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# Screening, evaluation and selection of phosphate-solubilising fungi as potential biofertiliser

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## Abstract

Phosphate-solubilising saprophytic fungi have a potential application in plant nutrition; therefore, the aim of this study was 1) to perform a screening and isolation of native phosphofungi from volcanic soils of southern Chile, 2) to select a liquid medium for the evaluation of these phosphofungi and 3) to test a selected phosphofungus as a biofertiliser in a volcanic soil.

The phosphofungi were screened using Martin medium (rose bengal-streptomycin agar) with calcium phosphate ($Ca_3(PO_4)_2$) or calcium phytate as the phosphorus source. Six promising strains (*Penicillium* sp.*, Penicillium albidum*, *Penicillium thomii, Penicillium restrictum, Penicillium frequentans* and *Gliocladium roseum*) were evaluated in the liquid media of Agnihotri, Asea-Wakelin, Pikovskaya and Nahas. The soluble phosphorus, acid phosphatase activity, pH and fungal biomass were determined.

In most soils, the greatest proportion of phosphofungi solubilised organic P. The Asea-Wakelin medium appears to be the medium of choice for the quantitative evaluation of phosphofungi isolated from the volcanic soils tested. *Penicillium albidum* was selected as a potential biofertiliser due to its capacity to solubilise both inorganic and organic P via its specific solubilising activity (64 mg P/g fungus), phosphatase secretion and enhancement of the growth and mineral nutrition of lettuce plants growing in a volcanic soil.

**Keywords:** phosphate solubilising-mineralising fungi; *Penicillium albidum*; phosphatase; lettuce

## 1. Introduction

Phosphorus (P) is incorporated in a series of fundamental cellular molecules, such as phospholipids, nucleic acids and nucleotides, making it an essential macronutrient for any organism. This nutrient is acquired by plants from the soil solution mainly in the form of $H_2PO_4^-$ and $HPO_4^{2-}$. Some soils, however, particularly volcanic soils, possess a high capacity to fix phosphate, thus limiting the bioavailability of P. This necessitates the application of significant doses of phosphate fertiliser to these soils each year, an expensive process that ultimately results in the accumulation of inorganic and organic P in significant quantities, the latter mainly in the form of monoester phosphate and diester phosphate (Borie and Rubio, 2003; Redel *et al.*, 2008). As a result, these soils are deficient in available P but contain high levels of total P, which increases annually as a result of the excessive fertilisation (Borie and Rubio, 2003).

It is crucial to take advantage of the accumulated P in the soil, improving the growth of vegetables while reducing fertiliser consumption. In this respect, the use of microorganisms in the rhizosphere that are capable of mobilising P into available forms as biofertilisers is feasible, particularly in sustainable agriculture. Hence, there is enormous interest in isolating phosphate-solubilising microorganisms, including phosphate-solubilising and -mineralising saprophytic fungi (phosphofungi), due to their large biomass, metabolic activity and ability to maintain their solubilising capacity for years; such phosphofungi have been isolated from various soils (Borie *et al.*, 1983; Pandey *et al.*, 2008). The mechanisms of inorganic phosphate solubilisation are associated mainly with the acidification of the medium and organic acid production, whereas these fungi must produce phosphatase enzymes to mineralise organic P. In addition, some of these fungi present other characteristics, such as a wide range of tolerance for temperature, pH and salt concentration (Pandey *et al.*, 2008) and phytohormone or siderophore production (Vassileva *et al.*, 2010).

Phosphofungi belong predominantly to the *Aspergillus* and *Penicillium* genera, and several strains have been identified that benefit the nutrient concentrations and yield of vegetables under both greenhouse and field conditions (Whitelaw, 2000). However, the beneficial effect on plants may be influenced by several factors, including the type of soil and its level of P, inadequate laboratory methods for selecting the fungi or a poor understanding of the interactions of the inoculated microorganisms with the soil. One strategy to overcome these limitations is the use of native phosphofungi adapted to the edaphoclimatic conditions of each region, an approach that would require the identification and isolation of native phosphofungi and their subsequent evaluation and would advance the knowledge of phosphofungus-soil-plant interrelationships.

Indeed, research in this area is becoming critical because the great majority of P fertilisers are obtained from phosphate rock, a non-renewable source of P that is expected to be exhausted in 30 to 50 years and because the exploited deposits are of increasingly lower quality, which results in increases in the cost of phosphate fertiliser production.

Thus, the use of phosphofungi in volcanic soils in Chile, alone or co-inoculated with mycorrhizal fungi, would be relevant for agriculture, particularly in the development of sustainable agriculture in which these types of microorganisms have great

potential. Given the large amount of agricultural activity that these types of soils maintain, this would also have significant financial and environmental impacts.

Therefore, the aim of this study was 1) to perform a screening and isolation of native phosphofungi from volcanic soils from the south of Chile, 2) to select a liquid medium to quantify the P-solubilising capacity of the phosphofungi, and 3) to test a phosphofungus as a biofertiliser in a volcanic soil.

## 2. Materials and methods

*2.1. Screening and isolation of phosphofungi*

Phosphofungi from 21 volcanic soils from southern Chile (Araucanía region) were screened. These soils included those that sustained crops with different agronomic management to increase the probability of isolating phosphofungi with high P-solubilising capacity. The main soil characteristics are shown in Table 1.

**Table 1.** Basic characteristics of the soils used.

| Site | Soil order (series) | Management system | pH ($H_2O$) | Olsen-P (mgP/kg) | O.M. (%) |
|---|---|---|---|---|---|
| 1 | Andisol (Freire) | Organic farming, maize | 5.60 | 16.9 | 15.3 |
| 2 | Andisol (Freire) | Natural pasture | 5.33 | 2.5 | 22.1 |
| 3 | Andisol (Temuco) | Organic farming, | | | |
| 4 | | garlic | 6.08 | 17.5 | 14.1 |
| | | pasture | | | |
| 5 | Andisol (Temuco) | Natural pasture | 5.62 | 8.4 | 16.4 |
| 6 | Andisol (Temuco) | Vegetable | 5.50 | 7.9 | 15.9 |
| 7 | Ultisol (Metrenco) | Organic farming, | | | |
| 8 | | natural pasture | 5.70 | 11.2 | 10.1 |
| | | wheat | 5.67 | 15.6 | 6.2 |
| 9 | Ultisol (Metrenco) | NT, lupine | 5.51 | 16.9 | 9.3 |
| 10 | Ultisol (Vilcún) | NT, wheat | 5.43 | 23.8 | 11.6 |
| 11 | | RT, wheat | 5.49 | 19.2 | 11.0 |
| 12 | | CTS, wheat | 5.26 | 21.9 | 8.6 |
| 13 | | CTB, wheat | 5.34 | 17.9 | 9.2 |

(O.M., organic matter; NT, no-tillage; RT, reduced tillage; CTS, conventional tillage with stubble retained; CTB, conventional tillage with stubble burned)

The phosphofungi were isolated from the rhizosphere using serial dilution and were plated on rose bengal-streptomycin agar (Martin, 1950), with calcium phosphate or calcium phytate added as an insoluble source of inorganic or organic P, respectively. On either medium, a halo around the colony indicates solubility. The plates were incubated for 5 days at 28 ºC, and the number of solubilising colonies, their total diameter (colony + halo), and the diameter of the colony were then determined. A solubilisation index was calculated using total / diameter of the colony ratio (Fankem *et al.*, 2006). Those colonies with the greatest diam-

eter and solubilisation index were selected to be re-evaluated using Martin medium. The total number of colonies was also determined.

### *2.2. Re-evaluation on Martin medium and identification of phosphofungi*

Selected colonies were replicated several times using Martin medium and re-evaluated for effects on calcium phosphate or calcium phytate. The medium for the organic P (the latter) had phenolphthalein phosphate added to detect phosphatase release; a red halo around the colony, as revealed with ammonia fumes, indicated phosphatase release (Barik *et al.*, 2001).

These fungi were identified through their growth rate for 5 days on 2% malt agar at 23 ± 2 ºC. To determine the reproductive, resistance and vegetative structures, fresh cultures were prepared from the colonies that had formed by the fifth day, and their characteristics were compared with taxonomic keys according to von Arx (1981) to identify the fungi at the genus level. To determine the species, each isolate was cultured on 2% CZpex agar and 2% potato dextrose agar and incubated at 23 ± 2 ºC for 5 days to determine the macroscopic characteristics of the colonies (colour, margin, texture, odour and height). The characteristics and measurements of the reproductive, resistance and vegetative structures were determined using microscopic preparations. All of the data obtained were compared with taxonomic keys (Ramírez 1982; Domsch *et al.*, 1995).

### *2.3. Quantification of the solubilising capacity in liquid medium*

Based on the solubility index, diameter of the colony, total diameter and capacity to release phosphatase in Martin medium, isolates were selected for evaluation in the 4 following liquid media using $Ca_3(PO_4)_2$ as the P source: 1) Agnihotri (1970), 2) Asea *et al.* (1988), modified by Wakelin *et al.* (2004), 3) Pikovskaya (1948), and 4) Nahas *et al.* (1994). The compositions of these media are provided in Table 2. Mycelium circles of 3 mm in diameter obtained from colonies growing on Martin agar were inoculated in 50 mL of liquid medium and cultivated in the dark at 20 ºC with intermittent orbital agitation at 100 rpm for 9 minutes every hour. At the end of 7 days, the liquid phase was separated by filtration, the pH and soluble P were determined using the method of Murphy and Riley (1962), and the acid phosphatase activity was determined according to Rubio *et al.* (1990). The fungal mass was determined in the solid phase as the difference between the mass at 60ºC for 24 hours and the mass of the residue after being subjected to calcination in a muffle furnace at 500ºC for 6 hours (Reyes *et al.*, 1999). The controls included the same treatments but without the phosphofungi. All of the treatments were performed in quadruplicate.

### *2.4. Inoculation of a selected fungus in lettuce-growing soil*

A fungus with a high capacity for solubilising phosphate and releasing phosphatase in liquid medium was selected to evaluate its effect on the development of lettuce (*Lactuca sativa* L.) *in situ*. This assay comprised a) a seedbed stage under greenhouse conditions and b) subsequent transplanting and development under shaded field conditions.

#### *Seedbed stage*

Lettuce seeds (*L. sativa)* were disinfected with water at 45 ºC for 24 hours and then allowed to germinate for 3 days. Each seedling was placed in the cell of a speedling tray containing 100 mL of a non-sterile mixture of soil (Andisol, pH = 5.7, available P = 8

mg $kg^{-1}$), sand, vermiculite and compost (4.5:4:0.5:1). At the beginning of the seedbed stage, the soil of ten plants was inoculated with 1.9 x $10^6$ CFU of the phosphofungus, and another 10 seedlings were controls without fungi. The inoculum consisted of a mycelium suspension obtained by culture in Sabouraud broth at 20 °C for five days, followed by filtration, washing and resuspension in sterile water. The speedling trays remained in the greenhouse; at the end of 2 months, the aerial and root weights of the plants and the soil Olsen-P were recorded for five replications of the inoculated and non-inoculated soil. P-solubilising colonies and the acid phosphatase activity (Rubio *et al.*, 1990) were also evaluated. The ten remaining plants (five control and five treated) were transplanted.

*Transplant stage*

At the end of the seedbed stage, the remaining plants were transplanted to 1 L pots containing the above-described soil, sand, vermiculite and compost mixture plus worm humus at a ratio of 60:40 and grown outdoors under shade. At five months after transplanting, the aerial weights of the plants (total, heart, stem, and leaves) were determined, and the nutrient concentrations (P, K, Ca, Mg, Mn, Cu, Zn, and Fe) were measured using 1 g of dry matter that was calcined in a muffle furnace at 480 ºC for 8 hours. The resulting ashes were treated with 2M HCl; the P was determined using spectrophotometry, and the remaining elements were analysed using atomic absorption (Sadzawka *et al.*, 2004).

*2.5. Statistical analysis*

For the statistical analysis of the liquid medium results, a one-way ANOVA was used, and the averages were compared using the LSD test ($p < 0.05$). A student's t-test ($p < 0.05$) was used for the correlation coefficients and the testing of the lettuce soil inoculation.

**Table 2.** Composition of the liquid media used to quantify the calcium phosphate solubilisation by the selected phosphofungi.

| Media component | Amount (g $L^{-1}$) | | | |
|---|---|---|---|---|
| | **Agnihotri** | **Asea-Wakelin** | **Pikovskaya** | **Nahas** |
| NaCl | | 0.1 | - | 0.1 |
| $NH_4Cl$ | | 0.4 | - | 1.0 |
| $KNO_3$ | | 0.78 | - | - |
| MgSO4 | 0.5 | 0.5 | - | - |
| $CaCl_2 \cdot 2H_2O$ | | 0.1 | - | 0.1 |
| Sucrose | | 10 | - | - |
| $NaNO_3$ | 5 | - | - | - |
| KCl | | - | 0.2 | 0.2 |
| $MgSO_4 \cdot 7H_2O$ | | - | 0.1 | 1.2 |
| Glucose | 15 | - | 10 | 10 |
| Yeast extract | | - | 0.5 | 0.5 |
| $FeSO_4 \cdot 7H_2O$ | | - | 0.002 | - |
| $MnSO_4 \cdot H_2O$ | | - | 0.002 | - |
| $(NH_4)_2SO_4$ | | - | 0.5 | - |
| $Ca_3(PO_4)_2$ | 2 | 2 | 2 | 2 |

## 3. Results and discussion

### *3.1. Screening and isolation of phosphofungi*

In all of the soils sampled, phosphofungi were found on the order of $10^4$ CFU per gram of soil, independent of the soil type (Table 3). In most soils, the organic phosphate-solubilising CFU represented between 10 and 35% of the total fungi, where the percentage of organic P solubilisers appeared to be higher than that of the inorganic P solubilisers (Table 3), results similar to those found in a previous screening (Borie *et al.*, 1983). The influence of the soil management on the amount of fungi was most evident in the Ultisol Vilcún series, with results showing that NT (no tillage) and RT (reduced tillage), compared to CT (conventional tillage), contributed to an increase in the total number of fungi and the percentage of phosphofungi (Table 3). Stubble burning also contributed to the abundance of fungi in the soil when compared to the soil without burning (Table 3), possibly due to an increased mineralisation and availability of some nutrients, favouring microbial growth, particularly phosphofungi. In contrast, Wang *et al.* (2010) did not find any effect of stubble burning on the saprophytic fungi in the soil; therefore, such factors require further study.

Most of the colonies detected had a low solubility index (≤1.15) (Figure 1). When this index increased, the number of solubilising colonies decreased, but the proportion of solubilising colonies of calcium phytate increased. In these soils, the phosphofungi solubilising organic P exceeded the percentage of the phosphofungi solubilising inorganic P, suggesting an adaptation of the fungi to such edaphic conditions with high contents of organic P. This adaptation was also suggested by Borie *et al.* (1983), who found that phosphofungi had a greater ability than bacteria to solubilise organic phosphates in volcanic soils.

**Table 3.** Total and solubilising fungi isolated from different volcanic soils.

| Site | Management system | Fungi on Martin medium (CFU/g.d.s) | | | | Main fungi isolated |
|---|---|---|---|---|---|---|
| | | + inorganic P | | + organic P | | |
| | | Total | solubilising | Total | solubilising | |
| 1 | Organic farming, maize | $6x10^5$ | $5x10^4$ (8) | $6x10^5$ | $6x10^4$ (10) | *Penicillium restrictum*<br>*Penicillium frequentans*<br>*Penicillium jensenii*<br>*Eupenicillum javanicum*<br>*Myrothecium roridum* |
| 2 | Natural pasture | $2.2x10^5$ | $5x10^4$ (23) | $2.2x10^5$ | $6x10^4$ (27) | *Penicillium* sp. |
| 3 | Organic farming, garlic | $8x10^4$ | $1x10^4$ (12) | $1x10^5$ | $3x10^4$ (30) | *Gliocladium roseum* |
| 4 | Organic farming, pasture | $1.7x10^5$ | $5x10^4$ (29) | $2.2x10^5$ | $7x10^4$ (32) | |
| 5 | Natural pasture | $1.8x10^5$ | $3x10^4$ (17) | $1.8x10^5$ | $2x10^4$ (11) | *Myrothecium roridum* |
| 6 | Vegetable | $1.5x10^5$ | $1x10^4$ (7) | $1.6x10^5$ | $2x10^4$ (13) | |
| 7 | Organic farming, pasture | $1.7x10^5$ | $4x10^4$ (24) | $1.8x10^5$ | $4x10^4$ (22) | |
| 8 | Organic farming, pasture | $1.8x10^5$ | $6x10^4$ (33) | $1.7x10^5$ | $6x10^4$ (35) | *Penicillium thomii* |
| 9 | NT, Lupine | $1.8x10^5$ | $2x10^4$ (11) | $1.9x10^5$ | $4x10^4$(21) | |
| 10 | NT, wheat | $2.4x10^5$ | $7x10^4$ (29) | $2.4x10^5$ | $8x10^4$ (33) | *Penicillium albidum* |
| 11 | RT, wheat | $2.3x10^5$ | $6x10^4$ (26) | $2.3x10^5$ | $7x10^4$ (30) | |
| 12 | CTS, wheat | $1.8x10^5$ | $3x10^4$ (17) | $1.9x10^5$ | $5x10^4$ (26) | |
| 13 | CTB, wheat | $2.1x10^5$ | $5x10^4$ (24) | $2.4x10^5$ | $8x10^4$ (33) | |

(Values in parentheses represent the percentage of solubilising fungi. NT, no-tillage; RT, reduced tillage; CTS, conventional tillage with stubble retained; CTB, conventional tillage with stubble burned; g.d.s., grams of dry soil).

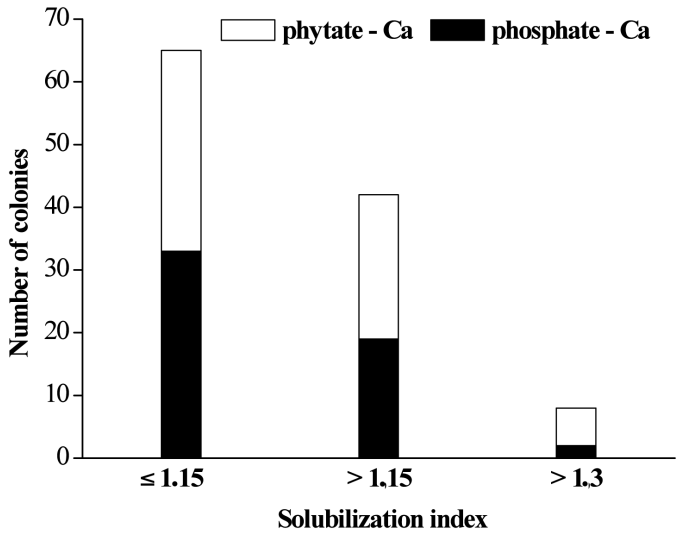


**Figure 1.** Number of phosphate- and calcium phytate-solubilising colonies in relation to the solubility index.

Based on the colony diameter and solubilisation index, the phosphofungi selected and purified from successive cultures on Martin agar were identified according to taxonomic keys as *Penicillium* sp., *P. albidum*, *P. thomii, P. restrictum, P. frequentans, Gliocladium roseum*, *Myrothecium roridum*, *P. jensenii* and *Eupenicillum javanicum* (Tables 3 and 4). Five of these species were isolated from an Andisol Freire series under organic agriculture and cropped with maize. However, only a single species was isolated from this same type of soil under natural pasture conditions in a nearby site (Table 3), suggesting that organic agriculture increases the diversity of these fungi, which is consistent with the reports on the fungal diversity (Mäder *et al.*, 2002), total fungi and active fungi (Shannon *et al.*, 2002) found in organic agriculture. Furthermore, the type of plant cultivated is also significant because, in this study, the soil used under organic agriculture with garlic as the main crop presented a low range of both total fungi and P solubilisers. It has been indicated previously that garlic possesses antimicrobial and anti-fungal substances (Irkin and Korukluoglu, 2009), which suggests that these substances are exuded into the soil through the root, decreasing the fungal population. One species (*P. albidum*) was isolated from a Ultisol Vilcún under NT, but none were found under RT or CT, probably due to a greater wealth and diversity of saprophytic fungi under NT soils compared with CT soils.

Of the phosphofungi selected at this stage, *P. thomii* and *P. restrictum* have been reported as phosphofungi, although it has also been indicated that the former produces plant growth inhibiters. In contrast, the remaining fungi have been reported in different studies, including those regarding volatile hydrocarbon production (*Gliocladium roseum*), a biological control agent against rotting of stone fruits (*P. frequentans*), the production of anti-fungal sub-

stances (*Eupenicillium javanicum*), or even antibiotic production (*P. albidum*). Therefore, such additional properties have acquired relevance with respect to the use of phosphofungi in the manufacture of multifunctional biofertilisers as well as in the treatment of agro-industrial wastes, composting or the control of phytopathogens (Vassileva *et al.*, 2010), indicating the broad biotechnological potential of these microorganisms. However, the presence of some fungi with pathogenic characteristics or growth inhibitors, such as *Myrothecium roridum*, a fungus pathogenic to some plants, emphasises the use of caution in applications.

The *P. albidum*, *P. thomii*, *P. restrictum*, *P. frequentans* and *Gliocladium roseum* isolates demonstrated two important characteristics in the mobilisation of P in volcanic soils, the solubilisation of inorganic P and the mineralisation of organic P (Table 4), which is consistent with the approach of producing biofertilisers with more than one function.

**Table 4.** Evaluation of the phosphate solubilising ability of the selected fungi on Martin medium.

| Species | Inorganic P | | | Organic P | | | P-ase |
|---|---|---|---|---|---|---|---|
| | T.D. | C.D. | S.I. | T.D. | C.D. | S.I. | |
| *Penicillium albidum* | 3.4 | 2.8 | 1.2 | 4.7 | 4.4 | 1.1 | ++ |
| *Penicillium thomii* | 3.4 | 2.8 | 1.2 | 4.0 | 3.8 | 1.1 | ++ |
| *Penicillium restrictum* | 5.0 | 4.0 | 1.3 | 4.0 | 3.0 | 1.3 | ++ |
| *Penicillium frequentans* | 2.1 | 1.9 | 1.1 | 2.2 | 1.7 | 1.3 | ++ |
| *Gliocladium roseum* | 3.0 | 2.6 | 1.2 | 5.4 | 5.0 | 1.1 | ++ |
| *Myrothecium roridum* | 2.5 | 1.9 | 1.3 | 3.5 | 2.0 | 1.8 | - |
| *Penicillium jensenii* | 1.6 | 1.4 | 1.1 | 2.0 | 1.8 | 1.1 | + |
| *Eupenicillium javanicum* | 1.7 | 1.4 | 1.2 | 2.4 | 2.0 | 1.2 | + |

(T.D. total diameter; C.D. colony diameter; S.I. solubility index; + colour intensity)

*3.2. Behaviour of the selected fungi in liquid media*

Therefore, for their capacity to solubilise phosphate and calcium phytate and produce phosphatase (P-ase) were quantitatively evaluated in liquid media the following isolates: *Penicillium albidum*, *Penicillium thomii, Penicillium restrictum, Penicillium frequentans, Gliocladium roseum*, and the *Penicillium* sp. The greatest total solubilising capacity of *P. albidum* and *P. thomii* was found in the Pikovskaya medium, whereas the *Penicillium* sp., *P. restrictum* and *P. frequentans* exhibited the highest level in the Asea-Wakelin medium and *Gliocladium roseum* exhibited the highest level in the Nahas medium (Figure 2A). Therefore, one half of the isolates solubilised the greatest amount of P in the Asea-Wakelin medium. We found no medium in which all of the fungi expressed their capacity to solubilise P with equal efficiency, similar to the results of Xie *et al.* (2009) for bacteria. Nevertheless, these results indicate that the majority of the native phosphofungi in southern Chile will manifest their ability to solubilise P best in Asea-Wakelin medium.

There was a general acidification of the liquid media (Figure 2B), and there have been reports of the independent and dependent mechanisms of $NH_4^+$ acidification (Asea *et al.*, 1988) that must be related to the solubilisation of P. In this study, *P. albidum*,

*P. frequentans* and *P. restrictum* even acidified the Agnihotri medium, which only contains N in the form of $NO_3^-$. The significant negative correlation ($p< 0.05$) between the variation in the pH of the medium and the solubilised total P (r = -0.91 in Asea-Wakelin, r = -0.91 in Pikovskaya, r = -0.90 in Nahas and r = -0.87 in Agnihotri) is also remarkable, i.e., the greater the P solubilisation was, the more the pH decreased. Accordingly, the acidification of the medium explains to a large extent the *in vitro* solubilisation of calcium phosphate, possibly due to the release of organic acids by these fungi (Wakelin *et al.*, 2004; Pandey *et al.*, 2008).

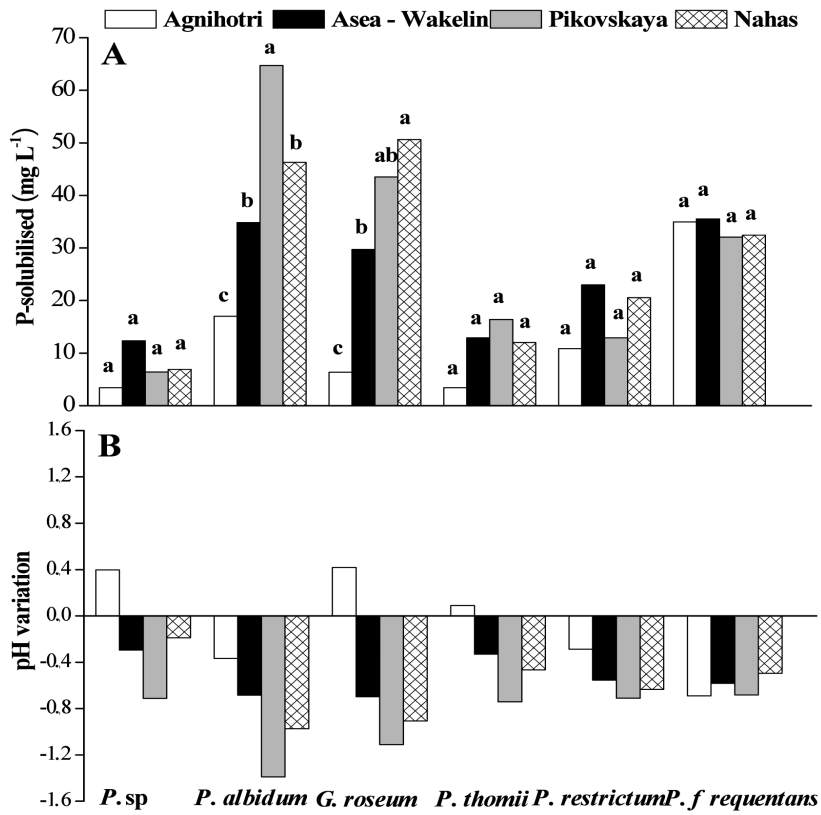


**Figure 2.** A) Total solubilised phosphorus and B) pH variation in the liquid media. Bars with different letters indicate significant differences between the culture media for the same fungus by LSD ($p < 0.05$)

When standardising the solubilised P with respect to the fungal mass (specific solubilising activity), it was observed that the phosphofungi exhibited a high specific solubilising activity in the Asea-Wakelin medium (Figure 3), with *P. albidum* (64 mg P/g fungus, $p < 0.05$) displaying a particularly high level. These results indicate that the metabolism of the fungi is aimed more toward the solubilisation of P in Asea-Wakelin medium, which is more restricted in nutrients compared with the Pikovskaya and Nahas media that contain yeast extract. Accordingly, it is possible that the fungi with a high specific solubilising activity in the Asea-Wakelin medium have a greater potential as biofertilisers, given that adverse conditions, such as the low availability of nutrients, is common to many soils.

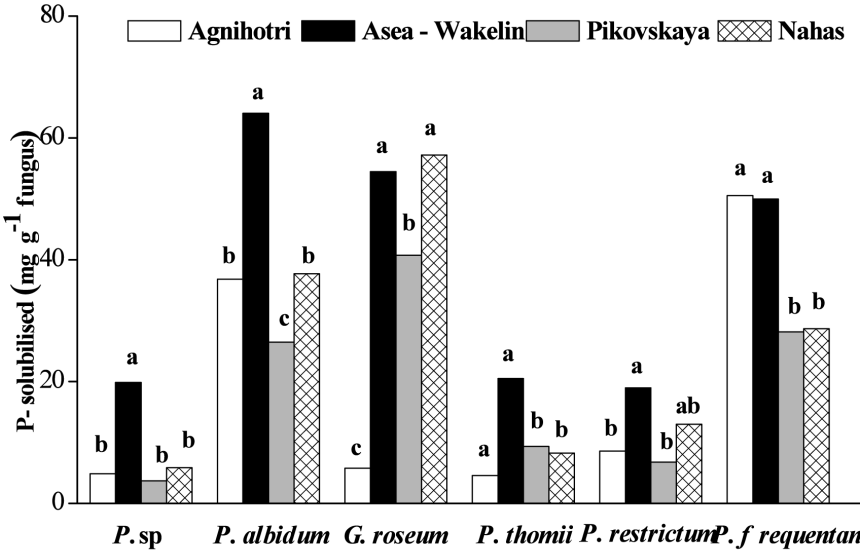


**Figure 3.** Specific solubilising activity in liquid media. Bars with different letters indicate significant differences between the culture media for the same fungus by LSD ($p < 0.05$)

The greatest acid phosphatase secretion per gram of fungus was observed in the Asea-Wakelin medium, reinforcing the idea that the metabolism of the phosphofungi is aimed more at the mobilisation of P in this medium. Again, *P. albidum* was prominent in this medium, together with the *Penicillium* sp. and *P. thomii* (Figure 4).

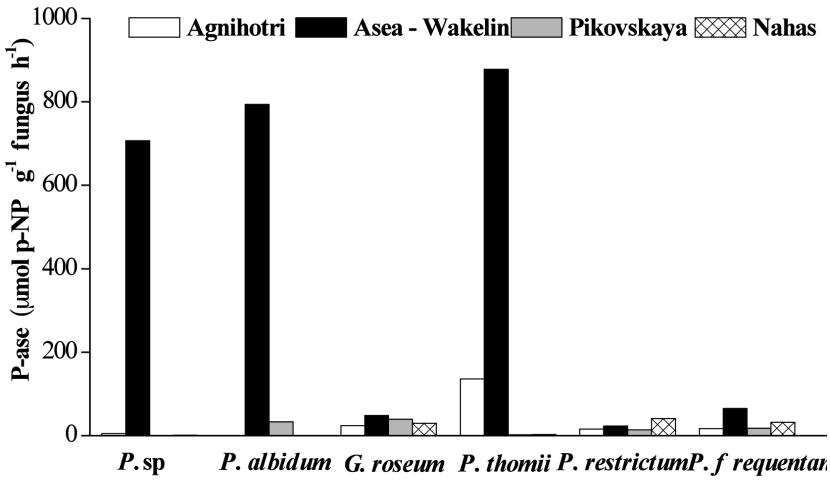


**Figure 4.** Extracellular acid phosphatase activity.

*3.3. Effect of inoculation*

Considering its capacity to solubilise both organic and inorganic P and its elevated P-ase activity, *P. albidum* appears to have the greatest potential to release P for the benefit of plants; therefore, it was selected to evaluate the *in situ* effect on lettuce growth. After two months of growth (the end of the seedbed stage), the results indicated that inoculation with *P. albidum* significantly increased both the aerial and the root weights (Table 5). These results are similar to those reported by Kohler *et al.* (2007) in lettuce inoculated with *Aspergillus niger*, among other microorganisms, after two months of growth.

**Table 5.** *Lactuca sativa* L. growing in Andisol inoculated with *Penicillium albidum.*

| | **Control** | ***P. albidum*** |
|---|---|---|
| At seedling production stage | | |
| Shoot fresh weight (mg plant$^{-1}$) | 53 b | 107 a |
| Root fresh weight (mg plant$^{-1}$) | 10 b | 37 a |
| Olsen-P (mg kg$^{-1}$) | 8.5 a | 9.7 a |
| P-ase (mg p-NP g$^{-1}$ soil h$^{-1}$) | 202 b | 251 a |
| Number of colonies (CFU) | $2x10^4$ b | $1x10^5$ a |
| At harvest | | |
| Heart fresh weight (g plant$^{-1}$) | 37 a | 44 a |
| Stem fresh weight (g plant$^{-1}$) | 8 a | 9 a |
| Leaf fresh weight (g plant$^{-1}$) | 23 a | 29 a |
| Total fresh weight (g plant$^{-1}$) | 68 b | 82 a |
| P in the foliage (mg kg$^{-1}$) | 11.0 a | 11.2 a |
| K (g 100 g$^{-1}$) | 7 a | 9 a |
| Ca (g 100 g$^{-1}$) | 0.35 b | 0.50 a |
| Mg (g 100 g$^{-1}$) | 0.27 b | 0.35 a |
| $Mn^{2+}$ (mg kg$^{-1}$) | 55 a | 38 b |
| $Cu^{2+}$ (mg kg$^{-1}$) | 6.0 a | 6.5 a |
| $Zn^{2+}$ (mg kg$^{-1}$) | 27 a | 29 a |
| $Fe^{3+}$ (mg kg$^{-1}$) | 426 b | 561 a |

(Different letters show significant differences between the inoculated and control plants. $p < 0.05$, Student's t-test).

The plants inoculated with *P. albidum* also showed a significant increase in the soil P-ase activity (Table 5), which was consistent with the high P-ase secretion by this fungus in liquid medium and the increased root development of the inoculated plants. It should be emphasised that fungal P-ase is more effective in mineralising P than plant-derived P-ase (Tarafdar *et al.*, 2001). Therefore, the acid P-ase produced by *P. albidum* may be relevant to organic P mobilisation in volcanic soils that have a high content of organic P (Borie and Rubio, 2003).

It is noteworthy that, after transplantation, the lettuce continued to grow until reaching maturity (the commercial stage, heart stage) five months after planting. At the end of this stage, the inoculated plants presented, on average, greater development in the heart, leaves and stems than those that were not inoculated, with a significant increase in the gross weight of approximately 20% over the control (Table 5). These results also indicate, however, that the effects of this fungus are more relevant at the seedling stage of lettuce.

In addition, with the exception of Mn, there was an increase in the concentration of macro- and micronutrients in the inoculated plants, particularly for Ca, Mg and Fe (Table 5), which indicates that *P. albidum*

favours the mineral nutrition of lettuce. Nevertheless, the slight increase in the P concentration in the inoculated plants, together with the notable increase in the fresh root weight at the seedling stage, suggests that mechanisms other than P solubilisation, such as the induction of root development, might have participated in the growth and mineral nutrition of the inoculated plants.

As reported in different studies (Whitelaw 2000; Yadav and Tarafdar, 2011), the favourable effect of P-solubilising fungi on the development of plants could be improved by co-inoculating the solubilising fungus with mycorrhizae (Kohler *et al.* 2007), which suggests that a joint inoculation of *P. albidum* with native mycorrhizae could have a synergic effect on the growth of lettuce. *P. albidum* was also able to promote the growth of clover in a volcanic soil under greenhouse conditions (Morales *et al.*, 2007). Therefore, *P. albidum* appears to be a potential biofertiliser in volcanic soils, and its effect on other plants, in addition to its distinctive properties in P mobilisation, are currently being evaluated in our laboratory.

## 4. Conclusions

For most of the soils tested, the proportion of phosphofungi solubilising organic P and their solubilisation index were greater than those solubilising inorganic P. The *in vitro* solubilisation of P was associated with the acidification of the medium.

Of those tested, the Asea-Wakelin medium appears to be best suited to evaluate the solubilising capacity of the phosphofungi isolated from the volcanic soils of southern Chile.

Due to its capacity to solubilise inorganic and organic P, its specific solubilising activity, the secretion of phosphatase and the benefits to the growth and mineral nutrition of lettuce, the *P. albidum* phosphofungus appears to have the most potential as a solubiliser and mineraliser of P in volcanic soils, and it is, therefore, appropriate to evaluate its effect on plants, either alone or co-inoculated with mycorrhizae.

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