Isolation, Characterization, Genotyping, Phenotyping of Fungi for their Plant Growth-Promotions and Production of Secondary Metabolites

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Abstract

Objectives: Isolation of endophytic fungi from the halophyte *porteresia coarctata* and observation of their plant growth promoting (PGP) activities under normal and salt-stressed conditions and bioassay of the secondary metabolites produced by them.

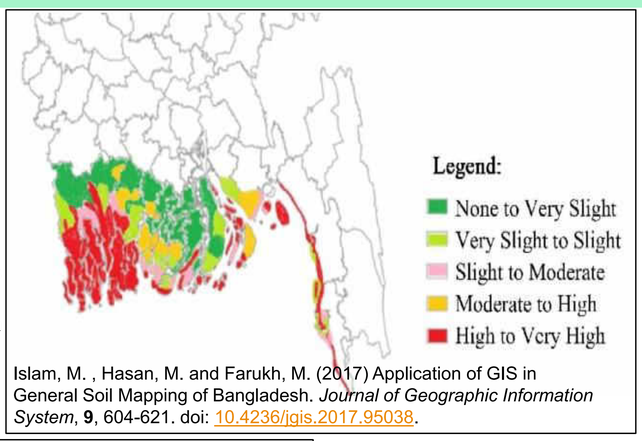
Methods: Endophytic fungi were isolated from the halophyte *Porteresia coarctata* and primarily differentiated by morphological analysis. Their amplified ITS regions intervening with the 5.8s rRNA gene were sequenced for molecular identification. Among them, one fungus which was identified as *Aspergillus niger* selected for further study according to nitrogen fixation and siderophore production activity, Phosphate solubilization, zinc solubilization activity test under normal and 900mM saline condition. To investigate their effects on plant growth, this fungal isolate with PGP activities was inoculated into the commercial rice variety BRRI dhan 28 under normal and salt stress condition. Shoot length and weight, root length and weight, chlorophyll content, malondialdehyde, H_2O_2 content, Na^+ /K^+ ratio were measured. This study was also conducted to isolate and characterize secondary metabolites from the fungi. Based on the results of TLC screening, antimicrobial and antioxidant activity tests of fungal crude extracts, again, *Aspergillus niger* was selected for compound isolation. Column chromatography followed by solvent treatment was conducted to purify compounds from the crude extract and ^{1}H NMR, ^{13}C NMR, DEPT-135, COSY, HSQC, and HMBC spectrometry were conducted to elucidate the structure of compound. *In-vitro* anti-proliferative activity was tested against lung cancer cell line a549.

Results: *Aspergillus niger* inoculated plants showed significantly better ability to combat salt stress, better growth, and better biomass production compared to non-inoculated plants. Crude extract of *Aspergillus niger* exhibited the strongest antioxidant activity among all. NMR spectrometry revealed that, totally a new compound was produced by this fungal isolate named 5-methy-l,8-demethoxy flavasperone and it showed significant anti proliferative activity against lung cancer cell line a549 *in-vitro*.

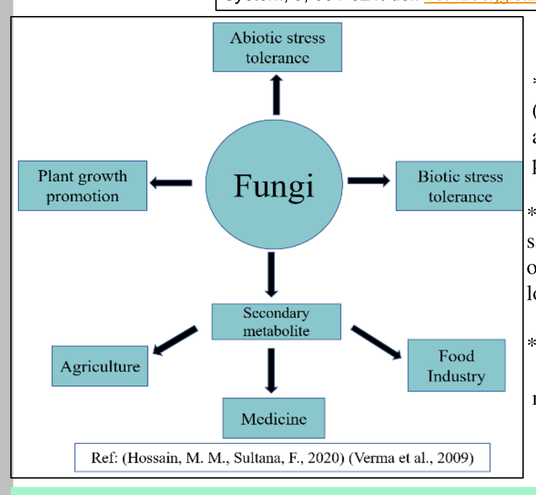
Conclusions: We expect that this plant growth promoting fungus- *Aspergillus niger* can be used as biofertilizer and it will help in the growth and yield of salt-sensitive plants under a higher level of salt stress. Additionally, compounds produced by this isolate may be used as a potential lead molecule in drug discovery.

Background

- Due to climate Change, soil salinity is increasing.
- Increased salt affected area hampers crop yield as well as rice production which is our staple food.



Islam, M. , Hasan, M. and Farukh, M. (2017) Application of GIS in General Soil Mapping of Bangladesh. *Journal of Geographic Information System*, **9**, 604-621. doi: 10.4236/jgis.2017.95038.



* Plant Growth Promoting (PGP) Endophytic Fungi (EF) are involve in plant growth promotion under stress.

*Endophytic fungi at high salt site enhance salt tolerance of inoculated plant than plant at low salt site.

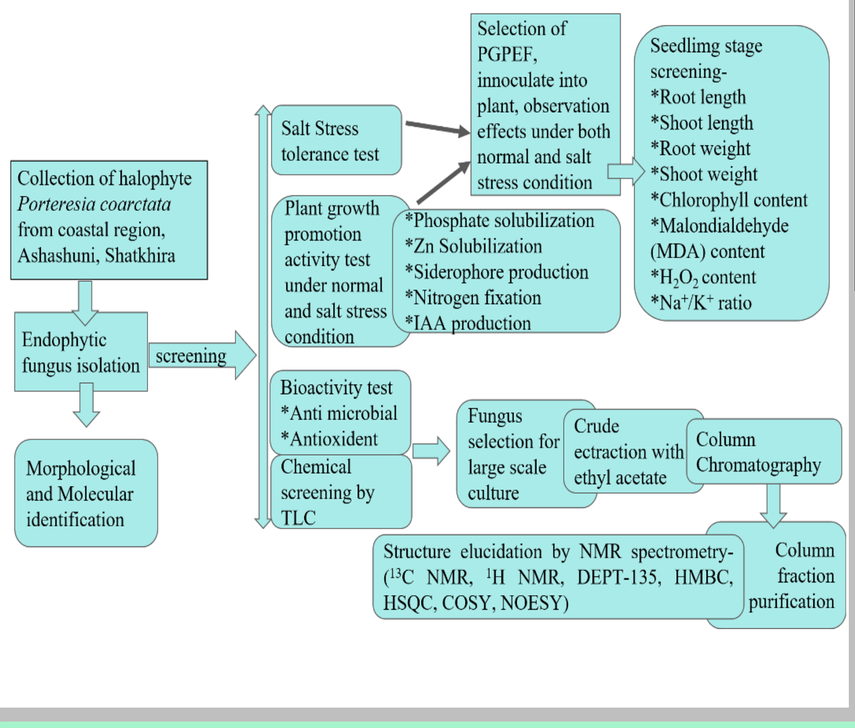
* Endophytic fungi also produce various bioactive secondary metabolites.

Aim of the study

Endophytic fungi isolation from *Porteresia coactata*

- **Characterization for PGP activity**
- **Observation of their PGP activities into rice plant under nomal and salt stress**
- **Screening for their bioactive secondary metabolites.**

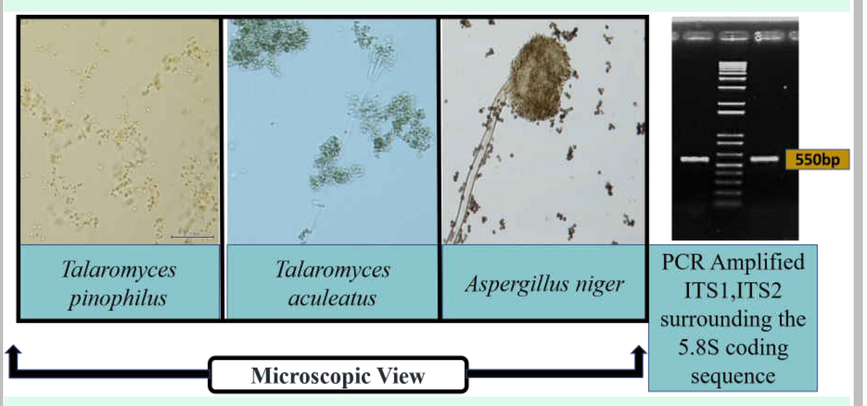
Workflow and methodology



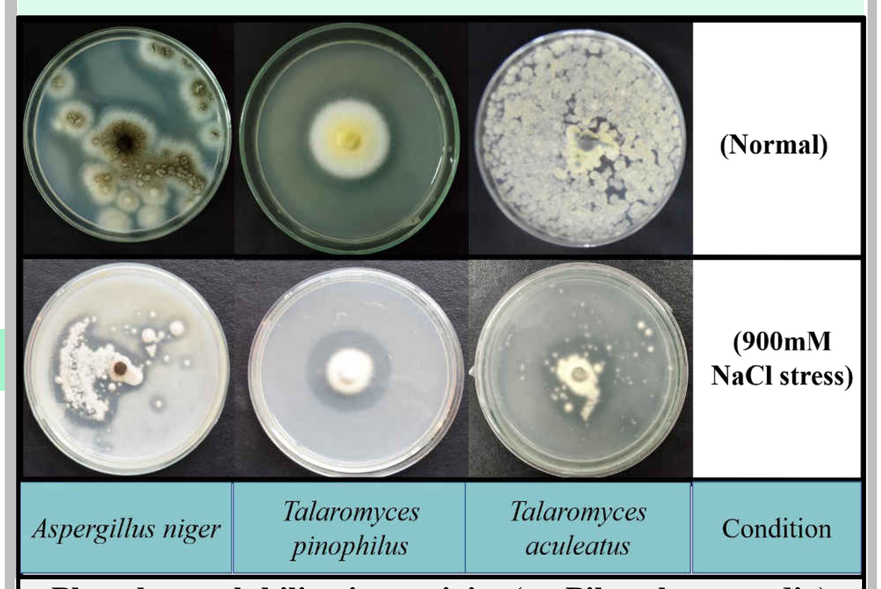
Result

- 9 endophytic fungi were isolated and were subjected to identification

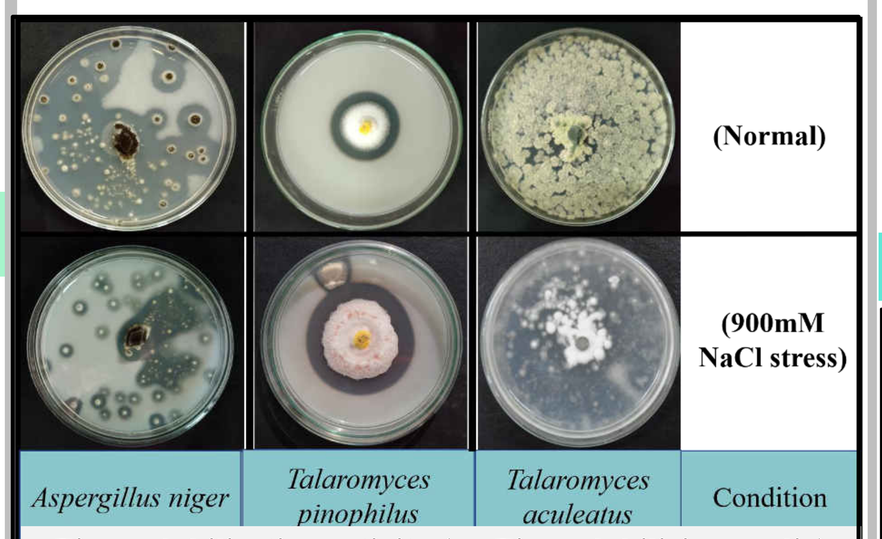
Morphological and Molecular Identification of the fungi



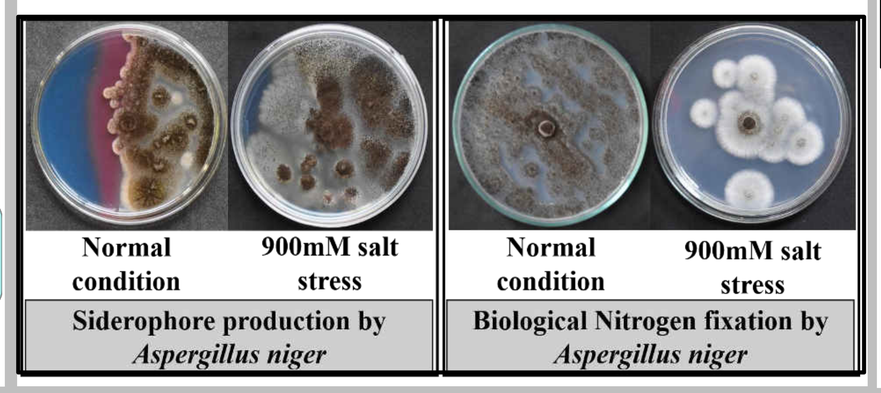
Screening for Plant Growth Promoting (PGP) activity



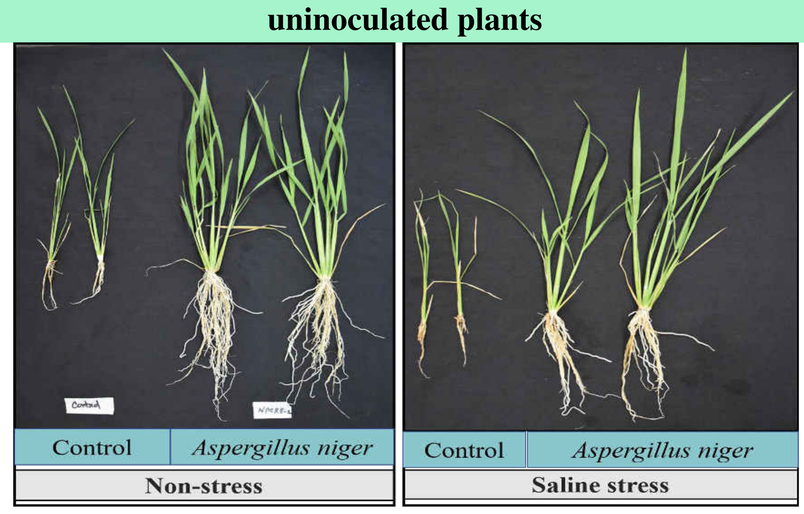
Phosphate solubilization activity (on Pikovskaya media)



Zinc solubilization activity (on Zinc solubilizing media)

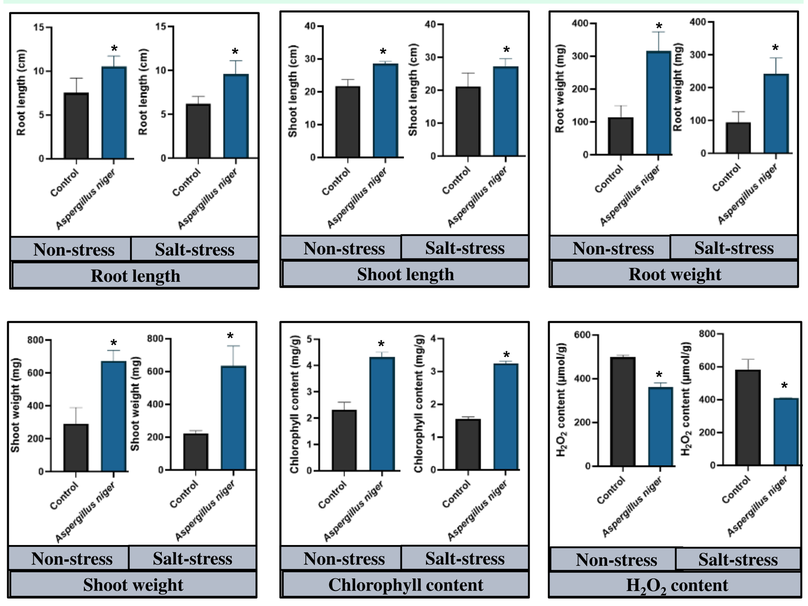


PGPEF inoculated plants perform much better than uninoculated plants

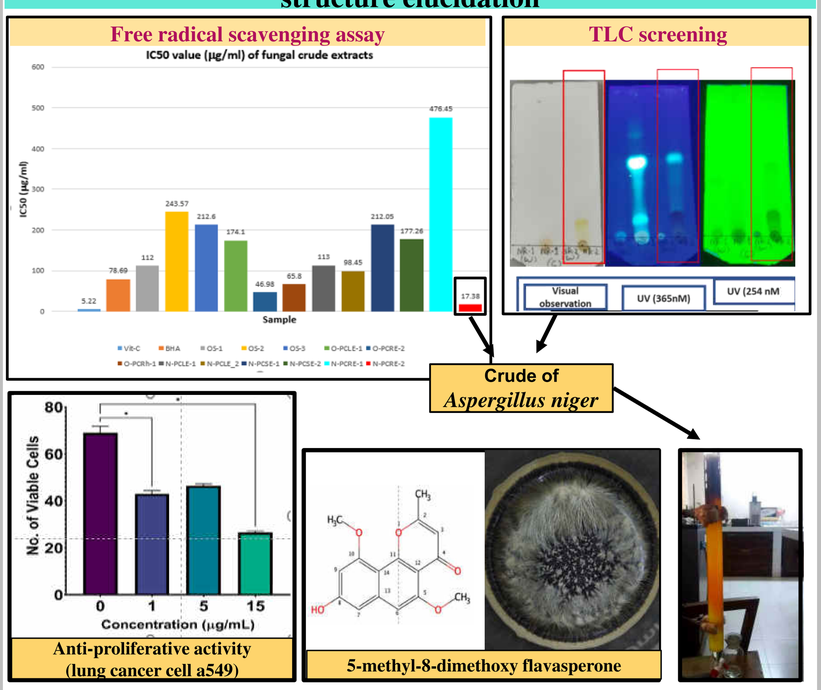


Analysis of agronomic traits and graphical representation

(* =P ≤ 0.05=Significant)



Screening for bioactive secondary metabolites, separation and structure elucidation



Conclusion

- Endophytic fungus inoculated plants performed better than control plants under both normal and salt stressed conditions.
- *Aspergillus niger* - a potential source of novel antioxidant products.
- 5-methyl 8-demethoxy flavasperone, new compound isolated from *Aspergillus niger,* showed significant anti proliferative activity against lung cancer cell line in-vitro.

Future prospects

- Comparative transcriptome analysis between fungus inoculated and uninoculated plants.
- Strain specific identification of *Aspergillus niger*.
- Formulation of a suitable natural, ecofriendly bio-fertilizer.
- Heterologous expression of the isolated new compound
- Remaining column fractions analysis for bioactive compound.

Acknowledgement

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