DOI: 10.21276/sajb.2016.4.9.8

Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2016; 4(9):738-744 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Original Research Article

Screening and optimization of indole 3 acetic acid producing non-heterocystous cyanobacteria isolated from saline soil

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Abstract: Cyanobacteria are diverse group of oxygenic photosynthetic prokaryotes. Indole 3 acetic acid is one of the major phytohormones for plant growth. To explore the Indole 3 Acetic Acid (IAA) production by cyanobacteria from saline soil 11 non-heterocystous cyanobacteria isolated from saline soil of Baramati region of Maharashtra. These isolates were evaluated for production of Indole-3-acetic acid in the presence of L-tryptophan. Out of 11 isolates, 9 isolates were able to produce IAA. Productivity of IAA was detected spectrophotometrically. IAA production of isolated cyanobacteria was evaluated in different pH, light photoperiods and various concentration of L-tryptophan, salt and nutrients. During 3 week incubation period TCC-1, TCC-4 and TCC-5 were producing higher amount of IAA (10.65µg/ml). TCC-1 and TCC-4 were found to produce higher amount (10.65µg/ml) of IAA in presence of 500,750 & 1000µg/ml of L-tryptophan concentrations. At pH 8, TCC-1 isolate was producing high amount (9.97µg/ml) of IAA. In the presence of light (24:00, L: D) conditions TCC-4 produces higher amount (9.42µg/ml) of IAA. In the presence of 200 mM and 400mM concentration of NaCl TCC-1, TCC-3 and TCC-4 produces IAA in higher amount respectively. Isolate TCC-5 was produces higher amount of IAA in presence and in absence of $NaNO_3$. TCC-1, TCC-4 and TCC-5 cyanobacterial isolates were producing higher amount of IAA in optimized conditions.
Keywords: Oxygenic photosynthetic prokaryotes, Non-heterocystous cyanobacteria, Indole-3-acetic acid (IAA), L-tryptophan, saline soil.

INTRODUCTION

There are five major classes of plant growth promoting substances i.e. Auxin, Gibberellic acid, Cytokinins, Ethylene and abscisic acid [1]. Whereas auxins are first and major class of hormones that are recognized mainly for root initiation and also for cell enlargement and bud formation. Auxins, especially Indole-3-acetic acid are commonly found in plants and produced by many organisms. Auxins, 1-Naphthalene acetic acid (NAA), Indole-3-Burtyric acid, Phenyl acetic acid and 4-Chloro-Indole acetic acid are also synthesized by various microorganisms and they play an important role in plant growth promotions[2, 3].

Plant growth-promoting organisms can play important role in improving plant growth viz. phytohormone production, phosphate solubilization, siderophore production, biological nitrogen fixation. These growth promoting substances are used instead of chemical fertilizers, pesticides and other supplement [4]. Microbes are used for synthesis of indole-3-acetic acid by using L-tryptophan as a precursor. The plant gives well response to the concentrations of indole-3-acetic acid produced by microbes. Production of Indole-3-acetic acid (IAA) is well-studied and reported in diverse bacterial and cyanobacterial species [5].

Cyanobacteria are considered to be potential source of secondary metabolites. They are used in mariculture, food, feed, fuel, fertilizer, medicine and cosmeceuticals industry [6, 7, 8]. Cyanobacteria are mainly known for their nitrogen fixing ability [9, 10]. Many nitrogen fixing cyanobacteria are used as biofertilizer in agriculture, where as cyanobacteria contribute 20-25 kg N/ha/season and also improve fertility of soil [11, 12].

Sergeeva *et. al.*, [13] reported that the free living and symbiotic cyanobacteria *Nostoc*, *Chlorogloeopsis*, *Calothrix*, *Plectonema*, *Gloeothece*, *Anabaena*, *Cylindrospermum*, and *Anabaenopsis* have an ability to synthesize indole-3-acetic acid. Whereas non heterocystous filamentous cyanobacteria *Oscillatoria*, *Phormidium*, *Lymnothrix*, *Pseudoanabaena* and unicellular cyanobacteria *Chroococcidiopsis*, *Synechocystis*; produced indole-3-acetic acid and enhance the plant growth [14, 15, 16].

Therefore, the present work is focused on screening of non heterocystous cyanobacteria for the production of IAA and optimizes the different environmental conditions for their production. In this study, we have taken previously isolated and characterized (pH and salt tolerance) cyanobacterial isolates from saline soil. The IAA producing cyanobacterial isolates are optimized for their production.

MATERIALS AND METHODS

Cultures and growth conditions

Previously isolated set of axenic cyanobacterial cultures were used for the present study [17]. Cultures were maintained in 150ml flask containing 100ml sterile BG-11 medium. The flasks were incubated at 24± 2ºC at 1800-2000 lux light.

Screening of Indole-3-aceitc acid production

The homogenized cell suspension of each cyanobacterial isolates (5ml) having optical density 0.5 at 600 nm was used for inoculation. A set of cyanobacterial cultures was grown in BG-11 medium supplemented with L-tryptophan (500µg/ml). The Isolates were incubated at 24±2ºC at 1800-2000 lux light for 21 days. After incubation the culture was centrifuged at 10,000rpm for 10min at 4°C. In 1ml supernatant 2ml Salkowaski reagent (2ml of 0.05M $FeCl_3$ mixed in 100ml of 35% Perchloric acid) was added. The mixture was incubated for 30 min in the dark at room temperature. Development of red/pink color indicates the presence of Indole 3-acetic acid (IAA), which was determined spectrophotometrically at 535nm against a control of 1ml culture medium and 2ml Salkowaski reagent. A standard curve drawn from known concentration of IAA was used to quantify IAA present in the culture supernatant [18].

Effect of different environmental parameters on production of IAA

Incubation time

IAA was estimated during different growth stages of cyanobacterial isolates. In order to achieve this cyanobacterial isolates inoculated in flasks containing 100ml of medium supplemented with L-tryptophan with a control. Cyanobacterial isolates were harvested after every week and were used for determination of IAA.

L-tryptophan

In this experiment cyanobacterial isolates were inoculated with different concentration of L-tryptophan in culture medium. The experiment was done using varying initial concentration of L-tryptophan in µg/ml (250, 500, 750, 1000 and 1250).

pH

Effect of pH on IAA production was studied by inoculating cyanobacterial isolates for three weeks in 100ml sterilized BG-11 medium supplemented with 500µg/ml L-tryptophan at various pH (5, 6, 7, 8 and 9).

Light

The effect of light (light: dark = 16:08, 08:16, 24:00, 00:24) on the production of the IAA was studied by incubating cyanobacterial isolates for 3 weeks in culture medium supplemented with 500µg/ml L-tryptophan.

Salt

To study the effect of salt (NaCl) concentration (200mM and 400mM) on the production of the IAA was studied by incubating cyanobacterial isolates for 3 weeks in culture medium supplemented with 500µg/ml L-tryptophan.

Sodium Nitrate ($NaNo_3$)

The effect of Sodium nitrate (Presence/Absence) on the production of the IAA was studied by incubating cyanobacterial isolates for 3 weeks in culture medium supplemented with 500µg/ml L-tryptophan.

RESULTS

Indole-3-aceitc acid production by cyanobacteria

The eleven non heterocystous cyanobacterial isolates were selected and their IAA production (µg/ml) details are given in Table 1.

Table 1: Set of cyanobacterial cultures used for screening of indole-3-acetic acid production

Sr. No.	Isolates	Filamentous	Taxonomy	IAA concentration (µg /ml)
1	TCC- 1	Filamentous	*Oscillatoria spp.*	10.65
2	TCC- 2	Filamentous	*Oscillatoria spp.*	8.09
3	TCC- 3	Filamentous	*Oscillatoria spp.*	6.26
4	TCC- 4	Filamentous	*Oscillatoria spp.*	10.65
5	TCC- 5	Filamentous	*Oscillatoria spp.*	10.65
6	TCC- 6	Filamentous	*Oscillatoria spp.*	0
7	TCC- 7	Filamentous	*Oscillatoria spp.*	0
8	TCC- 8	Filamentous	*Phormidium spp.*	4.35
9	TCC- 9	Filamentous	*Pseudanabaena spp.*	4.77
10	TCC- 10	Filamentous	*Limnothrix spp.*	4.35
11	TCC- 11	Filamentous	*Lyngbya spp.*	6.1

Out of eleven isolates, nine isolates synthesized IAA in the presence of L-tryptophan as precursor. Isolate TCC-1, TCC-4 and TCC-5 synthesized high amount of IAA. Three among the 9 isolates (TCC-1, TCC-4 and TCC-5) were found to be possibly efficient cultures for biotechnological applications in saline soil. These three isolates were optimized for IAA production in different environmental conditions.

Effect of different environmental parameters on production of IAA

Effect of incubation time

IAA was estimated during different growth stages of cyanobacterial isolates. In order to achieve this, cyanobacterial isolates inoculated in flasks containing 100 ml of medium supplemented with L-tryptophan with a control. Every week, cyanobacterial isolates were harvested for the production of IAA. Isolates, TCC-1, TCC-4 and TCC-5 were able to produce maximum amount of IAA during three weeks of incubation in sterile BG-11 medium (Figure 1).

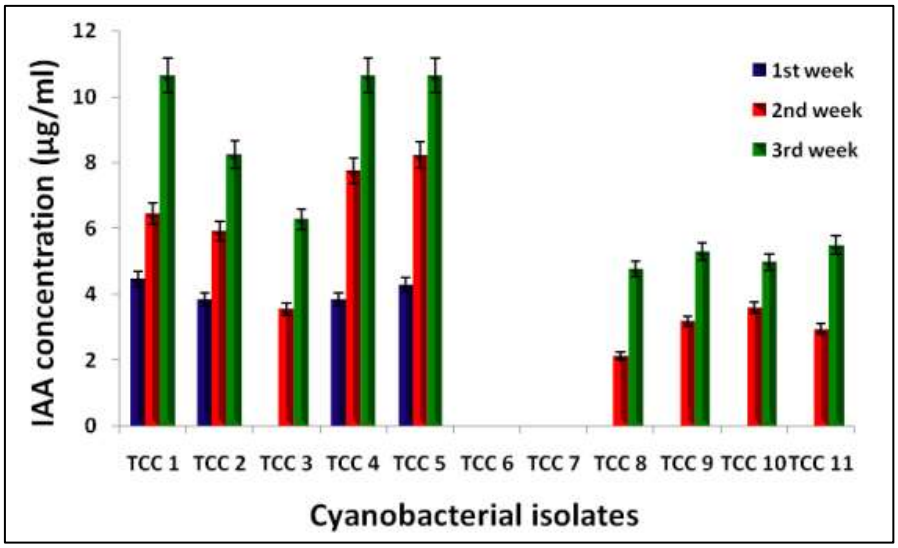

Fig-1: Effect of incubation time on production of IAA

Effect of L-Tryptophan concentration

In this experiment cyanobacterial isolates were inoculated with different L-tryptophan concentration in a BG-11 medium. The experiment was done using varying initial concentration of L-tryptophan (250, 500, 750, 1000 and 1250µg/ml). In presence of 500, 750, 1000µg/ml of L-tryptophan concentration, TCC-1 and TCC-4 isolate synthesize high amount of IAA (Figure 2).

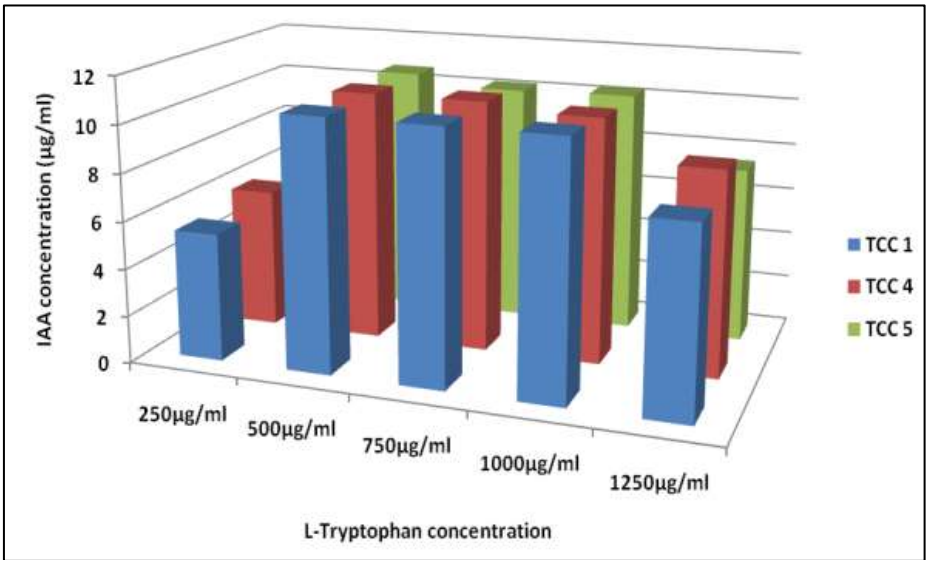

Fig-2: Effect of L-Tryptophan concentration on production of IAA

Effect of pH

At various pH of the medium; optimum pH for maximum production for TCC-1 and TCC-4 cyanobacterial culture was found to be 8 (Figure 3).

Effect of light (Photoperiod)

TCC-4 and TCC-5 show maximum production of IAA at 24 hrs light periods; however TCC-1 shows maximum IAA production at 8:16 light period (Figure 4).

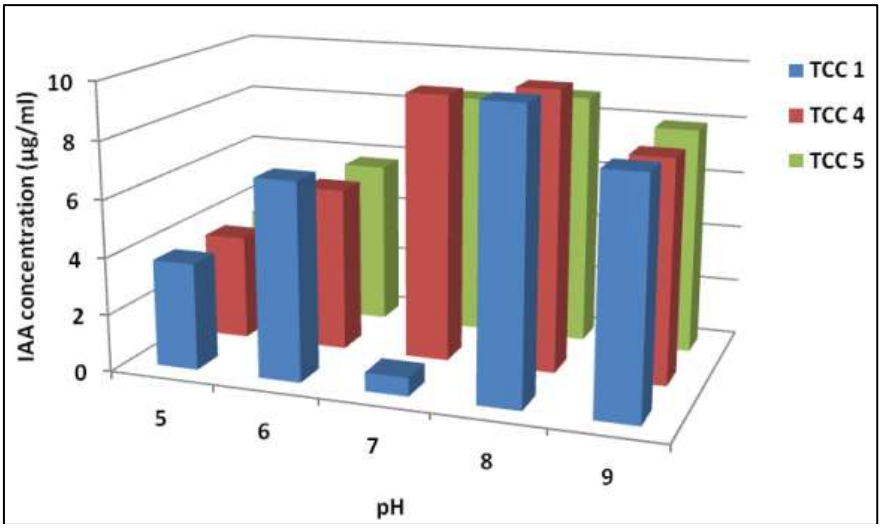

Fig-3: Effect of pH on production of IAA

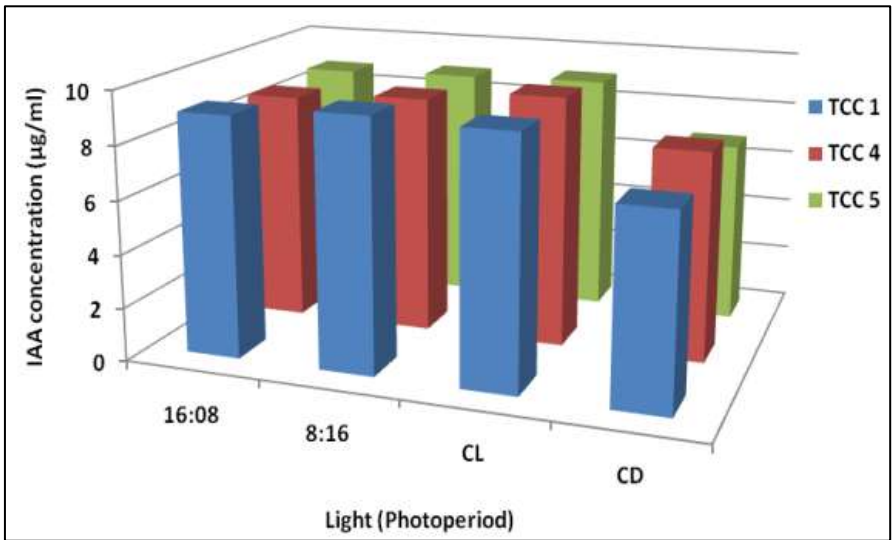

Fig-4: Effect of light on production of IAA

Effect of salt concentration

The effect of salt NaCl concentration on the production if IAA was studied by incubating cyanobacterial isolates for three weeks in BG-11 medium with L-tryptophan containing of 200 mM and 400 mM NaCl concentration (Figure 5). 200mM concentration of NaCl was found to suitable for TCC-1 and TCC-4 isolates.

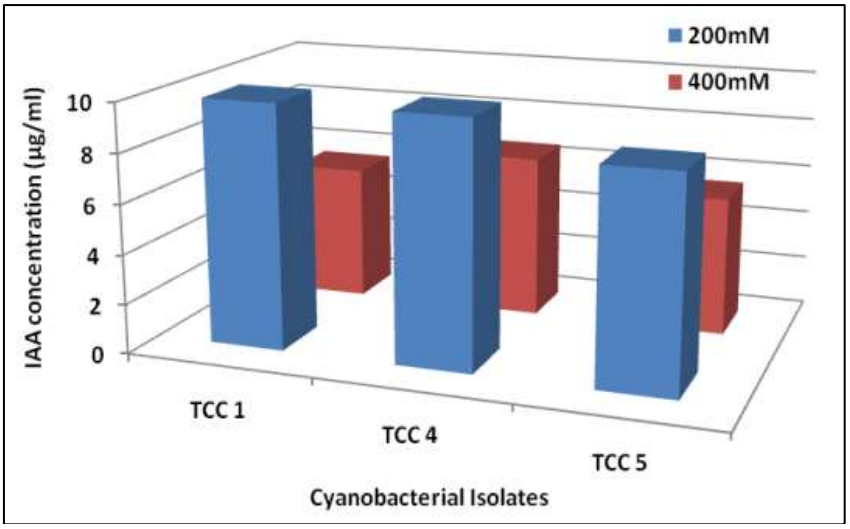

Fig-5: Effect of salt concentration on production of IAA

Effect of $NaNO_3$

The effect of nutrient concentration on the production of IAA was studied by incubating cyanobacterial isolates for three weeks in BG-11 medium with L-tryptophan in presence or absence of $NaNO_3$. TCC-5 produced maximum amount of IAA in presence and in absence of $NaNO_3$ as compared to TCC-1 and TCC-4 (Figure 6).

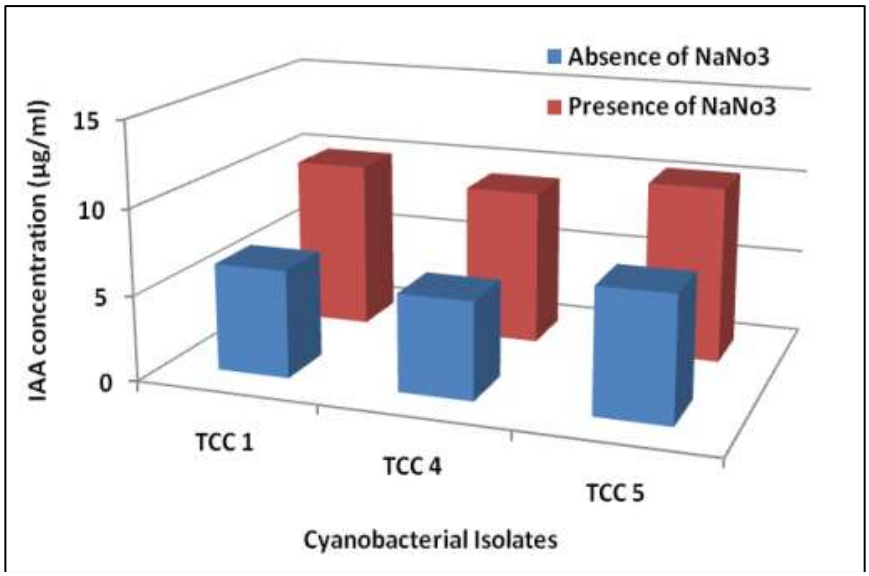

Fig-6: Effect of sodium nitrate concentration on production of IAA

DISCUSSION

A set of eleven non-heterocystous cyanobacterial isolates was used for the present investigation. A survey of rice field in India showed that non-heterocystous cyanobacterial population was much higher than the population of heterocystous strains [19, 20, 21]. *P. polymyxa* RC05, *P. putida* RC06 and *Bacillus* OSU-142 strains were isolated from the rhizosphere of field- grown crops which able to produced plant growth promoting phytohormones [22]. *Azotobacter* spp and *Pseudomonas* spp are N_2 fixing and phosphate solubilizing, respectively are able to produce IAA in presence of L-tryptophan. Its IAA production are optimizes at pH, temperature, and other concentration of medium ingredients [23]. Damam *et al*., reported that the *Pseudomonas* spp and *Pantoea* spp have ability to produce IAA ranging from 20-60 µg/ml in the presence of tryptophan and 7.7- 21.4 µg/ml without tryptophan [28].

In the present study out of eleven cyanobacterial isolates, nine were able to produce IAA in the presence of L-tryptophan. These isolates also belong to Oscillatoriaceae family. Several bacteria increase IAA production by increasing L-tryptophan concentration [24]. Cyanobacteria also synthesize IAA in the presence of L-tryptophan [18]. *Arthrospira platensis* is a filamentous cyanobacterium belongs to the family Oscillatoriacae. It showed L-tryptophan dependant production of IAA. Also Khan *et al*., reported that the addition of L-tryptophan are favored the enhancement in the production of IAA and

concentration of IAA in culture broth was measured using colorimetric assay [29].

All isolates were grown in the sterilized BG-11 medium at pH 7.4. IAA production is highest at pH 8 and lowest at pH 5, whereas *Kitasatospora* spp could survive high pH (9) but can't at low pH (4 and 5) in Free State [25]. After three weeks incubation period maximum amount of IAA production were seen in *Arthrospira platensis* strain MMG-9. It shows gradual increase in with respective time and L-tryptophan concentration provided [18]. Our results show that 24 hrs light conditions appeared to be optimum for TCC-4 and TCC-5; however TCC-1 shows maximum IAA production at 8:16 light period. It was reported that *Anabaena* sp. RP9 shows gradual increase in the IAA production within 21 days at continuous light period when supplemented with various concentration of L-tryptophan [26]. Salt tolerance was observed in between 300-500 mM NaCl in BG-11medium. IAA production was maximum at 200mM NaCl concentration. Biosynthesis of auxin was detectable in *Streptomyces* strain C and IAA production was found to be increased in the presence of salt [27].

CONCLUSION

Isolates from saline soil were able to tolerate high salt concentration ranges 200mM to 500mM. TCC-1, TCC-4 and TCC-5 isolates produces maximum IAA after three weeks of incubation, 500µg/ml L-Tryptophan, pH-8, continuous light period, 200mM NaCl salt concentration were the optimum environmental parameters in presence of sodium nitrate for maximum production of IAA. These cultures may be helpful in agriculture for better yield and might be for reclamation of saline soil or to improve the plant growth in it.

Acknowledgement

Authors are thankful to the Principal, Tuljaram Chaturchand College, Baramati for providing necessary facilities and constant help during the research period and also to Hon'ble Vice-Chancellor, North Maharashtra University, Jalgaon; Director, School of Life Sciences, NMU, Jalgaon. Dr. Sunil T. Pawar is grateful to UGC, New Delhi, for providing financial support [Major Research Project: F.No.39-199/2010 (SR)].

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