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, H₂O₂ 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 ¹H NMR, ¹³C 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-1,8-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. PGPEF inoculated plants perform much better than Result Background uninoculated plants 9 endophytic fungi were isolated and were subjected to identification Morphological and Molecular Identification of the fungi • Due to climate Legend: Change, soil salinity is increasing. None to Very Slight Very Slight to Slight Increased salt Slight to Moderate affected area hampers crop yield as well as Moderate to High rice production which High to Very High is our staple food. Islam, M., Hasan, M. and Farukh, M. (2017) Application of GIS in Cortad General Soil Mapping of Bangladesh. Journal of Geographic Information PCR Amplified Talaromyces *Talaromyces* Control Aspergillus niger Aspergillus niger System, 9, 604-621. doi: 10.4236/jgis.2017.95038. Control Aspergillus niger ITS1,ITS2 pinophilus aculeatus Saline stress Non-stress Abiotic stress urrounding the tolerance 5.8S coding **Analysis of agronomic traits and graphical representation**(* =P ≤ 0.05=Significant) * Plant Growth Promoting **Microscopic View** sequence (PGP) Endophytic Fungi (EF) **Screening for Plant Growth Promoting (PGP) activity** are involve in plant growth promotion under stress. Fungi promotion tolerance *Endophytic fungi at high salt site enhance salt tolerance of inoculated plant than plant at low salt site. (Normal) Secondary metabolite Endophytic fungi also produce Food Salt-stress Salt-stress Salt-stress **Non-stress Non-stress** various bioactive secondary Non-stress Industry **Root** weight **Shoot length Root length** metabolites. Medicine Ref: (Hossain, M. M., Sultana, F., 2020) (Verma et al., 2009) (900mM Aim of the study NaCl stress) Characteriza tion for PGP activity **Talaromyces Talaromyces Salt-stress** Salt-stress **Non-stress Salt-stress Non-stress Non-stress** Aspergillus niger Condition pinophilus **Shoot weight Chlorophyll content** H₂O₂ content aculeatus **Observation of** Phosphate solubilization activity (on Pikovskaya media) their PGP activities **Endophytic fungi** into rice plant isolation from under nomal and salt stress Porteresia coactata Screening for their bioactive (Normal) secondary metabolites. Non-stress Salt-stress Non-stress Salt-stress Non-stress Salt-stress Root Na+/K+ ratio Shoot Na+/K+ ratio Malondialdehyde content Workflow and methodology Screening for bioactive secondary metabolites, separation and structure elucidation Selection of (900mM **TLC screening** Seedling stage Free radical scavenging assay IC50 value (µg/ml) of fungal crude extracts screening-NaCl stress) innoculate into *Root length plant, observation *Shoot length Salt Stress effects under both *Root weight tolerance test normal and salt *Shoot weight Collection of halophyte stress condition Talaromyces Talaromyces *Chlorophyll content Porteresia coarctata Plant growth Aspergillus niger Condition *Phosphate solubilization *Malondialdehyde from coastal region, pinophilus aculeatus promotion *Zn Solubilization (MDA) content Ashashuni, Shatkhira activity test Zinc solubilization activity (on Zinc solubilizing media) *Siderophore production *H₂O₂ content under normal *Nitrogen fixation *Na+/K+ ratio and salt stress *IAA production condition Endophytic screening fungus isolation Bioactivity test Crude of Aspergillus niger *Anti microbial Fungus Crude Column *Antioxident selection for ectraction with Chromatography Chemical Morphological large scale ethyl acetate and Molecular screening by \ TLC identification

Conclusion

Endophytic fungus inoculated plants performed better than control plants under both normal and salt stressed conditions.

HSQC, COSY, NOESY)

Structure elucidation by NMR spectrometry-

(13C NMR, 1H NMR, DEPT-135, HMBC,

Column

fraction

purification

Normal

condition

- 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

900mM salt

stress

Siderophore production by

Aspergillus niger

Comparative transcriptome analysis between fungus inoculated and uninoculated plants.

Biological Nitrogen fixation by

Aspergillus niger

900mM salt

stress

Concentration (µg/mL)

Anti-proliferative activity

(lung cancer cell a549)

Strain specific identification of Aspergillus niger.

Normal

condition

- Formulation of a suitable natural, ecofriendly bio-fertilizer.
- Heterologous expression of the isolated new compound
 - Remaining column fractions analysis for bioactive compound.

Acknowledgement

5-methyl-8-dimethoxy flavasperone







