Isolation and Classification of Nitrogen Fixing and Phosphate Solubilizing Bacteria

Rattanah Mahal1, Marcus Schicklberger2, Romy Chakraborty2
1 Reedley College 2 Lawrence Berkeley National Laboratory

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Abstract
The elements N and P are essential elements for the growth and survival of plants. Yet plants are limited in their ability to fix elemental N from the atmosphere, as well as hydrolyze organic and inorganic phosphorus from insoluble compounds. To compensate for this shortcoming, plants form a mutualistic relationship with bacteria to obtain usable nitrogen and phosphate. Although the majority of plants that form nitrogen-fixing root nodules are in the legume family, new species of N₂-fixing bacteria have been discovered in association with non-nodulating crops. The goal of this research lies in the identification of beneficial bacteria capable of fixing nitrogen and solubilizing phosphate. In this study, High-Throughput Isolation (HTI) was used to identify N fixing and/or P solubilizing bacteria. Five different phylogenetic orders were identified: Enterobacteriales, Bacillales, Actinomycetales, Rhizobiales, and Sphingobacteriales. The strain Kosakonia Oryzae ola 51 from the order Enterobacteriales, in particular, was identified as a nitrogen fixer by amplifying the nifH gene; those results were then confirmed by the acetylene reduction assay. Ultimately, the goal of this research is to decrease fertilizer dependency by engineering plants to attract diazotrophic bacteria.

Bacteria Extraction

**Results**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Order</th>
<th>Closest Relative in the NCBI database</th>
<th>Similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1ManHGB</td>
<td>Enterobacteriales</td>
<td>Kosakonia Oryzae ola 51</td>
<td>99%</td>
</tr>
<tr>
<td>RS7OxaP1</td>
<td>Bacillales</td>
<td>Paenibacillus amyloyticus strain JCM 9906</td>
<td>99%</td>
</tr>
<tr>
<td>R4ManHGB</td>
<td>Actinomycetales</td>
<td>Isoperoica variabilis strain 225</td>
<td>99%</td>
</tr>
<tr>
<td>RS3FumNF</td>
<td>Rhizobiales</td>
<td>Ochrobactrum haematophilum strain CCUG 38531</td>
<td>99%</td>
</tr>
<tr>
<td>RP3GluNF</td>
<td>Sphingobacteriales</td>
<td>Sphingobacterium sp. 21 strain 21</td>
<td>99%</td>
</tr>
</tbody>
</table>

**Conclusion**

We were able to successfully isolate diazotrophic bacteria from Nicotiana tabacum. Most of the bacteria were from the strain Kosakonia Oryzae ola 51 which can fix nitrogen as evidenced by the nifH PCR and the acetylene assay. Future projects include measuring and comparing growth of re-inoculating hydroponic plants with the isolated bacteria. SDS-PAGE analysis on the isolated bacteria and IC of prospective phosphorous solubilizing bacteria to detect solubilization activity.

**Future Projects**

- Measuring and comparing growth of re-inoculating hydroponic plants with the isolated bacteria
- SDS-PAGE analysis on the isolated bacteria
- IC of prospective phosphorous solubilizing bacteria to detect solubilization activity

**Acknowledgments**

I would like to thank Romy Chakraborty and Lawrence National Berkeley Laboratory for hosting me his summer and allowing me to work on a cutting-edge project. Thanks to my mentor Marcus Schicklberger and Angelica Pettenato for being patience teachers. Thanks to Husna Yasin and Jeanine Parizio for being great lab partners and thanks to E’S and SynBERC for this opportunity to conduct research at UC Berkeley.

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**Contact Information**

Rattanah Mahal
Reedley College
Email: rattna.mahal15@gmail.com

**Screening for Nitrogen Fixers**

- Since the nifH gene is highly conserved, it was used as a screen to identify nitrogen fixing bacteria by amplifying the nifH gene in the extracted DNA by PCR.
- We used Pol F and Pol R primers as well as Adenosin as the positive control and DEPC water as the negative control.
- One assay used was the Acetylene Reduction Assay which takes advantage of the dinitrogenase’s property to reduce acetylene to ethylene.
- This graph shows the production of ethylene by Gass Chromatography analysis of the strain R1ManHGB.

**Screening for Phosphorous Solubilizers**

- Phosphorous solubilizing bacteria are known to release organic acid to solubilize phosphorus. That property was used to screen for phosphorous solubilizing bacteria by using phosphorous selective medium.
- Pikovskaya medium contains tricalcium phosphate and bromophenol blue (an indicator).
- Another medium used was the NMB medium which contains hydroyxypatite (Ca₅(PO₄)₃OH) and bromophenol blue.
- The starting pH of both medium is 7 and a change in color from blue to yellow indicates drop in pH which also indicates the presence of potential phosphorous solubilizing bacteria.

**Support Information**

We were able to successfully isolate diazotrophic bacteria from Nicotiana tabacum. Most of the bacteria were from the strain Kosakonia Oryzae ola 51 which can fix nitrogen as evidenced by the nifH PCR and the acetylene assay. An assay for screening for phosphate solubilizing bacteria is still in development.

**Future Projects**

- Measuring and comparing growth of re-inoculating hydroponic plants with the isolated bacteria
- SDS-PAGE analysis on the isolated bacteria
- IC of prospective phosphorous solubilizing bacteria to detect solubilization activity

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