Factors influencing the growth and production of auxin in two locally isolated *Synechocystis* species from variant rice field habitats

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Abstract

*Synechocystis* species have been assessed for their capacity to produce IAA (Indole acetic acid) in the rhizosphere and water of a rice field. Temp, acid levels, treatment time, and multiple tryptophan proportions were analyzed to see how they affected growth and phytohormones content. Different temperatures and pH levels ranges were preferred by habitat-isolated strains for development and auxin production. The ideal pH and temperature for water isolate growth were 8 and 30°C, respectively, while for rhizospheric isolate growth, 7 and 25°C. Both strains produced the most auxin at pH 6 and 35°C. The production of auxin by both isolates was positively related to incubation and tryptophan quantity.

Key Words: IAA, Cyanobacteria, *Synechocystis*

INTRODUCTION

The spread of cyanobacteria, Gram-negative photosynthesizing prokaryotes is worldwide. They can thrive in a variety of habitats, including aquatic and terrestrial environments. Numerous studies have shown that cyanobacteria can discharge a diverse range of organic and inorganic chemicals into their exterior, which include freshwater, sea, and soil environments (Khalid *et al.*, 2004, Prasanna *et al.*, 2009).

Cyanobacteria that live in agricultural areas have the potential to affect and impact plant development by producing a variety of secondary metabolites, including phytohormones. The potential of cyanobacteria to produce auxin has economic benefits over conventional synthetic phytohormones, with a broad range of action and effective amounts of biologically active
components suitable for typical plant growth and development. A lot of environmental factors, including pH, temp, and light, contribute to the control of the plant’s development and growth by regulating their hormonal hemostasis (Mattiello et al., 2010). As a result, several physical factors, such as incubation time, temperature, light and dark periods, pH of the culture medium, and affecting cyanobacterial development, may have a role in altering the cyanobacteria’s capacity to create auxin (Ahmed et al., 2010). In the current work, cyanobacteria *Synechocystis* strains SM-05 and SM-10 were incubated under a variety of different growth conditions in an effort to improve the circumstances for auxin synthesis.

**MATERIAL AND METHODS**

*Isolation and SM-10 Synechocystis sp. purifying*

It was necessary to take irrigation water and rhizospheric soil samples from a rice crop in order to isolate the *Synechocystis* strain. Root-associated soil was then collected in a polythene bag after uprooted rice plants were gently shaken to eliminate additional soil for the sample's isolation from the rhizospheric region. One gram of soil was agitated in 10 ml of sterile, distilled water using a Vortex-Mixer model SLV-6 to suspend the sediments. One cc of suspended soil and collected irrigation water samples were put to 250-milliliter conical flasks with 100 ml of properly sterilized BG11 (Rippka et al., 1979), bold basal (Kent and Triplett, 2002), Gorham (Hughes, 1958), Chu-10 (Chu, 1942), and D culture (Castenholz, 1981). Each flask was incubated at 25°C for 15 days, alternately receiving 12 hours of light (120 mol photons m\(^{-2}\) s\(^{-1}\)) and 12 hours of darkness. Samples from flasks were obtained after 15 days and diluted tenfold on BG11 medium plates using cyclohexamide (100 g/ml). All plates were cultured for two weeks at 25°C with a constant exposure to 18 mol photons m\(^{-2}\) s\(^{-1}\). Individual colonies were chosen and transferred to fresh BG11 plates under stereomicroscopy from mixed cultures of cyanobacteria grown somewhat on plates' surface. Once more, pure colonies were chosen and streaked onto fresh BG11. This strategy was used several times to establish an axenic civilization. The axenic status of the cultures was investigated by transferring portions of the agar block that contains the cyanobacterial cultures into potato dextrose agar medium plates and Luria Bertani agar (Gerhardt et al., 1994; Naicker et al., 2007).

The morphology of isolated cyanobacterial cultures was studied. Cell size, shape, colour, motility, and existence have been noted in order to research the morphology of cyanobacteria.
Tandeau de Marsac and Houmard (1988) technique was used to assess cyanobacterial growth. 16S rRNA sequencing was used to identify different cyanobacterial strains. With a few minor adjustments, the Srivastava et al. (2006) approach was used to isolate DNA from cyanobacterial cultures that had been in existence for 15 days. Extracted DNA was amplified with forward primer 27 F1 (5'-TAGTGTAAAACGGCCAGTAGTTGATCCTGGCTCAG-3') and reverse primer 409R (5'-TTACAACCCAGGGCCTTCTCCC-3') (Neilan et al., 2002). Amplified and purified DNA was sequenced using an automated sequencer (Applied Biosystem; Model 3100).

**Biosynthesis of IAA and growth measurement**

The impact of various parameters such as incubation period (5, 10, 15, 30, 40, 50, and 60 days), pH (4, 5, 6, 7, 8, 9, and 10), temperature (10, 15, 20, 25, and 30 C), light-dark duration (24:00, 20:04, 16:08, 12:12, 08:16, 04:20, and 0:24), and tryptophan presence on the growth and auxin production of *Synechocystis* strains was investigated. According to Tandeau de Marsac and Houmard's 1988 approach, the assessment of cyanobacterial cultures' chlorophyll-a concentration (g/ml) was used to gauge growth. Using the Salkowski reagent, auxin production in cyanobacterial strains was measured (Glickmann & Dessaux, 1995). IAA substances from cyanobacterial samples were examined using GC-MS in a selected ion monitoring mode, according to Barkawi et al. (2008).

**RESULTS**

*Synechocystis sp. SM-10 isolation and purification*

From a field of rice at Punjab University's agricultural region, two samples were taken. A sample was from irrigated water, and the other has been taken from rhizosphere. Five different kinds of media, including BG11, bold basal, Gorham, Chu-10, and D media, has been employed to enrich samples in the first stage. As BG11 medium showed the greatest growth, it was utilized for further purification and identification.

**Analyzing and identifying Synechocystis species SM-10**

One strain of unicellular bacteria was recovered from each sample. The 16S rRNA gene revealed that both isolated cultures belonged to same species, *Synechocystis*, but that their growth characteristics and auxin production varied greatly (Table 1). While SM-05 separated from water
grew more quickly but produced less auxin, SM-10 isolated from the rhizosphere exhibited the opposite tendency (Table 1). Following identification, light microscopy was used to characterize the morphology. Both strains of cells had very thin, colorless sheaths and were primarily solitary and in pairs. The cells in both strains were blue-green in hue and around 2 to 3 µm in diameter (Table 2).

**Table 1: Strains of *Synechocystis* with their accession number identified by 16S rRNA gene sequencing isolated from *Oryza sativa* field.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Identified as</th>
<th>Sequence length</th>
<th>Maximum homology</th>
<th>Accession number</th>
<th>Chl-a (µg/ml)*</th>
<th>IAA (µg/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM-05</td>
<td><em>Synechocystis</em></td>
<td>1266</td>
<td>96</td>
<td>JF703682</td>
<td>3.51</td>
<td>1.65</td>
</tr>
<tr>
<td>SM-10</td>
<td><em>Synechocystis</em></td>
<td>1395</td>
<td>98</td>
<td>JF703681</td>
<td>2.63</td>
<td>4.43</td>
</tr>
</tbody>
</table>

*Chlorophyll measured in laboratory after 30 days of incubation

**Table 2: Morphological characterization of *Synechocystis* strains.**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Morphotype</th>
<th>Color</th>
<th>Shape</th>
<th>Size</th>
<th>Growth pattern</th>
<th>Sheath</th>
<th>Gas vesicle</th>
<th>Motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM-05</td>
<td>Unicellular</td>
<td>Blue green</td>
<td>Circular</td>
<td>2-3 µm</td>
<td>Dispersed</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(solitary/pair)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM-10</td>
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<td>(solitary/pair)</td>
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<td></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

**Measuring growth and estimating auxin**

It was shown that longer incubation times resulted in more growth and auxin production. After 40 days, their growth became stationary, but they continued to produce more auxin for another 50 days. After 60 days, there was a significant decrease in the auxin concentration of *Synechocystis* SM-10, although there was just a little variation in *Synechocystis* SM-05. Both
*Synechocystis* strains have strong correlations between incubation time and auxin production (SM-05, r =0.830*, SM-10, r =0.820*) and growth (SM-05, r =0.952**, SM-10, r =0.943**).

The production of auxin and growth both had positive correlations [SM-05 (r =0.923**), SM-10 (r =0.884**)] (Figure 1A). More auxin production was seen in both strains at pH 6, but the optimal pH for their growth varied. At pH 8, SM-05 showed optimal growth, while SM-10 showed maximum growth (Figure 1B). Both strains [SM-05 (r =0.968**), SM-10 (r =0.966**)] showed good correlations between growth and production of auxin at all temperatures. At 3°C, both strains produced their most auxin and grew the fastest (Figure 1C). Both strains thrive best when exposed to a 16:08-12:12 light–dark cycle. At 08:16 light: dark phase, both strains generated their most auxin (Figure 1D). Both incubation period and higher tryptophan concentration have a positive link with auxin production, it was discovered when the combined effects of incubation time period and variable tryptophan concentration were examined on the auxin production of both strains (Table 3). To establish the synthesis of auxin, cyanobacterial samples were subjected to GC-MS analysis (Figure 2).

**DISCUSSION**

The current study concentrated on two unicellular *Synechocystis* strains that were obtained from two distinct rice field habitats. Regarding growth and auxin production, both strains demonstrated conflicting behavior. In comparison to the *Synechocystis* strain SM-05 isolated from water, SM-10 from the rhizospheric area was more effective in producing auxin. Many researchers have discovered that rhizospheric bacteria are more adept at possessing features that promote plant development (Dobbelaere *et al*., 2003, Khalid *et al*., 2004, Prasanna *et al*., 2009). Plant roots release a vast variety of potentially beneficial chemicals as a result of changes in root secretions in different root zones, which are frequently accountable for affecting the functional and structural diversification of rhizospheric microbes (Kent and Triplett, 2002; Das *et al*., 2010). With progressively longer incubation times, cyanobacterial strains produced more auxin and grew faster. More auxin was being released into the media as the cells grew, and this rise was consistently seen for 50 days. However, decline was afterwards noticed. The cause may be that auxin was being synthesized at a faster rate than it was being broken down when cells were actively developing, but as they moved into their stationary phase, auxin production may have stabilized while deprivation persisted. Ahmed *et al.* (2010) in *Arthospira* and Sergeeva *et al.*
Figure 1: Effect of different physical parameters on IAA production of cyanobacterial strains in media supplemented 250 µg/ml Tryptophan in BG11 media, (A) Incubation period (Days); (B) pH; (C) Temperature (ºC); (D) Light: Dark period (hours)
Table 3: IAA produced by cyanobacterial strains in media supplemented with varying amount of precursor L-tryptophan at different incubation period (days). (A) Synechocystis strain SM-05 (B) Synechocystis strain SM-10. Mean of five independent experiments.

r¹: Correlation coefficient between IAA production and initial tryptophan concentration

r²: Correlation coefficient between IAA production and incubation period in days

A

<table>
<thead>
<tr>
<th>Incubation period (Days)</th>
<th>Initial tryptophan concentration (µg/ml)</th>
<th>r¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>0±0.00</td>
<td>0.02±0.004</td>
</tr>
<tr>
<td>10</td>
<td>0.01±0.003</td>
<td>0.06±0.005</td>
</tr>
<tr>
<td>15</td>
<td>0.01±0.006</td>
<td>0.09±0.007</td>
</tr>
<tr>
<td>30</td>
<td>0.02±0.03</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>40</td>
<td>0.05±0.01</td>
<td>0.18±0.02</td>
</tr>
<tr>
<td>50</td>
<td>0.07±0.01</td>
<td>0.29±0.03</td>
</tr>
<tr>
<td>60</td>
<td>0.06±0.009</td>
<td>0.23±0.03</td>
</tr>
</tbody>
</table>

r²: 0.948**  0.936**   0.808*  0.805*  0.772*  0.830*  0.767*    0.765*       

B

<table>
<thead>
<tr>
<th>Incubation period (Days)</th>
<th>Initial tryptophan concentration (µg/ml)</th>
<th>r¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>0.02±0.01</td>
<td>0.21±0.03</td>
</tr>
<tr>
<td>10</td>
<td>0.05±0.01</td>
<td>0.26±0.03</td>
</tr>
<tr>
<td>15</td>
<td>0.08±0.01</td>
<td>0.33±0.04</td>
</tr>
<tr>
<td>30</td>
<td>0.13±0.01</td>
<td>0.56±0.04</td>
</tr>
<tr>
<td>40</td>
<td>0.28±0.03</td>
<td>0.84±0.07</td>
</tr>
<tr>
<td>50</td>
<td>0.47±0.02</td>
<td>1.03±0.06</td>
</tr>
<tr>
<td>60</td>
<td>0.19±0.03</td>
<td>0.65±0.06</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.765*</td>
<td>0.850*</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).
Figure 2: GC-MS analysis of the methylated cyanobacterial IAA sample (ions at m/z 130 and 189) containing the added $^{13}$C$_6$-IAA internal standard (ions at m/z 136 and 195) and its comparison with labeled and unlabeled IAA standard, (A) Standard of $^{13}$C$_6$-IAA, (B) Standard of unlabeled IAA, (C) Cyanobacterial sample *Synechocystis* SM-10.
(2002) in *Nostoc* cyanobacteria both observed an increase in auxin content with time. Both strains had distinct preferred pH and temperature ranges for optimum development; SM-05 exhibited best growth at pH of 8 and 30°C, whilst SM-10 showed best growth at pH 7 and 25°C. Both demonstrated the optimum release of auxin as far as temperature was concerned at 6 and 35°C. The percent increase (11-26%) in auxin production under the same circumstances was substantially connected with the 71-79% reduction in growth at 6 pH in comparison to their optimal growth pH (7-8). Numerous studies have demonstrated that an acidic environment causes genes to function in the synthesis of auxin and other phytohormones to become more active (Ryu and Patten, 2008; Mattiello *et al*., 2010; Fierer and Jackson, 2006; Yuan *et al*., 2008). The percent increase (12-70%) in auxin production was significantly connected with the decline (21-98%) in growth at temperature 35°C as compared to their most favorable growth temperatures (25 and 30°C). Due to the fact that extremely low and extremely high temperatures are shown to promote plant hypocotyl via increasing auxin synthesis, phytohormones production is boosted under stress situations (Gray et al, 1998; Stepanova *et al*., 2008). Different light and dark cycle priorities were displayed by the *Synechocystis* strains for maximum auxin synthesis and growth. They grew the fastest during the phase with the most light and secreted the most auxin during the phase with the least amount of light. Given that cyanobacteria are phototrophs, they may use a phase with roughly equal dark and light cycles for their development, which is one explanation for how this fascinating type of performance manifests (Litchman, 2003; Waterbury, 2006). Additionally, it has long been known that IAA is light-sensitive and that extended light periods may cause it to tarnish more quickly (Yuan *et al*., 2008). Distinct trends were seen in both strains of *Synechocystis* when the synchronized influence of incubation duration length and different quantity of tryptophan on production of auxin was investigated. Elevated auxin concentration with exogenous tryptophan up to 0.08% was detected in SM-05 after 40 to 50 days of incubation. However, additional tryptophan ingestion revealed decreased auxin levels. In contrast to SM-05, SM-10 displayed a decrease in auxin concentration with the addition of tryptophan at a rate of 0.06% during all incubation times. The fact that tryptophan becomes fatal at larger doses for their development may be the reason why they react in this way. Rawson noticed a decrease in cyanobacteria growth and their capacity to fix nitrogen with greater concentrations of tryptophan (1985). We deduced from the current investigation that the strain isolated from the rhizospheric area was more capable of generating auxin than the water
isolate. The ideal physical conditions for auxin synthesis and development varied between the two strains.

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