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Investigating the Gut Bacteria of Earthworm Inhabiting Natural Farming System for their Plant Growth-Promotional Traits

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Abstract

Earthworm gut is considered as ideal habitat for microorganisms and they increase microbial activities by providing their gut mucus and considerable physico-chemical conditions. Though earthworm-inhabiting microbes play an important role in soil fertility and decomposition, the diversity of microorganisms associated with earthworm needs to be studied and explored. In the present study, composition and diversity of the gut bacteria associated with earthworm inhabiting natural farming system was studied. Earthworm samples were collected from natural farming fields at distinct depth. The collected earthworms were carried to the laboratory for further studies. Based on the morphological features, the earthworms were identified as *Eudrilus eugeniae.* Their gut content was extracted by dissection method for bacterial isolation by standard plating techniques. A total forty-four gut bacterial isolates were obtained and they were morphologically characterized. Based on qualitative screening for their ability to produce siderophore and to solubilize phosphate and potassium, Ten superior gut bacterial isolates were selected for further screening for ammonia and phytohormone production. All the superior bacterial isolates were found positive for their nitrogen fixing ability and phytohormone production. The range of nitrogen-fixation ability of bacterial isolates were from 1.42 to 4.13 mg/L. EGNF-12 (35.23 µg/ml) and EGNF-9 (32.14 µg/ml) had the highest IAA production while EGNF-12 (6.14 µg/ml) and EGNF-15 (6.16 µg/ml) also produced the highest levels of gibberellic acid (GA), respectively. These findings suggest the potential of earthworm-associated microbes in enhancing soil fertility and promoting sustainable agricultural practices in natural farming systems.

Keywords : Gut bacteria of earthworm, Natural farming, Phytohormone

Earthworms are terrestrial invertebrates belonging to the phylum *Annelida*, known for their long, segmented bodies and role in soil ecosystems. These organisms, often referred to as 'nature's plough', burrow through the soil, enhancing its structure, aeration and water-holding capacity. They play a pivotal role in maintaining soil health and fertility, making them integral to natural farming systems. Earthworms are particularly beneficial in natural farming fields, as their activities promote nutrient cycling and help decompose organic matter, turning it into nutrient-rich humus that plants can easily absorb (Singh *et al.,* 2015). Additionally, their digestive tract, particularly their gut harbours a diverse community of microbes, that play a pivotal role in soil ecosystem function and productivity.

One of the key aspects of earthworm functioning is their gut microbiome, which harbours a variety of beneficial bacteria. As earthworms consume organic matter, these bacteria aid in breaking it down, further enhancing nutrient availability. Gut bacteria that have nitrogen-fixing and phosphate-solubilizing activities can directly promote plant growth by making essential

nutrients more accessible. Microbes associated with the earthworm gut also promote plant growth by stimulating various beneficial processes through several direct and indirect mechanisms. They produce plant growth-promoting hormones like auxins, cytokinins and gibberellins which stimulates root growth, enhance nutrient uptake and improve overall plant vigour (Houida *et al.,* 2022).

The gut microbiome of earthworm plays multifaceted roles in promoting soil fertility, enhancing nutrient cycling, suppressing diseases and promoting plant growth. However, the composition and function of these microbes vary depending on the type of earthworm's feeding habit, their habitat or farming system and associated soil environment (Jin *et al.,* 2022). In natural farming fields, high organic matter content and minimal disturbance favor a diverse microbial community in the earthworm gut. This diversity supports complex ecological interactions and promotes soil health, nutrient cycling and plant growth. This diversity of bacteria found in earthworm gut need to be investigated and studied. So, the present study was to isolate bacteria from gut of earthworm inhabiting natural farming system and to screen them for plant growth promoting traits.

Material and Methods

Collection, Identification and Extraction of Earthworm Gut Content

Earthworm along with soil samples were collected from natural farming field (13°35'49" N, 77°27'03" E) in Mudigere village of Chikkaballapur district, Karnataka, India. Three pits of size 1 cubic meter were burrowed in field to collect earthworms at distinct depth. Physico-chemical properties of soil like pH, EC, organic carbon, available nitrogen, phosphorus and potassium were estimated.

Collected earthworms along with soil were placed into sterile air-tight polythene bags and brought to Department of Agricultural Microbiology, UAS, GKVK, Bengaluru for further studies. The earthworm species was identified based on morphological features (Gates, 1972).

For gut extraction, the live earthworms were washed thoroughly and surface sterilized with 70 per cent ethanol. The skin was cut open to expose the gut from the dorsal site without puncturing the alimentary canal. The intestine was carefully cut open from post-clitellum to posterior end with a fine sterile blade following dissection method (Samanta and Das, 2016). The intestinal content was taken in Phosphate saline buffer and stored under refrigerator for further studies.

Isolation of Bacteria from Earthworm Gut

The intestinal content was serially diluted and standard plating technique was followed for isolating bacteria on nutrient agar media. The culture plates were incubated at 30°C for 48 hours in order to get distinct microbial colonies. The bacterial isolates obtained were purified by four-way streak plate method and preserved on agar slants at 4°C for further studies.

Qualitative Screening of Bacterial Isolates for Plant Growth Promotional Traits

The bacterial isolates obtained were qualitatively screened based on their ability to solubilize phosphate and potassium and siderophore production.

Phosphate Solubilization

Preliminary screening for phosphate solubilization was done by a plate assay method using Pikovskaya's agar medium (Pikovskaya, 1948) supplemented with tricalcium phosphate where bacterial isolates were spot inoculated and incubated up to 7 days at 30°C. Formation of distinct clear zones around bacterial colonies indicates solubilizing ability. The results were expressed as phosphate solubilization index (SI).

Potassium Solubilization

Preliminary screening for potassium solubilization was done by a plate assay method using Aleksandrov's agar medium supplemented with insoluble potassium bearing mineral (Mica) where bacterial isolates were spot inoculated and incubated up to 7 days at 30°C. Formation of distinct clear zones around bacterial colonies indicates solubilizing ability. The results

were expressed as potassium solubilization index (SI) (Hu *et al.,* 2006).

Siderophore Production

A qualitative assay of siderophore production was conducted in Chrome Azurol S (CAS) agar medium. CAS agar plates were prepared and spot-inoculated with test organism and incubated at 30°C for 3-5 days. Change of blue color of the medium surrounding the bacterial growth to fluorescent yellow indicated the production of siderophore (Schwyn and Neilands, 1987).

Quantitative Screening of Bacterial Isolates for Plant Growth Promotional Traits

After qualitative screening, the bacterial isolates were biochemically characterized and further screened for ammonia and phytohormone (Indole acetic acid and Gibberellic acid) production.

Biochemical Characterization

The biochemical characterization of the isolates was essentially carried out as per the procedures outlined by Cappuccino and Sherman (1992). The tests conducted are detailed below.

Catalase Test

Bacterial colonies (24-h-old) were taken on glass slides and one drop of H_2O_2 (30%) was added. Appearance of gas bubble indicated the presence of catalase enzyme.

Oxidase Test

A strip of filter paper was dipped in Kovac's reagent and air-dried. With the help of sterile wire loop, one day old colonies of bacterial cultures from agar plates were transferred on this filter paper strip. The oxidase positive colonies show lavender-colored, which become dark purple to black in color within 5 min.

Citrate Utilization Test

The citrate utilization test was performed by inoculating the microorganisms into Simmon's citrate agar, where sodium citrate was the only carbon and energy source. Bromothymol blue was added as an indicator. After incubation at 30°C for 48 h, the cultures were observed for the growth and colouration of the medium. A positive test is demonstrated by growth with a color change from green to intense blue along the slant.

Methyl Red Test

Tubes containing the sterilized MR-VP broth were inoculated with isolated bacterial strains and one tube as uninoculated comparative control and were incubated at 37°C for 48 hours. After incubation, five drops of methyl red indicator was added to each tube. When methyl red was added, it remained red which was indicative of positive test while turning of methyl red to yellow indicated the negative test.

Voges - Proskauer Test

The test cultures were inoculated to the pre-sterilized tubes containing MR-VP broth and were incubated for 48 hours at 37°C. After incubation, ten drops of Barrett's reagent A was added and gently shaken followed by addition of ten drops of Barrett's reagent B. The development of rose colour in the broth was considered as positive for the test.

Hydrogen Sulfide Production

Sulfide indole motility (SIM) agar stabs were inoculated with the bacterial isolates and incubated at 30°C for 48 hrs. Black coloration along the line of stab inoculation indicated H_2S production.

Ammonia Production

The bacteria were cultured in peptone water broth, shaken at 180 rpm and incubated at 30°C. After 48 hours of incubation, the broth was centrifuged at 10,000 rpm for 2 min at 4°C and the supernatant was collected. NH_4^+ concentration was determined by the Nessler method. After treatment with Nessler's reagent, sample developed a yellowish-brown colour. The colour intensity of solution corresponds to the amount of ammonia originally present. The concentration of ammonia was determined colorimetrically with Nessler's reagent at the wavelength of 420 nm (Ha *et al.,* 2018).

Indole Acetic Acid (IAA) Production

Production of Indole acetic acid (IAA) by bacterial isolates was determined by following the method described by Gordon and Paleg (1957). The bacterial cultures were inoculated in nutrient broth with tryptophan (5μg/mL) and incubated at 30°C for 5 days. After incubation, cultures were centrifuged at 6000 rpm for 15 min. Two ml of the supernatant was mixed with 2 drops of orthophosphoric acid and 4 mL of Salkowski's reagent (50 ml of 35 per cent perchloric acid + 1 ml 0.5 $FeCl_3$) and incubated in the dark for 25 minutes. Development of pink colour indicates indole-3-acetic acid (IAA) production. The optical density was measured at 530 nm using Spectrophotometer. The quantity of IAA production was estimated using standard IAA graph and expressed as micrograms per milliliter.

Gibberellic Acid Production

The gibberellic acid (GA) produced by bacterial isolates was determined by adopting the method described by Paleg (1965). The bacterial cultures were inoculated in nutrient broth and incubated at 30°C for 7 days. After seven days of incubation, 25 ml of the culture filtrate was collected in a test tube to which two ml of zinc acetate was added. Then, 2 ml of potassium ferrocyanide was added and centrifuged at 1,000 rpm for 15 minutes. Five ml of 30 per cent HCl was added to five ml of supernatant and incubated at 20°C for 75 minutes. The blank sample was treated with five per cent HCl. The absorbance of the samples as well as the blank was measured at 254 nm in a UV spectrophotometer. The amount of GA present in the extract was calculated from the standard curve and expressed as micrograms per ml of the medium.

Statistical Analysis

The data which are obtained from all the experiments were subjected to the statistical analysis to evaluate effects of treatments. Analysis was carried out by completely randomized design (CRD) using software WASP-2 tool (Duncan, 1995).

Results and Discussion

Earthworm along with soil samples were collected from natural farming field (13°35'49" N, 77°27'03" E) in Mudugere village of Chikkaballapur district, Karnataka, India. Physicochemical properties of the soil were estimated and presented in Table 1.

Table 1

Physicochemical properties of soil collected from natural farming field

Physicochemical properties of soil	
Texture	Red loamy
pH	6.4 ± 0.06
EC (dS m^{-1})	0.32 ± 0.005
OC (%)	0.74 ± 0.01
Available N (kg ha^{-1})	199.32 ± 3.15
Available P (kg ha^{-1})	19.01 ± 0.30
Available K (kg ha^{-1})	161.80 ± 2.55

Earthworms were collected from three randomly burrowed pits (1m^3) at distinct depth of soil profile. The population of earthworm at distinct depth was recorded and tabulated in Table 2. A total of 21 earthworms were collected from three different pits out of which, nine earthworms are from pit-1, six earthworms are from pit-2 and six are from pit-3.

The collected earthworms were carried to the laboratory for further studies. The population of earthworm in three different pits was found in between

Table 2

Population of earthworm inhabiting natural farming field at distinct depth

Soil profile at distinct depth (m)	Population of earthworm in three different pits		
	Pit-1	Pit-2	Pit-3
0 - 0.2	4	4	5
0.2 - 0.4	2	2	1
0.4 - 0.6	0	0	0
0.6 - 0.8	0	0	0
0.8 - 1.0	0	0	0

0-0.4m of soil profile. This indicates that the earthworms are epigeic (surface feeder) as their activity is high at surface level. An adult earthworm was selected randomly to study its morphological features. Based on the morphological features like surface feeding habit, >100mm size, epilobic mouth, pigmented body, lumbricine setal arrangement and intestinal origin at fifteenth segment, the earthworm was identified as *Eudrilus eugeniae* as per available literature of Blakemore, 2015.

After earthworm collection and identification, it was taken into dissection tray to extract gut content where the skin was cut open to expose the gut from the dorsal site without puncturing the alimentary canal (Plate 1).

The gut content was mixed with 1X phosphate saline buffer (pH=6.9) and it was vortexed. The gut content was serially diluted and standard plating technique was followed for isolating bacteria on nutrient agar media. From this, a total forty-four bacterial isolates were obtained. They were purified on culture media and stored at 4°C for further studies.

Morphological characteristics of earthworm gut bacterial isolates were recorded and presented in Table 3. Out of forty-four isolates, thirty-six isolates have circular shaped colonies and remaining eight has irregular shaped colonies. Isolates mainly exhibited circular colony shapes with colors ranging from white to creamy white and yellow. Most had a slimy texture, except for a few with rough or wrinkled surfaces. Majority of them were Gram-positive, rod shaped and found motile in nature.

Qualitative Screening of Bacterial Isolates for Plant Growth Promoting Traits

Forty-four gut bacterial isolates were subjected to qualitative screening for their ability to solubilize phosphate and potassium and also siderophore production (Table 4).

Out of forty-four, twenty-one isolates showed positive results for phosphate solubilization with solubilization indices ranging from 1.70 to 3.10. The highest phosphate solubilization index is seen in EGNF-8 with a value of 3.10. Similarly, twelve showed positive results for potassium solubilization. The solubilization indices ranged from 1.65 to 2.20, with EGNF-5 showing the highest potassium solubilization index of 2.20. Also, twenty-four showed positive results for siderophore production by

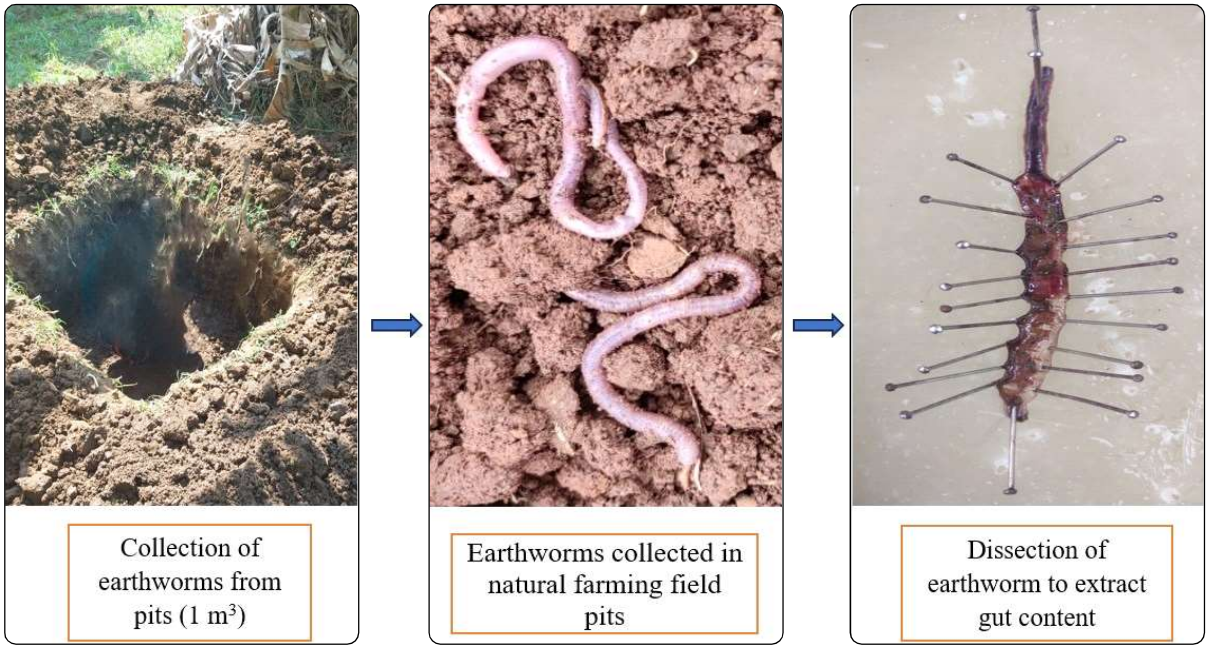

Plate 1 : Collection of earthworms from natural farming field and its dissection to extract gut content for isolation of gut bacteria

TABLE 3

Morphological Characteristics of bacteria isolated from gut of earthworm inhabiting natural farming system

Isolate code	Colony Morphology			Cell Morphology		
	Shape	Colour	Texture	Gram reaction	Cell shape	Motility
EGNF-1	Circular	white	Slimy	-	Rod	+
EGNF-2	Circular	creamy white	Slimy	+	Rod	+
EGNF-3	Circular	white	Slimy	+	Rod	+
EGNF-4	Circular	white	Slimy	+	Rod	-
EGNF-5	Circular	milky white	Slimy	+	Rod	+
EGNF-6	Irregular	white	Rough	+	Rod	+
EGNF-7	Circular	yellow	Rough	-	Rod	+
EGNF-8	Circular	white	Rough	+	Rod	-
EGNF-9	Circular	white	Slimy	-	Cocci	+
EGNF-10	Irregular	white	Wrinkled	+	Rod	+
EGNF-11	Irregular	white	Rough	+	Rod	+
EGNF-12	Irregular	white	Wrinkled	+	Rod	+
EGNF-13	Circular	creamy white	Slimy	-	Rod	+
EGNF-14	Circular	creamy white	Slimy	+	Rod	+
EGNF-15	Circular	white	Slimy	-	Rod	-
EGNF-16	Circular	milky white	Slimy	-	Cocci	+
EGNF-17	Circular	white	Rough	+	Rod	+
EGNF-18	Circular	white	Rough	+	Rod	-
EGNF-19	Circular	white	Rough	-	Rod	+
EGNF-20	Circular	white	Rough	+	Rod	+
EGNF-21	Irregular	white	Slimy	+	Rod	+
EGNF-22	Circular	creamy white	Slimy	-	Rod	+
EGNF-23	Circular	yellow	Slimy	-	Rod	+
EGNF-24	Circular	white	Slimy	+	Rod	+
EGNF-25	Circular	white	Slimy	+	Rod	-
EGNF-26	Circular	white	Slimy	-	Cocci	-
EGNF-27	Irregular	white	Rough	+	Rod	+
EGNF-28	Irregular	white	Wrinkled	+	Rod	+
EGNF-29	Circular	white	Rough	+	Rod	+
EGNF-30	Circular	creamy white	Slimy	-	Rod	+
EGNF-31	Circular	white	Slimy	+	Rod	+
EGNF-32	Circular	white	Slimy	-	Cocci	+
EGNF-33	Circular	white	Slimy	+	Rod	+
EGNF-34	Circular	white	Slimy	+	Rod	-
EGNF-35	Circular	white	Slimy	+	Rod	+

Continued....

TABLE 3 Continued....

Isolate code	Colony Morphology			Cell Morphology		
	Shape	Colour	Texture	Gram reaction	Cell shape	Motility
EGNF-36	Circular	white	Slimy	-	Rod	-
EGNF-37	Circular	white	Slimy	+	Rod	+
EGNF-38	Circular	white	Slimy	+	Rod	+
EGNF-39	Circular	white	Rough	-	Rod	+
EGNF-40	Circular	white	Rough	-	Rod	+
EGNF-41	Irregular	white	Rough	+	Rod	+
EGNF-42	Circular	white	Slimy	+	Rod	-
EGNF-43	Circular	white	Slimy	-	Cocci	+
EGNF-44	Circular	white	Slimy	+	Rod	+

Note : '+' - positive; '-' - negative

TABLE 4

Screening of gut bacterial isolates for phosphate and potassium solubilization

Isolate	P-solubilization		K-solubilization		Siderophore production
	Qualitative assay	Solubilization index	Qualitative assay	Solubilization index	
EGNF-1	-	0.00	-	0.00	-
EGNF-2	+	2.20	-	0.00	+
EGNF-3	+	2.15	+	1.80	+
EGNF-4	-	0.00	-	0.00	-
EGNF-5	-	0.00	+	2.20	+
EGNF-6	+	2.40	-	0.00	+
EGNF-7	-	0.00	-	0.00	-
EGNF-8	+	3.10	-	0.00	+
EGNF-9	+	1.95	+	2.10	+
EGNF-10	+	3.10	-	0.00	+
EGNF-11	-	0.00	-	0.00	-
EGNF-12	+	2.65	+	1.95	+
EGNF-13	+	2.30	-	0.00	+
EGNF-14	-	0.00	-	0.00	-
EGNF-15	+	2.35	+	0.00	+
EGNF-16	-	0.00	-	0.00	-
EGNF-17	+	2.55	+	1.65	+
EGNF-18	-	0.00	-	0.00	-
EGNF-19	+	1.95	+	1.85	+

Continued....

Table 4 Continued....

Isolate	P-solubilization		K-solubilization		Siderophore production
	Qualitative assay	Solubilization index	Qualitative assay	Solubilization index	
EGNF-20	+	2.45	+	1.80	+
EGNF-21	-	0.00	-	0.00	-
EGNF-22	-	0.00	-	0.00	-
EGNF-23	+	1.95	+	1.85	+
EGNF-24	+	1.80	-	0.00	+
EGNF-25	-	0.00	+	1.90	-
EGNF-26	+	2.15	-	0.00	+
EGNF-27	-	0.00	-	0.00	-
EGNF-28	-	0.00	+	1.70	+
EGNF-29	+	1.85	-	0.00	+
EGNF-30	-	0.00	-	0.00	-
EGNF-31	+	1.75	+	1.65	+
EGNF-32	+	1.95	-	0.00	+
EGNF-33	-	0.00	-	0.00	-
EGNF-34	-	0.00	-	0.00	-
EGNF-35	+	1.80	-	0.00	+
EGNF-36	-	0.00	+	1.90	+
EGNF-37	-	0.00	-	0.00	-
EGNF-38	+	1.95	-	0.00	+
EGNF-39	-	0.00	-	0.00	-
EGNF-40	+	1.85	-	0.00	+
EGNF-41	-	0.00	+	1.75	+
EGNF-42	+	1.70	-	0.00	+
EGNF-43	+	2.05	-	0.00	+
EGNF-44	-	0.00	-	0.00	-

Note: '+' - positive; '-' - negative

producing yellow transparent zones around bacterial colonies.

Bacteria that solubilize phosphate and potassium play a critical role in improving nutrient availability to plants (Nalini and Muthuraju, 2022). This is especially valuable in low-nutrient soils or natural farming systems. Siderophores assist in the sequestration of iron, an essential micronutrient for plants. Several studies highlight the role of phosphate and potassium-solubilizing bacteria in enhancing plant growth and yield which emphasizes the positive impact of these bacteria in sustainable agriculture.

Secondary Screening of Bacterial Isolates for Plant Growth Promotional Traits

Qualitative screening resulted in ten superior gut bacterial isolates *i.e.,* EGNF-3, EGNF-5, EGNF-9, EGNF-10, EGNF-12, EGNF-13, EGNF-15, EGNF-17, EGNF-19 and EGNF-20 were selected for further screening for ammonia and phytohormone (indole acetic acid and gibberellic acid) production.

TABLE 5

Biochemical characteristics of screened gut bacterial isolates

Isolate	Oxidase	Catalase	Citrate utilization	Methyl red	Voges Proskauer	H_2S production
EGNF-3	+	+	+	+	-	-
EGNF-5	+	+	+	+	+	-
EGNF-9	+	+	+	-	+	-
EGNF-10	+	+	+	-	-	-
EGNF-12	+	+	+	-	+	-
EGNF-13	-	+	+	-	-	-
EGNF-15	+	+	+	-	+	-
EGNF-17	+	+	+	+	-	-
EGNF-19	+	+	+	+	-	-
EGNF-20	-	+	+	+	+	-

Note : '+' - positive; '-' - negative

These ten superior bacterial isolates were biochemically characterized and are presented in Table 5. Bacterial isolates were tested for various enzymatic and metabolic traits, such as oxidase, catalase, citrate utilization, methyl red, Voges-Proskauer and hydrogen sulfide (H_2S) production. Isolates such as EGNF-3, EGNF-5 and EGNF-12 exhibited positive results for oxidase and catalase activity, indicating their aerobic metabolic potential. Citrate utilization and methyl red tests showed that most isolates were capable of utilizing citrate and fermenting glucose. EGNF-3, EGNF-12 and EGNF-15 were positive for most tests but negative for certain traits like Voges-Proskauer or H_2S production.

The positive results in catalase and citrate utilization tests indicate the bacteria's resilience and metabolic versatility. Catalase-positive isolates can survive oxidative stress in the soil, while citrate-utilizing bacteria can make use of citrate exudates from plant roots. Such traits make bacteria better candidates for plant growth promotion under natural farming systems (Bindushree and Shivaprakash, 2022).

After biochemical characterization, these bacterial isolates were assessed for production of ammonia (NH_4^+) and results are indicated in Fig. 1. All the bacterial isolates were found positive for their nitrogen fixing ability. The range of nitrogen-fixation ability

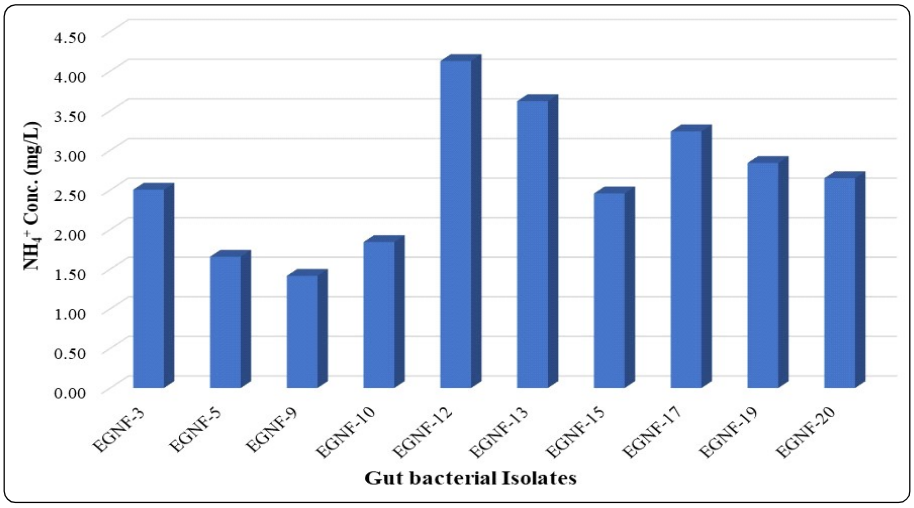

Fig. 1 : Ammonia production (NH_4^+) by superior gut bacterial isolates

of bacterial isolates were from 1.42 to 4.13mg/L. EGNF-12 could fix the highest amount of nitrogen (4.13mg/L).

Ammonia production is often linked with other beneficial traits like phosphate solubilization and siderophore production. The symbiotic relationship between earthworms and gut bacteria plays a pivotal role in nutrient cycling. Recent research by Houida *et al.* (2024), emphasized how earthworms, through their gut bacteria, can enhance nitrogen mineralization processes, leading to better plant nutrient availability.

Phytohormone Production by Gut Bacterial Isolates

Gut bacterial isolates were also tested for their ability to produce important plant hormones like Indole Acetic Acid (IAA) and Gibberellic Acid (GA), which are essential for plant growth and data is presented in Table 6. All the bacterial isolates were able to produce phytohormones. EGNF-12 and EGNF-9 had the highest IAA production with values of 35.23 µg/ml and 32.14 µg/ml. Similarly, EGNF-12 and EGNF-15 also produced the highest levels of gibberellic acid (GA), with concentrations of 6.14 µg/ml and 6.16 µg/ml, respectively.

Table 6

Estimation of phytohormone production by screened gut bacterial isolates

Isolate	IAA (µg/ml)	GA (µg/ml)
Control	0.00 ^{g}	0.00 ^{g}
EGNF3	17.26 ^{cd}	4.23 ^{cd}
EGNF5	14.56 ^{de}	5.21 ^{b}
EGNF9	32.14 ^{a}	3.95 ^{cd}
EGNF10	20.17 ^{bc}	3.12 ^{ef}
EGNF12	35.23 ^{a}	6.14 ^{a}
EGNF13	23.51 ^{b}	4.56 ^{bc}
EGNF15	18.21 ^{cd}	6.16 ^{a}
EGNF17	12.82 ^{ef}	3.08 ^{ef}
EGNF19	9.75 ^{f}	2.75 ^{f}
EGNF20	17.33 ^{cd}	3.54 ^{de}

Note : Numerical values are mean of three replicates. Treatments with the different superscripts represent a significant difference as determined by DMRT ($p \leq 0.05$)

IAA and GA production is directly linked to root elongation and shoot growth in plants. Earthworm gut bacteria's ability to produce phytohormones like IAA and GA is well documented in natural systems, where they enhance soil fertility and plant health. These results were in accordance with Biswas *et al.* (2018). The property of synthesizing IAA is considered as an effective tool for screening beneficial microorganisms suggesting that IAA producing bacteria have profound effects on plant growth.

The study provides detailed morphological, biochemical and functional profiles of earthworm gut bacterial isolates. Gut bacteria isolated from earthworms in natural farming systems exhibited plant growth-promotional traits, such as nutrient solubilization and production of growth hormones. These traits enhance soil fertility and support sustainable agriculture by promoting nutrient availability and improves plant health.

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