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ORIGINAL PAPER

Aspergillus tubingensis and *Talaromyces islandicus* Solubilize Rock Phosphate Under Saline and Fungicide Stress and Improve *Zea mays* Growth and Phosphorus Nutrition

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Abstract

The purpose of this study was to evaluate the capability of *Aspergillus tubingensis* and *Talaromyces islandicus* to solubilize inorganic phosphorus sources, their activity under abiotic stress, and the enhancement of P availability in soils and plant growth. The P-solubilizing capability and acidification mechanism of the strains were assessed in vitro using tricalcium phosphate and rock phosphate. Independent assays were conducted with rock phosphate under NaCl and fungicides carbendazim, chlorothalonil, and propamocarb hydrochloride using a factorial design. Thereafter, the effects of fungal inoculations in rock phosphate–amended soil and P nutrition of *Zea mays* were assessed in a greenhouse experiment. Both fungi solubilized P in vitro via acidification through the exudation of acetic, citric, lactic, malic, quinic, and succinic acids. The P-solubilizing efficiency of *A. tubingensis* was maintained above 97.5% under 0.5 to 3.0% NaCl, up to 28.7% in the treatment with carbendazim, up to 5.3% with chlorothalonil, and above 96.5% with propamocarb hydrochloride; while *T. islandicus* efficiency decreased to 45.2% in a NaCl concentration-dependent trend, and maintained it above 80% in the fungicide treatments. The inoculation with *A. tubingensis* increased the available P in the amended soil by up to 65% after 30 days and resulted in 87% higher foliar P content, 111% greater plant height, and 25% greater dry weight of maize shoots. Similarly, *T. islandicus* contributed to these parameters in 55, 67, 90, and 17%, respectively. These findings suggest their potential as qualified phosphorus solubilizing microorganisms to develop novel and sustainable approaches for P fertilization in agriculture.

Keywords Abiotic stress · Biofertilization · Low molecular weight organic acid · Plant growth · Sustainable agriculture · Tricalcium phosphate

1 Introduction

Phosphorus (P) availability in soils raises a challenge to the sustainability of agroecosystems. This element is essential for all living organisms and strongly influences crop productivity, so its deficiency is a common concern globally (Samaddar et al. 2019). To overcome the need for soluble P in agricultural soils, P fertilizers are frequently applied. However, the costs and environmental impacts that are associated with this practice are relevant issues (Amann et al. 2018). Furthermore, most of the P in fertilizers can be immobilized by calcium (Ca^{2+}), iron (Fe^{3+}), and aluminum (Al^{3+}) through the formation of the insoluble precipitates variscite ($AlPO_4{\cdot}2H_2O$), strenite ($FePO_4{\cdot}2H_2O$), and vivianite ($(Fe_3PO_4)_2{\cdot}8H_2O$) in acidic soils, and tricalcium phosphate ($Ca_3(PO4)_2$), dicalcium phosphate ($CaHPO_4$), fluorapatite ($Ca_5(PO_4)_3F$), and hydroxyapatite ($Ca_5(PO_4)_3OH$) in alkaline soils (Zhu et al. 2018). In fact, it has been stated that these would represent a potential reservoir of this nutrient, which is commonly described as "Legacy P" (Condron et al. 2013). Consequently, increased attention is being paid to finding alternative sources

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of P, such as organic amendments (Wu et al. 2019), agricultural wastes (Wang et al. 2015), and rock phosphate (Vassilev and Vassileva 2003), but the effectiveness of these amendments still needs to be improved.

Phosphorus solubilizing microorganisms (PSMs) are considered crucial in the transition to a sustainable agriculture (Mora et al. 2017), as they play a fundamental role in the P cycle due to the ability to mobilize this element from different sources (Musarrat and Khan 2014). In the last decades, studies on P-solubilizing bacteria have found that the main mechanisms involve acidification, chelation, and exchange reactions associated with the exudation of low molecular weight organic acids (Vazquez et al. 2000; Chen et al. 2006; Collavino et al. 2010; Lobo et al. 2019). However, several species of fungi have garnered attention in P solubilization for their higher efficiency when compared to bacteria (Chuang et al. 2007). Previous studies have demonstrated the potential of the fungal species belonging to the genera *Aspergillus*, *Penicillium*, and *Trichoderma* (Wakelin et al. 2004; Gaind 2016; Tandon et al. 2019).

The performance of beneficial microorganisms could be limited in stressful environments, when their capacity to establish, multiply, and spread through the soil is affected. In this respect, it is accepted that the solubilizing capability depends on the physical and chemical properties of soil, which are influenced by factors such as the salinity, temperature, element content, and xenobiotics (Herrera et al. 2019; Boroumand et al. 2020). However, some PSMs have been reported to tolerate different levels of abiotic stress and preserve their solubilizing activity. For example, *Eupenicillium parvum* was tolerant to desiccation, acidity, salinity, and aluminum toxicity (Vyas et al. 2007). Similarly, Xiao et al. (2013) demonstrated the capability of *Aspergillus niger* WHAK1 to solubilize rock phosphate over a wide range of temperature, pH, salinity, and desiccation. Srinivasan et al. (2012) demonstrated the capability of *Aspergillus terreus* to solubilize rock phosphate under salt stress, and Kanse et al. (2015) found a similar performance in *Talaromyces funiculosus* under different concentrations of NaCl, as well as a relatively high tolerance to the presence of fungicides. Therefore, stress-tolerant fungi are of particular interest to improve the availability of P in crops under changing environmental conditions (Babu and Reddy 2011; Xiao et al. 2013).

Most of the PSMs are commonly selected based on the test for solubilization of tricalcium phosphate under in vitro conditions. However, this criterion in itself would be relatively weak and unreliable to guarantee enhancing plant growth (Bashan et al. 2013). Further research is required regarding the interactions between PSMs, different sources of P, and abiotic stress conditions, as well as confirmatory studies of P uptake and plant growth through greenhouse or field-scale experiments (Musarrat and Khan 2014; Tandon et al. 2019). Previously, the potential P-solubilizing capability of *A. tubingensis* and *T. islandicus* has been suggested (Sudhakara Reddy et al. 2002; Mendes et al. 2013). In addition, Babu and Reddy (2011) reported that the co-inoculation of soil with *A. tubingensis* and arbuscular mycorrhizal fungi promoted the plant growth. However, the stress tolerance of these fungal strains and the effects of their inoculation into the soil on P nutrition in plants are not well clarified.

Thus, the hypothesis of the present study was that *A. tubingensis* and *T. islandicus* are suitable PSMs candidates and have the capability to solubilize inorganic P under saline or fungicide stressful conditions, improve P fertilization, and enhance plant growth. Therefore, the objectives were to evaluate the two fungal strains in (i) the efficiency and mechanisms when solubilizing tricalcium phosphate and rock phosphate, (ii) the capability to solubilize P under salinity or fungicide stress, and (iii) the improvement of P availability in soils amended with rock phosphate and the increase in P uptake by plants. This study forms part of our efforts to improve the selection of potential PSMs inoculants as a novel alternative for P management in agricultural soils.

2 Material and Methods

2.1 Experimental Overview

In this study, three different experimental approaches were employed to assess the PSMs characteristics of the fungi *A. tubingensis* and *T. islandicus*. First, an in vitro test was performed for 20 days to evaluate the P-solubilizing capability by the fungal strains using a liquid medium supplemented with tricalcium phosphate or rock phosphate. In addition, the medium pH and exudation of low molecular weight organic acids were quantified to describe the mechanisms involved in the inorganic P solubilization in the experiment. Second, two independent in vitro tests were conducted to assess the P-solubilizing capability of the strains under salt stress (NaCl) and in the presence of fungicides carbendazim, chlorothalonil, and propamocarb hydrochloride. Finally, a greenhouse pot experiment was performed with rock phosphate–amended soil to evaluate the effect of the inoculation with the fungal strains on the amount of available P in the soil, its uptake and growth of *Zea mays* after 30 days. Details of each of the methods and experiments are provided below.

2.2 Fungal Strains, Culture Media, and Inoculum Preparation

Fungal strains *A. tubingensis* and *T. islandicus* were obtained from the research group GRINBIO (Bioprospecting Program, Universidad de Medellín, Colombia). The molecular characterization is described in the supplementary information (Supplementary description 1, Fig. S1, and Fig. S2). Prior to

the experiments, a single-spore potato dextrose agar (PDA) culture of each fungal strain was grown for 7 days. After that, the inoculant of each strain was prepared at the concentration of 1×10^6 conidia mL^{-1} using a hemocytometer.

Rock phosphate (hydroxyapatite $Ca_5(PO_4)_3(OH)$, Rio Claro Tecnología en Agricultura S.A.S., Colombia) and tricalcium phosphate ($Ca_3(PO_4)_2$ Sigma-Aldrich) were used in the in vitro experiments as representative inorganic P sources with different complexities. In all of the assays, 1 mL of inoculum was added to 100 mL of NBRIP liquid medium (Shekhar Nautiyal 1999) supplemented with 5 g L^{-1} of each P source in 250 mL Erlenmeyer flasks. The general culture conditions were motion at 100 rpm in horizontal shaker (Innovaciones Actum S.A.S) at 30 °C in the darkness.

2.3 In Vitro Solubilization of Inorganic P Sources

The P-solubilizing capability of each of the fungal strains was assessed using a factorial design (2 × 2), giving the following treatments: *A. tubingensis* + rock phosphate, *A. tubingensis* + tricalcium phosphate, *T. islandicus* + rock phosphate, and *T. islandicus* + tricalcium phosphate. Uninoculated Erlenmeyer flasks were used as a control. Each treatment was prepared in triplicate and the experiment was carried out for 20 days. The liquid medium was sampled after 4, 5, 10, 15, and 20 days for P quantification, the pH determination, and analysis of low molecular weight organic acids. The time of sampling was selected according to preliminary observations, which showed that the pH in the medium decreased after 4 days of incubation.

2.4 In Vitro Assay for Rock Phosphate Solubilization Under Stress Conditions

2.4.1 Saline Stress Experiment

The interaction between the fungal strains and different levels of salinity during the rock phosphate solubilization process were analyzed using a factorial design (2 × 6). A culture was prepared in a liquid NBRIP medium supplemented with 5 g L^{-1} of rock phosphate, as already described (section 2.2.), and was enriched with sodium chloride (NaCl; TACChem®) to obtain final concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0% NaCl (*w/v*). Erlenmeyer flasks that had been inoculated with each strain without the addition of NaCl were used as a control. The treatments were tested in triplicate, and after 10 days, an aliquot of the liquid medium was obtained to determine the final amount of P solubilized.

2.4.2 Fungicide Stress Experiment

Changes in the solubilization of rock phosphate by the fungal strains in the presence of different fungicides were evaluated using a factorial design (2 × 3 × 3). Three different compounds, representative of current use fungicides (Lewis et al. 2016), were prepared from their commercial formulations: carbendazim (500 g L^{-1}; Colizym 500® (Colinagro, Colombia)), chlorothalonil (500 g L^{-1}; Control 500® (ADAMA Andina B.V., Colombia)), and propamocarb hydrochloride (700 g L^{-1}; Previcur® (Bayer S.A., Colombia)). The NBRIP liquid medium was supplemented with three concentrations of each fungicide, calculated on the active ingredient basis: 12, 25, and 50 mg L^{-1} of carbendazim; 77, 155, and 310 mg L^{-1} of chlorothalonil; and 52, 105, and 210 mg L^{-1} of propamocarb hydrochloride. Erlenmeyer flasks that had been inoculated with each strain without the addition of fungicides were used as a control. The treatments were tested in triplicate, and an aliquot of the liquid medium was taken after 10 days to determine the amount of soluble P.

2.5 Greenhouse Pot Experiment

The effects of fungal inoculation in soils with and without rock phosphate on the P availability, P uptake, and plant growth were evaluated using a factorial design. The strains and two rock phosphate levels (0 and 5% w/w) were used with three replicates per treatment. Non-sterile loamy soil (9 mg kg^{-1} P, pH 6.5) was used for the experiment. Soil samples were air-dried, ground, and sieved (< 2.0 mm) prior to the experiments. Commercial fertilizers urea (100 mg kg^{-1}) and KCl (150 mg kg^{-1}) were added to supply N and K respectively. Certified seeds of *Zea mays* var. ICA V-305 (Agrosemillas S.A.S., Colombia) were individually planted in pots containing 500 g of soil with the respective treatment of rock phosphate. Then, 1.0 mL of the inoculant of each strain was applied to the sown seeds. In addition, uninoculated seeds were planted in soil with or without rock phosphate (5% w/w) as controls, giving the following treatments: (i) uninoculated soil without rock phosphate, (ii) uninoculated soil with added rock phosphate, (iii) soil without rock phosphate inoculated with *A. tubingensis*, (iv) soil without rock phosphate inoculated with *T. islandicus*, (v) soil with the addition of rock phosphate inoculated with *A. tubingensis*, and (vi) soil with the addition of rock phosphate inoculated with *T. islandicus*.

The plants were grown under greenhouse conditions with an average temperature of 25 °C, a relative humidity of 50–60%, and a 12-h light and 12-h dark photoperiod using natural lighting. The moisture content of the soil was maintained at 60% of its maximum water-holding capability (WHC) to avoid nutrient leaching and root damage. After 30 days, soil samples were collected for pH measurement and available P quantification by the Olsen-P extraction method (Pansu and Gautheyrou 2006). *Z. mays* seedlings were harvested to determine root and shoot elongation, dry biomass, and P concentration in the leaves.

2.6 Chemical Analyses

2.6.1 Phosphorus Quantification

The concentration of P was measured in the liquid medium after the in vitro experiments and in the soil and leaves after the greenhouse experiment. For each in vitro treatment, an aliquot of the liquid medium was filtered through a 0.45-μm membrane prior to analysis. The bulk soil samples were prepared using sodium bicarbonate extraction, and the plant leaves were digested using 2.0 mL of 6.0 N HCl and 10.0 mL of 1.0 N HNO_3. Afterwards, the amount of P in each sample was quantified using the molybdenum blue colorimetric method with an UV-visible 8500 spectrophotometer at 800 nm (Murphy and Riley 1962).

2.6.2 Measurement of pH and Low Molecular Weight Organic Acids Quantification

A filtered aliquot of the liquid medium was used for pH measurement by a HQd portable pH meter with a glass electrode (HACH®). The pH in the bulk soil samples was measured using the saturation paste method (2:1 ratio). The low molecular weight organic acids were quantified in an aliquot of the liquid medium using high-performance liquid chromatography (HPLC). Quinic, acetic, and lactic acids were quantified with a Synergi 4 μm Hydro-RP 80 Å column at room temperature using a phosphoric acid buffer with sodium phosphate monohydrate (pH = 2.9) as the mobile phase and a flow rate of 0.7 mL min^{-1}. Malic, citric, and succinic acids were quantified with an HPX 87H column at a temperature of 55 °C, using 5 mM of sulfuric acid as the mobile phase and a flow rate of 1 mL min^{-1}. For both systems, an injection volume of 20 μL and a wavelength of 205 nm were used.

2.7 Statistical Analyses

All data are presented as mean values and standard deviation of three replicates. The significance of the differences between treatments were analyzed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test. The interaction between the fungal strains and rock phosphate amendment of the soil in the greenhouse pot experiments were analyzed using two-way ANOVA. Prior to analysis, Bartlett's test and the Shapiro-Wilk test were applied to verify the assumptions of homogeneity of variance and data normality, respectively. Pearson's correlation analysis was used to examine the relationships between the pH, solubilized P, and low molecular weight organic acids for in vitro tests results, and between soil pH, available P in soil, the plant growth parameters, and foliar P in greenhouse experiments. All statistical tests were performed using Statgraphics® Centurion XVI software, with $P \leq 0.05$ considered significant. In addition, the production of low molecular weight organic acids by the fungal strains was examined through cluster and heat-map analysis using the gplots 3.0.3 package in R software version 3.2.3.

3 Results

3.1 Phosphorus Solubilizing Capability and Exudation of Low Molecular Weight Organic Acids

Tables S1 and S2 show the changes in pH, soluble P, and low molecular weight organic acid concentrations in the tricalcium phosphate and rock phosphate solubilization by *A. tubingensis* and *T. islandicus*, respectively. There was a drastic decrease in the pH of the culture medium and an increase in soluble P concentration with both strains. When rock phosphate was used as a P source, *A. tubingensis* showed the highest concentration of soluble P on day 15 (510.2 mg L^{-1}), when the pH of the medium was 3.0, whereas *T. islandicus* reached the highest concentration on day 20 (493.2 mg L^{-1}), when the pH was 3.1. In the case of tricalcium phosphate, the maximum soluble P concentration was reached by *A. tubingensis* on day 20 (495.0 mg L^{-1}), corresponding to a pH of 3.5, and by *T. islandicus* on day 5 (497.9 mg L^{-1}), when the pH of the medium was 4.6. Both *A. tubingensis* and *T. islandicus* were more efficient in solubilizing rock phosphate.

The analysis by HPLC allowed the identification of low molecular weight organic acids quinic, acetic, lactic, malic, citric, and succinic during the solubilization process. The concentrations of low molecular weight organic acids varied with the incubation time, strain, and P source. When rock phosphate was used as P source, both strains produced more quinic acid than other low molecular weight organic acids, with the concentration tending to increase with time for *A. tubingensis* and decrease with time for *T. islandicus* (Table S1). In the case of tricalcium phosphate, *A. tubingensis* showed increased production of quinic and acetic acids over time, while *T. islandicus* showed an increase in the production of malic, acetic, and quinic acids over time (Table S2).

In the rock phosphate–supplemented medium that was inoculated with *A. tubingensis*, the highest concentration of low molecular weight organic acids was detected after 20 days (194.9 mg L^{-1}). The composition had 73% quinic acid (141.4 mg L^{-1}); 20% acetic acid (38.1 mg L^{-1}); 3% malic acid (6.5 mg L^{-1}); 3% citric acid (6.2 mg L^{-1}); and 1% lactic acid (1.8 mg L^{-1}) and succinic acid (0.9 mg L^{-1}). By contrast, the solubilization of rock phosphate by *T. islandicus* resulted in the highest concentration of low molecular weight organic acids on day 4 (274.4 mg L^{-1}). The composition was 93% quinic acid (255.3 mg L^{-1}); 3% acetic acid (8.1 mg L^{-1}); 2% malic acid (5.6 mg L^{-1}); and 2% lactic acid (4.8 mg L^{-1}) and succinic acid (0.6 mg L^{-1}).

In the tricalcium phosphate-supplemented medium that was inoculated with *A. tubingensis*, the highest concentration of low molecular weight organic acids was observed on day 20 (137.0 mg L^{-1}). The composition was 51% quinic acid (70.1 mg L^{-1}); 44% acetic acid (59.6 mg L^{-1}); 4% malic acid (6.0 mg L^{-1}); and 1% citric acid (0.9 mg L^{-1}), succinic acid (0.3 mg L^{-1}), and lactic acid (0.1 mg L^{-1}). Similarly, in the solubilization of tricalcium phosphate by *T. islandicus*, it was observed the highest concentration of low molecular weight organic acids on day 20 (333.3 mg L^{-1}). The composition was 71% malic acid (237.7 mg L^{-1}); 18% acetic acid (61.6 mg L^{-1}); 9% quinic acid (30.8 mg L^{-1}); and 2% lactic acid (1.3 mg L^{-1}), citric acid (1.1 mg L^{-1}), and succinic acid (0.8 mg L^{-1}). Pearson's correlation analysis suggested significant associations between solubilized P, pH, and concentration of low molecular weight organic acids (Table 1).

Cluster and heat-map analysis allowed underlying different stages in the P solubilization process, determined by the types and concentrations of low molecular weight organic acids produced by fungal strains over time using each inorganic P source. *A. tubingensis* showed a two-stage process in the solubilization of rock phosphate (Fig. 1a). The first one between days 4 to 15 was characterized by an average production of 96.7 mg L^{-1} of quinic acid and 7.3 mg L^{-1} of acetic acid. The second stage was characterized by a 5-fold increase of acetic acid and the maximum production of quinic acid after 20 days. In the case of the solubilization of tricalcium phosphate by *A. tubingensis* (Fig. 1b), the dendrogram showed that the time between 4 and 5 days was characterized by the absence of succinic acid and the lowest concentrations of the other low molecular weight organic acids, whereas between 10 and 15 days, the concentrations of quinic acid were 47.6 and 28.4 mg L^{-1}, respectively. The highest concentrations of quinic acid and acetic acid were observed after 20 days (70.1 and 37.5 mg L^{-1}). Rock phosphate solubilization by *T. islandicus* was carried out in two stages (Fig. 1c), which were characterized by maximum production of quinic acid (255.3 mg L^{-1}) during days 4 to 10, while from day 15 to 20, the low concentration of quinic acid (2.1 mg L^{-1}) was observed with an increase of citric acid (10.9 mg L^{-1}). The dendrogram showed three stages of solubilization of tricalcium phosphate by *T. islandicus* (Fig. 1d): the first stage corresponded to day 4, which was characterized by the lowest concentration of malic acid (11.3 mg L^{-1}). The second stage was between 5 and 10 days, which were characterized by the lowest values of quinic, acetic, lactic, citric, and succinic acids. Finally, the third stage occurred between 15 and 20 days, which were characterized by the maximum production of malic acid (171.5 and 237.7 mg L^{-1}).

3.2 Phosphorus Solubilization Under Stress Conditions

The effects of NaCl salinity and three different fungicides on the P solubilization by the strains *A. tubingensis* and *T. islandicus* were evaluated using liquid culture (Table 2). The presence of NaCl (0.5–3.0%) significantly reduced the efficiency of *T. islandicus* to solubilize P in a concentration-dependent pattern (45.2–97.4%), but had no significant effects on *A. tubingensis*. In regard to fungicides, *A. tubingensis* had a significant lower response solubilizing P in presence of carbendazim (5.5–28.7%) and chlorothalonil (3.1–5.3%), but not propamocarb hydrochloride (96.5–98.8%), while *T. islandicus* had a solubilization efficiency of about 55.0 to 99.9% in the different trials.

3.3 Contribution of Fungi in Phosphorus Availability in Soil, its Uptake, and Plant Growth

The inoculation with *A. tubingensis* or *T. islandicus* into the soil treatments reduced pH, increased the available P, foliar P content, and growth of *Z. mays* seedlings (Table 3). This parameters were significantly correlated (Table 4), and the observed changes were mainly due to the fungi inoculation

Table 1 Pearson's correlation analysis between solubilized P, pH, and concentration of low molecular weight organic acids during in vitro solubilization of inorganic P sources by *Aspergillus tubingensis* and *Talaromyces islandicus*

	pH	Solubilized P	Quinic acid	Acetic acid	Lactic acid	Malic acid	Citric acid	Succinic acid	Total organic acids
pH		−0.72**	−0.48*	−0.16	−0.33	−0.04	−0.05	0.08	−0.39
Solubilized P			0.01	0.39	0.177	0.20	0.24	0.17	0.27
Quinic acid				−0.03	0.37	−0.17	−0.07	−0.06	0.56*
Acetic acid					−0.00	0.71**	0.19	0.23	0.69**
Lactic acid						−0.02	0.58*	0.58*	0.34
Malic acid							−0.08	−0.02	0.68**
Citric acid								0.93***	0.10
Succinic acid									0.158
Total organic acids									

Statistical significance is represented by $*P < 0.05$, $**P < 0.001$, $***P < 0.0001$

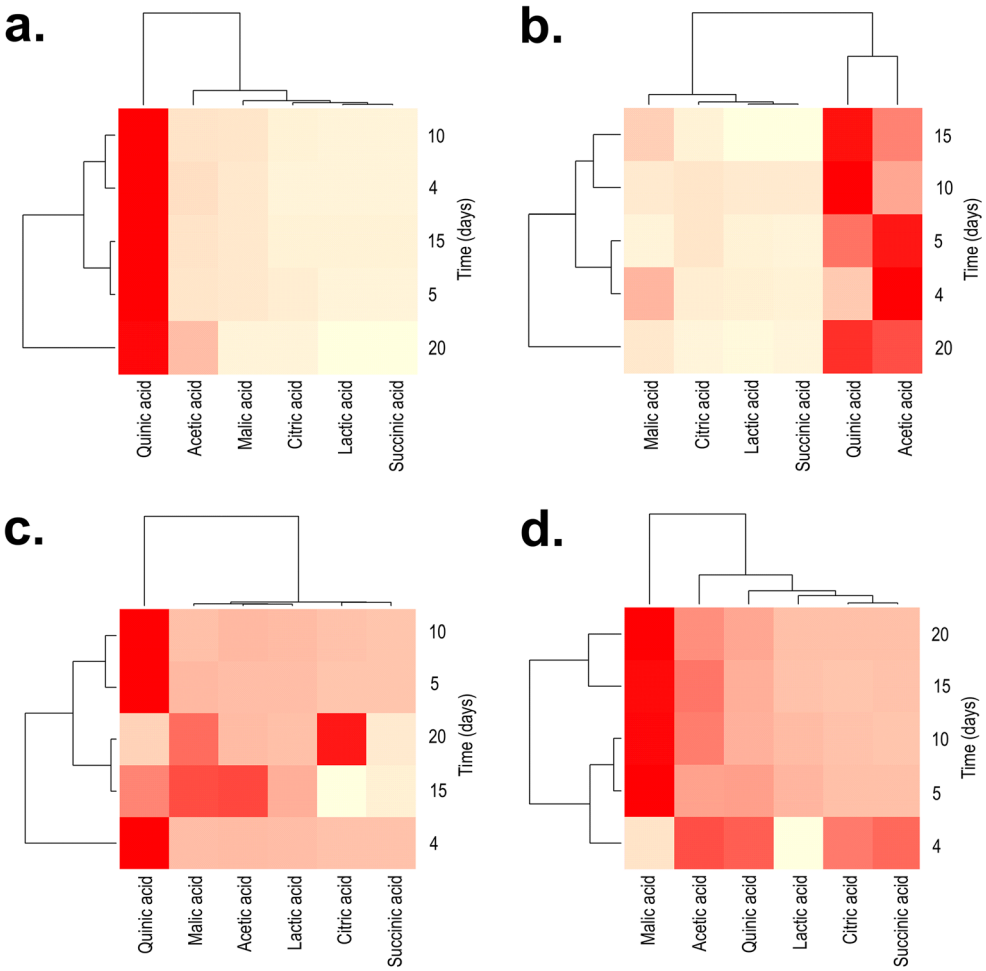

Fig. 1 Cluster and heat-map analysis of the concentration of low molecular weight organic acids during in vitro solubilization of inorganic P sources by *Aspergillus tubingensis* for **a** rock phosphate, **b** tricalcium phosphate; and by *Talaromyces islandicus* for **c** rock phosphate, and **d** tricalcium phosphate. White and red indicate normalized concentration gradient of concentration of low molecular weight organic acids from low to high, respectively. Concentrations of low molecular weight organic acids were quantified from in vitro culture at 4, 5, 10, 15, and 20 days

rather than the application of rock phosphate to the soil, as confirmed in the interaction analysis in the two-way ANOVA (Table S3). The available P concentration was 19.5 mg kg^{-1} for *A. tubingensis* and 18.3 mg kg^{-1} for *T. islandicus*, significantly different to the 11.8 mg kg^{-1} observed in the control treatment without inoculation (Table 3). *A. tubingensis* or *T. islandicus* also led a significant increase of the available P concentration in the soil that had not been amended with rock phosphate, resulting in 17.4 mg kg^{-1} P and 15.4 mg kg^{-1} P respectively, compared with the uninoculated control (10.3 mg kg^{-1}). The inoculation with *A. tubingensis* led a foliar P content in *Z. mays* seedlings of 0.28% and 0.19% in soils with and without rock phosphate (Table 3). Similarly, the foliar P content was 0.25% and 0.17% respectively, with *T. islandicus* inoculation.

4 Discussion

This study demonstrated the potential of *A. tubingensis* and *T. islandicus* as promising and efficient PSMs. Various relevant findings were identified in the study of these strains: (i) their efficiency in the solubilization of inorganic P was higher than the average reported fungi; also, the acidification mechanism by exudation of different low weight molecular organic acids was characterized; (ii) the fungal strains were able to modulate their capabilities to solubilize rock phosphate under the influence of salinity and fungicides; (iii) their capabilities to improve the availability of P in soils from added rock phosphate was demonstrated in greenhouse experiment, as well as the enhancement in P uptake and plant growth. The implications of these findings are herein discussed to contribute to the understanding of PSMs selection and support further practical applications in crop production under adverse environmental conditions.

The P-solubilizing capabilities of fungi belonging to the genera *Aspergillus*, *Talaromyces*, among others have been reported to range from 70.8 to 1075.0 mg L^{-1} for tricalcium phosphate and 43.3 to 296.2 mg L^{-1} for rock phosphate (Table 5). This indicates that the fungi strains evaluated in the present study are qualified candidates for solubilizing

Table 2 In vitro solubilization of rock phosphate by *Aspergillus tubingensis* and *Talaromyces islandicus* in liquid medium (NBRIP) supplemented with NaCl and fungicides after 30 days

Abiotic stress	*Aspergillus tubingensis*		*Talaromyces islandicus*	
	Solubilized P (mg L^{-1})	P solubilization efficiency (%)	Solubilized P (mg L^{-1})	P solubilization efficiency (%)
NaCl (% w/v)				
0.5	503.9 ± 6.0ab	99.3	456.2 ± 3.2e	97.4
1.0	498.1 ± 1.9ab	98.1	434.5 ± 1.9d	92.8
1.5	503.1 ± 1.0ab	99.1	314.1 ± 2.8c	67.1
2.0	496.5 ± 5.0a	97.8	240.4 ± 1.4b	51.3
3.0	495.1 ± 4.8a	97.5	211.8 ± 1.3a	45.2
Control (without NaCl)	507.6 ± 1.6b	100	468.2 ± 2.7e	100
Fungicides (mg L^{-1})				
Carbendazim				
25	150.3 ± 1.9c	28.7	458.3 ± 2.7c	97.3
50	65.9 ± 1.0b	12.6	432.8 ± 0.9b	91.9
100	28.6 ± 2.8a	5.5	389.5 ± 1.5a	82.7
Chlorothalonil				
155	27.6 ± 1.1b	5.3	450.2 ± 1.4a	95.5
310	24.5 ± 1.0b	4.7	385.1 ± 3.0b	81.7
620	16.5 ± 1.5a	3.1	259.5 ± 1.7a	55.0
Propamocarb hydrochloride				
75	518.5 ± 2.0bd	98.8	470.6 ± 3.1ad	99.9
150	512.4 ± 4.0ab	97.7	469.8 ± 5.0ad	99.7
300	506.3 ± 5.4a	96.5	460.5 ± 1.2a	97.7
Control (without fungicides)	524.6 ± 1.5d	100	471.2 ± 2.3d	100

The values represent the mean of three replicates ± standard deviation. Values that are followed by different letters within a column are significantly different ($P \leq 0.05$; Tukey's test)

inorganic P sources, particularly rock phosphate, which is commonly used to supply P in agricultural soils.

The efficiency in P solubilization was associated with the capacity to acidify the culture medium, which is explained by the low molecular weight organic acids exudation and protons excretion mechanisms (Prabhu et al. 2019). In this study, it was supported by the significant negative correlation ($r = -0.72$, $P < 0.001$) between pH and P solubilized (Table 1). The

Table 3 Effects of inoculation with *Aspergillus tubingensis* and *Talaromyces islandicus* in soils with and without rock phosphate (RP) on the pH, P availability, foliar P content, and growth of *Zea mays* seedlings after 30 days

Treatment	Soil pH	Available P (mg kg^{-1})	Root elongation (cm)	Plant height (cm)	Dry stem biomass (mg)	Dry root biomass (mg)	Foliar P content (%)
A. tubingensis							
Soil	6.5 ± 1.5a	17.4 ± 1.0b	18.9 ± 0.2b	20.5 ± 0.3d	180.6 ± 2.0d	260.5 ± 3.0c	0.19 ± 0.03c
Soil + RP	6.6 ± 1.0a	19.5 ± 1.3c	19.8 ± 0.1b	30.2 ± 2.3e	200.1 ± 0.3e	279.7 ± 3.0d	0.28 ± 0.02d
T. islandicus							
Soil	6.7 ± 0.4a	15.4 ± 2.0b	17.5 ± 0.2b	18.6 ± 0.1c	168.2 ± 0.9c	275.7 ± 2.4d	0.17 ± 0.05c
Soil + RP	6.8 ± 1.0a	18.3 ± 0.9c	16.4 ± 0.1a	27.2 ± 1.2e	185.8 ± 1.0d	298.3 ± 1.5e	0.25 ± 0.04d
Control							
Soil	7.2 ± 2.0a	10.3 ± 1.0a	15.8 ± 0.1a	9.9 ± 0.2a	135.5 ± 1.5a	243.1 ± 4.0a	0.11 ± 0.01a
Soil + RP	7.1 ± 1.0a	11.8 ± 1.2a	18.6 ± 0.3b	14.3 ± 0.1b	159.2 ± 3.0b	254.5 ± 5.0b	0.15 ± 0.02b

The values represent the mean of three replicates ± standard deviation. Values that are followed by different letters within a column are significantly different ($P \leq 0.05$; Tukey's test)

Table 4 Pearson's correlation analysis between soil pH, available P concentration in soil, root elongation, plant height, dry stem biomass, dry root biomass, and foliar P content in *Zea mays* seedlings after 30 days of greenhouse experiment

	Soil pH	Available P	Root elongation	Plant height	Dry stem biomass	Dry root biomass	Foliar P
Soil pH		− 0.84**	− 0.74**	− 0.71**	− 0.84**	− 0.75**	− 0.78**
Available P			0.75**	0.94***	0.97***	0.87**	0.96***
Root elongation				0.68*	0.81**	0.51	0.70**
Plant height					0.96***	0.80**	0.97***
Dry stem biomass						0.81**	0.96***
Dry root biomass							0.86**
Foliar P							

Statistical significance is represented by $*P < 0.05$, $**P < 0.001$, $***P < 0.0001$

results of HPLC analysis (Table S1 and S2) showed that *A. tubingensis* and *T. islandicus* produced quinic, acetic, lactic, malic, citric, and succinic acids; most of them related to the Krebs cycle (Li et al. 2016). The concentration and type of acids were different depending on time and the P source (Fig. 1), as reported in previous studies (Chuang et al. 2007; Xiao et al. 2013; de Oliveira Mendes et al. 2014; Liu et al. 2014). However, the correlation analysis showed a significant association between pH and quinic acid concentration ($r = -0.48$, $P < 0.05$) but not with the total organic acids (Table 1). Hence, it is important to mention that this phenomenon may not depend on the amount of acids produced but rather the

Table 5 Phosphorus solubilizing capability of *Aspergillus tubingensis*, *Talaromyces islandicus*, and other fungal strains reported in previous studies

Inorganic P source	Strain	Solubilized P (mg L^{-1})	Culture time (days)	Reference
Tricalcium phosphate	*Aspergillus niger*	1075.0	7	de Oliveira Mendes et al. (2014)
	Aspergillus niger	773.0 ± 0.13[a]	8	Jain et al. (2017)
	Penicillum sp.	711.0 ± 0.02[a]	10	Jain et al. (2017)
	Aspergillus tubingensis	495.0 ± 2.1[a]	20	This study
	Talaromyces islandicus	487.5 ± 2.1[a]	20	This study
	Candida krissii	238.5	7	Xiao et al. (2008)
	Penicillium pinophilum	217.5	28	Wahid and Mehana (2000)
	Talaromyces funiculosus	187.0	10	Kanse et al. (2015)
	Trichoderma koningiopsis	70.8 ± 1.65[a]	7	Tandon et al. (2019)
Rock phosphate	*Aspergillus tubingensis*	510.2 ± 3.1[a]	20	This study
	Talaromyces islandicus	493.2 ± 1.2[a]	20	This study
	Penicillium oxalicum	296.2 ± 4.7[b]	6	Singh and Reddy (2011)
	Penicillium bilaiae	101.7 ± 4.6[b]	7	Wakelin et al. (2004)
	Aspergillus niger	100.0	5	Xiao et al. (2013)
	Penicillium simplicissimum	58.8 ± 0.6[b]	7	Wakelin et al. (2004)
	Penicillium griseofulvum	56.1 ± 1.6[b]	7	Wakelin et al. (2004)
	Talaromyces flavus	48.6 ± 2.4[b]	7	Wakelin et al. (2004)
	Penicillium radicum	43.3 ± 8.7[b]	7	Wakelin et al. (2004)

[a] Standard deviation

[b] Standard error of mean

type of acid. It has been demonstrated that the acidification of the culture medium is related to the production of acids with higher dissociation constant (Li et al. 2016). In this study, the occurrence of acids of different intensity in variable concentrations in specific time (Fig. 1) could be associated with their role in the initial surface reaction with the P source and the subsequently P acquisition. The low molecular weight organic acids are involved in calcium complexation reactions (Plassard and Fransson 2009). Likewise, it has been reported that the solubilization of rock phosphate needed the action of low molecular weight organic acids on the surface functional groups like amine and bonded OH (Xiao et al. 2013). In addition, the culture medium acidification by the excretion of protons mechanism is associated with the use of ammonia as N source by fungi (Prabhu et al. 2019), which was used in the preparation of the NBRIP in this study. The fact that significant correlations were not observed for all the acids also could be explained by the co-occurring mechanisms. Similarly, it has been reported that no obvious trend was observed between the soluble P concentration in liquid medium and the total molar organic acid production by PSMs (Jiang et al. 2018).

Particularly, in this study, quinic acid was produced at higher concentrations, especially in rock phosphate–supplemented medium (Table S1 and S2). Quinic acid has been reported in the P solubilization from tricalcium phosphate by *Bacillus megaterium* (Kang et al. 2014). Its biosynthesis has been described in *Aspergillus nidulans*, involving the transformation of erythrose 4-phosphate and phosphoenol pyruvate to produce 3-deoxy-D-arabinoheptulosonate 7-phosphate (DAHP), precursor of dehydroquinic acid; also related to the shikimic acid pathway (Hawkins et al. 1993). The results of this study provided evidence of the role of quinic acid in the solubilization of inorganic P sources by *A. tubingensis* and *T. islandicus*, suggesting the association of these pathways in P solubilization mechanisms.

This research not only addressed the potential and mechanisms for P solubilization by *A. tubingensis* and *T. islandicus*, but also demonstrated that these two fungi differentially maintained their P-solubilizing capabilities under stress conditions. The bioassay with increased NaCl in the culture medium simulated saline soil levels, where the changes in osmotic potential can negatively affect the P-solubilizing capability of PSMs. Notably, *A. tubingensis* was able to solubilize rock phosphate with an efficiency above 97.5% under the range of 0.5 to 3.0% NaCl (Table 2), while *T. islandicus* had an efficiency above 92.8% in a lower range (0.5 to 1.0% NaCl). P-solubilizing capability under salt stress conditions has also been reported for *A.s niger* (Xiao et al. 2013), *A. terreus* (Srinivasan et al. 2012), and *T. funiculosus* (Kanse et al. 2015). The tolerance to saline stress has been associated to changes in plasma membrane fluidity and mechanisms for K^+/Na^+ exchange to regulate intracellular ion homeostasis (Gunde-Cimerman et al. 2009; Santander et al. 2019). Other responses involve the production of intracellular glycerol, that acts as osmo-protector, and modifications in the cell wall biogenesis and stability against osmotic pressure, which are activated by the kinase-regulated pathways of high-osmolarity glycerol (HOG) and cell wall integrity (CWI) respectively (Hayes et al. 2014).

The occurrence of xenobiotics in soils is an increasing concern as they exert toxicity on non-target organisms, compromising microbial functions like nutrient cycling. Fungicides, herbicides, and insecticides are widely used to support the agricultural production; however, their effects on beneficial soil microorganisms must be assessed (Herrera et al. 2019; Tortella et al. 2019), given that the tolerance to pesticides is an important step screening efficient PSMs. In this study, the P-solubilizing capabilities of *A. tubingensis* and *T. islandicus* were gradually affected when exposed to fungicides (Table 2). Chlorothalonil (155 mg L^{-1}) exerted a reduction to 5.3% in the solubilization efficiency of *A. tubingensis* but not of *T. islandicus* (95.5%). Both strains maintained their efficiency above 96.5% in the presence of propamocarb hydrochloride (75 to 300 mg L^{-1}). In addition, *A. tubingensis* reduced its P solubilization efficiency to 5.5% when exposed to 100 mg L^{-1} of carbendazim, while *T. islandicus* kept its performance above 82.7%. It has been reported that *T. funiculosus* had a P solubilization efficiency of 45.2% exposed to 12.5 mg L^{-1} of carbendazim (Kanse et al. 2015). In the same study, *T. funiculosus* was able to solubilize phosphate in the presence of mancozeb or hexaconazole. Fungicides interfere with a variety of cellular functions, which could activate responses through CWI or calcineurin pathways to induce alleviation mechanisms like the modulation of cell wall components, integral membrane proteins, antioxidants, and vacuolar functions (Hayes et al. 2014). The fungicides were evaluated in a range of concentrations consistent with their recommended application rate in agriculture. Therefore, although the decrease in solubilized P was generally less prominent in *T. islandicus*, both strains could still be considered efficient in P solubilization. Taking these results together with the salinity bioassay, the hypothesis of the suitability of *A. tubingensis* and *T. islandicus* as stress-tolerant PSMs is confirmed.

Although liquid medium assays are commonly used to assess the P solubilization capability by soil microorganisms, they do not correspond directly to the effect that these microorganisms may have on P availability in soil and its uptake by plants (Bashan et al. 2013; Kanse et al. 2015). This study obtained confirmatory results from greenhouse experiments about the effects of *A. tubingensis* and *T. islandicus* as inoculants in soil amended with rock phosphate and their contribution to P nutrition in *Z. mays* (Table 3).

A reduction in soil pH was observed when the fungal strains were used as inoculants. The decrease in the pH values (6.5–6.8) compared with the uninoculated control (7.1–7.2)

could be related with the acidification mechanisms in P solubilization through chelation and exchange reactions. A significant correlation between pH and available P was observed (Table 4); this indicated that acidification was an important mechanism to improve P solubilization, both in soils and in vitro conditions. Importantly, sole rock phosphate treatments had a lower contribution to plant growth and the effect in available P in soils was not significant (Table 3), as previously demonstrated by Khan et al. (2020). In addition, *A. tubingensis* and *T. islandicus* increased the concentration of available P in soil without rock phosphate treatments; it could be explained by the capability to desorb P from soil minerals. This mechanism has been demonstrated in *Mortierella* sp. (Osorio and Habte 2013), suggesting that legacy P in the soils is also a target for PSMs.

Available P in soil amended with rock phosphate increased to 55.1% by *T. islandicus* and 65.3% by *A. tubingensis* (Table 3). These results demonstrated that the inoculation with *A. tubingensis* and *T. islandicus* was effective in enhancing the fertilizer value of rock phosphate in soils. Consistently, the growth parameters of *Z. mays* and foliar P content significantly increased in soils with higher available P (Table 3). This capability has also been reported for *Penicillium oxalicum* in *Z. mays* (Singh and Reddy 2011), *Aspergillus fumigatus*, *A. niger*, *Penicillium pinophilum* (Wahid and Mehana 2000), *Trichoderma harzianum* (Gaind 2016), and *A. niger* (Xiao et al. 2013) in wheat plants. However, it is important to mention that several fungi could promote the plant growth by different mechanisms without increase available P in soil. In this regard, it has been advised to confirm PSMs activity with the assessment of foliar P content (Bashan et al. 2013). In this research, the greenhouse experiment provided results with a significant and positive correlation between the available P, foliar P, and general plant growth (Table 4), supporting the PSMs potential of the strains.

While this study provided the P-solubilizing profile of *A. tubingensis* and *T. islandicus* under stressful conditions and confirmed their contribution to plant P uptake in soils, ecological and physicochemical interactions of practical relevance should be considered for the successful development of biofertilizers. Hence, following the results of this study and its potential applications, it is recommended to address (i) the use of different P sources, even mixed; (ii) the confirmation of the increase of available P in soil and the enhancement of P uptake by plants; (iii) the assessment of biotic and abiotic stress, even in field conditions; (iv) the capabilities of the strains in alternative plant growth promotion mechanisms; and (v) the microbial interactions during colonization and its successful establishment when inoculated into the soil.

Novel fertilization approaches are essential to ensure a global sustainable agriculture. In this context, P has become an element of major concern at different latitudes due to its limited availability and agronomic efficiency, taking into account current changing environmental conditions. Among the different research advances to face this problem, the prospection of PSMs has gained a great relevance as an innovative solution to contribute integrally to the agricultural production and environmental sustainability (Barra et al. 2018, 2019). Overall, *A. tubingensis* and *T. islandicus* could be recommended for P biofertilization approaches in agricultural soils with deficiencies in available phosphorus.

5 Conclusions

This study confirmed the contribution of *A. tubingensis* and *T. islandicus* as PSMs. The fungal strains were also effective in solubilizing P from rock phosphate under salinity conditions and in the presence of fungicides, as well as enhanced P nutrition and plant growth, highlighting their *biotechnological relevance*. These fungi are of interest as novel inoculants to improve P management in agroecosystems.

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Authors' Contributions Julián E. López, Jorge L. Gallego: conceptualization, experimental design, methodology validation, assays development, data analysis, writing, review, and editing of the paper.

Alejandra Vargas-Ruiz, Amny Liceth Peña- Mosquera: soil samples collection, fungal strains maintenance, methodology validation, assays development, and data collection.

Arley David Zapata-Zapata: HPLC analysis and funding acquisition.

Idalia Jaqueline López-Sánchez: project administration and review of the paper.

Liliana Rocio Botero-Botero: conceptualization, funding acquisition, project administration, supervision, review and editing of the paper.

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Data Availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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