Journal of Soil Salinity and Water Quality 17(1), 15-26, 2025
DOI: https://doi.org/10.56093/jsswq.v17i1.162135

Effect of Salt and Nutrient Medium on Phosphorus Solubilization Potential of Fungi Isolated from Salt-affected Soils

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Abstract

Salinity is one of the most severe environmental constraints that limit agricultural productivity. Exploiting microorganisms with nutrient solubilizing and plant growth–promoting potential is an affordable natural approach to salinity alleviation. The current study was designed to isolate and screen salt–tolerant P–solubilizing fungi from salt–affected soils and optimize their growth parameters for future development as bioinoculants. Fifteen salt–tolerant P–solubilizing fungal isolates were evaluated. Fungi grown in potato dextrose medium (PDM) produced more biomass than those grown in Pikovskaya's medium, but the results varied depending on the type of fungi. The biomass of the isolates decreased as the NaCl concentration increased. The results show a wide range of significant variations in the P–solubilization efficiency of salt–tolerant fungi. Quantitative estimation of P–solubilization significantly affected the interaction between fungal isolates × medium, while all the isolates demonstrated high P–solubilization potential ($p \leq 0.05$). Fungal isolates also reduced the pH because of high organic acid production. The quantitative assay revealed the presence of intra/extracellular acid and alkaline phosphatase activity at variable titre. The study revealed the presence of salt–tolerant P–solubilizing fungi in the rhizosphere of crops growing in saline areas and PDB is a better medium for bulk production of fungi for developing bio–inoculants.

Key words: Phosphorus solubilisation, Salt-tolerant P-solubilizing fungi, Organic acid, Phosphatase activity, Bio-inoculants

Introduction

Salinity is one of the most stressful abiotic factors that significantly limits crop growth, yield and impedes soil health in arid and semi–arid regions. Plant metabolism and nutrient uptake are primarily affected by salinization (Basak *et al.*, 2020). The detrimental effects of salts on plants can slow down growth and development. As a result, the efficiency of plant nutrients remains underused additionally, the presence and activity of electrolytes cause nutritional imbalances in the soil. The dominance of Na^+ content in soil decreases the availability of Ca^{2+} and its transport and mobility onto the plant, which impacts the quality of the plant's reproductive and vegetative organs. Further, Na^+ can diminish K^+ uptake and Cl^- presence can lower NO_3^- uptake. Salinity reduces P uptake in crops because P availability predominantly affects saline soils (Rai *et al.*, 2022).

Phosphorus is the second vital plant nutrient needed for crop growth and productivity. The presence of exchangeable calcium in the solid and variable activity of Ca^{2+} in the soil solution phase alters P availability in saline soils (de Oliveira Mendes *et al.*, 2014; Li *et al.*, 2016). Therefore, due to the poor solubility and mobility of P in soil, plants can only access a limited amount of it. However, the overall P content of the soil typically exceeds what the plants need. Due to the unique characteristics of P in soil, viz., low solubility, poor mobility, and high fixation by the soil matrix, two significant processes primarily control P availability to plants: bioavailability and acquisition of P through rhizosphere–based chemical and biological processes (Sundha *et al.*, 2018). Using solubilization and mineralization processes, soil phosphate–solubilizers found in the rhizosphere can transform phosphate into a bioavailable form, providing plants with soluble phosphorus (Devi *et al.*, 2022). Through the processes of acidification, chelation, exchange reactions, and organic acid generation by phosphate solubilizing microorganisms (PSMs) (Alori *et al.*, 2017; Naik *et al.*, 2013), the insoluble phosphates are transformed into soluble forms. Fertilizers supplied to the soil are also mobilized by this mechanism for crops. PSMs dissolve phosphate and improve soil fertility, and benefit crops for food and feed production (Chandra *et al.*, 2021).

Microbial exploitation of soil P is one of the environmentally acceptable and potentially effective methods for sustainable P nutrition (Gyaneshwar *et al.*, 2002). Thus far, most studies have concentrated on tricalcium phosphate (TCP), a chemical form of inorganic P present in alkaline soils, and its solubilization. Many studies reported the occurrence of phosphate-solubilizing fungus (PSF) in developing bio–inoculants to restrain salt stress. A microbial strain grown in a medium that is optimally tolerant to salt must be used to create the bio–inoculant to maximize the benefits of inoculation in areas subject to salt stress (Verma *et al.*, 2023). It has been observed that PSFs are more effective than phosphate solubilizing bacteria (PSBs) at releasing P from insoluble inorganic substances (de Oliveira Mendes *et al.*, 2014). Furthermore, they are a good option for developing microbial inoculants due to their resistance to biotic and abiotic stress in a particular soil niche. The primary process for inorganic phosphate solubilization is thought to be the release of organic acid, and several reports indicate the potential of PSFs to produce organic acid (de Oliveira Mendes *et al.*, 2014).

Salt–tolerant fungi possess plant growth-promoting potential, and also interact and impart beneficial properties to plants under salt–stressed conditions (Manjunatha *et al.*, 2022). Fungal applications to plants for mitigation of salt stress establish new possibilities to enhance P availability and facilitate plant growth under salt–stressed conditions. Hence, the present study was planned to screen and characterize the salt–tolerant fungi inhabitants of high saline soils possessing P solubilizing potential. In addition, finding suitable medium and growth conditions for bulk production is a prerequisite to developing effective bioinoculants for SAS.

Material and Methods

Isolation of salt–tolerant phosphorus solubilizing fungi

The site of the study was the experimental farm of ICAR–Central Soil Salinity Research Institute (29°19′10″ N latitude and 76°47′30″ E longitude at 230 m above mean sea level) at Nain, Panipat, Haryana. This site possesses extensive soil salinity, EC_e from 4 to 30 dS m^{-1}, and $pH_{1:2}$ from 8.2 to 9.0. As a result, salt–tolerant varieties of wheat (*Triticum aestivum*), sorghum (*Sorghum bicolor*), mustard (*Brassica juncea*), and pearl millet (*Pennisetum glaucum* L.) are grown in soils with low to moderate salinity. The rhizosphere of such crops growing in saline soils was used source of salt–tolerant PSFs. The climate is semi–arid, subtropical, and monsoonal, with hot summers (May to June) and cold winters (December to January). The mean annual precipitation is 678 mm, with the most rain falling between July and November.

The soil was sandy loam in texture with sand, silt and clay per cent were 56.4, 25 and 18.6%.: Soil $pH_{1:2}$ (1:2 soil: water solution) was 7.70–8.86; electrical conductivity ($EC_{1:2}$; 1:2 soil: water) 1.43–3.3 dS m^{-1}; electrical conductivity of saturation paste extract (EC_e dS m^{-1}) 4.27–9.91 dS m^{-1}; organic carbon 3.10–0.47 g kg^{-1}; $KMnO_4$ oxidizable nitrogen 111.32–134.10 kg ha^{-1}; Olsen–P 12.48–13.79 kg ha^{-1}; NH_4OAc–K :221.01–238.9 kg ha^{-1}. The concentration of electrolytes in soil saturation extract parameters were Na^+: 78.1; K^+: 0.24; Ca^{2+}:16; Mg^{2+}: 2.5; CO_3^{2-}: non detectable; HCO_3^-:3.5; Cl^-: 15.75 me l^{-1}. As per USDA classification, the site soil is classified as *Hasplustepts* and is enclosed in thick salt efflorescence as well as the crust and complex saline and saline–sodic features. The isolation of PSFs was carried out from serially diluted rhizospheric soil collected from crops grown on saline soils (Chandra *et al.*, 2020) incubating at 28 ± 2°C on Pikovskaya's (PKM) agar medium supplemented with 5% NaCl (Pikovskaya, 1948) containing tricalcium phosphate (TCP) for two to seven days. Representative colonies of each fungus type with a clear halo around them were purified, subcultured, and maintained on PKM slants for future use. In the study, a total of 15 salt–tolerant PSF isolates were evaluated.

Salt tolerance

Homogenized cultures (3 days old) of fungal isolates were inoculated into 50 ml of potato dextrose broth (PDA) and PKM containing 1 and 5% NaCl and incubated at 28 ± 2°C for 5, 10, and 15 days. Following incubation, the cultures were filtered through pre–weighed Whatman No. 1 filter papers and dried to constant weight in a hot oven at 60°C. Each culture's dry mycelial weight was then recorded.

Phosphorus solubilizing potential

By inoculating isolated fungal strains on PKM supplemented with 1% NaCl again, the phosphorus solubilizing potential (P–sol) was confirmed. Under aseptic conditions, fungal culture was inoculated at the centre of PKM–containing petri dishes and incubated at 28 ± 2 °C for 5 days. P–sol was found in a clear zone that developed around the colonies. The results were expressed as zone diameter in millimetres (Pikovskaya, 1948).

Quantifying phosphorus solubilizing potential

The quantitative P–sol of the fungal isolates was studied under stationary conditions in two different mediums *i.e.* PKM and PDA liquid medium. 20 ml of growth medium supplemented with 1 and 5% NaCl (w/v) and also containing 0.1% (w/v) TCP in 50 ml flask were inoculated with the seven days old grown two discs (diameter 6mm) of mycelia of fungi. The flasks were incubated at 28 ± 2°C at static conditions for 15 days. For the quantitative estimation of P–sol, culture filtrate was obtained at regular 5-day intervals up to 15 day. P–sol was analyzed by measuring soluble P concentration colourimetrically by using the molybdenum blue method in the supernatant of the fungal–grown medium (Bray and Kurtz, 1945) The P–sol was expressed in mg ml^{-1}.

Production of organic acid and measurement of pH

Production of organic acid (OA) and change in pH concentration in the culture filtrate were also measured. The OA was calculated through titrating with 0.05 *M* NaOH and was expressed as g tartaric acid L^{-1} (Panda *et al.*, 2016). A digital pH meter estimated the change in pH concentration (Systronics ìpH system 362).

Estimation of phosphatase enzymes

Extracellular acid phosphatase (ACP) and alkaline phosphatase (ALP) were quantified in cell–free culture filtrate using modified universal buffer (MUB) (pH 11 and pH 6.5), substrate, *p*–nitrophenyl phosphate (*p*NP), and 0.5 ml culture filtrate. After one hour of incubation at 37°C, the enzyme activity was measured at 400 nm using a UV–vis Thermo Fischer Scientific Spectro–photometer. ACP and ALP activity was expressed as µg dry and fresh fungal biomass /g pNP ml^{-1} h^{-1} (Tabatabai and Bremner, 1969).

Statistical analysis

Statistical analysis was performed using SAS 9.3. The normality and variance heterogeneity were assessed through Shapiro-Wilk's and Bartlett's tests, respectively. A randomized complete block design was used in the analysis of variance (ANOVA) to evaluate the treatment effects.

Results

Fungal biomass

It was determined that the interaction effect between isolates and NaCl concentrations was significant. The biomass of the fungal isolates decreased as the NaCl concentration increased ($p \leq 0.05$). Higher biomass was found in fungi growing in PDM than PKM; however, results varied in different fungi, which indicated that PDM supported better growth in comparison to PKM for all the salt-tolerant fungal isolates. There was also a significant interaction between fungal isolates and medium ($p \leq 0.05$). Increased salt concentration inhibited fungi growth, as evidenced by a decrease in mycelia dry weight of all fungi at 5% NaCl concentration (Table 1).

Table 1. Salt-tolerance in terms of growth (dry wt g^{-1}) of fungal isolates at 1 and 5% NaCl concentration in different growth medium

Fungal isolates	Media I		Media II		Mean
	1%	5%	1%	5%	
N_1	8.66	7.23	6.20	6.00	7.02
N_2	8.61	7.48	6.29	6.03	7.10
N_3	8.22	7.33	6.69	6.28	7.13
N_4	8.82	7.51	6.61	6.29	7.31
N_5	10.66	8.94	7.61	7.67	8.72
N_6	9.23	7.19	6.42	5.92	7.19
N_7	10.46	9.57	7.28	7.28	8.64
N_8	11.47	9.67	7.53	7.98	9.16
N_9	11.73	9.39	7.19	8.16	9.12
N_{10}	9.72	7.61	6.88	6.32	7.63
N_{11}	8.72	7.13	6.21	6.48	7.14
N_{12}	11.69	9.07	7.94	7.92	9.15
N_{13}	9.55	7.73	6.61	5.97	7.47
N_{14}	9.43	7.98	6.48	6.44	7.58
N_{15}	9.23	7.71	6.48	6.20	7.41
Mean	9.75	8.10	6.83	6.73	
	Fungi (F)	Media (M)		F×M	
LSD (p=0.05)	0.29	0.15		0.57	

Table 2. Fungal biomass (dry wt g^{-1}) accumulation at various days of incubation

Fungal isolates	Incubation days		
	05	10	15
N_1	2.04	6.60	12.43
N_2	2.69	6.03	12.58
N_3	2.30	6.85	12.25
N_4	2.64	7.01	12.28
N_5	3.55	7.82	14.79
N_6	2.61	6.20	12.77
N_7	3.40	8.17	14.37
N_8	3.40	8.51	15.58
N_9	3.38	8.36	15.60
N_{10}	2.86	6.23	13.81
N_{11}	2.59	6.55	12.26
N_{12}	3.67	8.14	15.65
N_{13}	2.48	6.76	13.16
N_{14}	2.64	7.50	12.62
N_{15}	2.81	6.80	12.61
Mean	2.87	7.17	13.52
LSD (p=0.05)	Fungi (F)	Days (D)	F×D
	0.29	0.13	0.50

Different NaCl concentrations in various media show the data on the mycelia dry weight of selected fungal isolates. The time of incubation also had a significant effect on fungal biomass ($p \leq 0.05$). The growth of all the fungal isolates subsequently increased with the time of incubation, as observed by the results that the highest biomass was obtained at 15 days (Table 2). Different fungi have contrasting biomass at distinct incubation days.

Screening of phosphorus solubilization activity

The results show a wide range of significant variations in P–sol of salt–tolerant fungi. Fungal isolate N_9 (5.1 mm) had the largest zone of solubilization, followed by N_5 and N_8 (Table 2; Fig. 1; $p \leq 0.05$).

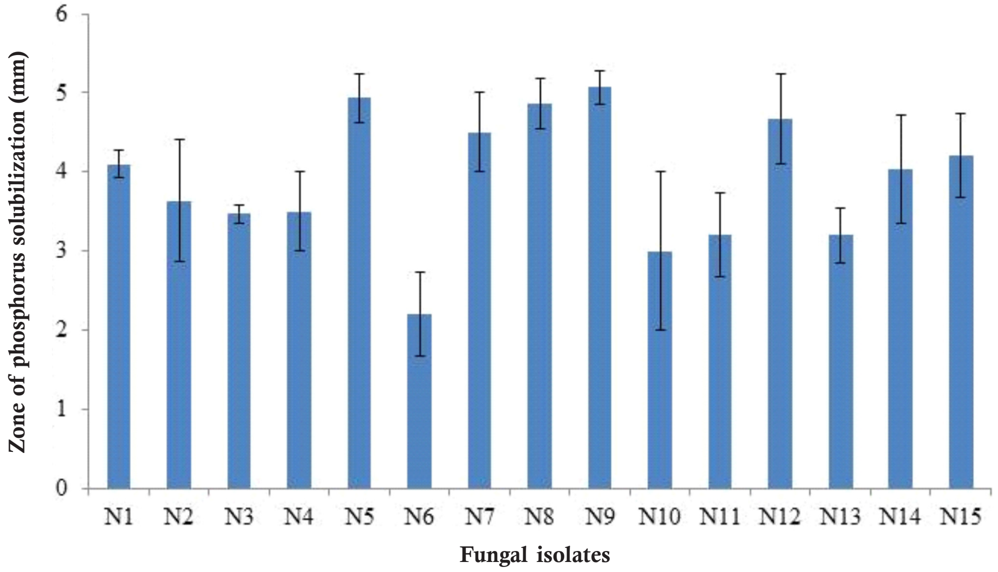

Fig. 1 Phosphorus solubilization activity of salt–tolerant fungal isolates

Quantitative activity

Phosphorous solubilization was significantly affected between fungal isolates (F) × medium (M) at day 5 of incubation and interaction between fungal isolates (F) × NaCl concentration (C) was also significant ($p \leq 0.05$) (Table 3). While, the interaction between medium (M) × NaCl concentration (C) and fungal isolates (F) × medium (M) × NaCl concentration (C) was non–significant. N_8 demonstrated the highest P–sol followed by N_9 at 1%, while at 5% NaCl concentration, N_5 was the highest. However, in PKM, N_9 showed the highest P–sol at both 1 and 5 % NaCl concentration.

At day 10 of incubation, P–sol was significantly affected by the interactions between fungal isolates (F) × medium (M), medium (M) × NaCl concentration (C) and medium (M) × NaCl concentration (C) ($P \leq 0.05$). However, fungal isolates (F) × medium (M) × NaCl concentration (C) interaction was non–significant. N_7 and N_{12} demonstrated maximum solubilization at 1 and 5% NaCl concentration in PDM, whereas N_9 performed best at both 1 and 5% NaCl concentration in PKM.

Similarly, at day 15, P–sol was significantly affected by interactions between fungal isolates (F) × medium (M), medium (M) × NaCl concentration (C), medium (M) × NaCl concentration (C) and fungal isolates (F) × medium (M) × NaCl concentration (C) ($p \leq 0.05$). The highest value of P–sol was observed at 15 days in all the isolates. In 1% NaCl supplemented PDM, N_8, N_9, and N_{12} showed the most elevated phosphate solubilizing activity, while 5% NaCl concentration N_{12} was superior. However, at 1% NaCl concentration, N_8, N_7, and N_9 showed the highest P–sol in PKM.

Lowering of pH

Fungal isolates (F), medium (M), NaCl concentration (C), and their interaction (F) × (M) significantly affected the pH of the medium at 5 days of incubation. N_8 and N_9 fungal isolates decreased the pH values at 1 and 5% NaCl concentration in PDM, whereas in PKM, N_5 was most efficient in reducing pH at both salt levels (Table 4). At day 10, fungal isolates (F), medium (M), and their interaction (F) × (M) significantly affected the pH of the medium. While interactions between fungal isolates (F) × NaCl concentration (C), medium (M) × NaCl concentration (C), and fungal isolates (F) × medium (M) × NaCl concentration (C) interaction was non–significant. N_8 was found to decrease the pH maximum at 1 and 5% NaCl concentration, respectively, in PDM, while in PKM, N_9 reduces the pH most at both 1 and 5% NaCl concentrations. Similarly, at day 15 of incubation, fungi isolates (F), medium (M), and their interaction (F) × (M) significantly affected the pH of the medium. NaCl concentration (C) and its interaction with medium (M) was also significant. N_8 and N_9 were most effective in reducing the pH in PDM and PKM, respectively, at both salt levels.

Organic acid production

All of the isolates endured a subsequent increase in organic acid (OA) release over time (Table 5). The results showed that N_7 released significant at $p \leq 0.05$) high organic acid on day 5 (48.3 meq L^{-1}), which gradually increased on day 10 (51.3 meq L^{-1}) and day 15 (57.5 meq L^{-1}). After that, N_{12} also demonstrated 47.9 meq L^{-1} of organic acid on day 5, which further increased on day 10 (50.7 meq L^{-1}) and day 15 (55.83 meq L^{-1}).

Phosphatases activity

The quantitative assay of intracellular ACP activity was highest in N_5 (100.26 μg g^{-1} dry wt pNP $ml^{-1}h^{-1}$ and 215 μg g^{-1} fresh wt pNP $ml^{-1}h^{-1}$), while extracellular ACP activity in cell–free culture filtrate was highest in N_9 (Fig. 2). A similar pattern was followed in ALP as N_5 (100.3 μg g^{-1} dry wt pNP $ml^{-1}h^{-1}$ and 215 μg g^{-1} fresh wt pNP $ml^{-1}h^{-1}$) possessed the highest intracellular ALP activity, and N_9 had the most increased extracellular activity.

Discussion

In the current investigation, sufficient amounts of salt–tolerant fungi were found in areas affected by high salinity, suggesting that the fungal isolates have a common resistance to sodium toxicity and water loss. The salt tolerance machanism prevents

Table 3. Phosphorus solubilizing activity (mg ml$^{"1}$) demonstrated by salt"tolerant fungal strains in different media and at different salt concentration

			Fungal isolates														
	NaCl	Control	N_1	N_2	N_3	N_4	N_5	N_6	N_7	N_8	N_9	N_{10}	N_{11}	N_{12}	N_{13}	N_{14}	N_{15}
05 Days																	
Media I	1%	4.9	7.4	6.4	8.3	20.6	28.8	10.1	27.7	35.2	30.6	26.0	19.5	31.3	22.6	26.2	25.8
	5%	4.2	5.6	10.9	6.9	17.4	27.3	8.5	25.6	23.5	26.4	19.7	15.9	27.1	15.4	19.8	17.7
Media II	1%	3.8	11.1	14.6	8.8	21.1	24.1	10.0	23.1	24.5	26.3	20.8	16.7	24.5	20.1	23.3	22.9
	5%	4.5	7.5	7.2	7.8	11.2	20.3	6.5	20.8	21.7	22.4	14.8	13.0	20.2	11.3	15.8	17.6
				Fungi (F)		Media (M)	NaCl (C)		F×M		F×C		M×C	F×M×C			
		Sem		0.60		0.21	0.21		0.85		0.85		0.30	1.20			
		LSD (*p*=0.05)		1.67		0.59	0.59		2.37		2.37		N/A	N/A			
10 Days																	
Media I	1%	5.2	11.5	12.6	20.8	24.2	32.8	20.3	37.9	36.5	35.8	29.3	20.3	37.6	29.2	30.6	29.2
	5%	4.7	8.1	10.5	11.8	18.9	27.1	13.7	28.2	24.9	28.9	22.2	17.5	28.2	24.2	20.8	21.7
Media II	1%	4.5	14.0	15.4	17.9	24.2	29.1	17.0	32.1	32.1	32.3	23.7	18.4	31.3	25.5	26.3	25.5
	5%	5.8	10.4	14.6	12.4	14.5	25.7	16.3	25.7	25.5	27.0	19.2	17.0	25.4	22.1	22.6	19.4
				Fungi (F)		Media (M)	NaCl (C)		F×M		F×C		M×C	F×M×C			
		Sem		0.57		0.20	0.20		0.81		0.81		0.29	1.15			
		LSD (*p*=0.05)		1.61		0.57	0.57		2.27		2.27		0.80	N/A			
15 Days																	
Media I	1%	6.5	19.7	18.6	23.6	25.3	35.3	21.4	38.2	40.2	40.4	30.2	22.3	40.5	36.0	34.3	35.2
	5%	5.2	15.2	14.6	18.3	20.7	28.9	19.6	32.5	30.5	32.0	24.2	18.4	33.8	30.0	21.4	22.1
Media II	1%	4.9	16.3	20.7	25.8	26.4	33.1	27.0	34.3	34.6	34.8	25.5	21.9	32.5	28.5	28.4	26.6
	5%	5.8	12.1	17.1	18.4	20.0	28.0	21.0	27.7	28.8	29.3	25.1	19.8	28.6	25.1	24.8	20.6
				Fungi (F)		Media (M)	NaCl (C)		F×M		F×C		M×C	F×M×C			
		Sem		0.56		0.20	0.20		0.79		0.79		0.28	1.12			
		LSD (*p*=0.05)		1.56		0.55	0.55		2.21		2.21		0.78	3.13			

Table 4. Changes in pH after fungal inoculation in different media at different salt concentration

	NaCl	Control	Fungal isolates N_1	N_2	N_3	N_4	N_5	N_6	N_7	N_8	N_9	N_{10}	N_{11}	N_{12}	N_{13}	N_{14}	N_{15}
05 Days																	
Media I	1%	8.13	6.03	5.72	5.75	5.28	5.57	5.86	5.31	4.75	4.89	5.96	5.87	5.70	6.17	5.67	5.80
	5%	8.10	5.92	5.62	6.01	6.15	5.24	5.97	5.49	4.66	4.77	5.95	5.97	5.17	5.77	5.72	5.95
Media II	1%	8.43	5.70	5.39	6.20	5.83	4.53	5.51	4.87	4.57	5.17	5.47	6.28	5.07	6.26	6.20	5.49
	5%	7.93	5.37	5.67	5.43	5.60	4.47	5.83	4.70	4.30	4.67	5.60	6.43	4.93	5.93	5.93	5.97
				Fungi (F)		Media (M)	NaCl (C)		F×M		F×C		M×C	F×M×C			
		Sem		0.097		0.034	0.034		0.137		0.137		0.049	0.194			
		LSD (p=0.05)		0.272		0.096	NS		0.38		NS		NS	NS			
10 Days																	
Media I	1%	7.90	5.47	5.58	5.67	5.72	5.17	5.82	5.10	4.57	4.72	5.78	5.67	5.03	5.83	5.60	5.73
	5%	8.00	5.83	5.31	5.48	5.97	5.15	5.69	5.31	4.67	4.53	5.75	5.68	5.07	5.53	5.38	5.60
Media II	1%	7.73	5.47	5.23	5.70	5.53	4.59	5.63	4.50	4.60	3.77	5.33	5.60	4.57	5.76	5.83	5.67
	5%	7.97	5.43	5.53	5.53	5.40	4.79	5.70	4.51	4.46	4.43	5.57	6.03	4.97	6.20	6.10	5.91
				Fungi (F)		Media (M)	NaCl (C)		F×M		F×C		M×C	F×M×C			
		Sem		0.111		0.04	0.04		0.16		0.16		0.07	0.222			
		LSD (p=0.05)		0.311		0.11	0.11		0.44		N/A		NS	N/A			
15 Days																	
Media I	1%	7.53	5.37	5.43	5.37	5.79	5.00	5.77	5.07	4.28	4.60	5.62	5.57	4.40	5.51	5.35	5.57
	5%	7.77	5.48	5.25	5.39	5.40	4.97	5.53	4.91	4.38	4.47	5.21	5.47	4.17	5.13	5.23	5.28
Media II	1%	7.67	5.27	4.50	5.40	5.20	4.24	5.63	3.50	3.46	3.31	5.13	5.43	3.37	5.20	5.27	5.13
	5%	8.10	5.37	5.83	6.00	5.50	4.07	5.50	4.13	3.90	4.03	5.57	5.47	5.07	5.67	5.71	5.83
				Fungi (F)		Media (M)	NaCl (C)		F×M		F×C		M×C	F×M×C			
		Sem		0.111		0.04	0.04		0.16		0.16		0.06	0.222			
		LSD (p=0.05)		0.311		0.11	0.11		0.44		N/A		0.15	N/A			

Table 5. Organic acid (meq L^{-1}) release by fungal strains at various incubation days

Fungal isolates	Incubation days		
	5	10	15
Control	25.0	27.9	29.58
N_1	35.4	38.8	42.92
N_2	34.6	39.2	42.92
N_3	34.2	38.3	44.17
N_4	33.3	36.7	45.42
N_5	47.1	49.6	53.33
N_6	37.5	41.3	44.17
N_7	48.3	51.3	57.50
N_8	47.9	49.6	53.75
N_9	44.2	47.9	50.42
N_{10}	36.7	40.8	42.92
N_{11}	35.4	38.8	42.50
N_{12}	47.9	50.7	55.83
N_{13}	37.5	40.4	45.00
N_{14}	35.0	39.6	42.50
N_{15}	39.6	42.1	47.08
Sem	1.82	2.18	2.24
LSD (p=0.05)	5.10	6.09	6.28

Na^+ from entering the cytoplasm since it is detrimental to the cell's ability to function, keeping their cytoplasm hyperosmotic to the external environment and generating the required turgor pressure (Oren *et al.*, 2002; Ventosa *et al.*, 1998). These salt–tolerant microorganisms have strong transport systems that remove Na^+ from the inside of the cell. This mechanisms is often dependent on Na^+/H^+ antiporters. To maintain a low concentration of Na^+ while achieving greater osmotic pressure in the cytoplasm, microbes employ two essentially distinct strategies: (i) the "salt–in" method refers to the ability of cells to maintain high intracellular salt concentrations (typically KCl), which will at least be equivalent to the exterior amounts osmotically. Then, every intracellular system ought to be adjusted to deal with the elevated quantities of salt; (ii) using small organic molecules referred to as compatible solutes (also known as the "compatible solute" method), cells can regulate the osmotic pressure of the medium and maintain low salt concentrations within their cytoplasm. Compatible solutes, as their name implies, do not impede the operations of cells, so a particular adaptation of the intracellular systems is not necessary (Oren *et al.*, 2002). Using this approach, many species can adapt to an extensive range of salinity concentrations. This is the second technique used by all known fungal species (Azad and Kaminskyj, 2016).

Fungi can persist at high salinity concentrations, but, as the results show, biomass falls as NaCl concentration rises (Fig. 3). The reduction in growth observed as the concentration of NaCl increases can be explained by the organisms being exposed to hyper–osmolar conditions, which causes decline in the activity of cytoplasmic water. Solutes like NaCl raise the osmolarity in the medium. Due to which, intracellular water content reduces and an simulataneously the osmolarity of the intracellular

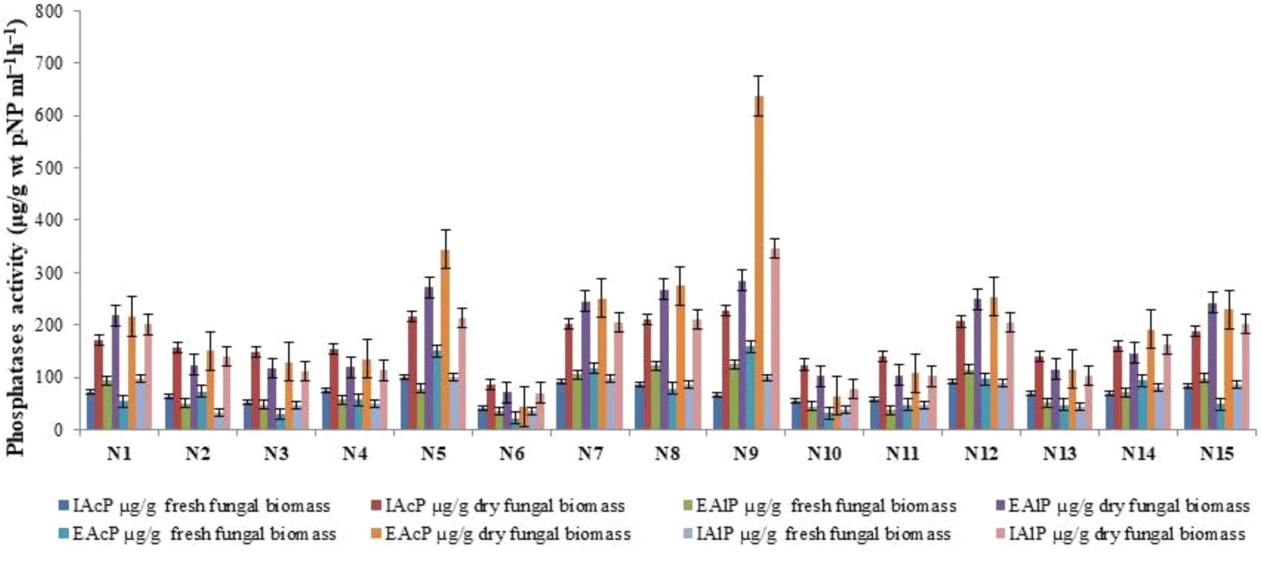

Fig. 2 Intracellular and extracellular phosphatase activity demonstrated by salt-tolerant fungal isolates

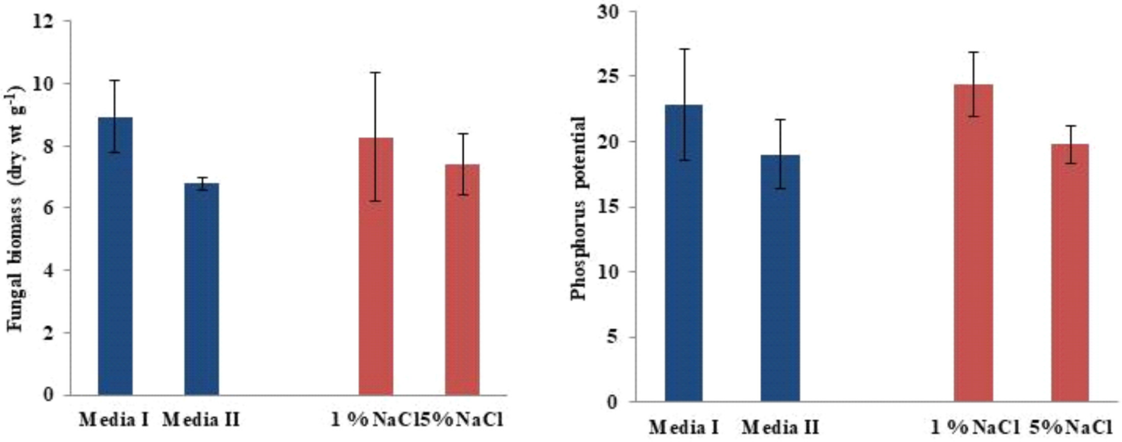

Fig. 3 Effect of nutrient medium and salt concentration on (a) fungal biomass and (b) phosphorus potential

contents increases (Srinivasan *et al.*, 2012). It seems likely that only specific normal ranges of water activity have been evolved by proteins (enzymes) and other biological macromolecules, beyond which some critical cellular functions become compromised (Bremer and Krämer, 2019; Gutierrez *et al.*, 1995). It was observed that several Rhizobium strains obtained from soils of high pH could withstand salt concentrations of up to 860 mM (5.0%) but that at 1290 mM (7.5%), growth was hindered (Surange *et al.*, 1997).

The present study was conducted to assess the ability of a selected group of efficient isolates to grow and solubilize tricalcium phosphate in a broth medium containing different concentrations of NaCl. These P-solubilizers were specifically isolated from soils affected by salt. In the presence of high salt concentrations, all PSF isolates showed discernible levels of phosphate solubilization. Several researchers suggests that strains isolated from extreme soils are naturally adapted to such harsh environments and possess genetic traits that enable them to solubilize phosphates even under significantly high salt stress.(Srinivasan *et al.*, 2012; Zhang *et al.*, 2023). PSF can transform insoluble Pi into soluble orthophosphate forms (PO_4^{3-}, HPO_4^{2-}, and $H_2PO_4^-$) by secreting various organic or inorganic acids. These acids release H^+, lowering the pH of the medium and facilitating phosphate solubilization..Organic acid carboxyl groups can bind P by either competing with cations for P adsorption sites or by replacing them, which increases Pi solubilization and PO_4^{3-} soil absorption. PSM isolates produce organic acids at notable amounts and perform well in P solubilization (Castagno *et al.*, 2011; Liu *et al.*, 2014). PSF that was isolated for this investigation also showed strong P-solubilizing capacity in addition to organic acid (Fig. 4a).

The study also noted a decrease in pH, which can be attributed to the fungi's release of organic acids into the medium (Fig. 4b). The inverse relationship between pH and soluble–phosphate concentration suggests that the strain's generation of organic acid contributes significantly to the medium's acidification, promoting phosphate's solubilization. The use of organic acid or the creation of alkaline substances may cause the pH to increase (Behera *et al.*, 2017; Zuluaga *et al.*, 2023). There has been prior research on the comparable inverse link between pH and soluble phosphate (Figure 4c) (Panda *et al.*, 2016).

The findings also indicated that P–solubilizing potential increased with incubation time, possibly because of improved adaptation of fungi to highly salinized conditions. Results also demonstrated that at every incubation period, a rise in NaCl content generally resulted in a decrease in the amount of Pi released. A disruption in the normal functioning of the synthesis pathways may cause the decreased development of fungi in the presence of NaCl. Research has demonstrated that specific mineral ions, including Na^+, harm growth by preventing certain enzymes from functioning. The presence of Na^+ may have impacted the enzymes necessary for the typical growth process of fungus (Pang *et al.*, 2020; Zhou *et al.*, 2023).

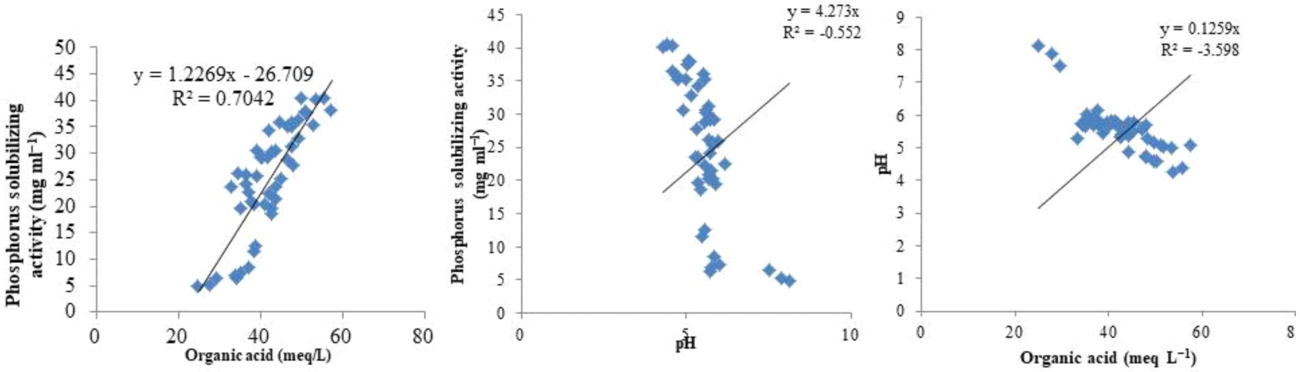

Fig. 4 Relationship between (a) production of organic acid and phosphorus solubilization activity; (b) production of organic acid and pH; (c) phosphorus solubilization activity and pH

To develop bio inoculants, fungal cultures are required to multiply in bulk, and suitable medium selection is required. In the present study, two mediums were studied, and results demonstrated that PDM supported fungi better than PKM in growth (Fig. 4) and P–solubilizing potential. Potato Dextrose Agar comprises dehydrated Potato Infusion and Dextrose that encourage luxuriant fungal growth. The bio–inoculant will be applied in natural conditions; hence, its ability to mineralize organic P needs to be investigated. In the present study, PSF isolated also has the potential to produce extracellular phosphatase enzyme, which can mineralize organic P to inorganic P.

Conclusion

This study demonstrates the presence of salt-tolerant phosphorus-solubilizing fungi, which are crucial for agriculture and are thriving in the challenging conditions of salt-affected soils. In conclusion, this study highlights the potential of salt-tolerant P-solubilizing fungi as a natural solution to mitigate the adverse effects of salinity on agricultural productivity. The findings demonstrate that fungal isolates exhibit significant variations in P-solubilization efficiency, with Potato Dextrose Medium (PDB) proving to be the optimal medium for bulk production of bio-inoculants. The ability of these fungi to produce organic acids and phosphatase enzymes further supports their role in enhancing nutrient availability in saline soils. These results pave the way for future research and practical applications in sustainable agriculture, offering an environmentally friendly approach to improving crop growth in salt-affected areas

Acknowledgements

The authors thank the Director, ICAR–Central Soil Salinity Research Institute, Karnal, India, for providing the necessary facilities to conduct the research work. This research article was approved by Prioritization, Monitoring and Evaluation Cell (PME), ICAR–CSSRI (Research Article No. 14/2022).

Funding

Indian Council of Agricultural Research, New Delhi (India) "Project: Development of endo-rhizospheric fungal consortia to increase salt tolerance in crops"; and Science and Engineering Research Board, Department of Science and Technology, Government of India (India) (EEQ/2021/000369); and Project (NRMACSSRI SIL201700600930): "Development of Arbuscular Mycorrhizal Fungi (AMF) based plant biostimulant to enhance the productivity of salt-affected soils".

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Received: December 9, 2024; Accepted: June 25, 2025