International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Optimization of P Sol ubilization Efficacy of Fungi Obtained from Mangrove Ecosystem

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Abstract

The current research study aims to optimize and assess phosphate solubilizing activity by fungal isolates derived from the mangrove ecosystem under a variety of environmental and nutritional circumstances. Fungal strains PSF-4 and PSF-6 were evaluated in Pikovskaya's medium under various phosphorus, nitrogen, and carbon sources, as well as pH levels, incubation times, and temperatures. PSF-4 had the greatest phosphate solubilization of all the parameters examined. Maximum solubility was achieved working with tricalcium phosphate 450 µg/ml, ammonium sulfate 390 µg/ml, and glucose 420 µg/ml as phosphorus, nitrogen, and carbon sources, respectively. Optimal environmental conditions were pH 6 460 µg/ml, 14 days of incubation 470 µg/ml, and 30°C temperature 445 µg/ml. PSF-6 had a lower activity level. This is the first thorough investigation on improving phosphate solubility in fungal isolates from a mangrove habitat. The results provide information on the effectiveness and adaptability of mangrove-derived fungus, showing their potential as eco-friendly biofertilizers in nutrient-deficient and saline-prone areas.

Keywords: Biofertilizer, Biogeochemical processes, Phosphate solubilization, Mangrove fungi, Tricalcium phosphate, Soil fertility, Sustainable agriculture, Plant growth-promoting fungi, Mangrove ecosystem, Sustainable agriculture, Phosphate bioavailability

Abbreviations: °C - Degrees Celsius, µg/ml - Micrograms per milliliter, NBRIP - National botanical research institute's phosphate, pH - Potential of hydrogen, PSF - Phosphate solubilizing fungus, PSM - Phosphate solubilizing microorganism, PVK - Pikovskaya's Medium, TCP - Tricalcium phosphate

Introduction

Phosphorus (P) represents a vital macronutrient for plants.crucial for energy transfer via ATP, nucleic acid and phospholipid synthesis, enzyme activation, and overall growth [1]. Despite its abundance in the soils, it is predominantly present in forms unavailable to plants due to low solubility, bound with calcium, aluminum, and iron, therefore limiting its utilization by plant, especially in tropical and subtropical regions [2]. This scarcity makes phosphorus a key limiting nutrient. While synthetic phosphate fertilizers are commonly used to address this deficiency, they are inefficient, with most of the applied phosphorus

becoming immobilized in the soil [3]. Overuse of chemical fertilizers also contributes to environmental issues like eutrophication, soil degradation, and water contamination [4].

Phosphate-solubilizing microorganisms (PSMs), especially phosphate-solubilizing fungi (PSF), Utilize an environmentally sustainable technique to increase available phosphorus for plant growth [5].Phosphate-solubilizing fungi enhance phosphorus availability by dissolving insoluble phosphates into soluble ions. accessible to plants via producing organic acids like gluconic, citric, oxalic, and lactic acids, thereby reducing soil pH and release phosphate ions [6]. PSF also secrete phosphatase enzymes that further break down organic phosphorus, enhancing its availability [7]. Fungi have distinct advantages over bacteria as P solubilizers due to their filamentous structure, which allows better soil penetration, and their potential to generate a wider spectrum of organic acids, boosting solubilization [8]. Fungal genera including *Aspergillus spp, Fusarium spp, Penicillium spp* and *Trichoderma spp* have shown promise by improving plant productivity and fertility of soil making them valuable in supporting environmentally friendly agriculture [9].

As agricultural practices evolve toward more sustainable methods, there has been an increased interest in exploring phosphate-solubilizing fungi from extreme and underexplored environments, such as the mangrove ecosystem [10]. Mangroves, which serve as an interface between terrestrial and marine ecosystems, present a unique and challenging environment for microorganisms because of their high saline conditions, Changing soil water regimes from tidal movements, low soil oxygen, and the breakdown of organic materials from plant residues [11]. These harsh conditions create selective pressure, resulting in specialized, stress-tolerant microorganisms, including fungi [12]. Mangrove ecosystems could harbor novel PSF strains with unique traits that allow them to function effectively under saline, acidic, or nutrient-deficient conditions [13].

Although the potential of PSF from mangrove ecosystems is promising, optimizing their phosphate-solubilization efficiency requires understanding the key factors that influence this process [14]. Phosphate solubilization is a dynamic process influenced by several environmental and nutritional conditions, involving temperature, pH, incubation period, in addition to the diversity of nitrogen and phosphorus sources available [15]. For instance, organic acid production by fungi is often pH-dependent, meaning that the solubility of different phosphorus sources can vary according to the acidity of the medium [16]. Similarly, the type of nitrogen available (such as ammonium, nitrate, or organic nitrogen) has a significant impact on fungal metabolism and, consequently, the efficiency of phosphorus solubilization [17]. Therefore, optimizing these factors is essential to maximizing the phosphorus solubilization potential of PSF [18].

Despite growing interest in PSF, research focusing on optimizing the phosphate-solubilizing abilities of fungi from mangrove ecosystems remains limited [19]. Fungi from these ecosystems may possess specialized physiological traits that enable them to thrive in environments with extreme salinity, acidity, and low nutrient availability conditions where conventional biofertilizers are less effective [20]. Exploring PSF from mangrove habitats offers a valuable opportunity to develop bioinoculants that are specifically tailored for use in saline, coastal, or degraded soils, where traditional agricultural practices may be ineffective [21].

Optimizing environmental and nutritional conditions for PSF is essential for both advancing research and practical agricultural use [22]. Cultivating PSF under optimal conditions improves their performance in variable field conditions, ensuring consistent results across different agricultural systems and reducing reliance on chemical fertilizers [23]. Optimized PSF formulations enhance phosphorus use efficiency,

improve soil health, and reduce nutrient leaching and runoff, addressing environmental concerns like water pollution and eutrophication [24]. Additionally, understanding the factors that affect PSF activity offers valuable insights into fungal physiology, nutrient solubilization, and soil-microbe interactions, which are crucial for developing effective nutrient management strategies.

The potential of PSF from mangrove ecosystems presents an exciting frontier in sustainable agriculture [25]. Given the unique environmental stresses present in mangrove habitats, PSF from these regions may offer solutions for phosphorus-deficient soils in coastal and degraded areas [26]. Through research and optimization, these fungi can be developed as biofertilizers that enhance agricultural efficiency and contribute for enhancing soil fertility and environmental integrity [27].

The current investigation aims of isolating and characterize phosphate-solubilizing fungus associated with mangrove ecosystems and assess their solubilization potential under various environmental and nutritional conditions. Key objectives include evaluating the effect of pH, temperature, incubation period, phosphorus sources, and nitrogen sources on phosphate-solubilizing efficiency. By optimizing these parameters, the study intends to identify the most favorable conditions for enhancing solubilization efficiency and develop sustainable fungal-based biofertilizers suitable for challenging soil environments.

Materials and methods

Optimization of parameters for phosphate solubilization

Evaluation of the solubilization efficiency in response to different phosphorus source

Based on previous studies, two fungal isolates, PSF-4 and PSF-6 were selected to further optimize the parameters of phosphate solubilization. The investigation performed by Aliat et al., in 2024 analyzed the effect of several phosphorus sources, including as rock phosphate, tricalcium phosphate, aluminium phosphate and zinc phosphate on NBRIP Broth [28].

The isolates were characterized based on their effectiveness in phosphorus solubilization through NBRIP Broth supplemented with various phosphorus sources. The inoculation was performed using a pure fungal mycelia. The sample was introduced to NBRIP Broth medium with incubation carried out at 28°C ± 2°C over a period of 7 days [29]. Fungal growth was observed upon incubation. An efficiency of phosphate solubilization was determined as previously described.

Impact of varying temperature on phosphate solubilizing efficiency

The compound that elicited the most favorable response from the fungi was employed in the media formulation. Fungi were introduced into the culture and kept at temperatures of 20 ºC, 25ºC, 30ºC, 35ºC, and 40ºC. The growth was then observed and documented as previously explained. The calculation of phosphate solubilization efficiency was performed.

Influence of varying pH levels phosphate solubilizing activity

The experiment used optimal medium and temperature conditions. However, the pH of the media was standardized to pH 4, 5, 6, 7 and 8 using NaOH and HCl alternatively. The growth was then measured as previously stated. The efficiency of phosphate solubilization was determined.

Effect of Incubation Period on efficacy of phosphate mineralization

In order to evaluate influence of incubation period, fungal isolates were inoculated on NBRIP medium and incubated at 28 ± 2 °C for varying durations, to evaluate their phosphate solubilizing capacity.

Observations were recorded at intervals of 4, 6, 8, 10, 12, 14, 16, 18, and 20 days post-inoculation.

Role of nitrogen source variation in modulating phosphate solubilizing efficiency

The impact arising from several nitrogen substrate, such as ammonium sulfate, Urea, sodium nitrate and Casein was examined on NBRIP broth. This isolates were studied in terms of their ability to solubilize phosphorus in NBRIP broth supplemented with various phosphorus sources. The inoculation was performed using a pure fungal colony obtained from a Potato Dextrose agar plate. The flask was kept at a temperature of 28°C ± 2°C for a duration for 7 days, as reported by Fasim et al. in 2002 [30]. Phosphate solubilization efficacy was determined as previously described.

Impact of various carbon source onphosphate solubilization activity

The effect of several carbon resources, including carbohydrates like lactose, glucose, sucrose, fructose and sucrose was investigated on NBRIP broth. The strains were examined regarding their ability to solubilize Tricalcium phosphate in NBRIP broth. The inoculation process included utilizing a pure fungal colony that had been cultivated in Potato Dextose Agar at temperatures of 28°C for 7 days, as suggested by Shah et al. (2022) [31]. The efficiency of phosphate solubilization was determined as previously described.

Results and discussion

Effect of pH on Phosphorus Solubilization

Phosphorus solubilization by fungal strains PSF-4 and PSF-6 varied significantly with pH as shown in figure 1. Both strains showed maximum activity at pH 6, where PSF-4 solubilized approximately 690 µg/ml and PSF-6 around 650 µg/ml. The lowest solubilization occurred at pH 4, with PSF-4 and PSF-6 reaching about 440 µg/ml and 450 µg/ml, respectively. PSF-4 consistently outperformed PSF-6 across most pH levels, particularly in mildly acidic conditions. The data suggests notably exceeding the values indicated by Coutinho et al. (2012) , where isolate PSF 145 achieved a maximum phosphorus solubilization of 51 µg/ml on the seventh day, accompanied by a pH decrease to 3.3 [32]. In other similar study researcher found that *Aspergillus neoniger* and *Talaromyces aurantiacus* exhibited optimal phosphorus solubilization in slightly acidic conditions, with solubilization peaking at pH 6. Also both fungi efficiently solubilized phosphorus across a range of pH values, with activity significantly decreasing at extreme acidic or alkaline conditions [33].

Effect of Temperature on Phosphorus Solubilization

Figure 2 indicates Phosphorus solubilization by PSF-4 and PSF-6 was highest at 30°C, with PSF-4 reaching 670 µg/ml and PSF-6 recorded approximate 650 µg/ml. Smallest activity was observed at 20°C, where PSF-4 and PSF-6 solubilized 480 µg/ml and 500 µg/ml, respectively. PSF-4 performed better at higher temperatures, particularly at 35°C. Both strains showed a decrease in solubilization at 40°C, indicating reduced efficiency at elevated temperatures. PSF-6 exhibited more sensitivity to temperature changes compared to PSF-4. Overall, moderate temperatures (25°C–30°C) were optimal for phosphorus solubilization. In other study it was observed that the ability of *Aspergillus spp*. to solubilize phosphate was highest at temperatures between 21°C and 28°C, with *A. niger* achieving peak solubilization (2354 µg/ml) at 21°C. Their study also highlighted that, while biomass production was optimized at lower temperatures (14°C to 21°C), solubilization of phosphate was most efficient under sub-optimal growth conditions [34]. In a latest investigation by sun et al. (2020), phosphorus (P) solubilization capacity

of *Aspergillus niger* strain Xj-2 was evaluated under various optimized conditions. The highest phosphorus solubilization recorded was 616.81 mg/l, showing the significant potential of *A. niger* in enhancing phosphorus availability [35].

Effect of Incubation period on Phosphorus Solubilization

Phosphorus solubilization by PSF-4 and PSF-6 increased with incubation time, peaking on day 14 at 690 µg/ml for PSF-4 and 640 µg/ml for PSF-6. The lowest solubilization was recorded on day 4, with PSF-4 at 400 µg/ml and PSF-6 at 380 µg/ml. A gradual decline in activity was observed after day 14, indicating reduced efficiency with prolonged incubation. PSF-4 consistently outperformed PSF-6 across the incubation period (Figure 3). In the study by Balogun et al. [36], indigenous rhizosphere fungi *Aspergillus niger, A. fumigatus,* and *A. flavus* were evaluated for their phosphate solubilization potential. Among them, *A. niger* showed the greatest solubilization index (1.72), followed by *A. fumigatus* (1.01) and *A. flavus* (0.95). Phosphate solubility increased with incubation time, peaking at day 11, with *A. niger* solubilizing 549 mg/l, *A. fumigatus* 430 mg/l, and *A. flavus* 379 mg/l. Sawdust proved to be a better biofertilizer carrier than charcoal, supporting higher fungal viability over time.

Effect of P source on Phosphorus Solubilization

Phosphorus solubilization was highest with tricalcium phosphate, where PSF-4 and PSF-6 achieved 690 and 650 µg/ml, respectively. Lowest solubilization occurred with zinc phosphate, with PSF-4 at 530 µg/ml and PSF-6 at 440 µg/ml. PSF-4 consistently showed greater efficiency across phosphate sources. Rock phosphate also supported high solubilization, particularly for PSF-4 (670 µg/ml). Aluminium phosphate resulted in moderate activity, especially for PSF-6 (figure 4). Wang et al. (2023) found that *Paecilomyces lilacinus* PSF7 showed effective solubilization of phosphorus, with tricalcium phosphate being a key phosphorus source. Under optimized conditions, the phosphorus soluble content reached 122.17 mg/l, closely matching the predicted value of 123.89 mg/l [37].

Effect of N source on Phosphorus Solubilization

Phosphorus solubilization was highest for PSF-4 with ammonium sulfate (680 µg/ml) and for PSF-6 with urea (640 µg/ml). The lowest solubilization was observed in PSF-6 with sodium nitrate (540 µg/ml). PSF-4 maintained relatively high solubilization even with less effective sources, such as sodium nitrate (630 µg/ml). Casein supported moderate activity for both strains. These results suggest that ammonium-based nitrogen sources enhance solubilization efficiency, particularly for PSF-4. PSF-6 showed a more variable response depending on the nitrogen source. Overall, PSF-4 demonstrated more consistent performance across all nitrogen treatments (Figure 5). Mpanga et al. (2019) [38] reported that phosphorus-solubilizing microorganisms (PSMs) exhibited significantly greater phosphorus solubilization when supplied with ammonium (NH_4) compared to nitrate (NO_3^-).Phosphorus solubilization was 19.5% higher with ammonium, and maize growth was improved by 13.5% compared to nitrate. This shows that ammonium was more effective in promoting phosphorus availability and maize growth.

Effect of C source on Phosphorus Solubilization

Phosphorus solubilization was highest for both PSF-4 and PSF-6 with glucose, reaching 685 µg/ml and 640 µg/ml, respectively. The lowest solubilization occurred with starch, where PSF-4 recorded 535 µg/ml and PSF-6 510 µg/ml. PSF-4 consistently outperformed PSF-6 across all carbon sources tested. Sucrose

supported moderately high solubilization in both strains (610 µg/ml), while lactose showed variable results, particularly lower in PSF-6 as indicated in figure 6. Kumari et al. (2024) [39] evaluate the influence of phosphorus solubility by *Aspergillus niger*. Glucose resulted in the highest phosphorus solubilization of 520 mg/l after 7 days, outperforming sucrose and fructose. In a study by Sang et al. (2022), *Mortierella* Strain L4 displayed the maximum phosphorus solubilizing capacity solubilization (123.72 mg/l) with glucose in form of carbon source, accompanied by lowest pH (5.08). Starch, maltose, and sucrose showed moderate effects, while mannitol resulted in no detectable solubilization. Phosphorus release was closely linked to pH reduction influenced by carbon type [40].

Conclusion

This research study concisely provides the immense Capacity of the fungus to mobilize insoluble phosphates isolated through the mangrove ecosystem, with isolate PSF-4 being the most efficient over a wide variety of environmental and nutritional conditions. The optimum circumstances, which included pH 6, a temperature of 30°C, a 14-day incubation period, and the usage of tricalcium phosphate, ammonium sulfate, and glucose, resulted in maximum phosphorus solubilization. These results improve the field study by exposing the resilient and adaptive nature of mangrove-associated fungus, which may be used to build effective biofertilizers for sustainable agriculture.

In comparison to previous research, the current study not only demonstrates the efficacy of fungal phosphate solubilizers, but also exhibits increased solubilization efficiency, emphasizing the unique isolates utilized. However, problems such as a lack of field validation and more extensive ecological sampling remains. Future study should focus on field experiments, molecular characterization of solubilization mechanisms, and the development of microbial consortium including PSF-4. The present research provides a valuable insight into the development of environmentally friendly soil fertility management systems and emerges novel opportunities for investigating microbial resources from underexplored environments such as mangroves.

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Figures:

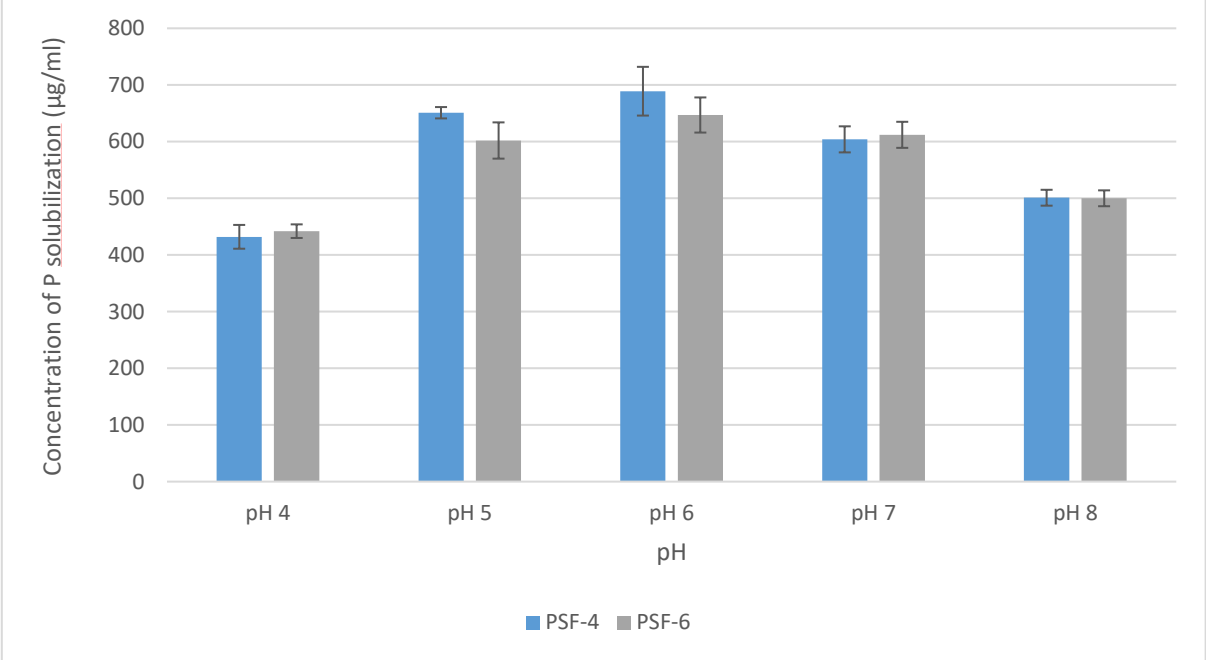

Figure 1. **Effect of pH on phosphorus solubilization by fungal strains PSF-4 and PSF-6**

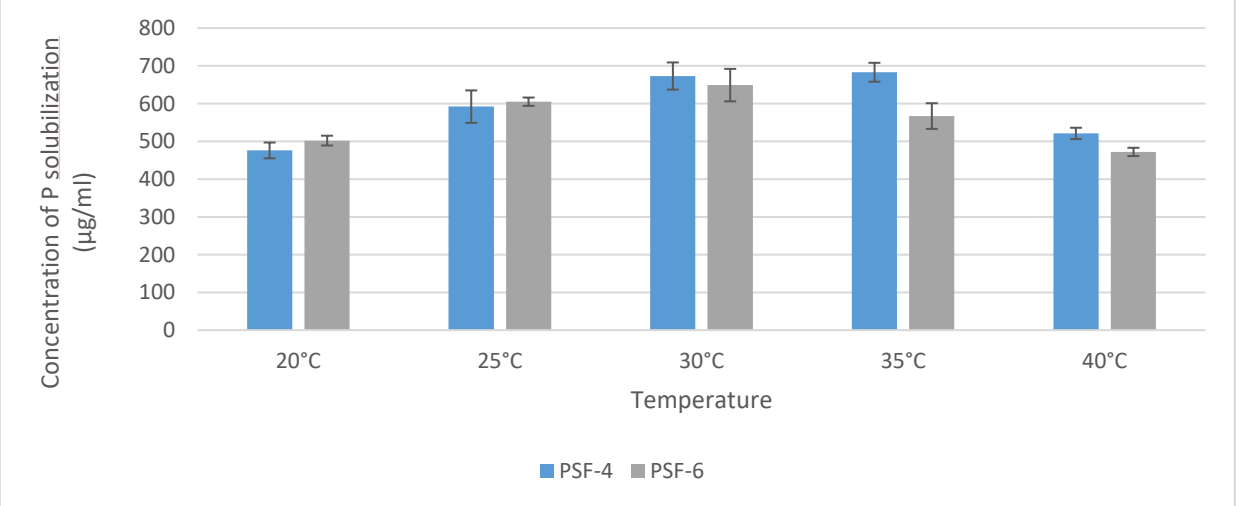

Figure 2. Effect of Temperature on phosphorus solubilization by fungal strains PSF-4 and PSF-6

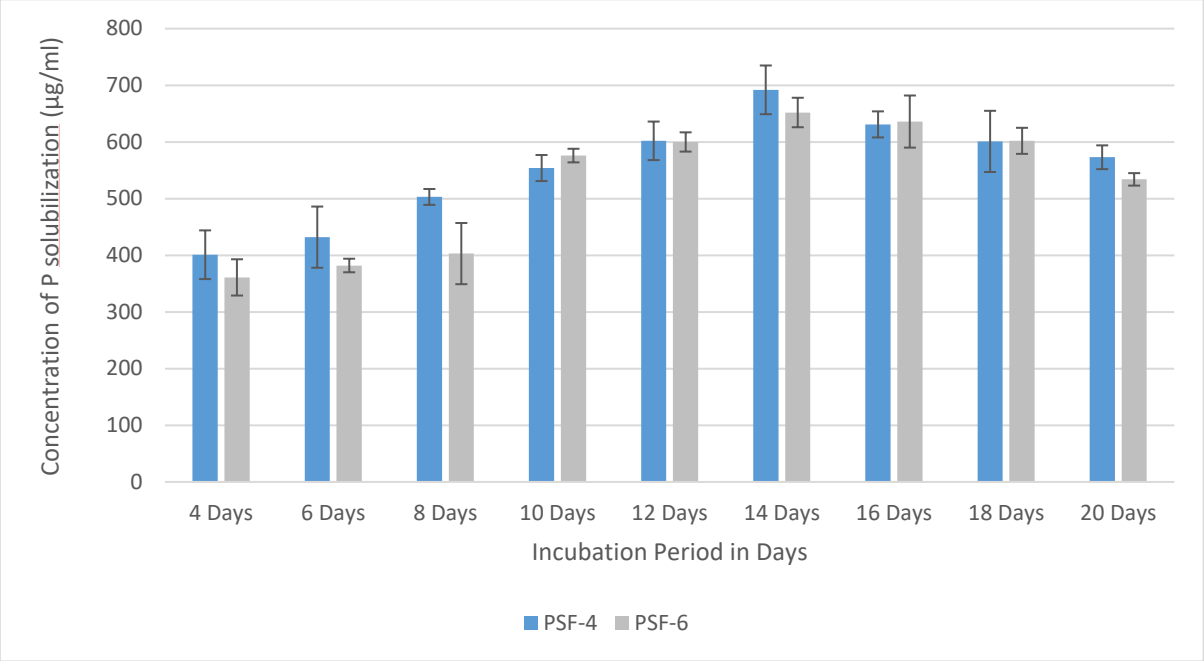

Figure 3. Effect of Incubation period on phosphorus solubilization by fungal strains PSF-4 and PSF-6

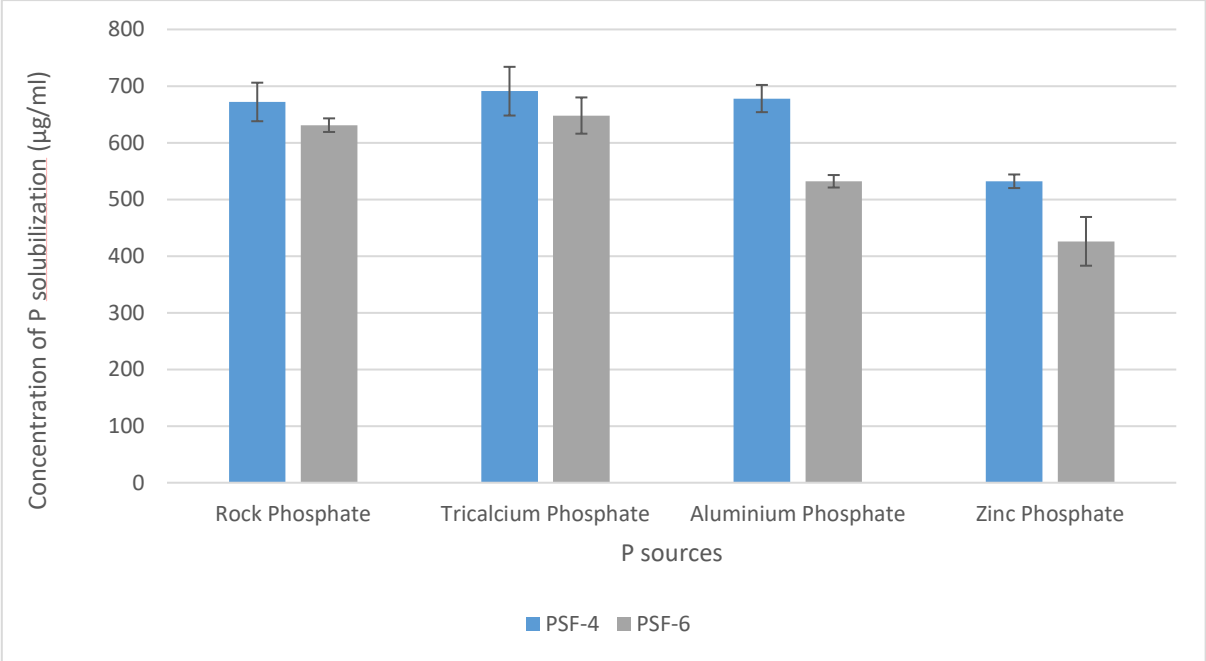

Figure 4. Effect of P source on phosphorus solubilization by fungal strains PSF-4 and PSF-6

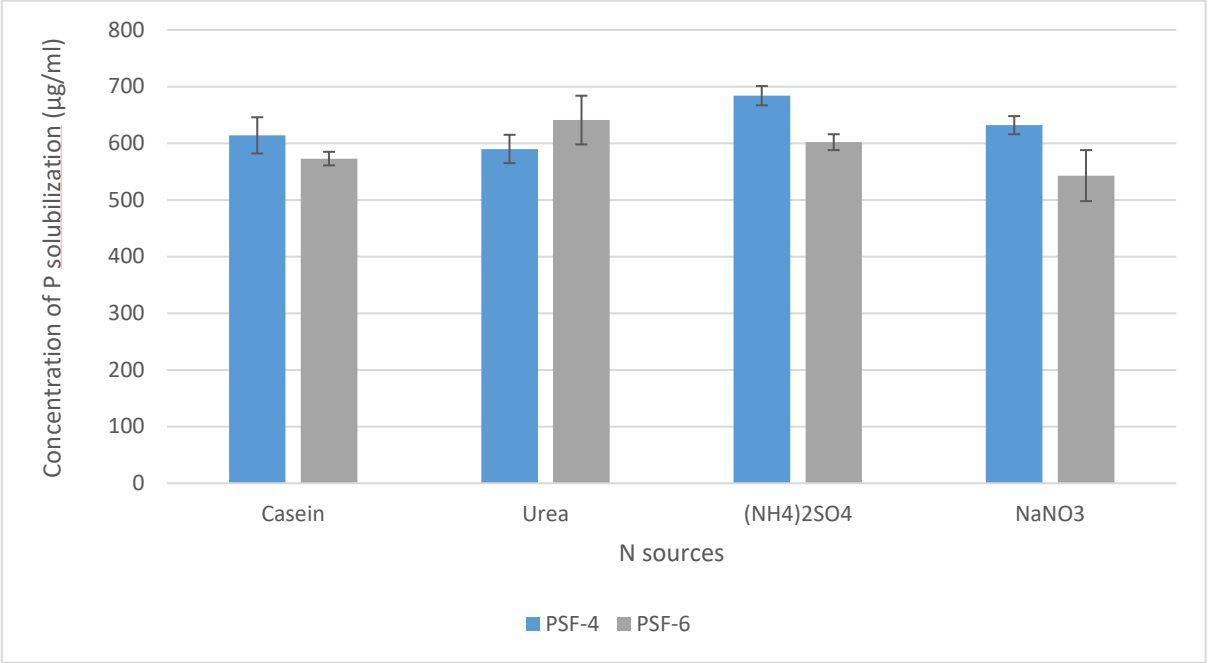

Figure 5. Effect of N source on phosphorus solubilization by fungal strains PSF-4 and PSF-6

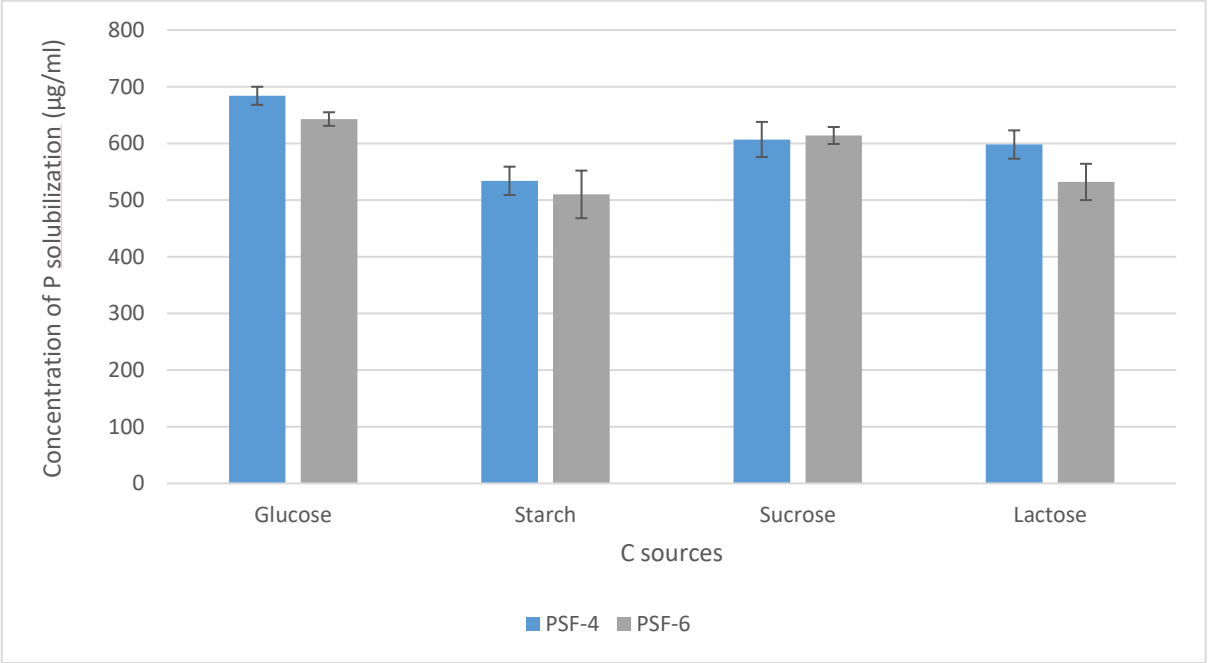

Figure 6. Effect of C source on phosphorus solubilization by fungal strains PSF-4 and PSF-6