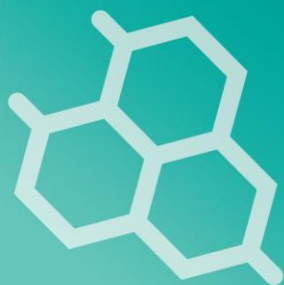


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INFLUENCE OF PH ON THE GROWTH OF PHOSPHATE SOLUBILIZING FUNGI ISOLATED FROM SOILS

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ABSTRACT

The pH profoundly influences phosphate-solubilizing fungi (PSF) by affecting their growth and survival, as each species has an optimal pH range for activity and survival. The influence of pH on the growth and survival of phosphate-solubilizing fungi were investigated. The three representative fungal strains were cultured for seven days with the initial pH in the medium ranging from 1.5 to 8.5. We estimated the fungal growth by measuring the dry matter of mycelial biomass. The growth-based measurements revealed that all the tested fungal strains were capable of growing and surviving in a wide range of pH (2.5-8.5). Among them, SI-10URAg (*A. niger*) enhanced the highest acidity in all tested pH values, followed by *P. oxalicum* (SI-16URAg) and SI-14URAg. Fungal growth primarily depends on the pH. SI-10URAg showed the highest growth (0.28g) at pH 3.5. Besides this, SI-14URAg and SI-16URAg showed the maximum growth (0.43g and 0.20g) when the initial pH value was 5.5 and 7.5, respectively. These findings suggested that *A. niger* have the strongest adaptability to acidic environment followed by *P. oxalicum*. Although these fungal strains could grow and survive in higher pH also. It may give an extra advantage to utilize these strains in any pH condition in the soil.

1. INTRODUCTION

The soil microbial community is responsible for most nutrient transformations in soil. Availability of these mineral nutrients limits soil fertility and plant productivity. The activities and survival of microbes are influenced by various properties such as biomass composition (De Ruyter et al.,1993), nutrient demand (De Vries et al., 2006; Rousk et al., 2007; Van et al., 2007), metal tolerance (Rajapaksha et al.,2004), temperature, and pH dependence (Pietikinen et al., 2005). Besides these, anthropogenic impacts, such as changes in nutrient input, climate change, and soil management, have

the potential to directly or indirectly affect the fungal composition, with consequent impacts on soil function.

Soil pH is one of the most influential factors affecting the microbial community. It also influences other abiotic factors, such as availability of carbon, nutrients and solubility of metals (Andersson et al., 2000; Kemmitt et al., 2006; Aciego et al., 2008; Firestone et al., 1983; Flis et al., 1993). In addition of these, soil pH may control the biomass composition of fungi and bacteria in both forest and agricultural soils (Fierer et al., 2006; Baath et al., 2003; Blagodatskaya et al., 1998; Arao et al., 1999; Bardgett et al., 2001). The growth of fungi could be affected by 'Hydrogen ion concentration' (pH) in a medium in which it grows, either directly by its action on the cell surfaces or indirectly by its effect on the availability of nutrients. However, the acid/alkaline requirement for growth of fungi is quite broad, ranging from pH 3.0 to more than pH 8.0, with an optimum around pH 5.0 if nutrient requirements are satisfied (Pardo et al., 2006). In general, a neutral to weak acidic environment was suitable for mycelial growth, with optimum pH 5.0 –7.0 and pH 5.0 –8.0 for conidial production (Zhao et al., 2010)

So, it is important to studying the influence of soil pH on growth and survival of fungi as an inherent problem that influences multiple parameters. Therefore, present study aimed to evaluate the influence of pH on the growth of selected fungal strains which were isolated from different soils in subtropical Okinwa, Japan as phosphate solubilizing fungi.

2. MATERIALS AND METHODS

Fungal Strains used in this study

In our previous study, sixteen phosphate-solubilizing fungal strains were isolated from different soils of Okinawa, Japan, and identified (Islam et al., 2019). The three representative fungal strains *Aspergillus niger* (SI-10URAgr), *Aspergillus flucosus* (SI-14URAgr) and *Penicillium oxalicum* (SI-16URAgr) were selected to determine the influence of pH on their growth and survival.

Preparation of inoculum for Biomass measurement of fungi under different pH conditions

Sporulated pure fungal cultures, slants of 10URAgr, SI-14URAgr, and SI-16URAgr were selected for preparation of spore suspension by using the standard procedure. A total volume of 5 ml sterile water with twin 80 was added in culture slants and the fungal colony surface was lightly scraped by sterile bamboo stick. The cultures were passing through a syringe with staff cotton. Spore count was done by a haemocytometer and the suspension was adjusted to approximately 10^6 spore's mL⁻¹. The potato dextrose liquid medium was precisely regulated to pH 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, 7.5 and 8.5 with adding hydrochloric acid for lower pH and sodium hydroxide for higher pH adjustment. Then sterilized at 121 °C for 20 min. The 1 ml spore suspension of each fungal strains were inoculated in Erlenmeyer flask containing 50 ml Potato Dextrose broth medium with various acidity (Fig. VI.1.). These flasks were incubated at 28 ± 2 °C for seven days under shaking speed 150 rpm. Then the cultures were filtered through Whatman No.2A filter paper and the mycelium was weighed after drying 24 h at 65 °C.

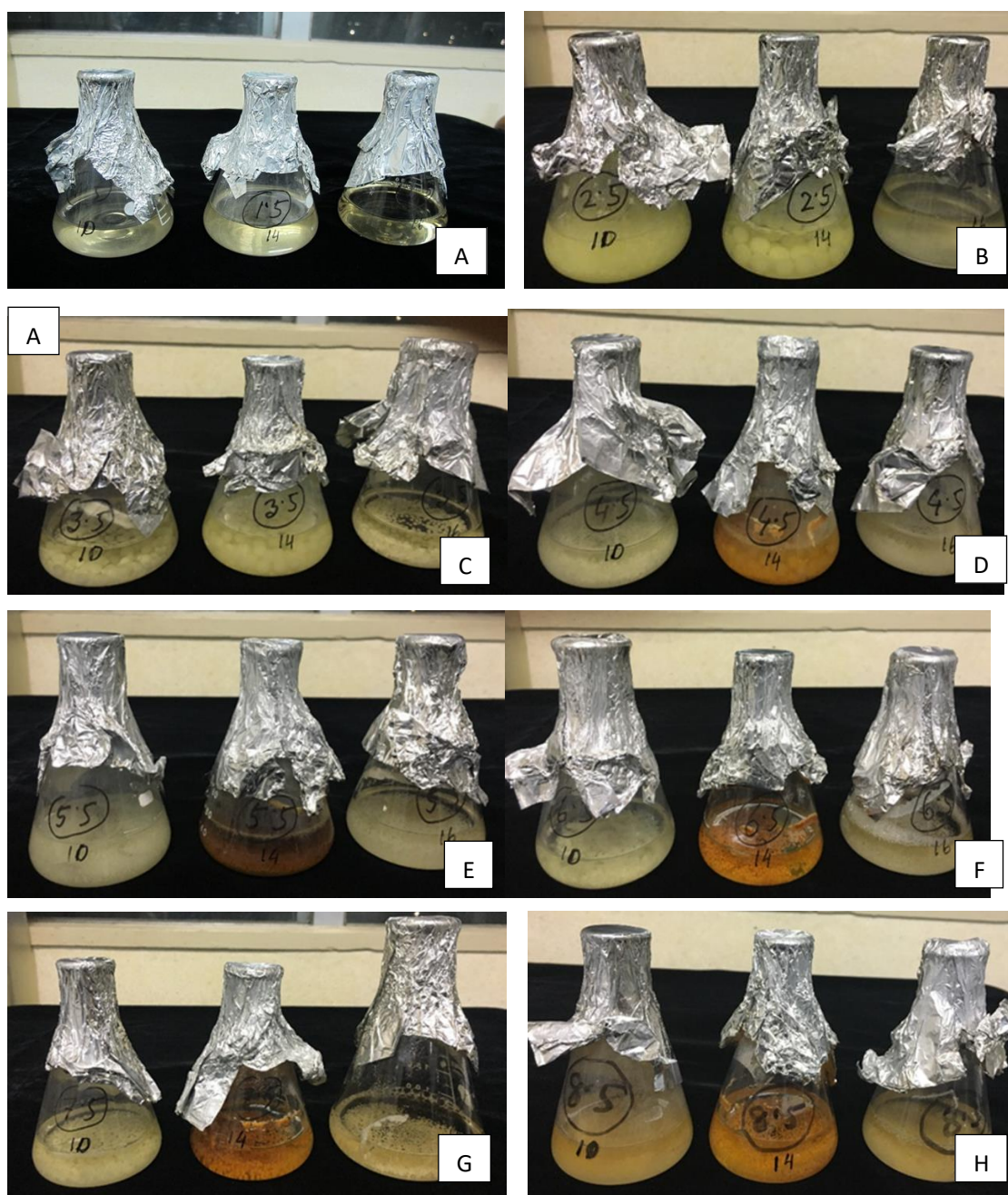


Figure VI.1: Effect of pH on the growth of phosphate solubilizing fungi isolated from subtropical soils in Okinawa, Japan (A: pH 1.5; B: pH 2.5; C: pH 3.5; D: pH 4.5; E: pH 5.5; F: pH 6.5; G: pH 7.5; H: pH 8.5).

pH value measurements

The pH values of fermented broth culture were measured after filtered through a 0.45 mm filter unit by a pH meter (Horiba, Japan) furnished with glass electrode.

Statistical analysis

All experiments were conducted in triplicate and data were analyzed using the Microsoft Excel program (version 2016). The mean values were compared by Tukey's Honest Significant Difference (HSD) Test at $p < 0.05$ level.

3. RESULTS

Influence of initial pH on the acidification ability of fungi

After seven days of incubation, the pH value of all culture filtrates were significantly decreased compared to original (8.5, 7.5, 6.5, 5.5, 4.5, 3.5 and 2.5). Results also showed that SI-10URAgr dropped the maximum pH when initial pH was 8.5, 7.5, 6.5, 5.5 and 4.5 followed by SI-6URAgr (Table VI.1). The final pH value was 1.70, 1.84, 1.86, 1.78 and 1.90 respectively. In the initial pH 7.7-5.5, all fungal strains have steady state condition in pH dropping (Fig.VI.2). Considering all the tested fungal strains, SI-10URAgr has the highest acid producing ability compare to other strain based on pH reduction.

Table VI.1. Variation of acidity of phosphate-solubilizing fungal strains in the culture medium isolated from soils

| Fungal strains | Original pH of the medium | | | | | | |
|----------------|------------------------------------|-------|-------|-------|-------|-------|-------|
| | 8.5 | 7.5 | 6.5 | 5.5 | 4.5 | 3.5 | 2.5 |
| | Final pH (After 7 days incubation) | | | | | | |
| SI-10URAgr | 1.70c | 1.84c | 1.86c | 1.78c | 1.90c | 1.86a | 1.96a |
| SI-14URAgr | 6.21a | 5.05a | 5.01a | 4.94a | 2.71a | 1.56b | 1.37c |
| SI-16URAgr | 2.38b | 2.36b | 2.33b | 2.40b | 2.12b | 1.91a | 1.87b |

Values given are the mean of three replicates. Means with the same letter are not significantly different according to Tukey's Honest Significant Difference (HSD) Test at $p < 0.05$ level.

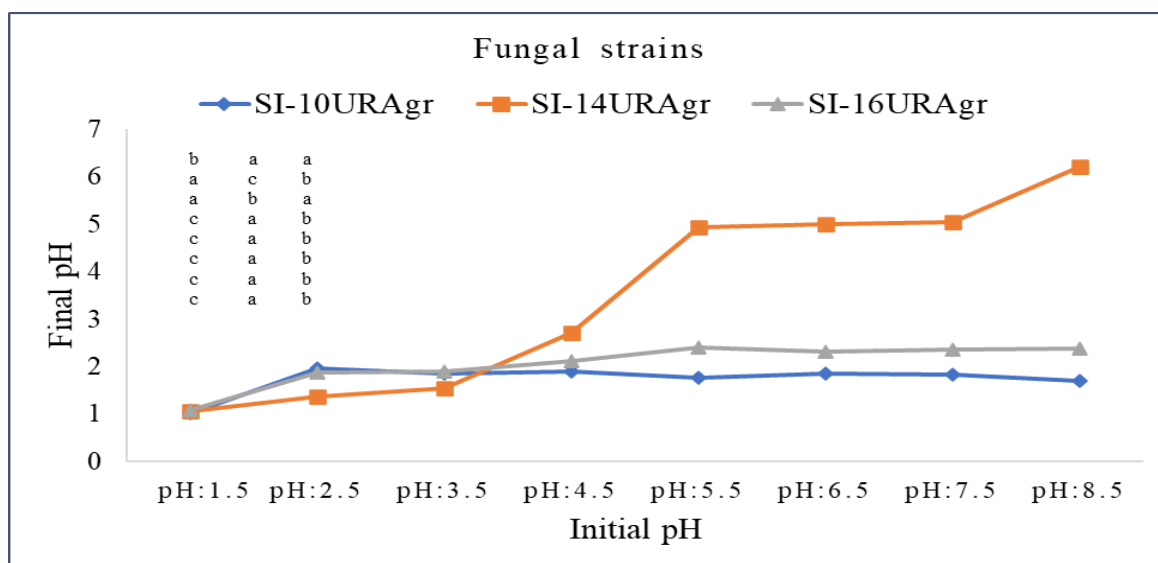


Fig. VI.2. Effect of pH on acidification ability of phosphate-solubilizing fungi isolated from soils.

Values given are the mean of three replicates. Means with the same letter are not significantly different according to Tukey's Honest Significant Difference (HSD) Test at $p < 0.05$ level.

Influence of pH on the growth of fungi in relation to their mycelial dry weight

All the tested fungal strains were cultured in the medium with initial pH value 8.5, 7.5, 6.5, 5.5, 4.5, 3.5, 2.5 and 1.5. Mycelial biomass is a significant parameter to directly evaluate the growth of fungi. The tested fungal strains showed distinct biomass change under various acidic environment (Table VI.2.). SI-10URAgr produced significantly the highest amount of biomass in extreme acidic environment (pH 3.5, 2.5 and 1.5) followed by SI-16URAgr and SI-14URAgr (Fig.VI.3). SI-14URAgr produced the significantly highest amount of biomass in pH 8.5, 7.5, 6.5, 5.5 and 4.5. This strain has better adaptability in both alkaline and acidic environments. Results also demonstrated that few dispersive mycelia can be identified at pH 1.5. The biomass of SI-10URAgr (0.283g), SI-14URAgr (0.433g) and SI-16URAgr (0.206g) arrived peak value when initial pH 3.5, 5.5 and 7.5 respectively (Fig.VI.3).

Table VI.2. Influence of pH on the growth of representative phosphate-solubilizing fungal strains isolated from subtropical soils.

| Fungal strains | pH of the medium | | | | | | | |
|----------------|--------------------------|---------|--------|--------|--------|--------|--------|--------|
| | 8.5 | 7.5 | 6.5 | 5.5 | 4.5 | 3.5 | 2.5 | 1.5 |
| | Produced biomass(g/50ml) | | | | | | | |
| SI-10URAgr | 0.16b | 0.183bc | 0.060c | 0.033c | 0.203b | 0.283a | 0.270a | 0.150a |
| SI-14URAgr | 0.246a | 0.22a | 0.230a | 0.433a | 0.266a | 0.200b | 0.160b | 0.100b |
| SI-16URAgr | 0.126c | 0.206b | 0.146b | 0.186b | 0.166b | 0.204b | 0.123b | 0.103b |

Values given are the mean of three replicates. Means with the same letter are not significantly different, according to Tukey's Honest Significant Difference (HSD) Test at $p < 0.05$ level.

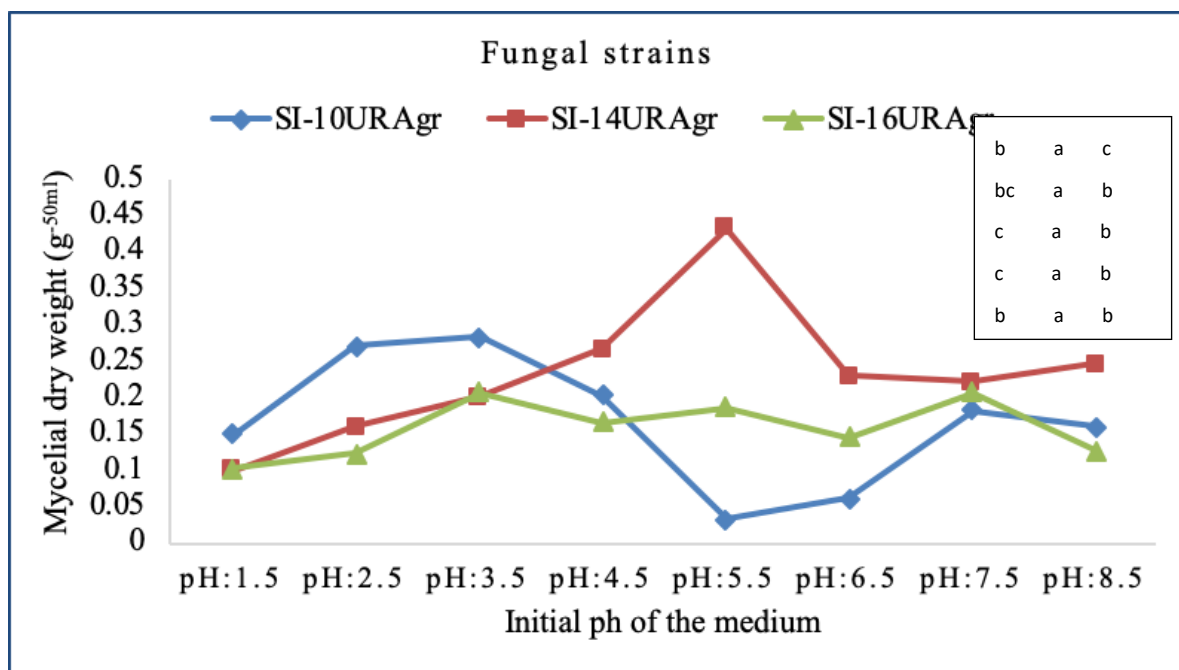


Fig.VI.3. Effect of pH on the growth of phosphate-solubilizing fungi isolated from soils.

Values given are the mean of three replicates. Means with the same letter are not significantly different according to Tukey's Honest Significant Difference (HSD) Test at $p < 0.05$ level.

4. DISCUSSION

The sixteen phosphate-solubilizing fungi were isolated from different soils in subtropical Okinawa, Japan and identified as *Aspergillus* spp. *Penicillium* spp. and *Talaromyces* spp. (Islam *et al.*, 2019). To investigate the mechanism of phosphate solubilization, organic acids were previously determined by HPLC. Considering phosphate solubilization in solid and broth medium amended with different insoluble phosphate compounds as well as their organic acid production potential, *Aspergillus niger* and *Penicillium oxalicum* were to be selected as prominent isolates followed by *Penicillium* sp. and *Aspergillus flucosus*. Three representative isolates were used in this study to evaluate their acid production potential and growth in different pH environments.

Seven days culture in this study showed that both SI-10URAg and SI-16URAg have impressive potentials in changing pH within such a short time compare with SI-14URAg. The organic acids secreted by fungi contribute to pH dropped. It indicated the higher phosphate-solubilizing ability of fungi. In the present study, *A. niger* and *P. oxalicum* were isolated from the soils in Okinawa, Japan and were identified as the fungi with prominent phosphorus-solubilizing ability in the pools of *Penicillium* and *Aspergillus* (in our lab) respectively.

The *A. niger* shows higher solubilizing ability and organic acids production potentials compared to *P. oxalicum* and *A. flucosus* (Islam *et al.*, 2019). Considering the biomass production, *A. niger* and *Penicillium oxalicum* produced a lower amount of biomass compared to that of *Aspergillus flucosus* (Table 2; Fig.VI.3.). *A. niger* and *P. oxalicum* shows high efficiency in acid secretion per unit of biomass (Zhen *et al.*, 2016). The pH reduction can be attributed to diffusion of various organic acids secreted by the PSF (Singh *et al.*, 2011). In soils, the organic acids are more adventitious compared to

inorganic acids because they have a low acidity constant (Zhen *et al.*, 2016).

A serial of organic acids has various acidity constants, which determine their ability in changing acidity of the environments. The acidity has great significance on the activities of microorganisms. The fungal species used in this study have eosinophilic characteristics, and their growth was relatively good in acidic environment (pH 3.5-5.5) but they also could grow in higher pH (8.5). However, under the extreme acidic condition (pH < 2.5), only the biomass of *A. niger* (SI-10URAgr) (Fig.VI.3.) was visualized. *Aspergillus niger* (SI-10URAgr) has more acid production potential and higher adaptability to acidic environments, compared to *Penicillium oxalicum* (SI-16URAgr) and *Aspergillus floccosus* (SI-14URAgr).

5. CONCLUSION

All the tested fungi could grow a wide range of pH (1.5-8.5) but pH values <2.5, their growth seriously hampered. Among the tested fungal strains, *Aspergillus niger* (SI-10URAgr) has more acid production potential and higher adaptability to acidic environment compared to *Penicillium oxalicum* (SI-16URAgr) and *Aspergillus floccosus* (SI-14URAgr). Besides this, the fungal strains also could grow well in higher pH levels. Finally, we concluded that *Aspergillus niger* is a better candidate followed by *Penicillium oxalicum*, as a bioinoculant for phosphate solubilization in phosphate deficient problem soils.

6. CONFLICT OF INTEREST

The authors declare that there is no existing conflict of interest

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