Studies on IAA Production by Marine Pseudomonas Species

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Abstract—Marine environment is treasure of microbes and their valued metabolites are explored in various fields including agriculture. Auxin is well known plant hormone has role in growth and development of plants. Inthepresentinvestigation42differentmicrobialstrains were isolated from marine water sample using Zobell agar media (hi media). From the 42 different microbial strains 18 marine *Pseudomonas* species were isolated using cetrimide agar. These isolated *Pseudomonas* species were screened for their inherent potential for the production of indoleacetic acid (IAA) by qualitative and quantitative studies using Salkowasky test. The marine *Pseudomonas* strains such as RSML 04, RSML 08, RSML 02 and RSML12 were found to produce IAA. Optimization studies of potential IAA producing isolate were conducted in reference with L-Tryptophan concentration, temperature and pH.

I. INTRODUCTION

Marine microorganisms are noteworthy producers of novel metabolites but still remain an untapped source for the discovery of new bioactive compounds. Marine micro organisms are unique in nature and vary in many aspects from their terrestrial counterparts. The majority of these microbes have never been cultured, identified or classified and their enormous chemical richness has remained unexploited (Iyer & Singhal 2009; Kiruthika, et. al., 2018).

The marine microbial flora significantly influences the health of the marine ecosystem and the regulation of the biogeochemical cycles. Metabolites derived from microbes and their use in different sectors, such as agriculture and medicine have great significance (Glockner et. al., 2012).

Pseudomonas, first discovered by Gessard in 1882, is a genus of Gram-negative bacteria belonging to the Gamma proteo bacteria. The *Pseudomonas* shows a great deal of metabolic diversity and consequently are able to colonize a wide range of habitat such as soil, water, air and also marine environments (Szoboszlay et.al, 2003; Pirnay et. al., 2005; Kimata et.al, 2004). IAA (indole3acetic acid) is most prevalent and important member of Auxin family (Vessey, JK2003). Auxin regulates a number of plant growth and developmental processes, including the elongation of roots, phototropic movements, embryonic development, leaf abscission, and fruit formation (Davies, P., 1995; Datta and Basu 2000), It is used in horticulture as a rooting hormone (Pattern and Glick, 2002). The tryptophan dependent biosynthesis of IAA is well understood and involves conversion of tryptophanintotheindole3acetamide (IAM)by tryptophan 2monooxygenase, indole3acetamide is further metabolized by IAM hydrolase to form IAA ((Zhao Y. 2010; Matsukawa et al., 2007).

Figure 1: Chemical structure of indole-3-acetic acid In the present investigation *Pseudomonas* were isolated from marine environment and screened for their IAA production ability.

II. MATERIALANDMETHODS

A. Sample collection: The marine water sample were collected from Alibagh beach, district: Raigad (Latitude:$18^0$38’29’’N, Longitude: $72^0$52’20’’E) Maharashtra, India in sterile water bottles and brought to laboratory within 24 hrs. The samples were stored at 4^{o}C till further use.

B. Isolation of marine bacteria:

For the isolation of marine bacteria, the standard tenfoldserialdilutionofthesamplewascarriedout.1 ml marine water sample was diluted in 9ml of sterile

water up to 10^{-7} and 0.1 ml of diluted sample was spread on sterile Zobell agar medium (Hi media) plates, and incubated for 3 days at 28°C.The morphological and biochemical characterization of isolates was carried out.
From isolates obtained, marine *Pseudomonas* were screened using cetrimide agar media and the isolated marine *Pseudomonas* were screened for their inherent potential of IAA production.

C. Characterization for IAA production: IAA production of isolated *Pseudomonas* species was studied using the method described by Brick et. al., using Salkowski reagent (Brick JM, Bostock RM, 1991). The isolates were grown in the sterile nutrient media (Hi media) containing tryptophan for 48hrs at 28°C. After incubation the broth was centrifuged at 10,000 rpm for 10 min at 28°C and supernatant was collected. To the 1ml of super natant 2ml of Salkovsky regent (2% of 0.5M Fecl3 in 50 ml of 35 % HCO4) was added, later one drops of Orthophosphoric acid was added and kept in dark for the color formation. Qualitative and quantities estimation was carried out based on spectral studies. The optical density (OD) was recorded at 535nm after 1 hour. Against control of (1ml of nutrient agar with one drop of orthophosphoric acid and 2 ml Salkovsky regent. Concentration of IAA produced was determined by usings landard plot (Mohite,2013). Standard plot was prepared using IAA (Hi media) in different concentrations in range of 10 to 50 ug/ml in distilled water and color was developed using Salkowasky reagent and orthrophosphoric acid, measuring OD at 535nmafter1hrindarkandstandardcurveprepared.

D. Extraction and purification of IAA: The isolated *Pseudomonas* were grown in the Nutrient broth amended with the tryptophan and incubated at 120 rpm at 28°C temperature. After incubation broth was centrifuged at 10,000 rpm for 10 min and the super natant was collected. The super natant was mixed water bottles and brought to laboratory within 24 hrs. The samples were stored at 4°C till further use.

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G. Extraction and purification of IAA: The isolated *Pseudomonas* were grown in the Nutrient broth amended with the tryptophan and incubated at 120 rpm at 28°C temperature. After incubation broth was centrifuged at 10,000 rpm for 10 min and the super natant was collected. The super natant was mixed with ethyl acetate (1:2) and were shaken continuously for10-15min.TheIAAwasextractedinsolventlayer.

H. Thin layer chromatography:
Thin layer chromatography of extracted sample was carried out in reference with standard IAA (10mg/100ml). The TLC plates were prepared using silica gel and calcium carbonate and mobile phase solvent system used was propanol in water (8:2). The

crude extract was spotted with capillary tube and solvent front was allowed to run for approximately 80% of the plate. The chromatogram was developed using Salkowski reagent (Kimata, et. al., 2004).

I. Optimization of IAA production by RSML 04: The IAA production was optimized by growing bacteria under various conditions of the culture media with various physicochemical parameters such as L-tryptophan concentration, pH and temperature. Effect of L-tryptophan concentration: The effect of L-Tryptophan concentration on IAA production of the isolate RSML 04 was studied by using different concentrations of L-tryptophan (100, 200, 300, 400 and500µg/ml) in the media. Quantitative production studies ware done based on standard plot (Hariharan et. al., 2014).

Effect of Temperature and pH on IAA production: The effect of temperature and pH on IAA production by the efficient IAA producing isolate RSML 04 was investigated. The effect of temperature was studied by growing the isolate at different temperatures such as 20,25,30,37,40°Cat120rpmfor48hrs.Quantitative production studies ware done based on standard plot (Hariharanet.al.,2014).The optimum pH for the production of IAA by the RSML04 was determined at different pH values such as 5,6,7,8 and 9 by adjusting the pH of nutrient broth amended with the Tryptophan. The flasks were incubated at 120rpm and 28°C for 48 hrs.

III. RESULTANDDISCUSSION

A. Isolation and Identification of marine isolates:

In the present investigation 42 different microbial strains were isolated from marine water sample using Zobell agar media (hi media). From the 42 different microbial strains 18 *Pseudomonas* strains were isolated using cetrimide agar. These isolates were screened for their IAA production potential

Detection of IAA by thin layer chromatography: Purified IAA sample was compared with standard IAA on TLC chromatograms. TLC of ethyl acetate extract showed pink colours pot at the Rf corresponding to the authentic IAA (0.57) as shown in Figure4. It confirmed IAA producing potential of marine isolates. Sridevietal,2008reportedTLCchromatogramof purified compound and standard IAA sprayed with salkowski reagent showed almost same Rf values.

B.Optimization of IAA production by RSML 04: Effect of L-tryptophan concentration on IAA production L-tryptophan is considered as a precursor for IAA production because its addition to medium increases IAA production (Ahmad et. al., 2005; Santi et.al., 2007; Sridevi et.al., 2007). The concentration of tryptophan required by bacteria varies, depending on the ability of the bacteria to synthesize tryptophan (Vincent, 1982). Based on the spectrophotometric analysis, the results showed that increasing production of IAA along with L-tryptophan increased concentrations up to 400 µg/ml and then started to decrease production of IAA. In the present investigation the maximum IAA production by marine *Pseudomonas* species RSML04 was found to be 47.52 µg/ml after 5 days of incubation at 400 µg/ml L-tryptophan concentration as shown in Fig. 5.

IV. CONCLUSION

Indole acetic acid (IAA) production studies using isolated marine *Pseudomonas* species revealed that RSML04isanefficientIAAproducer.Itshowedthe maximumproductionofIAAat400µg/ml Tryptophan concentration, at 30 °C temperature and pH 8. Future plan of this study is to explore marine *Pseudomonas* RSML 04 role in plant growth promoting studies and development of biofertilizer.

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