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Characterisation of plant growth promoting traits and stress tolerant properties of *Aspergillus terreus* (MCC 1819) isolated from the rhizosphere of *Aloe barbadensis* Mill. and its application

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Abstract
Plant growth-promoting fungi (PGPF) are a class of non-pathogenic soil-borne filamentous fungi that have beneficial effects on plants. It has been reported that PGPF increase the plant growth directly or indirectly by fixing nitrogen, producing phytohormones, inducing systemic resistance etc. The association of PGPF with plant roots has also been shown to modulate growth, morphology, nitrogen assimilation, resource allocation and mineral uptake of the host plant and also improves host reproductive fitness with subsequent increase in biomass and grain yield of crop plants. So, by considering the beneficial effects of PGPF and the growing demands of biofertilizers for sustainable agriculture, our focus was to isolate and characterize the PGPF from the rhizospheric soil of the plant *Aloe barbadensis* Mill. growing in Burdwan district, West Bengal, India. After isolation, the isolated PGPF were studied for their plant growth promoting traits like, Phosphate solubilising property, IAA production, ammonia production, HCN production etc. Bioactivities like antifungal activity as well as stress tolerance properties like, salinity stress and some heavy metal stress were also tested. The best potent strain was then selected and identified by 18s r-RNA sequencing. Subsequent preparation of the phylogenetic tree, it was confirmed that this PGPF fungal strain was *Aspergillus terreus* (MCC 1819). Furthermore, augmentation of growth and vigour of *Aloe barbadensis* by application of this PGPF has also been noticed.

Keywords: PGPF, IAA, HCN, ammonia, stress tolerance

Introduction
Chemical fertilizers cause health hazard and influence the microbial population in soil by demeaning the physical structure of the soil leading to scarcity of oxygen in the plant root zone and besides they are quite costly making the price of production high. Whereas, the bulk of the microorganisms dispersed around plant root surface have a function in the breakdown of organic matter and some may repress deleterious microorganisms, which could slow down plant growth. A few of the root-associated microorganisms can enhance plant growth and they have been called "plant growth-promoting fungi" (PGPF) [1]. PGPF are non-pathogenic saprophytes and are reported to suppress fungal and bacterial diseases of host plant [2, 3, 4]. The PGPF *Phoma* sp., which generally does not sporulate under natural conditions, has been found to improve plant growth, suppress plant pathogens and induce systemic resistance [5].

The PGPF association with roots of various plant species and infection has also been shown to modulate growth, morphology, nitrogen assimilation, resource allocation and mineral uptake of the host plant and also improves host reproductive fitness by enhancing plant growth, increase biomass and grain yield of crop plants [1].

The plant *Aloe barbadensis* Mill. belongs to the family Asphodelaceae (APG IV,2016) has great ethnomedicinal importance. *Aloe barbadensis* Mill. is one of the few known natural plant sources of Vitamin B12, and it contains many minerals vital to the growth process and healthy function of all the body's systems. *Aloe barbadensis* Mill. contains protein, calcium, magnesium, zinc, vitamins A, B12, C, E and essential fatty acids [6]. It was reported universally that *Aloe* is a common tonic for the immune system, it helps to fight against illness of many kinds. Various studies are underway to explore the potential of *Aloe barbadensis* Mill. components to enhance immunity and combat the HIV virus, and to treat certain types of cancer particularly leukemia [6].

Keeping these on mind present communication is focused on the interaction and effect of PGPF on the host plant *Aloe barbadensis* Mill.

Materials and Methods

The PGPF was first isolated by serial dilution technique after collecting the rhizospheric soil of *Aloe barbadensis* Mill. 1 gm of soil was taken from the plastic bag and then the soil was diluted to 10 ml of water in a test-tube which was labelled as stock solution. Remaining 9 test tubes were filled with 9 ml of water. Transferring 1 ml of water from the stock solution to 9 ml of sterilized distilled water with the help of pipettes yielded 10^{-1} dilutions and the series continued up to 10^{-6} dilutions. The fungal strains were first isolated in Pikovskaya agar medium and their further characterizations were performed. (Composition of agar Pikovskaya agar medium: Yeast extract-0.50 gm/ltr, Dextrose-10.00 gm/ltr, Calcium phosphate-5.00 gm/ltr, Ammonium phosphate-0.50 gm/ltr, Potassium chloride-0.20 gm/ltr, Magnesium sulphate-0.10 gm/ltr, Manganese sulphate-0.0001 gm/ltr, Ferrous sulphate-0.0001 gm/ltr, Agar-15.00 gm/ltr). Antibiotic (32 ppm) was applied in the medium as no bacterial colony was desired for the study.

A total number of 2 different colonies were isolated on Pikovskaya (PKV) Agar medium (Pikovskaya, 1948) [7] and the fungal strains for pure culturing were selected on the basis of the halo zone area that they produced in the same medium. The fungal isolates were designated as FSD1 and FSD2 (Fig.1) after pure culturing and the species level identification of the most potent strain (FSD2) was done following 18s rDNA sequencing.

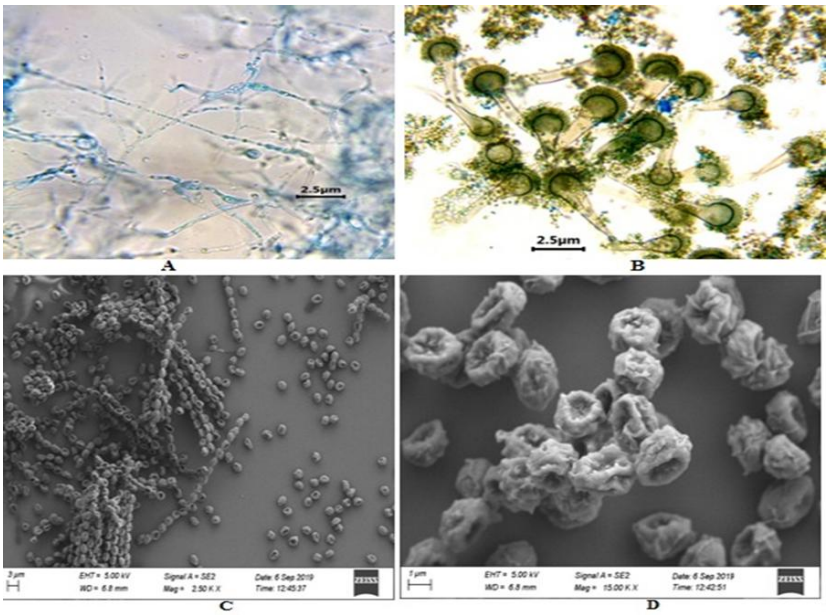


Fig 1 (A): Microscopic view of FSD1; (B) Microscopic view of FSD2; (C) SEM study shows the arrangement of conidia of the fungal strain FSD2; (D) SEM study shows the enlarged view of Conidia of the fungal strain FSD2.

Slants were prepared in the culture tubes with Potato Dextrose Agar (PDA) medium for further studies and the isolates were inoculated. Then the culture tubes were incubated at 30±2 ºC and observed at 24 hours intervals.

Characterization of PGPF traits

Quantitative test for phosphate solubilization: It was done following ammonium-molybdate method [8]. The fungal strains (FSD1 and FSD2) were inoculated into 60 ml of pikovskaya (PVK) broth medium and on rotary shaker and one control set were also taken. The fungal cultures were then filtrate after incubation. After that the filtrates were centrifuged at 10000 rpm for 15 minutes, centrifugation was repeated until the clear solution becomes available. 10 ml of aqueous filtrate was taken and 25ml Barton's reagent was added within it and the volume made up upto 50ml by adding distilled water. After 10 minutes absorbance was measured at 430 nm wavelength. Phosphate solubilization was determined using the standard curve of KH_2PO_4.

Estimation of Indole-3-acetic acid (IAA)

Fungal strains (FSD1 and FSD2) were inoculated in liquid pikovskaya (PVK) broth medium and kept for 3 days on a rotary shaker at 30±2ºc. After 72 hours of growth, the fungal cultures were centrifuged at 10,000 rpm for 15 minutes and then 1 ml of supernatant was taken in a test tube and add equal volume of Salkowski's reagent (35% of perchloric acid, 1 ml 0.5 M $FeCl_3$). Then the reaction mixture was kept at dark condition for 20 minutes. Appearance of pink colour in the test-tube indicated the production of IAA and the absorbance was taken at 535 nm. IAA estimation test was followed by the method of Brick *et al.* (1991) [9].

Assay of ammonia production

The fungal isolates were tested for ammonia production. Isolates was grown in peptone broth at 28 ºc for 3 days. After the incubation period, 0.5 ml of supernatant was taken and add 1ml of Nessler's reagent to the test tube. The development of faint yellow to dark orange colour indicated the positive result of ammonia production (Cappuccino and Sherman, 1992) [10].

HCN production

Hydrogen cyanide production was tested followed by the method of Lorck (1948) [11]. PDA was amended with glycine (4.4 g/l) and the fungal strains were streaked on modified agar plates. The solution of 2% sodium carbonate and 0.5% picric acid was made and the Whatman filter paper no. 1 was dipped into it and was impregnated at the inner surface of the lid of the Petridishes. Plates were covered with parafilm and incubated at 30 °C for 4 days. Development of orange to brown colour indicates that the fungal strains have the capability to produce hydrogen cyanide.

Nitrogen fixation test
The ability to fix atmospheric nitrogen was tested by inoculating the isolate in the DM media without ready source of nitrogen i.e. Ammonium sulphate and they are allowed to incubate at 30±2º C temperature for 3 days. Occurrence of fungal growth indicates its ability to fix atmospheric nitrogen.

Siderophore prodction
Siderophore production was detected by the universal method of Schwyn and Neilands (1987) [12] containing the dye chrom azurol S (CAS). Green halo zone around the colonies on blue plates were clear indication for siderophore production.

Assay of polysaccharide production
Polysaccharide production was tested following the Phenol Sulphuric Acid method [13]. The fungal strains were grown in Nutrient broth at 30ºc for 3 days. After 3 days, the fungal cultures were centrifuged at 6000 rpm for 10 minutes. 1 ml of supernatant was taken and 3 ml of Acetone was added within it. Then the precipitates were again re-precipitating with 3ml of acetone and centrifuged, this process were repeated several times. After that 1ml of precipitate was taken and add 1ml of 5% Phenol and 5ml of conc. Sulphuric Acid within it. This mixture was kept for 20 minutes. Development of orange-red colour indicates the presence of polysaccharide. O.D. was taken at 495nm and the concentrations were measured using the standard curve of glucose.

Production of organic acids
The productions of Organic Acids by the fungal Isolates (FSD1 and FSD2) were determined following the standard protocol of Jones *et al*. (1994) [14].

Salt tolerence activity
Fungal isolates were inoculated in different salt concentration (2%, 4%, 6%, 8%, 10%, 15%, 20%, 25%, 30%) and were allowed to incubate for 3 days at 30±2 ºC temperature. The growths of the fungal strains were observed after the incubation period.

Heavy metal tolerance activity
The fungal strains were assessed for its resistance against heavy metal. This was done following the method of agar dilution method (Cerventas *et al*., 1986) [15]. Freshly prepared Potato dextrose Agar media were amended with soluble heavy metals salt like, Zn ($ZnSO_4.7H_2O$) and Cr (CrO_3) at (5 -30) mM concentrations and allowed to grow at 30ºc for 72 hours. The growths of the fungal strains were recorded.
Zinc is a nutrient at low concentration but toxic at higher concentration. The solubilization of zinc might limit the growth of the microorganisms at higher level. Unless the cultures tolerate a higher level of zinc its solubilisation may not continue. Therefore the ability of selected isolate to tolerate solubilize zinc was determined under *in vitro* condition in nutrient broth containing different concentrations of soluble zinc ($ZnSO_4.7H2O$) [16].
The potato dextrose broth was prepared and splitterd in 10 ml aliquots in test tubes. $ZnSO_4$ and CrO_3 was incorporated separately into the broth in such a way that the final concentration of zinc and Chromium was 5,10,15,20,25,30 mM. Simultaneously, a control set was also maintained. The activities of the fungal strains were assessed by plating on potato dextrose agar media. The growth of fungal isolates in the zinc and chromium containing medium indicated their tolerance to those metals. The highest concentration at which poor growth was observed and taken as tolerance level.

Antibacterial activity of the fungal strains
Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts [17, 18]. Similar to the disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the bacterial inoculums (*Bacillus* sp. and *Escherichia coli*) over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer and the fungal strains (FSD1 and FSD2) are introduced into the well. Then, agar plates are incubated under suitable conditions for 48 hours. The fungal strains inhibited the growth of the bacterial strains tested and the zone of inhibition was then calculated.

Selection for best fungal strain on the basis of PGP traits
On the basis of plant growth promoting characters, FSD2 was selected as best performing strain among the two isolates and finally selected for the application process to the plant *Aloe barbadensis*.

Application of PGPF (FSD2) on growth and development of the plant *Aloe barbadensis* on pot culture
To study the effect of best PGP traits of the fungal isolate (FSD2), pot assay was performed. The seeds (collected from the Agricultural farm, The University of Burdwan) were surface sterilized by immersing in 95% ethanol for 30s and then in mercuric chloride (0.1%) for 3 min. To remove traces of mercuric chloride, the disinfected seeds were washed 3 times by sterile distilled water. The seeds were kept for 30-40 minutes in the broth culture of plant growth promoting fungi (FSD2) containing 4.4 X 10^6 colony forming unit (CFU)/ml. For germination, seeds were kept in dark for 2 days and then kept on a large table or workbench where it will receive eight to 10 hours of sunlight daily until the seeds germinate. After germination, the plantlets were planted in the earthen pots containing soil and sand mixture (soil: sand = 3:1). After 5 days of plantation, the fungal suspension (FSD2) was applied in the pot (as 10 ml/kg soil-sand mixture) for the study of its effect on the plant growth promoting parameters and marked the pot as treated set (PGPF) and simultaneously one control set was maintained where no fungal inoculum was added.

Effect of PGPF on different plant growth parameters
Effect on morphological parameters of plant
Plantlets were uprooted after 30 and 45 days and measured the
a) Shoot length (cm)
b) Root length cm)
c) Shoot weight (gm)
d) Root weight (gm)
e) Leaf number

Effect on biochemical parameters of plant
Extraction of chlorophyll from leaves
2g of fresh leaf tissue was taken from each replicates and homogenized with 80% acetone and subjected to centrifugation at 10,000 rpm for 8 minutes, this procedure was repeated for several times. Then supernatant was taken and OD value was recorded at 645nm and 663nm (Aron D,1949) [19]. The amount of chlorophyll was calculated by this formula:
Chlorophyll a: 12.7(A663) – 2.69(A645)
Chlorophyll b: 22.9(A645) – 4.68(A663)
Total Chlorophyll: 20.2(A645) + 8.02(A663)

Estimation of total protein

To estimate the total protein, pipetted out 0.2 ml of plant extract and add 2 ml of alkaline copper sulphate reagent. Shaken well and kept the mixture for 15 minutes. After 10 minutes, add 0.2 ml of reagent Folin Ciocalteau solution was added and were thoroughly mixed. The mixture was kept for another 30 minutes. The absorbance was measured at 660 nm in Beckman DU-64 spectrophotometer. The concentration of protein was determined by comparing the absorbance with a standard curve prepared [20].

Estimation of total carbohydrate

1 gm plant material was weighted in a tube. The sample was hydrolyzed by keeping it in boiling water bath for two hours with 5 ml 2.5 N HCL and then cooled in room temperature. Then it was neutralized with solid sodium carbonate until the effervescence stopped. The volume was made up to 100ml and then centrifuged. The supernatant was collected and 0.5ml aliquot was taken for analysis. The volume of the tube was made up to 1ml by the addition of distilled water, to it 4ml of Anthrone reagent was added and are allowed to heat for 8 minutes in a boiling water bath. After that the test tubes were rapidly cooled and read the green to dark green colour at 630 nm in the Beckman DU-64 spectrophotometer. The concentration of total carbohydrate in solution was measured from the standard curve [21].

Results and Discussion

Response of the fungal strains FSD1 and FSD2 to the following plant growth promoting traits

After establishing the pure culture, investigation on plant growth promoting traits were performed.

Following Nitrogen, Phosphorus is the second most important nutrient required for plant growth. A greater portion of phosphorus in soil is in the form of insoluble phosphates and cannot be used directly by the plants [22]. In our present study, both FSD1 and FSD2 isolates were found to confer clear zone on Pikovskaya agar medium containing insoluble mineral phosphate (tri-calcium phosphate). In the Quantitative Assay of Phosphate, FSD2 solubilize more phosphate than FSD1. The strain FSD1 solubilize 54 ppm of phosphate per ml whereas the strain FSD2 solubilize 350 ppm of phosphate per ml (Table 2).

IAA is usually considered to be the most vital phytohormone that functions as important signal molecule in the regulation of plant growth and development processes. It has been reported that more efficient auxin producers are commonly associated with rhizosphere soil as comparison to bulk soil[23]. In present study, both the isolates FSD1 and FSD2 showed positive results in IAA qualitative assay (Table 1). However, the isolate FSD1 produces lesser amount of IAA compared to FSD2 (Table 2). The strain FSD1 produce 256 ppm of IAA/ml whereas, the strain FSD2 produce 490 ppm of IAA/ml. (Fig.2)

While test for Ammonia production, both the isolates FSD1 and FSD2 again conferred positive results (Table 1).

Table 1: Qualitative study of Plant growth promoting traits of fungal isolates

Tests performed	FSD1	FSD2
Phosphate Solubilization	+	+
IAA production	+	++
Ammonia production	+	+
HCN production	-	+
Nitrogen fixing Ability	-	+
Siderophore Producing Ability	-	+
Exopolysaccharide Production	-	+

The result of the qualitative tests done for the isolated PGPFs. '+' sign indicated that the test is positive, '++'signs means more positive. '- 'sign indicated that the test is negative. All data are mean value of five replicates.

Table 2: Quantitative study of Plant growth promoting traits of fungal isolates

Characters	FSD 1	FSD 2
Phosphate solublization	54 ± 0.030 ppm/ml	350 ± 0.019 ppm/ml
IAA production	256 ± 0.027 ppm/ml	490 ± 0.032 ppm/ml

Data's are mean value of five replicates. [Values are mean ± SD (n = 5); $P<0.05$, significant, compared to control.]

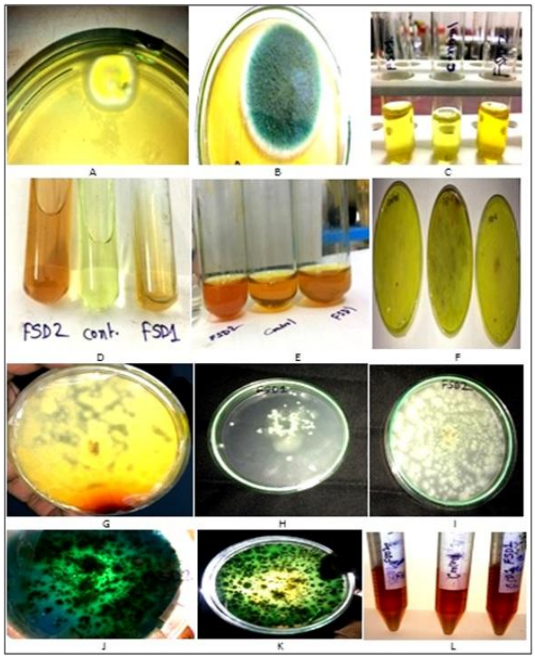
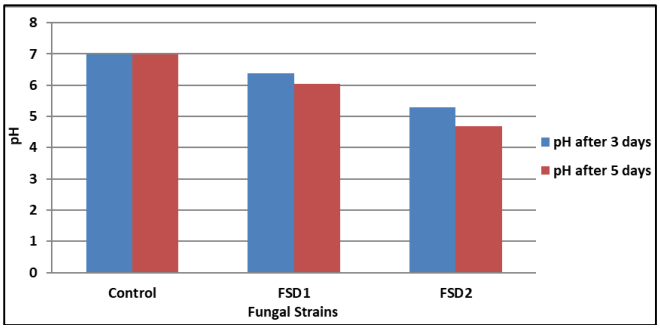


Fig 2: (A) Phosphate solubilization by FSD1; (B) Phosphate solubilization by FSD2; (C) Quantitative test for phosphate solubilization by FSD1 and FSD2; (D)IAA production; (E) Ammonia production; (F) HCN production by FSD1 and FSD2; (G) HCN production by FSD2; (H) Nitrogen fixation by FSD2;(I) Nitrogen fixation by FSD1;(J) Siderophore production by FSD2 (after 3 days);(K) Siderophore production by FSD2 (after 6 days); (L) Exopolysaccharide production.

Siderophore production by rhizobacteria acts as biocontrol tool under iron limiting condition [24]. FSD2 demonstrated positive activity for siderophore production and it is confirmed by the development of green halo zones (Fig. 2) on blue agar medium, but the strain FSD1 didn't show any positive response (Table 1).

Besides these, there are various mechanisms of biocontrol including production of HCN in plants [24]. In addition, HCN is an another important trait that indirectly influences growth of plants by playing essential role in the biological control of plant pathogens or acting as an inducer of plant resistance [25]. In present study, FSD2 produces HCN (Fig.2) certainly whereas FSD1 did not produce HCN (Table 1). Moreover, FSD2 has the ability to fix Nitrogen whereas FSD1 did not respond to this test (Table 1). The role of organic acids in the mobilization of plant nutrients from the rhizosphere was assessed in seven contrasting soil types [14]. In present study, FSD2 produces more amount of organic acids than FSD1 (Graph 1).



Graph 1: Graphical representation of the production of organic acid by the fungal strains

Soil salinity in arid regions is frequently an important limiting factor for cultivating agricultural crops. Although many technologies have been concerned in the improvement of salt tolerance, one of them is the use of biological organism in tolerance against salt stresses [26]. FSD2 tolerate 10% salt concentration (Fig.3) while the strain FSD1 could not tolerate even 2% of salt concentration (Table 3).

Table 3: Salt tolerance of the Fungal Isolates

Concentration of salts	FSD 1	FSD 2
0%	+	++
2%	-	++
4%	-	++
6%	-	++
8%	-	+
10%	-	+
15%	-	-
20%	-	-
25%	-	-
30%	-	-

The result of the qualitative tests done for the isolated PGPFs. '+' sign indicated that the test is positive, '++'signs mean more positive. '- 'sign indicated that the test is negative. All data are mean value of five replicates.

While heavy metal stress was concerned, the isolate FSD2 confirmed Zn and Cr tolerance upto 20 ppm (Table 4) and 10 ppm (Table 5) respectively. In contrast, the isolate FSD1 exhibited 15ppm tolerance of Zn (Table 4) whereas it does not tolerate any concentration of chromium. (Table 5).

Table 4: Zinc tolerance of the Fungal Isolates

Concentration of zinc	FSD 1	FSD 2
0	+	++
5mM	+	++
10mM	+	+
15mM	+	+
20mM	-	+
25mM	-	-
30mM	-	-

The result of the qualitative tests done for the isolated PGPFs. '+' sign indicated that the test is positive, '++'signs means more positive. '- 'sign indicated that the test is negative. All data are mean value of five replicates.

Table 5: Chromium tolerance of the fungal isolates

Concentration of Chromium	FSD1	FSD2
0	+	+
5mM	-	+
10mM	-	+
15mM	-	-
20mM	-	-
25mM	-	-
30mM	-	-

The result of the qualitative tests done for the isolated PGPFs. '+' sign indicated that the test is positive, '++'signs means more positive. '- 'sign indicated the test is negative. All data are mean value of five replicates.

Fungal strain FSD2 responded well to demonstrate its antibacterial activity against *Bacillus sp* and *Escherichia coli*. However, it formed larger zone of inhibition in case of *Escherichia coli* (Fig.3) than in case of *Bacillus sp* (Table 6). On the other hand, FSD1 does not exhibit any zone of inhibition against both the strains.

Table 6: Antibacterial Activity of the Fungal Strain FSD2

Fungal Strain	Against *Bacillus* sp.	Against *Escherichia coli*
FSD2	12.5±0.4cm^2	37.4±0.7 cm^2

Data's are mean value of five replicates. [Values are mean ± SD (n = 5); P<0.05, significant, compared to control.]

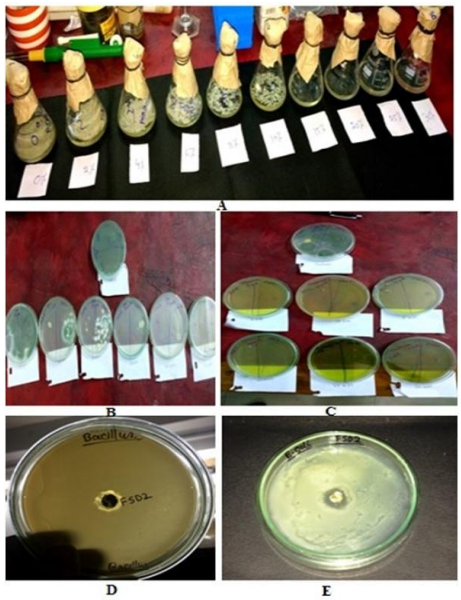
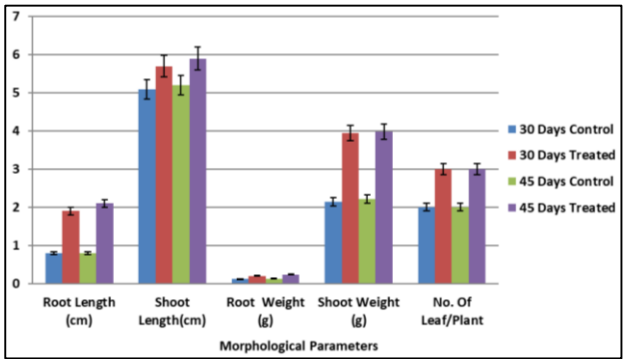


Fig 3: (A) Salt tolerance Activity; (B) Zinc tolerance activity; (C) Cromium tolerance activity; (D) Antibacterial activity against *Bacillus* sp.; (E) Antibacterial activity against *Escherichia coli*.

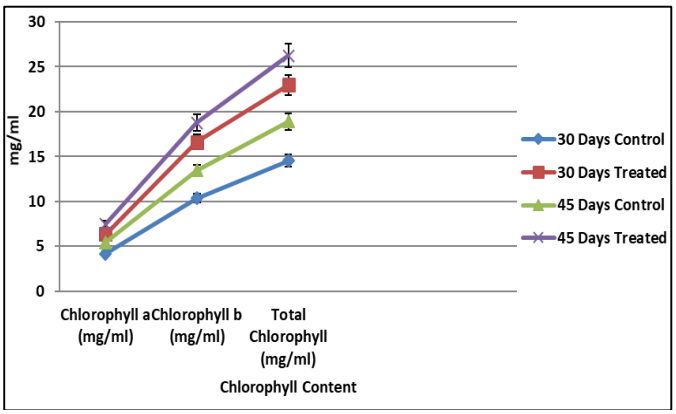
The fungal strain FSD2 enhances the growth of the plant *Aloe barbadensis* Mill.

After 30 days and 45 days of treatment, the effect of PGPF strain FSD2 on morphological and biochemical parameters of the plant *Aloe barbadensis* Mill. were recorded. It has been evidenced from the results (Graph 2) that the treated set displayed higher root length, shoot length, root weight, shoot weight and number of leaves per plant than the control set after 30 and 45 days of treatment (Fig.4).



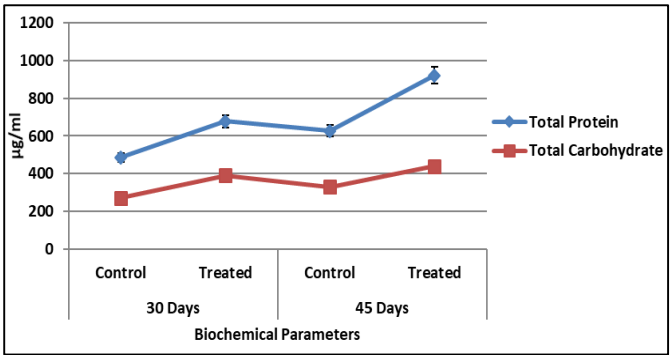
Graph 2: Morphological growth parameters in control and treated plants

The chlorophyll-a, chlorophyll-b and total chlorophyll content was elevated as a result of treatment after 30 and 45 days (Graph 3).



Graph 3: Chlorophyll content of control and treated plants

Total carbohydrate and total Protein content in *Aloe barbadensis* Mill. have been increased significantly in plants inoculated with PGPF (FSD2) compared to that of the untreated plants (Graph 4).



Graph 4: Biochemical growth parameters in control and treated plant

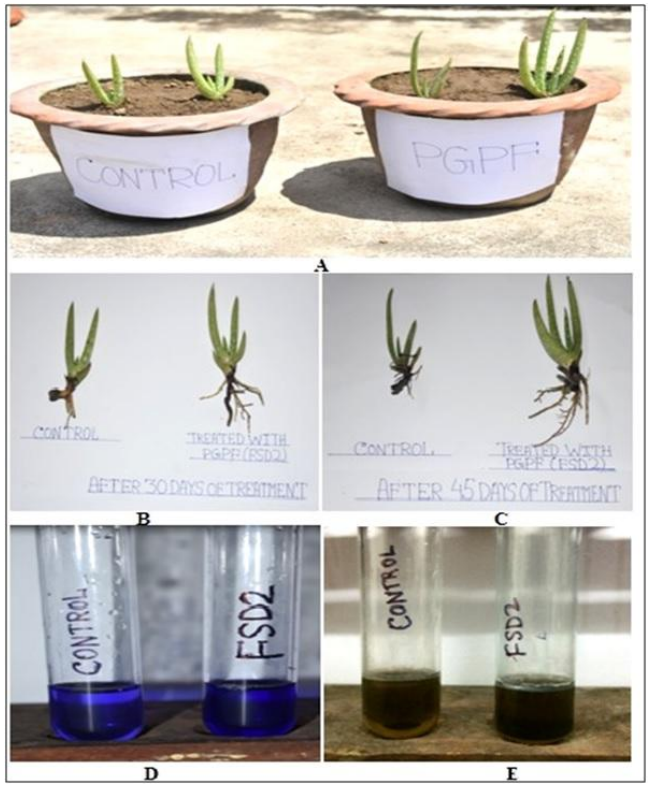


Fig 4: (A) Plants in the controlled and treated sets; (B) Plants uprooted after 30 days of Treatment from both the sets; (C) Plants uprooted after 45 days of Treatment from both the sets; (D) Estimation of Total Protein; (E) Estimation of Total Carbohydrate.

DNA Sequencing of the fungal strain FSD2.

Based on 18S rDNA sequence homology, the strain FSD2 showed 100% pair wise similarity with *Aspergillus terreus* (Table 7). The phylogenetic tree revealed that the FSD2 strain formed the cluster with *Aspergillus terreus*. The 18 S rDNA of FSD2 strain is given below in FASTA format and strain accession number of this strain obtained from MCC is MCC 1819.

Table 7: Molecular identification of FSD 2

Strain designation	Closest neighbour	% similarity	Accession number
FSD2	*Aspergillus terreus*	99	MCC 1819

18S rDNA sequencing (FASTA format)

TCATTAAATCAGTTATCGTTTATTTGATAGTACCTTA
CTACATGGATACCTGTGGTAATTCTAGAGCTAATAC
ATGCTAAAAACCTCGACTTCGGAAGGGGTGTATTTA
TTAGATAAAAAACCGATGCCCTTCGGGGCTCCTTGG
TGAATCATAATAACTTAACGAATCGCATGGCCTTGC
GCCGGCGATGGTTCATTCAAATTTCTGCCCTATCAAC
TTTCGATGGTAGGATAGTGGCCTACCATGGTGGCAA
CGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGG
GAGCCTGAGAAACGGCTACCACATCCAAGGAAGGC
AGCAGGCGCGCAAATTACCCAATCCCGACACGGGG
AGGTAGTGACAATAAATACTGATACGGGGCTCTTTT
GGGTCTCGTAATTGGAATGAGTACAATCTAAATCCC
TTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCA
GCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATT
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TTGGTGGAGTGATTTGTCTGCTTAATTGCGATAACGA
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TGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACAC
TGACAGGGCCAGCGAGTACATCACCTTGGCCGAGAG
GTCTGGGTAATCTTGTTAAACCCTGTCGTGCTGGGGA
TAGAGCATTGCAATTATTGCTCTTCAACGAGGAATG
CCTAGTAGGCACGAGTCATCAGCTCGTGCCGATTAC
GTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTAC
CGATTGAATGGCTCGGTGAGGCCTTCGGACTGGCTC
AGGGGAGTTGGCAACGACTCCCCAGAGC

Construction of phylogenetic tree

Construction of phylogenetic tree based on 18S rDNA sequences of valid strains obtained from National Centre for Biotechnology Identification (NCBI) sequence database a phylogenetic tree of FSD2 strain was made (Fig. 5). The evolutionary history was inferred using the Neighbour Joining method. Evolutionary analyses were conducted in MEGA7 (Kumar *et al*., 2016) [27].

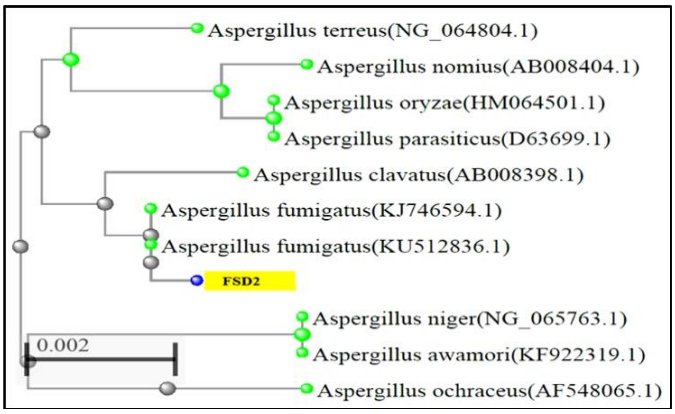

Fig 5: Construction of phylogenetic tree using mega 7 software

Conclusion

In the present study, the fungal strain FSD2 which was isolated from the rhizosphere of *Aloe barbadensis* Mill. proved to be a potent fungus in terms of its growth promoting traits. Furthermore, application of this strain *Aspergillus terreus* (FSD2) to *Aloe barbadensis* Mill. demonstrated significant improvement of growth of the treated plants. Very few research works have been done so far in this field. There are lot of studies regarding the interactions of *Aloe barbadensis* Mill. and rhizospheric bacteria [28, 29], on the other hand, almost no information is available regarding the cross talk of *Aloe barbadensis* and plant growth promoting fungi (PGPF).

Moreover, it can be concluded from the present study that this fungal strain, *Aspergillus terreus* (FSD2) may indeed be considered as a potent PGPF which promotes the growth of *Aloe barbadensis* Mill. and be able to use as a prospective biofertilizer for augmenting growth of this medicinal plants.

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