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Studies of Impact on Bioactive Molecules of SB_2 Isolate and their PGPR Activities from the Marine Environment

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ABSTRACT

Marine microbes are important sources for recovering bioactive substances. One of the marine isolates, SB_2, used a variety of media, with tryptic soy broth showing the best growth and yellow coloring. The largest amount of biomass was produced after 72 hours when the strain SB_2 was cultured on glucose, molasses, and peptone, a yeast extract containing carbon and nitrogen sources. Yellow pigment must be extracted using methanol, and the isolate SB_2 produced the most of this color. At a concentration of 100 g ml^{-1} of several bacterial pathogens, the recovered pigment from the SB_2 isolate exhibited antibacterial action. The maximum production of IAA and GA is shown in TS broth, which is additionally augmented by the siderophore synthesis of catechol and salicylate. The SB_2 also produces IAA and GA. In contrast, the highest level of ACC deaminase activity was found in nutrient broth. Due to the generation of pigment, the strain SB_2 may be essential for Plant Growth Promoting Rhizobacteria (PGPR) and antibacterial properties.

Keywords SB_2 isolate, Antibacterial activity, IAA, GA, ACC deaminase, Siderophore, Yellow pigment.

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INTRODUCTION

Marine-pigmented bacteria are ubiquitous everywhere, from soil to the mountain, earth to the atmosphere and water to the marine environment (Du *et al.* 2006). Many heterotrophic bacteria have synthesized carotenoids isolated from coastal and oceanic waters. It is also the ability to synthesize pigments in cheap culture the medium through faster and manageable growth. Sowmya and Sachindra (2011) reported that carotenoid synthesis from marine resources possesses various health-beneficial activities. According to the Fortune Business Insights report, the carotenoid fetched market value in 2019 is 1.44 billion dollars which were expected to reach 2027 approximately 1.84 billion dollars, and the forecast period from 2020 to 2027 has a CAGR of 3.4%. The demand for beta carotene was estimated in 2010 at a value of 261 million dollars, and the annual growth rate is 3.1% which was expected to reach 2018 approximately 334 million dollars (Zarandi *et al.* 2019).

Most plants require growth-promoting substances like IAA, GA and cytokinin for their growth which occurs in the rhizosphere microorganisms commonly called plant growth-promoting rhizobacteria (PGPR). It also acts as a biocontrol agent by suppressing

soil pathogen's hydrogen cyanide and siderophore production. In the seedling stage, the higher concentration of ethylene production in plants affects their growth. The production of ACC deaminase rhizobacteria could decrease ethylene concentration and improve crop growth (Kang *et al.* 2009, Madhaiyan *et al.* 2006). The availability of nutrition in the soil could be altered through the ability to fix nitrogen into the soil. The capability to solubilize minerals and the chelation of iron compounds are the direct mechanisms of PGPR activity (Hariprasad and Niranjana 2009). The activity of bacteria reduces ethylene concentration, and the organism acts as growth regulators, producing siderophore and suppressing soil pathogens, which could reduce environmental stress. Auxin induces cell elongation and cell division in plants. Manulis *et al.* (1994) were studied by synthesizing its pathway and producing IAA by *Streptomyces* spp. El-Tarabily *et al.* (2019) revealed that the growth could be increased by the production of IAA and ACC deaminase produced by *S. atrovirens.* With the earlier reports on carotenoid pigment from marine sources and its importance in industrial application, the present study aims to assess bacterial pigment against antimicrobial activity and PGPR production.

MATERIALS AND METHODS

The isolate were grown on different media

The yellow-pigmented bacterial isolate SB_2 was isolated from marine water (Aroumougame 2022). The bacterial isolate SB_2 (SB-Saline Bacteria) was grown on 100 ml of Nutrient broth, Zobell marine broth, Luria Bertani broth and Tryptic soy broth supplemented with kenamycin was added into it. They were inoculated and incubated at 30ºC in a rotary shaker for one week. The higher growth (pigmentation) was recorded for each culture media at 530 nm using spectrophotometer.

Growth on carbon sources

The nutrient broth was prepared in a conical flask containing carbon nutrient sources viz., glucose, cellobiose, galactose, glycerol, mannitol, sorbitol, lactose, sucrose, cellulose, xylose, molasses, olive oil and malic acid (0.2%) were sterilized and the pigmented isolate was inoculated into nutrient broth containing carbon source and incubated at 30ºC. After one week, the pigment production (mg l^{-1}) was observed.

Growth of SB_2 isolate by various nitrogen sources

The ammonium sulphate, ammonium nitrate, yeast extract, tryptamine, citrulline and peptone (0.2%) were added in nutrient broth as nitrogen sources into the flask and sterilized it. The 24 hrs pigment culture was inoculated into various nitrogen sources containing media and kept for incubation at 30ºC for one week. After one week, the pigment production was recorded in terms of mg l^{-1}.

Isolate grown at various periods for production of biomass

The yellow colorants strain was studied for various incubation times, the isolates ability to grow and production of pigment in a specific period has been recorded. The Tryptic soy broth was prepared and sterilized. The isolated strain was inoculated (1 ml) and the broth was kept for incubation for 20 to 160 hours at 30ºC. After incubation, the pigment was extracted using methanol solvent at 20, 40, 60, 80, 100, 120 and 160 hours and the higher biomass were recorded in g l^{-1} for specific incubation hours (Trivedi *et al.* 2017).

Test for carotenoid

The pigments were extracted from the bacterial strain which was isolated from the marine water mixed with 80% (H_2SO_4). The result of the experiment confirmed with carotenoid presence indicates of blue color.

Extraction of pigment using different solvents

The TS broth was prepared for pigment production, sterilize the broth and inoculate the SB_2 strain and incubate at the incubator. After one week, the pigment was extracted using different solvents viz., chloroform, acetic acid, ethanol, petroleum ether and ethyl lactate. The result was recorded at higher carotenoid production (mg l^{-1}) with different solvents (Mezzomo *et al.* 2011).

The pigmented isolates were checked for antimicrobial activities

The isolate SB_2 were checked for antibacterial activity to determine the Minimum Inhibitory Concentration against the pathogenic bacteria. The nutrient agar medium was poured on to the plate allow for solidifying. Then, the medium was swab with bacterial pathogens (*Staphylococcus aureus, Pseudomonas aeruginosa, E. coli*) and the plates were made well. The different concentration of yellow pigment 0.5, 1, 10 and 100 µg ml^{-1} was poured on to the well. The clear zone of growth inhibition of bacteria and the diameter was measured (mm) after 24 hrs incubation at 30ºC (Hamed *et al.* 2020).

Indole acetic acid production by SB_2 isolate

TS broth, NB, LB broth and ZM broth was prepared in two sets and sterilized in an autoclave, add 0.2% of L-tryotophan filter-sterilized into one set of each medium and another set of the medium was maintained as the control without the addition of L-tryptophan. The SB_2 isolate inoculated into two sets of each flask were kept for incubation at 30°C for one week in darkroom. The culture was taken for further analysis after one week of incubation. Each broth was separately centrifuged. The supernatant was collected and pellets were removed and it was acidified with 1N HCl to adjusted pH 2.8. The equal volume of diethyl ether mixed with acidified supernatant was taken into the conical flask and kept for 4 hrs in dark. The same process was applied in each media in the different flask. The separating funnel was used for extraction of IAA using diethyl ether in each media. The IAA can be separated into the solvent phase and the organic phase was discarded (Tien *et al.* 1979). The solvents were evaporated; the dried residues were added in 2 ml methanol. SB_2 culture in each media of methanolic extract (0.5 ml) was taken, add 4 ml of salpers reagent in each media, mixed with distilled water (1.5 ml) kept in dark at one hour. The intensity of pink color measured at 535 nm and expressed as µg ml^{-1}. A known concentration of IAA prepared from the standard curve (Gorden and Paleg 1957).

The SB_2 isolates for gibberellic acid production

TS broth, NB, LB broth and ZM broth was prepared, they were sterilized and the SB_2 culture was inoculated into each broth incubated for one week at 30ºC. All the cultures were centrifuged and the supernatant was collected. The cell pellets were mixed with phosphate buffer (pH 8.0), it was re-extracted and again centrifuged. The same process was done for each media separately. Both supernatants were pooled and acidified with 5N hydrochloric acid (pH 2.0) mixed with ethyl acetate for extraction. Each media of the solvent phase was evaporated add two ml of zinc acetate solution and 2 ml of distilled water containing 0.05% of tween 80 in residue, in each media after two minutes two ml of potassium ferro cyanide solution was mixed and centrifuged at 4000 rpm. The 5 ml of supernatant of the different media mixed with 5 ml of 30% hydrochloric acid and kept for 75 min, measured at 254 nm and expressed in terms of µg ml^{-1} by the different media of SB_2 culture was calculated (Tien *et al.* 1979).

Determination for siderophore production of the pigmented isolate

TS broth, NB, LB broth and ZM broth were prepared in two sets of the flask for salicylate and catechol type of siderophore and sterilized in an autoclave. The SB_2 isolate was added into each medium and incubated for one week at 30ºC. The broth culture of different media was centrifuged and SB_2 culture supernatant (20 ml) of the various media was adjusted with pH 3.0 mixed with an equal volume of ethyl acetate in different media separately which was repeated. The residue of each medium was dissolved using distilled water. Salicylate was estimated using solvent extracted of various media of SB_2 culture 5 ml mixed with 5 ml of Hathway reagent and absorbance at 560 nm with sodium salicylate as standard whereas catechol was estimated using solvent extracted from various media of SB_2 culture 5 ml was added into 5 ml of Hathway reagent measured at 700 nm with 2, 3, di hydroxybenzoic acid as standard which can be expressed in terms of µ moles ml^{-1} (Reeves *et al.* 1983).

The pigmented isolate produce ACC deaminase activity

TS broth, NB, LB broth and ZM broth was prepared and sterilized it. SB_2 bacterial culture was inoculated

into each media. It was kept at 25-30ºC under a rotary water bath (200 rpm). After 24 hrs of incubation SB_2 bacterial culture was inoculated to TS broth, nutrient broth, LB broth and ZM broth and incubated for 24 hrs in a rotary water bath (200 rpm) at 25 and 30ºC. Following this incubation, the population of SB_2 was enriched. One ml aliquot of SB_2 the enriched culture was inoculated into TS broth, NB, LB broth and ZM broth containing 3.0 mM ACC deaminase. The culture of various media incubated under a rotary water bath for 24 hrs at 25-30ºC. The various media grown on SB_2 culture were centrifuged and the cell pellets were washed by suspending 5 ml 0.1 M Tris-HCl, pH 7.6, it was estimated at 540 nm and expressed in n moles of α-ketobutyrate mg^{-1} h^{-1} (Siddikee *et al.* 2010).

RESULTS AND DISCUSSION

The pigment production ability of the isolate

The isolate SB_2 was found most effective as they grew well in Nutrient broth, Zobell marine broth, Luria Bertani broth (LB) and Tryptic soy broth (TS) as shown in Fig. 1. in which better growth was noticed on TS and LB broth. All the media were observed optimum growth though the isolate recovered from marine water but slight variation was observed in ZM broth of pigment production. The isolate SB_2 was found most effective as it grew well in tryptic soy agar and LB media. *Micrococcus* sp. showed maximum pigment production isolated from soil environment using trypticase soy broth medium (Mohana *et al.* 2013).

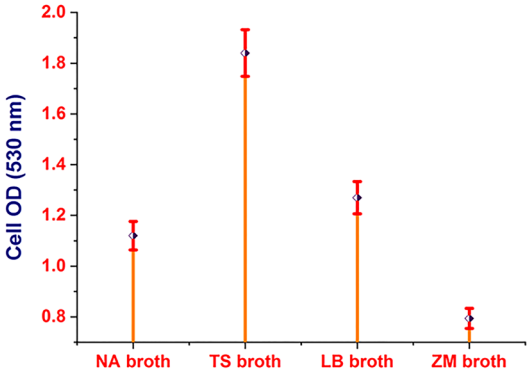

Fig. 1. Pigmented isolates grown on various media.

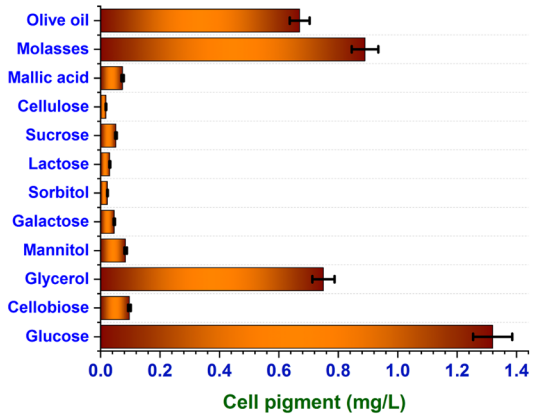

Fig. 2. Development of pigment from various carbon sources.

Growth on carbon sources

A variety of carbon nutrients utilized by SB_2 isolate for their growth and pigment development, the stain SB_2 preferred specifically glucose, molasses, glycerol and olive oil for its growth and pigment development, the result was presented in Fig. 2. Bacteria utilized various carbon nutrients for their growth and development, and the stain SB_2 preferred glucose, molasses, glycerol and olive oil for its growth and pigment development. Glycerol has the best source of carotenoid formation, which is the backbone of C30 and C40 structures (Kim *et al.* 2010).

Effect on different nitrogen source

The different nutrient sources were checked for the culture SB_2 in which, the results presented in Fig. 3. were observed that yeast extract and peptone as

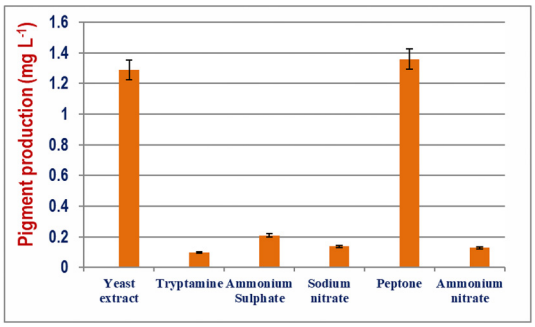

Fig. 3. The different nitrogen sources utilized for the isolate SB_2.

prominent sources of nitrogen used for the production of pigment. Pigmented colonies readily survive in the medium's peptone concentration, even if it can drive nutrients from a low-peptone concentration. The cultivation of bacteria varies depending on the samples used. Further, adding peptone to the production medium may alter the growth and pigment production of SB_2 culture. The yeast extract and peptone were added as a nitrogen source in the medium. The production of violacein, L-tryptophan and molecular oxygen are required (Momen and Hoshino 2000). The peptone was incorporated into the growth medium, highest pigment production obtained from methanolic extracts (Hamed *et al.* 2020).

Biomass production of isolate for the incubation period

The yellow-pigmented isolated SB_2 strain was observed on various time periods from 20 to 160 hours. The pigment production starts from 48 hrs and its attained maximum production at 72 hrs showed in Fig. 4. This isolates start to grow and slowly develops pigment because it is secondary metabolites after 48 hrs biomass were observed and higher growth and biomass was noticed gradually decrease when time was increased up to 120 hrs. Optimum yellow production was observed at 48 hr by *Exiguobacterium aurantiacum* FH, both growth and pigment production decline after 48 hrs. SB_2 strain was observed pigment production from 48 hrs and maximum production occurs in 72 hrs. The non-pathogenic strain of pigment-producing *Pseudomonas fluorescens* showed maximum pigment production in 48 hr was isolated from soil.

Carotenoid pigment test

The blue color shown in the result to confirm the strain produce carotenoid pigment was extracted from the bacteria. The presence of carotenoids indicated the blue color in addition to H_2SO_4 into the extracted pigment. The same results were obtained extracted pigment from isolated strain SB_2 and the result were also studied and reported by Ajayi *et al.* (2016).

Extraction of pigment from different solvents

The isolate was grown for pigment production which can be separated from bacterial cell need with different solvents viz., chloroform, acetic acid, ethanol, petroleum ether and ethyl acetate. In present experiment, the SB_2 isolate showed that methanol solvents produce higher pigment followed by ethanol and ethyl ether (Fig. 5). The solvents were able to attach to the bacterial cells and separate pigment from the cell due to the extraction time that may affect the yield of the compound. Some compounds are destroyed by high temperature, solvent play a critical role in the extraction of the pigment. The extraction of pigments from bacterial species was screened for chloroform, acetic acid, ethanol, petroleum ether and ethyl lactate (Enrıquez *et al.* 2013, Ishida and Chapman 2009). The maximum pigment production was observed by SB_2 strain using methanol solvent. Based on the affinity

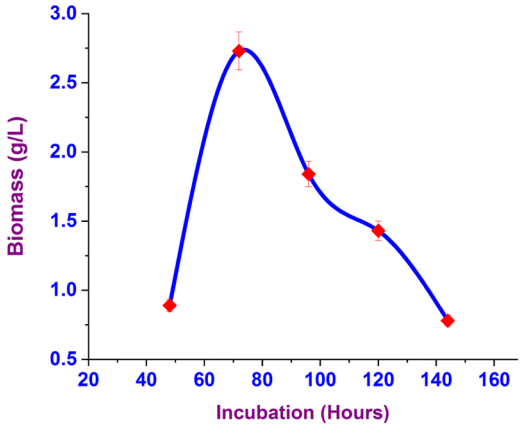

Fig. 4. Incubation time for biomass production of the isolates.

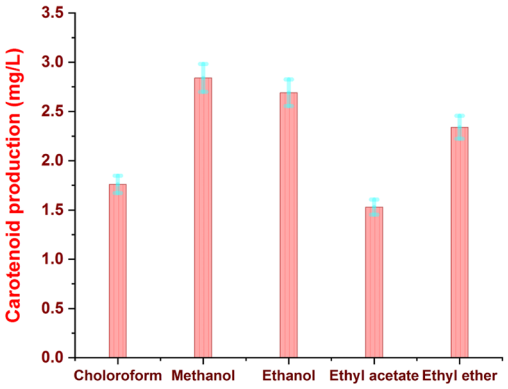

Fig. 5. Extraction of pigment from different solvents.

with the solvent, it has the ability to separate from a mixture of compounds is one of the important processes for the recovery of pigment. It was observed at acetic acid (Enrıquez *et al*. 2013).

The isolate capability to exhibit antimicrobial activity

It was used to check the organism acting as antimicrobial agents. The result of Fig. 6 observed that the SB_2 culture produce higher antibacterial activity, MIC concentration (100 µg ml^{-1}) of the bacterial pathogens. The different concentrations of yellow-pigmented isolate activity of the pathogens were reduced drastically. If it increases the concentration of the pigment decrease the bacterial activity due to the toxic property of the pigment. The pigment treated with bacterial pathogens which show antimicrobial activity in terms of zone of inhibition. The yellow-pigmented SB_2 strain found to be exhibited potential antibacterial activity for *Pseudomonas aerogenosa, E. coli,* and *Staphylococcus aureus.* Similar results were observed on pigmented marine bacteria *Micrococcus luteus* from seawater (Umadevi and Krishnaveni 2013). The *S. aureus, E.coli and B. subtilis* were exhibited antimicrobial activity for pigment. The *Stenotrophomonas maltophilia* is ubiquitous with potential antimicrobial activities capability to colonize plants, humans and marine environments. Carotenoid pigments extracted from marine bacteria and *Micrococcus luteus* exhibit antibacterial activity (Umadevi and Krishnaveni 2013). Seaweed is a source of *Halolactibacillus al-*

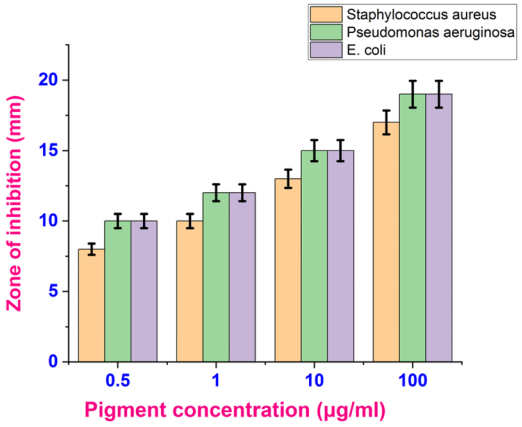

Fig. 6. Antibacterial activity of the pigmented isolates SB_2.

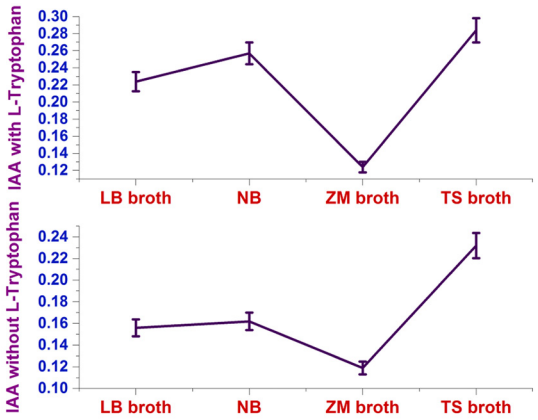

Fig. 7. Production of IAA by pigment SB_2 isolate.

kaliphilus MSRD1 was extracted from red pigment effective for antibacterial activity (Suresh *et al.* 2015).

IAA recovered from pigmented SB_2 isolate

The concentration of IAA production may vary due to microbial growth suitable for environmental condition. Phosphobacteria is also called phosphate solubilising bacteria which is able to synthesis IAA as well as to solubilise phosphate belongs to PGPR. The higher production of IAA was observed at YEMA medium by *Bacillus siamensis* supplemented with L-tryptophan (Suliasih and Widawati 2020) and IAA production was increased by *Streptomyces fradiae* (Myo *et al.* 2019). The isolate SB_2 was evaluated for IAA production (Fig. 7). The isolate SB_2 was observed higher production of IAA in TS broth (Tryptic soy broth) followed by NB (Nutrient broth) and LB broth (Luria Bertani broth). Microorganisms produce various plant growth-promoting substances called phytohormone from the rhizosphere to the endosymbiont of the crop plants. IAA play a critical role in the growth and development of plants by altering the plant metabolisms through the synthesis of a growth regulator called auxin. Earlier, *Rhizobium* was reported to produce IAA which is also able to produce cytokinin are the growth regulators used for plant development. Certain bacteria ability tolerate high salt concentration, survive in contaminated soil and resistant to drought by the production of IAA (Kudoyarova *et al.* 2019). The *Micrococcus yunna-*

nensis isolate SB_2 were observed great production of IAA on various media and methanol extraction was showed highest production in TS broth similar result was noticed from *B. brasilense* and *Herbaspirillumas. Azotobacter* is a free-living organism present on soil act as both nitrogen fixation and plant growth regulator (Barea and Brown 1974). *Azospirillum* fixes nitrogen to the soil apart from that it also synthesis IAA used for plant growth (Jain and Patriquin, (1985). Mordukhova *et al.* (1991) were also supported the *Burkholderia* isolates produce IAA and cytokinin, growth regulators. Some of the root colonizing bacteria were observed in the production of auxin and cytokinin by *Pseudomonas* sp. IAA production was observed on different types of microorganisms and one of the dominant groups was *Streptomyces.* The isolate SB_2 was recorded higher production of IAA in TS and NB media. The *M. yunnanensis* WI 60 yielded more IAA production. IAA contribute important function of plants by apical dominance, seed germination, stress tolerance, it improve growth and yield of the different crops (Maheshwari *et al.* 2015). The auxin production was increased in presence of salt by *Streptomyces* in wheat crop (Sadeghi *et al.* 2012).

Determination of gibberellic acid production

The isolate SB_2 have grown all the media and it ability to produce GA. The lowest recovery of GA was observed in ZM broth inoculated with SB_2 and the highest production noticed at TS broth followed by NB and LB (Fig. 8). When a sufficient carbon concentration is available in the substrate and nitrogen is depleted, the production of gibberellins occurs. The 136 types of GA were identified and studied among them GA_3 contribute increase the crop growth. The GA groups contribute to the yield of soybean crops by the isolate *Bacillus tequilensis* and reduce superoxide dismutase activity by synthesis of various amino acids, flavonoids and polyphenol in tomato plants (Kang *et al.* 2019, Kang *et al.* 2017). The isolates, SB_2 recorded higher quantity of GA in TS followed by NB and LB. *B. licheniformis* WI 90, followed by *Micrococcus* sp. strains WI 91 and *M. luteus* WI 80 were observed maximum gibberellic acid (GA) production. All the isolates showed optimum GA_3 production after 48 hours of incubation at various temperatures (20-40°C) at 30°C. All the endophytic, epiphytic and rhizospheric bacteria are capable to produce gibberellins (Mitter *et al.* 2002). Bacteria convert plant-available essential nutrients and produce plant growth hormones (Hakim *et al.* 2021). Gibberellic acid production promotes the growth and development of crops (Binenbaum *et al.* 2018).

Siderophore production observed from pigmented isolate

Siderophore production was evaluated from the isolate SB_2 which recovered two different types of siderophore (salicylate and catechol). The isolate ready to grow all the media and potential to produce siderophore, the higher production of salicylate showed in TS broth and the lowest production observed at ZM broth. The culture was exhibit better growth in NB broth followed by LB. Catechol type

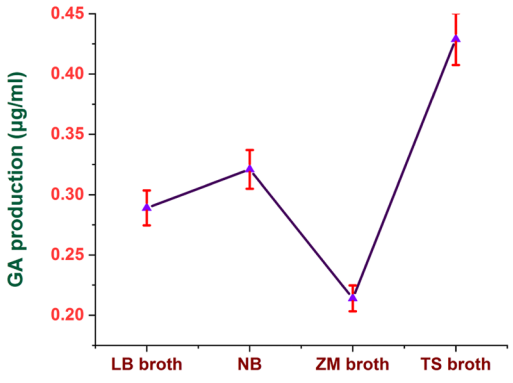

Fig. 8. GA produced by pigmented isolate.

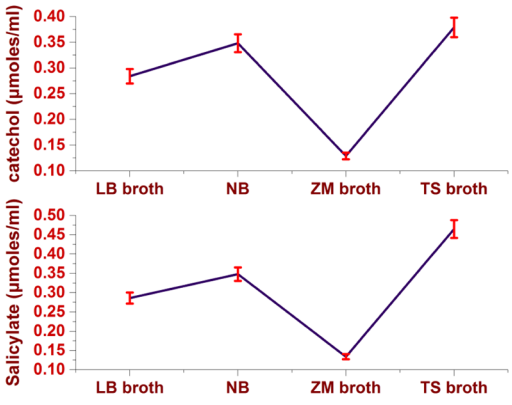

Fig. 9. Iron chelating activity of the isolate SB_2.

of siderophore produced by the isolate SB_2 on various media was higher production recorded at TS broth followed by NB broth and LB broth (Fig. 9). Siderophore is chelate iron from the soil that belongs to the activity of PGPR. The different types of siderophores are derived from soil viz., salicylate, catecholate, hydroxycarboxylic and hydroxamate acid and more than 500 compounds were identified so far. The low molecular weight compounds are used for soil pathogens for synthesizing hydrogen cyanide toxin applied for disease development (Boukhalfa and Crumbliss 2002). Some of the different types of salicylic acid and pyochelin siderophore were produced by *B. cepacia* isolates colonizing from rhizosphere soil and the higher production of siderophore was observed in *M. luteus* WI 12 (Bevivino *et al.* 1994). The SB_2 isolates were produced salicylate and catechol type of siderophores from various media in which higher production was noticed in TS media (salicylate and catechol) followed by NB media and the same result was reported by Neilands (1982). *Azospirillum* were the potential to produce salicylate and catechol type of siderophore. The marine environment depends on the bacteria which had the capability to phytoplankton for the availability of iron (Cordero *et al.* 2012). *Burkholderia cepacia* was able to produce various siderophore (Darling *et al.* 1998). Ornibactins was produced by *B. cepacia* from soil. Microorganisms other metal ions are the key factor for siderophore production (Gaonkar and Bhosle 2013). Catechol type of siderophore produces yellow color was treated with nitrous oxide for easy identification of this compound. *Streptomyces acidiscabies* was used to identify the siderophore production through mass spectrometry (Dimpka *et al.* 2009). *Micrococcus yunnanensi*s (SB_2 strain) was potential to produce catechol and salicylate type of siderophore. Certain phytoplankton population was altered through low concentration iron in oceanic water, the growth and development of marine organism require iron as a micronutrient (Gledhill and Buck 2012). *Alteromonas* sp. was produced alterobactin type of siderophore derived from the marine environment. Pyoverdine was a type of fluorescent siderophore produced from pigmented *Pseudomonas aeroginosa* (Gaonkar *et al.* 2012). **Bacillus megaterium** was ability to produce siderophore under alkaline condition. The hydroxomate and catechol type of siderophore produced by

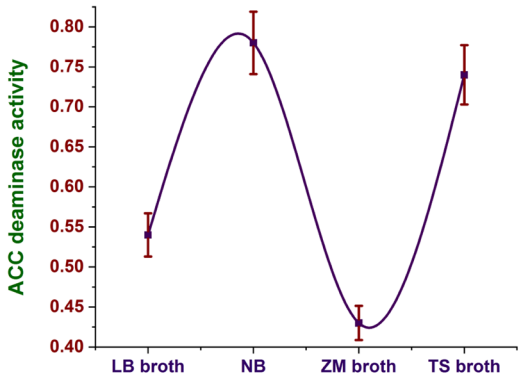

Fig. 10. ACC deaminase activity produced by pigmented isolate.

Bacillus sp. and *Pseudomonas* sp. from roots of the plant which is also used for plant nutrition (Grobelak and Hiller 2017, Ferreira *et al.* 2019).

The pigmented isolate and its ACC deaminase activity

The ACC deaminase activity occurs in all the media inoculated with SB_2 isolate, the highest activity was recorded in NB followed by TS broth and LB broth of the isolate SB_2 (Fig. 10). The overproduction of ethylene in plants at the time seedling stage inhibit the growth of root length leads to affect the plant growth in which PGPR organism play critical role in the reduction of ethylene by ACC deaminase (Glick *et al.* 2007). ACC deaminases protect the plant damage caused by the concentration of ethylene under salt stress environmental condition (Naing *et al.* 2021). The high quantity of ethylene is used for breaking the seed dormancy though it is used for beneficial purpose other side ethylene inhibit the root elongation in seed germination (El-Tarabily *et al.* 2019). Bacterial cells are degraded to provide nitrogen to the crop by the activity of ACC producing bacteria and the stress caused by the ethylene (Saravanakumar 2011). To overcome this process, the ACC deaminase enzyme produced by PGPR organisms adhere to the seed coat while growing the enzyme act on it to decrease the ethylene production as a result the root growth develops on the seedling. The isolate SB_2 using 1-aminocyclopropne-1-carboxylic acid (ACC) was

evaluated on TS, NB, LB and ZMB media supplemented with ACC (Caballero-Mellado *et al.* 2007). The highest production was recorded of NB broth followed by TS and LB of the isolate SB2. Ethylene concentration reduced due to ACC present in crop plants induced by rhizosphere microorganisms (Gamalero *et al*. 2023). ACC deaminase has a potential to reduce ethylene level in the plant to the extent the plant growth by their mechanisms of its action which enhance productivity in Agriculture and Horticultural crops (Penrose and Glick 2003). The ACC deaminase in the plants reduces environmental stress.

CONCLUSION

The SB_2 strain was effectively grown on various media in which the highest growth and yellow pigmentation occurred at TS broth, also enhanced by glucose, molasses, peptone and yeast extract added into the nutrients medium incubated at 72 hrs using methanol for extraction. The extracted yellow pigment showed antibacterial activity at 100 µg ml^{-1} confirmed that carotenoid pigment. The strain SB_2 also exhibits PGPR activities, the IAA, GA and siderophore production was observed in TS broth, while nutrient broth exhibits ACC deaminase activity.

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