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Diversity and Distribution of Indole Acetic Acid Producing Marine Sponge Associated Bacteria from Gulf of Mannar, Southeast Coast of India

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Abstract: Many microbes promote plant growth and many microbial products that stimulate plant growth have been marketed. In coastal agriculture system most of the IAA producing terrestrial or plant symbiotic microbes were used as growth stimulating agent. Those microbes were not withstand high salinity, nutrients etc., so that present study focused on this study. Sponge samples were collected from Gulf of Mannar, Southeast coast of India and identified. About four sponges were collected namely *Callyspongia diffusa*, *Hyattella Cribriformis, Sigmadocia carnosa, Spongia officinalis var. ceylonensis*. Sponge associated bacteria were isolated and identified.Then screened for Indole acetic acid production. Among the tested strains maximum indole acetic acid production was observed with *Callyspongia diffusa* associated *P.fluorescens.*

Key words: IAA · Sponges · Gulf of Mannar · Bacteria

INTRODUCTION

Indole-3 acetic acid (IAA) is a phyto hormone which is essential for plant growth and development. The ability to produce the plant hormone indole-3-acetic acid (IAA) is widespread among fungi and bacteria. Some microorganisms which inhabit the aerial or surfaces of plants are capable of IAA synthesis. Such microbes include rhizobia [1].

It has been reported that in some sponge species as much as 40% of animal biomass is attributed to bacteria, which exceeds the bacterial population of seawater by 2 orders of magnitude [2].

There are earlier works reported that marine bacteria have the ability to produce auxins IAA[3], The production of auxins by marine bacteria may offer a chance to use these bacteria as biofertilizers to improve the growth and yield of agricultural crops in coastal saline influenced lands in coastal agricultural system. Strains from marine environment which produce IAA may be used for the production of marine agro friendly biofertilizer production.

There are many studies regarding the synthesis of IAA by many terrestrial bacteria. IAA production by freshwater wetland rhizosphere bacteria [4] but, reports on auxin production by marine sponge associated bacterial strains is very scanty. Hence this study was carried out.

MATERIALS AND METHODS

Collection and Identification of Sponges: Sponges were collected from the Gulf of Mannar, Southeast coast of India (Lat 9°5’ N; Long 79°5’ E).The sponge sample soon after collection was transferred to a sterile polyethylene bag and transported under frozen condition to the laboratory.It was identified by through microscopic and macroscopic analyses.

Isolation of Bacteria Associated with Marine Sponges: The sponge samples soon after collection was transferred to a sterile polyethylene bag and transported at 4ºC to the laboratory for the isolation of associated microbes. On reaching the laboratory, the invertebrate was brought to room temperature and cut aseptically into small pieces (2 × 2 cm) using a sterile scissors. The pieces were freed from adhering particles by vortexing twice for 20 sec. with 2 ml of sterile seawater. The seawater was decanted, which was once again replaced with sterile seawater with continued vortexing between washings. Finally, sample in sterile seawater was homogenized using sterilized mortar and pestle in a Laminar flow chamber. The homogenate was serially diluted up to 10^{-6} dilutions and then spread plated on Zobell marine agar plates. The plates were incubated at room temperature for 24-48 hrs. Based on the colony morphology strains were collected and stored [5].

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Identification of Sponge Associated Bacteria: All associated bacterial strains which were selected based on morphology were identified biochemically. Morphological characters identified up to the species level by following Bergey's manual of determinative bacteriology [6].

Screening for Indole Acetic Acid (IAA) Producing Potential Strain: Screening was done by using Luria broth supplemented with L-tryptophan.A loop full of strains were inoculated in 10 ml of Luria broth supplemented with L-tryptophan containing tubes and incubated for 72 hrs at 30°C. Then, the cultures were centrifuged at 10,000 x g for 10 min. and the supernatant was collected.

One ml of supernatant was allowed to react with 2 ml of Salkowsky reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) at 30°C for 30 min.,. Pink colour development indicated the presence of IAA [7].

Estimation of IAA: The cultures were centrifuged at13000 x g for 10 min. and the supernatant was collected. The presence of IAA was measured in a spectrophotometer by adding 2 ml of Salkowsky reagent to 1 ml of supernatant, incubated for 30 min.The optical density was read at 530 nm. The recorded OD values were plotted in a standard curve prepared from commercially available IAA (8) and their concentration was calculated.

RESULTS AND DISCUSSIONS

Density of Bacteria Associated with Sponges: The sponges viz., *Callyspongia diffusa*, *Hyattella Cribriformis, Sigmadocia carnosa, Spongia officinalis var. ceylonensis* were analysed for associated bacterial population. In *Callyspongia diffusa* bacterial density was in the range of 7.68x10^3 CFU/g to 1.1 x 10^7 CFU/g, whereas in the other three species i.e., *Hyattella Cribriformis, Sigmadocia carnosa*, *Spongia officinalis var. ceylonensis* respectively 3.13 x10^{3}CFU/g to 1.6x 10^{7}CFU/g, 6.77 x10^{3}CFU/g to 1.5 x 10^{7}CFU/g, 2.69 x10^{3}CFU/g to 1.4 x 0^{7}CFU/g were the bacterial density found. Likewise, about 10 marine bacterial strains were isolated from the marine sponge *Callyspongia diffusa* by Kalirajan *et al.* [9]. Krishnan *et al.*[10] got 6.5 x 10^{6}cfu/ gm^{-2} total heterotrophic bacteria associated with the sponge, *Stylissa* sp. Boobathy *et al.* [11] extracted the bioactive protein from *P.aeruginosa, E.coli*, *V.cholera, V.heamolyticus* which was associated with *Callyspongia diffusa.*

Ravikumar *et al*. [12] isolated Cynobacterial symbionts from the sponge *Sigmodocia carnosa.*

In the present study most of the bacterial strains which were associated with sponges were indole acetic acid producers. *Callyspongia diffusa* associated bacterial strains were produced indole acetic acid. Among the strains *P.fluorescens* (0.41 µg/ml) followed by *A. hydrophila* (0.22 µg/ml), *P. putida (*0.21 µg/ml), *B. subtilis (*0.13 µg/ml) *B.licheniformis* (0.12 µg/ml), *A.faecalis*(0.11 µg/ml), *P. aeruginosa* (0.11 µg/ml) (Fig.1). In *Hyattella Cribriformis* associated bacteria maximum IAA produced with *B. subtilis* (0.26 µg/ml) followed by *P. putida* (0.21 µg/ml), *B.megaterium*(0.2 µg/ml), *C.kutcheri*(0.13 µg/ml) *B. cereus, L. plantarum* (0.1 µg/ml) (Fig.2).

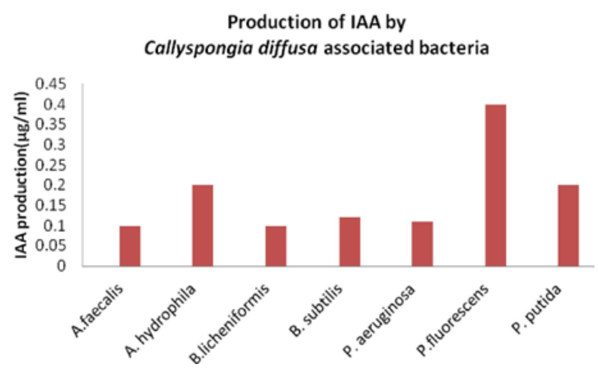

Fig. 1: IAA by *Callyspongia diffusa* associated bacteria

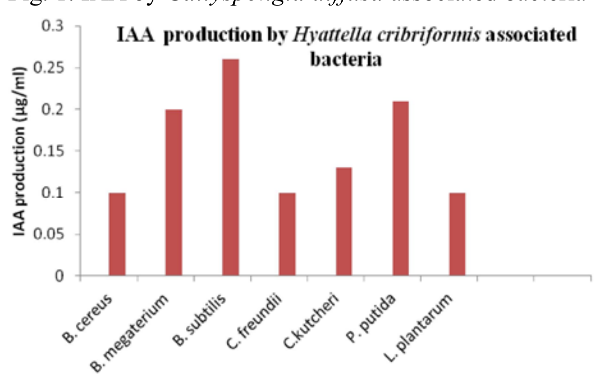

Fig. 2: IAA by *Hyattella cribriformis* associated bacteria

In *Sigmadocia carnosa* maximum IAA production was observed with *P. putida*(0.28 µg/ml), *B. pumilis* (0.24 µg/ml), *B. megaterium, S. marcescens* (0.23 µg/ml), *B. cereus,P.aeruginosa*(0.21 µg/ml), *L. divergens* (0.14 µg/ml), *B.brivis(0.12* µg/ml). (Fig.3).Maximum IAA production was observed with *B. subtilis* (0.3 µg/ml) *B. megaterium, C.glutamicum*(0.28 µg/ml), *E.coli* (0.27 µg/ml) minimum was observed with (*B. brevis* 0.21 µg/ml) in *Spongia officinalis var. ceylonensis* associated bacteria (Fig.4).

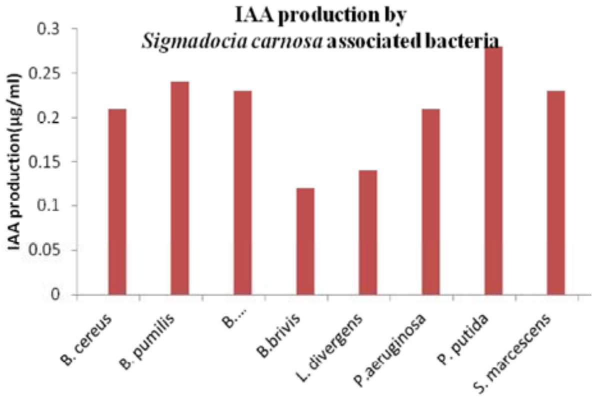

Fig. 3: IAA production by *Sigmadocia carnosa* associated bacteria

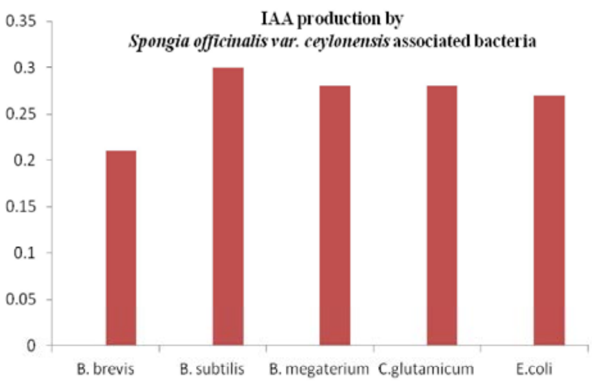

Fig. 4: IAA production by *Spongia officinalis var. ceylonensis* associated bacteria

Among the associated bacteria *P.fluorescens* from *Callyspongia diffusa* is a novel Iaa producer. Sergeeva *et al*.. [13] stated that 83% of the symbiotic isolates tested positive production of auxin-like compounds compared to 38% of the free-living ones, Prabha Devi *et al*. [14] collected the sponge *Halichondria* sp., from the Gujarat coast of the Indo Pacific region and they isolated the *Bacillus* sp. SAB1, extracted indole (1), 3-phenylpropionic acid.

Jayaprakashvel *et al.* [15] studied the IAA production from Halotolerent bacteria associated with rhizospheres of *Sueveda* sp. along the Kelambakkam salterns, Tamil Nadu. The plant growth-promoting rhizobacterial strain *Pseudomonas putida* Rs-198 was isolated from salinized soils from Xinjiang Province produce the IAA [16].

The present study thus proved the potential of the marine sponge associated bacteria has potent to produce indole acetic acid. In future it can be specially used in coastal agricultural system.

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