Zooplankton Surface Attached Bacteria Potentially Usable as A Plant Growth Promoter- an In-Vitro Study

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ABSTRACT

Present study analysed the diversity and ecology of zooplankton, hydrophytes and plankton associate bacteria of wetlands of freshwater ecosystem. In course of studies, we found four orders (cyclopoida, calanoida, podocopida&anomopoda) of zooplankton under two phyla (Arthropoda, rotifer). Distribution patterns of zooplankton species composition revealed discontinuous distribution in the study sites. In study sites SS1 and SS5 calanoida population are absent, where highest diversity of calanoida was found in study sites-3 (SS3). A total of sixty bacterial isolates were screened from zooplankton population of six different study sites, based on their colony morphology (shape, size and arrangement) and gram nature reaction nine native bacterial isolates were selected for further investigation during the study periods. These isolates have also been treated as plant growth promoting (PGPR) bacteria against five seeds like cauliflower, cabbage, tomato, ladies finger and mungbean seed, also finding few PGPR factors like indole acetic acid (IAA), salicylic acid (SA) HCN and NH_3.Overall, all strains are grown at a broad range of pH (3-9), temperature (25°C - 50°C) and salt concentration (1% - 9%) in in-vitro condition. The PGPR factors study revealed that all the isolate actively produces all types of tested plant growth promoting factors i.e., IAA, SA, HCN and NH_3. Among 9 bacterial strainsS1, S5 and S7 are three potent isolates, which have the strong plant growth promotion (PGPR) activity. With respect to the control, S1 actively promote the growth of cauliflower (VI- 416.67), ladies' finger (VI- 1369.33) and mungbean (VI-2516.56) seeds, where S5 and S7 isolates were actively work against cabbage (VI- 737.33) and tomato (VI- 937.67), respectively. So, the present research findings have unearthed some new and interesting research information pertaining to the trophic interactions in between zooplankton and bacteria in freshwater aquatic ecosystem and role of plankton associate bacteria as a PGPR.

1. Introduction:

Aquatic ecosystem provides a habitat for aquatic organisms. Aquatic organisms can be of two types macroorganisms like zooplankton, phytoplankton, bacteria etc and microorganisms like macrophytes, fishes and other large organisms. Zooplankton plays an important intermediate role in food chain between phytoplankton and small fish and helps in transferring the energy to higher trophic level, they also helps in regulating the self-purification capacity of the waterbody (Midya et al., 2018). Environmental heterogeneity effects the zooplankton population and create a certain distribution pattern that varies with different habitats, temperature, salinity, conductivity has immense effect on zooplankton diversity and abundance (Midya et al., 2018). Climatic condition of West Bengal is extremely diverse and create considerable effect on zooplankton diversity.

In an aquatic system bacterial population can be seen as planktonic form (free floating) or can form biofilm that remains attached with the different part of zooplankton (Lawrene et al., 1987). Bacterial attachment can be seen on the exoskeleton as well as on the gut lining of copepods which act as a favorable growth surface for the zooplankton associated bacteria (Nagasawa and Nemoto, 1988; Pruzzo et al., 1996; Carman and Dobbs, 1997). This interactive association must help the bacteria to have a protective shelter and can provide organic carbon comes from chitinous appendages of copepods (Verschoor et al., 2000a, b). This process of bacterial attachment can also be observed in non- crustaceans' zooplankton like Appendicularia's (Flood, 1991), jellyfish (Schuett and Doepke, 2009) and rotifers (Selim, 2001). These bacterial population can have some antifungal properties. Fungal inhibition can be useful in so many ways, it may have a medicinal prospective, can also be used in prevention of food spoilage and bio-preservation.

In times of rapid climate change, there is an urgent need for a proper understanding of both zooplankton–bacteria interactions and how they would react to future climate scenarios. The approach used in this study explains the screening of freshwater zooplanktonic bacteria, which possess plant growth promoting activity.

2. Materials and methods

2.1. Study Site:

The present investigation was carried out from aquatic ecosystems of freshwater lotic zone, and around certain wetlands of West Bengal, India. The samples were collected from six study sites i.e., Gope Palace (lat- 22.429564 & long- 87.290623), Medinipur (lat- 22.41402 & long- 87.323168), Khargapur (lat- 22.347953 & long- 87.276495), Mecheda (lat- 22.40.5004 & long- 87.898614), Jhargram (lat- 22.432885 & long- 87.001219) and Tamluk

(lat- 22.278367 & long- 87.91933) (Fig. 1) The samples are collected in 2022 in March, than the other study site. It effects on the growth and development of the planktons.

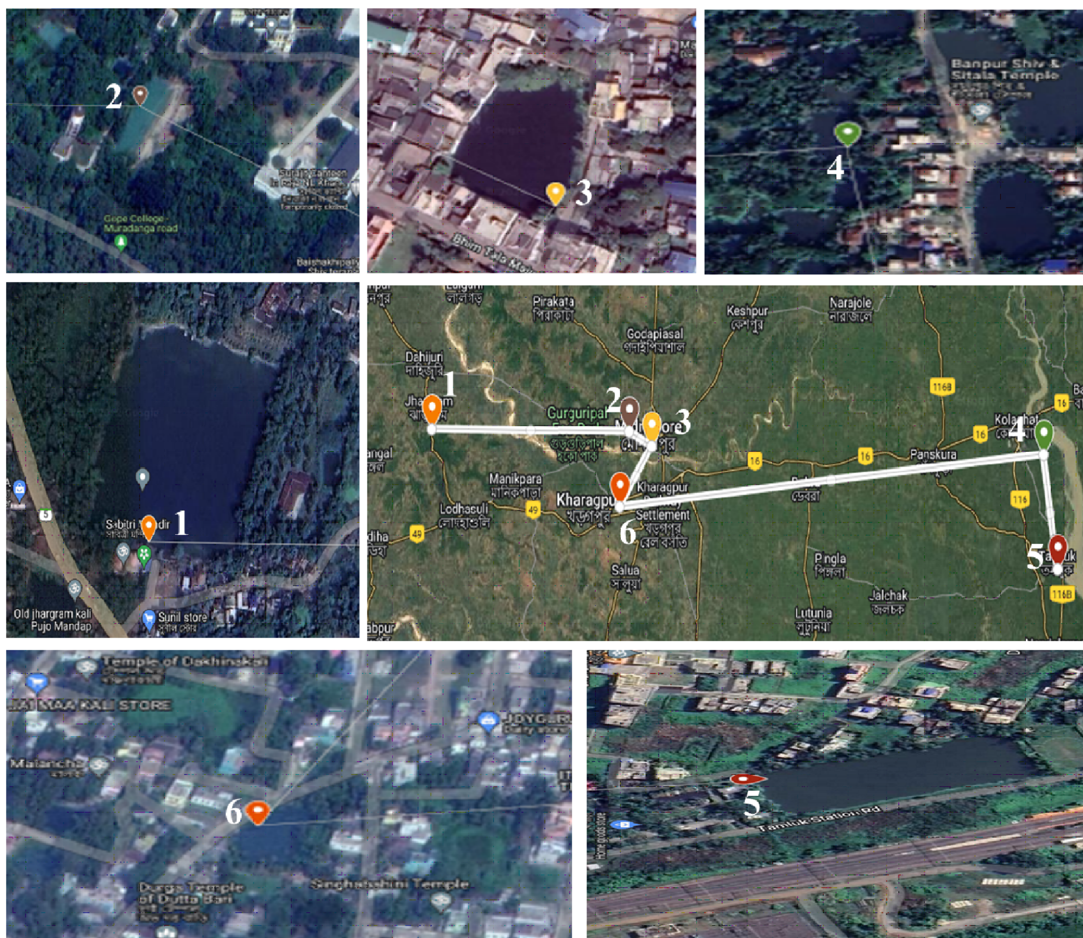

Figure-1: Geographical barrier of study sites prepared by online my map websites.

April and May mainly in early monsoon which is the most suitable period for Rotifera breeding. These ponds are mainly used in domestic purpose and sometimes used to cultivate vegetables on their edges. Study site in Kharagpur is mainly in the industrial area, much more chemicals also present in this site

2.2.Zooplankton diversity study:

Planktons mainly zooplanktons were collected scientifically from surface and sub surface zone of the pond and the wetlands. A 50 µm sized Nylobolt plankton net is used to collect the plankton. According to standard literature zooplanktons are the group lowest taxonomic

level (Battish,1992; Dussart and Dafeye,2001).The quantification of the zooplankton was done by Sedgwick-rafter cell counter under the phase contrast microscope. Planktons are collected in the cubet with formalin solution. The collected zooplanktons are numbering per amount of litter. The formula used to calculate the Sedgewick rafter cell is: Individuals/ml={A*(n/v)}/L.

2.3. Hydrophytes diversity:

Hydrophytes, the aquatic plants which possessed an adaptation power to survive in aquatic ecosystem. Various types of hydrophytic plants play an important role to balance the aquatic ecosystem (Mahalik, 2019). They are adapted themselves bysubmerging partially, totally or occasionally in water as *Utricularia sp.*, Nymphaeaceae (water lillies) etc. (Lefor, 1999). Hydrophytes can increase their surface area thereby increasing their gaseous exchange efficacy (Lefor, 1999). Different species of hydrophytes are observed in the margins of the ponds and industrial belts from sixdifferent study sites.

2.4. Isolation and screening of surface attachment bacteria:

The collected planktons and also their parts which are stored in 5% formalin. They are homogenised with a sterilized glass rod and incubated with Alkaline Peptone Water almost favourable temperature 37°C for 18-24 hours (Midya et.al., 2019). Bacterial isolates are isolated from APW medium and transferred to nutrient agar media (Media composition-4.2g agar nutrient, 0.7 g agar powder, 150 ml water) via spread technique and incubated for 72 hours at 30°. Then the particular bacterial colonies from them were screened and isolated in another fresh nutrient agar by streaking technique and incubating for 3 days at 37°. These selective bacterial colonies are used for further experiments.

2.5.Morphological characteristics:

The isolated bacteria are cultured in the petridish and the resulting colonies are identified. Zooplankton associated bacteria exhibit various type of morphological characters. They are varied in colony characteristic and also show variation among shape and gram character.

Colony characteristics: colony characteristics were examined visually and results were observed. The bacterial colony shape, margin, elevation, size all are measured. The shape of S1 and S8 colonies are only circular. Other colonies are irregular in shape. S2 showed umbonate elevation whereas many colonies (S1, S6, S8, S9) are raised in their elevation and some are flat. Most of the colonies exhibit shiny and smooth appearance. The texture of S2, S4, S7 colonies are dry but others are moist. Only S1 and S2 are resulted as yellowish and pinkish colonies respectively, other colonies exhibit white pigmented.

Shape and gram characters of isolates: slide preparation for gram staining consist some clean slides where bacterial film was drawn from 24-hour old culture and stained with gram

staining method for observation. observation done under light microscope. In bacteriology gram staining method (differential staining) is very much important because it helps in differentiating bacteria into two general classes, they are gram positive bacteria (bacteria retaining crystal violet, the primary stain) and gram-negative bacteria (bacteria that become colourless after detaining, counter stain safranin can be retained by these) (Goszczynska et al.,2000).

2.6. Effect of pH, temperature and salt concentration on isolates

2.6.1. ***Effect of pH:***

pH determined the nature of the culture media either it's acidic or alkaline. Acidity is maintained by adding conc. H_2SO_4 and alkalinity is controlled by adding proper amount of NaOH. The culture media is transformed into 5 petridishes with different pH values (1,3,5,7,9). Then the total 9 bacteria isolate i.e., S1 to S9 were transferred into nutrient agar mediaand incubated at 37°C for 24hrs, after that the result were recorded.

2.6.2. ***Effect of temperature:***

The bacteria are grown under several temperature in incubator. The effect of different temperature 10°C, 25°C, 37°C, 50°C and 60°C was determined by the growth of the native bacterial isolates in between of 24-48 hrs and the results are recorded.

2.6.3. ***Effect of salt concentration:***

The selected bacterial isolates were incubated separately with different salt concentration (1%,3%,5%,7%,9%) maintained in culture media (nutrient agar media). Culture media with different salt concentration was added to 5 Petridishes. Each petridish contains all isolates of bacteria (previously selected 9 bacterial isolates). Results were observed after 2 to 3 days.

2.7. ***Secondary metabolites production of native isolates***

2.7.1. ***Production of ammonia (NH_3):***

Ammonia production test was carried out with Peptone water medium [HiMedia, India: having composition (g/l) of Peptic digests of animal tissue, 10.0; Sodium chloride, 5.0; pH, 7.2±0.2]. The cultures were grown in peptone water in tubes and then the tubes were incubated at 30°C for 4 days. After incubation, 1 ml Nessler's reagent [HiMedia, India: having composition (g/l) of Mercuric chloride, 10.0; Potassium iodide, 7.0; Sodium hydroxide, 16.0; pH (at 25°C), 13.2±0.05], was added in each tube. Presence of a faint yellow colour indicates small amount of ammonia and deep yellow to brownishcolour indicates maximum production of ammonia, appearance of colour indicates the ammonia production (Glick et al., 1995).

2.7.2. ***Production of HCN:***

HCN production test was carried out with picric acid [HiMedia, India: having composition (g/l) of Picric acid, 2.5; Na_2CO_3, 12.5] solution, King's B broth [HiMedia, India: having composition (g/l) of peptone, 15.0; magnesium sulphate, 1.5; di-potassium phosphate, 1.5;

glycerol, 10 {ml/l}; pH, 7]. Amended with glycine at 4.4 g/l. The bacteria were inoculated on King's B broth amended with glycine. Sterile filter paper saturated with picric acid solution was placed in the top of the conical flask between the glass and cotton. The flasks were incubated at 28°C for 48 hrs. A change of colour of the filter paper from yellow to light brown, brown or reddish-brown was recorded as weak (+), moderate (++) or strong (+++) reaction respectively. Appearance of colour changes indicates the production of HCN (Bakker et al., 1987).

2.7.3. *Production of salicylic acid:*

Salicylic acid production was carried out by King's broth medium [HiMedia, India: having composition (g/l): peptone, 15.0; magnesium sulphate, 1.5; di-potassium phosphate, 1.5; glycerol, 10 {ml/l}; pH, 7]. Bacterial isolates were grown at room temperature (28±2°C) for 48 hours on a rotary shaker in 250 ml conical flask containing 50 ml of the King's broth medium. Cells were then collected by centrifugation at 10,000 rpm 10 minutes and 4 ml of cell free culture filtrate was acidified with 1N HCl to pH 2.0 and salicylic acid (SA) were extracted with equal volume of chloroform. To the pooled chloroform extracts, 4 ml of distilled water and 5 ml of 2 M $FeCl_3$ were added. The absorbance of the purple iron – SA complex, which was developed in the aqueous phase, was read at 527 nm with the help of spectrophotometer. A standard curve was prepared with SA dissolved in King's B broth medium. The quantity of SA in the culture filtrate was expressed as mg ml^{-1} (Meyer et al., 1992).

2.7.4. *Production of IAA:*

A loopful of culture of each isolate was inoculated in 25 ml of sterilized Nutrient broth [HiMedia, India: having composition (g/l) of beef extract, 3.0; peptone, 5.0] supplemented with L-Tryptophan (0.1g/l) and then incubated for 24 hours at 28°C on rotary shaker. Cultures were then centrifuged at 10,000 rpm for 15 minutes. Two ml of supernatant was taken and in it 2 drops of O-phosphoric acid was added. Four ml of the reagent Salkowski (2% 0.5 $FeCl_3$ in 35% perchloric acid solution) was added to the aliquot. The resulted sample was incubated for 25 minutes at room temperature and absorbance was read at 535 nm with the help of spectrophotometer (Gordon et al., 1951). Concentration of IAA produced by cultures was measured with the help of standard graph of IAA (Hi-Media) obtained in the range of 10–120 μg/ml.

2.8. Seed germination assay supplemented with isolated microorganisms:

2.8.1. *Seed surface sterilization:*

Seed surface sterilized by 70% ethyl alcohol. At first lentil seed was dissolved in 70% ethyl alcohol for 2 mins. Then the seed wash twice to thrice time with sterile distilled water. Extra surface water of sterile seed soaked by sterilized Whatman filter paper and it's ready for seed germination.

2.8.2. *Seed germination:*

The isolated microorganisms were bio assayed for their ability to promote and/or inhibit seedling growth using the method ESTA as described by Elliot et al., (1985) with few modifications. For seed germination assay growing (Nutrient Broth) young culture was spin at 10000 rpm for 6 mins and discard supernatant take the pellet. Then the pellet was dissolved in sterile distilled water, soak the surface sterilized seed in dissolved pellet for 30 mins. Extra water removed from seed and placed on water agar media (0.6% agar).Seeds treated with sterilized water alone were placed on control plates. Incubation at room temperature for 5 days and after seed germination measured the plant root length & shoot length also record their germinate percentage and vigour index.

2.9. *Statistical analysis*

Univariate description of variables based on calculation of sample statistics such as mean, SD, maximum and minimum values have been done on pooled dataset.Species abundance relation was calculated in terms of diversity index. The common indices calculated was relative abundance using the software XLSTAT.

3. Result and discussion:

3.1.Abiotic characteristics:

Ecological variables resulted a high variation in the wetlands, ponds, industrial belts among the study sites. The average pH value of the samples is in the range within pH-6.30 to pH-6.90, slightly acidic which is favourable for aquatic ecosystem. Water sample temperature is near about 30°C to 35°C. Site 2 and site 3 samples are mostly polluted due to high interference of local people and the drainage from industry respectively as the value of dissolved O_2 is very low.

3.2. Zooplankton Diversity

In course of studies, we found four orders (cyclopoida, calanoida, podocopida & anomopoda) under two phyla (Arthropoda, rotifer). Distribution patterns of zooplankton species composition revealed discontinuous distribution in the study sites. In study sites SS1 and SS5 calanoida population are absent, where highest diversity of calanoida was found in study sites-3 (SS3).Among the six study sites the diversity index i.e., abundance (A) and relative abundance (RA) was highest in rotifera population (A- 0.31 & RA-31.32) than podocopida (A- 0.23 & RA-23.39), cyclopoida (A- 0.21 & RA-21.13), anomopoda(A- 0.17 & RA-17.36) and calanoida (A- 0.06 & RA-6.79) (Figure- 2).

3.3. Hydrophyte diversity

The species list of hydrophytes along with their growth forms is given in table no-1. All types of growth forms were found in this study. Total seventeen hydrophytes' species of ten family were found in the six different study sites. Out of ten families three family i.e., araceae, poaceae and hydrocharidaceae are the dominant

family of these study sites (Figure-3).

3.4.Bacterial diversity study:

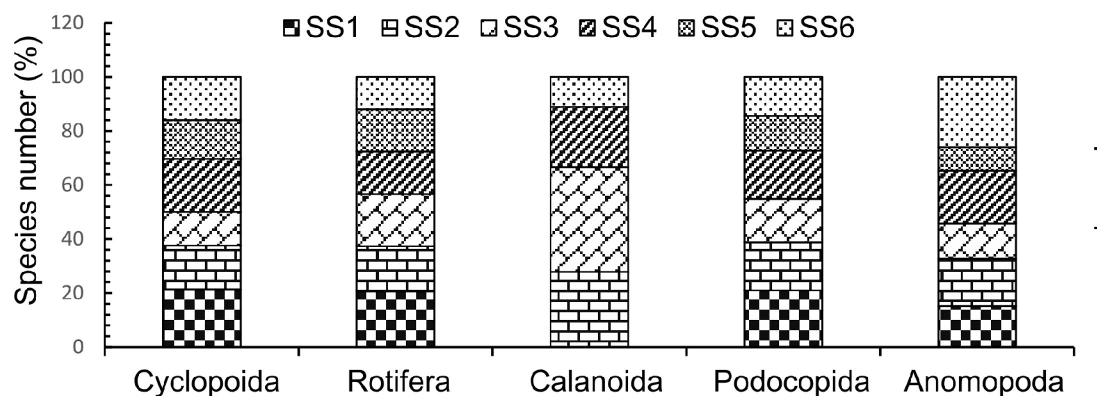

Figure-2: Study site wise diversity index of zooplankton population.

Dynamics of culturable microbiological parameter was interestingly varied with respect to their abundance i.e., the culturable total bacterial CFU/ml was higher in S5(6.35±0.52) than S2(6.27±0.63), S6(5.34±0.24), S1 (5.34±0.45), S4 (4.34±0.24) & S3 (4.31±0.38). So, the present study reveals that the microbial load was higher in case of S_5 study site than

Table-1: List of hydrophytes observed in different study sites.

Sl. No.	Species Name	Family	Growth Forms
1	*Colocasia esculenta*	Araceae	Rooted Emergent
2	*Wolffia arrhizal*	Araceae	Free Floating
3	*Lemna major*	Araceae	Free Floating
4	*Pistia stratiotes*	Araceae	Floating
5	*Peltandra virginica*	Araceae	Rooted
6	*Ipomoea aquatica*	Convolvulaceae	Rooted Floating
7	*Eleocharis dulcis*	Cyperaceae	Rooted Floating
8	*Eichhornniacarssipes*	Hydrocharidaceae	Free Floating
9	*Hydrilla sp.*	Hydrocharitaceae	Submerged
10	*Nymphaeaceae sp.*	Nymphaeaceae	Rooted Free Floating
11	*Dactylocteniumaegyptium*	Poaceae	Rooted
12	*Glyceriafluitans*	Poaceae	Floating (Rooted)
13	*Phalaris arundinacea*	Poaceae	Rooted Submerged
14	*Heterantherareniformis*	Pontederiaceae	Rooted Emergent
15	*Nelumbo nucifera*	Proteales	Submerged
16	*Bacopa monnieri*	Ptantagiraceae	Semi Aquatic
17	*Typha sp.*	Typhaceae	Rooted Submerged

Figure-3: Hydrophyte diversity showed in study sites(A-*Colocasia esculenta*, B-*Eichhornia crassipes,* C-*Phalaris arundinacea*, D-*Setariaviridis*, E-*Bacopa monrium*, F-*Ipomoea aquotica*, G-*Typha sp.*, H-*Dactyloctenium sp.*, I-*Nymphaeceae sp.* and J-*Lemna major*).

S2, S6, S1, S4 & S3 (Figure-4).

3.5. Morphological study of newly native isolates:

The most abundant and representative bacterial colonies are taken and gram stain to show their shape, size and arrangements. Among them S9, S-3, S-2, S1 & S-8 bacterial isolates were found as gram positive bacteria, whereas S4, S5, S6 & S7 bacterial isolates were found as gram negative. Among these bacterial isolates S1 & S2 isolates were round (i.e., in coccus form) and arranged in singly and rests are rod shaped (i.e., in bacillus form) (Table-2).

3.6. Effect of pH on bacterial growth:

Study of bacterial growth depending upon pH, demonstrate that the newly isolated bacterial isolates best grow at pH -7. Majority of the bacteria were reported to grow at neutral or slightly basic pH (False and Panda, 2000; Vaidya et al., 2001). Comparison between different pH concentration reveals that pH-9 was optimum for newly isolated bacterial stain S2, S6, S8 and S9 and the favourable pH for these bacterial isolates ranges from (pH3-pH9)

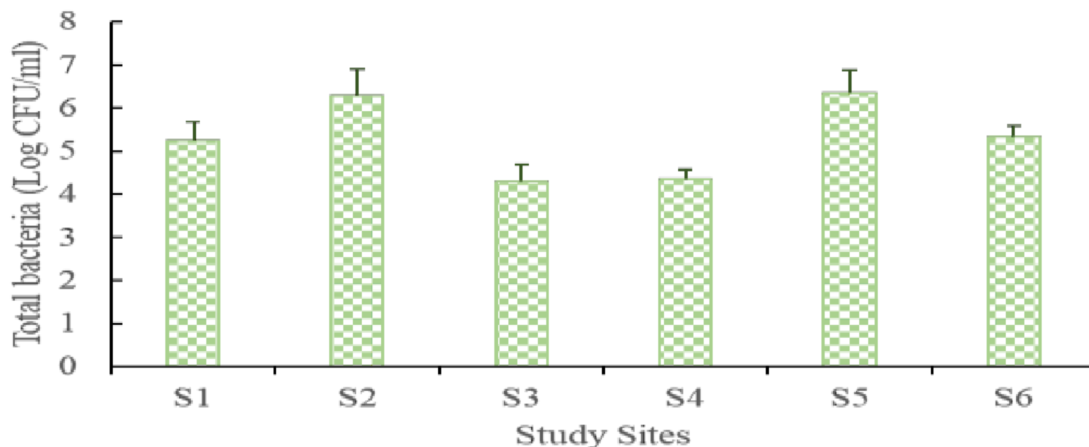

Figure-4: - Total bacterial diversity of six different study sites.

for S2 and (pH7-pH9) for S6, S8andS9. pH - 7favour the growth of all the newly isolated bacterial isolates S1, S2, S3, S4, S5, S6, S7, S8 and S9. Bacterial isolates S1, S3, S9 grow at (pH5-pH7) and the isolates S4, S5 and S7 grow only at pH 7. Extreme low or extreme high pH value can alter the structural configuration of the enzyme and protein thus inhibiting the bacterial growth at pH1 and pH 9 for most of the isolates.

Table-2: Morphology of selected native isolates.

Isolates	Shape	Margin	Elevation	Size	Texture	Optical properties	Appearance	Pigmentation
S1	Circular	Smooth	Raised	Small	Moist	Opaque	Shiny, Smooth	Yellowish
S2	Irregular	Irregular	Umbonate	Small	Dry	Opaque	Shiny, Smooth	Pinkish
S3	Irregular	Irregular	Flat	Small	Moist	Opaque	Shiny, Smooth	White
S4	Irregular	Wevy	Flat	Small	Dry	Opaque	Shiny, Smooth	White
S5	Irregular	Wevy	Flat	Small	Dry	Opaque	Shiny, Smooth	White
S6	Irregular	Wevy	Raised	Small	Moist	Opaque	Shiny, Smooth	White
S7	Irregular	Smooth	Flat	Small	Dry	Opaque	Shiny, Smooth	White
S8	Circular	Smooth	Raised	Small	Moist	Opaque	Shiny, Smooth	White
S9	Irregular	Smooth	Raised	Small	Moist	Opaque	Shiny, Smooth	White

3.7. Effect of salt concentration on bacterial growth:

Depending upon salinity the study of bacterial growth resulted that out of nine native isolates some are halotolerant (can tolerate salt concentration). S2, S4, S5, S6 and S8 these five bacterial isolates are growing simultaneously upto 9% salt concentration. But S3 and S7 native isolates produced colonies upto 3% to 9% salt concentration, respectively.

All nine native isolates gave minimum results under 1% and 3% salt concentration. S1, S2, S4, S5, S6, S7 and S8 these isolates grow comparatively higher salt concentration under 5% as well as in 7% except S7 isolates. In case of 9% salt concentration some isolates like S2, S4, S5, S6 and S8 showed their highest growth whereas this treatment (high salt concentration) become fatal for the rest (Kuddus et.al,2013).

3.8. *Effect of temperature on bacterial growth:*

The study of bacterial growth under selective temperature demonstrates significant result. No results were found under the 10°C and 60°C treatment as very low and very high temperature. The growth of all nine native bacterial isolates gradually increases with the 25°C and 37°C. As the temperature comparatively increases in 50°Conly a few bacterial growths could observe, these are S1, S2, S3 and S6 (Midya et.al.,2020).

From the above analysis it can be concluded that S1 could show its optimum growth under 50°C temperature, salt concentration 7% and at the pH 7. S2 highest growth was resulted under 50°C temperature and 9% salt concertation at the pH 9. S6 would show highest activity at 9% salt concentration and pH 9 at optimum temperature 50°C. At 3% salt concentration S3 would show optimum growth at pH 7 at 50°C temperature. S4 and S5 having maximum activity at 37°C at the pH 7 in 9% salt concentration. The maximum growth in case of S7, S8, S9 were resulted at 37°C temperature but the pH value and salt concentrationare different from each other (Figure-5).

3.9. Secondary metabolites activity of native bacterial isolates

All the plankton associate bacterial isolates in the present studies were tested for their active production of secondary metabolites in in-vitro condition. Besides stimulating plant growth by direct mechanisms; native bacterial isolates can also indirectly induce plant growth by protecting plants against plant pathogens.

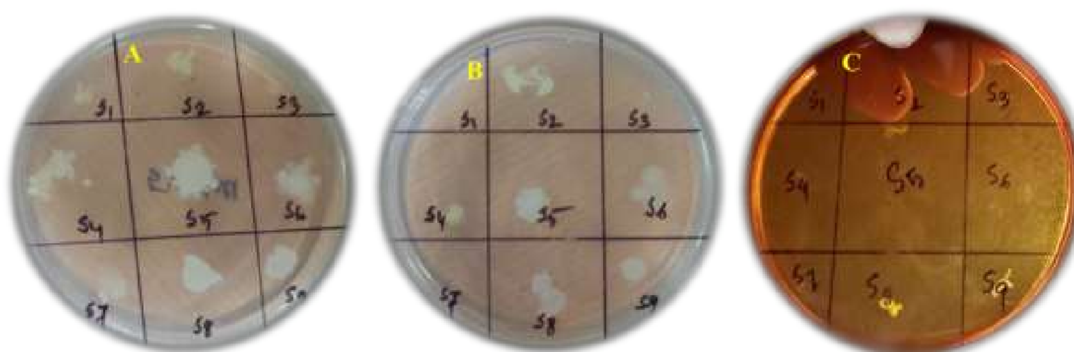

Figure-5: Effect of temperature, salt concentration and pH on native isolates.

Nineplankton associate bacterial isolates were selected for the secondary metabolite's trait analysis. They were tested for their ability to produce phyto-hormones- like IAA, HCN, Salicylic acid (SA) and as well as NH_3 activity. All the isolates were found to be IAA, HCN, and NH_3 positive. Out of these nine isolates S7 (110.47 µg/ml) produces highest amount of IAA plant hormones followed by S5 (106.47 µg/ml) and S6 (103.80 µg/ml). Higher salicylic acid production was found to be in isolates S7 (13.02 µg/ml) followed by S5 (11.77 µg/ml) and S6 (10.94 µg/ml). Maximum NH_3 and HCN production were found in isolates S4, S7 and S5, respectively (Table-3).

3.10. ***Plant growth promotion (PGPR) activity of different potential native bacterial isolates:*** Cauliflower, cabbage, ladies finger, tomato and mung bean seeds were treated with biotic elicitors and after five days of seed bacterization, the germination percentage, root length and shoot length of seedlings were recorded. Analysis of the observed data revealed that groundnut expressed best performance in plant growth promoting activity associated with seed germinability, root length and shoot length as well as vigour index in all treatments over check. Among the 9 bacterial isolates, the isolate S1 was found to be best plant growth promoter compared to other soil bacterial isolates. From the data, it was found that seeds bacterized with S1 and S5 (83.33 %) gave highest per cent seed germination, which was followed by S7, S4 and S6. Root length (S1- 3.17) and shoot length (S5- 2.30) of seedlings were found maximum for those isolates, respectively. The calculated vigour index based on germination percentage, root length and shoot length was also recorded maximum for the isolate S1 (416.67) followed by S5 (369.44) (Table-4).

Table-3: Biochemical quantification observed from the different native bacteria isolates.

Isolates	IAA (µg/ml)	SA (µg/ml)	NH_3	HCN
S1	95.47	8.33	++	+
S2	70.13	0.42	+	+
S3	81.47	3.96	++	+
S4	99.80	9.69	+++	++
S5	106.47	11.77	++	+ + +
S6	103.80	10.94	++	+ +
S7	110.47	13.02	+++	+
S8	79.13	3.23	+	+ +
S9	94.47	8.02	++	++

Among the 9 bacterial isolates, the isolate S5 was found to be best plant growth promoter compared to other soil bacterial isolates. From

the data, it was found that seeds bacterized with S4 and S9 (96.67 %) gave highest per cent seed germination, which was followed by S5 andS7 (93.33%), respectively. Root length (S1& S7- 4.13cm) and shoot length (S2- 4.40cm) of seedlings were found maximum for those isolates, respectively. The calculated vigour index based on germination percentage, root length and shoot length was also recorded maximum for the isolate S5 (737.33) followed by S7 (718.67) and S1 (707.78) (Table-4).Among the 9 bacterial isolates, the isolate S7 was found to be best plant growth promoter compared to other soil bacterial isolates. From the data, it was found that seeds bacterized with S7 and S5 (96.67 %) gave highest percent seed germination, which was followed by S9 and S7 (93.33%), respectively. Root length (S3- 4.13cm) and shoot length (S5- 7.13cm) of seedlings were found maximum for those isolates, respectively. The calculated vigour index based on germination percentage, root length and shoot length was also recorded maximum for the isolate S7 (937.67) followed by S9 (868.00) and S5 (863.78) (Table-4). Among the 9 bacterial isolates, the isolate S1 was found to be best plant growth promoter compared to other soil bacterial isolates. From the data, it was found that seeds bacterized with and S5 (96.67 %) gave highest per cent seed germination, which was followed by S1, S7 and S9 (86.67%), respectively. Root length (S1- 6.67cm) and shoot length (S4- 9.87cm) of seedlings were found maximum for those isolates, respectively. The calculated vigour index based on germination percentage, root

Table-4: Observed plant growth promotion (PGPR) activity of different potential native bacterial isolates against the selected seeds.

	Isolates	Control	S1	S2	S3	S4	S5	S6	S7	S8	S9	Sem (±)	CD (<0.05)
Cauliflower	GI%	50.00	83.33	26.67	30.00	76.67	83.33	73.33	80.00	30.00	70.00	7.49	15.20
	Y	1.20	1.83	1.53	2.13	0.87	2.30	0.87	0.73	0.90	1.40	0.18	0.36
	Z	0.80	3.17	1.90	1.13	1.43	2.13	0.50	0.53	0.57	0.67	0.28	0.57
	VI*	100.00^{7}	416.67^{1}	91.56^{9}	98.00^{8}	176.33^{3}	369.44^{2}	100.22^{6}	101.33^{5}	44.00^{10}	144.67^{4}	39.81	80.75
Cabbage	GI%	56.67	86.67	23.33	16.67	96.67	93.33	83.33	93.33	16.67	96.67	10.99	22.30
	Y	1.80	4.13	4.40	4.17	3.53	4.13	2.47	3.57	2.47	2.17	0.31	0.62
	Z	1.90	4.03	3.47	4.13	3.10	3.77	3.63	4.13	3.13	3.50	0.21	0.43
	VI*	209.67^{7}	707.78^{3}	183.56^{8}	138.33^{9}	641.22^{4}	737.33^{1}	508.33^{6}	718.67^{2}	93.33^{10}	547.78^{5}	83.25	168.84
Tomato	GI%	43.33	83.33	23.33	16.67	96.67	86.67	76.67	96.67	13.33	93.33	11.02	22.34
	Y	3.80	5.67	4.63	5.13	5.57	7.13	4.73	6.17	4.90	6.13	0.30	0.61
	Z	1.90	3.83	2.47	4.13	2.10	2.83	2.37	3.53	2.30	3.17	0.24	0.49
	VI*	247.00^{7}	791.67^{4}	165.67^{8}	154.44^{9}	741.11^{5}	863.78^{3}	544.33^{6}	937.67^{1}	96.00^{10}	868.00^{2}	107.81	218.65
Ladies finger	GI%	43.33	86.67	13.33	23.33	73.33	96.67	83.33	86.67	23.33	86.67	10.17	20.63
	Y	2.90	9.13	7.37	6.53	9.87	6.27	4.47	3.77	2.83	4.10	0.79	1.61
	Z	2.45	6.67	2.67	2.67	5.80	3.73	3.70	3.27	2.47	2.20	0.48	0.97
	VI*	231.83^{7}	1369.33^{1}	133.78^{9}	214.67^{8}	1148.89^{2}	966.67^{3}	680.56^{4}	609.56^{5}	123.67^{10}	546.00^{6}	140.05	284.03
Mung bean	GI%	53.33	96.67	6.67	16.67	93.33	83.33	80.00	90.00	13.33	93.33	11.68	23.69
	Y	9.70	18.60	15.63	19.67	20.80	20.20	15.13	9.60	12.67	17.37	1.31	2.66
	Z	3.20	7.43	5.90	4.57	3.33	4.67	6.90	8.47	7.90	5.70	0.59	1.19
	VI*	688.00^{7}	2516.56^{1}	143.56^{10}	403.89^{8}	2252.44^{2}	2072.22^{4}	1762.67^{5}	1626.00^{6}	274.22^{9}	2152.89^{3}	289.03	586.19

Germination% (GI%), Shoot Length (Y), Root Length (Z), Vigour Index (VI), *Rank.

length and shoot length was also recorded maximum for the isolate S1 (1369.33) followed by S4 (1148.89) (Table-4).Among the 9 bacterial isolates, the isolate S1 was found to be best plant growth promoter compared to other soil bacterial isolates. From the data, it was found that seeds bacterized with and S5 (96.67 %) gave highest per cent seed germination, which was followed by S4 and S9 (93.33%), respectively. Root length (S7- 8.47cm) and shoot length (S4- 20.87cm) of seedlings were found maximum for those isolates, respectively. The calculated vigor index based on germination percentage, root length and shoot length was also recorded maximum for the isolate S1 (2516.56) followed by S4 (2252.44) (Table-4).Similar findings were also reported by Yeole and Dube, where seed bacterization with soil bacterial isolates was found to increase germination percentage, root length and shoot length of cotton, groundnut, chilli and soybean. There were reports that seed bacterization with fluorescent *Pseudomonas* and *Bacillussp* enhanced growth and yield of field crops like potato, sugar beets (Suslow et al., 1982) and wheat.

Germination% (GI%), Shoot Length (Y), Root Length (Z), Vigour Index (VI), *Rank.4.

Discussion:

Beneficial effects of the bacterial isolates on seedlings with respect to plant growth promotion can be explained from the above results. In the present study, nine PGPR isolates (S1, S2, S3, S4, S5, S6. S7, S8, S9) were evaluated for their effect on plant growth, yield and nutrient uptake by investigating their involvement in production of essential plant growth promoters namely IAA, SA, HCN and NH_3. It can be derived that, amongst them S7 (110.47 µg/ml) produces highest amount of IAA plant hormones followed by S5 (106.47 µg/ml) and S6 (103.80 µg/ml), hence possess better IAA-producing trait and development of seedlings under both petridish (water-agar) and soil-pot media (Kloepper et. al., 1988; Jacobson et. al., 1994; Glick et al., 1995; Li et. al., 2000; Penrose and Glick, 2001); as it was previously cited that IAA is involved in enhancing growth and yield, plant height and biomass (Barbieri and Galli, 1993; Patten and Glick, 2002). The beneficial effect of PGPR in maintaining adequate levels of mineral nutrients especially the phosphorus in crop production had been previously reported (Rodriguez and Fraga, 1999; Saravanan et. al., 2007). It has been reported that higher concentrations of phosphate-solubilizing bacteria are commonly found in the rhizosphere soil as compared to non-rhizosphericsoil. IAA is one of the most important phytohormone and function as important signal molecule in the regulation of plant development. It has been reported that IAA production by PGPR can vary among different species and strains, and also influenced by culture conditions, growth stage and substrate availability (Mirza et.al., 2001). Higher level of IAA production by

Pseudomonas was recorded by other research workers (Glick et. al.,1996).

There are some reports that rhizobacteria that overproduce IAA inhibit root elongation, which is attributed to the stimulation of ethylene synthesis by IAA (Xie et. al., 1996; Glick et. al., 1998). The variation in the ability of PGPR to produce IAA found in the present study had also been reported earlier (Mansour et. al., 1994; Zahir et. al., 2000). This variation is attributed to the various biosynthetic pathways, location of the genes involved, regulatory sequences, and the presence of enzymes to convert active free IAA into conjugated forms (Patten and Glick 1996). Both cauliflower and cabbage seeds out of the six, exhibited shorter root length in the soil media, where S1, S5 and S7 showed maximum potency, showing correspondence to the IAA producing isolates. The beneficial effect of PGPR in maintaining adequate levels of mineral nutrients especially the P in crop production had been previously reported (Rodriguez and Fraga, 1999; Saravanan et. al., 2007). In most cases, SA application to plants has only a local effect on pathogens, SA is considered to mediate plant responses to pathogens (Delanay et.al.,1994) and is associated with pathogen-induced SAR (Chasan, 1995). SA was found to be essential for induction of resistance to plant pathogen in bean by the *rhizobacterium P.aeruginosa* 7NSK2. Systemic SA transport from roots to leaves is one possibility, but bacterial SA could also induce signals for systemic resistance at the root level (Meyer and Monica, 1997). This study presented S7, S5 and S6 respectively, with their strong ability to produce SA, followed by isolates S1 and then S9. Regarding seed germination, S1, S5 and S7 has consistently induced the highest percentage of the same, rightly followed by S4, S6 and S9 isolates – showing a similar homogeneous pattern like IAA production by the bacteria and its resultant effect on plant development and additional roles in the same.

Whether microbial sources plant growth substances have negative or positive effects on plants depends on their total and relative concentrations. Recent studies demonstrated the negative effect of indol acetic acid by rhizosphere-introduced pseudomonads on root elongation of sugar-beet seedlings (Loper and Schroth 1986). Many other metabolites rhizosphere microorganisms, including antibiotics (Brian, 1957; Norman, 1959), are toxic to plant roots. Most of these metabolites are organic acids (Lynch, 1976), e.g., HCN (Bakker and Schippers, 1987) may question the in vivo effects on roots of microbial substances such as HCN, which is easily inactivated by soil components or assimilated by soil microorganisms (Castdc, 1981).

Isolates S5, S4 and S6 in respective order, were found to produce the highest amount of HCN, with S8 and S9 showing moderate production. Amongst them, S4, S5 and S9 were found to show highest germination of cauliflower, ladies' finger and mung bean seedlings,

dictating the relation between the two aspects. Seed bacterization with the aforementioned nine isolates increased the root length, plant biomass, plant height and pod yield in pots and water-agar media significantly over control and consistently over the brief five days under potted conditions with demineralized and nutrition-less soil-sand mix provided with autoclaved-water and petridish media, devoid of any additional nutrition. These isolates also helped in better nitrogen fixation as revealed from significantly higher N content in shoot and in kernels in peanut (Dey et. Al.,2004).

Nitrogen is an essential nutrient known for the growth and development of plants and its fixation by soil bacteria is considered one of the main mechanisms by which plants benefit from the microbial association. Isolates producing PGPR plant hormones (indole acetic acid), solubilizing the phosphate and ammonia significantly improve plant growth (Moustaine et.al., 2017). S4 and S7 showed to produce highest proportion of NH_3 in the present study, followed by S1, S5, S6, S9 which again corresponds to the capacity of them as plant growth boosting PGPR inoculants, with mostly S3 here, lacking consistency in the results. PGPR colonize roots of plant and promote plant growth and development through a variety of mechanisms. The exact mechanism by which PGPR stimulate plant growth is not clearly known, although several mechanisms such as production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilization and promotion of the mineral nutrient uptake are usually believed to be involved in plant growth promotion (Glick, 1995; Lalande et.al., 1989). Through the above analysis the study also highlights similarities between the PGPR rhizobacterias and these nine isolates under study pointing their possible strains. Hence, amongst the participation of nine bacterial isolates, S1 and S5, following S7, S6, S4, S8, S9 showed maximal inducing properties as PGPR in the respective order and can be further studied for elaborative results via prolonged field trials and understanding additional potent roles.

5. Conclusion:

From the above study it can be concluded that S1, S5 and S7 are three potent isolates among 9 bacterial strains, which have the strong plant growth promotion (PGPR) activity. With respect to the control, S1 actively promote the growth of cauliflower, ladies' finger and mung bean seeds, where S5 and S7 isolates were actively work against cabbage and tomato, respectively. All the isolate actively produces various types of plant growth promoting factors like as IAA, SA, HCN and NH_3.

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