PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 10(1): 210–223
https://doi.org/10.14719/pst.1991

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Characterization of a potent plant growth promoting fungal strain *Aspergillus fumigatus* MCC 1721 with special reference to indole-3 -acetic acid production

Biyas Mukherjee[1,2], Sanchali Roy[1], Nasrin Parvin[1], Santanu Tarafdar[1], Sikha Dutta[1*]

[1] Molecular Plant Pathology and Fungal Biotechnology Laboratory, Department of Botany, The University of Burdwan, Purba Bardhaman 713104, West Bengal, India
[2] Department of Botany, East Calcutta Girls' College, P 237, Lake Town Road, Block B, Sreebhumi, Lake Town, Kolkata 700089, West Bengal, India

*Email:sikha.bu.academia@gmail.com

OPEN ACCESS

ARTICLE HISTORY

Received: 08 July 2022
Accepted: 21 October 2022

Available online
Version 1.0 : 19 November 2022
Version 2.0 : 01 January 2023

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS etc.
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Mukherjee B, Roy S, Parvin N, Tarafdar S, Dutta S. Characterization of a potent plant growth promoting fungal strain *Aspergillus fumigatus* MCC 1721 with special reference to indole-3-acetic acid production. Plant Science Today. 2023; 10(1): 210–223. https://doi.org/10.14719/pst.1991

Abstract

In the present study, indole-3-acetic acid (IAA) producing plant growth promoting fungus was isolated from rice field of Purba Bardhaman district, West Bengal, India. Among the isolated 6 strains, AP2 (*Aspergillus fumigatus*) was selected as best-performing plant growth promoting fungal strain as it was an efficient indole-3-acetic acid producer as well as exhibits different plant growth promoting ability viz, phosphate solubilization, siderophore production, ammonia and hydrogen cyanide production etc. Media and different growth conditions (pH, temperature, concentration of sodium chloride) were optimized for augmentation of the indole-3-acetic acid production. The genus of the selected isolate AP2 was identified as *Aspergillus fumigatus* both by 18S rDNA sequence-based homology and MALDI-TOF analyses of ribosomal protein. Plant growth promoting ability of *Aspergillus fumigatus* has been confirmed by measuring different morphological and biochemical growth parameters in *Trigonella foenum-graecum* L. So, AP2 (*Aspergillus fumigatus*) can be considered as novel plant growth promoting fungal strain that can be applied as bio-inoculants on agricultural field.

Keywords

Aspergillus, IAA producing fungi, indole-3-acetic acid, plant growth promoting fungi

Introduction

Now-a-days, pesticides and chemical fertilizers are excessively used in crop production (1). Although, these chemical fertilizers may increase crop nutrient in adverse condition but it has many negative impact in our environment (2). Chemical fertilizer made up of phosphate, potassium, nitrate salts are potential source of heavy metals and radio-active elements that may accumulate in soil and may enter into plant body (2). In this point, there is an extreme thrust for an alternative eco-friendly and environmentally sustainable method. A sustainable agricultural practice significantly reduces the use of hazardous chemical input to the agricultural field to ensure protection of the environment but should maintain the nutrient quality of crops (1).

Current research has focused on different soil borne plant growth promoting microbes that can be a good alternative of the harmful chemical fertilizers (1). Several rhizosphere fungi plays important role in growth and productivity of host plant (3). Different fungal stains that inhabit in rhizosphere region of host plants are able to increase the plant growth and productivity in various ways (4). Some commonly reported plant growth

promoting fungi (PGPF) strains are *Fusarium, Trichoderma, Aspergillus, Pho-ma, Penicillium* that can increases plant growth in considerable amount (5-7). Plant growth promoting fungi can solublize insoluble form of different nutrient like, phosphate, zinc, potassium, sulphur, iron that stored in the soil and make it available to the plant by producing organic acid (4). Many PGPF strains have reported to play important role in plant disease management viz, *Aspergillus* sp. reduce pathogenicity of plant root knot nematode *Meloidogyne incognita* (8). Apart from that, PGPF stimulate plant growth by secreting secondary metabolites like hydrogen cyanide, ammonia and plant hormones, mainly indole-3-acetic acid (IAA) (9). IAA has a great role in cell divisions, cell elongation, lateral root formation and root hair development that helps in nutrient uptake (9). IAA produced by fungal strains can build up immune response of plant and make the plant resistant against pathogenic microbes (9, 10). Considering the novel applications of PGPF in plant growth, our main objective was to search some potent plant growth promoting fungal strains and to check the potentiality of isolated fungal strain as plant growth promoter so that it can be used as a potent biofertilizer in crop field. Our present work deals with isolation, screening, characterization and identification of plant growth promoting fungal strain from rice field of Purba Bardhaman district in West Bengal, India; with special emphasis on their IAA production ability. Growth promotion capacity of isolated PGPF strains has been checked on *Trigonella foenum-graecum* L.

Materials and Methods

Collection of rhizosphere soil sample

Rhizosphere soil sample was collected from rice field of Purba Bardhaman district (Latitude- N 23°14'20.86", Longitude- E 87°51'45.743"). The rice plants were uprooted and the soil attached to the roots was stored in sterile polythene bags.

Characterization of collected soil sample

Physico-chemical properties of collected rhizosphere soil were checked using the standard method (11). Organic carbon, total nitrogen, total phosphorus, total sulphur and salinity, pH, electrical conductivity was estimated.

Isolation of plant growth promoting fungi (PGPF)

Potato dextrose agar media [Potatoes; infusion from-200 $g.L^{-1}$, Dextrose- 20 $g.L^{-1}$, agar- 20 $g.L^{-1}$, pH-6.5±0.2] was used to obtain pure culture by dilution plate technique (12). 0.5 g of collected soil was diluted to 10 ml of distilled water in a test tube which served as stock solution. 9 ml of water were added to remaining 10 test tubes. 1 ml of stock solution was transferred to 9 ml of sterilized distilled water yielded 10^{-1} dilutions and the series continued up to 10^{-7}dilutions. Then, the plates were incubated at 30 °C for 48 hr. After the incubation, fungal colonies of distinct morphology were selected for further study.

Morphological characterization of isolated PGPF strains

Colony features like shape, surface, colour and margin were recorded of the 3-day old colony.

In vitro characterization of plant growth promoting traits

Estimation of indole acetic acid production

Fungal strains were allowed to grow in potato dextrose broth [Potatoes infusion-200 $g.L^{-1}$, Dextrose- 20 $g.L^{-1}$, pH-6.5±0.2] supplemented with 0.5% L-tryptophan for 96 hr. After that, cultures were centrifuged at 6000 rpm for 15 min. 3-4 drops of orthophosphoric acid was added to 2 mL supernatant of each sample along with 2 ml of Salkowsky reagent [1% 0.5 M $FeCl_3$ in 35% perchloric acid]. Then, the reaction mixture was kept at dark condition in the incubator (27 °C ± 2 °C) for 20 min. Appearance of pink colour indicated the production of IAA and the absorbance of each sample was recorded at 533 nm (13). The concentration of IAA was estimated against the standard curve of IAA (Sigma Aldrich).

Nitrogen fixing ability of the fungal isolates

The ability to fix atmospheric nitrogen was tested by inoculating the isolate in the Asbay's mannitol agar media [Mannitol-20 $g.L^{-1}$, di-potassium phosphate-0.2 $g.L^{-1}$, magnesium sulphate-0.2 $g.L^{-1}$, sodium chloride-0.2 $g.L^{-1}$, potassium sulphate-0.1 $g.L^{-1}$, calcium carbonate-5.0 $g.L^{-1}$, agar-20 $g.L^{-1}$, final pH (at 25 °C) 7.4±0.2] because Ashby's mannitol agar media is devoid of any ready nitrogen source. Isolates were allowed to incubate at 27±2 °C temperature for 96 hr. Occurrence of fungal colony indicates its ability to fix atmospheric nitrogen.

Quantitative estimation of phosphate solubilization of insoluble calcium phosphate

This was done following ammonium-molybdate method (14). 96 hr old fungal culture grown in Pikovskaya broth media was used. After centrifugation at 6000 rpm for 15 min, 1 ml of culture filtrate was taken out and mixed with 2.5 ml Barton's reagent [solution A: ammonium molybdate (25 g) was dissolved in 400 ml distilled water; Solution B: ammonium metavanadate (1.25 g) in 300 ml boil distilled water and cooled then 250 ml concentrated HNO_3 was added. Solution A and B were mixed and the volume was made up to 1000 ml with distilled water] and volume made up to 5 ml. After 20 min, OD value of each sample was taken at 430 nm wave length and concentration of solubilized phosphate were determined with the standard curve of KH_2PO_4.

Hydrogen cyanide (HCN) production

Potato dextrose agar media [potatoes; infusion from-200 $g.L^{-1}$, dextrose- 20 $g.L^{-1}$, agar- 20 $g.L^{-1}$, pH-6.5±0.2] supplemented with 4.4 $g.L^{-1}$ Glycine was used to determine HCN producing ability of fungal isolates. Fungal strains were streaked into the plates and Whatman no.1 filter paper was dipped in 2% sodium carbonate in 0.5% picric acid solution which was placed at the inner surface of the lid of the Petri dishes. Plates were carefully covered with parafilm and incubated at 27±2 °C for 7 days. Development of orange to brown color in the filter paper indicated that the isolate have the capability to produce hydrogen cyanide (15).

Ammonia production

Fungal isolates were inoculated in peptone water and incubated for 96 hr at 27±2 °C, after that, 0.5 ml of Nesseler reagent was added to 1 ml of each culture. The development of yellow to dark orange color indicated the positive result of ammonia production (16).

Siderophore production

The siderophore activity of selected isolates was determined following the Chrome Azural S (CAS) agar plate method (17). To prepare 1 l of blue agar, solution I [6.05 mg CAS was dissolved in 5 mL of distilled water and mixed with 1 ml of iron (III) solution (1 mM $FeCl_3$, $6H_2O$ in 10 mM HCl) with stirring. This solution was slowly added to 7.29 mg HDTMA dissolved in 4 ml water] and solution II [90 ml CAAB media (casamino acid- 5 $g.L^{-1}$, K_2HPO_4-1.18 $g.L^{-1}$, $MgSO_4.7H_2O$- 0.25 $g.L^{-1}$, pH-5.6); was prepared and 2g.90mL $^{-1}$ agar was added after adjustment of the pH of CAAB medium. 3.024 g PIPES buffer powder was mixed with the 90 ml media]. Solution I and II were autoclaved separately. After that, both solutions were cooled down to 50 °C and solution I was added along the glass wall of the Erlenmeyer's flask with enough agitation to achieve mixing without the generation of foam. Then, finally prepared blue-CAS gar media poured in petridishes maintaining the volume of 25 ml and fungal strains were inoculated in the petridish. Siderophore production was quantitatively estimated by the CAS-shuttle assay of Pyne (18). The percentage of siderophore production was calculated by the following formula:

% of siderophore production = $\left[\frac{Ar-As}{Ar}\right] \times 100$

Where, Ar = absorbance of reference (blank minimal media + CAS assay solution) and As = absorbance of sample.

Exo-polysaccharide (EPS) production

EPS production was estimated following phenol sulphuric acid method (19). Fungal isolates were inoculated in potato dextrose broth [potatoes infusion from-200 $g.L^{-1}$, dextrose- 20 $g.L^{-1}$, pH-6.5±0.2], after incubation of 96 hr, cultures were centrifuged at 6000 rpm for 15 min. Then, the supernatant were again resuspended with 3 ml of Acetone and re-centrifuged, this process were repeated for 3 times. After that, supernatant was suspended with distilled water. 1 ml of 5% aqueous phenol was added to 1 ml of this suspension along with 5 ml concentrated H_2SO_4 and kept for 15 min. Development of orange red colour indicates the presence of exo-polysaccharides. OD value was measured at 490 nm wave length and concentration was determined using the standard curve of glucose.

Selection of best-performing PGPF strain

Considering the above performed test, one best-performing fungal strain has been selected and further experiments carried out with that best PGPF strain only.

Different parameter influencing IAA producing ability

IAA production with growth pattern of the selected isolate

Growth curve of the selected isolate was prepared by taking the dry weight of biomass in 2 day interval up to 10 days. IAA production was measured (13) simultaneously with the dry weight of biomass content of the selected best-performing PGPF strain to study the changes in IAA production in relation to its growth pattern.

IAA production with different L-Tryptophan concentration

Fungal isolate was inoculated to potato dextrose broth [potatoes; infusion from-200 $g.L^{-1}$, dextrose - 20 $g.L^{-1}$, pH-6.5±0.2] supplemented with different concentration of L-tryptophan (0.5%, 1%, 1.5%, and 2%) and IAA was measured in each sample of 8 day culture (13).

Media optimization influencing IAA producing ability of the selected PGPF strain

To optimize the carbon source of the media for highest production of IAA, different sugars of monosaccharide (glucose, galactose, arabinose and fructose) disaccharide (lactose, sucrose, maltose) and polysaccharide (cellulose and starch) were used in Czapex Dox broth media. All sugars were given at 3% concentration in Czapex Dox media. For, furthermore optimization of the most suitable carbon source, glucose was supplemented with 0.5%-3% concentration in the Czapex Dox broth media. Same as that, for the selection of the best nitrogen source to get highest amount of IAA, different nitrogen source were used (peptone, asparagine, glycine, L-glutamic acid, ammonium sulphate, sodium nitrate and potassium nitrate) in 0.2% concentration instead of sodium nitrate. Again peptone was supplemented with 0.2% to 1% concentration in the media as peptone was found to be the most suitable nitrogen source. IAA was measured in each condition following the standard method (13).

Optimization of growth parameter of the selected strain

The selected best-performing fungal strain was allowed to grow in different pH (4-8) and temperature (4 °C, 20 °C, 25 °C, 30 °C, 37 °C, 50 °C) and in different NaCl concentrations (2%, 4%, 6%, 8%, 10%) to optimize the growth parameter and simultaneously production of IAA by the selected strain was also checked.

Identification of the selected best-performing strain

18S rDNA sequencing

The selected fungal strain was identified using 18S rDNA sequencing method. Standard phenol/chloroform extraction method (20) was used to isolate genomic DNA, followed by PCR amplification of the 18S rRNA gene using universal primers 16F27 [5'-CCAGAGTTT GATCMTGGCTCAG -3'] and 16R1492 [5'-TACGGYTACCTTGTTACGACTT-3']. The amplified 18S rRNA gene PCR product was purified by PEG-NaCl precipitation and directly sequenced on an ABI® 3730XL automated DNA sequencer. Sequencing was carried out from both ends using additional internal primers so that each position was read at least twice. Assembly was carried out using Laser gene package followed by identification using the NCBI database. Jukes and Cantor's methods was used for constructing phylogenetic tree in MEGA X software. The significance of branch point was calculated by 1000 boostrap resamplings of the data (21). This strain was sent to National Centre for Microbial Resource (NCMR), Pune for general deposition.

MALDI-TOF analysis

MALDI - TOF analysis was done following the standard method (22). The spectra were calibrated externally using the standard calibration mixture (*Escherichia coli* extracts including the additional proteins RNase A and myoglobin , according to the instruction manual of Bruker Daltonik GmbH, Germany).MALDI Biotyper 3.1 (Bruker Daltonik GmbH, Germany) and FlexAnalysis version 3.4 (Bruker Daltonik GmbH, Germany) software was used for strain identification. The strain showing ≥1.7 log value with strain in database were confirmed as the member of that genus and strains showing ≥2.0 log values were confirmed as the member of that species.

Plant growth promoting assay on Fenugreek (*Trigonella foenum-graecum* L.) using the selected

Mature Fenugreek (*Trigonella foenum-graecum* L.) seeds were surface sterilized with 1.5% sodium hypochlorite (NaOCl) for 10 min, followed by successive washing with sterile distilled water and then the water was decanted. The seeds were then kept on sterilized moist blotting paper and incubated in dark at 25±2 °C for 2 days and then those are transferred in a growth room [RH- 70-80%, temp. 27 °C ±2 °C, illumination 270 mE m^{-2} s^{-1} (for 12 hr)]. 5 ml of conidial suspension at a cell density of 1×10^8 spore.mL^{-1} of AP2 (*Aspergillus fumigatus*) and PS1 was separately added in two sets of petridish (treated set). Then, the inoculated seeds were placed in rotary shaker for 6 hr at 27 °C ±2 °C to facilitate the penetration of inducer inside the treated seeds. Non-inoculated seeds were treated with sterile distilled water.

Several plant growth parameters like, germination percentage, dry weight and fresh weight of the seedling, root and shoot length, amount of chlorophyll were measured in 15 days seedling in each set.

Germination percentage (GP) was calculated following this formula:

$$GP = \left[\frac{Total\ no.of\ germinated\ seeds}{Total\ no.of\ seeds\ in\ all\ replicates}\right] \times 100$$

For extraction of chlorophyll, 2g of fresh leaf tissue was taken from each set and ground with 80% acetone and then centrifuged at 10000 rpm for 10 min, this process was repeated for 3 times until the precipitate become colourless. Then, 3ml of supernatant was taken out and absorbance was taken against acetone at 645 nm and 663 nm (23). The amount of chlorophyll a, chlorophyll b and total chlorophyll was calculated by this formula:

Total Chlorophyll (mg.g^{-1} F.W):

$$\frac{[20.2(A645) + 8.02(A663)] \times\ total\ volume\ of\ filtrate}{1000} \times\ tissue\ weight$$

Chlorophyll a (mg.g^{-1} F.W):

$$\frac{[12.7(A663) - 2.69(A645)] \times\ total\ volume\ of\ filtrate}{1000} \times\ tissue\ weight$$

Chlorophyll b (mg.g^{-1} F.W):

$$\frac{[22.9(A645) - 4.68(A633)] \times\ total\ volume\ of\ filtrate}{1000} \times\ tissue\ weight$$

Estimation of IAA, total sugar and total protein of the seedling

For extraction, 1 g of seedling from each set was homogenized in 10 mM ice-cold 50 mM Na-phosphate buffer (pH-6.8±0.2) containing 0.1 mM EDTA, 0.1 mM phenylmethanesulfonyl fluoride (PMSF) and 1% polyvinylpolypyrrolidone phosphate (PVPP). After centrifugation at 10000 rpm for 15 min, concentration of IAA and total sugar were estimated by the standard method (13, 19). Total protein content was measured (24). Then, 5 ml of Bradford reagent was added to 0.1 ml of supernatant. Development of blue colour occurs and OD taken at 595 nm. Known quantity of Bovine serum albumin was used to prepare standard curve of protein.

Statistical analysis

All data presented in the result were the means of 3 replicates. All the means was analyzed by single way ANOVA and compared at 5% significance level.

Results

Isolation and screening of PGPF

Six fungal colonies (AP1, AP2, PS1, PS2, PS3 and PS4) with distinct morphology were obtained from the rhizosphere soil of rice plant. The physico-chemical characters of soil indicate the soil was slightly saline and neutral (Table 1). Morphological colony characteristics of 6 isolated fungal colonies were recorded (Table 2). Different plant growth promoting traits like solubilisation of phosphate, IAA production, N_2 fixation, NH_3 and HCN production, siderophore production were tested in the six isolated strains (Table 3). AP2 (*Aspergillus fumigatus*) was found to produce a copious amount of IAA followed by PS1 and AP1 (Fig. 1A). AP2 (*Aspergillus fumigatus*) solubilize the insoluble calcium phosphate of Pikovskaya media most efficiently followed by PS1 (Fig. 1B). Siderophore and polysaccharide production of AP2 (*Aspergillus fumigatus*) and PS3 was highest compared to other four isolates (Fig. 1C, 1D). AP2 (*Aspergillus fumigatus*) was selected as best PGPF strain as it showed best result in all above mentioned plant growth promoting traits (Table 3).

Table 1. Physio-chemical properties of the soil.

Characters	Soil sample
Electrical conductivity (dS.m^{-1})	0.045
pH	7.3
Organic carbon (%)	0.68
Total nitrogen (µg. g^{-1} dry weight)	0.74
Total phosphorus (µg. g^{-1} dry weight)	0.48
Total sulphate (mg. g^{-1} dry weight)	0.65

Parameter influencing IAA production of AP2 (*Aspergillus fumigatus*)

When monitoring IAA production ability with dry weight of AP2 (*Aspergillus fumigatus*), it was found that the IAA production after a little lag phase increased simultaneously with the biomass content of AP2 (*Aspergillus fumigatus*)

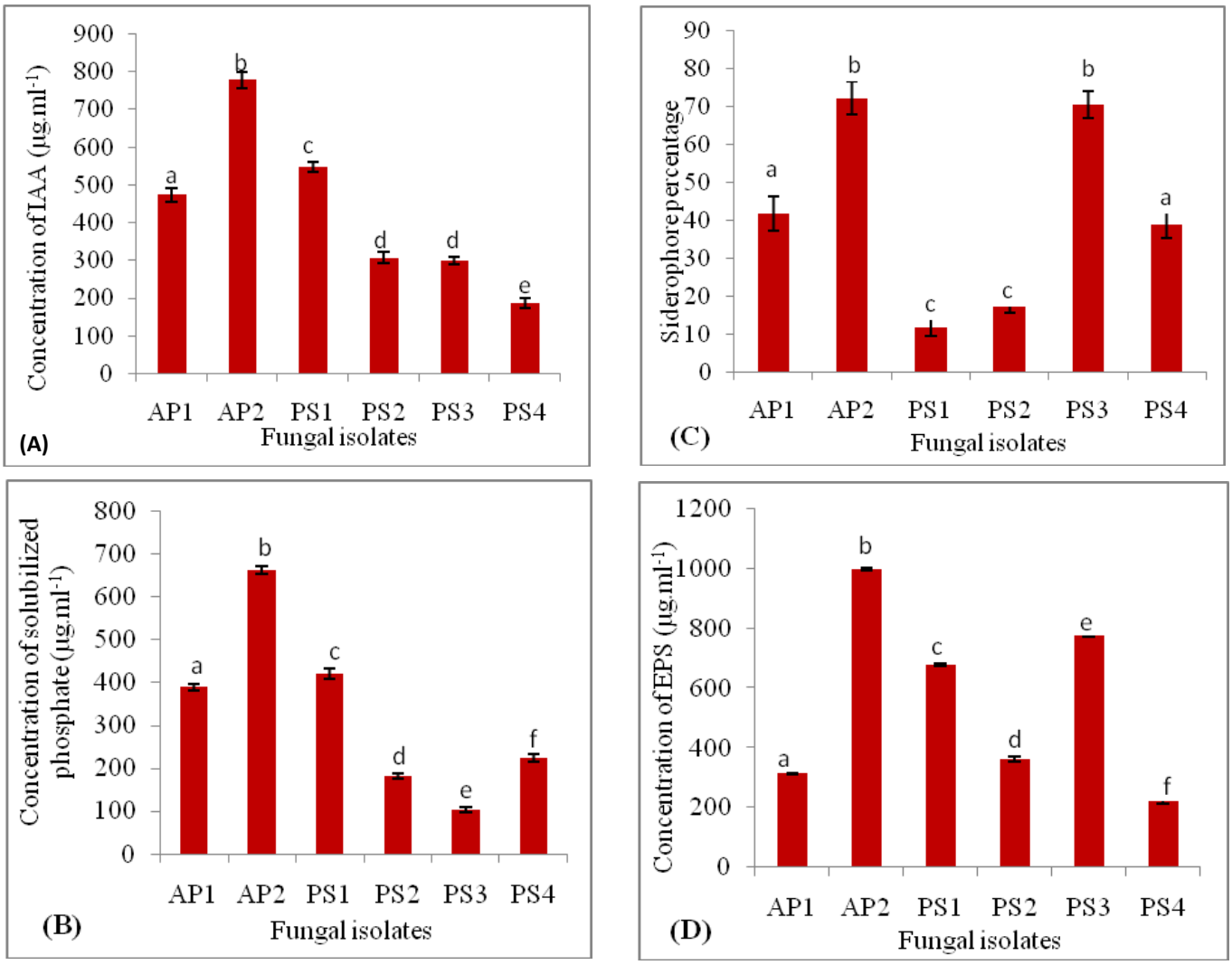

Fig. 1. Different plant growth ability of six isolates [(A) IAA production; (B) Solubilisation of insoluble phosphate; (C) Siderophore production; (D) EPS production]. Different letters indicate statistically significant differences between groups (Mean ± SD, n=3, one way ANOVA, $p < 0.05$).

Table 2. Colony characteristics of the fungal isolates.

Fungal	Shape	Surface	Margin	Colour
AP1	Round	Smooth	Smooth	Deep brown
AP2	Round	Rough	Wavy	Green
PS1	Irregular	Smooth	Wavy	Reddish-brown
PS2	Round	Rough	Wavy	Light Green
PS3	Round	Smooth	Smooth	White
PS4	Irregular	Smooth	Smooth	Reddish-brown

Table 3. Plant growth promoting traits shown by fungal isolates.

Fungal isolates	Nitrogen fixing ability	Ammonia production	HCN production	EPS production
AP1	N	P	N	P
AP2	P	P	P	P
PS1	N	P	N	P
PS2	N	N	N	N
PS3	P	P	N	N
PS4	N	P	P	N

(*'P' indicates positive result;'N' indicates negative result)

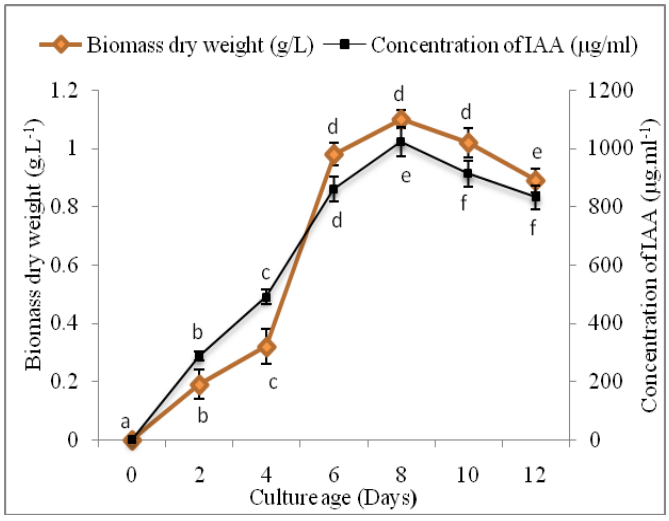

Fig. 2. Growth curve of selected AP2 strain (*Aspergillus fumigatus*) and Indole -3-acetic acid (IAA) production (µg.ml^{-1}) by the same up to 12 days in 2-day interval. Different letters indicate statistically significant differences between groups (Mean ± SD, n=3, one way ANOVA, $p < 0.05$).

upto 8th day. In the 8th day, biomasses as well as amount of IAA production were highest, and then both started to decline (Fig. 2). It has been seen that, while dealing with the media optimization for IAA production, AP2 (*Aspergillus fumigatus*)

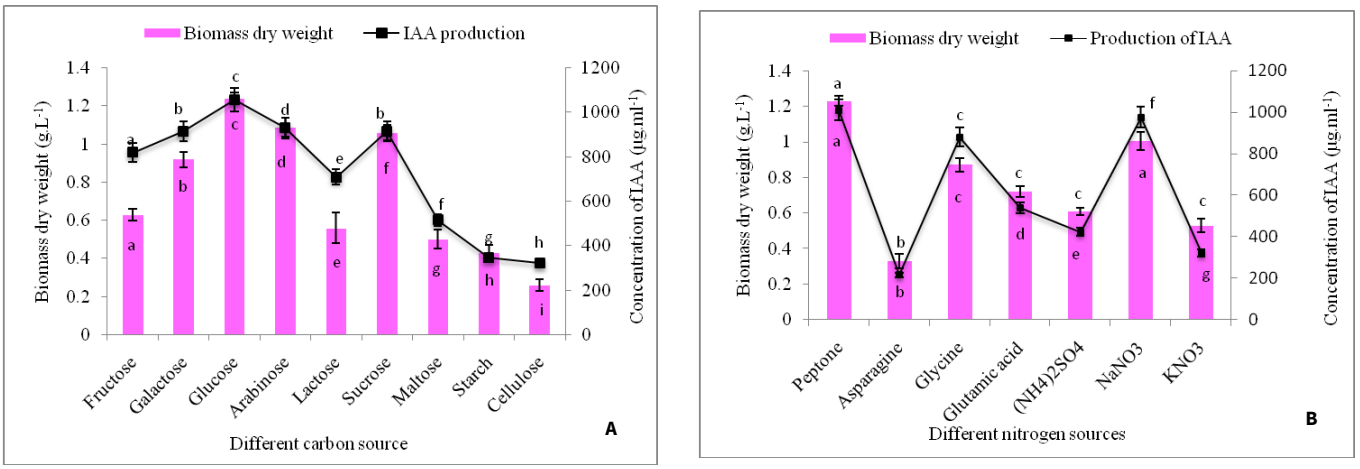

Fig. 3. Media optimization of AP2 (*Aspergillus fumigatus*) for IAA production [(A) with different carbon sources; (B) with different nitrogen sources]. Different letters indicate statistically significant differences between groups (Mean ± SD, n=3, one way ANOVA, $p < 0.05$).

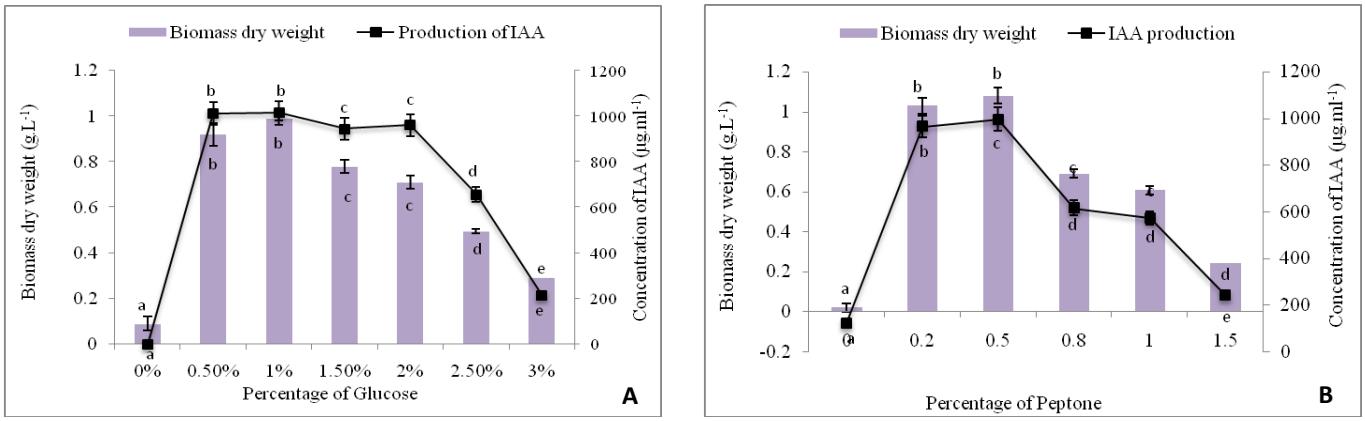

Fig. 4. Optimization of selected carbon and nitrogen source percentage for IAA production [(A) percentage of glucose in media; (B) percentage of peptone in media]. Different letters indicate statistically significant differences between groups (Mean ± SD, n=3, one way ANOVA, $p < 0.05$).

used glucose as carbon source most efficiently followed by arabinose; peptone was found to be as best nitrogen source for production of IAA (Fig. 3A-B). The production of IAA was more in 1% glucose and 0.5% peptone supplemented media (Fig. 4A-B).

Optimizing growth parameter of AP2 (*Aspergillus fumigatus*)

IAA production was accelerated with increasing its precursor L-tryptophan concentration and the production of indole acetic acid was highest using 1.5% concentration of L-tryptophan, but above this concentration of L-tryptophan, the production of IAA started to decreased (Fig. 5A).Optimum temperature for AP2 (*Aspergillus fumigatus*) growth was 25 °C though it can grow in temperature range of 20 °C to 30 °C. (Fig. 5B). AP2 (*Aspergillus fumigatus*) had pH tolerance range of 4-8, but pH 7 was optimum for its growth (Fig. 5C). AP2 can tolerate NaCl upto concentration of 6%, after which AP2 (*Aspergillus fumigatus*) failed to grow further (Fig. 5D). IAA production was decreasing with the increasing concentration of NaCl. In 2% NaCl concentration, IAA production was somewhat similar to control set, after that production was sharply declined (Fig. 5D).

Identification and phylogenetic analyses of AP2 as an *Aspergillus* sp.

Microscopic view of fungal isolate AP2 (Fig. 6A) showed entire, smooth, hyaline hyphae with conidiophore (width 3.8-5.6 µm), round vesicle (radius 10.4-13.41 µm) and phialide (length 2.88-7.1µm); Conidia were round, smooth with radius 0.98-1.31µm resembling the genus *Aspergillus*. Scanning electron microscopy of AP2 was shown in Fig. 6B-C. The molecular characteristics of AP2 were given in Table 4. The length of 18S rDNA sequence of the AP2 strain is 756 nucleotide bp. The 756 bp nucleotide were compared to recorded data in the nucleotide bank showed the closest neighbour of the AP2 strain was *Aspergillus fumigatus* ATCC 1022 (NR_121481.1) having 99% sequence similarity. MALDI -TOF analysis of ribosomal protein indicated that the strain AP2 belongs to the genus *Aspergillus* sp. (Fig. 7A) as it showed log value above 1.7 confirming its genus identity as *Aspergillus* sp. 18S rDNA sequence from type strains of all *Aspergillus* sp. with more than 98% sequence similarity was used to carried out phylogenetic analysis and it has been indicated the position of AP2 within the genus *Aspergillus*. (Fig. 7B). AP2 in the phylogenetic tree formed a clade within the cluster represented by *Aspergillus fumigatus* (HQ026746) and *Aspergillus fumigatus* (KR023997). The strain AP2 showed 100% sequence similarity to *Aspergillus fumigatus* (MT297633), *Aspergillus fumigatus* (KR023997), *Aspergillus* sp.(KF367498) and *Aspergillus fumigatus* (HQ026746) followed by 99.86% sequence similarity to *Aspergillus* sp. (MG674824) *and Aspergillus fumigatus.* (MK439477). The strain AP2 was deposited in Microbial Culture Centre (MCC) with the accession number MCC 1721.

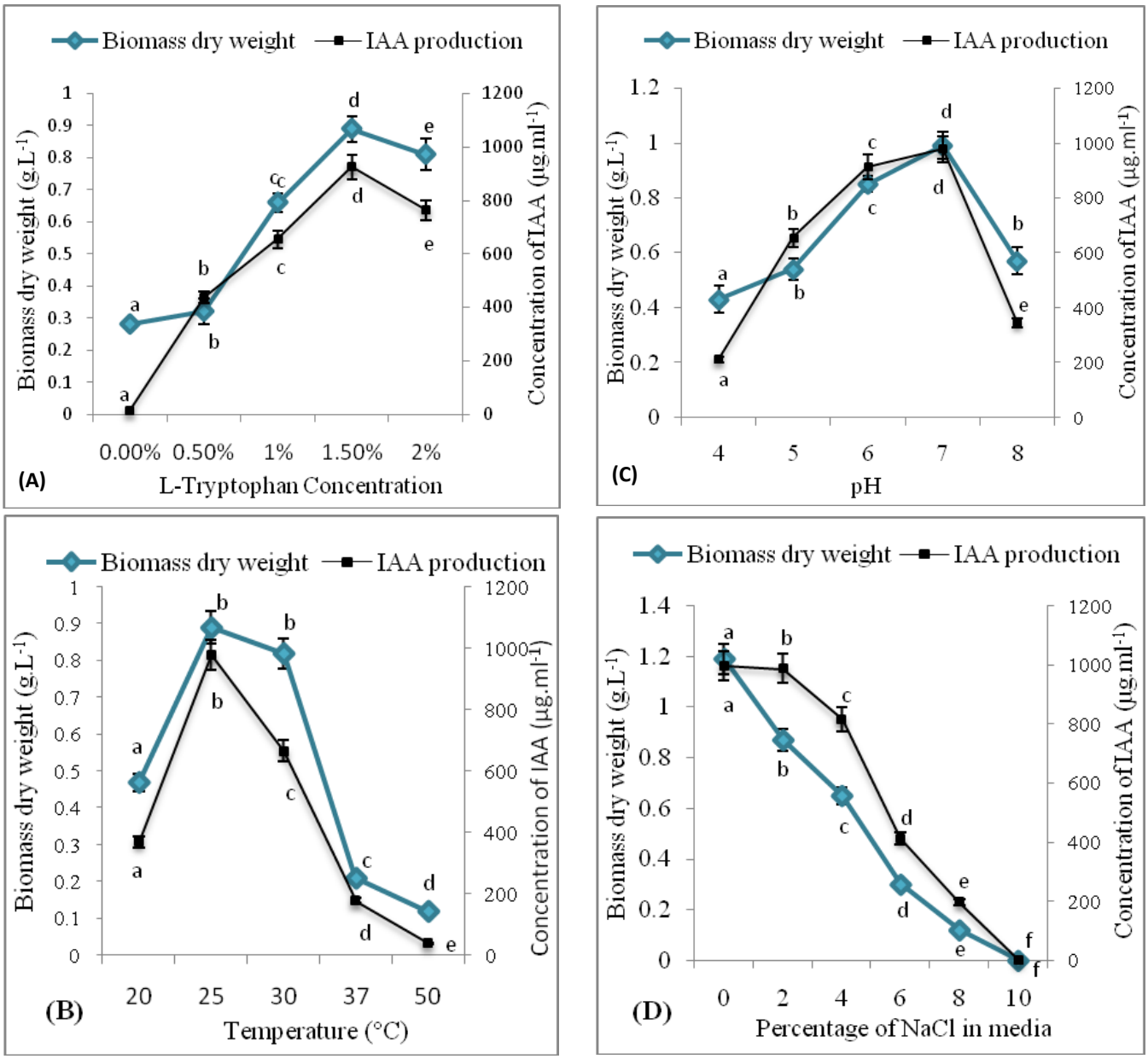

Fig. 5. Effect of different factors on growth and IAA production of AP2 isolate (*Aspergillus fumigatus*) [(A) Effect of different L-tryptophan concentration; (B) Effect of temperature; (C) Effect of pH; (D) Effect of NaCl]. Different letters indicate statistically significant differences between groups (Mean ± SD, n=3, one way ANOVA, $p < 0.05$).

Table 4. Molecular characteristics of AP2.

Molecular characteristics of AP2 strain						
MALDI-TOF analysis of ribosomal protein				Molecular identification by 18S rDNA		
Identification of the strain (best match)	MALDI score of the strain (second match)	Identification of the strain (second match)	MALDI score of the strain (second match)	Closest similarity [EzTaxon seq. acc. no.]	Pairwise similarity (%)	Strain sequence accession
Aspergillus fumigatus 45_II VML	1.753	*Aspergillus fumigatus 43_d VML*	1.726	*Aspergillus fumigatus* ATCC1022	99	MCC 1721

Response of *T. foenum-graecum* L. seedling after inoculation with AP2 (*Aspergillus fumigatus*) strain compared to non-inoculated and PS1-inoculated seedlings

The inoculation of AP2 (*Aspergillus fumigatus*) on Fenugreek seedling showed satisfactory promotion in terms of plant growth compared to non-inoculated and PS1-inoculated seedlings. The germination percentage, morphological parameter of root (root fresh weight, root dry weight, root length), shoot morphological parameters (shoot fresh weight, shoot dry weight, shoot length) and chlorophyll content showed highest result in *Aspergillus fumigatus* (AP2)-inoculated seedling followed by the seedlings treated with PS1 strain, summarized in Fig. 8A-D. In case of IAA, total sugar and total protein content, all parameters are increased in seedlings that were treated with selected AP2 strain (*Aspergillus fumigatus*) and PS1 strain compared to control set, but AP2-treated seedling gave most promising result (Fig. 9A-C).

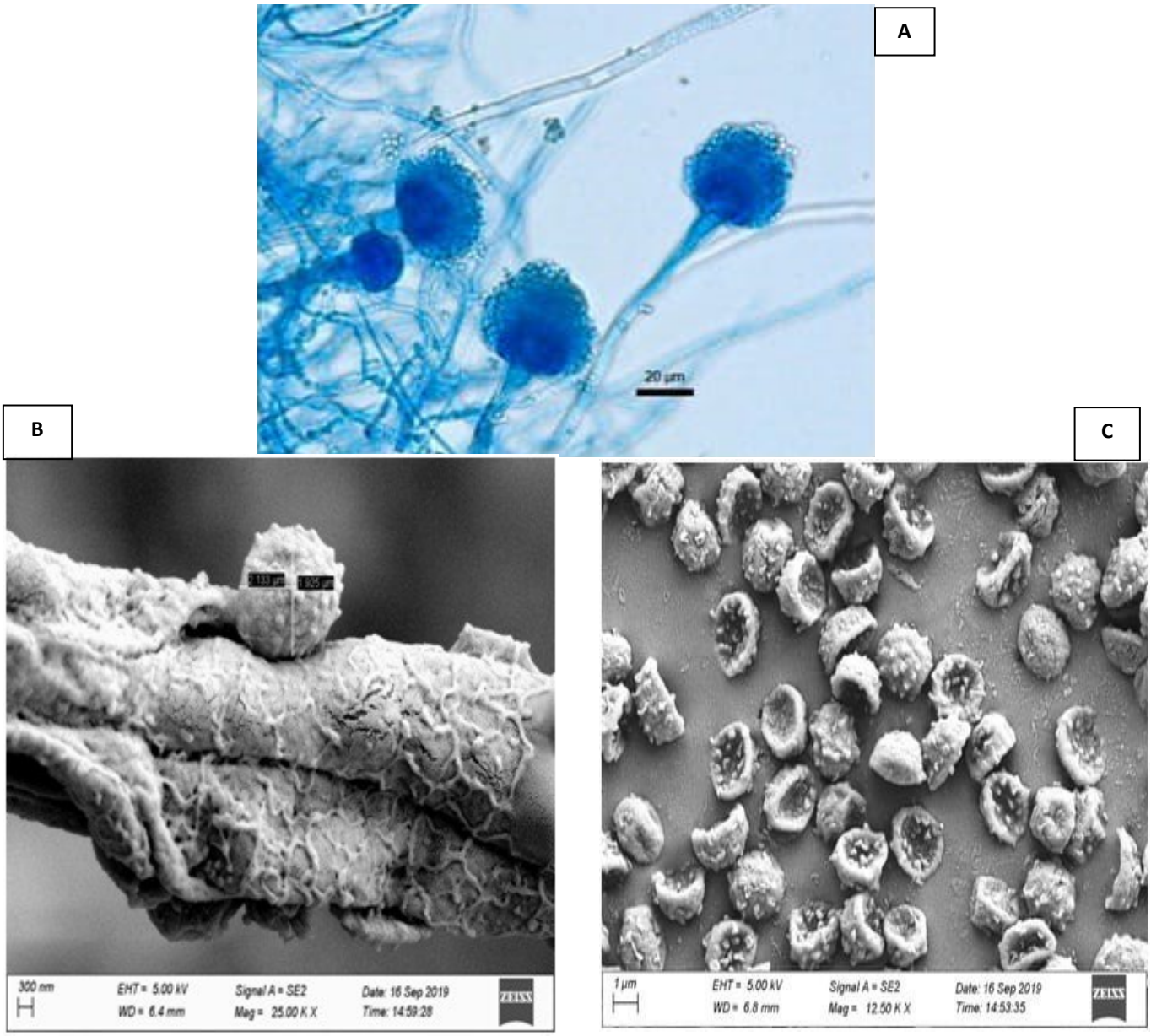

Fig. 6. Morphological characteristics of AP2 (*Aspergillus fumigatus*) [(A) light microscopic view; (B) Scanning electron microscopic view of hyphae with condiophore; (C) scanning electron microscopic view of condia].

(A)

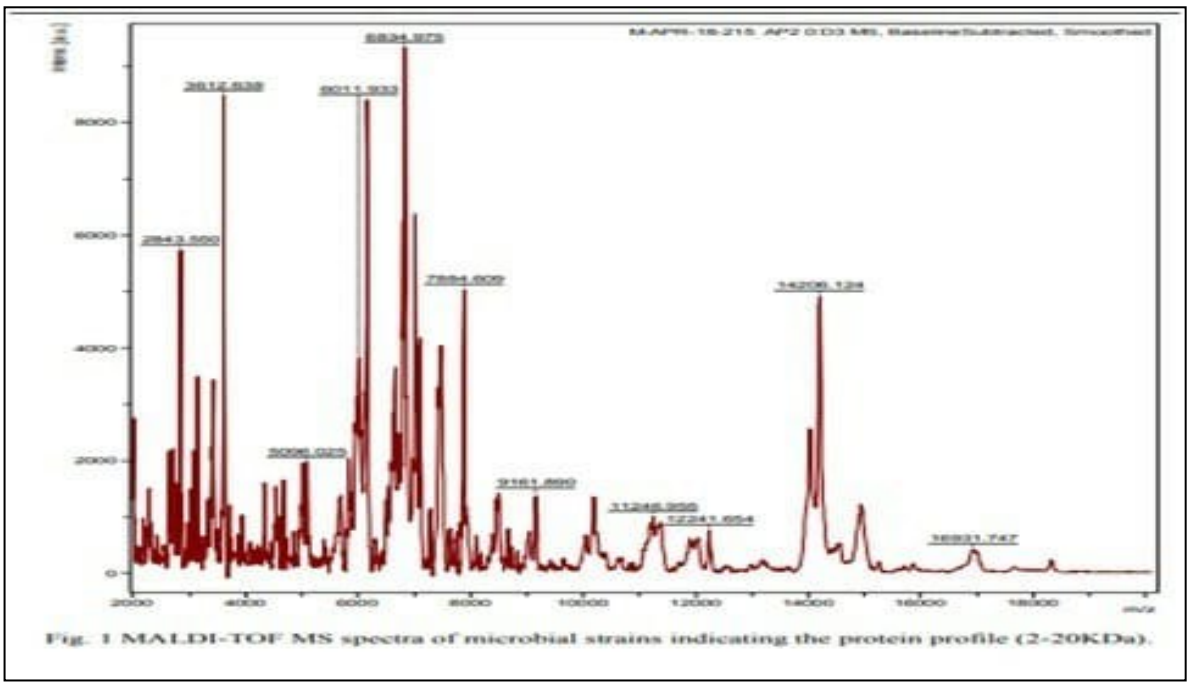

Fig. 1 MALDI-TOF MS spectra of microbial strains indicating the protein profile (2-20KDa).

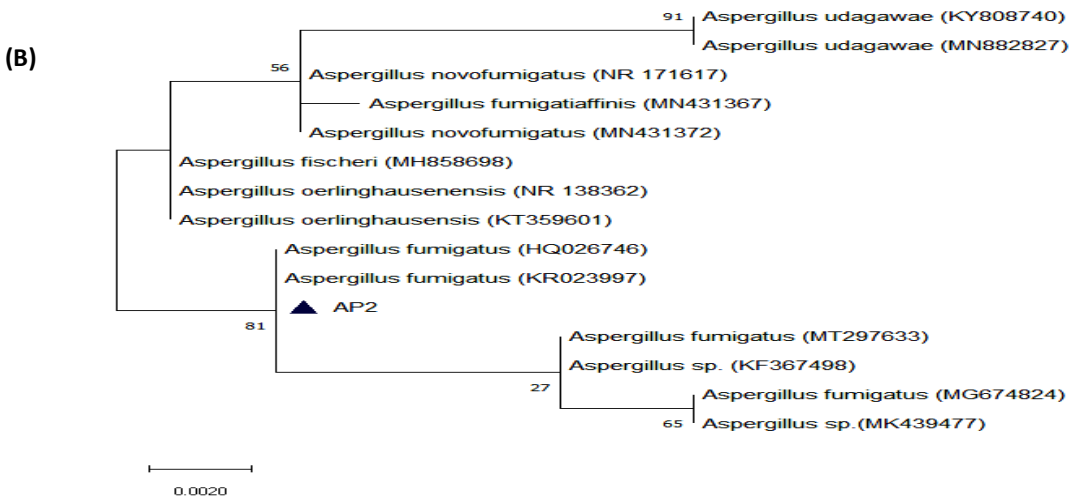

Fig. 7. (A) MALDI-TOF spectra analysis of the ribosomal protein of AP2 (*Aspergillus fumigatus*). (B) Maximum Likelihood phylogenetic tree showing the position of *Aspergillus* strain AP2 among the related taxa based on 18SrRNA gene sequences. The tree was generated using MEGA X software with Jukes and Cantor's correction (1969). Bootstrap values of 1000 replications expressed as percentages are given at branch points and strain AP2 formed a tight cluster with *Aspergillus fumigatus* strain (HQ026746 and KR023997). Bar 0.002 substitutions per site.

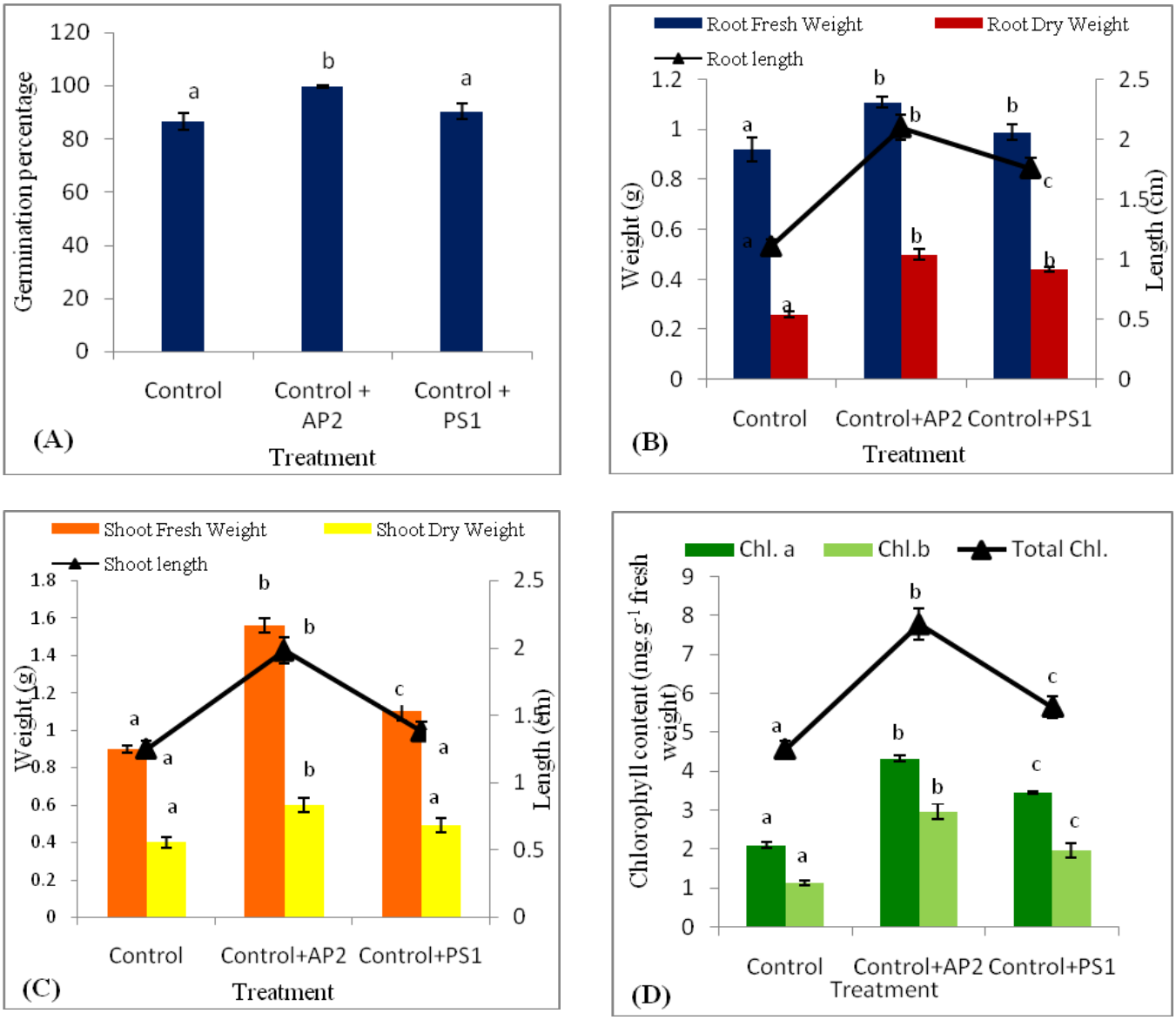

Fig. 8. Different plant growth promoting effect of Fenugreek seedlings with and without AP2 (*Aspergillus fumigatus*) and PS1 inoculation in control condition [(A) germination percentage; (B) root length, fresh weight & dry weight (C) shoot length, fresh weight & dry weight (D) chlorophyll content (Chl a, Chl b and total Chl). Different letters indicate statistically significant differences between groups (Mean ± SD, n=3, one way ANOVA, $p < 0.05$).

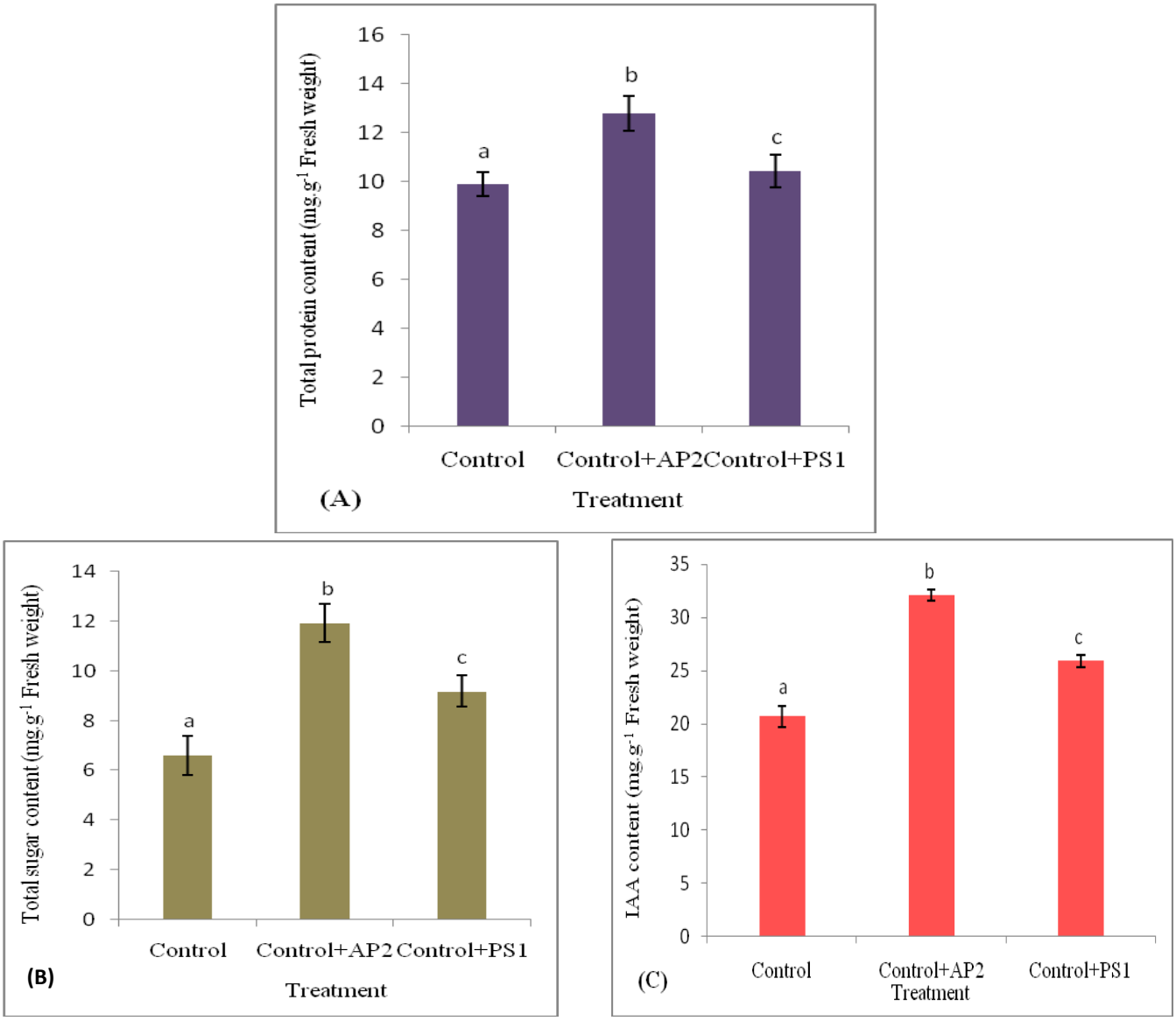

Fig. 9. Total protein, total sugar, and total IAA content of Fenugreek seedling under control, inoculated with AP2 (*Aspergillus fumigatus*), inoculated with PS1 [(A) total protein; (B) total sugar; (C) total IAA]. Different letters indicate statistically significant differences between groups (Mean ± SD, n=3, one way ANOVA, $p < 0.05$).

Discussion

Excessive use of chemical fertilizers accelerates rapid growth of plant but nutrition become very poor in plants grown in this way (25). In recent years, a technique has become popular to use plant growth promoting microbes (mostly bacteria and fungi) in agricultural field to promote plant growth and also to increase stress tolerance response (26). Though plant growth promoting bacteria are extensively studied in recent years but the investigation on fungal strains as plant growth promoter is limited. The Rhizosphere is the biologically active zone where plant-microbe interactions take place (27). Plant roots releases a wide range of chemical exudates that is the key factor of the rhizosphere plant-microbial interaction (27). Plant growth promoting fungi (PGPF) are soil-borne fungi that mimic the extensively-studied plant growth-promoting rhizobacteria (PGPR) by increasing plant growth and development by various means (26, 28-30). PGPFs are able to solubilize insoluble forms of phosphate, producing siderophore and IAA, HCN, ammonia production etc., thereby directly or indirectly contributing to the plant growth enhancement (29-31). IAA contributes to plant growth to large extent, and also helps in microbial colonization by loosening the cell wall of plant (32-34), thereby while screening for selection of best-performing fungal strain, the ability of *in vitro* IAA production can be taken as an important criterion.

In our study, AP2 (*Aspergillus fumigatus*) was selected as ideal PGPF strain among the six isolated fungal strains on the basis of its huge IAA producing ability as well as considering other plant growth promoting abilities. The growth of AP2 (*Aspergillus fumigatus*) was highest in 8th day of incubation and IAA production was also reached the maximum level at the same day. Many other reports supported this outcome as there also maximum amount of IAA was recorded in stationary phase of fungal growth curve (10, 35-36).

In this study, optimum temperature for maximum IAA production by AP2 (*Aspergillus fumigatus*) was 25 °C that showed the similarity with previous other reports that revealed that optimum temperature of IAA production by fungi lies between 25 °C to 30 °C (35, 37-41).

In our study, IAA production by AP2(*Aspergillus*

fumigatus) was at peak at pH 7, our result is in accordance with an earlier study (42), where maximum IAA production was at pH 7.5 in case of *Pseudomonus putida* UB-1 and in a study (43), maximum production was observed in pH 7.2. In one study (44), pH 8 was the optimum pH for highest IAA production which is very similar to our result.

In our study, it was found that, higher amount of IAA was obtained at 0% and 2% NaCl concentration and higher amount of NaCl tend to inhibit the IAA production as well as fungal growth. This report is concordant with an earlier studies (45, 46), in which IAA production was decreased with increasing salinity.

Several studies revealed that, L-tryptophan is the precursor of IAA, so that, adding the L-tryptophan in the culture media increase the production of IAA (47 -49). In this study, IAA production was highest with 1.5% L-tryptophan concentration after that the production rate slows down. This report matches many previous study reports that also proved that IAA biosynthesis is greatly affected by L-tryptophan concentration as in study (40), in which the amount of IAA production by *Colletotrichum fructicola* CMU-AU 006 and *Tulasnella* sp. CMU-SLP 007 was increased with increment of L-tryptophan concentration upto a certain level and then decreased. The optimum concentration of L-tryptophan was found to be 1 $mg.mL^{-1}$ for production of IAA by *Pleurotus ostreatus* (50).

Different carbon sources in culture media were reported to affect the amount of microbial IAA production (42, 46, 48, 51). In this study, different monosaccharide (Glucose, galactose, Arabinose, fructose) disaccharide (lactose, sucrose, maltose) and polysaccharide (cellulose and starch) were used to check their efficiency to accelerate IAA production, among all glucose was recorded to be the best-used carbon source at 1% concentration by AP2 (*Aspergillus fumigatus*) for IAA production. It was revealed that, sucrose was the most efficiently used carbon source by *Pseudomonus putida* for IAA production (42). Similar to our study, another study showed that, for IAA production, glucose was reported to be the best carbon source for strain MJHN10 and other 2 strains MJHN1 and MBN3 used sucrose and mannitol most preferably as carbon source for maximum IAA production (52). In another report (51), it was found that PGPR strain *Rhizobium* used mannitol and L-glutamic acid most suitably.

Many study reported that, like carbon sources, nitrogen sources in culture media also influence microbial IAA production (51-53). In our study, different nitrogen sources (peptone, asparagine, glycine, L-glutamic acid, ammonium sulphate, sodium nitrate and potassium nitrate) were used to evaluate their effect on amount of IAA production and peptone at 0.5% concentration was used most preferably by AP2 (*Aspergillus fumigatus*) as nitrogen source for maximum IAA production. Yeast extract was most suitable nitrogen source (54-56) and ammonium sulphate was in an earlier study (42). Study report on media optimization with different carbon and nitrogen sources for highest production of IAA by fungal sources are very limited.

Apart from IAA producing ability, AP2 (*Aspergillus fumigatus*) also found to be a tremendous phosphate solubilizer, it can solubilize 677±8.14 3µg/ml insoluble phosphate. This finding was supported by the study of Banerjee et al. where it proved that PGPF can solubilise insoluble phosphates (12). Phosphate solubilization by PGPF was also supported by an earlier report (57). AP2 (*Aspergillus fumigatus*) can grow in Ashby's mannitol agar media which is devoid of nitrogen source indicated that AP2 (*Aspergillus fumigatus*) can fix atmospheric nitrogen. Nitrogen fixation by fungal strain was also found in an earlier study (19). *Aspergillus fumigatus* produced ammonia and HCN; these traits of PGPF were also reported (12). AP2 secreted a good amount of exo-polysaccharides (998±6.11 $\mu g.ml^{-1}$) that may help to tolerate stress (58-60). According to the reports, fungal strains can produce good amount of exo-polysaccharides (19, 61). The fungal strain AP2 (*Aspergillus fumigatus*) synthesized a huge amount of iron chelating siderophore (72±4.35 $\mu g.ml^{-1}$) that helps in iron uptake of plants (62). The ability of fungi to produce siderophores was proved earlier (19, 61).

The selected PGPF strain AP2 has been identified as *Aspergillus sp.* on the basis of its microscopic characteristics, MALDI-TOF analyses and 18S rDNA sequence homology. Previously, many reports proved that different species of *Aspergillus* are capable of plant growth promotion. (63-64).

Inoculating the seeds of *Trigonella foenum-graecum* L. with AP2 (*Aspergillus fumigatus*) strains showed overwhelming enhancement in germinability, root length, dry and fresh weight of root, shoot length, dry and fresh weight of shoot and chlorophyll content of the seedlings. As AP2 (*Aspergillus fumigatus*) produced huge amount of IAA, it helps in root growth (65). Total IAA content of seedlings was enhanced significantly in AP2 (*Aspergillus fumigatus*) treated set, microbial effect on IAA content was also reported in an earlier study (66). Total sugar, total protein content of the host plant was more in case of PGPF treated set compared to control set, this finding was supported by the report, where also enhanced nutritive content was seen in the fungi-treated (*Aspergillus japonicas*) set (67).

In conclusion, AP2 (*Aspergillus fumigatus*) was IAA producing novel PGPF *Aspergillus* sp. having different plant growth promoting trait viz., phosphate solubilization, Ammonia production, HCN production, siderophore production etc. *Aspergillus fumigatus* successfully promoted seed germination, seedling growth and nutritive values of the host plant. Hence, AP2 (*Aspergillus fumigatus*) can be a good alternative of chemical fertilizers and pesticides minimizing the environmental pollution.

Conclusion

Considering the result, AP2 which was identified as *Aspergillus fumigatus,* can be easily considered as a potent PGPF strain that has many plant growth promoting characters proved both in *in vitro* condition and in plant growth promoting assay on *Trigonella foenum-graecum* L. *Aspergillus fumigatus* produce copious amount of IAA influencing the growth of the plant. As now-a-days, crop science is focussing on biofertilizers considering the harmful effects of chemical fertilizers, in that case, AP2 can be used as efficient biofertilizer in agricultural field for development of sustainable way of crop production.

Acknowledgements

We are very much grateful to Department of Botany, The University of Burdwan for providing required facilities for carried out this work. We are also grateful to the members of East Calcutta Girls' College for helping us throughout our study.

Authors contributions

BM and SD have framed the research work. SD gave the main conceptual ideas of this study. BM, SR and NP have carried out all laboratory work and statistical analysis. Datas are checked and evaluated by all authors. Results are discussed among the authors and final write up of the manuscript was prepared by BM and ST.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

References

1. Hossain MM, Sultana F, Islam S. Plant Growth-Promoting Fungi (PGPF): Phytostimulation and Induced Systemic Resistance. In: Singh D., Singh H., Prabha R. (eds) Plant-Microbe Interactions in Agro-Ecological Perspectives. Springer, Singapore. 2017; p. 135-91. https://doi.org/10.1007/978-981-10-6593-4_6
2. Savci S. Investigation of Effect of Chemical Fertilizers on Environment. APCBEE Procedia 1. 2012; 287-92. https://doi.org/10.1016/j.apcbee.2012.03.047
3. Hyakumachi M. Plant growth promoting fungi from turfgrass rhizosphere with potential for disease suppression. Soil Microorgan. 1994; 44:53-68. https://doi.org/10.18946/JSSM.44.0_53
4. Maiyappan S, Amalraj ELD, Santhosh A, Peter AJ. Isolation, evaluation and formulation of selected microbial consortia for sustainable agriculture. J Biofertil Biopestici. 2010;2:109. https://doi.org/10.4172/2155-6202.1000109
5. Hossain MM, Sultana F, Kubota M et al. The plant growth-promoting fungus *Penicillium simplicissimum* GP17-2 induces resistance in *Arabidopsis thaliana* by activation of multiple defense signals. Plant Cell Physiol. 2007; 48(12):1724-36. https://doi.org/10.1093/pcp/pcm144.
6. Hossain MM, Sultana F, Miyazawa M et al. The plant growth promoting fungi *Penicillium* spp. GP15-1 enhances growth and confers protection against damping-off and anthracnose in the cucumber. J Oleo Sci. 2014;63(4):391-400. https://doi.org/10.5650/jos.ess13143
7. Shoresh M, Harman GE, Mastouri F. Induced systemic resistance and plant responses to fungal biocontrol agents. Annu Rev Phytopathol. 2010;48: 21-43. https://doi.org/10.1146/annurev-phyto-073009-114450
8. Lee HR, Jung J, Riu M, Ryu CM. A new frontier for biological control against plant pathogenic nematodes and insect pests I: by microbes. Res Plant Dis. 2017;23: 114-49. https://doi.org/10.5423/RPD.2017.23.2.114
9. Mehmood A, Khan N, Irshad M et al. IAA producing endopytic fungus *Fusariun oxysporum* wlw colonize maize roots and promoted maize growth under hydroponic condition. Eur Exp Biol. 2018;8(4):24. https://doi.org/10.21767/2248-9215.100065
10. .Kumla J, Suwannarach N, Bussaban B et al. Indole-3-acetic acid production, solubilization of insoluble metal minerals and metal tolerance of some sclerodermatoid fungi collected from northern Thailand. Annals of Microbiology. 2014;64:707-20. https://doi.org/10.1007/s13213-013-0706-x
11. Spark DL, Page AL, Summer ME, Tabatabai MA, Helmke PA. Methods of soil analysis, part 3, chemical methods. USA: American Society of Agronomy. 1996. https://doi.org/10.2136/sssabookser5.3
12. Banerjee S, Mukherjee B, Dutta S. Characterization of plant growth promoting rhizobacterial strain *Pseudomonas aeruginosa* MCC 3877 with special reference to indole acetic acid (iaa) production. Int J Pharma Bio Sci. 2019; 10(3):80-88. https://doi.org/10.22376/ijpbs.2019.10.3.b80-88
13. Brick JM, Bostock RM, Silverstone SE. Rapid insitu assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. Appl Environ Microbiol. 1991; 57:535-38. https://doi.org/10.1128/aem.57.2.535-538.1991
14. Yoon SJ, Choi YJ, Min K, Cho KK, Kim JW, Lee SC et al. Isolation and identification of phytase producing bacterium, *Enterobacter* sp. 4 and enzymatic properties of phytase enzyme. Enzym Microb Technol. 1996; 18:449-54. https://doi.org/10.1016/0141-0229(95)00131-X
15. Lorck H. Production of hydrocyanic acid by bacteria. Plant Physiol. 1948; 1:142-46. https://doi.org/10.1111/j.1399-3054.1948.tb07118.x
16. Cappuccino JC, Sherman N. In: Microbiology, A Laboratory Manual, 3rd ed., Benjamin/Cummings Pub. Co., New York. 1992.
17. Schwyn B, Neilands JB. Universal chemical assay for the detection and determination of siderophores. Anal Biochem. 1987; 160:47-56. https://doi.org/10.1016/0003-2697(87)90612-9
18. Payne SM. Detection, isolation and characterization of siderophores. Methods Enzymol. 1994;235:329-44. https://doi.org/10.1016/0076-6879(94)35151-1
19. Roy S, Mukherjee B, Dutta S. Isolation of an endophytic fungus *Colletotrichum* sp. and study of its plant growth promoting traits. The Pharma Innovation. 2021; 10(4):1038-44. https://doi.org/10.22271/tpi.2021.v10.i4o.6129
20. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning a laboratory manual, Cold Spring Harbor Laboratory Press, New York. 1989.
21. Felsenstein J. Phylogenesis and comparative methods. Am Nat. 1985;125:1-15. https://doi.org/10.1086/284325
22. Pulcrano G, Lula VD, Vollaro A et al. Rapid and reliable MALDI-TOF mass spectrometry identification of *Candida non-albicans* isolates from bloodstream infections. J Microbiol Methods. 2013;94:294-96. https://doi.org/10.1016/j.mimet.2013.07.001
23. Aron D. Copper enzymes isolated chloroplasts, polyphenol oxidase in *Beta vulgaris*. Plant Physiology. 1949; 24:1-15. https://doi.org/10.1104/pp.24.1.1
24. Bradford M. Rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54. https://doi.org/10.1016/0003-2697(76)90527-3
25. Chandini, Kumar R, Kumar R, Prakash O. The impact of chemical fertilizers on our environment and ecosystem. Research Trends in Environmental Sciences. 2019;2(5):69-86.

26. Zhang S, Gan Y and Xu B. Application of Plant-Growth- Promoting Fungi *Trichoderma longibrachiatum* T6 Enhances Tolerance of Wheat to Salt Stress through Improvement of Antioxidative Defense System and Gene Expression. Front. Plant Sci. 2016;7:1405. https://doi.org/10.3389/fpls.2016.01405

27. Gahan J, Schmalenberger A. The role of bacteria and mycorrhiza in plant sulfur supply. Front. Plant Sci. 2014;5:723. https://doi.org/10.3389/fpls.2014.00723

28. Hossain MM, Sultana F, Hyakumachi M. Role of ethylene signaling in growth and systemic resistance induction by the plant growth promoting fungus *Penicillium viridicatum* in *Arabidopsis*. J. Phytopathol. 2017;165:432-41. https://doi.org/10.1111/jph.12577

29. Muslim A, Hyakumachi M, Kageyama K et al. Induction of systemic resistance in cucumber by hypovirulent binucleate *Rhizoctonia* against anthracnose caused by *Colletotrichum orbiculare*. Trop Life Sci Res. 2019;30:109. https://doi.org/10.21315/tlsr2019.30.1.7

30. Zhang Y, Chen FS, Wu XQ et al. Isolation and characterization of two phosphate-solubilizing fungi from rhizosphere soil of moso bamboo and their functional capacities when exposed to different phosphorus sources and pH environments. PLoS ONE. 2018;13(7):e0199625. https://doi.org/10.1371/journal.pone.0199625

31. Jogaiah S, Abdelrahman M, Tran LSP et al. Characterization of rhizosphere fungi that mediate resistance in tomato against bacterial wilt disease. J Exp Bot. 2013; 64:3829-42. https://doi.org/10.1093/jxb/ert212

32. James EK, Gyaneshwar P, Mathan N et al. Infection and colonization of rice seedlings by the plant growth-promoting bacterium *Herbaspirillum seropedicae* Z 67. Mol. Plant. 1Microbiol Interact. 2002;15:894-906. https://doi.org/10.1094/MPMI.2002.15.9.894

33. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. 2003;255:571-86. https://doi.org/10.1023/A:1026037216893

34. Chi F, Shen SH, Cheng HP et al. Ascending migration of endophytic rhizobia, from roots to leaves inside rice plants and assessment of benefits to rice growth physiology. Appl Environ Microbiol. 2005;71:7271-78. https://doi.org/10.1128/AEM.71.11.7271-7278.2005

35. Khan AL, Hamayun M, Kang SM, Kim YH, Jung HY, Lee JH, Lee IJ. Endophytic fungal association via gibberellins and indole acetic acid can improve plant growth under abiotic stress: an example of *Paecilomyces formosus* LHL10. BMC Microbiol. 2012; 12: 3. https://doi.org/10.1186/1471-2180-12-3

36. Tsavkelova EA, Cherdyntseva TA, Botina SG et al. Bacteria associated with orchid roots and microbial production of auxin. Microbiol Res. 2007; 162: 69-76. https://doi.org/10.1016/j.micres.2006.07.014

37. Suwannarach N, Kumla J, Matsui K et al. Characterization and efficacy of *Muscodor cinnamomi* in promoting plant growth and controlling *Rhizoctonia* root rot in tomatoes. Biol Control. 2015; 90:25-33. https://doi.org/10.1016/j.biocontrol.2015.05.008

38. Waqas M, Khan AL, Lee IJ. Bioactive chemical constituents produced by endophytes and effects on rice plant growth. J Plant Interact. 2014; 9: 478-87. https://doi.org/10.1080/17429145.2013.860562

39. Khan AR, Ullah I, Waqas M et al. Plant growth promoting potential of endophytic fungi isolated from *Solanum nigrum* leaves. World J Microbiol Biotechnol. 2015;31:1461-66. https://doi.org/10.1007/s11274-015-1888-0

40. Chutima R, Lumyong S. Production of indole-3-acetic acid by Thai native orchid-associated fungi. Symbiosis. 2012;56:35-44. https://doi.org/10.1007/s13199-012-0158-2

41. Pedraza RO, Ramirez-Mata A, Xiqui ML, Baca BE. Aromatic amino acid aminotransferase activity and indole-3-acetic acid production by associative nitrogen-fixing bacteria. FEMS Microbiol Lett. 2004;233:15-21. https://doi.org/10.1016/j.femsle.2004.01.047

42. Bharucha U, Trivedi UB, Patel K. Optimization of indole acetic acid production by Pseudomonas putida UB1 and its effect as plant growth -promoting rhizobacteria on Mustard (*Brassica nigra*). Agric Res. 2013;2 (3):215-22. https://doi.org/10.1007/s40003-013-0065-7

43. Shanti M, Keshab C, Dey S. Optimization of cultural and nutritional conditions for indole acetic acid production by a *Rhizobium* sp. isolated from root nodules of *Vigna mungo* (L.) Hepper. Res J Microbiol. 2007;2:239-46. https://doi.org/10.3923/jm.2007.239.246

44. Sachdev DP, Chaudhari HG, Kasture VM et al. Isolation and characterization of indole acetic acid (IAA) producing *Klebsiella pneumonia* strains from rhizosphere of wheat (*Triticum aestivum*) and their effect on plant growth. Ind J Exp Boil. 2009;47:993-1000.

45. Lebrazi S, Niehaus K, Bednarz H et al. Screening and optimization of indole-3- acetic acid production and phosphate solubilization by rhizobacterial strains isolated from *Acacia cyanophylla* root nodules and their effects on its plant growth. Journal of Genetic Engineering and Biotechnology. 2020;18:71. https://doi.org/10.1186/s43141-020-00090-2

46. Wang Y, Mopper S, Hasenstein KH. Effects of salinity on endogenous ABA, IAA, JA and SA in Iris hexagona. J Chem Ecol. 2001; 27(2):327-42. https://doi.org/10.1023/A:1005632506230

47. Ahmad F, Ahmad I, Khan MS. Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan. Turk J Biol. 2005; 29:29-34.

48. Santi M, Keshab C, Dey S, Pati BR. Optimization of cultural and nutritional conditions for indole acetic acid production by a *Rhizobium* sp. isolated from root nodules of *Vigna mungo* (L.) Hepper. Res J Microbiol. 2007;2:239-46. https://doi.org/10.3923/jm.2007.239.246

49. Sridevi M, Mallaiah KV. Bioproduction of indole acetic acid by *Rhizobium* strains isolated from root nodules of green manure crop, *Sesbania sesban* (L.). Merr Iran J Biotechnol. 2007;5:178-82.

50. Bose A, Shah D, Keharia H. Production of indole-3-acetic-acid (IAA) by white rot fungus *Pleurotus ostreatus* under submerged condition of *Jatropha* seedcake. Mycology. 2014; 4: 103-11. https://doi.org/10.1080/21501203.2013.823891

51. Sridevi M, Yadav NCS., Mallaiah KV. Production of indole acetic acid by *Rhizobium* isolates from *Crolataria* species. Res J Microbiol. 2008; 3 (4):276-81. https://doi.org/10.3923/jm.2008.276.281

52. Bhutani N, Maheshwari R, Negi M et al. Optimization of IAA production by endophytic *Bacillus* spp. from *Vigna radiata* for their potential use as plant growth promoters. Israel journal of plant sciences. 2018; 65(1). https://doi.org/10.1163/22238980-00001025

53. Leelahawonge C, Pongsilp A, Nuntagij A. Factors Influencing Indole-3-Acetic Acid Biosynthesis of Root-Nodule Bacteria Isolated from Various Leguminous Plants. Thammasat International Journal of Science and Technology. 2009;14:1-12.

54. Balaji N, Lavanya SS, Muthamizhselvi S, Tamilarasan K. Optimization of fermentation condition for indole acetic acid production by *Pseudomonas* species. Int J Adv Biotechnol Res. 2002;3: 797-803.

55. Nutarata P, Monprasit A, Srisuk N. High-yield production of indole-3-acetic acid by Enterobacter sp. DMKU-RP206, a rice phyllosphere bacterium that possesses plant growth-promoting traits. 3. Biotech. 2017;7:305. https://doi.org/10.1007/s13205-017-0937-9

56. Nguyen Ngoc Lan, Vu Van Dung, Nguyen Thi Kim Lien et al. Isolation and identification of indole acetic acid producing bacteria from the coasts of Ben Tre and Tra Vinh Provinces. Tap chi Sinh hoc (Journal of Biology). 2019;41(4):55-67. https://doi.org/10.15625/0866-7160/v41n4.13869

57. Naziya B, Murali M and Amruthesh KN. Plant growth-promoting fungi (PGPF) instigate plant growth and induce disease resistance in *Capsicum annuum* L. upon infection with *Colletotrichum capsici* (Syd.) Butler & Bisby. Biomolecules. 2019; 10(1):41. https://doi.org/10.3390/biom10010041

58. Nadeem SM, Zahair ZA, Naveed M, Asghar HN, Asghar M. Rhizobacteria capable of producing ACC-deaminase may mitigate salt stress in wheat. Soil Sci Soc Am. 2010; 74:533-42. https://doi.org/10.2136/sssaj2008.0240

59. Upadhyay SK, Singh JS, Singh DP. Exopolysaccharide producing PGPR under salinity condition. *Pedosphere*. 2011; 21(2):214-22. https://doi.org/10.1016/S1002-0160(11)60120-3

60. Habib SH, Kaussar H, Saud HM. Plant growth-promoting rhizobacteria enhance salinity stress tolerance in okra through ROS-scavenging enzymes. Bio Med Res Int. 2016; 1-10. https://doi.org/10.1155/2016/6284547

61. Banerjee S and Dutta S. Plant growth promoting activities of a fungal strain *Penicillium commune* MCC 1720 and its effect on growth of black gram. The Pharma Innovation. 2019; 8(12):121-27.

62. Maheshwari DK. Bacteria in agrobiology: stress management. USA: Springer. 2012. https://doi.org/10.1007/978-3-662-45795-5

63. Hung R, Rutgers SL. Applications of *Aspergillus* in Plant Growth Promotion. New and Future Developments in Microbial Biotechnology and Bioaengineering. *Aspergillus* System Properties and Applications. 2016; 223-27. https://doi.org/10.1016/B978-0-444-63505-1.00018-X

64. Pandya ND, Desai PV, Jadhav HP et al. Plant growth promoting potential of *Aspergillus* sp. NPF7, isolated from wheat rhizosphere in South Gujarat, India. Environmental Sustainability. 2018;1:245-52. https://doi.org/10.1007/s42398-018-0025-z

65. Kim K, Jang YJ, Lee SM, Oh BT, Chae JC, Lee KJ. Alleviation of salt stress by *Enterobacter* sp. EJ01 in tomato and *Arabidopsis* is accompanied by up regulation of conserved salinity responsive factors in plants. Mol Cell. 2014; 37(2):109-17. https://doi.org/10.14348/molcells.2014.2239

66. Sarkar A, Ghosh PK, Pramanik K, Mitra S, Soren T, Pandey S, Mondal MH, Maiti TK. halotolerant *Enterobacter* sp. displaying ACC deaminase activity promotes rice seedling growth under salt stress. Research in Microbiology. 2018; 169:20-32. https://doi.org/10.1016/j.resmic.2017.08.005

67. Ismail MH, Hussain A, Iqbal A, Khan SA, Lee IJ. Endophytic fungus *Aspergillus japonicus* mediates host plant growth under normal and heat stress conditions. BioMed Research International. 2018; p.11. https://doi.org/10.1155/2018/7696831

§§§