International Journal of Engineering Science Invention
ISSN (Online): 2319 – 6734, ISSN (Print): 2319 – 6726
www.ijesi.org ||Volume 6 Issue 11|| November 2017 || PP. 13-18

Optimization of phytostimulatory potential in *Bacillus toyonensis* isolated from tea plant rhizosphere soil of Nilgiri Hills, India

Payel Choudhury[1], Arshad Jawed[2] and PradiptaSaha[1]*
[1]*Department of Microbiology, The University of Burdwan, Golapbag, Burdwan-713104, WB, India*
[2]*College of Nursing and Allied Health Sciences, Jazan University, Jazan, Saudi Arabia, 45142*
** Corresponding Author: Email: psaha@microbio.buruniv.ac.in*

Abstract: *A Gram positive, rod shaped bacterial strain, designated NHPC4, was selected from a pool of bacterial cultures isolated from tea garden rhizospheric soil, collected from Nilgiri hills, India. Using phenotypic characters and 16S rRNA gene based molecular phylogenetic approach, it was identified as Bacillus toyonensis. The strain possessed several plant growth promoting (PGP) traits including production of siderophore, ammonia, solubilization of zinc, fixation of nitrogen and auxin production. Production of Indole-3-acetic acid (IAA) from L-tryptophan by the strain was determined through bioassay and effect of various parameters (precursor concentration, temperature, pH, percentage of sodium chloride, various nitrogen and carbon sources) on its production was investigated. Maximum yield of IAA was recorded at 218 h of growth 37°C and pH 7. Optimised IAA production was recorded on a mineral medium containing L-tryptophan (1%, w/ v), supplemented with maltose, 0.20% and ammonium sulphate, 0.01%. The strain could also produce several hydrolytic enzymes like amylase, caseinase and gelatinase.*

Keywords: *Plant growth promoting rhizobacteria, Indole-3-acetic acid, Bacillus toyonensis*

Date of Submission: 23-10-2017 Date of acceptance: 07-11-2017

I. Introduction

The use of plant growth promoting rhizobacteria (PGPR) or microbial inoculants for sustainable agricultural production is being widely accepted in many parts of the world [1]. The PGPR are rhizosphere dwelling bacteria that have the ability to colonize plant roots and exert a beneficial effect on plant growth [2]. Currently, most agricultural fields are supplemented with chemical fertilizers and toxic agrochemicals such as herbicides, pesticides to enhance and maintain crop productivity. In fact, pesticides have become a primary input in farming for greater crop yields [3]. Henceforth, sources of renewable energy, economic issues and most importantly environmental concerns necessitate the use of biological alternatives [4]. Biosynthesis of IAA is an important criterion for the screening of potential PGPR [5]. IAA plays a predominant role in regulating plant growth and bacterial IAA producers stimulate plant growth by adding to the endogenous pool of host plant IAA [6]. L-tryptophan (L-Trp) is a physiological precursor for biosynthesis of IAA in plants and microbes [7].

In the present work, an attempt was made to explore bacteria having PGP traits from tea plant rhizospheric soil of Nilgiri Hills (NH), India. A strain designated, NHPC4 capable of producing IAA was isolated and characterized. The strain was identified as *Bacillus toyonensis*, based on 16S rRNA gene sequence similarity. Although, plant enhancing effect of *B. toyonensis* has been studied in two plants [8], production and optimization of the phytohormone, IAA has not been reported earlier in *B. toyonensis*. NHPC4 is endowed with other PGP traits and capable of producing hydrolytic enzymes. Henceforth, the strain might have biotechnological potential and can be part of phytostimulant and bio-fertilizer formulation, which might find application for better crop productivity.

II. Materials And Methods

2.1 Isolation and Identification of the strain

Tea plant rhizospheric soil samples were collected from different tea gardens located in Ooty (11.41°N 76.70°E) and Coonoor (11.35°N 76.82°E) hill stations of Tamil Nadu, India. Many bacteria were isolated and among them, a bacterial strain, NHPC4 was characterized phenotypically [9], screened for presence of PGP traits, and hydrolytic enzyme activities. This was followed by quantification and optimization of IAA production in the strain. Analysis based on 16S rRNA gene sequence was carried out at EzTaxon server; www.ezbiocloud.net/eztaxon [10].

2.2 Screening for extracellular hydrolytic enzymes

The strain was screened for the presence of various hydrolytic enzyme activities adopting methods as described in the standard manual [11].

2.3 Screening for plant growth promoting (PGP) attributes

NHPC4 was screened for the presence of several PGP traits. The ability to solubilise phosphate was checked on Pikovskaya's agar (Himedia) medium. Production of siderophore was detected using Chrome azurol S agar medium [12]. Peptone broth was used for checking ammonia production [13]. The method of Lorck (1948) was adapted for detecting the production of hydrogen cyanide. Nitrogen-fixing ability was assessed using nitrogen-free Ashby's agar medium, and growth after repeated transfer (5 to 7) was recorded as presumptively positive for fixation of nitrogen. IAA production by the strain, in the absence and presence of the precursor, L-Trp (1%) was determined by following the Salkowski's method [15]. Following growth, the bacterial culture was centrifuged at 10,000 x g for 10 minutes. 1 mL of the cell-free culture supernatant was mixed with 2 mL of Salkowski's reagent (0.5 M $FeCl_3$, 2 mL; 35% perchloric acid, 98 mL) and incubated in dark for 30 minutes. The intensity of resultant pinkish colour was recorded at 530 nm by using an UV-Vis spectrophotometer (Cary50, Varian). Quantification of IAA was carried out against a standard curved prepared from authentic IAA (Sigma-Aldrich, India).

2.4 Effect of physicochemical parameters on IAA production

For optimization of IAA production process parameters**,** the factorial design of experiments, the 'one factor at a time' method was adopted. The optimum pH for IAA production was assessed by adjusting the initial pH of the medium to 4, 5, 6, 7 and 8. IAA production was quantified at various temperature ranges (10, 20, 28, 37, 45°C), sodium chloride (NaCl) concentrations [0.5, 1.0, 2.0, 3.0, 4.0, 5.0%, (w/v)] and, shaking vs static condition for optimized period of time.

2.5 Effect of nutritional parameters

The effect of precursor on IAA production was determined by incorporating different concentrations of L-Trp [0.01, 0.03, 0.05, 0.1, 0.5, 1.0% (w/v)] into the production medium. The effect of different organic (beef extract, peptone, tryptone, yeast extract) and inorganic [ammonium chloride (NH_4Cl), ammonium dihydrogen phosphate ($NH_4H_2PO_4$), ammonium bicarbonate (NH_4HCO_3), diammonium hydrogen orthophosphate $(NH_4)_2HPO_4$, ammonium sulphate $(NH_4)SO_4$, ammonium iron (II) sulphatehexahydrate $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$, sodium nitrite ($NaNO_2$)] nitrogen (N) sources on the production of IAA was checked in M9 minimal medium. The amount of the best N supplement was varied [0.001, 0.005, 0.01, 0.05, 0.1, 0.2, 0.25, 0.5%, (w/v)]. Different sugars such as glucose, maltose, mannose, D-ribose, glycerol, mannitol, D-sorbitol, adonitol, xylitol were incorporated in M9 minimal medium to analyze their effect on IAA production. The concentration of the best carbon source was varied [0.01, 0.05, 0.2, 0.5, 1.0, 2.0, 2.5, 5.0% (w/v)]. The M9 minimal medium used for studying the effect of carbon and nitrogen source on IAA production was exogenously supplied with L-Trp (1%).

2.6 Time course assay of IAA production

A time course assay for monitoring IAA production was performed. Starting from the 0^{th} h of incubation till the 224^{th} h, IAA production was monitored at regular time intervals of 3 h. Optimum parameters for IAA production obtained from previous experiments were used for the time course assay.

III. RESULTS

3.1 Characterization and identification of the strain

The cultural, morphological, and biochemical characteristics along with the plant growth promoting traits of the strain, NHPC4 are documented in **Table 1**. With reference to 16S rRNA gene sequence, it showed closest sequence identity with *Bacillus toyonensis* BCT-7112^T (100%) followed by *Bacillus thuringiensis* ATCC 10792^T (99.93%), CM000739-s AH1271 (99.93%) and *Bacillus wiedmannii* FSL W8-0169^T (99.79%). The partial 16S rRNA gene sequence of the strain was deposited in GenBank database; its accession number is KP229423. The strain was deposited in Microbial Culture Collection (MCC), National Centre for Cell Science, Pune, India; (accession number, MCC 3302) for its general availability to scientific community.

3.2 Optimization studies for IAA production

Optimization studies using the strain recorded maximum IAA production at temperature of 37°C and a pH of 7 (**Fig. 5**). There was a linear rise in the IAA production with the increase in L-Trp concentration (**Fig. 1**). Addition of L-Trp to production medium has been reported to enhance IAA production [16]. Higher yield of IAA was recorded under shaking condition in comparison to incubation under static condition (**Fig. 7**). There was a linear rise in the IAA production with the increase in L-Trp concentration (**Fig. 1**). In comparison to the organic N supplements, the inorganic N sources supported higher IAA production (**Fig. 3**). Ammonium sulphate (0.01%) was recorded to be the best N source. Among tested C sources, maltose (0.20%) was best, followed by glycerol and adonitol (**Fig. 2**). The amount of IAA produced was 54.6 μg mL^{-1} and 49.2 μg mL^{-1} using the

optimal concentration of maltose and ammonium sulphate, respectively (**Fig. 4**). No IAA could be detected even at the lowest concentration of NaCl.

3.3 Time course of IAA production

Production of IAA in the medium begun from 48 h (41.32 µg mL^{-1}) and it increased gradually with time. Maximum IAA production was recorded at 218 h (127.56 µg mL^{-1}) (**Fig. 6**). In a previous study [17], IAA production was reported to increase progressively from two to eight days and decreased later with a decrease in the growth of bacteria in the production medium.

3.4 PGP attributes and hydrolytic enzyme properties of the strain

The strain also possessed other PGP traits like siderophore production, ability to fix atmospheric nitrogen, ammonia production, and solubilization of zinc; produced hydrolytic enzymes like amylase, gelatinase, and caseinase.

IV. Discussion

The IAA synthesis by bacteria dwelling in the rhizosphere can act concurrently with the endogenous IAA in plants, thereby, promoting enhanced root system development and uptake of nutrients by plants from the soil [18]. This eventually leads to better plant productivity. IAA synthesized by the PGP microbes is considered more effective by virtue of their continuous slow release [18]. The strain in the present work produced considerable amount of IAA, when compared to reports of IAA production by previous authors [1, 16, 17, 19, 20]. To the best of our knowledge, *B. toyonensis* has never been quantified and optimized for production of IAA in any earlier work report. There lies significant scope of the strain being developed into a bio-inoculant and being explored for the production of the agroactive compound, auxin.

V. Conclusion

A propitious strategy for the replacement and/or supplementing the activity of chemical fertilizers is the use of "go green" technology of using PGP bacteria used individually or part of multi-strain bacterial consortium. These PGP microorganisms are potential candidates to be developed into commercial biofertilizer candidates.

References

[1] A. Majeed, M.A. Abbasi, S. Hameed, A. Imran, N. Rahim, Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion, *Front Microbiol*, 6, 2015, 1-10.

[2] M. Ahemad, M. Kibret, Mechanisms and applications of plant growth promoting rhizobacteria: current perspective, *J King Saud Univ Sci*, 26, 2014, 1–20.

[3] B. Neupane, P. Thapa, Assessment of Pesticide Use and Heavy Metal Analysis of Well Water in JhikuKhola Watershed, Kavrepalanchowk, Nepal . *Int Res J Environ Sci*, 3, 2014, 79-83.

[4] S. Gopalakrishnan, A. Sathya, R. Vijayabharathi, R. K. Varshney, C. L. L. Gowda, L. Krishnamurthy, *3 Biotech*, 5, 2015, 355–377.

[5] M. Khalid, M. Arshad, Z.A. Zahir, Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat, *J Appl Microbiol*, 96, 2004, 473–480.

[6] S. Spaepen, J. Vanderleyden, R. Remans, Indole3-acetic acid in microbial and microorganism-plant signaling, *FEMS Microbiol Rev*, 31, 2007, 425–448.

[7] M. Arshad, W.T. Frankenberger, Plant growth regulating substances in the rhizosphere: microbial production and functions, *Adv Agron*, 62, 1998, 146–151.

[8] B. Rahmoune, A. Morsli, M. Khelifi-Slaoui, L. Khelifi, A. Strueh, A. Erban, J. Kopka, J. Prell, J.T.V. Dongen, Isolation and characterization of three new PGPR and their effects on the growth of *Arabidopsis* and *Datura* plants, *J Plant Interact*, 12, 2017, 1-6.

[9] R.M. Smibert, N.R. Krieg, Phenotypic characterization, in P.Gerhardt, R.G.Murray, W.A.Wood, N.R.Krieg (Eds), *Methods for General and Molecular Bacteriology* (American Society for Microbiology, Washington DC, 1994) 607-654.

[10] J. Chun et al, EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol*. 57, 2007, 2259–61.

[11] K.R. Aneja, Experiments in Microbiology, Plant pathology and biotechnology *(*New Age Int P Ltd, New Delhi, 2003, 4th ed).

[12] B. Schwyn, J.B. Neilands, Universal chemical assay for the detection and determination of siderophores, *Anal Biochem*, 160, 1987, 47–56.

[13] P.J. Thokal, B.L. Shelar, S.H. Shaikh, N.N. Adhapure, Microbial optimized production of indole acetic acid and assessment of other plant growth promoting activities, *Int J Sci Nat*, 4, 2013, 627–32.

[14] H. Lorck, Production of hydrocyanic acid by bacteria, *Physiol Plant*, 1, 1948, 142–146.

[15] J.G. Holt, N.R. Krieg, P.A.P. Sneath, Bergy's Manual of Determinative Bacteriology (Williams and Wilkins Pub, Baltimore, 1994, 9th ed).

[16] F. Ahmad, I. Ahmad, M.S. Khan, Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan, *Turk J Biol*, 29, 2005, 29–34.

[17] M. Swain, S. Naskar, R. Ray, Indole-3-acetic acid production and effect on sprouting of yam (*Dioscorea rotundata* L.) minisets by *Bacillus subtilis* isolated from culturable cow dung microflora, *Po. J Microbiol*, 56, 2007, 103–110.

[18] G. Gupta, S.S. Parihar, N.K. Ahirwar, S.K. Snehi, V. Singh, Plant Growth Promoting Rhizobacteria (PGPR): Current and Future Prospects for Development of Sustainable Agriculture, *J Microb Biochem Technol*, 7, 2015, 96-102.

[19] S. Poonguzhali, M. Madhaiyan, T. Sa, Isolation and identification of phosphate solubilizing bacteria from chinese cabbage and their effect on growth and phosphorus utilization of plants, J Microbiol Biotechnol, 18, 2008, 773–777.

[20] J. Dutta, P.J. Handique, D. Thakur, Assessment of culturable tea rhizobacteria isolated from tea estates of Assam, India for growth promotion in commercial tea cultivars, *Front Microbiol*, 6, 2015, 1-13.

Table 1. Phenotypic **c**haracters of Bacillus toyonensis strain NHPC4
Abbreviations used: R, rod shape; +, positive reaction; ++, strong positive; –, negative reaction; +/-, acid production from carbohydrate/no gas production from carbohydrate; -/-, no acid/no gas production

Cultural and morphological characteristics	
Colony morphology	Irregular, curled, rough, opaque, turbid white, umbonate elevation
Vegetative cells	R
Gram reaction	+
KOH reaction	-
Endospores	+
Motility	+
Colony colour	-
Aerobic growth	+
Temperature optimum	10–45°C (37°C)
pH optimum	5.5–10 (7.0)
Biochemical characteristics	
Catalase test	+
Oxidase test	+
Indole production	-
Methyl red test	-
Voges Proskauer test	+
oxidative / fermentative /negative	Fermentative
Nitrate reduction	+
Growth on MacConkey agar medium	-
Growth on blood agar	-
Starch hydrolysis	+
Cellulose ,,	-
Casein ,,	+
Gelatin ,,	+
Pectin ,,	-
Chitin ,,	-
DNA ,,	-
Acid and gas production from carbohydrates	
Mannitol	-/-
Glucose	+/-
Sucrose	+/-
Plant growth promoting traits	
Siderophore production	++
Ammonia production	+
Presumptive nitrogen fixation	+
Solubilization of phosphate	-
Hydrogen cyanide production	-

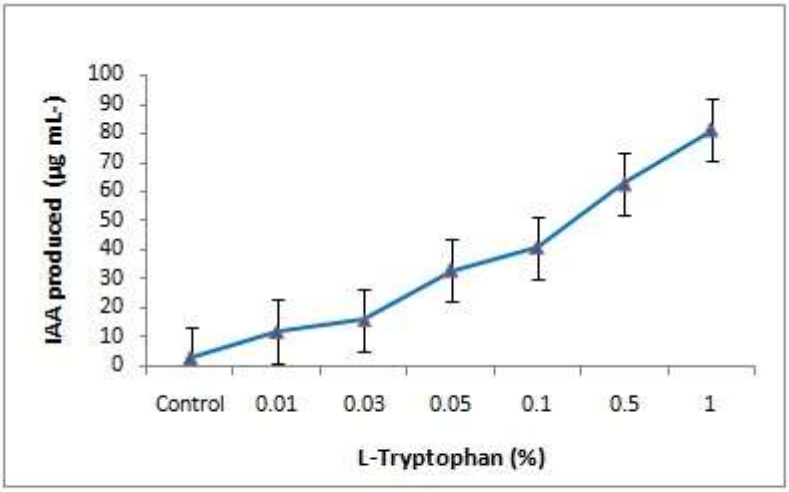

Fig. 1 Effect of L-Tryptophan concentration

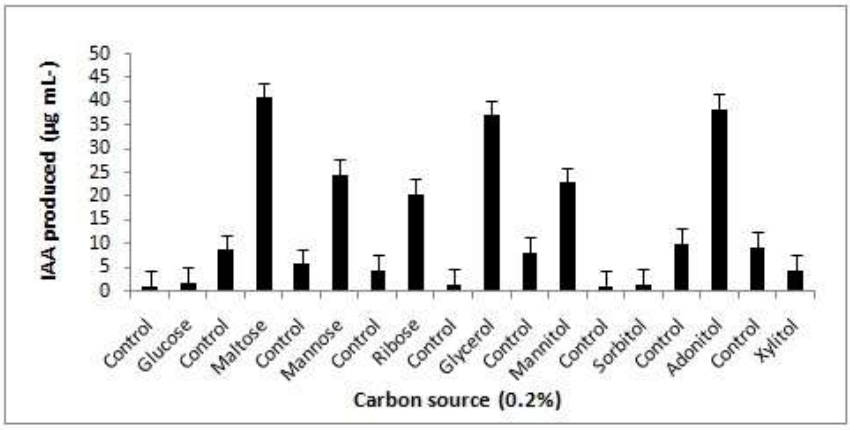

Fig. 2 Effect of various carbon sources

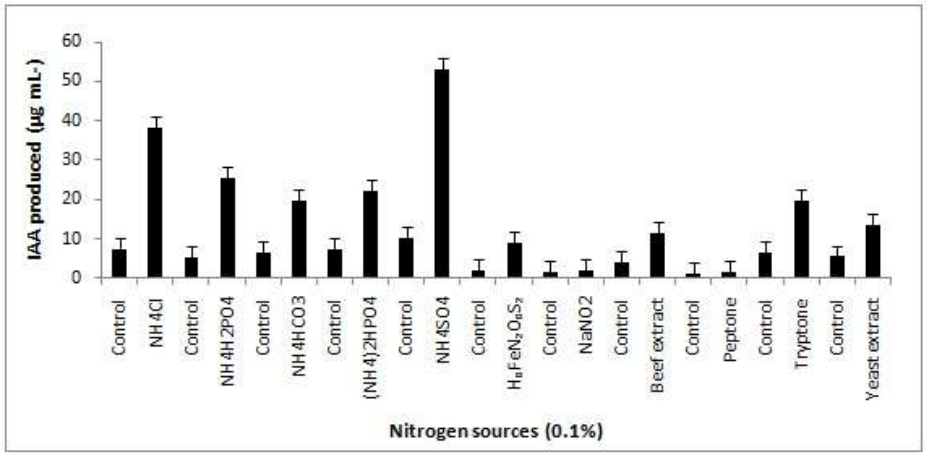

Fig. 3 Effect of various nitrogen sources

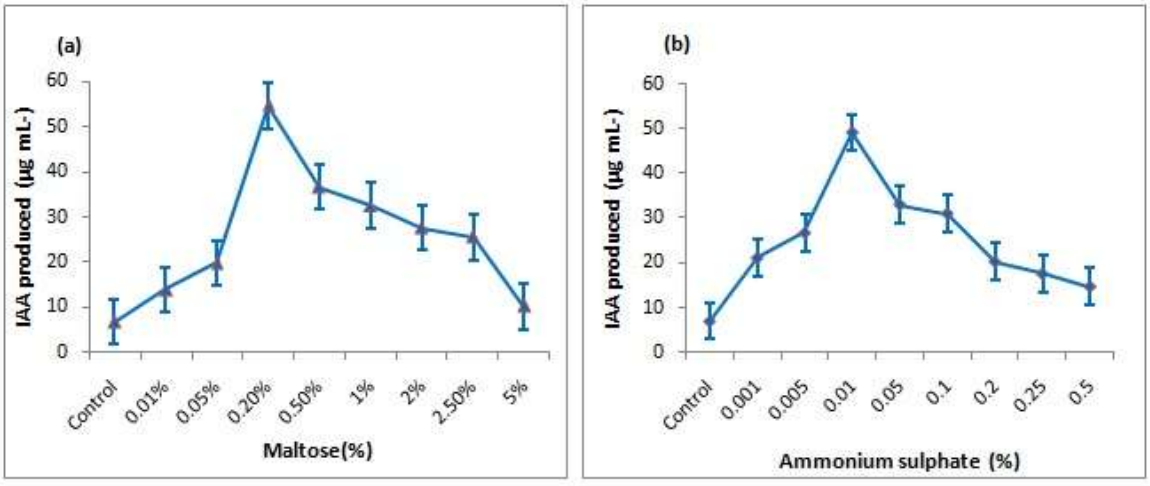

Fig. 4 (a) Effect of maltose; **(b)** Effect of ammonium sulphate

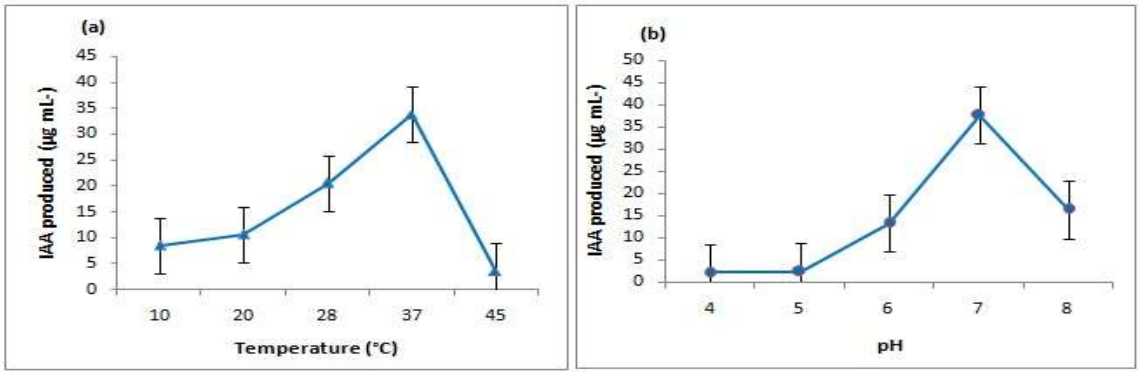

Fig. 5 (a) Effect of temperature; **(b)** Effect of pH

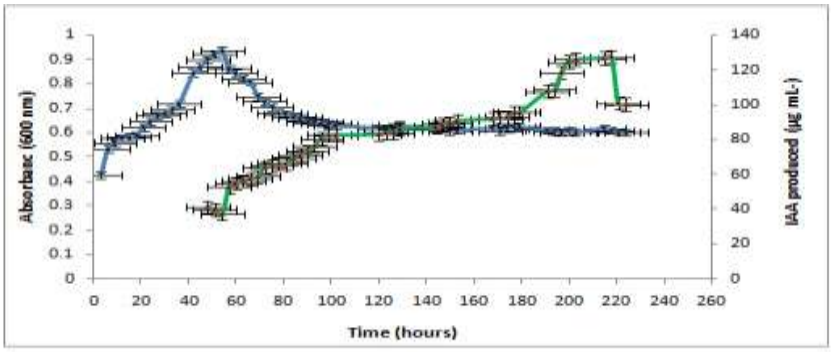

Fig. 6 Time course of IAA production

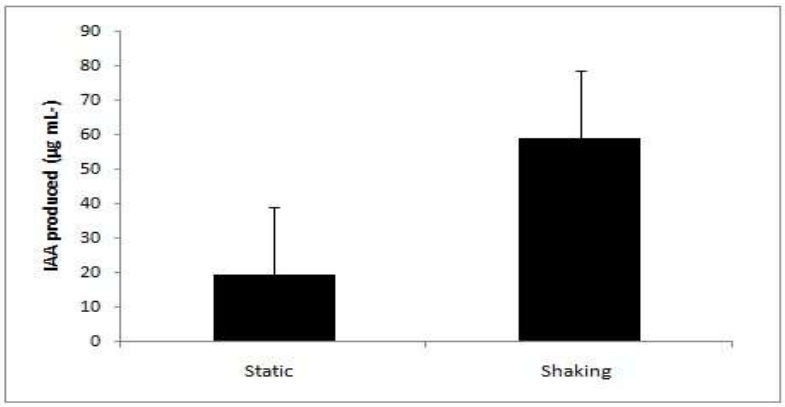

Fig. 7 Effect of shaking vs static condition

International Journal of Engineering Science Invention (IJESI) is UGC approved Journal with Sl. No. 3822, Journal no. 43302.

Payel Choudhury Optimization of phytostimulatory potential in Bacillus toyonensis isolated from tea plant rhizosphere soil of Nilgiri Hills, India." International Journal of Engineering Science Invention(IJESI), vol. 6, no. 11, 2017, pp. 13-18.