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ORIGINAL ARTICLE

Optimization of Indole Acetic Acid Production by *Bacillus lichiniformis ISM1193* Endosymbiotic Bacteria Isolated from *Gracilaria Corticata (J. Agardh)*

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ABSTRACT
Endosymbiotic bacteria Bacillus lichiniformis ISM1193 isolated from Gracilaria corticata (J. Agardh) produces a significant amount of IAA (Indole Acetic Acid) Production parameters such as pH, temperature, salinity, carbon, nitrogen source and concentration of a tryptophan and time of incubation were optimized for the maximum production of IAA. The present study revealed that remarkable production of IAA was observed when cultural conditions were maintained at 37C (104.57 µg/ml), pH 7 (109.5 µg/ml), salinity 6% (108.µg/ml), and tryptophan concentration1.5 µg/ml (123 µg/ml). The maximum IAA was produced when glucose (119.60 µg/ml) and yeast extract (89.4. µg/ml) were used as carbon and nitrogen sources for 6 days (111.52 µg/ml).
Keywords: *Endosymbiotic bacteria, Bacillus lichiniformis, Gracilaria corticata, IAA.*

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INTRODUCTION

The rhizobacteria Bacillus was considered the most effective plant growth promoting rhizobacteria (PGPRs) that inhabit the rhizosphere and enhance the plant growth parameters by different direct and indirect methods [1]. Different species of Bacillus increase plant growth by producing plant growth stimulants such as gibberellic acid [2], Auxins [3] and cytokinin [4]. In previous studies, the scientists revealed that the use of IAA producing bacteria as biofertilizers improved plant growth and yield by promoting root length and root hairs and enhancing the growth of lateral roots which enables the plant to absorb nutrients effectively and promote overall plant growth [5, 6]. Recent studies [7] showed that *Bacillus lichiniformis* can be used in saline soils as a green bioinoculant to combat the salt stress on plant growth. The use of IAA producing symbiotic bacteria to increase plant growth which colonizes terrestrial plants was well established but the use of marine symbiotic bacteria as a biofertilizer has not been thoroughly investigated. Recent studies showed that Marine bacteria associated with Marine algae will experience drastic biotic and abiotic stress to combat and survive in this harsh environment they produce secondary metabolites [8, 9] which can be exploited for the wellbeing of mankind. The present study focused on the optimization of Physical and chemical parameters for the optimum IAA production by *Bacillus lichiniformis ISM1193* isolated from *Gracilaria corticata (J. Agardh)*. As *Bacillus lichiniformis ISM1193* was a halo tolerant bacterium it can be used as a PGPB for sustainable agriculture in arid and semiarid regions.

MATERIAL AND METHODS

In the present study, six Indole acetic acid producing endosymbiotic bacteria were isolated from *Gracilaria corticata (J. Agardh).* The highest IAA producing bacteria was subjected to taxonomical characterization and it was identified as *Bacillus licheniformis ISM1193.* Further studies were carried out to maximize the IAA production by optimizing media parameters such as Optimization of carbon, nitrogen source, tryptophan concentration, and process parameters such as pH, temperature, and salinity were considered to maximize IAA production from the selected strain [10].

Effect of pH on IAA production

To observe the effect of pH on the production of IAA by *Bacillus licheniformis ISM1193* at different pH (pH 3, 5, 7, and 9) levels were set up with 50ml of Yeast malt Dextrose broth supplemented with L-tryptophan. Cultures were incubated for 3 days with 200 rev/min in a water bath shaker, Cell free extract of the Bacterial culture broth of 72 hours old was unasked-for IAA by adding Salkowski reagent and measured the absorbance by spectrophotometer at 530 nm. The O.D. values were converted into the concentration of IAA by interpreting the values from the IAA standard graph [11].

Effect of temperature on IAA production

To assess the optimum temperature for the production of IAA by *Bacillus licheniformis ISM1193,* experiments were performed on above said cultures keeping the flasks incubated at different temperatures viz., 25, 30, 35, 40 and 45°C.

Effect of different carbon sources

Carbohydrates in the culture media provide the carbon source for the growth of the bacteria. In general, dextrose acts as a carbon source but different bacteria show differences in the production of IAA in the presence of 1% of arabinose, fructose, and mannose. To check which carbon source efficiently promotes the production of a high amount of IAA comparatively was observed by inoculating the 100µl of 24 hrs old *Bacillus licheniformis* in Yeast Malt Dextrose media, by replacing dextrose with sucrose and maltose [12, 13].

Effect of different nitrogen sources

IAA production was checked in the presence of different nitrogen sources like yeast extract, beef extract, peptone and Tryptone. Media was supplemented with 0.5% of the different nitrogen sources and measured the production of IAA.

Effect of L-tryptophan on IAA production

250 ml Erlenmeyer flasks containing 50 ml of Yeast malt dextrose broth were inoculated with 100 µl bacterial suspension ($1x10^6$ CFU/ml) and supplemented with each of L-tryptophan concentrations 0.5, 1, 1.5,2 and 2.5 mg/100ml respectively. The flasks were kept for 72 hours in a shaker incubator at 30^0C with 120 rev/min. At the end of the 3rd day, the culture filtrate was used to quantify the amount of IAA spectrophotometrically [14].

Effect of salinity on IAA production

IAA producers were grown in 50 ml of Yeast malt dextrose medium and 100 µl containing 24 hours aged cultures in triplicates. The salt content was adjusted with distilled water to 2%, 4%, 6%, 8% and 10% salinities and incubated at 30˚C for 72 hours. The tubes were centrifuged and the concentration of IAA in the supernatant was quantified spectrophotometrically as mentioned above [15].

Effect of incubation period on IAA production

To estimate the time course for the higher production of IAA 2 up to 10 days observation was done. The concentration of IAA produced was checked for every 2 day intervals up to the 10th day. 50 ml of Yeast Malt Dextrose broth was prepared with the addition of 0.75mg of L-tryptophan, 3g of NaCl and pH 7. One ml of inoculum *Bacillus licheniformis* was added to the flasks (the experiment was carried out in triplicate) and kept in a water bath shaker at 35 ± 2^0c with 120 rev/min. Culture filtrate was collected by centrifugation every 48 hours. IAA produced was estimated by the abovementioned method continuously for 10 days.

RESULTS AND DISCUSSION

Production of IAA by bacteria is influenced by multiple factors such as pH, temperature, salinity, carbon and nitrogen source, concentration of tryptophan and time of incubation. Effects of these parameters on the production of IAA were identified.

Effect of temperature on IAA production

The optimum temperature for the maximum production of IAA was 35^0C where it produced 104.57 µg/ml of IAA. The production of IAA(101.28 µg/ml) was also significant at 30^0C but substantially decreased to 27.49 µg/ml. It produced 38.4 µg/ml of IAA only at 40^0C (Figure 1). Values are depicted as means ± the standard error of the mean (SEM) and N=3.

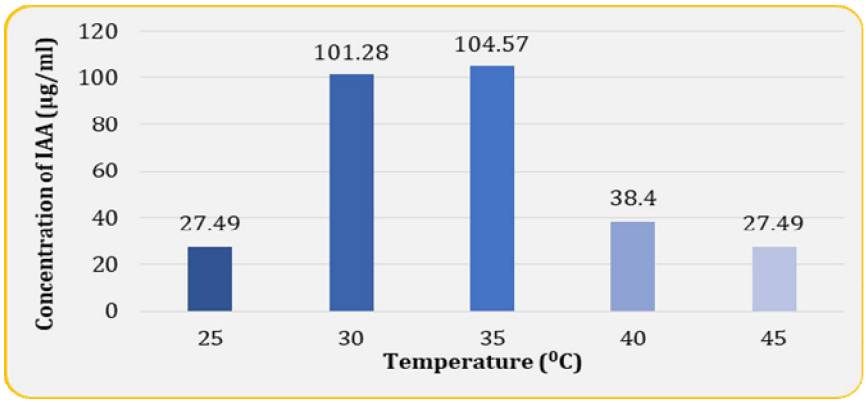

Figure 1: Concentration of IAA produced by *Bacillus licheniformis ISM1193* at different temperatures.

Effect of pH on IAA production

The highest and lowest values of IAA 109.5 µg/ml and 20.12 µg/ml were obtained at pH 7.0 and 3.0 respectively (Figure 2). Values are depicted as means ± the standard error of the mean (SEM) and N=3.

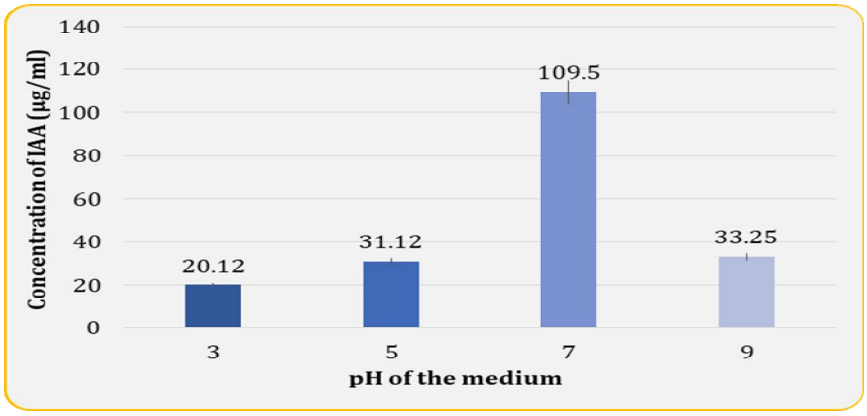

Figure 2: Quantity of IAA produced by *Bacillus licheniformisISM1193* at different pH value.

Effect of sodium chloride on IAA production

The maximum amount of IAA production was 108.µg/ml at 6% of NaCl concentration. The amount of IAA production at 4% was 98.47 µg/ml. IAA production was 42.45 µg/ml, 12.72 µg/ml respectively at 8% and 10% of NaCl concentrations. It indicated that higher salt concentrations decreased the production of IAA. The optimum salt concentration was 6% for the highest production of IAA (Figure 3). Values are depicted as means ± the standard error of the mean (SEM) and N=3.

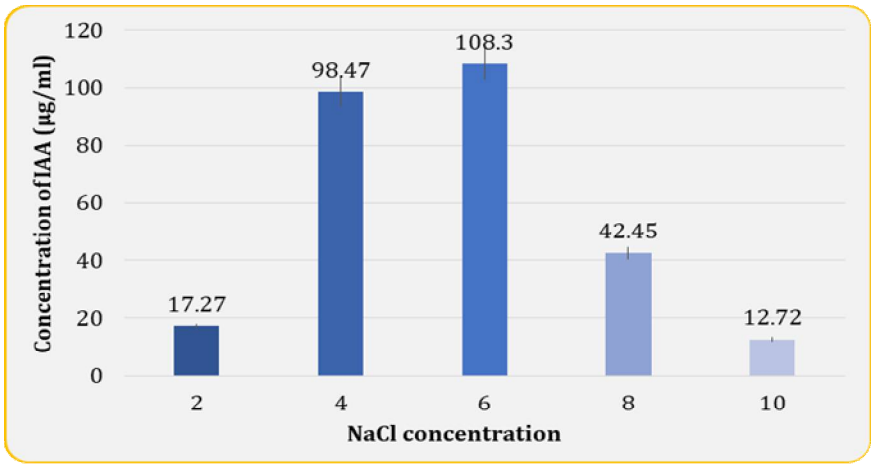

Figure 3: Concentration of IAA produced by *Bacillus licheniformisISM1193* at different NaCl concentrations.

Effect of tryptophan on IAA production

The optimum concentration of L-tryptophan for the production of IAA was 1.5 µg/ml. The production of IAA was good in the range of 1 to 2 µg/ml. The amount of IAA produced was 123, 95.68, and 83.2µg/ml when supplemented with the concentrations of 1.5,1 and 2 µg/ml respectively (Figure 4). Values are depicted as means ± the standard error of the mean (SEM) and N=3.

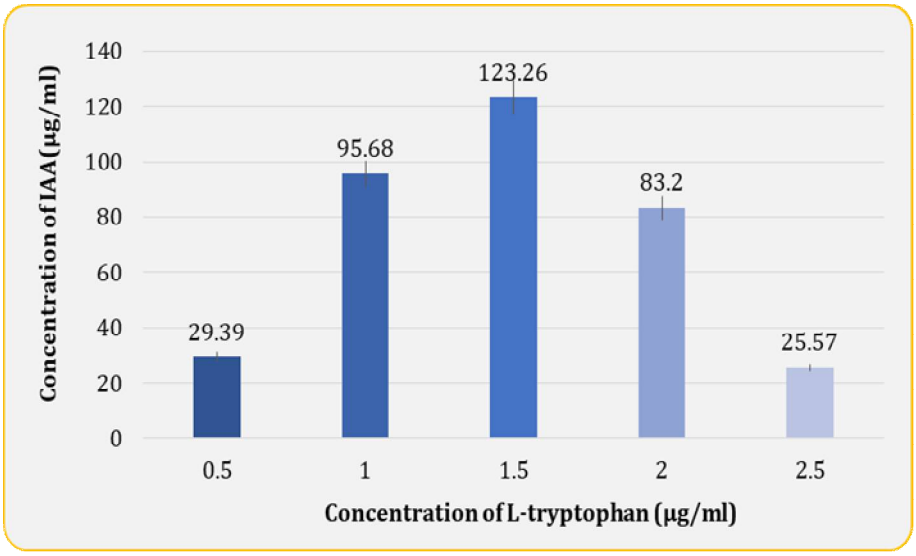

Figure 4: Concentration of IAA produced by *Bacillus licheniformis ISM 1193* at different tryptophan concentrations.

Effect of carbon source on IAA production

Glucose was observed as the good carbon source for the IAA production (119.60 µg/ml) followed by Fructose (50.5 µg/ml), Maltose (27.55 µg/ml), and arabinose (17.17 µg/ml) respectively (Figure 5). Values are depicted as means ± the standard error of the mean (SEM) and N=3.

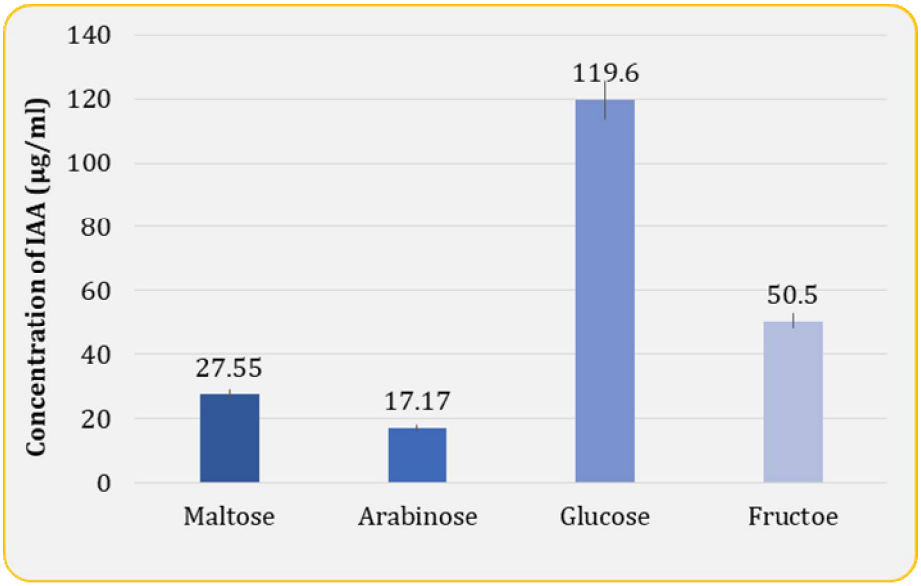

Figure 5: Amount of IAA produced by *B. licheniformis ISM1193* in the presence of different carbon sources.

Effect of nitrogen source on IAA production

The amount of IAA produced was 89.4. µg/ml in the presence of yeast extract. The amount of IAA produced in different nitrogen sources such as Yeast extract, Tryptone Beef extract, and peptone was 89.4 µg/ml, 54.58 µg/ml, 50.5 µg/ml, and 42.45 µg/ml respectively (Figure 6). Values are depicted as means ± the standard error of the mean (SEM) and N=3.

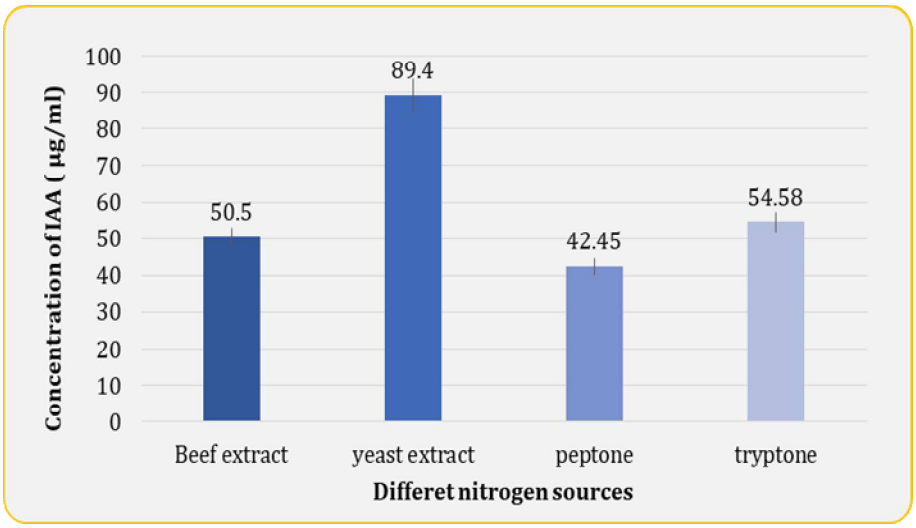

Figure 6: Concentration of IAA produced by *B. licheniformis ISM1193* in different nitrogen sources.

Effect of time on IAA production

The maximum amount of IAA was produced on the 6th day of incubation and decreased by the end of the 10th day. Production of IAA was observed from the 2nd day onwards and it produced good amounts of IAA on the 4th and 6th day. The amount of IAA produced on day 4th and day 6th were 109.52 and 111.52 µg/ml respectively (Figure 7). Values are depicted as means ± the standard error of the mean (SEM) and N=3.

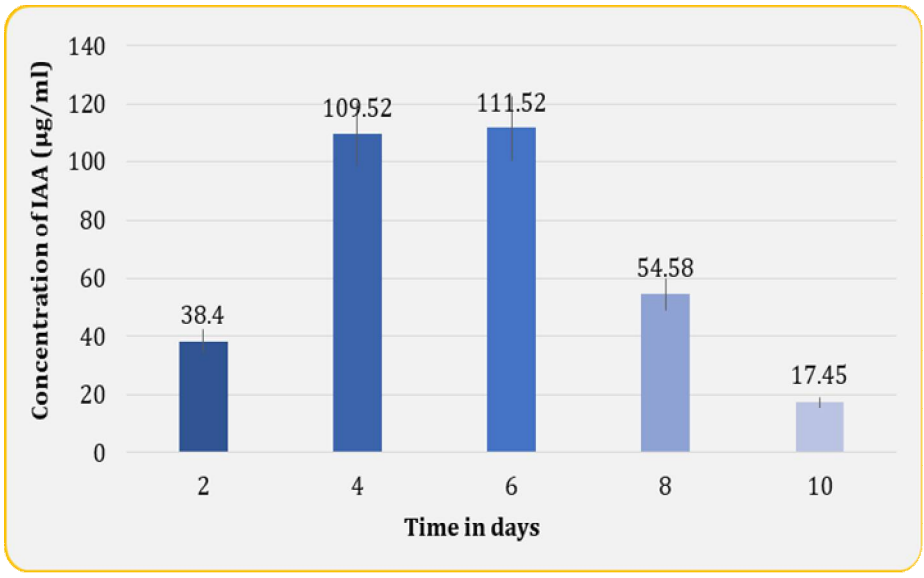

Figure 7: Concentration of IAA produced by *B. licheniformis ISM1193* at different time intervals.

IAA production by the bacterial isolates was influenced by various factors. The yield of IAA by the *Bacillus licheniformis ISM1193*was remarkably maximized when cultural conditions were optimized. The present study revealed that *Bacillus licheniformis ISM1193* produces IAA over a temperature range from 25 to 40 °C (Fig-1), with the highest yield at 35°C(Fig-1). (104.57 ± 0.06µg/ml) A similar finding was reported in previous studies [11, 16, 17, 18]. The results of [19] also follow our findings. The enzymes involved in IAA biosynthesis were affected by the temperature [20]. Beyond 45°C the production of Auxin was reduced. The pH of the medium considerably affected the bacterial auxin biosynthesis, producing higher amounts of auxin at neutral pH. The present study also reported high amount of IAA production by the *Bacillus licheniformis ISM1193*at neutral pH (109.5 ± 0.13 µg/mL).

Studies by [11, 18] also reported that *Bacillus spp*. produced a maximum amount of auxin at neutral pH. *Bacillus lichenoformisMML2501*isolated by [18] produced a notable amount of IAA 23 µg/ml at pH 7.Which are consistent with our results. Production of IAA was increased in acidic media and decreased in alkaline media. (Fig. 2). The strain produced maximum amount of IAA in a medium that has Glucose as the

major carbon source (Fig. 3). [14] Reported that Plant Growth Promoting Characteristics of *Bacillus Licheniformis DS3* isolated from Agriculture field Soil also showed maximum IAA production in a glucose medium.

The varying levels of NaCl also affected the IAA production. *Bacillus licheniformis ISM1193* produced the optimum level of auxin at 6% NaCl concentration (Fig. 3). [21] reported that Maximum IAA production is observed at 6% of NaCl (86.5mg/ ml). Similarly, *Bacillus licheniformis* strain A2 and *Kocuriaturfanensis* 2M4 isolated from saline soil showed PGPR traits determined by in vitro biochemical tests that could enhance the growth of *Arachis hypogaea (L)*(ground nut) [22]. It has recently been shown that the salt-tolerant *A. Brasilense isolate NH* produces IAA during salt-stressed conditions, and it was hypothesized that this production may considerably contribute to the increase in salt tolerance of inoculated wheat plants [23]. Salinity can increase the rate of ethylene biosynthesis via elevated levels of 1-aminocyclopropane-1-carboxylic acid (ACC), which may lead to physiological changes in plant tissue [24]. Thus, *Bacillus licheniformis ISM1193* can be considered as the salt tolerant PGPR that can be effectively used in saline soils to improve crop productivity. Availability of Nutrients is another important factor that may affect the production of the IAA by bacteria. In the present study, Glucose supplementation in the medium significantly enhanced the concentration of auxin in the culture filtrate. Bacteria use tryptophan as a precursor for auxin biosynthesis. The presence of Tryptophan in the growth media stimulates auxin production [25, 26]. Bacteria can synthesize IAA through tryptophan dependent and tryptophan independent pathways, and a high degree of similarity between biosynthesis of IAA in plants and bacteria have been reported [27, 28]. The present study also revealed that with the increase in L-Tryptophan concentration increased the production of IAA. The studies of the [29] proved that Tryptophan in the medium plays a vital role in the maximum IAA release (174.72 µg/ml) by Bacillus licheniformis ML3.The concentration of auxin increased in the culture supernatant with incubation time, as it was a secondary metabolite [17]. Present observations revealed that the highest amount of IAA was obtained on the 6^{th} day at optimal conditions (pH-7, 35^0C, 6% NaCl, and 1.5mg of Tryptophan). A significant affiliation was observed between bacterial growth and IAA production from our findings. The IAA concentration in culture filtrate decreased in later stages due to degradation of IAA by the IAA degrading enzymes such as IAA oxidase, and peroxidase by the bacteria [30, 31].

CONCLUSION

Bacillus licheniformis ISM1193 exhibits promising potential as a sustainable bioinoculant for saline agricultural soils. The isolated strain has salt tolerance combined with remarkable IAA production trait is well suited to increase crop yield in salt stressed conditions. The application of *Bacillus licheniformis* ISM1193 as a biofertilizer reduces the use of chemical fertilizers and keeps the environment safe offering an approach to sustainable and ecofriendly agricultural practices.

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