Isolation and Characterisation of Seaweed Endophytic Fungi as an Efficient Phosphate Solubiizers

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Phosphate-solubilizing fungi (PSF) generally enhance the availability of phosphorus (P) released from soil, which contributes to plants' P requirement, especially in P-limiting regions. In this study we isolated endophytic fungi from seaweeds and screened for phosphate solubilizing in both solid and liquid culture and estimated the solubilizing index and enzyme activity. Six fungus of Penicillium oxalicum, P. citrinums and Aspergillus sp. shows maximum phosphate solubilizing activity. Hence Seaweed endophytic fungus isolated from chlorophyceae express as an alternate source to replace chemical fertilizer.

Keywords: Chlorophyceae; Penicillium Oxalicum; Aspergillus sp.

Phosphorus (P) is the next important nutrients after nitrogen which have an impact on plant growth and its metabolic processes (Widawati S & Suliasih D, 2006). Phosphorus is essential to promote photosynthesis, energy regulation, sugar production, synthesis of nucleic acid, and enhancing N2 fixation in leguminous plants (Saber et al., 2005). It also plays an important role in strengthening straw of the cereal plant and quality of the crop, boost flower formation and increase the fruit production, stimulates root development and essential for seed formation, maturity, and also to develop resistance against diseases (Sharma et al., 2011, Richardson, 2007).

Generally, the mobility of phosphate ions in the soil is very low due to high retention. Stevenson and Holford reported in 1986 that the plants utilize P from soil in the rate of about 10-30% (Stevenson, 1986) and the remaining 70-90% of Phosphorus is accumulated in the soil as immobilize form or it is bounded with Al or Fe in acid soils or Ca and Mg in alkaline soils (Prochnow et al., 2006; Yang et al., 2010)

This insoluble form of Phosphorus is converted to soluble form by the microorganisms which play a key role in dissolving both free and bound phosphate in the soil that is environmentally friendly and sustainable (Khan et al., 2007). Microorganisms including fungi, bacteria and actinomycetes are known to be efficient as fixed P solubiizes (Sundara et al., 2002).

Seaweeds are considered as a well-endowed determinant for nutrients that ultimately leads to high competition between different microbial communities (Burgess et al., 1999; Armstrong et al., 2001; Penesyan et al., 2009).
The second largest diverse assemblage of marine fungi is from seaweeds (Bugni and Ireland 2004; Schulz et al. 2008; Suryanarayanan et al. 2010; Godinho et al. 2013). Seaweeds encompassing genera belonging to *Phaeophyceae, Rhodophyceae* and *Chlorophyceae* have been broadly studied worldwide for their fungal associations and the study revealed that the dominant species of fungal endosymbionts were ascomycetes and anamorphic fungi (Schulz et al. 2008; Zuccaro et al. 2008; Suryanarayanan et al. 2010; Godinho et al. 2013.). The fungi associated with seaweeds are mostly parasites, saprobes or asymptomatic fungal endosymbionts (Bugni and Ireland 2004; Loque et al. 2010; Suryanarayanan et al. 2010). The diverse fungal population in Red and Brown seaweeds were comparatively high compared to that of Green seaweed (Suryanarayanan et al. 2010). This is due to short lifecycle of Green seaweeds and characteristically slow growth of the endosymbionts could together be responsible for low fungal diversity (Zuccaro and Mitchell 2005). Fungal genera of *Acremonium, Alternaria, Arthrinium, Aspergillus, Cladosporium, Fusarium, Geomyces, Penicillium*, and *Phoma* (Zuccaro et al. 2003; Loque et al. 2010; Suryanarayanan et al. 2010; Flewelling et al. 2013; Godinho et al. 2013; Furbino et al. 2014) were the most common fungal endosymbionts.

On comparing with bacteria fungi have greater ability to solubilize insoluble phosphate (Nahas, 1996). Endophytic fungi from Marine plants and Marine algae are gaining interest because of their existence in an ecosystem renowned by resource inconstancy such as temperature, salinity, osmotic stress, light vailability (Debbab et al., 2011; Oliveira et al., 2012).

Therefore, the objective of this study is to search for the seaweed in order to select the endophytic fungi which can solubilize phosphate. The results may provide insights into potential fertilizer in acidic soils and P-deficit soils. Microbial phosphate solubilization has an impact on plant growth promotion. There are several reports regarding plant growth promotion due to inoculation of phosphate solubilizing microorganisms. Screening and characterization of phosphate solubilizing microorganisms are important for proper utilization of their beneficial effects to increase the crop production and sustain agricultural productivity of the country without contaminating environments.

**MATERIALS AND METHODS**

**Collection of sample**

Seaweeds were collected from Rameswarem coastal region. Abundant seaweeds are selected for the isolation of endophytic fungus. *Cauerapa racemosa, Halimeda macrooba* (Chlorophyceae), *Turbinaria conoides, Sargassum sp, Padina sp* (Phaeophyceae), *Gracilaria sp., Portieria sp.* (Rhodophyceae). Seaweeds were collected in sterile bags and processed in laboratory. Freshly collected plant materials were utilized for the isolation of the endophytic fungi to reduce the chance of contamination.

**Isolation of endophytic fungi from seaweeds**

Healthy thallus of the seaweeds were thoroughly washed in seawater followed by running tap water, then surface sterilized by a modified method of [Raviraja, 2005]. The selected thallus segments were immersed in 95% ethanol for 30 sec, 4% sodium hypochlorite solution for 3 min and 95% ethanol for 30 sec followed by rinsing with sterile distilled water three times and allowed to surface dry under sterile conditions. After drying, each thallus segment was cut into approximately 0.5 cm and placed on petri plates containing potato dextrose agar medium (PDA) supplemented with Chloramphenicol (100 mg/L) to suppress the bacterial growth. Petri plates were sealed with parafilm and incubated at 30°C in a light chamber for up to one week. They were monitored every day for growth of endophytic fungal colonies. Fungi growing out from the samples were subsequently transferred onto fresh PDA plates to isolate pure colonies.

**Identification by morphological characteristics of endophytic fungi**

Sporulating fungi were identified based on colony morphology, conidiospore and conidiophore characteristics. The microscopic identification of the isolates was carried out by lacto phenol staining technique [Nagamani et al., 2006].

**Screening of Phosphate solubilizing in Solid Medium**

Phosphate solubilizing ability of the isolates was confirmed by incubating them on Pikovskaya’s agar medium incorporated with
P-fungal isolates Phosphate Solubilizing Index (PSI) mm

<table>
<thead>
<tr>
<th>No</th>
<th>Fungal Isolates</th>
<th>7th Day</th>
<th>14th day</th>
<th>21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Pencillium oxallicum</em></td>
<td>3.5</td>
<td>5.2</td>
<td>7.1</td>
</tr>
<tr>
<td>2</td>
<td><em>P. Purpuranogenum</em></td>
<td>2.5</td>
<td>3</td>
<td>3.5</td>
</tr>
<tr>
<td>3</td>
<td><em>P. citrinum</em></td>
<td>2.6</td>
<td>3.2</td>
<td>3.7</td>
</tr>
<tr>
<td>4</td>
<td><em>P. aurantio-griesum</em></td>
<td>1.3</td>
<td>2.4</td>
<td>2.9</td>
</tr>
<tr>
<td>5</td>
<td><em>Aspergillus niger</em></td>
<td>1.7</td>
<td>2.6</td>
<td>3.1</td>
</tr>
<tr>
<td>6</td>
<td><em>Aspergillus sp.</em></td>
<td>3.2</td>
<td>5.7</td>
<td>7.9</td>
</tr>
</tbody>
</table>

**Fig. 1. Solubilizing index**
RESULTS AND DISCUSSIONS

Seven seaweeds belonging to Clorophyceae, Rhodophyceae and Phaeophyceae were selected for the isolation of endophytic fungi. Totally 18 endophytic fungi were isolated and screened for Phosphate solubilization. Of that six species shows better result which is discussed in this paper.

Phosphate solubilization Solid Medium

Endophytic fungal isolates showed phosphate Solubilizing activities as detected in Pikovskaya’s agar amended with tri-calcium phosphate in the medium by the appearance of halos around the inoculum on the medium. Aspergillus sp, P. oxalicum, P. citrinum, superior solubilization index (PSI) 5.7, 5.2 and 3.2 respectively in 15 days incubation.

Phosphatase solubilization in liquid culture

Phosphate solubilization by six species of fungal strains was carried out at, The fungal broth were weekly withdrawn to estimate solubilization index and the corresponding changes in the pH of the broth are presented in Fig. 3.

Phosphate solubilization was estimated on the 7th day giving the values of *Penicillium oxalicicum* 220 µg mL⁻¹ with the pH 3.9, by pH 3.3 it solubilize 110 µg mL⁻¹ *P. aurantio-griseum*, 230 µg mL⁻¹ by *P. citrinum* with an pH 3.7, 210 µg mL⁻¹ by *P. purpurogenum* pH 3.7 and *Aspergillus niger* with the pH 4 it solubilize phosphate 190 µg mL⁻¹ and *Aspergillus sp.* by 195 µg mL⁻¹ with the pH 3.5.

There is a increase in solubilization rate and decrease in the pH at the 15th day of incubation. The values were recorded as pH 3.3 with 310 µg

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Fig. 2. Phosphate soubilization in pH of broth at 21°C estimated upto day 30 days at Weekly intervals
mL⁻¹ *P. oxalicum* and 425 µg mL⁻¹ with the pH 3.1 *P. citrinum* and at pH 3.1 *P. purpurogenum* solubilizes 391 µg mL⁻¹ for *Aspergillus niger* at 3.9 pH it solubilizes 410 µg mL⁻¹ and *Aspergillus sp.* with pH 3.5 solubilizes 325 µg mL⁻¹ If the strains attained the maximum solubilization rate at 400 µg mL⁻¹ decline values were recorded at 21st day. *P. oxalicum* attain its maximum solubilization at 30th day of incubation.

Decrease in pH of the broth coinciding with increase in phosphate solubilization estimations which indicates the production of organic acids in the broth, recorded in all the strains were noticed. Various mechanisms have been reported for phosphate solubilization, the most recognized one is through the production of organic acids (Nahas, 1996). Production of organic acids, like citric, gluconic and oxalic acid, have been recognized for phosphate solubilization by several species of *Penicillium*, namely, *P. bilaii*, *P. radicum*, *P. rugulosum*, *P. variabile* (Asea et al., 1988; Cunningham and Kuiack 1992; Vassilev et al. 1996). Omar (1998) reported phosphate solubilization activity by *Aspergillus niger* and *Penicillium citrinum* causing remarkable drop in pH of liquid culture.

**Quantitative estimation enzyme phosphatase**

The acidic and alkaline phosphatase activity exhibited by six fungal stains were incubated at 21°C for 30 days and estimated at weekly interval is presented in Fig. 4. All the species showed acidic phosphatase activity higher (1.5–2.0 times) in comparison to the alkaline phosphatase. The maximum phosphatase activity

Fig. 3. Acid and Alkaine phosphatase activity of Fungal species estimated weekly intervals upto 4 weeks
21.23 (Aspergillus sp.), 23.37 (P. oxalicum) and 19.13 (Aspergillus niger), 16.32 (P. aurantiogriseum) was recorded at the week. After week 2 and 28.83 (P. citrinum), 24.32 (Aspergillus sp.), 24.67 (P. oxalicum), 21.34 (P. purpurogenum) and 22.41 (Aspergillus niger) after week 3 of growth. Alkaline phosphatase activity ranged between 9.67 (Aspergilus sp.) and 11.21 (P. citrinum) after week 3 of incubation. Tarafdar et al. (2003), reported the efficiency of seven fungal phosphatase producing fungi and in which the acid phosphatase was three times higher than the alkaline phosphatase. Naturally occurring phosphate solubilizing microorganisms have been recognized as a source of P fertilizer. Amongst fungi, maximum Penicillium species have been reported for various properties of biotechnological applications.

**CONCLUSION**

Therefore, these strains can be a good candidate and exploited as bio fertilizers through further evaluation and optimization test to increase agricultural productivity. Other strains such as Aspergillus niger, P. aurantiogriseum, P. purpurogenum were positive for phosphate solubilization efficiency. P. oxalicum was the superior among the isolated fungi in solubilizing index 5.3 after further evaluation on **invitro** test, green house and field trials as bio fertilizer. The rise in the cost of chemical fertilizer, the lack of fertilizer industries in developing countries and the growing environmental issue and biodiversity loss using chemical fertilizer timely important concern using alternative ecofriendly bio fertilizer to increase yield and productivity.

Fungus from marine ecosystems are distinct from those of terrestrial environments. The most efficient phosphate solubilizing strains *penicillium sp* and *Aspergillus sp.* were identified from green seaweed and to solubilise inorganic phosphate to organic form. Phosphate solubilizing fungus can effectively replace chemical fertilizer.

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