

# Indole -3-Acetic Acid Production by the Bacterial Strains Isolated from Vermicomposts in the Presence and Absence of Tryptophan

B.Sivasankari\*

Department of Biology, Gandhigram Rural Institute- Deemed University, Gandhigram, Dindigul (Dt), Tamilnadu,  
India

**ABSTRACT:** Vermicompost was prepared from leaf materials such as *Gliricidia sepium* Jacq, *Leucaena leucocephala* (Lam.) De Wit and *Cassia auriculata* Linn and earthworms, *Eudrilus eugeniae* (Kinberg) and *Eisenia fetida* (Savigny) along with cowdung in six Treatments. Fortnightly analyses (0, 15, 30, 45 and 60 d) of the colony forming units (CFU) of bacteria in vermicomposts, vermicasts and earthworm guts (fore, mid and hind) showed that vermicompost prepared using *C.auriculata* + *L.leucocephala* + Cowdung mixture with *E.eugeniae* is the best of all six treatments. Nine bacterial strains which have the capability to produce Indole-3-acetic acid (IAA) were screened they were *Streptococcus* spp. (18.78 µg/ml), *Micrococcus* spp. (5.99 µg/ml), *Klebsiella* spp. (5.69 µg/ml), *Bacillus* spp. (18.99 µg/ml), *Enterobacter* spp. 6.10 µg/ml), *Escherichia* spp. 6.38 µg/ml), *Alcaligenes* spp. (10.75 µg/ml), *Erwinia* spp. (15.77 µg/ml), and *Pseudomonas* spp. (18.67 µg/ml). The quantities of IAA produced by all these nine bacterial strains were estimated using spectrophotometer. Among all the nine bacterial strains tested one strain (i.e) *Bacillus* spp. Showed significantly higher quantity of IAA production. Hence the bacterial strain was taken for further molecular analysis and identified as *Bacillus cereus*. Attempt has also been made to standardize the production of IAA by *B.cereus* in different tryptophan concentrations (1, 2, 3, 4 and 5mg/ml), at different pH (5, 7 and 9), at different temperatures (28 37 and 45<sup>0</sup> C) and at different day (1 to 7 day) intervals. The results showed that the tryptophan concentration at which IAA production was higher was in the order of 5>4>3>2>1 mg/ml at pH 7 followed by 5 and 9. The suitable temperature was 37<sup>0</sup>C followed by 28 and 45<sup>0</sup>C. The number of days required for higher rate of production of IAA was in the order of 7>6>5>4>2>1 compared to others.

**KEYWORDS:** Vermicompost, *Bacillus cereus*, Tryptophan, Indole-3-acetic acid

## I. INTRODUCTION

Sustainability of agricultural systems has become an important issue all over the world. Many of the issues of sustainability are related to soil quality and its change with time. It is well known that intensive cultivation has led to a rapid decline in organic matter and nutrient levels besides affecting soil physical properties. Conversely, management practices with organic materials influence agricultural sustainability by improving physical, chemical and biological properties of soils (Karlen *et al.*, 1997) [1]. The importance of organically grown products is well known. Under the present conditions of environmental degradation, loss of soil fertility and declining crop yields, use of organic manures to meet the nutrient requirement of crop would be an inevitable practice for sustainable agriculture. In recent day's vermicompost as organic manure is being popularized. It has gained potential importance because it is believed to produce nutritious, tasty and toxic residue free product. In view of increasing reports on the adverse effect of long term use of chemical fertilizers on soil fertility, the immediate need is to make proper use of available unutilized organic wastes into vermicompost within a minimum time span. Earthworms are aptly called 'Cinderella of organic farming', due to their vital role in sustainable organic farming. Earthworms help to solve some of the problems facing civilization today in rebuilding the soil and restoring the earth to immediate usable form for safe healthy food production and clean

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 5, May 2016

environment. Vermicomposting of biowastes not only minimizes the pollution effects, but also helps to get safe, healthier and higher yields, at a relatively low-cost without deterioration of the soil health. The earthworms are considered as 'ecosystem-engineers' which can be efficiently employed as nature's unpaid labour force not only in the compost manufacturing or in the 'decomposer-industry' but also in situ development by selected species of earthworms in cultivated lands, which are not ploughed (Sharma and Agarwal, 2004)[2].

Vermicompost contains plant-growth regulating materials, such as humic acids (Masciandaro *et al.*, 1997[3]) auxins, gibberellins and cytokinins (Krishnamoorthy and Vajranabhaiah, 1986[4]). These plant growth-regulating materials are produced by the action of microbes like fungi, bacteria and actinomycetes (Edwards, 1998[5]) and earthworms. Many free living nitrogen fixing bacteria are good producers of growth regulating substances. Microorganisms mineralize complex substances, releasing into soil available nutrients for plants and on the other hand, synthesize as a consequence of their secondary metabolism, a whole series of substances, many of which are biologically active. (Marinissen and Dexter, 1990[6]). There have been many studies on the production of plant growth-regulating substances by mixed microbial populations in soil, but relatively few investigations into their availability to plants, persistence and fate in soils or effects on plant growth (Arshad and Frankenberger, 1991[7]) have been observed. Additionally, there are a number of references in the literature that show that plant growth regulators, such as indole-3-acetic acids (auxins), gibberellins and cytokinins are produced by microorganisms and there have been suggestions that the promotion of microbial activity in organic matter by earthworms would result in the production of significant quantities of plant growth regulators (Edwards, 1998[5]). Earthworm activity accelerates the humification of organic matter, and their influence in increasing microbial populations enhances the presence of auxins and gibberellins-like substances as well as humic acids. Moreover, humic acids have also been shown to stimulate plant growth in auxin, gibberellin and cytokinin bioassays. The widely studied *Bacillus* genus represents one of the most diverse genera in the *Bacilli* group. Numerous *Bacillus* and *Paenibacillus* strains express plant growth promoting (PGP) activities and a number of these strains have already been commercially developed as biological fungicides, insecticides, and nematicides or generic plant growth promoters. The use of these strains in agriculture has recently been reviewed (Gardener, 2004[8]). Those strains that besides having several PGP properties can also fix nitrogen and also do phosphate solubilization. IAA is a common product of L-tryptophan metabolism by several microorganisms (Lynch, 1985[9]). Microorganisms inhabiting rhizosphere of various plants are likely to synthesize and release auxin as secondary metabolite because of the rich supplies of substrates exuded from the roots compared with non-rhizospheric soils.

## II. METHODS

### 1. Vermicompost preparation

In the present study epigeic earthworms, *Eudrilus eugeniae* (Kinberg) and *Eisenia fetida* (Savigny) were collected from the breeding stock of the Department of Biology, Gandhigram Rural Institute-Deemed University, Gandhigram, Tamilnadu, India and leaf materials of *Gliricidia sepium* Jacq, *Leucaena lucocephala* (Lam.) De Wit and *Cassia auriculata* Linn were collected from Gandhigram campus. The leaf materials were separately subjected to predigestion for 15 days by sprinkling water on the heap and covering it with gunny bag and turning it periodically in order to release out the initial heat produced during decomposition of organic material. The changes in temperature was observed every three days. The vermibeds were prepared in plastic containers of 45x35x15 cm size and the substrate was moistened to hold 60-80 percent moisture and kept for 24 hours stabilization. 20 numbers of healthy clitellate *E. eugeniae* and 30 numbers of *E. fetida* were separately introduced in the vermibeds. The vermicomposting trials were carried out in the rearing room with the relative humidity and the temperature of 75-85 percent and 26-28<sup>0</sup> C respectively. The substrate was turned (mixed) once in a week and maintained up to 60 days. The experiment was carried out with three replicates for each substrate with proper control. (Daniel and Karmegam, 2000[10]).

### 2. Microbial study

The total microbial counts in terms of colony forming units (CFU) of bacteria in the vermicomposts, vermicasts and earthworm (fore, mid and hind) guts were determined every 15 days (0, 15, 30, 45 and 60 d) using standard plate count method, Nagarathinam *et al.*, 2000[11]). From the total colony forming units, only those bacterial

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 5, May 2016

colonies (19 bacterial) which showed predominant growth were restreaked on to appropriate agar medium to obtain pure cultures and subjected to characterization and identification.

### 3. Screening of bacterial strains for production of plant growth regulator (IAA)

All the nineteen bacterial strains were screened for the production of Indole-3-acetic acid (IAA). The strains were separately inoculated in the nutrient broth medium with tryptophan (1 mg/ml) and incubated at  $28\pm 2^{\circ}\text{C}$  for one week. After a week the cultures were centrifuged at 3000 rpm for 30 min. Two milliliters of the supernatant was mixed with 2 drops of orthophosphoric acid and 4ml of Salkowski reagent (50 ml of 35 percent perchloric acid + 1 ml of 0.5M  $\text{FeCl}_3$ ). Development of a pink colour indicates IAA production. OD was read at 530 nm using spectronic 20D<sup>+</sup>. The level of IAA produced was estimated by a standard IAA graph (Loper and Schroth, 1986[12]). Among the nineteen colonies tested, only nine colonies were able to produce IAA. From these nine colonies only one colony produced a higher rate of IAA and that is *Bacillus* spp. This organism (*Bacillus* spp.) was selected for further molecular and plant growth studies.

### 4. Molecular Studies of *Bacillus* spp. and *Pseudomonas* spp.

The selected bacterial strain i.e. *Bacillus* spp. which showed greater rate of PGR producing activity was subjected to the following molecular studies. DNA Extraction, 16S rDNA sequencing, Nucleotide sequence accession, Phylogenetic analysis, Prediction of RNA secondary structure, Restriction site analysis in 16S r DNA.

### 5. Screening of bacterial strains for production of plant growth regulator (IAA)

All the nineteen bacterial strains were screened for the production of Indole-3-acetic acid (IAA). The strains were separately inoculated in the nutrient broth medium with tryptophan (1 mg/ml) and incubated at  $28\pm 2^{\circ}\text{C}$  for one week. After a week the cultures were centrifuged at 3000 rpm for 30 min. Two milliliters of the supernatant was mixed with 2 drops of orthophosphoric acid and 4ml of Salkowski reagent (50 ml of 35 percent perchloric acid + 1 ml of 0.5M  $\text{FeCl}_3$ ). Development of a pink colour indicates IAA production. OD was read at 530 nm using spectronic 20D<sup>+</sup>. The level of IAA produced was estimated by a standard IAA graph (Loper and Schroth, 1986[12]). Among the nineteen colonies tested, only nine colonies were able to produce IAA. From these nine colonies only one colony produced a higher rate of IAA and that is *Bacillus* spp. This organism (*Bacillus* spp.) was selected for further molecular studies.

### 6. Standardization of tryptophan concentration, pH, temperature and number of days required for IAA production:

The selected *Bacillus* spp. was inoculated in nutrient broth containing five different concentration of tryptophan i.e. 1 to 5 mg/ml of nutrient broth, at three different pH: the acidic (pH 5.0), the neutral (pH 7.0) and the alkaline (pH 9.0) and incubated at three different temperatures i.e. 28, 37 and  $45^{\circ}\text{C}$  for 1 week. The rate of production of IAA was estimated for every 24 hours, for one week, following the procedure given in 4.A.

## III. RESULTS AND DISCUSSION

### A. Screening of bacterial strains for Indole -3- acetic acid (IAA) Production

The nineteen bacterial strains which showed predominant growth from vermicompost, vermicast and earthworm (fore, mid and hind) guts were given in Table 1. Nine bacterial strains which showed positive result for IAA production among nineteen strains isolated from vermisources were given in Table 2. The observation on the quantity of IAA produced by the nine strains is given in Table 3. The microbial synthesis of plant growth regulators (gibberellin and auxin) is an important factor in soil fertility (Kampert and Strzelczyk 1975[13]). Indole-3 acetic acid (IAA) and Gibberellin (GA) are secondary metabolites, which are important biotechnological products, produced by many of the microorganisms (Okon and Kapulnik, 1986[14]). A total of 21 isolates of *Azotobacter* and fluorescent *Pseudomonas* sp. were isolated from rhizospheric soil and tentatively identified on the basis of biochemical tests. These bacterial isolates were screened for their ability to produce plant growth regulator, IAA. Prokryl *et al* (1985)[15] have reported about the production of IAA and some other auxins in liquid culture of *Pseudomonas cepacia* and *P.fluorescens* isolated from maize and bean rhizosphere. Production of growth substances such as auxin (indole-3-acetic acid) by bacteria has also

## International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 5, May 2016

been confirmed in other studies (Frankenberger and Arshad, 1995[16]). The partial 16S rRNA sequences carried out in the present study for *Bacillus* spp. covered a stretch of approximately 1500 nucleotides for each. About half of the sequences found in the clone library showed only slight relationship to other known sequences, while the other half were highly similar (approximately 95 percent sequence identity) to other database entries for *Bacillus* spp. Less than 0.5 percent of all nucleotides was found to be unique within the conserved regions of the cloned sequence and could almost always be related to reading errors in ambiguous regions of the sequencing gel. The *Bacillus* spp. is identified as *Bacillus cereus*. Beneduzi *et al* (2008) [17] had isolated the plant growth promoting strain SVPR30 and identified by 16S rRNA gene sequence as *Bacillus* spp. and they had tested it for plant growth through in vivo experiments. This strain was characterized as a high IAA producer, able to solubilize phosphate and also fix a considerably high amount of nitrogen. The inoculation of rice with *Bacillus* spp. SVPR30 strain showed a significant increase in the root and shoot parts when compared with the controls within 15 and 30 days after sprouting. Numerous *Bacillus* and *Paenibacillus* strains expressed plant growth promoting (PGP) activities and a number of these strains have been commercially developed as generic plant growth promoters.

**Table1. Predominant bacterial strains isolated from vermisources**

S.No	Sample source	Name of the strain
1.	Vermicompost (T2)	<i>Staphylococcus</i> spp.
2.	Vermicompost (T3)	<i>Corynebacterium</i> spp.
3.	Vermicompost (T5)	<i>Bacillus</i> spp.
4.	Vermicompost (T6)	<i>Micrococcus</i> spp.
5.	Vermicast (T3)	<i>Klebsiella</i> spp.
6.	Vermicast (T5)	<i>Streptococcus</i> spp.
7.	Vermicast (T6)	<i>Enterobacter</i> spp.
8.	Earthworm hind gut (T2)	<i>Tricococcus</i> spp.
9.	Earthworm hind gut (T3)	<i>Yersinia</i> spp.
10.	Earthworm hind gut (T5)	<i>Micrococcus</i> spp.
11.	Vermicompost (T1)	<i>Escherichia</i> spp.
12.	Vermicompost (T2)	<i>Proteus</i> spp.
13.	Vermicompost (T4)	<i>Paracoccus</i> spp.
14.	Vermicompost (T5)	<i>Pseudomonas</i> spp.
15.	Vermicast (T2)	<i>Citrobacter</i> spp.
16.	Vermicast (T3)	<i>Erwinia</i> spp.
17.	Vermicast (T5)	<i>Alcaligenes</i> spp.
18.	Vermicast (T6)	<i>Serratia</i> spp.
19.	Earthworm mid gut (T2)	<i>Pediococcus</i> spp.

T1-Control (1) - *G.sepium* + *L.leucocephala* + Cowdung in (1:1:2)(worm free), T2-*G.sepium* + *L.leucocephala* + Cowdung in (1:1:2) + *E.eugeniae*, T3-*G.sepium* + *L.leucocephala* + Cowdung in (1:1:2) + *E.fetida*, T4-Control (2) - *C.auriculata* + *L.leucocephala* + Cowdung in (1:1:2) (worm free), T5-*C.auriculata* + *L.leucocephala* + Cowdung in (1:1:2) + *E.eugeniae*, T6-*C.auriculata* + *L.leucocephala* + Cowdung in (1:1:2) + *E.fetida*

## International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 5, May 2016

**Table2. List of bacterial strains isolated from vermisources and which showed IAA producing activity**

S.No	Name of the organism	IAA production
1.	<i>Streptococcus</i> spp.	Positive
2.	<i>Micrococcus</i> spp.	Positive
3.	<i>Klebsiella</i> spp.	Positive
4.	<i>Bacillus</i> spp.	Positive
5.	<i>Enterobacter</i> spp.	Positive
6.	<i>Escherichia</i> spp.	Positive
7.	<i>Alcaligenes</i> spp.	Positive
8.	<i>Erwinia</i> spp.	Positive
9.	<i>Pseudomonas</i> spp.	Positive

**Table3. Quantity of IAA produced by various bacterial strains isolated from vermisources**

S.No	Name of the organism	Quantity of IAA produced (µg/ml)
1.	<i>Streptococcus</i> spp.	18.78
2.	<i>Micrococcus</i> spp.	5.99
3.	<i>Klebsiella</i> spp.	5.69
4.	<i>Bacillus</i> spp.	18.99
5.	<i>Enterobacter</i> spp.	6.10
6.	<i>Escherichia</i> spp.	6.38
7.	<i>Alcaligenes</i> spp.	10.75
8.	<i>Erwinia</i> spp.	15.77
9.	<i>Pseudomonas</i> spp.	18.67

### B. Estimation of IAA production by bacterial isolate

Tryptophan concentration, pH, temperature and number of days play a very significant role in the production of IAA. Since the tryptophan is a precursor in IAA synthesis, in the present study also when the tryptophan concentration (1 to 5 mg/ml) get increased the production level of IAA by the bacterial strain also get increased. pH is a very important factor which plays a crucial role in the IAA synthesis. In the present study pH played a very important role, when the pH get decreased (pH 5) the amount of production of IAA also get decreased when the pH at neutral level (pH 7) the production of IAA get increased, when the pH level get increased (pH 9) the level of production of IAA get decreased. Next important factor temperature which determines the bacterial growth and development. In the present study also when the temperature falls down (28<sup>0</sup>C) it affected the production of IAA, at optimum temperature (37<sup>0</sup>C) the production of IAA get increased when the temperature get raised (45<sup>0</sup>C) the level of production of IAA was significantly increased. Another important factor which determines the production of IAA is number of days. During log phase the bacterial strains will start to produce IAA at stationary phase the retain the production of IAA at when decline phase comes using the dead cells as their food the new cells produce significantly higher amount of IAA. In the present study also at day 1 the production of IAA was very less when the days go up to 7 the level of production of IAA was also get increased. In the present study at 1mg/ml tryptophan concentration, at pH 7, at 37<sup>0</sup>C and on 7<sup>th</sup> day the bacterial strain produced significantly higher concentration of IAA (19.39 µg/ml) compared to pH 5 and pH 9, at 28<sup>0</sup>C and 45<sup>0</sup>C and on 1, 2, 3, 4, 5, 6 days given in Figure 1. Swain *et al* (2007) [18] showed the effect of L-tryptophan concentrations on IAA production by *Bacillus* CM4 and CM5 strains. With the increase in L-tryptophan concentration from 0 to 2g/l, there was a linear increase in IAA production in the case of *Bacillus* CM4. However, in the case of *Bacillus* CM5, an increase in IAA production was observed only up to a concentration of 1g/l of tryptophan and there was a slight decrease at higher concentrations. Similar result was reported for *Azospirillum brasilense* by Tien *et al* (1979) [19]. At 2mg /ml of tryptophan concentration at pH 7, at 37<sup>0</sup>C and on 7<sup>th</sup> day the bacterial strain in the present study produced significantly higher concentration of IAA (32.79 µg/ml) compared to pH 5 and pH 9, at 28<sup>0</sup>C and 45<sup>0</sup>C

## International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 5, May 2016

and on 1, 2, 3, 4, 5, 6 days given in Figure 2. Husen (2003)[20] reported that *Bacillus cereus* UW 85 and *Azotobacter vinelandi* Mac 259 produced 4.28 and 2.09 micro mol ml<sup>-1</sup>, at 5 mM L-tryptophan concentration level after 48 hours. Microbial production of IAA is known to result from different pathways (Persello-Cartieaux *et al.*, 2003[21]). Although tryptophan-independent biosynthesis pathways have been identified in several microorganisms, tryptophan remains the most common precursor of microbial IAA production (Patten and Glick, 1996[22]).

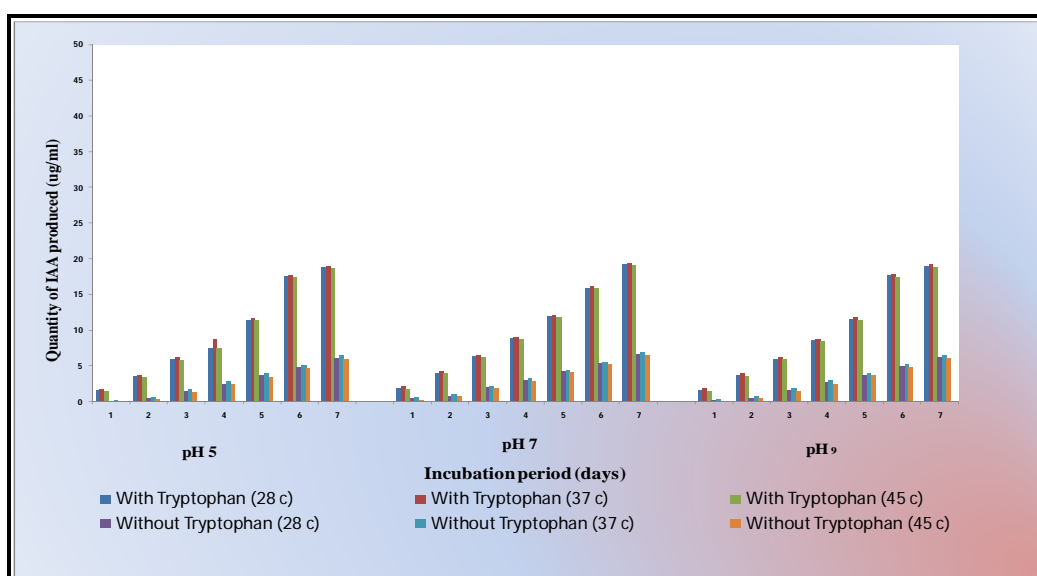


Fig 1. Quantity of IAA produced by *Bacillus cereus*. in the medium supplemented with 1mg/ml of tryptophan, at various pH (5, 7 and 9), at various temperatures (28, 37 and 48°C) and at various day intervals (1 to 7)

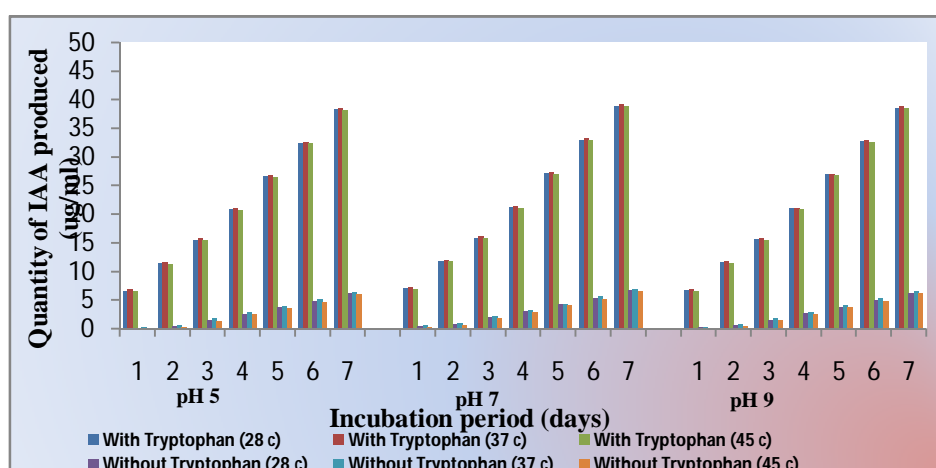


Fig 2. Quantity of IAA produced by *Bacillus cereus*. in the medium supplemented with 2mg/ml of tryptophan, at various pH (5, 7 and 9), at various temperatures (28, 37 and 48°C) and at various day intervals (1 to 7)

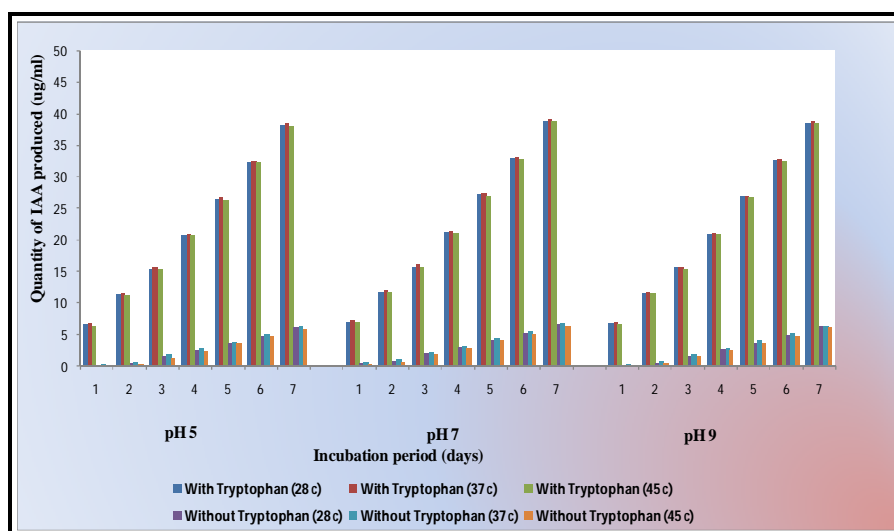
Tryptophan concentration at 3mg /ml at pH 7, at 37°C and on 7<sup>th</sup> day the bacterial strain in the present investigation produced significantly higher concentration of IAA (39.11 µg/ml) compared to pH 5 and pH 9, at 28°C and 45°C and on 1, 2, 3, 4, 5, 6 days given in Figure 3. Varying levels of IAA production were recorded with different concentrations of tryptophan i.e. 0, 1, 2, 3, 4, 5 mg/ml. The range of IAA production in *Azotobacter* isolates increased in the presence of 1, 2 and 5 mg/ml of tryptophan, i.e. 1.47-11.88 mg/ml, 5.99-24.8 mg/ml and 7.3-32.8 mg/ml

## International Journal of Innovative Research in Science, Engineering and Technology

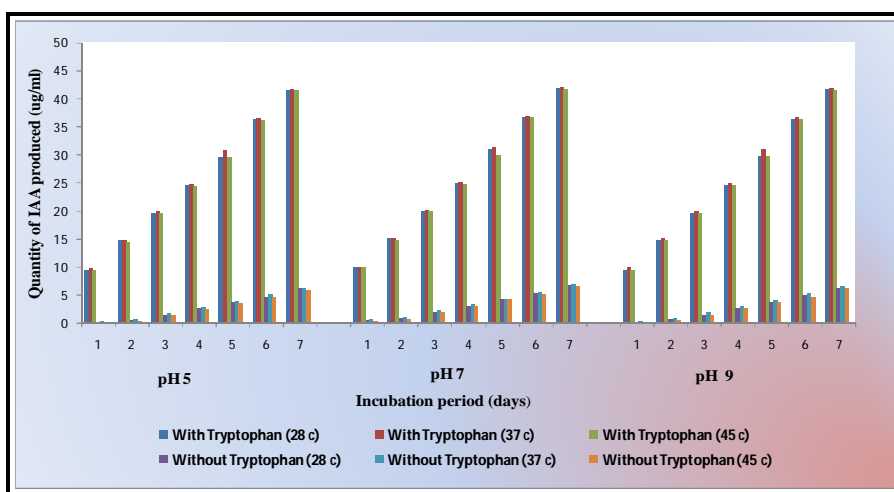
(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 5, May 2016

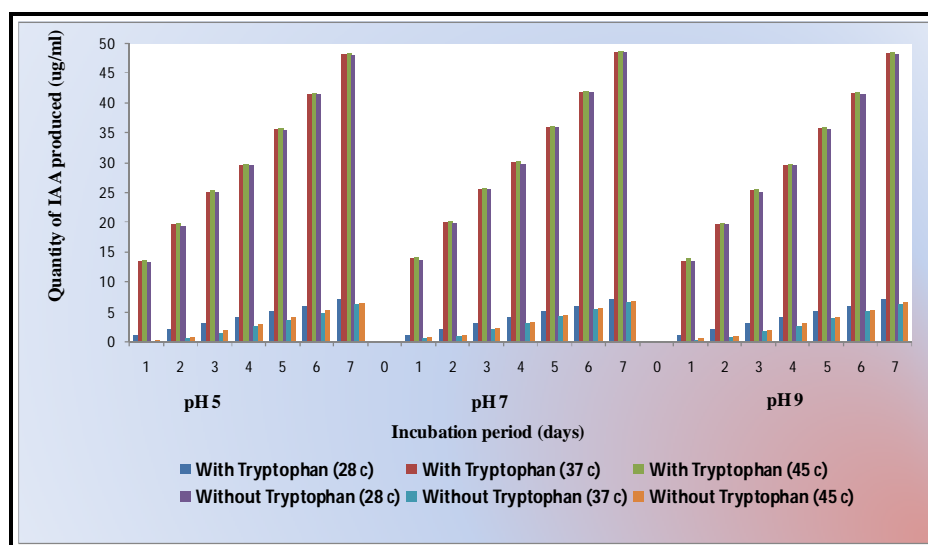
respectively as reported by Lopez and Vela (1981)[23]. At 4mg /ml of tryptophan concentration at pH 7, at 37<sup>0</sup>C and on 7<sup>th</sup> day the bacterial strain in the present study produced significantly higher concentration of IAA (42.12 µg/ml) compared to pH 5 and pH 9, at 28<sup>0</sup>C and 45<sup>0</sup> C and on 1, 2, 3, 4, 5, 6 days given in Figure 4. Prokryl *et al* (1985)[15] have reported about the production of IAA and some other auxins in liquid culture of *Pseudomonas cepacia* and *P.fluorescens* isolated from maize and bean rhizosphere. Production of growth substances such as auxin (indole-3-acetic acid) by bacteria has also been confirmed in other studies (Frankenberger and Arshad, 1995)[16]. In the present study at 5mg/ml tryptophan concentration, at pH 7, at 37<sup>0</sup>C and on 7<sup>th</sup> day the bacterial strain produced significantly higher concentration of IAA (48.77 µg/ml) compared to pH 5 and pH 9, at 28<sup>0</sup>C and 45<sup>0</sup> C and on 1, 2, 3, 4, 5, 6 days given in Figure 5. The production of IAA was almost linear from 2 to 7 days and after that the IAA biosynthesis marginally decreased.



**Fig 3.** Quantity of IAA produced by *Bacillus cereus*. in the medium supplemented with 3 mg/ml of tryptophan, at various pH (5, 7 and 9), at various temperatures (28, 37 and 48<sup>0</sup>C) and at various day intervals (1 to 7)



**Fig 4.** Quantity of IAA produced by *Bacillus cereus*. in the medium supplemented with 4mg/ml of tryptophan, at various pH (5, 7 and 9), at various temperatures (28, 37 and 48<sup>0</sup>C) and at various day intervals (1 to 7)



**Fig.5. Quantity of IAA produced by *Bacillus cereus* . in the medium supplemented with 5mg/ml of tryptophan, at various pH (5, 7 and 9), at various temperatures (28, 37 and 48°C) and at various day intervals (1 to 7)**

This is in concomitant with the growth of *B.subtilis* in tryptophan supplemented medium reported by Swain *et al* (2007). Unyayar *et al* (2000)[24] had reported that *Pseudomonas* spp. synthesized maximum amount of IAA only during stationary phase of growth. The reason hypothesized was that during stationary phase the bacterium might be able get maximum tryptophan from the dead bacterial mass which had resulted in the production of more IAA.

#### IV. CONCLUSION

In the present study Nine bacterial strains (*Streptococcus* spp., *Micrococcus* spp., *Klebsiella* spp., *Bacillus* spp., *Enterobacter* spp., *Escherichia* spp., *Alcaligenes* spp., *Erwinia* spp. and *Pseudomonas* spp.) were identified as IAA producing microorganisms. The quantity of IAA produced by all these nine bacterial strains were estimated using spectrophotometer. Also attempt has been made to standardize the production of IAA by *Bacillus* spp. in different tryptophan concentrations (1, 2, 3, 4 and 5 mg/ml), at different pH (5, 7 and 9), at different temperatures (28, 37 and 45°C) and at different day intervals (1 to 7) and the IAA produced was measured using spectrophotometer. The results showed that the tryptophan concentration at which IAA production was higher was in the order of 5 > 4 > 3 > 2 > 1mg/ml at pH 7 followed by 5 and 9. The suitable temperature was 37°C followed by 28 and 45°C. The number of days required for higher rate of production of IAA was in the order of 7 > 6 > 5 > 4 > 3 > 2 > 1. The findings of the present investigation highlighted that IAA producing bacteria from vermicompost could easily be isolated and exploited after strain improvement for local use. However, further studies using IAA mutant strains of these isolates are needed to explore the exact contribution of IAA production in the promotion of plant growth as well as the contribution of other PGR traits.

#### REFERENCES

- [1] Karlen, D.L., Mausbach, M.J., Dorah, J.W., Cline, R.G., Harris, R.F. and Schuman, G.E. 1997. Soil quality: A concept, definition and framework for evaluation-soil science society. *America Journal*. 61:4-10.
- [2] Sharma, A. and Agarwal, A.K. 2004. Organic farming: Today's revolution, tomorrow's prosperity, *Agrobios Newsletter*. 3:16-18.
- [3] Masciandaro, G., Ceccanti, B. and Garcia, C. 1997. Soil agro-ecological management fertirrigation and vermicompost treatments. *Bioresource Tecnology*. 39:119-206.
- [4] Krishnamoorthy, R.V. and Vajranabhaiah, S.N. 1986. Biological activity of earthworm casts: an assessment of plant growth promoter levels in the casts. *Proc.Indian Acad.Sci. (Anim.Sci.)*. 95:341-351.



# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 5, May 2016

- [5] Edwards, C.A. 1998. The use of earthworms in the breakdown and management of organic wastes. In: The influence of humic acids derived from earthworm-processed organic wastes on plant growth. (Ed.). Atiyeh, R., Lee, S., Edwards, C.A., Arancon, N.Q. and Metzger, J.D. Bioresource Technology. 84:7-14.
- [6] Marinissen, J.C.Y. and Dexter, A.R. 1990. Mechanisms of solubilization of earthworm casts and artificial casts. Boil. Fertil. Soils. 9:163-167.
- [7] Arshad, M. and Frankenberger, Jr. W.T. 1991. Microbial production of plant hormones. Plant Soil. 133:1-8.
- [8] Gardener, B.B.M. 2004. Ecology of *Bacillus* and *Paenibacillus* spp. In: Agricultural Systems Symposium: The Nature and Application of Biocontrol Microbes: *Bacillus* spp. Phytopathology. 94:1252-1258.
- [9] Lynch, J.M. and Wood, D.A. 1985. Controlled microbial degradation of lignocellulose: the basis for existing and novel approaches to composting. In: the effect of lignin on biodegradability. (EDS). Richard, T. Science and Engineering.
- [10] Daniel, T. and Karmegam, N. 2000. Bio-conversion of selected leaf litters using an African epigeic Earthworm, *Eudrilus eugeniae*. Ecol. Env. and Cons. 5 (3):273-277.
- [11] Nagarathinam, B., Karmegam, N. and Daniel, T. 2000. Microbial changes in some organic materials subjected to earthworm action. J. Ecobiol. 12(1):45-48.
- [12] Loper, J.E. and Schroth, M.N. 1986. Influence of bacterial source of indole-3-acetic acid for root elongation of sugar beet. Phytopathol. 76:386-389.
- [13] Kampert, M. and Strzelczyk, E. 1975. Synthesis of auxins by fungi isolated from the roots of pine seedlings (*Pinus silvestries* L.) and from soil. Acta Microbiol. Polon. Ser. B. 7:223-230.
- [14] Okon, Y. and Kapulnik, Y. 1986. Development and function of *Azospirillum*-inoculated Roots. Plant and Soil. 90:3-16.
- [15] Prokryl, Z., Vancura, V. and Wurst, M. 1985. Auxin formation by rhizosphere bacteria as a factor of root growth. Biologin Plantarum. 27:159-163.
- [16] Frankenberger, Jr., W.T. and Arshad, M. (Ed.). 1995. Phytohormones in soils microbial production and function. Marchel Dekker. New York. Pp.503.
- [17] Beneduzi, A., Peres, D., Vargas, L.K., Bodanese, M.H., Maria, L.P. and Passaglia. 2008. Evaluation of genetic diversity and plant growth promoting activities of nitrogen-fixing bacilli isolated from rice fields in South Brazil. Applied Soil Ecology. 39:311-320.
- [18] Swain, M., Amir, S., Naskar, K. and Ramesh, C. and Ray. 2007. Indole-3-acetic acid production and effect on sprouting of yam (*Dioscorea roturdata* L.) minisets by *Bacillus subtilis* isolated from culturable cowdung microflora. Polish journal of microbiology. 56(2):103-110
- [19] Tien, T.M., Garkins, M.H. and Hubbell, D.H. 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl Millet *Pennisetum americanum* (L.). Appl. Environ. Microbiol. 37:1016-1024.
- [20] Husen, E. 2003. Screening of soil bacteria for plant growth promotion activities in vitro. Journal of Agricultural Science. 4(1):27:31.
- [21] Persello-Cartieaux, F., Nussaume, L. and Robaglia. 2003. Tales from the underground: molecular plant-rhizobacteria interactions. Plant, cell and environment. 26:189-199.
- [22] Pattern, C.L. and Glick, B.R. 1996. Bacterial biosynthesis of indole-3-acetic acid. Canadian Journal of microbiology. 42:207-220.
- [23] Lopez, G.Z. and Vela, G.R. 1981. True Morphology of the Azotobacteraceae filterable bacteria Nature. 289:588-590.
- [24] Unyayar, S., Unyayar, A. and Uhal. E. 2000. Production of auxin and abscisic acid by phanerochaete *Chroosporium* Me44 b immobilized on polyurethane foam. Turk. J. Biol. 24:769-774.