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Isolation of Indole acetic acid producing bacteria from digester effluent and their effect on plant growth promotion

V.S. Patil
Department of Microbiology, Lal Bahadur Shastri College of Arts, Science and Commerce, Satara-415002, MS, India
vishwasp15@yahoo.com

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Abstract

The synthesis and release of indole acetic acid is an important property of bacteria that play key role in stimulating growth of crops. Indole acetic acid is phytohormone involved in growth and development in plants. The study aimed for isolation and to identify IAA producing bacteria from digester effluent of vegetable waste based biomethanation plant and to test its growth stimulatory effect on crops. Seventeen bacteria were isolated from digester effluent. They were tested to determine production ability for IAA. Two most potent IAA producing bacteria were selected further to test its stimulatory effect on growth of crops by pot assay method. The potent IAA producing plant growth promoting bacterial isolates were identified by molecular characterization using 16 S rRNA gene analyses. The results obtained from pot experiment demonstrated that two most potent IAA producing bacterial isolates cause significant increase in plant development parameters of inoculated crop plants by comparing with control. The study suggests two potent IAA producing bacteria from digester effluent can serve as efficient biofertilizer inoculants to enhance soil fertility and plant growth promotion.

Keywords: Digester effluent, IAA, biofertilizers, plant growth, PGPR, etc.

Introduction

Indole 3 acetic acid, abbreviated as IAA is a growth hormone synthesized and excreted from the plant roots and also by bacteria. IAA is secondary metabolic product synthesized from Tryptophan by several dependant and independent Tryptophan pathways[1,2].

IAA is important physiologically active auxin and enhances growth and development of plants significantly. The IAA stimulates proliferation of roots resulting in increased nutrient uptake rate[3,4]. Further, it influences several processes at cellular and physiological level[5,6].

Several microbial species are noted to be involved in producing IAA by many researchers[7,8]. Among soil microorganisms, bacteria found in rhizospheric area, colonizing the plant roots and living freely in soil have ability to synthesize IAA and hence play beneficial role in growth of plants and hence called as PGPR[8]. These bacteria have been isolated previously from soil and rhizosphere by several researchers[9,10].

Biomethanation is a widely used technology for waste management. The process converts waste into biogas and digester effluent[11]. The use of digester effluent increases crop yield[12-14]. There are very few reports for isolation of these bacteria from digester effluent and studies on their stimulatory effect on the growth of crops. Thus, the study was aimed for isolation of potent IAA positive bacteria isolated from digester effluent of vegetable waste biomethanation plant and to test their stimulatory influence on test crops growth parameters.

Materials and methods

Biomethanation of vegetable waste: Biomethanation study has performed in 5 litre capacity locally fabricated digesters. The digesters were operated with feeding of the vegetable waste slurry (consisting of equal mixture of potato, onion, tomato, brinjal, cauliflower and cabbage wastes) at organic loading rate 0.320g volatile solids /l.d, and pH was 7.0 under ambient temperature conditions with two cycles of 20 days hydraulic retention time.

Isolation of bacteria from digester effluent: Bacteria were isolated by spread plate technique using Nutrient agar. The representative distinct bacterial isolates were preserved at refrigeration temperature.

Screening of bacteria for production of IAA: This capacity was tested in 0.2% L-Tryptophan containing nutrient broth. The presence of IAA in broth was detected by Salkowski method[15,16].

Confirmation of IAA: The aliquots of centrifuged culture broth were extracted with ethyl acetate in 1:2 proportions and confirmed by using thin layer chromatography (TLC) and comparing with standard IAA. The TLC plates were developed with the Salkowaski reagent[17].

Quantitative measurement of IAA: Spectrophotometer was used for this measurement at 535nm wavelength[15,16].

Effect of inoculation of potent IAA positive isolates: Pot assay method was selected for this purpose. Healthy plant seeds

of Maize (*Zea mays* L.), Jowar (*Sorghum bicolor*), Wheat (*Triticum aestivum* L.) and Groundnut (*Arachis hypogea*) were surface disinfected.

The 0.5ml individual bacterial suspension (OD at 600nm=0.9) was used for coating the seeds and further dried. Further, these seeds were put in pots for sowing. Control set was with uncoated seeds. After the growth of seedlings, 0.1g Try/ kg soil in aqueous was inoculated into all the pots. The pots were irrigated with sterile plant nutritive solution daily and were placed in sunlight. The C labeled pot was control and contained only soil, B-1 contained soil+B1 isolate, and B-2 contained soil+ B2 isolate.

After 2 weeks, uprooted seedlings were subjected for measurement of lengths of shoots and roots. Further, roots and shoots were subjected to determine their fresh and dry weights. Statistical analysis of test and control data was carried out to test for significant effect.

Screening of potent IAA producing isolates for other PGPR traits: The production of siderophores, ammonia, catalase and hydrogen cyanide were detected in selected potent IAA producing bacterial isolates as per standard methods[18-20].

Identification of bacterial isolates: The preliminary identification of the potent IAA producing bacterial isolates from digester effluent upto species level was carried out by using standard literature[21,22]. Further, the confirmation of these isolates was done by molecular characterization.

Results and discussion

Biomethanation of vegetable waste: The biogas yield for mixture of six vegetable wastes at ambient temperature conditions was found to be 510-1340 (mL/d). The average yield was 0.633L biogas /g VS.d and methane % was found to be 59%.

Isolation of bacteria from digester effluent: The seventeen different bacterial isolates obtained were maintained in refrigeration conditions.

Screening for IAA production ability: The seventeen bacteria obtained from digester effluent were subjected to determining IAA production ability. Bacterial isolates B1 and B2 were found to be efficient IAA producers as compared to others (Table-1 and Figure-1).

Confirmation of IAA: The extracted sample containing IAA was run on TLC plates and compared with known IAA. The pink coloured spots were observed on TLC plate at the Rf value 0.9 similar to the standard IAA.

Quantitative measurement of IAA: The IAA yield in liquid media by B1 and B2 was found to be 15mg/L and 62.5mg/L respectively.

Effect of potent IAA positive bacteria on crop plants: The growth response exhibited by crop plants to the selected IAA producers is shown as per Table-2 and Figure-2-13.

Screening of potent IAA producers for other PGPR traits: The results are represented as per Table-1.

Identification of bacterial isolates: The morphological, cultural and biochemical characterization of potent bacterial isolates B1 and B2 revealed the identity as *Bacillus amyloliquifaciens* and *Brevundimonas diminuta* respectively. The 16 S rRNA gene analyses of selected bacterial isolates confirmed the identification results.

Table-1: Detection of IAA and other PGPR traits**.**

Test	Result	
	Isolate B1	Isolate B2
IAA production		
Qualitative	+	+
Quantitative (mg/L)	15	62.5
Siderophore production	+	+
Ammonia production	+	+
HCN production	+	+
Catalase production	+	+

Table-2: Effect of potent IAA positive bacteria on crop growth.

Treatment	Maize		Jowar		Wheat		Groundnut	
	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)
Control	31.45± 2.5252	20.625± 1.8875	13.5667± 2.2368	8.6±2	24.55± 0.9469	15.325± 2.4595	12.0667± 2.2723	11.3333± 2.4786
B-1	34.225± 2.1626	24± 1.7493	18.7667± 2.3861	15.7333± 2.3459	28.275± 2.5025	11.775± 2.0694	13.7667± 1.4012	13.8667± 2.0502
P value	0.2646	0.159299	0.0615	0.0688	0.0450	0.1734	0.4887	0.412387
B-2	35.625± 2.168	28.275± 1.4997	17.8333± 1.0214	12.8± 2.1656	29.05± 1.4295	16.35± 2.2038	16.0333± 0.8021	15.4667± 3.0354
P value	0.0799	0.06189	0.0604	0.1299	0.0164	0.5355	0.078681	0.0411

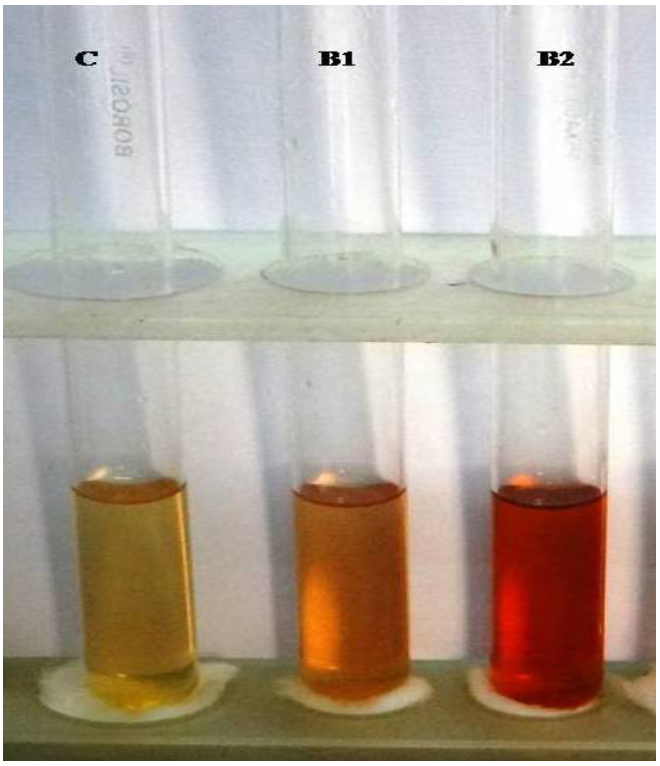

Figure-1: Detection of IAA producing ability in bacterial isolates.

Figure-3: Effect of potent IAA producing bacteria on the Maize growth development.

Figure-2: Effect of potent IAA producing bacteria on Maize growth.

Figure-4: Effect of potent IAA producing bacteria on Jowar growth.

Figure-5: Effect of potent IAA producing bacteria on Jowar growth development.

Figure-7: Effect potent IAA producing bacteria on Wheat growth development.

Figure-6: Effect of potent IAA producing bacteria on Wheat growth.

Figure-8: Effect of potent IAA producing bacteria Groundnut plant growth.

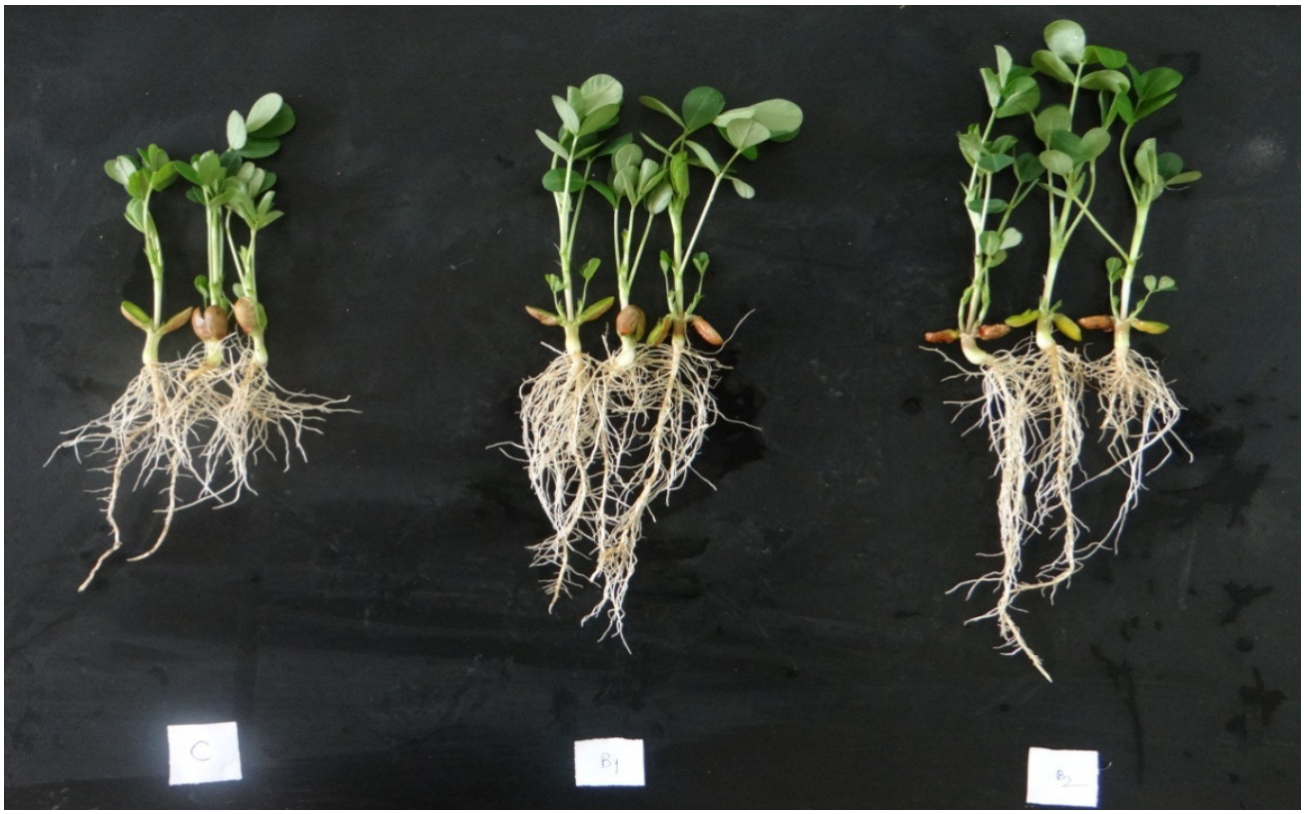

Figure-9: Effect of potent IAA producing bacteria on Groundnut plant growth development.

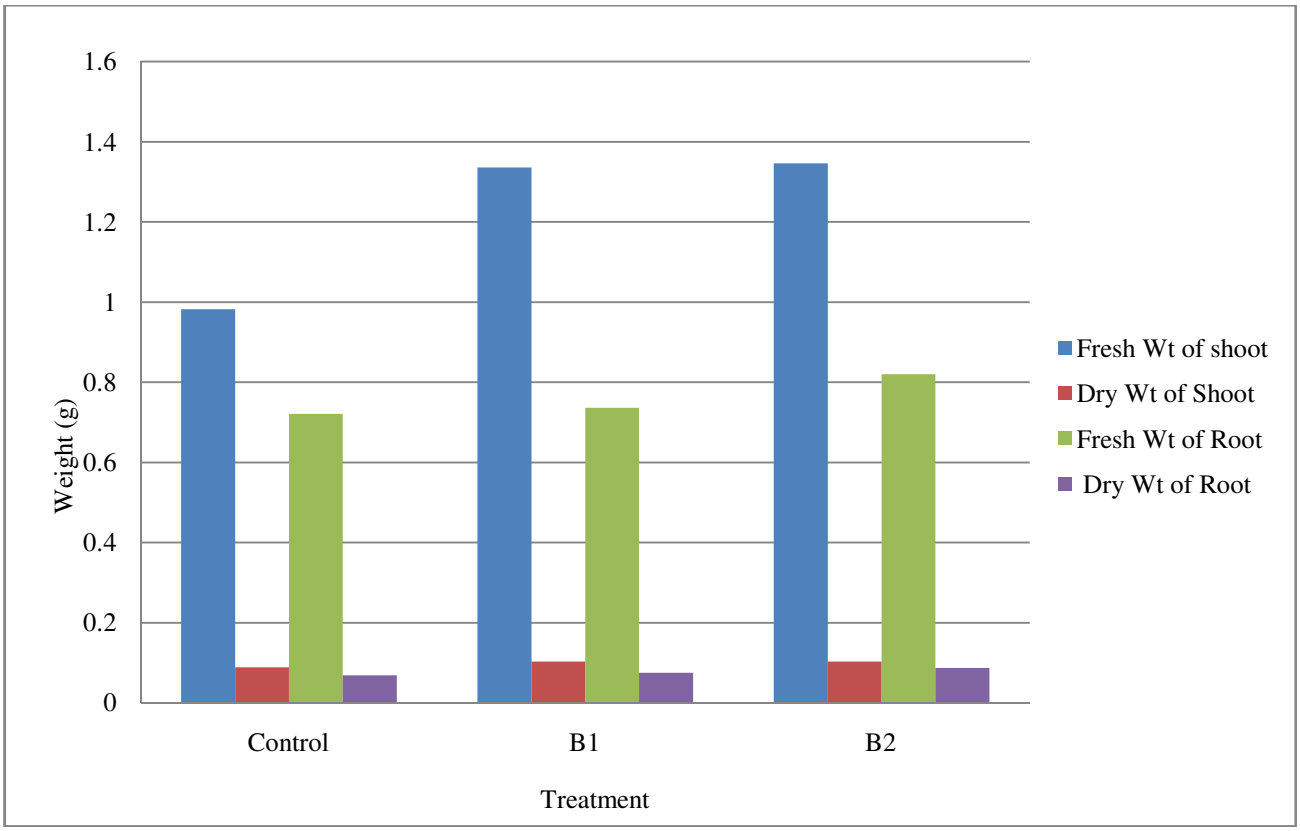

Figure-10: Effect of potent IAA producing bacteria on Maize growth development.

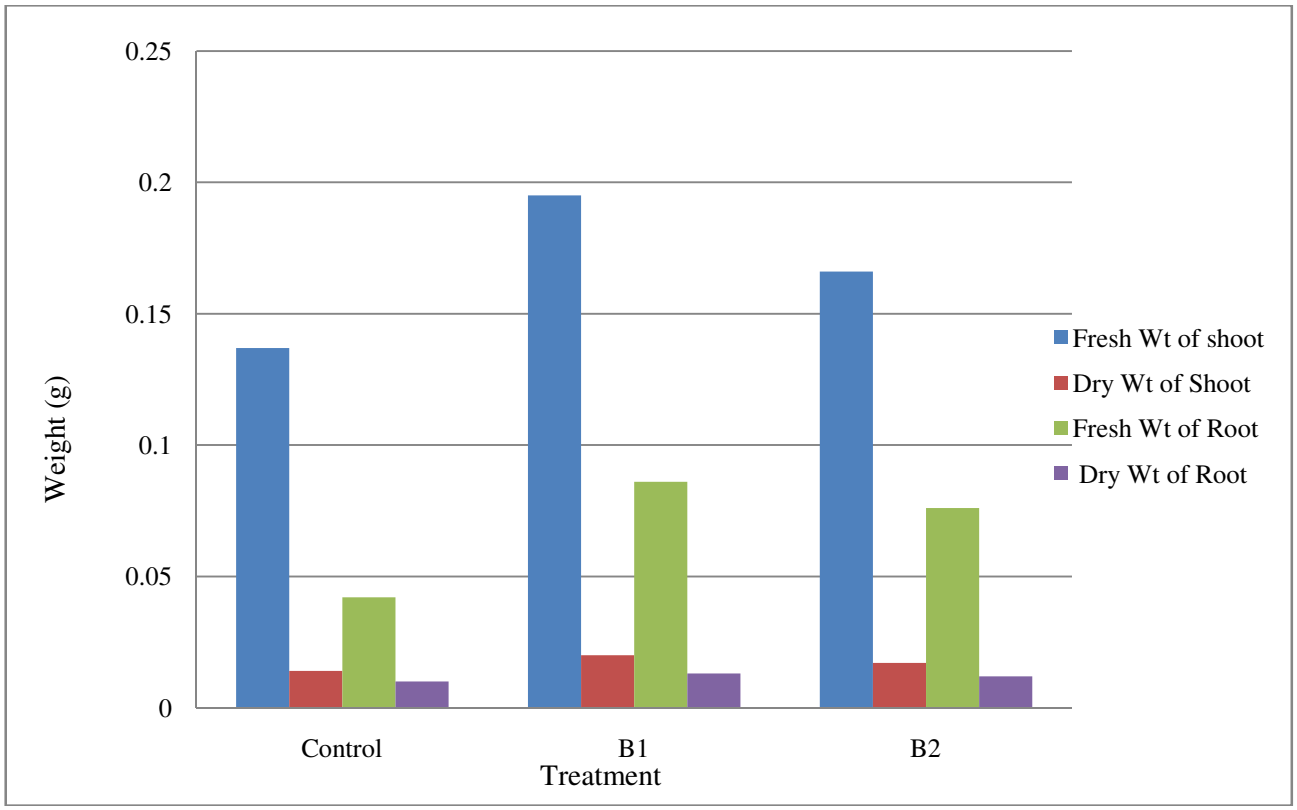

Figure-11: Effect of potent IAA producing bacteria on Jowar growth development.

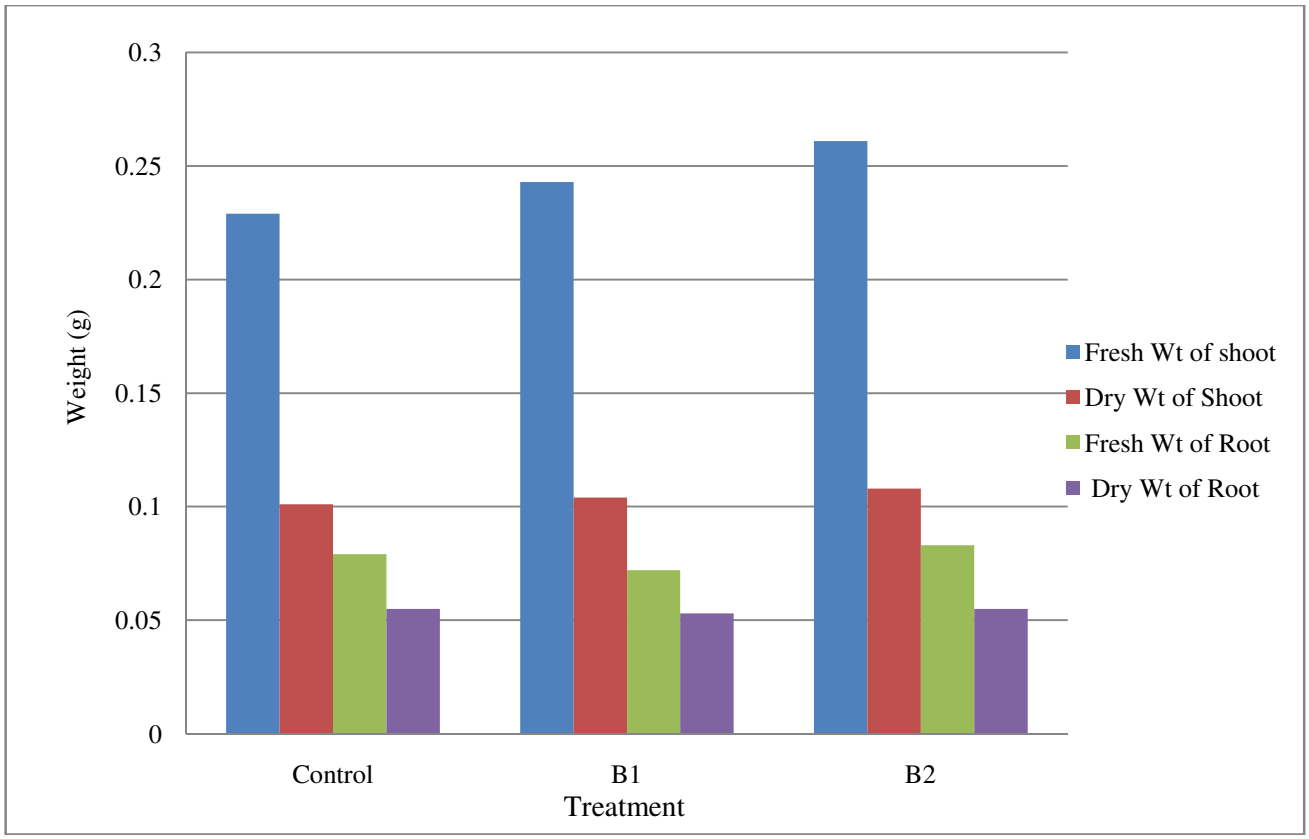

Figure-12: Effect of potent IAA producing bacteria on Wheat growth development.

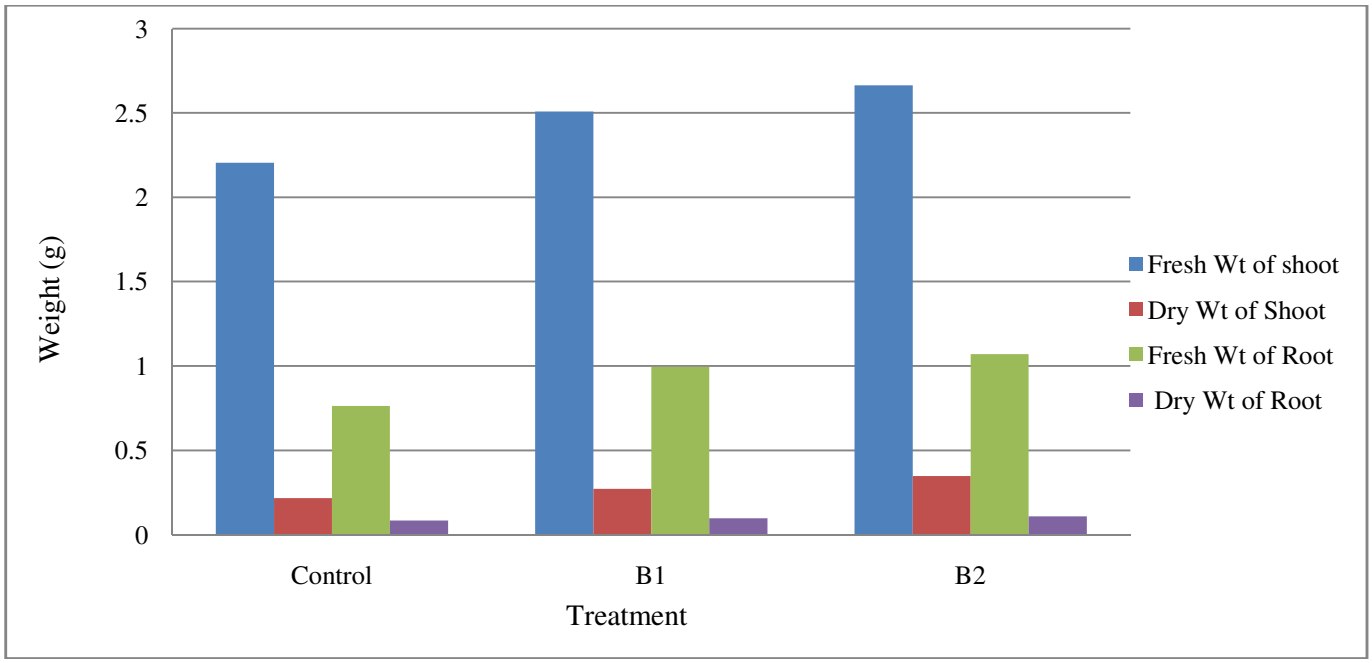

Figure-13: Effect of potent IAA producing bacteria on Groundnut plant growth development.

Discussion: IAA production is an important trait to screen beneficial microorganisms and IAA producing bacteria have been found to have stimulatory effect on plant growth[23]. The seventeen bacteria were isolated from digester effluent by using standard microbiological methods. These isolates were further screened to detect their ability to produce IAA. Two bacterial isolates namely *Bacillus amyloliquefaciens*-B1 and *Brevundimonas diminuta*-B2 produced IAA as 15 mg/L and 62.5mg/L respectively. Among these two, *Brevundimonas diminuta* was the best IAA producer. Earlier studies have reported that Gram negative bacteria are most common IAA producers with the very few bacterial members from Gram positive group including Bacillus genus[23]. There are variations in bacterial IAA production ability with reference to several cultural and environmental conditions[24]. IAA production by rhizobacteria was reported by Lwin et al[25] in the range 15-97.2 mg/L. Malik and Sindhu[26] reported IAA yield as 10.2 to 31.2mg/L in different *Pseudomonas species*. The range of IAA production in *Azotobacter species* and *Pseudomonas species* was reported to be 7.3-32.8 and 23.4-53.2mg/L respectively[27]. The present result matches with the previous findings.

The two potent IAA producing bacterial isolates were selected for testing their plant growth promotion ability by pot assay method. The plant growth development throughout the experiment was noted in the form of length, fresh and dry weight of uprooted shoots and roots separately. It was found to be increased in test crop in significant level. In Maize plants, as compared to control, bacterial isolate-1 (B1) stimulated the development of shoot by 8.82, 36.05 and 15.73% respectively whereas stimulated development of roots by 16.36, 2.08 and 8.70% respectively. Bacterial isolate-2(B2) was found to be more stimulatory than B1. As compared to control, B2 isolate stimulated the development of shoot by 13.28, 37.07 and 15.73% respectively whereas stimulated development of roots by 37.09, 13.73 and 26.09% respectively.

In case of Jowar plants, as compared to control, B1 isolate stimulated the development of shoot by 38.30, 42.34 and 42.86% respectively whereas stimulated development of of roots by 82.95, 104.76 and 30% respectively. The B2 was found to have low efficiency in stimulation than B1 in Jowar plants. As compared to control, B2 isolate stimulated the development of shoot by 31.45, 21.17 and 21.43% respectively whereas stimulated development of roots by 48.84, 80.95 and 20% respectively.

In Wheat plants, B1 isolate stimulated the development of of shoot by 15.17, 6.11 and 2.97% respectively whereas no stimulatory effect was seen on root development. As compared to control, B2 isolate stimulated the development of shoot by 18.33, 13.97 and 6.93% respectively whereas stimulated development of roots by 6.69, 5.06 and 7.27% respectively.

In Groundnut plants, B1 isolate stimulated the development of shoot by 14.09, 13.79 and 25.46% respectively whereas stimulated development of roots by 22.36, 30.45 and 15.66% respectively. As compared to control, B2 isolate stimulated the development of shoot by 32.87, 20.87 and 61.11% respectively whereas stimulated development of roots by 36.47, 40.42 and 31.33% respectively.

The B2 isolate was found to be superior to B1 in plant growth promotion in tested crop plants except Jowar. Statistical analysis of the results by P test revealed that these isolates stimulate the plant growth significantly than control. Further, these two isolates produced siderophores and other PGPR traits.

The digester effluent is known to enhance the soil fertility for crop production[12,13]. Inoculation with IAA positive bacteria showed increased development of root and shoot for inoculated plants[28]. *Bacillus species* and *Pseudomonas species* are known as excellent plant growth promoters[12,13]. *B. amyloliquefaciens* strains have gain attention as PGPR since it produces several plant growth promoting compounds[29]. *Brevundimonas diminuta (*formerly classified as *Pseudomonas diminuta)* isolated from digester effluent having plant growth promoting ability. These bacteria also produced siderophores and cyanide that have ability to promote plant growth indirectly by pathogen elimination[30].

Conclusion

The two efficient IAA producing bacteria were isolated from biogas digester run on vegetable waste. These isolates stimulated development of roots and shoots in crops as revealed by pot assay. The identification of these isolates was confirmed by 16 S rRNA gene analyses as *Bacillus amyloliquifaciens-B1* and *Brevundimonas diminuta-B2*. It is evident that digester effluent can be a rich source bacteria producing IAA. These isolates also possess other plant growth promoting traits as well. The study suggests the IAA producing bacteria isolated from digester effluent can be used as efficient biofertilizer inoculants in sustainable development of agriculture and will play important role in prevention of environmental pollution by avoiding excessive applications chemical fertilizers.

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