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Phosphate solubilization by stress-tolerant soil fungus *Talaromyces funiculosus* SLS8 isolated from the Neem rhizosphere

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Abstract A promising biotechnological strategy in the management of phosphorus (P) fertilization is the use of phosphate-solubilizing fungi to solubilize rock phosphates and allow the recovery of unavailable P fixed to soil particles. Phosphate-solubilizing rhizosphere fungus, *Talaromyces funiculosus* SLS8, isolated from Neem (*Azadirachta indica*) on saline soil, was tolerant to environmental stressors, salinity and agricultural systemic fungicides. Phosphate solubilization under different nutritional conditions was investigated by culturing *T. funiculosus* SLS8 in Pikovskaya liquid medium containing different nitrogen sources (ammonium sulfate, casein, urea, potassium nitrate or sodium nitrate) and carbon sources (glucose, fructose, galactose or sucrose), NaCl, and three systemic fungicides. The highest concentration of solubilised phosphate (187 mg P L^{-1}) was achieved after 5 days of incubation in the medium with glucose and ammonium sulphate. The culture pH decreased from 6.5 to 4.2 and HPLC demonstrated organic acid production. Phosphate solubilized was highly negatively correlated with pH ($r=-0.96$). Increasing salinity had no effect on phosphate solubilization. The maximum tolerance limits to systemic fungicides carbendazim, mancozeb, and hexaconazole were 12.5 µg mL^{-1}, 2,000 µg mL^{-1} and 250 µl mL^{-1} respectively. At these concentrations carbendazim, mancozeb and hexaconazole were found to decrease phosphate solubilization by 55 %, 37 %, and 30 %, respectively. Our results indicate that *T. funiculosus* SLS8 may be a potential candidate for the development of a biofertilizer for maintaining available phosphate levels in environmentally stressed soils such as saline agricultural soils impacted by systemic fungicide application or seed treatment.

Keywords Systemic fungicides · P solubilization · *Penicillium funiculosum* · Salinity · *Talaromyces funiculosus*

Introduction

Phosphorus (P) is an important macronutrient required for plant growth and development and is a major limiting factor for yield in most crop species. However, much of the soluble P applied as fertilizer may react with the soil and be 'fixed' or converted into sparingly soluble forms so that they are unavailable to the plant (Whitelaw 2000). Consequently, farmers need to apply a surplus of P to ensure that there is sufficient available P in the soil solution for plant uptake (Goldstein 1986). The most widely used fertilizers are obtained from the acidification of rock phosphates with strong acids, an expensive process that involves high environmental damage (Vassilev et al. 2006).

Phosphate-solubilizing microorganisms play an important role in supplying relatively unavailable phosphate to plants. The important microbial groups of phosphate-solubilizers include bacteria (Nautiyal et al. 2000; Hwangbo et al. 2003; Park et al. 2010), actinomycetes (Palaniyandi et al. 2013) and fungi (Whitelaw 2000; Mittal et al. 2008). Among the soil bacterial communities, *Pseudomonas*, *Bacillus*, *Burkholderia*, *Enterobacter*, and *Rhizobium* spp. solubilize phosphate by releasing organic acids such as keto-gluconic acid and gluconic acid (Park et al. 2010). Soil fungal species belonging to the genera *Penicillium*, *Aspergillus*, *Rhizopus*, and *Fusarium* also solubilize phosphate by releasing organic acids such as gluconic, oxalic, citric, formic, acetic, propionic, lactic

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and succinic acid (Whitelaw et al. 1999; Rashid et al. 2004). *Penicillium* species, such as *Penicillium bilaiae*, *Penicillium rugulosum*, *Penicillium oxalicum*, *Penicillium purpurogenum*, and *Penicillium radicum*, can be important components of the root microbiota of a diverse range of plant species. These fungal species are involved in P cycling by secreting organic acids that can directly dissolve P precipitates or chelate P precipitating cations, which results in a release of available phosphate (Gadd 1999; Whitelaw 2000; Scervino et al. 2010).

Soil microorganisms play an important role in enhancing nutrient availability via a wide range of activities such as the decomposition of plant residues, solubilization of phosphate, mineralization, and biological nitrogen fixation. However, in modern agricultural practices, beneficial microorganisms in the soil are affected deleteriously by excessive soil salinity, often caused by inappropriate irrigation. Soil salinity has an adverse effect on plant growth, and restricts crop yields on 32 million hectares of dryland farming and 45 million hectares of irrigated land worldwide (Munns and Tester 2008). Indian alkaline soils may have salt concentrations as high as 2 % (Nautiyal et al. 2000). Beneficial soil microbes are also affected by increased use of chemical fertilizers, organic pesticides, insecticides, and herbicides. Many fungicides have a harmful impact on soil organisms, decreasing soil microorganism populations and efficiency of organic matter breakdown (Corden and Young 1965; Rasool and Reshi 2010; Imfeld and Vuilleumier 2012).

Biotechnology may offer sustainable solutions to mitigate the problems of plant P nutrition in the light of the finite, non-renewable nature of P fertilizers (Vassilev et al. 2012). The object of this study was to screen and isolate microorganisms from the rhizosphere of Neem plants growing in saline soils, with the view that such conditions would select microorganisms able to solubilize phosphate in saline agricultural soils. The effect of the carbon source, N source, salinity (NaCl) and systemic fungicides (carbendazim, mancozeb, and hexaconazole) on phosphate solubilization activity were also investigated.

Materials and methods

Isolation of phosphate-solubilizing microorganisms and determination of phosphate solubilization index

Soil and roots were collected from underneath Neem trees (0–10 cm depth) in the campus area of Swami Ramanand Teerth Marathwada University, Nanded, India. The soil pH (H_2O) was 7.5 and salinity (total soluble salts) was 0.64 % of the soil solution (APHA 1998). Soil adhering to roots was collected, placed in sterile polythene bags and immediately stored at 4ºC. Soil sub-samples (10 g) were added to 100 mL sterile distilled water, thoroughly mixed by orbital shaker for 30 min, and serially diluted onto Pikovskaya agar medium (Pikovskaya 1948) (glucose 10 g L^{-1}; $Ca_3(PO_4)_2$, 5 g L^{-1}; $(NH_4)SO_4$, 0.5 g L^{-1}; NaCl, 0.2 g L^{-1}; $MgSO_4 \cdot 7H_2O$, 0.1 g L^{-1}; KCl, 0.2 g L^{-1}; NaCl, 0.2 g L^{-1}; $MnSO_4 \cdot 7H_2O$, 0.002 g L^{-1}; $FeSO_4 \cdot 7H_2O$, 0.002 g L^{-1}; yeast extract 0.5 g L^{-1}), with and without 20 µg mL^{-1} tetracycline, and incubated at 30ºC in darkness.

Qualitative phosphate solubilization potential was checked by spot inoculation of each isolate on Pikovskaya plates, which were incubated for 10 days at 30ºC in darkness. Phosphate Solubilization Index was determined daily from days 3 to 8 by using following formula (Edi–Premono et al. 1996):

Solubilization Index = (colony plus halo) diameter/colony diameter.

Identification

Fungal isolate 'SLS8' showing the highest Solubilization Index was selected and identified on the basis of its colony morphology and microscopic observations on Czapek yeast autolysate agar, and malt extract agar (Pitt 1988; Mukadam 1997) plus 18S rDNA sequencing. The isolate *Talaromyces funiculosus* SLS8 has been deposited in the culture collection of National Bureau of Agriculturally Important Microorganisms (NBAIM), Uttar Pradesh, India (Accession number NAIMCC-F-03105). The 18S rDNA gene sequence was deposited in Genbank with accession number JX456460.

18S rDNA sequencing

A 4-mm^2 mycelial plug was taken from a single-spore potato dextrose agar (PDA) culture of SLS8 and transferred into a 50 mL polypropylene tube containing 22 mL of 1:5 diluted V8 juice (Campbell Soup Co., Camden, NJ, USA) with 0.3 % $CaCO_3$. The tube was agitated at 150 rpm for 4 days at 25 °C in darkness. The resulting fungal mycelium was transferred to a sterile polypropylene tube and centrifuged at $18,000\,g$ for 5 min. The supernatant was removed and the mycelium was collected, frozen in liquid nitrogen, and stored at −80 °C until required for DNA extraction. Fungal DNA was extracted from the frozen mycelium using the DNeasy Plant Mini Kit (Qiagen) and concentration was determined by absorbance at 260 nm. DNA concentrations were adjusted to 12.5 ng μL^{-1} and the samples were stored at −20 °C.

The 18S rDNA gene fragment was amplified by PCR using the universal primers ITS1 TCCGTAGGTGAACCTGCGG and ITS4 TCCTCCGCTTATTGATATGC (White et al. 1990). Sequencing was performed by ABI 3730XL sequencing machine (National Center for Cell Science, Pune, India). The Basic Local Alignment Search Tool (BLAST) was used to compare the sequences with those of known fungi archived at

the National Centre for Biotechnology Information (NCBI) nucleotide database. The 18 s rDNA sequence for SLS8 was submitted to the NCBI GenBank and was allotted accession no. JX456460.

Effects of NaCl and different carbon and nitrogen sources on phosphate solubilization

The ability of fungal isolate SLS8 to solubilize phosphate under different nutrient and salinity conditions was tested in liquid culture. Pikovskaya liquid medium (50 mL) was prepared in 250 mL bottles with 1 g P L^{-1} as $Ca_3(PO_4)_2$. NaCl (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 % w/v) was added to test the ability of the fungus to solubilize phosphate under saline conditions. Glucose, fructose, galactose or sucrose (4 g C L^{-1}) was added as the carbon source with ammonium sulfate as the N source at concentration of 106 mg N L^{-1}. Nitrogen sources were evaluated similarly by including ammonium sulfate, casein, urea, potassium nitrate or sodium nitrate (106 mg N L^{-1}) as the sole N source with glucose as the C source at concentration of 4 g C L^{-1}. The sugar, phosphate and nitrogen sources were sterilized separately by autoclaving and added to the media.

Pure cultures of SLS8 were maintained on Pikovskaya agar slants at 5 °C and fresh inoculum was prepared by inoculating Pikovskaya liquid medium and incubating at 30 ºC for 1 week in darkness. A 1 mL aliquot of this medium, consisting of a suspension of conidia and mycelium at 2×10^6 colony forming units (CFU) mL^{-1}, was used as inoculum. The bottles were incubated for 10 days in an orbital shaker at 30 ºC and 150 rpm in darkness. There were three replicates of all treatments. Controls consisted of uninoculated Pikovskaya liquid medium.

Phosphate, pH determination and organic acid determination

Samples of the cultivation medium were collected every 24 h for 10 days. The samples were centrifuged at 6,000 rpm for 20 min and the pH and concentrations of released phosphate (phosphomolybdic blue colour method, Jackson 1973) were determined in the cell free supernatant.

Organic acids produced by SLS8 after 7 days incubation were determined by passing the liquid medium cultures through 10 μm membrane filters for injection (20 μL) into HPLC (Perkin Elmer, Waltham, MA USA) with an X-terra RP-18 column (4.6 mm×250 mm) of particle size 5 μm. Solvent A was KH_2PO_4 buffer (0.03 M, pH 3.2) and solvent B was acetonitrile: water (1:1) and the flow rate was 1 mL min^{-1}. A gradient program was employed whereby solvent A and cumulative time were 80 % at 0 min, 80 % at 5 min, 30 % at 12 min, 30 % at 20 min, 80 % at 25 min and 80% at 30 min. The retention time of each signal was recorded at 210 nm wavelength. HPLC profiles of culture filtrates were analyzed by comparison with the elution profiles of standard organic acids (Chen et al. 2006).

Sensitivity to systemic fungicides

Fungal isolate SLS8 was tested further for its sensitivity to three systemic fungicides (carbendazim, mancozeb, and hexaconazole) at different concentrations by agar well diffusion assay on Pikovskaya agar plates. The highest concentration tolerated by the fungus was noted as the maximum tolerance limit of the fungicide. To study further the effect of these fungicides on phosphate solubilization activity, the isolate was inoculated in Pikovskaya liquid medium containing varying concentrations of the fungicides. The flasks were incubated for 7 days at 30 ºC in darkness with shaking (150 rpm). After incubation, the pH and concentration of soluble phosphate were determined in the cell free supernatant as previously described.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using Genstat for Windows, 15th Edition. Least significant differences (*LSD*) and correlation analyses were performed by Genstat for Windows, 15th Edition.

Results

Six phosphate-solubilizing microorganisms from the Neem rhizospheric soil samples were isolated on Pikovskaya agar plates. These were purified by sub-culturing and their phosphate Solubilization Index was determined on Pikovskaya plates (Fig. 1). The Solubilization Index for four of the six isolates increased up to the fourth day of incubation before

Fig. 1 Phosphate solubilizing fungal isolate *Talaromyces* sp. SLS8: Colonies on Pikovskaya agar showing zone of clearance around the colonies after (**a**) 3 days, (**b**) 4 days and (**c**) 5 days incubation at 30 °C in darkness

Fig. 2 Phosphate Solubilization Index of fungal isolates on Pikovskaya agar over 8 days of incubation. Values with dissimilar letters are significantly different (*LSD*, *P*<0.05)
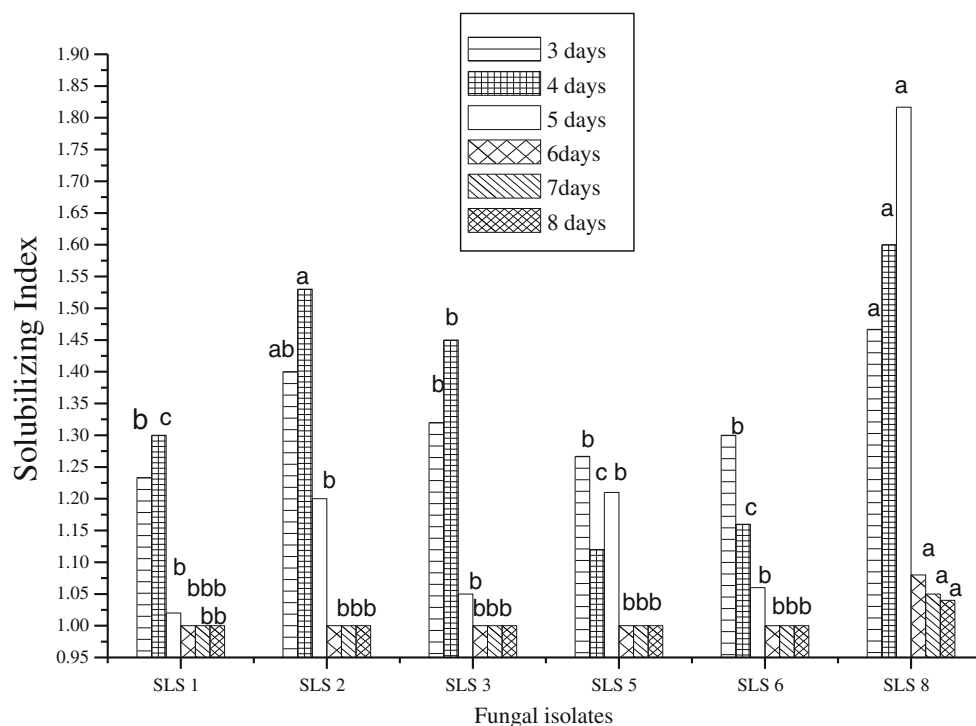
decreasing to 1, whereas the Solubilization Index for isolate SLS8 was maintained above 1 for 8 days (Fig. 2).

Based upon their colony morphology, spore characteristics and microscopic studies the phosphate solubilizing isolates were tentatively identified as species of *Penicillium* or *Talaromyces* (SLS2, SLS 3, SLS 8), *Aspergillus* (SLS1, SLS6) and *Rhizobium* (SLS5) (data not shown). As the Solubilization Index was highest, and phosphate solubilization ability of SLS8 was retained for the longest period of time (Fig. 2), SLS8 was selected for further study.

Identification

Fungal isolate SLS8 formed colonies on malt extract agar (Oxoid) of 45 mm diameter after 7 days at 25 ºC in darkness. Colonies were funiculose (hyphae aggregated into rope-like strands) with white mycelium and conidiogenesis was grey-

blue at the margins and dull green elsewhere (Fig. 3a). The reverse was pale brown. Colonies on CYA were funiculose with white mycelium, moderate dull green conidiogenesis, clear exudate droplets (Fig. 3b) and colony diameter of 45 mm after 7 days. Microscopically, conidiophores were biverticillate, with verticils of appressed metulae. From the 18S rDNA sequence, SLS8 presented a similarity of 98 % with *Talaromyces funiculosus* strain MUCL 38968 (Lopez-Villavicencio et al. 2010). As the colony morphology also matched that of *Talaromyces funiculosus* (formerly *Penicillium funiculosum*) (Pitt 1988), SLS8 was identified as *T. funiculosus*.

Phosphate solubilization

The ability of *T. funiculosus* SLS8 to solubilize phosphate was tested by inoculating Pikovskaya liquid media containing four

Fig. 3 *Talaromyces* sp. SLS8: (**a**) Conspicuously funiculose colony on malt extract agar, 45 mm diameter after 7 days incubation at 25 °C in darkness; (**b**) clear exudate droplets on a Czapek yeast autolysate agar colony
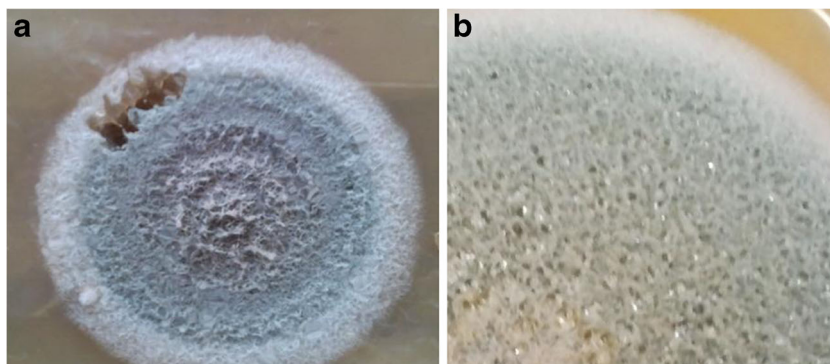
C and five N sources and incubating for 7 days. Phosphate solubilization with different N sources followed the order: ammonium sulfate = potassium nitrate > sodium nitrate > casein > urea. The extent of acidification followed the order: ammonium sulfate > casein > potassium nitrate > urea = sodium nitrate (Fig. 4a). There was a negative correlation ($r=-0.70$, $P<0.001$) between phosphate solubilized and the medium pH.

When comparing carbon sources, the maximum phosphate released after 7 days incubation (180 mg L^{-1}) was obtained with glucose as C source and ammonium sulfate as N source. With each treatment, significant amounts of phosphate were released with concurrent acidification of the medium (Fig. 4b). A negative correlation ($r=-0.96$, $P<0.001$) was observed between the amount of phosphate solubilized and pH of the medium.

The kinetics of phosphate solubilizing activity by *T. funiculosus* SLS8 was investigated in liquid Pikovskaya solution culture with glucose as the C source and ammonium sulfate as the N source. Phosphate released from $Ca_3(PO_4)_2$ increased to a peak of 187 mg L^{-1} by the fifth day before decreasing, while pH decreased from 6.5 to 4.2 by the fifth day before increasing again (Fig. 5). A negative correlation ($r=-0.94$, $P<0.05$) was observed between the amount of phosphate solubilized and pH of the medium indicating maximum activity at low pH.

The finding that solubilization of phosphate by *T. funiculosus* SLS8 in Pikovskaya liquid medium occurred with decreased pH indicated the fungus may have secreted organic acids. HPLC analysis showed that *T. funiculosus* SLS8 produced tartaric acid, lactic acid, and an unknown organic acid. The retention times were 2.419 min, 2.742 min, and 7.166 min for tartaric acid, lactic acid, and unknown acid respectively.

The rate of phosphate solubilization by *T. funiculosus* SLS8 was further studied in the presence of different concentrations of NaCl in Pikovskaya liquid medium. None of the NaCl concentrations had any significant effect on phosphate solubilization by *T. funiculosus* SLS8 (Fig. 6).

The phosphate solubilization activity of *T. funiculosus* SLS8 was decreased by the presence of systemic fungicides carbendazim, mancozeb, and hexaconazole (Table 1). *T. funiculosus* SLS8 exhibited a higher maximum tolerance limit to mancozeb (2,000 μg mL^{-1}) and hexaconazole (250 μg mL^{-1}) than to carbendazim (12.5 μg mL^{-1}) (data not shown). At the maximum tolerance limit concentrations of carbendazim (12.5 μg mL^{-1}), mancozeb (2,000 μg mL^{-1}), and hexaconazole (250 μg mL^{-1}), *T. funiculosus* SLS8 retained 45 %, 63 %, and 70 % of its maximum phosphate-solubilizing activity respectively (Table 1).

Discussion

The maximum phosphate Solubilization Index values for the six Neem rhizosphere isolates in this study (Solubilization Index 1.3 to 1.6) lie within the published Solubilization Index range for 66 fungal isolates from sugarcane and sugar beet rhizospheres (Solubilization Index 1.13 to 1.59) (Mahamuni et al. 2012). Similarly, the Solubilization Index range for fungal cultures isolated from maize rhizosphere ranged from 1.53 to 1.80 (Alam et al. 2002). *T. funiculosus* SLS8, isolated from Neem rhizosphere soil, was able to solubilize significant amounts of phosphate from $Ca_3(PO_4)_2$ under different conditions of N and C nutrition. Our results are in conformity with previous reports of *Penicillium* and *Talaromyces* fungi playing roles in P solubilization (Asea et al. 1988; Whitelaw et al. 1999; Mittal et al. 2008; Matias et al. 2009; Wakelin et al. 2007). The negative correlations between phosphate solubilization by *T. funiculosus* and culture pH are also consistent with those of many similar fungal phosphate

Fig. 4 Effect of nitrogen and carbon sources on *Talaromyces funiculosus* SLS8 phosphate solubilization activity (from $Ca_3(PO_4)_2$) after 7 days of incubation. (a) Nitrogen sources with glucose (4 g C L^{-1}) as the C source; (b) carbon sources with ammonium sulfate (106 mg N L^{-1}) as the N source. Means for solubilized phosphate and pH followed by different letters (upper case and lower case, respectively) are significantly different (*LSD*, $P<0.05$)
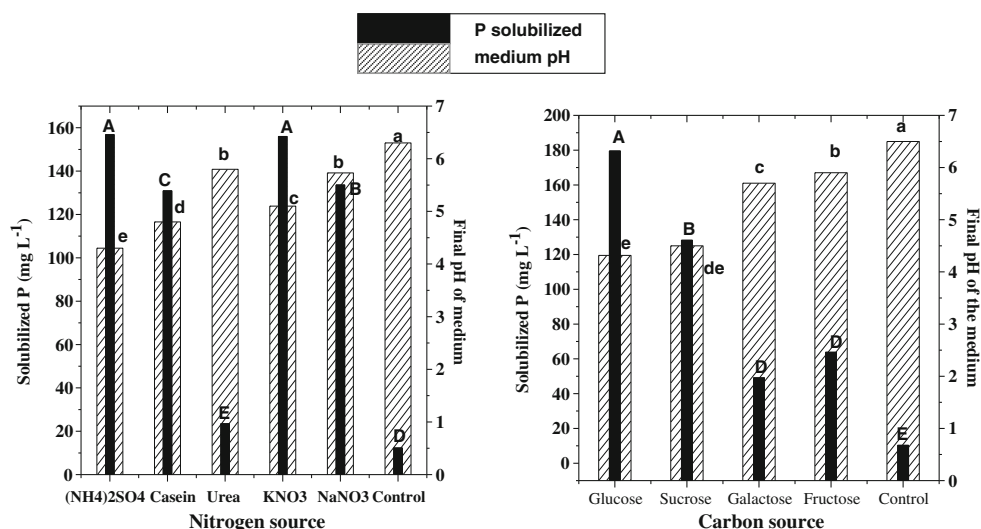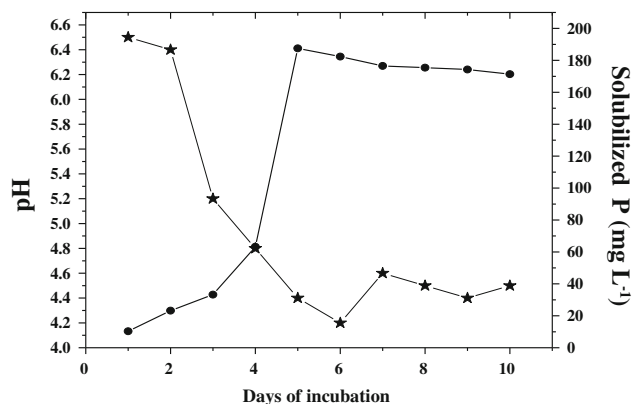
Fig. 5 Changes in pH and concentration of solubilized P during solubilization of Ca$_3$(PO$_4$)$_2$ by *Talaromyces funiculosus* SLS8 in PVK solution culture over 10 days with ammonium sulphate (106 mg N L^{-1}) as the nitrogen source and glucose (4 g C L^{-1}) as the carbon source

solubilization investigations (Asea et al. 1988; Whitelaw et al. 1999; Matias et al. 2009).

Although we found that the ammonium and nitrate sources caused equivalent phosphate release by *T. funiculosus* SLS8, the assimilation of ammonium caused greater acidification. Increased acidification with ammonium has also been reported for phosphate solubilization by *P. bilaiae* (Asea et al. 1988), *Penicillium aurantiogriseum* and *Penicillium simplicissimum* (Illmer et al. 1995), *P. radicum* (Whitelaw et al. 1999), *Aspergillus aculeatus* (Narsian and Patel 2000); *Aspergillus niger* (Srividya et al. 2009), *Aspergillus awamori* (Jain et al. 2012) and *Aspergillus* sp. (Pradhan and Sukla 2005).

Lowering of pH during phosphate solubilization is probably caused by production of H$^+$ and organic acids by the fungal hyphae (Jacobs et al. 2002). The relatively greater reduction in pH with ammonium indicates possible H$^+$ efflux from fungal hyphae during ammonium uptake (Roos and Luckner 1984). Ammonium has commonly been reported as
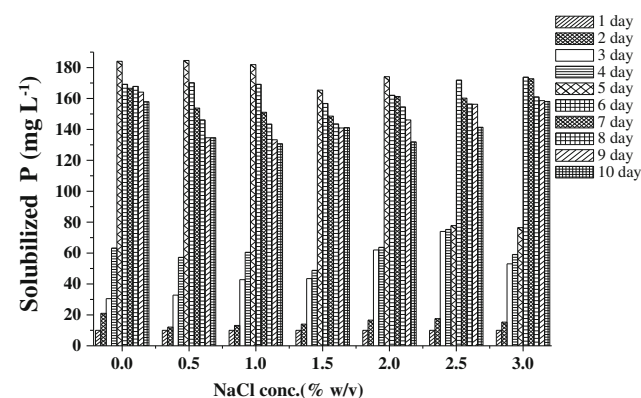
the best N source for phosphate solubilization (Whitelaw et al. 1999; Narsian and Patel 2000; Jacobs et al. 2002; Pradhan and Sukla 2005; Matias et al. 2009; Srividya et al. 2009), although in some cases nitrate caused the greatest phosphate solubilization: e.g. *Aspergillus tubingensis* (Relwani et al. 2008), *P. rugulosum* (Reyes et al. 1999) and *A. niger* (Seshadri et al. 2004).

The greatest phosphate release by *T. funiculosus* SLS8 occurred with glucose as the sole carbon source. Other studies also have shown glucose to be the preferred carbon source for phosphate solubilization of calcium phosphates by *Penicillium* spp. (Chai et al. 2011; Scervino et al. 2011; Yadav et al. 2011) although sucrose was preferred by *P. rugulosum* (Reyes et al. 1999). Within *Aspergillus* spp. the greatest phosphate release has been reported with mannitol (Seshadri et al. 2004; Barroso et al. 2006), maltose (Srividya et al. 2009; Jain et al. 2012), arabinose (Narsian and Patel 2000), as well as glucose (Pradhan and Sukla 2005) and sucrose (Relwani et al. 2008).

Talaromyces funiculosus SLS8 produced tartaric acid and lactic acid when solubilizing Ca$_3$(PO$_4$)$_2$. Similarly, many *Aspergillus* sp. produce tartaric acid (Gaur 1990; Singal et al. 1994) and lactic acid was produced by *Talaromyces flavus* and *Penicillium janthinellum* (Scervino et al. 2010). However, various other organic acids can also be produced during phosphate solubilization by *Penicillium* or *Aspergillus* spp. *P. radicum* and *P. purpurogenum* produced gluconic acid during P solubilization (Whitelaw 2000; Scervino et al. 2010), although a later study showed that gluconic acid may actually contribute little to P solubilization (Mendes et al. 2013). *A. tubingensis* produced succinic, acetic and oxalic acids (Relwani et al. 2008) and *A. niger* produced citric, lactic, gluconic, lactic, maleic,

Fig. 6 Effect of NaCl concentration on phosphate solubilization by *Talaromyces funiculosus* SLS8 in Pikovskaya liquid medium containing 1 g P L^{-1} as Ca$_3$(PO$_4$)$_2$ with ammonium sulphate (106 mg N L^{-1}) as the nitrogen source and glucose (4 g C L^{-1}) as the carbon source. NaCl had no effect on phosphate solubilization on any day (based on *LSD*, *P<0.05*)

Table 1 Effect of fungicides on phosphate solubilization activity of *Talaromyces funiculosus* SLS8

Fungicides (μg/mL)	Phosphate solubilized (mg L^{-1})	Phosphate solubilization activity (%)
Carbendazim		
3.12	152±0.6	90.5
6.25	88±4.0	52.4
12.5 [a]	76±12	45.2
Mancozeb		
1000	144±13.6	85.7
1500	130±2.8	77.4
2000 [a]	106±10.0	63.1
Hexaconazole		
150	164±5.6	97.6
200	124±16.0	73.8
250 [a]	118±2.0	70.2
Control (nil fungicide)	168±46.0	100

[a] Maximum tolerance limits for *T. funiculosus* SLS8

oxalic and tartaric acids (Barroso et al. 2006; Schneider et al. 2009). Mendes et al. (2013) demonstrated that the most important organic acid produced by *A. niger* FS1 during solubilization of $AlPO_4$ and $FePO_4$ was oxalic acid. They concluded that acidification effectively causes solubilization of $Ca_3(PO_4)2$, but for compounds such as $AlPO_4$ and $FePO_4$ or more complex structures, like the rock phosphates, the production of chelating organic acids is more important. Future work should involve testing of *T. funiculosus* SLS8 for solubilization of these more insoluble phosphate minerals.

Increasing salinity (NaCl from 1 % to 3 % w/v) did not decrease phosphate solubilization by *T. funiculosus* SLS8, indicating that the fungus may be of benefit in maintaining available phosphate levels in saline soils. This is in agreement with Khan et al. (2011), who also reported that growth promotion of soybean by *T. funiculosus* LHL06 was not affected by salinity stress. A *Penicillium citrinum* isolate from sugarcane rhizosphere was also able to solubilize phosphate under saline conditions, although that study did not investigate NaCl concentrations higher than 1 % (Yadav et al. 2011). *Eupenicillium parvum* (Vyas et al. 2007) and some *Aspergillus* spp. (Narsian and Patel 2000; Rinu and Pandey 2010; Srinivasan et al. 2012) also are able to solubilize phosphate at various levels of salinity. The mechanism for such salinity tolerance is unknown, although a study on arbuscular mycorrhizal fungal strain, *Glomus intraradices* CdG, from saline soil showed that adaptation to salinity was related to up-regulation of fungal genes encoding chaperones or aquaporins (Estrada et al. 2013). The possibility that chaperones and aquaporins may have a role in the salinity tolerance of phosphate solubilizing fungi is a research area that needs investigation.

In vitro phosphate solubilization activity of *T. funiculosus* SLS8 was decreased by systemic fungicides carbendazim, mancozeb, and hexaconazole. Similarly, plant growth promoting bacterium *Enterobacter asburiae* PS2, from mustard plant rhizosphere, solubilized less phosphate in the presence of systemic fungicides tebuconazole, hexaconazole, metalaxyl, and kitazin (Ahemad and Khan 2010). A Canadian study also showed that treatment of pea and chickpea seed with systemic fungicides metalaxyl; fludioxinil and metalaxyl; carbathiin and thiram; carbathiin and thiabendazole; and trifloxystrobin and metalaxyl restricted mycorrhizal fungal colonization, host growth and P uptake to different levels (Jin et al. 2013). The negative effect on phosphate solubilization by *T. funiculosus* SLS8 of commonly used systemic fungicides shows that the use of systemic fungicides in agriculture is probably deleterious to the natural solubilization of inorganic phosphates in soil, thus affecting soil fertility. However, *T. funiculosus* SLS8 retained significant phosphate solubilization activity at fungicide levels higher than found in most agricultural soils, indicating that the fungus may be of benefit in maintaining

available phosphate levels in agricultural soil affected by systemic fungicides.

Although liquid medium assays are commonly used to test phosphate solubilization by soil microorganisms, they do not correspond directly to phosphate solubilization in soils. Liquid medium assays use high C and N concentrations, and they lack the interactions with other microbes (competition/facilitation) and sorption/desorption processes of P that occur in soil (Wang et al. 2012). In addition, many studies have shown that plants inoculated with a mixed inoculum containing free-living phosphate-solubilizing fungi and mycorrhizal fungi received greater benefit from rock phosphate or soil P than plants receiving only one of the microorganisms (Zaidi and Khan 2007; Matias et al. 2009; Osorio and Habte 2001). These synergistic interactions likely occur because of the efficiency with which mycorrhizal fungi take up P in solution and translocate it to the roots, thus preventing re-immobilization by soil of phosphate released by the free-living phosphate-solubilising fungi (Osorio and Habte 2001). Thus, the ability of *T. funiculosus* SLS8 to solubilize poorly available P in soils, with and without mycorrhizal fungal inoculation, is a promising strategy and will be the subject of further investigation.

Conclusion

In conclusion, as it is able to solubilize phosphate in vitro under saline conditions and in the presence of three commonly used systemic fungicides, *Talaromyces funiculosus* SLS8 is considered a suitable candidate for further testing in soil conditions with a view towards development as a biofertilizer capable of maintaining available phosphate levels in environmentally stressed soil such as saline agricultural soils impacted by systemic fungicides.

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