Isolation Of Siderophore Producing Bacteria From Seaweed

(Solieria robusta, Kappaphycus alvarezii,Gracilaria edulis, Turbinaria ornata)

Hema.S and Poongothai.M

[1]*PG and Research Department of Biotechnology, Dr. N. G. P Arts and Science College, Coimbatore-641048, Tamil Nadu, India.*

Corresponding author mail id: hemamahesh369@gmail.com

Mobile no: +91 6380150755

ABSTRACT:

Seaweed is a term applied to multicellular, marine algae which are large enough to be seen by the naked eyes. Some seaweeds are grown up to 60 meters in length, it includes member of the red, brown and green algae. They belong to the kingdom protista that is they are not plants. Some species of seaweeds are used for nutritional, bio-medicinal and also for bio-remediation process. So these seaweeds are used as food, fodder, bio-fertilizers and for bio-fuel production. From the seaweed (*Solieria robusta, Kappaphycus alvarezii,Gracilaria edulis, Turbinaria ornata*), endophytic bacteria were isolated and they were checked for siderophore activity. Siderophores (from the Greek: "iron carriers") are defined as relatively low molecular weight, ferric ion specific chelating agent elaborated by bacteria and fungi growing under low iron stress. The role of these compounds is to scavenge iron from the environment and to make the mineral, which is almost always essential, available to the microbial cells. From the seaweed samples (*Solieria robusta, Kappaphycus alvarezii,Gracilaria edulis, Turbinaria ornata*) totally 10 organisms were observed in the culture plates. The 10 different isolates were characterized by Gram staining and its shows both gram positive and gram negative results. Among 10 isolates, 4 isolates were gram positive bacteria and 6 isolates were gram negative. The isolates 1, 2, 8, 9, & 10 showed rod shaped colonies and remaining 5 were cocci in shape. Biochemical studies were done for the isolated 10 bacterial strains. Biochemical tests such as MR&VP, citrate, Indole, urease, carbohydrate fermentation test etc., Siderophore producing bacteria is used as plant growth promoting agent.

Key Words: Seaweed, Siderophore, nutritional, bio-fertilizer and bio-fuel

1.INTRODUCTION:

Iron is that the fourth most abundant element in Earth's crust. It is a transition metal which can exist in two oxidation states, Fe (III) and Fe (II). The variable valence of iron allows it to play a key role within the oxidation-reduction reactions. Iron is required in several metabolic processes including tricarboxylic acid cycle, electron transport chain, biological process and photosynthesis. It also regulates the biosynthesis of porphyrins, vitamins, antibiotics, toxins, cytochromes, siderophores, pigments, and aromatic compounds, and macromolecule synthesis. At physiological pH (7.35–7.40), the ferrous form (Fe_2+) of iron is soluble, while the ferric form (Fe_3+) is insoluble (1).

Recently it has been observed that iron plays an important role within the microbial bioflim formation because it regulates the surface motility of microorganism and produces certain compounds with low molecular mass called siderophore (2). Siderophore (sideros meaning iron and phores means bearer) are the metal chelating agents that primarily function to capture the insoluble kind of iron from different sources (3). Iron is known to be a very important microelement influencing metabolism of bacteria, fungi and plants. Siderophores are divided into four main types: hydroxamate, catecholate, salicylate and carboxylate.

Siderophore which assimilates Fe from environments are to be used by the plants and other living system and involves symbiosis. It's especially essential within the time of environment stress resulting from the inadequate amount of iron within the soil, which mainly leads to the inhibition of plant growth and in distributing their functions (4). Pseudomonas sp which produce psedubactin increase growth and yield of varied plant in agriculture once they are inoculated within the soil.

Siderophores not only contributes to provide nutrition to plant and microorganism but also in other environmental applications such as soil mineral weathering, bio-geochemical cycling of Fe in oceans, and biotechnological applications like enhancing growth and pathogen bio-control of plants, bio-control of fish pathogens, microbial ecology and taxonomy, bioremediation of environmental pollutants, petroleum hydrocarbons, nuclear fuel reprocessing, optical biosensor, bio- bleaching of pulp (2)

2.MATERIALS AND METHODS:

2.1SAMPLE COLLECTION:

Algal sample (*Solieria robusta, Kappaphycus alvarezii,Gracilaria edulis, Turbinaria ornata*) was collected from the Mandapam, and sealed with a fresh polythene bag, to avoid contamination and it had been delivered to the laboratory. Then the collected sample has been authenticated in Botanical Survey of India T.N.A.U. Campus, Coimbatore.

2.2.ISOLATION OF BACTERIA FROM SEAWEED SAMPLE:

Marine agar medium was prepared and autoclaved at 121ºC for 15minutes. The plates were prepared. After Surface sterilization 100mg of algal samples (*Solieria robusta, Kappaphycus alvarezii,Gracilaria edulis, Turbinaria ornata*) was weighed and cover the prepared plates and incubated at 37ºC for 12-24 hours. After the incubation, colonies were observed. (5) Initially bacterial culture was characterizes by morphological and biochemical tests (6)

2.3.ISOLATION OF SIDEROPHORE PRODUCING BACTERIA:

Siderophore production is confirmed by using CAS agar medium. It's a selective media. The medium was prepared and autoclaved at 121ºC for 20 minutes. The various isolates were streaked into the plates and incubated at 37ºC for 24hrs. After incubation, the isolates which produces cream colour colonies were confirmed as siderophore producing organisms. Those organisms were isolated and used for siderophore production (7) and therefore the confirmed siderophore producing isolates were selected and identified.

2.4.CHARACTERIZATION AND ESTIMATION OF SIDEROPHORE BY IRON PERCOLATE ASSAY:

Ferric chloride test: 1ml of 2%aqueous $FeCl_3$ solution was added to 1ml of culture filtrate of the bacterial isolates , and examined for the looked for orange or sepia colour which indicates positive test for siderophore production(8)

The Kings B medium was prepared and autoclaved at 121ºC for 20 minutes. In the prepared medium the cultures were inoculated and incubated at 37ºC for 48hours. After incubation, 1ml of the culture filtrate was taken into the microfuge tube then centrifuged at 6000rpm for 15mins. After centrifugation, 1ml of supernatant was transferred into the test tubes, then 1ml of ferric chloride was added and mixed properly. For blank 1ml of water was added and incubated at room temperature for 5minutes. After incubation, the observation of scarlet colour indicates the presence of siderophore and estimated the quantity of siderophore produced by the culture by visual method based on the intensity of the colour formation. The activity of siderophore within the sample was measured by observing the OD at 480nm using a colorimeter (9)

2.5.PRODUCTION OF IAA:

10 ml of LB broth was prepared and autoclaved. 200µl of bacterial culture was inoculated in LB broth and incubated at 28°C for 1-2 days. After every 24hrs 5ml of broth culture was withdrawn and centrifuged at 3000 rpm for 30 minutes. After centrifugation, 2ml supernatant was removed and 10mM phosphoric acid and 4ml of Salkowski reagent was added. Mixture was incubated for 25minutes at room temperature and the pink colour developed it indicated the IAA production. (10)

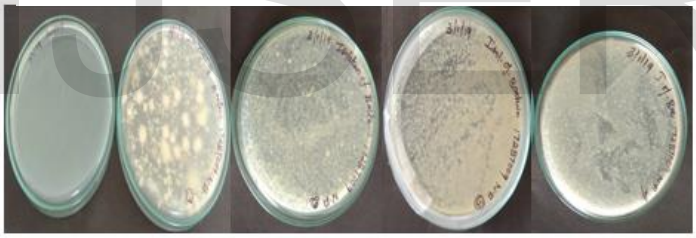
3. RESULTS AND DISCUSSION:

The study focused on the isolation of siderophore producing organism from the seaweeds sample (*Solieria robusta, Kappaphycus alvarezii,Gracilaria edulis, Turbinaria ornata*). Iron is the essential element for the plant growth. So, in future the studies focussing on the use of siderophore compound for the promotion of plant growth are to be explored in detail.

3.1.ISOLATION AND CHARACTERIZATION OF BACTERIAL COLONIES:

From the seaweed samples (*Solieria robusta, Kappaphycus alvarezii,Gracilaria edulis, Turbinaria ornata*) totally 10 organisms were observed in the culture plates. The 10 different isolates were characterized by Gram staining and it shows both gram positive and gram negative results. Among 10 isolates, 4 isolates showed gram positive bacteria and 6 isolates showed gram negative. The isolates 1, 2, 8, 9, & 10 showed rod shaped colonies and remaining 5 showed cocci in shape. Biochemical studies were done for the isolated 10 bacterial strains. Biochemical test such as MR&VP, citrate, Indole, urease, carbohydrate fermentation test etc., (11) were reported. Isolated colonies from seaweed samples were confirmed according to the Berge's manual of systematic bacteriology and the result was shown in *fig :1*

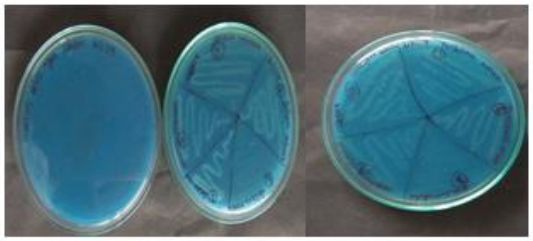
Fig:1 Isolation of bacteria from seaweeds on Marine Agar medium



3.2.SCREENING THE BACTERIAL ISOLATES FOR THE SIDEROPHORE PRODUCTION:

The 10 isolates from sea weeds were streaked on the CAS agar medium and left it for incubation at 37°C for 12-24 hours. After incubation the isolates 1, 8 and 10 showed the cream colour colonies on the plate. So, this proves that isolates from the seaweeds are able to produce the siderophore compound. The result was show in the *fig.(2)*

Fig:2 Screening of Siderophore producing bacteria



3.3. CULTURE IDENTIFICATION:

Based on the siderophore production the isolates were selected and sent for identification. Isolates 1,8 and 10 were selected because they produce cream colour on the CAS agar plates.

The isolates were identified by MALDI-TOF, as

• Isolate1: *Stenotrophomonas maltophilia*

• Isolate2: *Pseudomonas sp.*

• Isolate3: *Bacillus cereus*

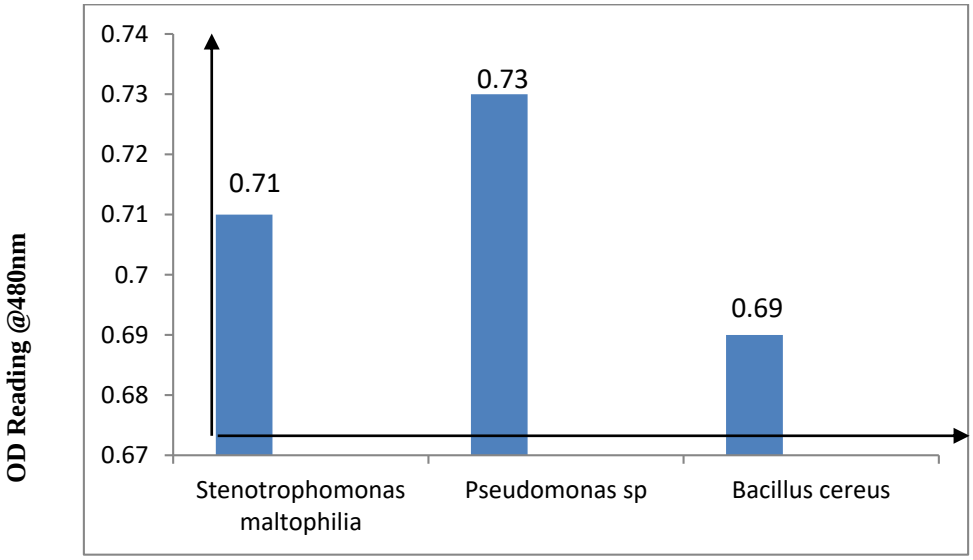
Stentrophomonas maltophilia was isolated from *Turbrina ornata* and *pseudomonas sp.* and *Bacillus cereus* was isolated from *Kapphaphycus alverezii.*

3.4. ESTIMATION OF SIDEROPHORE BY IRON PERCOLATE ASSAY:

Siderophore producing bacterial strains were inoculated in Kings B broth and kept for incubation. After incubation the broth turned turbid, it showed the bacterial culture grown well in the broth, finally all the three isolates were centrifuged at 5000rpm and the supernatant is collected.

Siderophore estimation was carried out for all the three isolates. Formation of orange red colour was observed at 480nm. The isolates 1, 8 and 10 showed the high amount of siderophore content (0.71, 0.73 and 0.69). The results for concentration were shown in graph(1) Our study agreed with the siderophore production and optimization condition is in agreement with result obtained by(12)

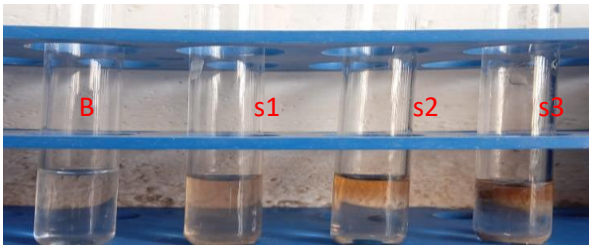
Graph (1): Estimation of siderophore by iron percolate assay



3.5. PRODUCTION OF INDOLE ACETIC ACID :

(*Stenotrophomonas maltophilia, Pseudomonas sp. and Bacillus cereus)* were used in IAA production. Pink colour formation in the broth were observed which showed that the bacteria are able to produce the hormone like IAA and they are able to enhance the plant growth. In these three species Pseudomonas sp showed more IAA production. This is especially true for *Pseudomonas* and Bacillus sp. which are popular biocontrol agents (13).The result with different concentrations was shown in the (*fig:3*)

Fig 3: Production of Indole Acetic Acid



4. SUMMARY AND CONCUSION

The study focused on the isolation of siderophore producing organism from the seaweed. From the seaweed sample 10 different endophytic bacterial isolates were isolated. Those isolates were morphologically and biochemically tested. Among those isolates1, 2, 8, 9 &10 showed cocci shape and 2,4,5,6 and 7 showed rod shapes. The isolates 3, 7 & 10 showed positive result for MR and isolates 3,5,6 &7 showed negative result for VP. All the isolates were checked in CAS Agar Medium. In the conformation test, isolates 1, 8 &10 had shown creamy coloniesans they were confirmed, again the siderophore producing isolates were checked for the iron percolate test for Siderophore Concentration. The isolate 1, 8&10 showed the maximum amount of siderophore. Then they were given for culture identification. Isolates from the seaweed *Kapphaphycus alverezii.* showed highest amount of siderophore content and it has the ability to produce IAA. So, with the help of *Pseudomonas* sp. we could produce siderophore compound and it can be used as plant growth promoting agent.

5. ACKNOWLEDGEMENT:

The author thanks to the management, principal, Deans, head of the department, guide and other biotechnology staff members in Dr. N.G.P. Arts and Science College (Autonomous), Coimbatore. The author acknowledges the DBT Star College Scheme & DST-FIST Scheme. Communication number: DrNGPASC 2020-21 BS032

6. REFERENCE:

1. Neilands JB. (1995) Siderophores: structure and function of microbial iron transport compounds. *J Biol Chem*. 270:26723-26726.
2. Ahmed E, Holmström SJM. (2014), Siderophore in environmental research: roles and applications. *Microbial Biotechnology*. 7:196-208.
3. Nagoba B, Vedpathak D (2011), Medical applications of siderophores. *Eur J Gen Med* 8:229–235
4. Kloepper JW, Leong J, Teintze M, Schroth MN. (1980), Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. *Nature*. 286:885-886.
5. Dhanya K. I., Swati V. I., Vanka Kanth Swaroop, and Osborne W. J., (2015). Antimicrobial Activity of *Ulva reticulata* and Its Endophytes .*J. Ocean Univ. China* (Oceanic and Coastal Sea Research),15 (2): 363-369

6. Cappuccino, James G., Sherman, Natalie., (2014). Microbiology: A Laboratory Manual, Pearson Publication, 10th edition, ISBN: 0321840224, 9780321840226.
7. Chaudhary Yogita D, Gosav Pratiki and Durve-Gupta Annika, Isolation and application of siderophore producing bacteria. *International Journal of Applied Research* (2017); 3(4): 246-250.
8. Gull Munazza, Hafeez Fauzia Yusuf, (2012), Characterization of siderophore producing bacterial strain *Pseudomonas fluorescens* Mst 8.2 as plant growth promoting and biocontrol agent in wheat. *African Journal of Microbiology Research*, 6.33: 6308-6318
9. Atkin, C.L, Neiland, J.B, Phaff, H.F(1970). J Bacteriol, 103,722
10. Chandra Shella, Askari Kazim, and Kumari Madhumita, (2018) Optimization of Indole acetic acid production by isolated bacteria from *Stevia rebaudiana* rhizosphere and its effect on plant growth, *Journal of Genetic Engineering and Biotechnology*. 16: 581-586
11. Kannahi,M and Senbagam, N., (2014), Studies on Siderophore production by microbial isolates obtained from rhizosphere soil and its antibacterial activity. ,*Journal of chemical and pharmaceutical research.* 6: 1142-1145
12. Yeole,R.D., Dave B.P., and Dube H.C., (2001), Siderophore production by *Fuorescent pseudomonas* colonizing root of certain crop plant. Ind. J. Exp. Biol., 39: 464-468.
13. Beneduzi A, Ambrosini A, Passaglia LMP. (2012) Plant growth promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. *Genet Mol Biol* 35:1044-1051.