Bio-solubilization of Rock Phosphate and Plant Growth Promotion by *Aspergillus niger* TMPS1 in Ultisol and Vertisol

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Main Concern Related to Phosphorus Research

- **Agricultural point of view**
  - ✓ Phosphorus requirement for sustainable crop production

- **Environmental point of view**
  - ✓ Soil or fertilizer phosphorus contribution to eutrophication of aquatic environments
Facts

- P is an essential plant nutrient
- Global Rock Phosphate (RPs) Production >160 mmt (2008)
- Most RPs are not suitable for phosphatic fertilizer production due to low reactivity and impurities present in them
- Low-grade rock phosphate can be utilized as direct application P fertilizer with or without processing
- Application of rock phosphate in conjunction with phosphate solubilizing microorganisms
Soil Phosphorus Cycle

Exportations in Plant and animal Products

Mineral Fertilizers

Organic fertilizers

Inorganic P in the soil solution

Roots

Aerial Parts

Labile Organic P

Stable Organic P

Inorganic P in primary minerals

Other form of Inorganic P

Microbial solubilization

Microbial P

Losses by erosion, run-off, leaching
The overall objective of this study was to evaluate the efficacy of a fungus *Aspergillus niger* strain TMPS1 isolated from mangrove rhizosphere as phosphate solubilizing bio-fertilizer under greenhouse conditions using $^{32}$p isotope dilution technique.
Isolation of Fungus from Soil

- Soil: Rhizospheric soil of Mangrove
- Media: Pikovskaya’s agar plates
- Formation of a clear halo around the fungal growth after 5 days of incubation
Aspergillus niger TMPS1
Molecular Identification of Fungus

DNA Isolation

Amplification of 18S rRNA gene
(NS5 [5’-AACCTAAAGGAATTGACGGAAG-3’] and NS8 [5’-CCGCAGGTTACCTACGGA-3’])

Cloning

pT257R/T

18S rRNA sequencing and sequence analysis
M13 primer (TGTAAAACGACGGCCAGT)

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Length of ITS region Sequenced (bp)</th>
<th>Identification by ITS region sequencing</th>
<th>Gene identity (%)</th>
<th>GenBank Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMPS1</td>
<td>560</td>
<td>Aspergillus niger</td>
<td>98</td>
<td>DQ316605</td>
</tr>
</tbody>
</table>
Radiotracers for phosphorus

- $^{32}\text{P}$
- $T_{1/2} = 14.3$ DAYS
- $\beta$-EMITTER
- $E_{\text{max.}} = 1.71$ MeV
- COUNTING
  - i) GM
  - ii) Cerenkov or LSC

- $^{33}\text{P}$
- $T_{1/2} = 25$ DAYS
- $\beta$-EMITTER
- $E_{\text{max.}} = 0.248$ MeV
- COUNTING
  - i) LSC
Greenhouse experimental details

- **CROP**: Wheat (Triticum aestivum)
- **VARIETY**: PbW 343
- **SOIL**: 2 kg soil per pot
  - Black soil (Vertisol)
  - Red Soil (Ultisol)

**TREATMENTS**
- Control (No P)
- Soil+ $^{32}$P
- Soil+ TMPS1+ $^{32}$P
- Soil + LRP+ $^{32}$P
- Soil + LRP + TMPS 1+ $^{32}$P
- Soil + PRP + $^{32}$P
- Soil+ PRP+ TMPS 1 + $^{32}$P

**EXPERIMENTAL DESIGN**
- CRD

**REPLICATIONS**
- 04

**Activity applied**
- 5 MBq $^{32}$P kg$^{-1}$ Soil
## Characteristics of rock phosphates

<table>
<thead>
<tr>
<th>Rock phosphate</th>
<th>Total P (%)</th>
<th>Citric Acid Soluble P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purulia (PRP)</td>
<td>14.4</td>
<td>1.623</td>
</tr>
<tr>
<td>Lalitpur (LRP)</td>
<td>9.8</td>
<td>0.425</td>
</tr>
<tr>
<td>Sr. No.</td>
<td>Characteristics</td>
<td>Value</td>
</tr>
<tr>
<td>---------</td>
<td>------------------------------------------</td>
<td>----------------</td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Texture</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>pH ((1:2)) (soil:water)</td>
<td>8.20</td>
</tr>
<tr>
<td>3</td>
<td>Free CaCO(_3) (g kg(^{-1}))</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
<td>0.05M CaCl(_2) Extractable Al (mg kg(^{-1}))</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>Moisture equivalent (%)</td>
<td>32.0</td>
</tr>
<tr>
<td>6</td>
<td>Organic carbon (g kg(^{-1}))</td>
<td>5.10</td>
</tr>
<tr>
<td>7</td>
<td>CEC (C mol (P+) kg(^{-1}))</td>
<td>51.13</td>
</tr>
<tr>
<td>8</td>
<td>Total N (g kg(^{-1}))</td>
<td>0.6</td>
</tr>
<tr>
<td>9</td>
<td>Available P (mg kg(^{-1}))</td>
<td>3.7 (Olsen’s)</td>
</tr>
<tr>
<td>10</td>
<td>P fixing capacity (g kg(^{-1}))</td>
<td>620</td>
</tr>
</tbody>
</table>
Parameters studied

- Dry matter yield (DMY) (g pot\(^{-1}\))
- Total phosphorus uptake (mg pot\(^{-1}\))
- Specific Activity (Bq mgP\(^{-1}\))
- Phosphorus derived from labeled soil (bioavailable P) and rock phosphate (mg pot\(^{-1}\))
• Total P uptake by plant (mg P Pot\(^{-1}\)) \( U_{TP} = DMY \times (\% P/100) \)
• DMY- Dry matter yield of plant
• Specific Activity (Bq mg P\(^{-1}\)) = \( Bq\, g\,^{-1}\,\text{plant}/mg\, P\, g^{-1}\,\text{plant} \)
• %PdfS -- Fraction of P in the plant derived from the soil

\[
\% \, PdFS = \frac{\text{SA in Plant (Bq/mg P) in presence of RP}}{\text{SA in plant (Bq/ mg P) in absence of RP}} \times 100
\]

• %PdfRP----Fraction of P in the plant derived from the rock phosphate

\[
\% \, PdFRP = 1 - \frac{\text{SA in Plant (Bq/mg P) in presence of RP}}{\text{SA in plant (Bq/ mg P) in absence of RP}} \times 100
\]

• P uptake from labeled source (mgP Pot\(^{-1}\)) = \( U_{TP} \times (%PdfS/100) \)
• P uptake from rock phosphate (mgP Pot\(^{-1}\)) = \( U_{TP} \times (%PdfRP/100) \)
Effect of fungus inoculation on shoot dry matter yield and P uptake of wheat in ultisol and vertisol.

Dry Matter Yield

P uptake

Vertisol
Ultisol
Effect of fungus inoculation on specific activity (Bq mgP⁻¹) of wheat in ultisol and vertisol
Effect of fungus inoculation on percentage of P derived from soil and rock phosphates in ultisol

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatments</th>
<th>Ultisol</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>PdfS</td>
<td>PdfRP</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>mgP pot⁻¹</td>
<td>%</td>
<td>mgP pot⁻¹</td>
</tr>
<tr>
<td>1</td>
<td>Soil + LRP+³²P</td>
<td>90.4</td>
<td>0.932</td>
<td>9.6</td>
<td>0.100</td>
</tr>
<tr>
<td>2</td>
<td>Soil + LRP + TMPS 1 +³²P</td>
<td>66.7</td>
<td>1.210</td>
<td>33.3</td>
<td>0.604</td>
</tr>
<tr>
<td>3</td>
<td>Soil + PRP +³²P</td>
<td>95.3</td>
<td>0.906</td>
<td>4.7</td>
<td>0.045</td>
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<tr>
<td>4</td>
<td>Soil + PRP + TMPS1+³²P</td>
<td>70.3</td>
<td>1.063</td>
<td>29.7</td>
<td>0.449</td>
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<tr>
<td></td>
<td>LSD (P&lt;0.05)</td>
<td>0.126</td>
<td></td>
<td>0.076</td>
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</table>
Effect of fungus inoculation on percentage of P derived from soil and rock phosphates in vertisol

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<thead>
<tr>
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<th>Vertisol</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>PdfS</td>
<td>PdfRP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>mgP pot⁻¹</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>Soil + LRP +³²P</td>
<td>98.5</td>
<td>1.355</td>
<td>1.5</td>
</tr>
<tr>
<td>2</td>
<td>Soil + LRP + TMPS 1 +³²P</td>
<td>81.1</td>
<td>3.338</td>
<td>18.9</td>
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<tr>
<td>3</td>
<td>Soil + PRP +³²P</td>
<td>99.3</td>
<td>1.356</td>
<td>0.7</td>
</tr>
<tr>
<td>4</td>
<td>Soil + PRP + TMPS1 +³²P</td>
<td>82.7</td>
<td>3.425</td>
<td>17.3</td>
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<td>LSD (P&lt;0.05)</td>
<td>0.825</td>
<td>0.095</td>
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Conclusions

- Potential use of *Aspergillus niger* TMPS1 isolate as a phosphate solubilizer in ultisol as well as vertisol.

- In general, rock phosphates solubilization in alkaline vertisol does not occur, but this fungus solubilized native unavailable soil P as well as rock phosphate in vertisol.

- This study showed the advantages of using the $^{32}$P isotope in distinguishing the contribution of bio-available native soil P and P from rock phosphates to P nutrition in plant–microbe interaction.

- This fungus should be evaluated as bio-fertilizer under field condition in different agro climatic regions for different crops.
Acknowledgement

- BRIT, DAE for providing $^{32}\text{P}$ radioisotope

- Head, NABTD and Director BMG, BARC, Mumbai, India
THANK-YOU