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Research Article

INDOLE-3-ACETIC ACID (IAA) PRODUCTION BY *ENDOPHYTIC BACTERIA* ISOLATED FROM SALINE DESSERT, THE LITTLE RUNN OF KUTCH

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ABSTRACT
A total of 13 isolates were screened for their potential to produce IAA and all isolates were able to utilize tryptophan and produce the IAA. The two isolates with high IAA production were secondary screened to select the most highest producer. Isolation of microorganisms was carried out from root samples of different plants collected from saline desert, Little Rann of Kachchh. Isolation was carried out by enrichment isolation technique using YEM (Yeast Extract Mannitol) agar and ASM (Ashby's Mannitol) Agar. TLC and FTIR analysis confirmed the IAA production in the cell filtrates of the strain. Sequencing of the 5AY isolate had 16S rRNA gene with 99% nucleotides identity to that of *Pseudomonas stutzeri*, and 10AY isolate had 16S rRNA gene with 99% nucleotides identity to that of *Bacillus sp. TA_AJ2*. IAA production by *Pseudomonas stutzeri* and *Bacillus sp. TA_AJ2*was optimized by studying some factors and the results revealed that the maximum IAA value was obtained when the isolate cultivated in tryptone yeast extract broth medium supplemented by tryptophane 200µg/ml, incubated at 37°C for 168 hours. These results suggest that IAA-producing *Pseudomonas stutzeri* could be a promising candidate for utilization in growth improvement of plants of economic and agricultural value.

Keywords*: Indole-3-Acetic Acid; Pseudomonas Stutzeri; TLC; HPLC; 1 6S rRNA; Optimization*

INTRODUCTION
There are many endophytic and epiphytic bacteria are directly or indirectly involved in plant growth and development. Endophytic bacteria live in plant tissues without causing substantive harm to the host or gaining any benefit other than a noncompetitive environment inside host. It has recently been demonstrated that bacterial endophytes may also have beneficial effects on host plants, such as growth promotion and biological control of pathogens (Downing and Thomson 2000; Ryu *et al.,* 2005; Sturz *et al.,* 1999).
Some studies have indicated that the plant growth-promoting potential of endophytes is higher than that of rhizosphere microbes (Reiter *et al.,* 2002; Van *et al.,* 1993), but the role of bacterial endophytes in plant growth are not yet fully understood. Most of these microorganisms are not pathogenic to the host plant. Moreover, the association between the plant and its endophytes is very often mutualistic. In 1926, endophytic growth was recognized as a particular stage in the life of bacteria, described as an advanced stage if infection and as having a close relationship with mutualistic symbiosis (Perotti, 1926). Since then, endophytes have been defined as microorganisms that could be isolated form surface-sterilized plant organ (Henning and Villforth, 1940). Although the presence of bacterial endophytes in plants is variable and, occasionally transient (Overbeek and Elsas, 2008), they are also often capable of eliciting drastic physiological changes that modulate the growth and development in the plant (Conrath, 2006).
Little attention has been devoted on the functions of the microbial populations which impact soil processes and other life forms. Studies on the role of microorganisms and the interactions among them could allow an enhanced knowledge for a better understanding that enable life to continue (Gesheva, 2002; Miller *et al.,* 1990). The rhizosphere is an ecological niche in which develop microbial communities develop.
Many bacteria are intimately associated with plant roots. Rhizodeposition of various exudates provide an important substrate for the soil microbial community and there is a complex interplay between this community and the quantity and type of compounds released (Kandeler *et al.,* 2002; Marschner and Baumann, 2003; Ramakrishnan *et al.,* 2009). However, the composition and quantity of root exudates

varies depending on the plant species (Smith, 1976) and the physical environment such as humidity and temperature (Martin and Kemp, 1980).

There are several plant growth-promoting rhizobacteria (PGPR) inoculants currently commercialized that seem to promote growth through at least one mechanism of the following: suppression of plant disease (termed Bioprotectants), improved nutrient acquisition (Biofertilizers), or phytohormone production (Biostimulants). Commercial applications of PGPR are being tested and are frequently successful; however, a better understanding of the microbial interactions that result in plant growth increases will greatly increase the success rate of field applications (Burr *et al.,* 1984).

The auxins are a group of indole ring compounds which have the ability to improve plant growth by stimulating cell elongation, root initiation, seed germination and seedling growth (Tarabily, 2008). Indole-3-acetic acid (IAA) is the main member of the auxinIndole-3-acetic acid (IAA) production by *Streptomyces atrovirens* family that controls many important physiological processes including cell enlargement and division, tissue differentiation, and responses to light and gravity (Teale *et al.,* 2006). IAA also serves as a regulating agent for microbial cell differentiation, for example, it stimulates spore germination and mycelia elongation in the *Streptomyces* (Matsukawa *et al.,* 2007). Several *Streptomyces* species, such as *S. olivaceoviridis*, *S. rimosus*, *S. rochei*and *Streptomyces* spp. From the tomato rhizosphere, have the ability to produce IAA and improve plant growth by increased seed germination, root elongation and root dry weight (Tarabily, 2008). Microbial isolates recovered from the rhizosphere of different crops appear to have a greater potential to synthesize and release IAA as secondary metabolites. Production of IAA by microbial isolates varies greatly among different species and strains and depends on the availability of substrate (s). Many bacteria isolated from the rhizosphere have the capacity to synthesize IAA in vitro in the presence or absence of physiological precursors such as tryptophan (Davies, 1998). Different bacterial pathways to synthesize IAA have been identified and a high degree of similarity between IAA biosynthesis pathways in plants and bacteria was observed (Spaepen *et al.,* 2007). Tryptophan is believed to be the primary precursor for the formation of IAA in plants and microorganisms (Monteiro *et al.,* 1988). However, work with tryptophan-auxotrophic mutants and isotope labeling has established that IAA biosynthesis can occur via a tryptophan independent route (Normanly, 1997; Venis and Napier, 1991), although in the presence of tryptophan, microbes release greater quantities of IAA and related compounds. There is evidence that the growth hormones produced by the bacteria can in some instances increase growth rates and improve yields of the host plants (Sarwar and Frankenberger, 1994). In vivo, the effect of culture filtrates on maize and cowpea seed germination and root elongation was evaluated and compared with the commercial IAA (Thangapandian *et al.,* 2007). Nowadays, some endophytes are studied and developed as a commercial product.

The aim of this study was to investigate IAA production of endophytic bacteria isolated from root of plants from little runn of kutch.

MATERIALS AND METHODS

Sampling

Endophytic bacteria were randomely collected according to the plants in different areas of saline desert From Little Rann of Kutch such as Dhutbet, Pungbet, Vithroi and Kodadha from Gujarat, India.

Cultivation and Isolation of Entophytes

Plant root was washed with sterile distilled water and then disinfected with diluted $HgCl_2$. Rinsed again with distilled water to remove $HgCl_2$, finally roots were added with normal saline and Macerated with sterile mortar and pastle. Then inoculated into ASM (Ashby''smannitol) broth, and YEM (Yeast Extract Mannitol) by enrichment culture technique with following composition with additional 5% w/v NaCl and at pH 9.

Inoculated Samples were incubated for 3-4 days at 37 ^{0}C under shaking conditions with regular monitoring for growth. After appearance of cell density, a loop full of the enriched culture was streaked on the YEM agar media, well isolated colonies were selected and pure cultures were obtained by subsequent streaking on the respected agar plate.

Composition of ASM Agar (Gms/litre)

Mannitol: 20.00, Dipotassium phosphate: 0.20, Magnesium sulphate: 0.20, Sodium chloride: 0.20, Potassium sulphate: 0.10, Calcium carbonate: 5.00, Agar: 15.00, Final PH (at25 c), 7.4± 0.2,

Composition of YEM Agar (Gms/litre)

Yeast extract 1.000, Mannitol 10.000, Dipotassium phosphate0.500, Magnesium sulphate 0.200, Sodium chloride 0.100, Calcium carbonate 0.100, Agar 15.000 Final pH 6.8±0.2 (25°C)

Preservation of Culture

The isolates were maintained on the YEM (pH 9; NaCl, 5% w/v) slant of medium, and stored at 4o C. The organisms were sub cultured at monthly.

Microscopic and Macroscopic Observation of Isolates

In order to characterize the organism's microscopic and morphological examinations viz., cell shape and arrangement gram nature (Cappuccino and Sharman, 2002). Whereas, Macroscopic characters of colony was recorded by streaking the isolates on the YEM agar like size margin, Evaluation, Texture, Pigmentation, etc of endophyte.

Biochemical Characterization

The isolates were inoculated in the different biochemical media like Thiosulphate iron(TSI) agar slant, Peptone Nitrate Broth (PNB), 1% Tryptone broth, Glucose Phosphate Broth (GPB), Simmon citrate agar slant, Urea broth, N-agar slant, Gelatin agar tube, Starch agar plate, Tributyrene agar plate, Skim milk agar plate.

Screening of IAA Production from Bacterial Isolates

Qualitative assay of IAA production: Prepared Selective media such as Yeast Extract Mannitol (YEM) and Ashby's Mannitol broth containing 0.51 gm L-Tryptophan. Add 5% NaCl and pH 9 adjust by NaOH. Isolated bacterial strains were inoculated in a respective media. Incubate all tubes in a shaking condition at 150 rpm (room temperature). After 3 days incubation the broth was centrifuged at 2500 rpm for 25 min. The cell free supernatant was collected and used for estimation of IAA production.

2 ml Supernatant was collected and add 2-3 drop of ortho-phosphoric acid and 4 ml of Salkowski's reagent (2ml of 0.5M FeCl, + 98 ml 35% Perchloric acid) and incubated in dark for 1 hr. Absorbance was measured at 530 nm by using spectrophotometer.

Preparation of Standard Graph of IAA

Range between standard of IAA: - 10 – 100 µg/ml

Different IAA concentrations are prepared as aqueous solution of IAA range from 10 microgram/ml to 100 micrograms/ml. make the 2 ml of the standard working solution, and add 2-3 drop of ortho-phosphoric acid and 4 ml of 2% 0.5 M FeCl3 in 35% perchloric acid i.e. Salkowski's reagent is added and readings are taken after 25 minutes at 530 nm by spectrophotometer. Standard graph is prepared by plotting concentration of IAA in micrograms/ml Vs Optical Density at 530 nm.

Standard graph of IAA

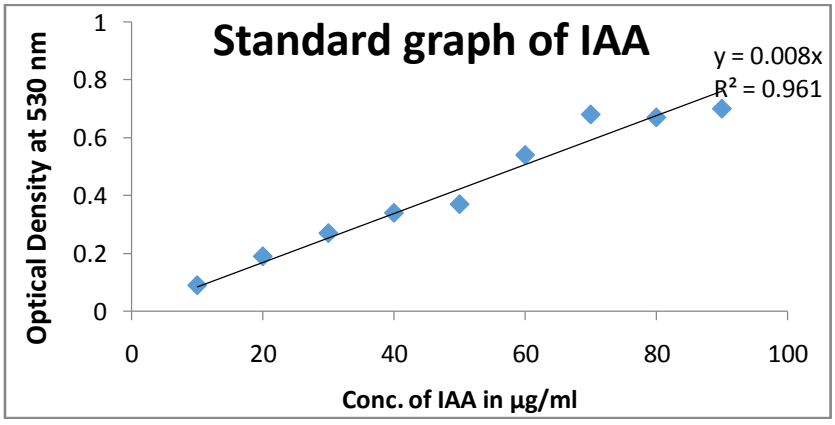


Figure 1: Standard graph of IAA

Optimization of IAA Production for Isolates

Effect of Tryptophan Concentration:

To check the effect of tryptophan on IAA production, YEM broth Supplemented with 10 mg/ml L-Tryptophan. To make the three different concentration of working solution are 100 µg/ml, 200 µg/ml, and 300 µg/ml and this working solution and bacterial isolates was added in a different YEM broth. Incubate all flasks in shaking condition at 150 rpm at normal condition (room temperature).After 72 hrs check the production of IAA by salkowski's method.

Effect of Different Concentration of Production Media:

Media optimization for maximum indole acetic acid production was carried out by statistical approach. Plackett-Burman design software (Design expert version 9.0.2.0., Stat-Ease Corporation,USA). The Plackett-Burman was employed for initial screening of the factors, potentially influencing the response. Followed by selection of factors based on regression coefficient value and selected factor with range were subjected to RSM (Response surface methodology) for obtaining the optimum concentrations of individual variables.

Response surface methodology Based on the value of Regression Coefficient further statistical analysis was carried out using RSM. In the both responses, negative value indicating factors are excluded in the Central Composite Design (CCD). So total six factors including Yeast extract, Mannitol, MgSO4.7H2O, K2HPO4, NaCl and L-Tryptophan were fed to a 2nfactorial CCD and 52 experiments were generated which were performed accordingly.

Extraction of IAA

After 72 hours, the broth was centrifuged at 7000 rpm for 10 min. Then pH of broth brought to 2.5 to 3.0 with 1N HCl and the sample were extracted three times with ethyl acetate. The organic phase was concentrated to dryness and then diluted with 0.5 ml methanol or ethanol.

Purification of IAA by TLC

This solution along with the standard IAA and IAA sample were spotted on aluminum-backed silica gel G plate and TLC was run by using the mobile phase isopropanol: ammonia: water (16:3:1 [v/v]). After running, TLC foils were dried and Spots were allowed to developed in observed under 256- and 360-nm UV light.

FTIR Analysis for Confirmation of IAA Production

In order to confirm the production of IAA by bacterial isolate based on information about its chemical bonds and molecular structure, FTIR analysis was carried out. Methanolic extract was completely dried and mixed with spectral grade potassium bromide, and FTIR spectral analysis of IAA was recorded at the transmission mode from 400–4000 cm−1.

Identification of Bacterial Isolate Using 16S rRNA Gene Sequence

Identification of bacterial strain at species level was performed with 16s RNA gene seqence homology method. The 16S rRNA gene sequencing was done from chromous biotech. The obtained partial sequence was analyzed by NCBI BLAST. The sequence was found 100% similar to the sequence of isolates. Multiple sequence alignment was done using CLUSTALW software.

RESULTS

Isolation of Microorganisms

Isolation of microorganisms was carried out from root samples of different plants collected from saline desert Little Rann of Kachchh. Isolation was carried out by enrichment isolation technique using YEM (Yeast Extract Mannitol) agar and ASM (Ashby's Mannitol) Agar. On YEM Plate seven isolates were isolated while on ASM six isolates were isolated.

Microscopic and macroscopic observation of Isolates:

Out of 13 isolates seven isolates were gram positive and six isolates were gram negative and short rod. All the colonies were having round shape with mostly entire margin and smooth texture. The colony size was ranging between 2 to 6 mm. except 10AY with yellow pigmentation all remaining isolates colonies were dirty white.

Biochemical Characterization

All the isolates were negative for indole production, Urease, Gelatin liquification and Lipid hydrolysis and positive for citrate utilization. While, variable response was evident in remaining biochemical characteristics (Table 1& 2).

Table 1: Biochemical Test for YEM isolates

Biochemical Test	Name of medium	Isolates from YEM						
		2Y	3Y	5AY	7Y	8Y	10Y	10AY
H_2S production	TSI agar slant	+	-	-	+	+	+	-
Nitrate reduction	PNB broth	-	+	-	+	+	+	-
Indole production	1% Tryptone broth	-	-	-	-	-	-	-
MR Reaction	GPB broth	+	+	+	+	+	-	-
VP Reaction	GPB broth	+	-	-	-	-	+	+
Citrate Utilization	Simmon citrate agar slant	+	+	+	+	+	+	+
Urease activity	Urea broth	-	-	-	-	-	-	-
Catalase activity	N-agar slant	+	+	+	+	+	-	+
Gelatin liquification	Gelatin agar tube	-	-	-	-	-	-	-
Starch hydrolysis	Starch agar plate	-	-	-	-	-	-	-
Lipid hydrolysis	Tributyrene agar plate	-	-	-	-	-	-	-
Ammonia production	PNB broth	-	+	+	-	+	+	+
Casein hydrolysis	Skim milk agar plate	-	-	+	-	-	-	-

Table 2: Biochemical Test for ASM isolates

Biochemical Test	Name of medium	Isolates for ASM					
		4A	11	11A	11B	11D	13A
H_2S production	TSI agar slant	+	+	-	-	-	-
Nitrate reduction	PNB broth	+	+	+	+	+	-
Indole production	1% Tryptone broth	-	-	-	-	-	-
Methyl Red (MR)Test	GPB broth	-	+	+	-	+	-
Voges-Proskauer Test	GPB broth	-	-	-	-	-	-
Citrate Utilization Test	Simmon citrate agar slant	+	+	+	+	+	+
Urea Hydrolysis Test	Urea broth	-	-	-	-	-	-
Catalase Test	N-agar slant	+	+	+	+	+	+
Gelatin liquification Test	Gelatin agar tube	-	-	-	-	-	-
Starch hydrolysis Test	Starch agar plate	+	+	-	-	+	-
Lipid hydrolysis Test	Tributyrene agar plate	-	-	-	-	-	-
Ammonia production	PNB broth	-	-	-	+	-	+
Casein hydrolysis	Skim milk agar plate	-	-	+	-	-	-

Indole-Acetic Acid (IAA) Production

Screening of Bacterial Isolates for IAA Production:

The screening for IAA production was carried out by salkowsky'smethod for all isolates after incubation of 72 hrs, 144 hrs and 168n hrs. All the isolates reported IAA producer. Qualitative comparison indicated the highest production of IAA by isolate 5AY after incubation of 168 hrs (Table 3).

Table 3: Screening of bacterial isolates for IAA production

Isolate	Medium	Incubation Time After 72 hrs.	After 144 hrs.	After 168 hrs.
2Y	Yeast Extract mannitol broth with L-tryptophan	+	++	++
3Y		+	++	++
5AY		+	++	+++
7Y		+	++	++
8Y		+	+	++
10Y		+	++	+
10AY		+	++	++
4A	Ashby's mannitol broth with L-tryptophan	+	+	+
11		++	+	+
11(A)		++	+	++
11(B)		+	+	+
11(D)		+	+	+
13(A)		+	++	+

Optimization of IAA Production:

Effect of Tryptophan Concentration

The majority of the isolates were producing IAA in presence of tryptophan. As the concentration of tryptophanincreases, the production of IAA also increases.Two isolates was found to be the efficient producer of IAA amongst all the isolates. Using three different concentration such as 100 µg/ml, 200 µg/ml, and 300 µg/ml of tryptophan in the medium, After 72 hrs, IAA was obtain in 5AY and 10AY bacterial isolates in increase IAA production with increased tryptophan concentration.

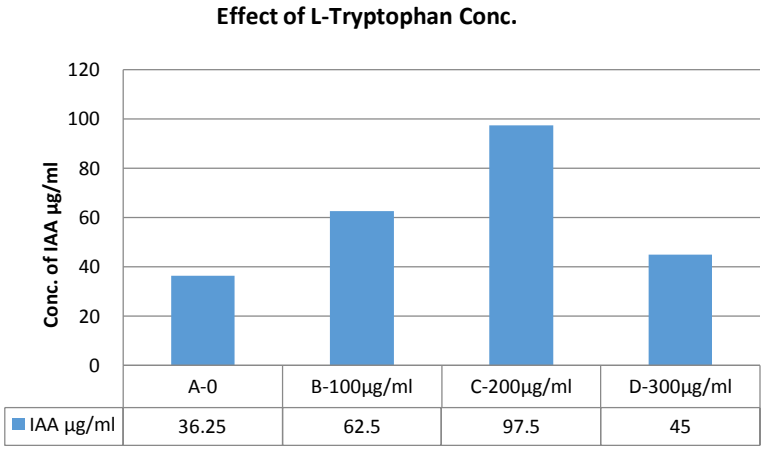


Figure 2: Effect of L-Tryptophan Conc. on IAA production by 5AY isolate

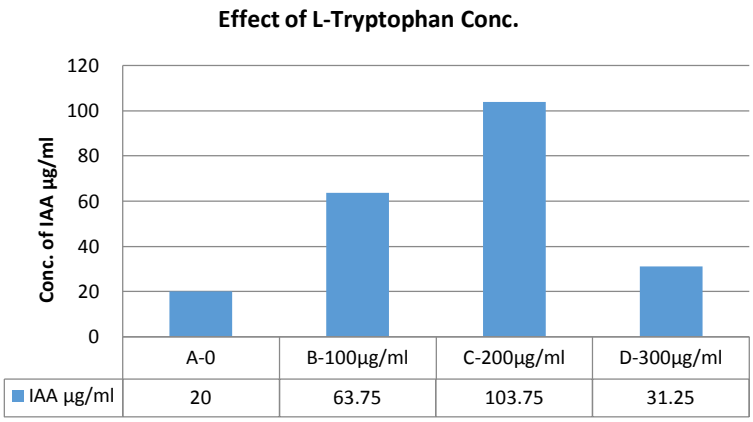


Figure 3: Effect of L-Tryptophan Conc. on IAA production by 10AY isolate

Effect of Different Concentration of Medium Composition

Twelve experiments generated by the Plackett-Burman design were carried out accordingly. All the factors were used in gm and dissolved in 50 ml of media. And responses namely the growth of isolate and IAA production were measured (Table 4).

Table 4: Plackett-Burman experimental design for indole acetic acid production

Run	Factor 1 A:Yeast extract %	Factor 2 B:Mannitol %	Factor 3 C:MgSO4 %	Factor 4 D:K2HPO4 %	Factor 5 E:NaCl %	Factor 6 F:LTryptophan %	Response 1 Growth O.D	Response 2 IAA µg/ml
1	0.01	10	0.02	5	10	0.1	0.049	9
2	1	0.1	2	5	0.5	1	1.79	27.5
3	0.01	0.1	0.02	5	0.5	1	0.254	35.625
4	0.01	10	2	0.05	10	1	0.143	44.125
5	1	10	0.02	5	10	1	0.52	63.25
6	1	10	0.02	0.05	0.5	1	1.865	141.125
7	0.01	0.1	2	0.05	10	1	0.468	79.25
8	0.01	0.1	0.02	0.05	0.5	0.1	0.85	136.625
9	1	10	2	0.05	0.5	0.1	1.892	161.875
10	1	0.1	0.02	0.05	10	0.1	0.674	124.25
11	1	0.1	2	5	10	0.1	1	123.5
12	0.01	10	2	5	0.5	0.1	1.858	148.875

Optimization by RSM

Optimization was very important to scale up the bulk production of IAA at industrial scale. The data in the Table 5 indicated that there was a wide variation in IAA production in 52 runs. All the factors were used in gm and dissolved in 10 ml of media. This variation reflects the importance of media optimization to attain higher yields.

RSM generated 52 experiments with variable concentration of Yeast extract; Mannitol, MgSO4.7H2O, K2HPO4, NaCl and L-Tryptophan.

IAA production in standard YEM medium was 36.25 µg/ml. In optimization study with response surface methodology for different composition of YEM medium variable response was observed. In selected combination in which the IAA production was observed more than standard medium was in range of 1.22 to 4.45 fold increase. The top three maximum production was 4.45, 4.41and 3.9 fold respectively for combination no 6, 9 and 3 respectively. Comparative analysis for all three top production reveled that

Mannitol 10%, and NaCl 0.5 % was favorable for better IAA production while for remaining component different concentration in different composition was found effective (Table 5).

Table 5: Comparison of IAA production in different medium condition

No	Yeast Extract	Mannitol	MgSO4. 7H2O	K_2HPO_4	NaCl	Tryptophan	Increased IAA conc. µg/ml After Optimization	Fold increment	O.D Growth
IAA production in standard YEM composition									
YEM	0.1	1	0.02	0.05	5	0.3	36.25	-	
IAA production in Different composition									
1	0.01	10	2	0.05	10	1	44.125	1.24	0.143
2	1	10	0.02	5	10	1	63.25	1.75	0.52
3	1	10	0.02	0.05	0.5	1	141.125	3.9	1.865
4	0.01	0.1	2	0.05	10	1	79.25	2.2	0.468
5	0.01	0.1	0.02	0.05	0.5	0.1	136.625	3.77	0.85
6	1	10	2	0.05	0.5	0.1	161.875	4.45	1.892
7	1	0.1	0.02	0.05	10	0.1	124.25	3.43	0.674
8	1	0.1	2	5	10	0.1	123.5	3.4	1
9	0.01	10	2	5	0.5	0.1	148.875	4.1	1.858
10	0.505	5.05	1.01	6.3986	5.25	0.55	44.375	1.22	0.77
11	0.01	10	0.02	0.05	10	0.1	58.625	1.6	0.025

Result of TLC Analysis

Development of spots on TLC foil is observed under UV light. Separation and possible identification of natural and synthetic indole derivatives. Separation showed RF value of standard IAA 0.69. The same RF value was obtained from IAA produced by the isolates. Standard IAA showed RF value of sample 5AY 1 is 0.67 and 5AY 2 is 0.66.

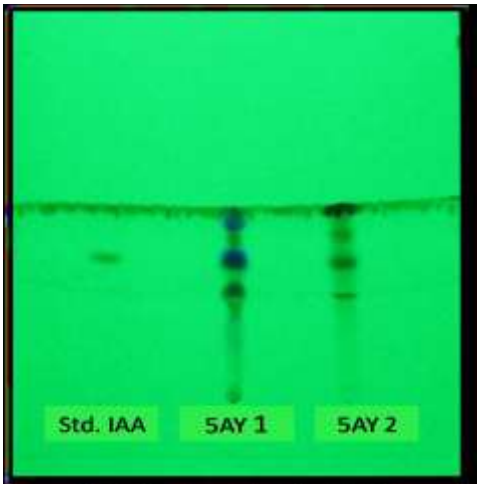


Figure 4: TLC Analysis

Result of FTIR analysis

Characterization of IAA based on FTIR spectra was performed as per Kamnev *et al.*, (2001). Figure shows the FTIR spectra of Extracted IAA from 5AY. Characteristic (N-H) Streching of indolemoity is observed at 3339.22 cm-1(N–H) bending, and wagging was observed at 1642.32 cm−1and 524.06 cm−1, respectively. Alkyl (–CH2) asymmetric stretching, symmetric stretching, and bending was observed at 2979.51 cm−1 and 1453.11 cm−1, respectively. FTIR spectra confirm the presence of IAA.

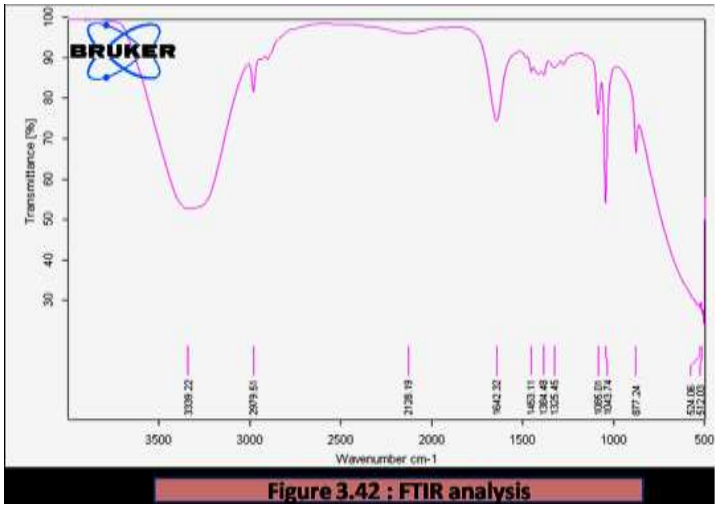
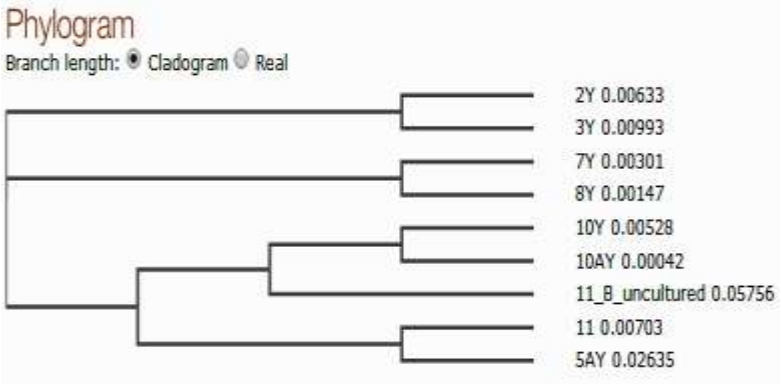


Figure 5: FTIR analysis

16 S r RNA homology based identification and details of similar isolates from gene bank reports:

Identification of isolates was done by 16 S r RNA sequencing followed by similarity search with NCBI blast. Isolates 2Y, 3Y, 5AY, 7Y, 8Y, 10Y, 10AY, 11 and 11B were found 99% similar to *Bacillus tequilensis, Bacillus licheniformis, Pseudomonas stutzeri, Bacillus safensis, Bacillus sp. TA_FJ1, Bacillus sp. TA_AJ2, Bacillus sp. TA_AJ2 and Pseudomonas sp. WS-1, uncultured* Bacillus sp. Isolates were found isolated from the diverse habitat and endophytic nature and PGPR activity was found reported in literature.

Multiple sequence alignment of all the isolates was carried out with on line clustal W tool available at EBI all the isolates were found different and clustering of *Bacillus* and *Pseudomonas group* was found.



Phylogram of all Isolates

DISCUSSION

Plant hormones are chemical messengers that affect a plant ability to respond to its environment. IAA is the member of the group of phytohormone and is generally considered the most important native auxin

(Kloepper *et al.,* 2004). It functions as an important signal molecule in the regulation of plant development including organogenesis, tropic responses, cellular responses such as cell expansion, division, differentiation and gene regulation (Ryu and Patten, 2008). Diverse endophytic bacteria possess the ability to produce the auxinphytohormone IAA, these endophytic bacteria produce auxins in the presence of a suitable precursor such as L-tryptophane. Total 13 isolates which collected from 15 plant root samples in this investigation were tested for their ability to produce IAA. I t is worth mentioning that all isolates had the ability to produce IAA. Several reports have shown that different actinomycetes species from many crop rhizosphere soils have this ability (Tarabily and Sivasithamparamb, 2006; Tsavkelova *et al.,* 2006). Two isolates were able to produce high concentrations of IAA ranged between 97.5µg/ml and 103.75 µg/ml respectively. It is possible that the high levels of tryptophan will be present in root exudates of the two plants and enhance IAA biosynthesis in endophytic bacteria isolated from the plant root. This observation came in a good agreement with those reported by other researcher (Khamna *et al.,* 2010). The culture filtrate of the highest active isolate *Pseudomonas stutzeri*, 5AY was used to extract IAA for characterization by TLC. Chromatograms of culture extracts and standard IAA, sprayed with Salkowski's reagent, showed almost the same Rf values. The TLC findings are in agreement with other reports (Xie *et al.,* 1996). IAA production by *Pseudomonas stutzeri* and*Bacillus sp. TA_AJ2* was optimized by studying some factors and the results revealed that the maximum IAA value was obtained when the isolate cultivated in tryptone yeast extract broth medium supplemented by tryptophane 200µg/ml, incubated at 37°C for 168 hours. However, a higher concentrationof L-tryptophane exerts an adverse effect on production. IAA production in standard YEM medium was 36.25 µg/ml. In optimization study with response surface methodology for different composition of YEM medium variable response was observed. In selected combination in which the IAA production was observed more than standard medium was in range of 1.22 to 4.45 fold increase. The top three maximum production was 4.45, 4.41and 3.9 fold respectively for combination no 6, 9 and 3 respectively. Comparative analysis for all three top production reveled that Mannitol 10%, and NaCl 0.5 % was favorable for better IAA production while for remaining component different concentration in different composition was found effective. It has been reported that IAA production by plant growth promoting rhizobacteria can vary among different species and it is also influenced by culture condition, growth stage and substrate ability (Mirza *et al.,* 2001). IAA can increase colonization of plant surfaces by the epiphytic an endophytic bacteria (Lindow and Brandl, 1883; Lin *et al.,* 2013) that enhances plant growth and yield.

Conclusion

In present work an attempt has been carried out for isolation of endophytic microorganism from the saline desert, little Rann of Kachchh. Wide spread presence and different bacillus and Pseudomonas species were found to have IAA production ability in variable nature. Significant increase in IAA production was obtained after optimization for isolate 5AY similar to *Pseudomonas stutzeri*. Ability of Isolates to grow in robust condition and PGPR activity suggest that isolates can be potential candidate for further studies for biofertilizer application.

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