Production of Gibberellic Acid by *Bacillus siamensis* BE 76 Isolated from Banana Plant (*musa spp*)

M. S. Ambawade¹, G. R. Pathade²

¹Microbiology Department, Vasantdada Sugar Institute, Manjari, Pune (MH) India
²The Principal, H. V. Desai, College, Pune (MH) India

**Abstract:** Based on morphological, cultural, biochemical and 16s rRNA gene sequencing a newly isolated endophytic bacteria from the banana plant (*musa spp*) was identified as *Bacillus siamensis* and designated as BE 76. Fifteen bacterial isolates were screened out for their productivity of Gibberellic acid by spectrophotometric method. Out of these isolates BE-76 isolate showed high amount of Gibberellic acid production supplemented with and without L tryptophan in medium(0.180 and 0.240 mg/mL, respectively). Gibberellic acid production of BE-76 was further confirmed by high performance liquid chromatography (198.16 and 254.29 ppm, respectively) with and without L-tryptophan in medium for more accuracy.

**Keywords:** Banana, *Bacillus siamensis*, Gibberellic acid, 16s rRNA gene sequencing, HPLC

**1. Introduction**

Banana (*Musa paradisica* L.) is one of the leading tropical fruit crops. It ranks next to mango in both area and production in India [1]. Endophytes are defined as ‘microbes that colonize living, internal tissues of plants without causing any immediate, overt negative effects’. Endophytes are diverse microorganisms inhabiting in internal plant tissues [13]. *Bacillus* and *Pseudomonas* *spp* were reported as endophytes [8]. Plant growth promoting bacteria (PGPB) are defined as free-living soil, rhizosphere, rhizoplane, endophytic, and phyllosphere bacteria that, under certain conditions, are beneficial for plants [2]. The strains with PGPR activity, belonging to genera *Azospirillum*, *Azotobacter*, *Arthrobacter*, *Bacillus*, *Clostridium*, *Enterobacter*, *Gluconacetobacter*, *Pseudomonas*, and *Serratia*, have been reported [10]. Phytohormones (Indole acetic acid, Gibberellins) are plant growth regulators, which have stimulatory effects on plant growth [18]. It is also very likely that growth promoting effects of various PGPRs are due to bacterial production of plant growth regulators such as indole-3-acetic acid (IAA), gibberellins, and cytokinins [4], [3]. Gibberellic acid production was confirmed by *B. pumilus* and *B. licheniformis* [7]. The present investigation was conducted to demonstrate the Gibberellic acid production of *Bacillus siamensis* BE 76 isolated from stem of banana (*musa spp*) with and without L-tryptophan supplement in medium .Such bacteria are further useful for production biofertilizers to enhance growth and productivity of banana as well as to decrease use of harmful chemical fertilizers.

**2. Materials and Methods**

**2.1. Collection of Sample**

Banana plant sample (stems) was collected from village Zari, district of Parbhani, Maharashtra (India).

**2.2. Isolation of endophytic bacteria from banana plant:**

**2.2.1. Surface disinfection:** The banana stem samples were thoroughly washed in running tap water. They were then surface-disinfected using 70% ethanol for 2 min and immersed in 150 ml of 1.5% sodium hypochlorite plus a few drops of Tween 20 for 5 min with shaking. The samples were then rinsed thoroughly in five changes of sterile distilled water and dried in sterile paper towels [15].

**2.2.2. Isolation of Endophytic bacterial isolates:** After Surface disinfection samples were macerated with a sterile mortar and pestle and then serially diluted in 12.5 mM potassium phosphate buffer at pH 7. For isolation of Gibberellic (GA₃) producing endophytic bacterial isolates several types of media were used such as nitrogen-free media - NFb [16], MacConkey’s, Congo red [17], YEM agar [20] and nutrient agar [5]. Total 15 isolates were obtained from stems of banana plant. The isolates were further checked for Gibberellic acid production capability.

**2.2.3. Morphological, cultural and biochemical characterization of isolates:**

Morphological and cultural characterization was done on the basis of colony size, shape, color, margin, opacity, consistency, elevation, motility and gram staining, Endospore, capsule staining and based on the colony morphotypes selection of representative isolates was done. Biochemical tests performed were oxidase, amylase , gelatinase and catalase like enzyme production, citrate utilization, indole test, Vogus Proskauer test, methyl red test, H₂S production, sugars (Glucose, Sucrose, Lactose, Xylose and Mannitol) fermentation, Triple sugar iron (TSI) test, nitrate reduction, urease test etc. [9].
2.2.4. 16s rRNA gene sequencing:

Bacterial genomic DNA was isolated using geneO-spin Microbial DNA isolation kit (geneOmbio technologies, Pune; India). Partially a 16S rRNA gene was sequenced. Bacterial 16S region gene was amplified using standard PCR (Machine by Applied Biosystems 2720) reaction. The primer pair 27F (AGAGTTTGATCMTGGCTCAG3) and 1492R (TACCTTGTAGCACCT) as a universal primer were used in a PCR reaction with an annealing temperature of 57°C. After amplification, products were purified by using a geneO-spin PCR product Purification kit (geneOmbio technologies, Pune; India) After PCR is completed, the PCR products were checked on 1% Agarose gel. Agarose gel spied with Ethidium bromide at a final concentration of 0.5 µg/ml was prepared using Agarose (LE, Analytical Grade, Promega Corp., Madison, WI 53711 USA) in 0.5X TBE buffer. 5.0 µl of PCR product was mixed with 1% of 6X Gel tracking dye. 5µl of g Scale 100bp size standard (geneOmbio technologies, Pune; India) was loaded in one lane for confirmation of size of the amplicon using reference ladder. 1% agarose gel was run at 5V/cm until the tracking dye is 2/3 distance away from the lane within the gel. bands were detected under a UV Trans illuminator. Gel images were recorded using BIO-RAD Gel Doc XR gel documentation system. The PCR product of size 1450bp was generated through this reaction and directly sequenced using an ABI PRISM Big Dye Terminator V3.1 kit (Applied Biosystems, USA). The sequences were analyzed using Sequencing Analysis 5.2 software. DNA sequencing was performed using one of the PCR primers [11]. BLAST analysis was performed at Blast N site at NCBI server and matching of sequence was compared using reference ladder. The DNA molecules were resolved at 20 min/sample. The wavelength used for detection of GA was at 254 nm [12], [Department of chemistry, Fergusson College, Pune].

3. Results and Discussion

3.1. Morphological, cultural and biochemical characterization of isolates:

The isolation of bacteria from surface disinfected stems of banana normally allows the recovery of putative endophytic bacteria. All isolates obtained from banana and selected by these combined criteria were grouped by morphological similarities and phenotypic characteristics [14]. In the present study total 15 N2 fixing Endophytic bacterial isolates were isolated from stems of banana plant which showed different culture and morphological characteristics in which eleven isolates showed gram negative rods in nature, three were gram positive rods and remaining gram positive cocci. The sporulation, capsulation and motility as well as biochemical characteristics were also studied (Table 3.1.1).

![Table 3.1.1: Biochemical characterization of promising isolate](https://www.ijsr.net)

### Table 3.1.1: Biochemical characterization of promising isolate:

<table>
<thead>
<tr>
<th>S.N</th>
<th>Biochemical Tests</th>
<th>Results of Isolate BE- 76</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oxidase test</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Amylase test</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Gelatinase liquidation test</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Catalase test</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>indole test</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>methyl red test</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Vogus Froskat test</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>citrate utilization</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>H2S production</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Glucose</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Sucrose</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>Xylose</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>Mannitol</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>Lactose</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>Triple sugar iron (TSI) test</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>nitrate reduction</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>Urease test</td>
<td>-</td>
</tr>
</tbody>
</table>

3.2.16s rRNA gene sequencing of isolate BE 76: As described in more detail in methods we devised and implemented a method to extract the bacterial SSU rRNA 27f and 1492r primer-binding site sequences from the data in the RDP and the Sargasso Sea metagenomic data. A key point of the method is that it assumes only a 50% sequence identity between the region containing the primer-binding site in the sequence being analyzed and at least one member of a diverse set of “reference” sequences. The method is general and can be used to extract any portion of a sequence that is sufficiently conserved or at least is flanked by conserved sequences [11]. As per partially 16s rRNA gene sequencing of the isolate 575 bases sequenced are as follows:

---

**Volume 4 Issue 7, July 2015**

[www.ijsr.net](http://www.ijsr.net)  
Licensed Under Creative Commons Attribution CC BY
(GTCTGACCCGACATGGGCTAGTGATGAGGCTACCAAGGCAGA
CGATCGCAGTGAGGGAATCTCCGCAATGACGAA
AAAGTCTGAGCGAGCAAGCCTGAAGGGTGATCGGCA
ACTGAGAGGCTACCAAGGCGAACGATGCGTAGCCGAC
CGATGCGTAGCCGACCTGAGAGGGTGATCGGCCAC
CACTGGGACTGAGACACGGCCCAGACTCCTA
CGCAGCCGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGA
AAGTCTGACGGAGCAACGCCGCTGAGTGATGAA
GGTTTTCGATCGTAAAGCCTGTTGTTAGGGAAG
ACAAAGTGCCCAGTTCAAATAGGGCGGCACCTTGAC
GGTGGAATACGTAGGTGGCAAGCGTTGCTCCGGA
ATTATTGGGCGTAAAGGGCTCGCAGG
GGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCA
ACCGGGAGGGTTGCAAACGGAGTGGAATTCCACG
GGTAAAGAGGAGAGTGGAATTCCACG
GGTGAAATGCGTAGAGATGTGGAGGAACACCAGT
GGCGAAGGCGACTCTCTGGTCTGTAA) After NCBI
BLAST analysis these bases 99% matched with Bacillus
siamensis belong the family Bacillaceae and hence this
isolate was identified as Bacillus siamensis based
on Bergey’s manual, and 16s rRNA gene sequencing and was
designated as BE 76.

3.3. Gibberellic acid production potential of banana
dendophytic bacterial isolates:

Fifteen isolates were screened out for their productivity of
gibberellic acid on spectrophotometer. Out of these 15
isolates six isolates (BE-63, BE-66, BE 79, BE-71, BE-74
and BE-76) showed ability to produce gibberellic acid
(GA$_3$) without L-tryptophan supplement in medium at 75
minutes. From these gibberellic acid producing isolates,
BE-76 produced high amount of gibberellic acid (0.180 and
0.240 mg/ml) supplemented with and without L-tryptophan
in medium at 254nm (Fig.3.3.1). Productivity of gibberellic
acid of BE-76 isolate was further estimated and confirmed
on high-performance liquid chromatography (HPLC).

![Figure 3.4.1: Chromatogram of Gibberellic acid produced by banana Endophytic bacterial isolate (BE-76) without tryptophan in medium](image1)

![Figure 3.4.2: Chromatogram of Gibberellic acid produced by banana Endophytic bacterial isolate (BE-76) with 0.1% tryptophan in medium](image2)

4. Conclusions

From the present investigation, it is clear that endophytic
bacteria isolated from stems of banana can provide a rich
source of Gibberellic acid and has the ability to produce a
significant amount of Gibberellic acid with and without
tryptophan-supplemented in media. Out of 15 isolates six
isolates showed ability to produce Gibberellic acid with and without L-tryptophan supplement in the medium. BE-76 showed high amount of Gibberellic acid production which was estimated on Spectrophotometer and for more accuracy the productivity of Gibberellic acid of BE 76 isolates HPLC was used. Based on morphological, cultural, biochemical and 16s rRNA gene sequencing this isolate was identified as Bacillus siamensis and designated as BE 76. It is concluded that presence of such growth promoting Endophytic bacteria (Bacillus siamensis) were accountable for the beneficial effects on crop growth and yield. Nitrogen fixation, plant growth promotion and improved nutrient absorption are important criteria for achieving a sustainable banana production system. The Gibberellic acid producing Bacillus siamensis BE 76 will promote the growth at the field level and prevent environmental pollution by avoiding excessive applications of chemical fertilizers and add to development of liquid bioinoculant for sustainable agriculture.

5. Acknowledgements

The authors are thankful to Vasantdada Sugar Institute, Pune and Fergusson College, Pune for their continuous support and encouragement.

References


Author Profile

**Dr. G. R. Pathade** is working as Principal, H. V. Desai, College, Pune (India). He has completed M.Sc., M.Phil, Ph D in Microbiology. He has more than thirty years’ academic, administrative and research experience. He is recognized research guide for M.Phil & PhD in Microbiology and Environmental Sciences of Savitribai Phule Pune University. Fourteen students (6 PhD & 8 M.Phil) have completed their research work under his guidance. He has also published more than 110 research papers in national and international research journals. He received Bharat Shikhsha Ratan Award – 2012 (New Delhi).

**Mr. M. S. Ambawade** is pursuing PhD in Microbiology from VSI Pune (Savitribai Phule Pune University). He has completed M.Sc., M.Phil in Microbiology. He has also published six research papers in national and international research journals.