can be stable for certain periods of time and induce adverse effects to the surrounding tissues acting as chemical messengers.

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Indole-3-acetic acid production by the cyanobacterium *Fisherella muscicola* NDUPC001

S. K. Mishra, Jyoti Singh, Astha Raj Pandey and N. Dwivedi*

Department of Botany, U.P. College (Autonomous),
Varanasi 221 002, India

Fisherella muscicola* NDUPC001 was isolated from agricultural fields of Varanasi, India. The cyanobacterial strain was characterized by morphological as well as molecular methods (16S rRNA gene with accession no. JX876898.2) and was deposited at NAIMCC (NBAIM), Mau, Uttar Pradesh, India (accession no. NAIMCC-C-000121). The cyanobacterial strain produced tryptophan-dependent indole-3-acetic acid (IAA), which was identified by thin-layer chromatography and quantitative determination was done by Salkowski's colorimetric method. The maximum amount of IAA production was 286.82 µg/ml on the 19th day in culture medium supplemented with 5 mg/ml of L-tryptophan. The cyanobacterial extract increased the length of radicle, plumule and number of adventitious roots of rice several times in comparison to control to state the IAA production by *Fisherel-

*For correspondence. (e-mail: drnagendra.dwivedi@gmail.com)

***la muscicola*. Also, the production of IAA by this strain is highest among cyanobacteria reported so far.**

Keywords: Agricultural fields, cyanobacterial strain, *Fisherella muscicola*, indole-3-acetic acid, tryptophan.

THE cyanobacteria are morphologically diverse, oxygenic photosynthesis, prokaryotes and have a cosmopolitan distribution[1]. They fix approximately 2.32×10^{14} g of carbon on a global scale, accounting for 1/2000 of global biomass[2]. Many strains of cyanobacteria fix atmospheric nitrogen and are used as biofertilizers.

The cyanobacteria are a rich source of biologically active secondary metabolites[3,4]. They commonly induce plant growth through the release of fixed nitrogen, phosphate solubilization and phytohormone and siderophores production. Auxin, a phytohormone is also produced by pathogenic as well as non-pathogenic microbes[5]. Indole-3-acetic acid (IAA) is a well-known auxin which induces plant growth by regulating cell division, elongation, differentiation, root elongation and tropic response. Various cyanobacteria have shown the ability to produce IAA[6–8]. Cyanobacterial extracts having IAA induce rooting and shooting in callus[9]. Tryptophan-dependent and tryptophan-independent production of IAA have been reported in cyanobacteria[7,10]. Also, Cyanobacteria are important flora of agricultural fields and their role in the fertility of agricultural fields is well established. Hence, evaluation of IAA production of cyanobacteria is necessary. In this study, cyanobacterium was isolated, characterized and screened for IAA production.

The cyanobacterium was isolated as described by Singh *et al.*[11]. Powdered soil samples (collected from agricultural fields of Varanasi, Uttar Pradesh, India) were placed in autoclaved petri plates and moistened with nitrogen-free BG-11 medium. The petri plates were kept in culture room and the development of cyanobacterial colonies was monitored. The cyanobacterial strain was isolated and purified by streaking method[12]. The purified strain was grown in nitrogen-free BG-11 medium. The culture room was maintained at a temperature of 28° ± 2°C and light illumination of 12 wm^{-2}.

Morphological features of the strain, i.e. nature of filament, shape and size of vegetative cells, heterocysts and spores were observed at 400× and 1000× using a microscope (Olympus 21Xi, Japan). Magnus PRO micromeasurement and image analysis software was used to analyse morphological features. The strain was identified to cyanobacterial species according to taxonomic descriptions available in the literature[13–16].

Genomic DNA of the cyanobacteria was isolated according to Singh *et al.*[17] with some modifications. The cyanobacteria were harvested from 25 ml liquid culture by centrifugation at 6000 *g* for 5 min (centrifuge CPR-30, Remi, Mumbai, India) and pellets were used for isolation of DNA. The qualitative analysis of genomic DNA was done by electrophoresis on 0.8% agarose gel and the quantity was determined by a UV–Vis spectrophotometer (Perkin Elmer 2380, USA). The purity of genomic DNA was determined by the ratio between absorption at 260–280 nm.

16S rDNA was amplified by PCR using primers for _5/-GAGTT(CT)GATCCTGGCTCAGGA-3/ and Rev_ 5/-TCCAGCCGCACCTTCCAGTA-3/ (ref. 17). The PCR products were analysed by electrophoresis on 1.4% agarose gel and purified using a PureLink® PCR purification kit (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. The purified PCR product was sequenced on an automated capillary sequencer (ABI 3130 Genetic Analyser, Applied Biosystems, Foster City, CA, USA) at the Indian Institute of Vegetable Research, Varanasi and a partial (1300–1400 bp) 16S rRNA gene was obtained. The sequence of the strain was compared with 16S rRNA gene sequences available in GenBank/EMBL/DDBJ using BLASTn searches.

For IAA detection, *Fisherella muscicola* NDUPC001 was inoculated in BG-11 nitrogen-free medium supplemented without and with 5 mg/ml of tryptophan. After incubation for 14 days, IAA production was determined by Salkowski's colorimetric method[18].

IAA production was measured by using the Salkowski's colorimetric method[18]. *Fisherella muscicola* NDUPC001 was inoculated in BG-11 nitrogen-free medium supplemented with 3, 4, 5 and 6 mg/ml of tryptophan respectively. Growth and IAA in the extract were determined after an interval of two days up to 21 days. Then 5 ml culture was centrifuged at 10,000 *g* for 10 min at 4°C, the supernatant was sterilized by filtration (Millipore filter 0.25 µm (Axiva Sichem Biotech, New Delhi)) and Salkowski reagent (1 ml of 0.05 M $FeCl_3$ mixed in 50 ml of 35% perchloric acid) was added to the supernatant in the ratio of 1 : 2 (v/v). Incubation of the mixture was done for 30 min in the dark at room temperature; red colour was seen to develop. Optical density (OD) of red colour was measured at 535 nm against culture medium and Salkowaski reagent mixture in ratio of 1 : 2 (v/v). Calibration curve was prepared from IAA stock solution of 10–100 µg/ml.

Indole-3-butyric acid (IBA) (1 mg/ml) and IAA (1 mg/ml) were spotted along with filtrate (15 µl each) of *F. muscicola* NDUPC001 culture on silica gel plate (silica gel-60, Merck) and placed in chloroform and acetic acid: 95 : 5 solvent system[19]. Van Urk's reagent (1 g 4-dimethyl amino benzaldehyde dissolved in 50 ml diluted HCl 1 : 19) was sprayed on the plate after 3 h and Rf values were compared with standard IAA.

Certified seeds of rice (*Oryza sativa*, IC-545295) were obtained from the Institute of Agricultural Science, Banaras Hindu University, Varanasi. The seeds were surface-sterilized following standard procedure. Ten seeds of rice were placed in each petri plate containing Whatman filter paper moistened with 10 ml of sterilized distilled water,

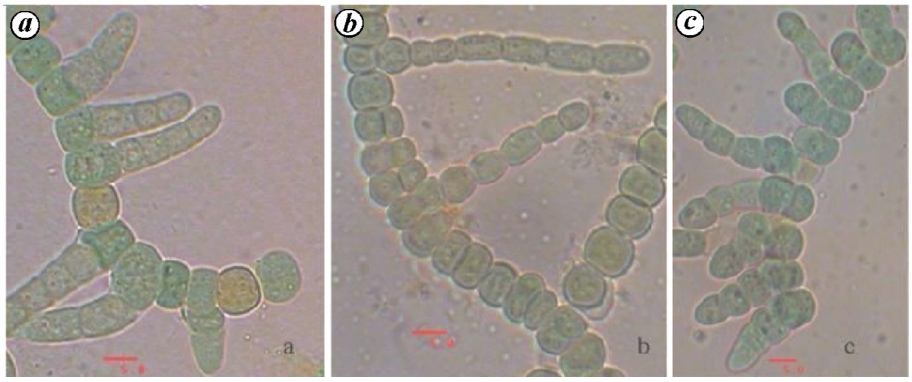

Figure 1. Photomicrograph of *Fisherella muscicola* NDUPC001 (scale bar = 5 µm). ***a***, Young filament with intercalary heterocyst and branching. ***b***, Old filament with cells in two rows. ***c***, Young filament with cells in two rows and branching.

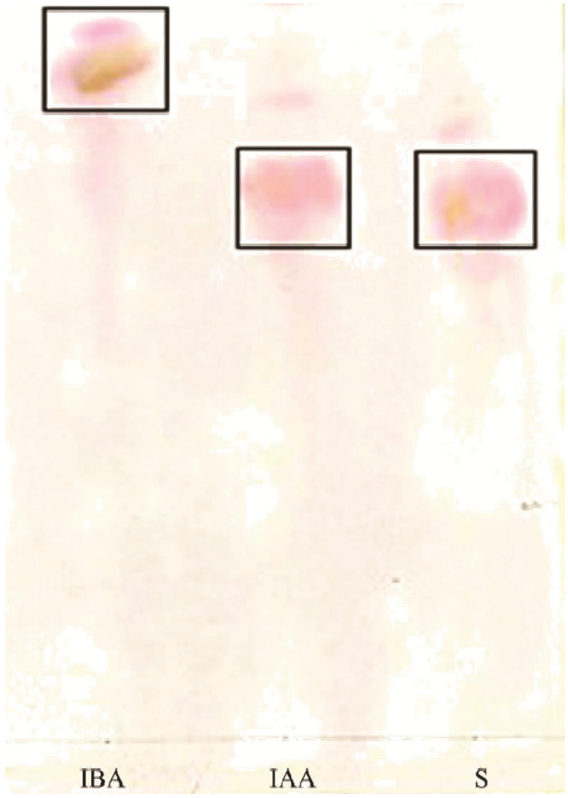

Figure 2. Thin layer chromatogram showing spots produced by IBA, IAA and cyanobacterial extract (S).

10 ml of sterilized BG-11 medium and 10 ml of sterilized distilled water mixed with 20, 30, 40, 50, 60 and 70 µl of cyanobacterial extract (culture medium having 5 mg/ml of tryptophan) respectively. Petri plates were placed in an incubator for 36 h at 30°C and then transferred to culture room maintained at 30° ± 2°C and 16/8 h light/dark cycles. Germination percentage of seeds was noted after incubation of 36 h. The length of coleoptile and radicle was measured after seven days of growth of seedlings.

The data for IAA production were recorded in triplicate; mean and standard error were calculated.

The cyanobacterial strain is filamentous, branched and heterocystous. Filament dark green, branched, the main filament creeping, flexuous, interwoven and most portions of the filament with cells in two rows. Cells of the main filament are subquadrate, subspherical, 6.16–8.04 µm broad and 6.58–7.42 µm long, constricted at the cross wall (Figure 1). The sheath of the filament is close to the trichome. Lateral branches are erect, more or less straight, distinct from the main filament, cells rectangular, closely compressed, light-coloured, not constricted, apical portion rounded. Cells are 3.57–5.05 µm broad and 4.65–7.11 µm long (Figure 1). Heterocysts are intercalary in both main filament as well as branches, subspherical, yellowish, 7.04–8.4 µm broad and 5.45–8.35 µm long (Figure 1). Hormogones long as well as club-shaped. Morphological characters closely match with *Fisherella muscicola* (Thuret) Gom[14]; hence the strain was identified as *Fisherella muscicola* with strain NDUPC001. Identity of the isolate was further confirmed by molecular means. Partial 16S rRNA gene of the strain was sequenced and submitted to GenBank of NCBI with accession no. JX876898.2. Blasting of the partial sequence of 16S rRNA gene of the strain was performed to the NCBI sequence databank (GenBank). The sequence of strain NDUPC001 was 99% identical with *Fisherella muscicola* SAG2027, which confirms the identity of the isolate. The strain was deposited at NAIMCC (ICAR-NBAIM), Mau, Uttar Pradesh (accession no. NAIMCC-C-000121).

Fisherella muscicola NDUPC001 produced tryptophan-dependent IAA. The IAA produced by *F. muscicola* NDUPC001 was characterized by TLC using commercial IAA and IBA as reference (Figure 2). Rf values of commercial IAA and IAA of cyanobacterial strain were the same (0.66). Effect of tryptophan concentration on IAA production by cyanobacterial strain was studied (Figure 3). The cyanobacterial strain produced maximum amount of IAA (286.82 µg/ml) on the 19th day in culture supplemented with 5 mg/ml tryptophan (Figure 3).

Table 1. Effect of extracellular filtrate of *Fischerella muscicola* NDUPC001 on seed germination, coleoptile and radicle growth of rice

Treatment	Percent germination	Length of coleoptiles (cm)	Length of radicle (cm)	No. of lateral roots
(a) Sterile water	90	1	0.8	Nil
(b) BG-11 medium	90	3	0.8	Nil
(c) 20 µl extract	100	1.2	2	1
(d) 30 µl extract	90	1.8	2.5	3
(e) 40 µl extract	90	5.7	2.8	5
(f) 50 µl extract	90	5.1	2.9	7
(g) 60 µl extract	90	6.5	3.8	7
(h) 70 µl extract	90	7.1	7.5	8

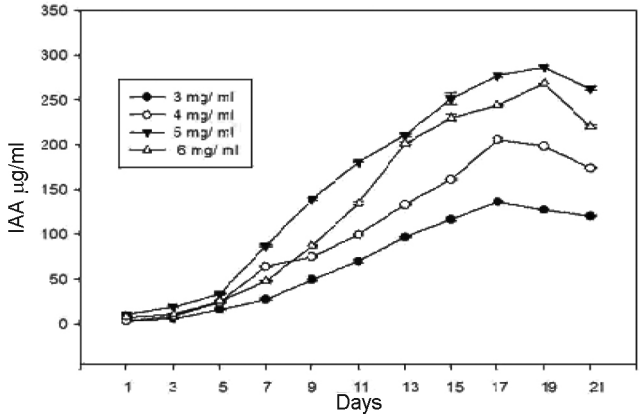

Figure 3. Indole-3-acetic acid (IAA) production in culture medium supplemented with varying amounts of L-tryptophan.

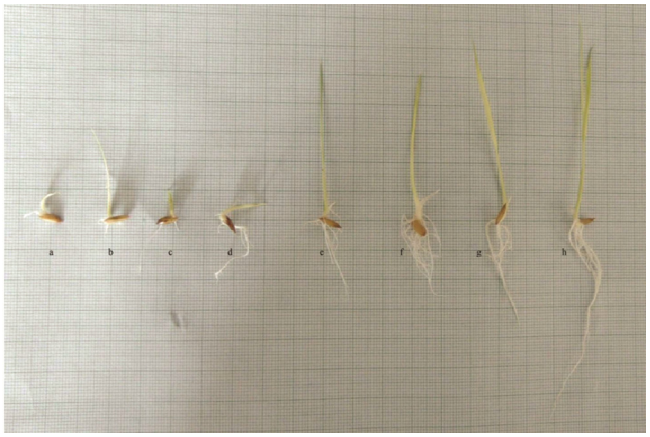

Figure 4. Effect of different concentrations of cyanobacterial extract supplemented with 5 mg/ml of tryptophan. a, sterile water; b, BG-11 medium; c, 20 µl extract; d, 30 µl extract; e, 40 µl extract; f, 50 µl extract; g, 60 µl extract and h, 70 µl extract.

Effect of cell-free filtrates of culture supplemented with 5 mg/ml of tryptophan on germination, number of lateral roots, and radical and plumule length of rice was studied. Maximum eight lateral roots were formed in 70 µl of extract treatment and 90% germination was observed in all the treated extracts, except in 20 µl of extract treatment where it was 100% (Table 1). The length of coleoptiles and radical was also induced in treated extract, i.e. coleoptile was 7.1 cm long in 70 µl of extract treatment in comparison to 1 and 3 cm in sterile distilled water and BG-11 medium respectively (Table 1). Radicle was also induced in treated extract, i.e. radical was 7.5 cm long in 70 µl of extract treatment in comparison to 0.8 cm in sterile distilled water and BG-11 medium treatments (Table 1).

F. muscicola NDUPC001 produced tryptophan-dependent IAA and maximum amount of IAA (286.82 µg/ml) was produced on the 19th day in culture medium supplemented with 5 mg/ml L-tryptophan. Evidence of IAA was proved by TLC analysis, as a spot of the extract had the same Rf value as standard IAA. Cyanobacteria produce both tryptophan-dependent as well as tryptophan-independent IAA[7,6]. Amount and duration of IAA production are mainly dependent on the concentration of tryptophan in the medium[6]. Similar trends of IAA production have been reported in several bacteria, e.g. *Azotobacter* sp., *Pseudomonas* sp., *Rhizobium* sp., etc.[20,21].

Tryptophan is a well-known precursor for auxin biosynthesis in plants and microorganisms[22] and L-tryptophan supplemented in the culture medium is utilized by the bacteria and cyanobacteria for IAA biosynthesis[6]. Findings of this experiment also suggest that *F. muscicola* NDUPC001 is continuously using tryptophan for IAA biosynthesis.

Bioassay of the cyanobacterial extract on germination, length of radical and plumule and number of adventitious roots of rice showed that germination was slightly induced in 20 µl of extract treatment, whereas length of coleoptile, radical and number of lateral roots were induced several times in comparison to control in 70 µl of extract treatment. Several features of cyanobacteria, i.e. secretion of growth regulators, nitrogen fixation, ammonium secretion, polysaccharide production, phosphate solubilization, etc. prove that it is a suitable biofertilizer. A number of cyanobacteria have been reported to produce IAA, e.g. *Anabaena* sp. CW1 (maximum production of 11.43 µg/ml)[7], *Anabaena* sp. RP9 (maximum production of 11.43 µg/ml)[7], *Cylindrospermum stagnale* (maximum

production of 95.6 μg/ml)[23], *Lyptolingbya* sp. (maximum production of 51.06 μg/ml)[8], *Gietlerinema* sp. (maximum production of 67.87 μg/ml)[8], *Oscillatoria* sp. TCC4 (maximum production of 10.65 μg/ml)[24] and *Arthrospira platensis* strain MMG-9 (maximum production of 113 μg/ml)[6]. The production of IAA by *F. muscicola* is highest (maximum production of 286.82 μg/ml) among the bacteria and cyanobacteria reported so far. Hence, the extract of this strain promotes growth of rice seedlings several times in comparison to control. This cyanobacterium can be a good biofertilizer and the extract can be used instead of synthetic agents for organogenesis induction in tissue culture.

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Pedotransfer functions for predicting soil hydraulic properties in semi-arid regions of Karnataka Plateau, India

S. Dharumarajan[1,*], Rajendra Hegde[1], M. Lalitha[1], B. Kalaiselvi[1] and S. K. Singh[2]

[1]ICAR-National Bureau of Soil Survey and Land Use Planning, Regional Centre, Hebbal, Bengaluru 560 024, India
[2]ICAR-National Bureau of Soil Survey and Land Use Planning, Amaravati Road, Nagpur 440 033, India

Soil hydraulic properties are important for irrigation scheduling and proper land-use planning. Field capacity, permanent wilting point and infiltration rate are the three vital hydraulic properties which determine the availability and retention of water for crop growth. These properties are difficult to measure and time-consuming, but can be easily predicted from the available information like soil texture, bulk density,

*For correspondence. (e-mail: sdharmag@gmail.com)