement of soil fungal populations to assess their impact. P transformation experiments conducted in hermetic jars showed that CM1 had the highest CO2 release (approximately 7115.30 mg CO2/100 g soil) and the highest soluble P levels at the final sampling time (78.85 mg/L), thus outperforming the other strains. Furthermore, in soil hermetic jars, CM1 (reaching up to 26$×$104 CFU (colony forming units)/g soil) and C4 significantly enhanced soil microbial activity and P bioavailability. These results clearly highlight the potential of the selected fungal strains as biofertilizers to improve P availability and boost crop productivity in P-deficient soils.

Keywords: phosphorus bioavailability; biofertilizers; fungi; soil; sustainable agriculture Citation: Rim WERHENI AMMERI, Yasmine OCHI, Maroua

OUESLETI, Hassen ABDENNACEUR, Najla SADFI ZOUAOUI. 2026. Phosphate-solubilizing fungi: Isolation, characterization, and impact on soil as potential biofertilizers.

Journal of Arid Land, 18(2): 339–352. https://doi.org/10.1016/j.jaridl.2026.02.007; https://cstr.cn/32276.14.JAL.20250361

1 Introduction

Arid soils, often linked with poverty and land degradation, face severe challenges in nutrient content (Naorem et al., 2023) and microbial functioning (Li et al., 2024). Increasing aridity leads ∗Corresponding author: Rim WERHENI AMMERI (E-mail: rim.werheni@gmail.com) Received 2025-08-07; revised 2025-11-29; accepted 2025-12-12 http://jal.xjegi.com; https://www.keaipublishing.com/en/journals/journal-of-arid-land/ JOURNAL OF ARID LAND 2026 Vol. 18 No. 2 to lower soil fertility (Pratibha et al., 2023), reduced microbial diversity, and impaired nutrient cycling, which together threaten ecosystem productivity and resilience (Zhang et al., 2024).

Lower microbial biomass and simplified microbial networks result in decreased rates of carbon and nitrogen mineralization, enzyme activities, and overall soil functionality, which weakens nutrient cycling and soil health (Bogati and Walczak, 2022). Bacteria and fungi break down soil organic matter, converting complex compounds into simpler and plant-available nutrients. Fungi excel at decomposing tough materials like lignin and cellulose, while bacteria rapidly process simpler substrates (Wang et al., 2024). Bacteria and fungi are central to the transformation and cycling of phosphorus (P), nitrogen (N), and potassium (K) in soils, directly influencing soil fertility and plant productivity. Their activities drive nutrient availability through decomposition, solubilization, mineralization, and symbiotic interactions (Chen et al., 2024).

Bacteria (e.g., Bacillus, Pseudomonas, and Nocardioides) and fungi (e.g., Penicillium and mycorrhizal fungi) solubilize insoluble phosphates, making P available to plants. Genes like gcd and phoD in bacteria are crucial for P cycling, and their abundance is influenced by fertilization and soil pH (Luo et al., 2024).

Fungi, especially arbuscular mycorrhizal (AM) and saprotrophic species, play a vital role in transforming soil P from unavailable forms into plant-accessible nutrients. Their activities are crucial for soil fertility, plant growth, and sustainable agriculture, particularly in P-limited or degraded soils (Zhang et al., 2023). Many fungi (e.g., Penicillium, Aspergillus, Trichoderma, and Rhizopus) secrete organic acids (like oxalic and citric acid) that lower soil pH and dissolve mineral phosphates, releasing soluble P for plant uptake (Bononi et al., 2020). Fungi

transform soil P through organic acid secretion (Arias et al., 2023), enzymatic mineralization (Pang et al., 2024), mycorrhizal symbiosis (Wang et al., 2023), storage/release (Arias et al., 2023), and microbial interactions. These mechanisms are essential for maintaining soil fertility and plant nutrition, especially in P-limited environments (Wang et al., 2023).

Glucose, temperature, and pH are key environmental factors that significantly influence the ability of fungi to transform and solubilize P in soil. These factors affect fungal metabolism, enzyme production, and the efficiency of P release from insoluble sources. Glucose serves as an easily metabolizable carbon source, enhancing fungal growth and the production of organic acids (e.g., gluconic and citric acids) that lower pH and solubilize inorganic phosphates (Jin et al., 2024). Most phosphate-solubilizing fungi show optimal P transformation at temperatures between 25°C and 35°C. Phosphate solubilization is noticeable across a range of temperatures, but efficiency drops outside the optimal range due to reduced fungal metabolism and enzyme activity (Gaind, 2016). Fungal-mediated phosphate solubilization is strongly linked to a decrease in soil pH, primarily due to organic acid secretion. Many fungi can tolerate and function in acidic environments (pH in a range of 3–6), with phosphate solubilization often negatively correlated with pH (Zhang et al., 2018). Some fungi maintain biomass across a wide pH range, but phosphate-solubilizing efficiency and the type of P source can influence outcomes (Gaind, 2016).

Fungi, especially phosphate-solubilizing fungi (PSF) and arbuscular mycorrhizal fungi (AMF), are increasingly used as biofertilizers to enhance P availability and uptake in crops, offering a sustainable alternative to chemical fertilizers (Fu et al., 2024). Fungi used as biofertilizers are effective, eco-friendly tools for improving soil P transformation and plant nutrition. Their application supports sustainable agriculture by enhancing P availability, crop yields, and soil health while reducing dependence on chemical fertilizers (Luo et al., 2024). The objectives of the present study are: (1) to isolate and select fungal strains capable of converting insoluble soil P into plant-available forms; (2) to identify the selected strains using morphological and molecular techniques; and (3) to assess the efficiency of these strains in transforming P during incubation in sealed jars containing soil from arid areas.

2.1 Isolation, selection, and identification of phosphate-solubilizing fungi

Fungal strains were isolated from agricultural soils and composts. The soil collected from arid Rim WERHENI AMMERI et al.: Phosphate-solubilizing fungi: Isolation···soil was situated within the Chenchou perimeter, near El Hamma of Gabes, Tunisia (33°53′N, 10°07′E). The location is characterized by an arid climate, receiving an average annual precipitation of 191 mm. The annual average temperature is 24°C, with July being the warmest month of the year, reaching an average temperature of 32°C. The arid area near El Hamma of Gabes is characterized by xerophytic and halophytic vegetation (Cherif et al., 2015), with spon-

taneous species such as saltbush (Atriplex) and white wormwood (Artemisia herba-alba Asso). The cultivated date palm (Phoenix dactylifera L.) is central to oasis agriculture, creating a microclimate that supports diverse crops and ecological processes (Saadaoui et al., 2019).

The isolation was performed using the serial dilution method, in which 10 g of soil or compost was mixed with 90 mL of sterile distilled water to detach fungal spores and hyphae. The suspension was shaken at 150 r/m, and tenfold serial dilutions ranging from 10–1 to 10–4 were prepared. From each dilution, a 0.2-mL aliquot was plated on potato dextrose agar (PDA) supplemented with 50 mg/L of antibiotics (streptomycin) to inhibit bacterial growth (Hidri et al., 2021). We selected fungal isolates based on their ability to solubilize P in both liquid and solid media containing Ca3(PO4)2.

Fungal strains were inoculated onto Pikovskaya (PVK) agar plates (Nelofer et al., 2016) supplemented with glucose (10.0 g/L) and agar (18.0 g/L). Ca3(PO4)2 served as the sole insoluble P source. Following 7 d of incubation at 25°C, phosphate solubilization capacity was determined by the formation of a clear halo around the fungal colonies against the opaque medium (Souchie et al., 2007). Uninoculated PVK plates acted as negative controls. The solubilization index (SI) was calculated as the ratio of the total diameter (colony plus halo) to the colony diameter (Berraquero et al., 1976). We classified SI values as low (<2), medium (2–3), or high (>3) based on the criteria established by Silva Filho and Vidor (2000). All strains were tested in triplicate.

Soluble P quantification was performed following culture of fungal strains in a modified PVK liquid medium (Nelofer et al., 2016) using Ca3(PO4)2 as the sole insoluble P source, glucose (10.0 g/L), (NH)4)2SO4 (0.5 g/L), and yeast extract (0.5 g/L). Cultures were incubated for 7 d at 25°C under shaking (150 r/m) before centrifugation (8000 r/m at 4°C). The resulting supernatant was collected for analysis, while the fungal pellet was dried at 80°C for 24 h to determine dry cell mass. Soluble P (free orthophosphate) was quantified colorimetrically using the Murphy and Riley (1962) method, primarily relying on the formation of a blue-colored phospho-molybdenum complex via the reaction of molybdate and ascorbic acid. Additionally, the vanado-molybdate method was applied as a complementary technique.

2.2 Fungal identification

Fungal identification was conducted through a combination of macroscopic and microscopic morphological examination, following Samson et al. (2014), supplemented by biochemical and physiological characterization as described by Fossi et al. (2005) and Benaouida (2008). Genomic deoxyribonucleic acid (DNA) was extracted from 7-d fungal cultures grown on agar plates using the protocol by Liu et al. (2000). Molecular identification targeted the internal transcribed spacer (ITS) region, amplified with universal primers ITS4 (5′-CCTCCGCTTATTGATATGC-3′) and ITS5

(5′-GGAAGTAAAAGTCGTAACAAGG-3′) according to White et al. (1999). Sequences were compared against related taxa retrieved from the National Center for Biotechnology Information GenBank database using Jukes and Cantor (1969) model for phylogenetic analysis.

2.3 Effect of growing conditions on phosphate solubilization by the selected strain

The study examined the effects of temperature, glucose concentration, ammonium sulfate concentration, and medium pH on maximum phosphate solubilization in liquid PVK medium.

Experimental ranges were as follows: temperature (25°C and 35°C), glucose (5.0 and 25.0 g/L), ammonium sulfate (0.5 and 2.5 g/L), and pH (3 and 8). All treatments were released during 7-d incubation under shaking conditions (150 r/m). Each parameter was varied individually while keeping the others constant, following the methodology of Nelofer et al. (2016).

In February 2023, topsoil samples (0–20 cm depth) were collected from an arid area of Tunisia.

The samples were stored in sterilized bags at 4°C until further analysis. Soil pH and electrical JOURNAL OF ARID LAND 2026 Vol. 18 No. 2 conductivity (EC) were measured in soil-to-water suspensions at ratios of 1.0:2.5 and 1.0:5.0, respectively. Available P was extracted using the bicarbonate method described by Olsen and Sommers (1982). The organic carbon and organic matter content were determined using a modified Walkley–Black titration method. Particle size distribution was analyzed using a laser particle sizer (Analysette 22 NeXT, Fritsch, Idar-Oberstein, Germany). Finally, we measured the soil K2O (potassium oxide) content according to standard methods (APHA, 1998).

2.4 Evaluation of the efficiency of selected fungi in hermetic storage conditions

The efficiency of fungal strains in P mineralization was assessed via an anaerobic incubation assay conducted in 500 mL hermetically sealed glass jars (Werheni Ammeri et al., 2021). About 100.0 g aliquots of soil were triple-sterilized (120°C for 45 min) and adjusted to 75.00% of the soil' s field capacity before inoculation. Each jar was inoculated with a specific fungal strain ($×$104 CFU (colony forming units)/g soil). The inocula were prepared by centrifuging mycelial biomass (6000 r/m for 15 min) grown in potato dextrose broth (PDB) for 5–7 d. Treatments included supplementation with or without added Ca3(PO4)2. The jars were incubated at a constant temperature of 28°C. Phosphate solubilization, CO2 released, and fungal activity were monitored over a 23-d period.

The amount of CO2 released was measured daily during the incubation period. For measurement, a forced stream of CO2 free air was circulated through the flasks for 2 h and then bubbled into a 0.1-N NaOH solution to trap the displaced

CO2. The resulting sodium hydroxide solution was subsequently titrated with a 1.0-N HCl solution in the presence of 5 mL of 0.1 N BaCl2 and phenolphthalein as an indicator (Saidi et al., 2009). We used the titrimetric results to calculate the released CO2, based on the principle that (V0–V1)=1 mL of 0.1 N HCl, which corresponds to a release of 1.2 mg of C. In this equation, V0 (mL) is the volume of titrant needed to neutralize the initial amount of base in the control flask and V1 (mL) is the volume of titrant needed to neutralize the remaining excess base in the sample flask .The soil mineralizing activity (C), expressed as mg CO2/100 g soil, is calculated using the following formula:

C=(V0−V1)$×$1.2. Soil available P was determined using the Olsen method (Olsen and Sommers, 1982). Soil samples, first sieved by the 2-mm sieve, were extracted with Duval' s reagent. The mixture was stirred for 30 min and filtered. Concentrated H2SO4 was then added to the filtrate, followed by light agitation and 1 h of degassing. For final colorimetric quantification, 1 mL of Duval' s reagent and 10 mL of 1.00% ascorbic acid solution were added, and the sample was incubated in a water bath at 80°C for 45 min. The final optical density was measured using a spectrophotometer at 660 nm after cooling.

Fungi in the soil sample were enumerated using the serial dilution method plated onto potato dextrose agar (PDA) (Hidri et al., 2021). Quantification was performed by counting only plates containing discrete and countable colonies (Pane et al., 2022).

2.5 Statistical analysis

The experimental setup investigating biofertilization and irrigation with non-conventional water was illustrated using the BioRender tool. All experiments were conducted in triplicate, and the results are expressed as mean values±standard deviation (SD). To assess treatment effects, we performed an analysis of variance (ANOVA) on the measured P content and fungal population in soil under different treatments. Differences between treatments were evaluated using Duncan' s multiple range test at a significance level of $P<0.05$. All statistical analyses were conducted using the SPSS v.21.0 software (IBM Corp., IBM SPSS Statistics for Windows, Armonk, New York, USA).

3.1 Isolation, selection, and identification of phosphate-solubilizing fungi

Analysis of the four fungal strains Penicillium crustosum (C3), Penicillium fellutanum (C4), Rim WERHENI AMMERI et al.: Phosphate-solubilizing fungi: Isolation···Aspergillus flavus (CM1), and Metarhizium robertsii (J1) isolated from compost allowed for the characterization of their phosphate solubilization potential on PVK medium at 25°C (Table 1).

Strain CM1 proved to be the most efficient in the qualitative test, exhibiting the largest halo diameter ($4.00\pm0.01mm$), $indicating strong excretion of organic acids capable of releasing phosphat$

Figure 1

Figure 1: Figure 1

mg/L), strains CM1 and J1 displayed very similar values, highlighting the overall efficiency of these three isolates. Conversely, strain C3 showed the lowest performance, both in terms of qualitative solubilization and biomass production, making it the least suitable for further research. In conclusion, strain CM1 is considered the best candidate for in-depth study, thanks to its optimal combination of solubilization capacity and growth viability.

These results underscore the potential of specific fungal strains as efficient agents for biofertilization and sustainable P management in soil systems.

Macroscopic characterization of the selected fungal isolates enabled the differentiation of four distinct colony morphotypes. The strains C3, C4, CM1, and J1 displayed characteristic colony colors and textures: C3 and CM1 initially developed white colonies that later turned green and black, respectively; C4 formed brown colonies, while J1 exhibited a transition from white to dark pigmentation. The textures ranged from cottony C3, to powdery C4, to woolly CM1 and J1, and all four strains demonstrated rapid radial growth, exceeding 3 cm in diameter within 7 d of incubation, indicating their fast-growing nature.

Table 1 Selection of phosphate-solubilizing fungal strains in Pikovskaya (PVK) liquid and agar media supplemented with P at 25°C Strain Halo diameter (mm) P content (mg/L) Biomass of fungi (g) 3.00$±0.123.50±0.104.00±0.012.50±0.201.63±0.121.64±0.141.62±0.211 $J1, Metarhiziumrobertsii; C4, Penicilliumfellutanum; CM1, Aspergillusflavus; C3, Penicilliumcrustosum$ n=3.

Microscopic examination further supported species-level differentiation. Strain CM1 displayed globose vesicles with brown to black conidia bearing a rough, spherical morphology, characteristic of Aspergillus niger. Strain C4 showed singular, variably sized conidiophores with divergent phialides producing long chains of globose conidia, suggesting its affiliation with the Penicillium genus. In contrast, strains C3 and J1 exhibited hyphal and reproductive structures resembling those of the Trichoderma genus. Subsequent molecular identification confirmed the taxonomic identity of the four isolates, as illustrated in Figure 1

.

3.2 Influence of culture conditions on phosphate solubilization efficiency of selected fungal

strains The effect of various culture conditions (pH, temperature, ammonium, and glucose) on phosphate solubilization by the selected fungal strains is presented in Table 2 . Temperature showed a notable impact, with all four strains exhibiting increased solubilization at higher temperatures. Among them, C4 achieved the highest concentration of soluble P, approximately 370.37

($\pm$2.50)$mg/L, at 35°C, where assignificantly lower levels were observed across all strains at 25°C with a value 6.52(
3). In contrast, C3 recorded the lowest solubilization at alkaline pH with a value 7.61($\pm$\$0.98)
mg/L, reinforcing the notion that acidic environments favor the release of P, likely due to the enhanced secretion of organic acids or acid phosphatases.

Regarding the carbon source, particularly glucose, the fungal isolates responded distinctly to concentrations of 5.0 and 25.0 g/L. C3 displayed the highest solubilization capacity at the JOURNAL OF ARID LAND 2026 Vol. 18 No. 2 Fig. 1 Molecular evolutionary genetics analysis of fungal isolates. The number of 48, 56, 96, and 100 means bootstrap value, which indicates the strength of the evidence supporting the grouping of the isolates.

Table 2 Effects of temperature, pH, glucose, and ammonium on phosphorus (P) and pellet weight formation for fungal strains in PVK liquid medium Fungal strain Temperature (°C) Glucose (g/L) Ammonium (g/L) P (mg/L)
152.10\$$\pm$12.00300.72$\pm$12.308.64$\pm$2.309.52$\pm$1.0273.48$\pm$1.3655.89$\pm$2.3011.75$\pm$1.017.25$\pm$0.98 Pellet (g) 17.68$\pm$8.2
$Mean \pm SD; n = 3$. elevated glucose concentration, reaching 130.37($\pm$15.10)mg/L, indicating that increased carb
mg/L of soluble P, while J1 demonstrated the lowest output under the same conditions.

The analyzed sample exhibits a predominantly sandy texture, evidenced by the high percentage of sand (78.00%), resulting in a coarse soil that is highly draining but possesses low water and nutrient retention capacity. Chemically, the soil is near neutral (pH=7) and non-saline (EC= 1.27 mS/cm). Although soil organic matter (1.45%) and carbon (0.75%) levels are low, which is typical for sandy soils, the sample shows exceptional mineral fertility, with both P (121.132 mg/L) and potassium (K) (432.36 mg/L) contents classified as very high. In summary, the soil is chemically balanced but structurally poor in soil organic matter, yet it is highly enriched in major nutrients, minimizing the need for P and K fertilization.

3.3 Evaluation of the efficiency of selected fungi in hermetic storage conditions

Microbial respiration, quantified as CO2 emissions (mg CO2/100 g soil), showed a consistent Rim WERHENI AMMERI et al.: Phosphate-solubilizing fungi: Isolation···three-phase trend: the initial phase (1–5 d) displayed a sharp spike known as the "flush effect" , driven by soil rewetting and rapid mineralization of labile soil organic matter (Fig. 2 [FIGURE:2]). Following this, the intermediate phase (6–19 d) featured a gradual decline in emission, reflecting the depletion of easily degradable compounds and a shift toward the slower utilization of more recalcitrant carbon sources. Finally, the terminal phase (20–23 d) reached a plateau (exhaustion stage), indicating limited microbial degradation of remaining stable organic residues.

Among the assessed strains, CM1 exhibited the highest respiratory activity, recording a maximum CO2 emission of approximately 7115.30 mg CO2/100 g soil with P vs. 3856.30 mg CO2/100 g soil without P. This result reflects strong

metabolic activity and the potential to enhance soil organic matter turnover. The other strains (C3, C4, and J1) showed comparatively lower rates. This variable capacity highlights the potential of CM1 as an effective agent for improving soil biological functionality in biofertilization applications (Fig. 2).

Fig. 2 Daily CO2 release from soil inoculated with fungal strains C4, C3, J1, and CM1, without (a) or with (b) phosphorus (P) addition. CM1, Aspergillus flavus; C4, Penicillium fellutanum; J1, Metarhizium robertsii; C3, Penicillium crustosum.

As shown in Figure 3 [FIGURE:3], the fungal population increased in P-enriched soils. Strain CM1 consistently maintained the highest fungal density across all treatments, reaching up to the maximum of 26$×$104 CFU/g soil. These results highlight its strong adaptability and responsiveness to increased P availability. This substantial impact and its confirmed efficiency in P-transformation support the hypothesis of a synergistic interaction between fungal activity and P supplementation, reaffirming its potential as a promising biofertilizer candidate for P mobilization.

P contents in the soil varied substantially across treatments, ranging widely from 100.00 to 850.00 mg/L (Fig. 4 [FIGURE:4]). Treatments combining fungal inoculation with Ca3(PO4)2 supplementation significantly enhanced P availability, especially at the initial sampling time (T0). Furthermore, this addition enhanced P availability (above 100.00 mg P2O5), confirming the agronomic efficacy of these interventions. Ultimately, fungal strain CM1 proved the most effective phosphate solubilizer at the final sampling time (Tf), achieving soluble P content of approximately 78.85 mg/L.

3.4 Principal component analysis (PCA)

To summarize the variability observed in different treatments of arid soil microcosms, we performed PCA. The procedure allowed the principal components reported as regression factor scores (Fig. 5 [FIGURE:5]). The main component accounted for 90.33% of the total variance. In this study, the heat map displays the correlation matrix among key experimental variables, namely temperature at 25°C and 35°C, pH levels of 3 and 8, glucose concentrations of 5.0 and 25.0 g/L, and ammonium sulfate concentrations of 1.5 and 2.5 g/L, as illustrated in Figure 5a and b.

JOURNAL OF ARID LAND 2026 Vol. 18 No. 2 Fig. 3 Effect of P on fungal abundance in soil inoculated with strains C4, C3, J1, and CM1 with or without P. T0, initial sampling time, Tf, final sampling time; CFU, colony forming units. Different lowercase letters within the same treatment indicate significant differences among different strains at $P<0.05$ level. Bars are standard deviations.

Fig. 4 Variations in P content in soil amended with Ca3(PO4)2 under different treatments. Different lowercase letters within the same addition and the same sapling time indicate significant differences among different treatments at

P<0.05 level. Bars are standard deviations.

Additionally, the correlation matrix highlights meaningful associations among the variables, fungal density, soluble P, and CO2 evolution as a proxy for microbial respiration. A particularly strong positive correlation was detected between fungal density and soluble P concentration, suggesting that higher fungal populations are closely linked to enhanced phosphate solubilization.

An inverse correlation was observed between soluble P and CO2, potentially reflecting the temporal decline in microbial respiration following P mineralization or the differing metabolic demands associated with nutrient transformation versus respiration. These parameters likely enhance the metabolic activity and/or enzymatic production responsible for P release, suggesting that these parameters most favorably influence P bioavailability. Although these correlation patterns offer valuable insights into the interdependencies of abiotic and biotic factors influencing phosphate solubilization, it is important to interpret these findings within a broader experimental context. Further inferential statistical analyses are warranted to validate the observed relationships and account for confounding factors, as illustrated in Figure 5.

4 Discussion

P deficiency is a major constraint, limiting crop productivity in arid and semi-arid soils, where both low P bioavailability and poor microbial diversity restrict nutrient cycling and plant growth.

In these environments, the application of phosphate-solubilizing microorganisms represents a sustainable strategy to improve P nutrition by converting insoluble phosphate forms into plant- available orthophosphates. Among P-solubilizing microorganisms (PSMs), fungi have been reported to possess superior solubilization capacities compared with bacteria, primarily due to their ability to secrete a wide range of organic acids and P (Nelofer et al., 2016).

Rim WERHENI AMMERI et al.: Phosphate-solubilizing fungi: Isolation···Fig. 5 Principal component analysis (PCA) of fungal responses under different experimental conditions. (a), liquid medium; (b), soil condition. PC, principal component; T, temperature; Glu, glucose; NH4, ammonium sulfate.

The present study aimed to isolate and identify fungal strains with high potential for phosphate solubilization, initially under controlled laboratory conditions using both solid and liquid media, and subsequently in soil systems. The primary screening was conducted on solid agar medium supplemented with poorly soluble Ca3HPO4. In this medium, phosphate-solubilizing efficiency was assessed by measuring the diameter of solubilization halos surrounding fungal colonies, which serve as qualitative indicators of phosphate mobilization capacity. However, our findings revealed that halo formation on solid media is not a reliable criterion for effective selection. Many isolates that failed to form visible halos were later found to exhibit considerable phosphate solubilization activity in liq-

uid media. Consequently, we retained only four promising fungal strains based on their combined performance in solid and liquid assays. To verify their efficiency, we subsequently cultured these strains in PVK liquid medium enriched with Ca3HPO$_4$. Soluble P concentrations were quantified, and fungal biomass was measured to evaluate phosphate transformation efficiency, under protocols established in previous studies (Nelofer et al., 2016).

Among the selected strains, those belonging to the genera Aspergillus and Penicillium demonstrated notably high phosphate solubilization activity. This result aligns with existing literature, where Aspergillus species, such as A. niger and A. flavus, are consistently cited as efficient P-solubilizing fungi, and also for their enzymatic versatility and strong cellulolytic capabilities (Kitamoto et al., 1996; Fujita et al., 2002). Similarly, Penicillium species—including P. fellutanum and P. notatum—are recognized for their metabolic versatility, notably in their production of hydrolytic enzymes such as α-amylase, which may contribute indirectly to phosphate mobilization via enhanced soil organic matter decomposition (Kathiresan and Manivannan, 2006; Balkan and Ertan, 2007). This study highlights the limitations of halo-based qualitative screening on solid media and emphasizes the importance of incorporating quantitative evaluations in liquid culture for the reliable identification of high-performance P-solubilizing fungal strains suitable for biofertilization in arid agroecosystems. Fungal genera such as JOURNAL OF ARID LAND 2026 Vol. 18 No. 2 Trichoderma (Sheir-Neiss and Montenecourt, 1984; Nogawa et al., 2001), alongside the previously mentioned Aspergillus and Penicillium, are widely recognized for their ecological adaptability and significant contributions to soil organic matter decomposition and nutrient cycling through both cellulolytic and phosphatase-mediated activities. Extensive research has demonstrated the efficacy of these groups in mobilizing insoluble P forms from soil matrices (Achal et al., 2007; Vassilev et al., 2007; Xiao et al., 2018; Fan et al., 2020).

The process of phosphate solubilization is not only solely dependent on fungal genetics, but also significantly influenced by environmental and nutritional factors such as pH, temperature, and the availability of carbon and nitrogen sources. Aspergillus spp. exhibit optimal growth and metabolic activity within a temperature range of 30°C–35°C (Leong et al., 2006), while the enzymatic machinery responsible for phosphate solubilization, particularly acid phosphatases, shows peak activity near 29°C (Jena et al., 2014). The present study, which recorded the highest levels of soluble P at approximately 30°C, corroborates these findings, affirming that optimal solubilization aligns closely with conditions favoring both fungal proliferation and enzyme expression.

Carbon availability, particularly as glucose, plays a pivotal role in regulating fungal metabolic pathways, including organic acid production—an essential mechanism for phosphate mobilization.

While literature reports considerable variation in optimal glucose concentrations among Aspergillus strains—ranging from 10.0 g/L (Gokhale et al., 1991) to 100.0 g/L (Khurshid et al., 2013)—our findings identify 25.0 g/L as the most effective

concentration for promoting phosphate solubilization. However, it is noteworthy that elevated glucose levels can suppress the production of certain catabolic enzymes via carbon catabolite repression, a phenomenon previously observed in filamentous fungi (Gokhale et al., 1991).

Ammonium sulfate was selected as the primary nitrogen source in this study due to its economic feasibility compared with complex organic sources such as yeast extract. Nitrogen concentrations ranging from 1.0 to 5.0 g/L were assessed, with 2.5 g/L emerging as the optimal dose for phosphate solubilization, consistent with earlier reports (Dixon-Hardy et al., 1998; Seshadri et al., 2004; Jena et al., 2014). This result supports the assertion that moderate levels of ammonium nitrogen enhance microbial metabolism and acidification processes that are conducive to phosphate release.

Finally, it is essential to recognize that while phosphate supplementation is often employed to boost P availability, excessive application—particularly in systems amended with compost—may lead to P saturation, posing environmental risks such as runoff and eutrophication (Lompo et al., 2009). Therefore, careful calibration of both microbial inoculation and nutrient supplementation strategies is imperative to achieve agronomically beneficial and ecologically sustainable outcomes.

The application of PSMs represents a promising strategy to improve P bioavailability in soils, especially in areas where P is a limiting factor for plant growth. These microorganisms play a central role in modulating soil P dynamics by transforming insoluble P compounds into forms that are readily available for plant uptake (Islam and Hossain, 2012; Kafle et al., 2019). PSMs employ a range of mechanisms—most notably the secretion of organic acids and extracellular enzymes such as phosphatases—to mediate this transformation (Sarr et al., 2020; Zúñiga-Silgado et al., 2020).

A wide array of organic acids has been identified as key agents in phosphate solubilization, including glycolic, 2-ketogluconic, acetic, citric, propionic, succinic, tartaric, formic, fumaric, lactic, malic, butyric, gluconic, valeric, oxalic, and citric acids (Zhu et al., 2012; Jog et al., 2014; Mehta et al., 2015; Yadav et al., 2017). These organic acids chelate metal cations (e.g., Ca2+, Fe3+, and Al3+), thereby releasing phosphate ions into the soil solution. The treatments investigated in the current study facilitated the mobilization of P compounds through interactions with iron and aluminum oxides and soil organic matter, ultimately enhancing their solubility. Specifically, P mobilized from Ca3(PO4)2 became bioavailable, as previously reported by Lompo et al. (2009).

Additionally, the surplus P introduced into the soil, especially in the presence of phosphate amendments, significantly influenced the assimilation of carbon and nitrogen by soil microbial Rim WERHENI AMMERI et al.: Phosphate-solubilizing fungi: Isolation···communities (Lompo et al., 2009).

The impact of P enrichment on microbial activity is further supported by studies on P removal systems. Mulkerrins et al. (2004) emphasized that most bi-

ological P removal systems have been developed for soils with low P content. Sudiana et al. (1999) further suggested that limited phosphate availability may constrain the growth and functionality of phosphate-accumulating organisms, thereby reducing the efficacy of bioremediation or nutrient cycling strategies in such systems.

Biological soil respiration, often monitored through CO2 emission rates, provides an important indicator of microbial activity and substrate utilization efficiency. The daily emission of CO2 varies considerably during incubation and is closely related to the treatments applied (Lompo et al., 2009). According to Dilly (2005), CO2 concentrations in cultivated soils typically range between 0.50 and 10.00 mg CO2/100 g soil. Elevated CO2 emissions may reflect poor substrate quality or inefficient microbial metabolism (Fliebach et al., 2007), whereas moderate to high emissions under nutrient-enriched conditions are generally associated with heightened microbial activity and effective nutrient cycling.

Fungi, in particular, exhibit many beneficial traits, such as mineral solubilization, antagonism against plant pathogens, and secondary metabolite production, which make them effective plant growth-promoting organisms when associated with the rhizosphere (Khan et al., 2010). The integration of such fungal strains into biofertilization regimes has emerged as a sustainable pathway to enhance agricultural productivity and to minimize the dependence on synthetic inputs.

As emphasized by Reis et al. (2021), the accessibility and large-scale deployment of microbial bio-inputs, including PSMs, are crucial to advancing sustainable agriculture. The use of fungi isolated from compost as biofertilizers for P soil enrichment is gaining attention due to their ability to solubilize phosphate and promote plant growth. However, the literature provides limited direct evidence on standardized toxicity testing for these fungal strains before their application in agriculture (Lucchetta, 2025). Despite the demonstrated benefits, there is a notable gap in the literature regarding formal toxicity testing of these fungi prior to their use as biofertilizers. Most studies focus on their efficacy in promoting plant growth and soil health, but do not report on toxicity assays for non-target organisms, humans, or environments (Mącik et al., 2020). Some research notes the presence of potentially pathogenic fungi in soil, but also observes that beneficial strains can suppress these pathogens (Timofeeva et al., 2022). For example, the application of beneficial bacteria and fungi reduced the abundance of known pathogenic fungi, suggesting a possible indirect safety benefit (Yang et al., 2025).

Nevertheless, the global availability of microbial strains, particularly for plant protection products, remains limited due to challenges in the selection and commercialization of effective strains. Many candidates exhibit strong potential under in vitro conditions but fail to deliver consistent results in field applications, underscoring the need for more robust screening protocols that consider both laboratory performance and agroecological adaptability.

5 Conclusions

The valorization of phosphate-solubilizing fungi represents a promising, sustainable strategy to mitigate the reliance on conventional chemical inputs in agriculture. This study notably validates the dual functional potential of specific fungal strains, particularly CM1 and C4, confirming their roles both as effective biofertilizers and active agents for soil rehabilitation. These selected strains exhibit a high capacity for phosphate solubilization, successfully converting insoluble compounds into plant-available forms, which directly contributes to addressing nutrient limitations in the soil.

Crucially, the application of these microbial bio-inputs led to a significant enhancement in the growth and productivity of plant in our trials, thereby validating their efficacy and potential for practical agricultural deployment. Finally, the integrated evaluation model established in this work offers a robust and reliable framework for the targeted screening of similar microbial agents, positioning this research as a foundation for developing effective microbial interventions in sustainable agricultural practices.

JOURNAL OF ARID LAND 2026 Vol. 18 No. 2 Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the team at the Laboratory of Mycology, Pathologies and Biomarkers, Faculty of Sciences of Tunis, University Tunis El Manar, Tunisa.

Author contributions Conceptualization: Rim WERHENI AMMERI; Methodology: Rim WERHENI AMMERI; Yasmine OCHI, Maroua OUESLETI; Writing - original draft preparation: Rim WERHENI AMMERI, Hassen ABDENNACEUR, Najla SADFI ZOUAOUI. All authors approved the manuscript.

References

Achal V, Savant V V, Reddy M S. 2007. Phosphate solubilization by a wild type strain and UV-induced mutants of Aspergillus tubingensis. Soil Biology and Biochemistry, 39(2): 695–699.

APHA (American Public Health Association). 1998. Standard Methods for the Examination of Water and Wastewater (20th ed.).

Washington D.C.: American Public Health Association.

Arias R, Abarca G, Del Carmen Perea Rojas Y, et al. 2023. Selection and characterization of phosphate-solubilizing fungi and their effects on coffee plantations. Plants, 12(19): 3395, doi: 10.3390/plants12193395.

Balkan B, Ertan F. 2007. Production of α-amylase from Penicillium chrysogenum under solid-state fermentation by using some agricultural by-products. Food Technology and Biotechnology, 45(4): 439–442.

Benaouida K. 2008. Study of alpha-amylase from yeasts isolated from an extreme ecosystem (soil surrounding thermal springs) and cultivated on a whey-based medium. MSc Thesis. Constantine: Mentouri University. (in French) Berraquero F R, Baya A M, Cormenzana A R. 1976. Establishing indicators to study phosphate solubilization by soil bacteria.

Ars Pharmceutica, 17(4): 399–406. (in Spanish) Bogati K, Walczak M. 2022. The impact of drought stress on soil microbial community, enzyme activities and plants. Agronomy, 12(1): 189, doi: 10.3390/agronomy12010189.

Bononi L, Chiaramonte J, Pansa C, et al. 2020. Phosphorus-solubilizing Trichoderma spp. from Amazon soils improve soybean plant growth. Scientific Reports, 10: 2858, doi: 10.1038/s41598-020-59793-8.

Chen J, Xu H, Seven J, et al. 2024. Microbial phosphorus recycling in soil by intra- and extra-cellular mechanisms. ISME Communications, 3: 135, doi: 10.1038/s43705-023-00340-7.

Cherif H, Marasco R, Rolli E, et al. 2015. Oasis desert farming selects environment-specific date palm root endophytic communities and cultivable bacteria that promote resistance to drought. Environmental Microbiology Reports, 7(4): 668–678.

Dilly O. 2005. Microbial energetics in soils. In: Buscot F, Varma A. Microorganisms in Soils: Roles in Genesis and Functions.

Heidelberg: Springer, 123–138. Dixon-Hardy J E, Karamushka V I, Gruzina T G, et al. 1998. Influence of the carbon, nitrogen and phosphorus source on the solubilization of insoluble metal compounds by Aspergillus niger. Mycological Research, 102(9): 1050–1054.

Fan D W, Jing Y J, Zhu Y L, et al. 2020. Toluene induces hormetic response of soil alkaline phosphatase and the potential enzyme kinetic mechanism. Ecotoxicology and Environmental Safety, 206: 111123, doi: 10.1016/j.ecoenv.2020.111123.

Fliebach A, Oberholzer H R, Gunst L, et al. 2007. Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agriculture, Ecosystems and Environment, 118(1–4): 273–284.

Fossi B T, Tavea F, Ndjouenkeu R. 2005. Production and partial characterization of a thermostable alpha amylase from ascomycete yeast strain isolated from starchy soil. African Journal of Biotechnology, 4(1): 14–18.

Fu S F, Balasubramanian V, Chen C L, et al. 2024. The phosphate-solubilising fungi in sustainable agriculture: Unleashing the potential of fungal biofertilisers for plant growth. Folia Microbiologica, 69(4): 697–712.

Fujita M, Ike M, Kusunoki K, et al. 2002. Removal of color and estrogenic substances by fungal reactor equipped with ultrafiltration unit. Water Science and Technology: Water Supply, 2(5-6): 353-358.

Gaind S. 2016. Phosphate dissolving fungi: Mechanism and application in alleviation of salt stress in wheat. Microbiological Research, 193: 94-102.

Gokhale M, Holmes W, Kopser A, et al. 1991. Building and using a highly parallel programmable logic array. Computer, 24(1):

Hidri Y, Hibar K, Bchir A, et al. 2021. Changes in the microbial properties of olive cultivated soils under short, medium and Rim WERHENI AMMERI et al.: Phosphate-solubilizing fungi: Isolation···long-term irrigation with treated wastewater. Asian Soil Research Journal, 5(1): 1-20.

Islam M T, Hossain M M. 2012. Plant probiotics in phosphorus nutrition in crops, with special reference to rice. In: Maheshwari D K. Bacteria in Agrobiology, Plant Probiotics. Heidelberg: Springer, 325-363.

Jena A B, Goldman D, Weaver L, et al. 2014. Opioid prescribing by multiple providers in Medicare: Retrospective observational study of insurance claims. BMJ, 348: g1393, doi: 10.1136/bmj.g1393.

Jin Z X, Wang G W, George T, et al. 2024. Potential role of sugars in the hyphosphere of arbuscular mycorrhizal fungi to enhance organic phosphorus mobilization. Journal of Fungi, 10(3): 226, doi: 10.3390/jof10030226.

Jog R, Pandya M, Nareshkumar G, et al. 2014. Mechanism of phosphate solubilization and antifungal activity of Streptomyces spp. isolated from wheat roots and rhizosphere and their application in improving plant growth. Microbiology, 160(4):

Jukes T H, Cantor C R. 1969. Evolution of protein molecules. Mammalian Protein Metabolism, 3: 121-132.

Kafle A, Cope K R, Raths R, et al. 2019. Harnessing soil microbes to improve plant phosphate efficiency in cropping systems.

Agronomy, 9(3): 127, doi: 10.3390/agronomy9030127.

Kathiresan K, Manivannan S. 2006. Amylase production by Penicillium fellutanum isolated from mangrove rhizosphere soil.

African Journal of Biotechnology, 7(5): 1-4. Khan M S, Zaidi A, Ahemad M, et al. 2010. Plant growth promotion by phosphate solubilizing fungi-current perspective.

Archives of Agronomy and Soil Science, 56(1): 73-98.

Khurshid H, Li W F, Chandra S, et al. 2013. Mechanism and controlled growth of shape and size variant core/shell FeO/Fe3O4 nanoparticles. Nanoscale, 5(17): 7942-7952.

Kitamoto N, Go M, Shibayama T, et al. 1996. Molecular cloning, purification and characterization of two endo-1, 4-β-glucanases from Aspergillus oryzae KBN616. Applied Microbiology and Biotechnology, 46(5-6): 538-544.

Leong S L L, Hocking A D, Pitt J I, et al. 2006. Australian research on ochratoxigenic fungi and ochratoxin A. International Journal of Food Microbiology, 111(Suppl. 1): 10-17.

Li Y, Jiang L M, Yuan H F, et al. 2024. The impact of artificial afforestation on the soil microbial community and function in desertified areas of NW China. Forests, 15(7): 1140, doi: 10.3390/f15071140.

Liu D, Coloe S, Baird R, et al. 2000. Rapid mini-preparation of fungal DNA for PCR. Journal of Clinical Microbiology, 38(1):

Lompo F, Segda Z, Gnankambary Z, et al. 2009. Effect of natural phosphate on the quality and biodegradation of corn straw compost. Tropicultura, 27(2): 105-109. (in French) Lucchetta M.

2025. Crisis-proofing

[2025-08-01]. https://www.unive.it/web/fileadmin/user_{upload}/dipartimenti/DEC/doc/Pubblicazioni_{sc
{{{DSE}}}{{lucchetta}}}*{{{08}}}*{{25}}}.pdf. banks. Department heterogeneous Economics.

Luo D, Shi J, Li M, et al. 2024. Consortium of phosphorus-solubilizing bacteria promotes maize growth and changes the microbial community composition of rhizosphere soil. Agronomy, 14(7): 1535, doi: 10.3390/agronomy14071535.

Mącik M, Gryta A, Frąc M. 2020. Chapter two-Biofertilizers in agriculture: An overview on concepts, strategies and effects on soil microorganisms. In: Sparks D L. Advances in Agronomy. New York: Academic Press, 31-87.

Mehta R A, Stiénon M, Xu P. 2015. The Atiyah class of a dg-vector bundle. Comptes Rendus Mathematique, 353(4): 357-362.

Mulkerrins D, O' Connor E, Lawlee B, et al. 2004. Assessing the feasibility of achieving biological nutrient removal from wastewater at an Irish food processing factory. Bioresource Technology, 91(2): 207-214.

Murphy J, Riley J P. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta, 27: 31-36.

Naorem A, Jayaraman S, Dang Y P, et al. 2023. Soil constraints in an arid environment-challenges, prospects, and implications.

Agronomy, 13(1): 220, doi: 10.3390/agronomy13010220.

Nelofer R, Syed Q, Nadeem M, et al. 2016. Isolation of phosphorus-solubilizing fungus from soil to supplement biofertilizer.

Arabian Journal for Science and Engineering, 41: 2131–2138.

Nogawa T, Kamano Y, Yamashita A, et al. 2001. Isolation and structure of five new cancer cell growth inhibitory bufadienolides from the Chinese traditional drug Ch' an Su. Journal of Natural Products, 64(9): 1148–1152.

Olsen S R, Sommers L E. 1982. Phosphorus. In: Page A L. Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties. Madison: American Society of Agronomy, 403–430.

Pane R D P, Farrasati R, Darlan N H, et al. 2022. Study of soil bacteria and fungi population in oil palm with big hole planting system. Berkala Penelitian Hayati, 27(2): 91–97.

Pang F, Li Q, Solanki M K, et al. 2024. Soil phosphorus transformation and plant uptake driven by phosphate-solubilizing microorganisms. Frontiers in Microbiology, 15: 1383813, doi: 10.3389/fmicb.2024.1383813.

Pratibha G, Manjunath M, Raju B, et al. 2023. Soil bacterial community structure and functioning in a long-term conservation agriculture experiment under semi-arid rainfed production system. Frontiers in Microbiology, 14: 1102682, doi:

JOURNAL OF ARID LAND 2026 Vol. 18 No. 2 10.3389/fmicb.2023.1102682.

Reis B O, Prakki A, Stavroullakis A T, et al. 2021. Analysis of permeability and biological properties of dentin treated with experimental bioactive glasses. Journal of Dentistry, 111: 103719, doi: 10.1016/j.jdent.2021.103719.

Saadaoui I, Sedky R, Rasheed R, et al. 2019. Assessment of the algae-based biofertilizer influence on date palm (Phoenix dactylifera L.) cultivation. Journal of Applied Phycology, 31: 457–463.

Saidi Y, Finka A, Muriset M. 2009. The heat shock response in moss plants is regulated by specific calcium-permeable channels in the plasma membrane. The Plant Cell, 21(9): 2829–2843.

Samson R A, Visagie C M, Houbraken J, et al. 2014. Phylogeny, identification and nomenclature of the genus Aspergillus.

Studies in Mycology, 78(1): 141–173. Sarr P S, Tibiri E B, Fukuda M, et al. 2020. Phosphate-solubilizing fungi and alkaline phosphatase trigger the P solubilization during the co-composting of sorghum straw residues with Burkina Faso phosphate rock. Frontiers in Environmental Science, 8: 559195, doi: 10.3389/fenvs.2020.559195.

Seshadri S, Ignacimuthu S, Lakshminarasimhan C. 2004. Effect of nitrogen and carbon sources on the inorganic phosphate solubilization by different Aspergillus niger strains. Chemical Engineering Communications, 191(8): 1043–1052.

Sheir-Neiss G, Montenecourt B S. 1984. Characterization of the secreted cellulases of Trichoderma reesei wild type and mutants during controlled fermenta-

tions. Applied Microbiology and Biotechnology, 20: 46–53.

Silva Filho G N, Vidor C. 2000. Phosphate solubilization by microorganisms in the presence of different carbon sources.

Brazilian Journal of Soil Science, 24(2): 311–319.

Souchie E L, Barea Navarro J M, Saggin-Júnior O J, et al. 2007. Indolacetic acid production by P-solubilizing microorganisms and interaction with arbuscular mycorrhizal fungi. Acta Scientiarum Biological Sciences, 29(3): 315–320.

Sudiana I M, Mino T, Satoh H, et al. 1999. Metabolism of enhanced biological phosphorus removal and non-enhanced biological phosphorus removal sludge with acetate and glucose as carbon source. Water Science and Technology, 39(6):

Timofeeva A M, Galyamova M R, Sedykh S E. 2022. Bacterial siderophores: Classification, biosynthesis, perspectives of use in agriculture. Plants, 11(22): 3065, doi: 10.3390/plants11223065.

Vassilev A, Hadid H B, Botton V, et al. 2007. Experimental and numerical studies on the topology of gas/liquid interfaces. In:

CFM 2007–8th Conference French of Mechanics. Grenoble: Association of French Mechanics, 27–31. (in French) Wang G W, Jin Z X, George T, et al. 2023. Arbuscular mycorrhizal fungi enhance plant phosphorus uptake through stimulating hyphosphere soil microbiome functional profiles for phosphorus turnover. New Phytologist, 238(6): 2578–2593.

Wang X L, Chi Y K, Song S Z. 2024. Important soil microbiota's effects on plants and soils: A comprehensive 30-year systematic literature review. Frontiers in Microbiology, 15: 1347745, doi: 10.3389/fmicb.2024.1347745.

Werheni Ammeri R, Kraiem K, Riahi K, et al. 2021. Removal of pentachlorophenol from contaminated wastewater using phytoremediation and bioaugmentation processes. Water Science and Technology, 84(10–11): 3091–3103.

White R J, Ghez A M, Reid I N, et al. 1999. A test of pre-main-sequence evolutionary models across the stellar/substellar boundary based on spectra of the young quadruple GG Tauri. The Astrophysical Journal, 520(2): 811–821.

Xiao W, Chen X, Jing X, et al. 2018. A meta-analysis of soil extracellular enzyme activities in response to global change. Soil Biology and Biochemistry, 123: 21–32.

Yadav G S, Babu S, Meena R S, et al. 2017. Effects of godawariphos gold and single super phosphate on groundnut (Arachis hypogaea) productivity, phosphorus uptake, phosphorus use efficiency and economics. The Indian Journal of Agricultural Sciences, 87(9): 1165–1169.

Yang B, Yang S, Wang X M, et al. 2025. Inhibition of RNase to attenuate fungal-manipulated rhizosphere microbiome and diseases. Advanced Science, 12(40), e03146, doi: 10.1002/advs.202503146.

Zhang C, Lei S L, Wu H Y, et al. 2024. Simplified microbial network reduced microbial structure stability and soil functionality in alpine grassland along a natural aridity gradient. Soil Biology and Biochemistry, 191: 109366, doi: 10.1016/j.soilbio.

Zhang X, Rajendran A, Grimm S, et al. 2023. Screening of calcium- and iron-targeted phosphorus solubilizing fungi for agriculture production. Rhizosphere, 26: 100689, doi: 10.1016/j.rhisph.2023.100689.

Zhang Y, Chen F S, Wu X Q, et al. 2018. Isolation and characterization of two phosphate-solubilizing fungi from rhizosphere soil of moso bamboo and their functional capacities when exposed to different phosphorus sources and pH environments.

PLoS ONE, 13(7): e0199625, doi: 10.1371/journal.pone.0199625.

Zhu X K, Li C Y, Jiang Z Q, et al. 2012. Responses of phosphorus use efficiency, grain yield, and quality to phosphorus application amount of weak-gluten wheat. Journal of Integrative Agriculture, 11(7): 1103–1110.

Zúñiga-Silgado D, Rivera-Leyva J C, Coleman J J, et al. 2020. Soil type affects organic acid production and phosphorus solubilization efficiency mediated by several native fungal strains from Mexico. Microorganisms, 8(9): 1337, doi: 10.3390/microorganisms8091337.