

## Detection of GFP Expression and Colonization of Wheat by two Endophytic Bacteria tagged with GFP Gene

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Received: 19.01.2017 | Revised: 30.01.2017 | Accepted: 1.02.2017

### ABSTRACT

The two endophytic bacteria had 92% similarity with *Pseudomonas synxantha* strain NBRC103159 and 96% with *Pseudomonas cedrina* strain CFML96-198 were identified using 16S ribosomal DNA sequence and isolated from stems of *Eleusine indica*. Its endophytic colonization were investigated microscopically using green fluorescent protein introduced by vector pGLO containing ampicillin resistance gene and arabinose sugar. The strains were able to colonize wheat tissues and localized in the roots, stems and leaves.

**Key words:** *Pseudomonas* spp., pGLO vector, GFP, Fluorescent microscopy, Wheat

### INTRODUCTION

Endophytic bacteria colonize the host tissue internally, sometimes in high numbers, without damaging the host or eliciting symptoms of plant disease. They are beneficial for plant in various ways like IAA production, gibberellins production, cytokines production, ACC deaminase activity and nitrogen fixation. It has been reported that some of bacteria colonize sugarcane and sweet potato<sup>1</sup>, wheat<sup>2</sup>, rice and Maize<sup>3</sup>. Wheat is an important crop in india and Plant-microbe interactions that promote plant development and plant health have been the subject of considerable interest. Endophyte enters in plants primarily through the root tips and spread throughout the plants including seeds. Colonization of endophytes is very important in the practical use of plant

growth promotion activity. Reporter gene like green fluorescent protein (GFP) has become a valuable tool for interaction and colonization studies of endophytes with their host plants. This gene does not required ATP and any cofactor for its activation<sup>4</sup>.

The goal of the study was to examine the colonizing patterns of two strain of endophytes which were tagged with GFP inside the wheat.

### MATERIALS AND METHODS

#### Isolation of Endophytic bacteria

Bacteria were isolated from stems of *Eleusine indica* that is a one type of grass from Banni region of Kutch. The 1 cm stems were surface sterilized and the endophytic bacteria were isolated using Nutrient agar media<sup>5</sup>.

**Cite this article:** Akbari, D.L. and Akbari, L.F., Detection of GFP Expression and Colonization of Wheat by two Endophytic Bacteria tagged with GFP Gene, *Int. J. Pure App. Biosci.* 5(1): 844-848 (2017). doi: <http://dx.doi.org/10.18782/2320-7051.2476>

## Molecular Characterization of endophytic bacteria

The isolates were grown in Nutrient broth medium for 24 h and total DNA was isolated using invitrogen charge Switch® gDNA Mini Bacteria Kit. The 16S rRNA was amplified using the primers 907R (5'-CCGTC AATTCCTTTRAGTTT-3') and 27F (5'-AGAGTTTGATCCTGGCTCAG-3')<sup>6</sup>.

The PCR condition was one cycle of denaturation at 95°C for 7min, 35 cycles at 95°C for 30 s, annealing at 48°C for 30 s, elongation 72°C for 45 s and final elongation at 72°C for 5 min. The amplified DNA was partially sequenced using forward primer 27 F on a 3130xl capillary sequencer (Applied Biosystem). The 16S rRNA sequences of isolates were compared to the NCBI database using BLASTn.

## Incorporation of GFP gene into endophytic bacteria

For the colonization studies, endophytic bacteria were tagged with green fluorescent protein using pGLO™ Bacterial Transformation Kit (Catalogue Number 166-0003EDU BIO-RAD). The pGLO plasmid contain ampicillin resistance gene and arabinose sugar for activate the GFP gene. The incorporation of GFP gene was carried out by transformation using  $CaCl_2$  solution. Heat shock gave at 47 °C for exactly 2 min and incubation on ice before heat shock was 15 min and after for 5 minutes. The plasmid is stably maintained in a number of Gram-negative bacteria. After transfer of the plasmid, cultures were spread on LB media containing ampicillin and arabinose. The colonies those were uptake pGLO plasmid showing the green fluorescence under UV light. Certified seeds of wheat GW-366 were obtained from Wheat Research Station, Junagadh Agriculture University, Junagadh were surface sterilized with 0.1%  $HgCl_2$  for 5 min followed by 3 times washing with sterile distilled water and also washed with 70% ethanol for 3 min followed by 2 times washing with sterile distilled water. Sterile seeds of wheat were inoculated in gfp trasformed

endophytic bacteria in nutrient broth had a arabinose (600mg/ml) and ampicillin (30mg/ml) for 1 hour. Colonization of endophytic bacteria was checked on wheat seedlings by providing the natural system of soil under the controlled conditions in an environmental chamber (22-24°C, 10 h day/light) for 15 days. Soil used in process were autoclaved three times after intervals of 24 hours<sup>1,7</sup>.

## Examination of GFP-tagged endophytic bacteria in wheat seedlings

After 15 days of growth in an environmental chamber (22-24°C, 10 h day/light), wheat seedlings were removed from the tubes and washed in running tap water, placed separately on blotting paper for absorbing access water then sections were cut. Hand cut section of live leaves, stem and roots were examined using Axiomager-Z fluorescence Microscope (ZEISS) and images were captured with a camera, using the software Zen. The filter set in Zeiss with a 450-490 nm band-pass excitation and 550 nm emission was used for the GFP examination.

## RESULTS AND DISCUSSION

### Isolation and selection of endophytic bacteria for GFP

A total 25 endophytic bacteria were isolated from sterilized stem of *Eleusine indica* which were further selected on the basis of their plant growth promoting activities. The two strains which gave positive results for IAA production, ACC deaminase activity, siderophores production, phosphate solubilisation and nitrate reduction were selected for 16S rRNA sequencing and tagging with GFP.

### Identification of Endophytic bacterial strain

The isolate 1 and 2 showed 92% similarity with *Pseudomonas synxantha* strain NBRC103159 (Acc no- NR113583.1) and 96% with *Pseudomonas cedrina* strain CFML96-198 (Acc no- NR042147.1), respectively using 16S rRNA sequences. The

*Pseudomonas* spp. had been earlier identified as an endophytic bacteria from poplar tree<sup>8</sup>, *Sopora alopecuroides*<sup>9</sup>, rice<sup>10</sup> and sand dune<sup>11</sup>.

### Colonization and localization of endophytic bacteria in wheat tissue

*Pseudomonas synxantha* strain NBRC103159 appears in root tip and intra cellular spaces (fig 1 A & E) of wheat root, also localize in xylem vessels and inter & intracellular spaces of wheat stem (fig 1 B, C & F). This bacterium also colonize intercellular spaces of wheat leaves (fig 1 D). *Pseudomonas cedrina* strain CFML96-198 colonized pericycle, exodermis cells and vascular bundles of wheat root (fig 2 A & B) and stem (fig 2 C & D) while able to colonize stomata wall and inter and intracellular spaces of wheat leaves (fig 2 E & F). The rapid spread of gfp-derived strains in wheat plant indicates that the vascular system is the probable route for systemic colonization. The effects of gfp-tagged *P. putida* W619 and

*Enterobacter* sp. strain 638 endophytic bacteria were reported on poplar roots<sup>8</sup> and Zong 1 (*Pseudomonas* sp.) colonized in roots of *Sophora alopecuroides*<sup>9</sup>.

Among Gram negative bacteria, *Pseudomonas* is the most abundant genus in the plants and beneficial for them by producing plant growth promoting traits and also used as biocontrol agents producing a different enzymes and antibiotics. Another feature is that *Pseudomonas* strains were used as a vector to transfer different enzymes in plant and they are able to colonize and localized inside the different wheat tissues and promoting the growth. The present results also confirmed that stems of the plants also harbour similar valuable endophytic bacteria as the roots have. The experiment has demonstrated that GFP-tagged cells can be used to detect and locate the position of endophytic bacteria into the wheat tissues.

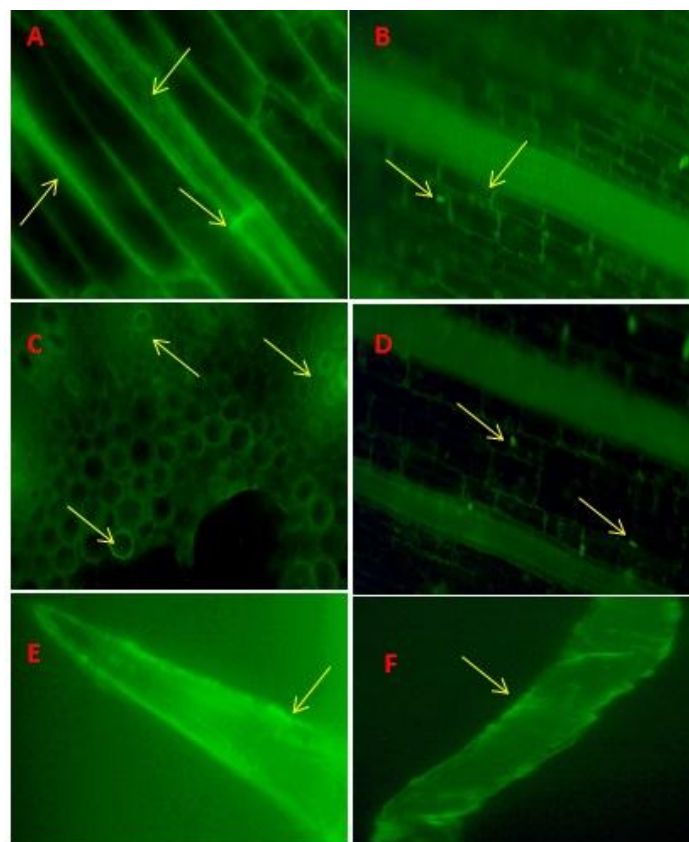


Fig 1 :Colonization of wheat seedlings by *Pseudomonas synxantha* strain NBRC103159 strain tagged with gfp. A, B, C, D, E and F – Transverse and longitudinal sections of root, stem and leaf.

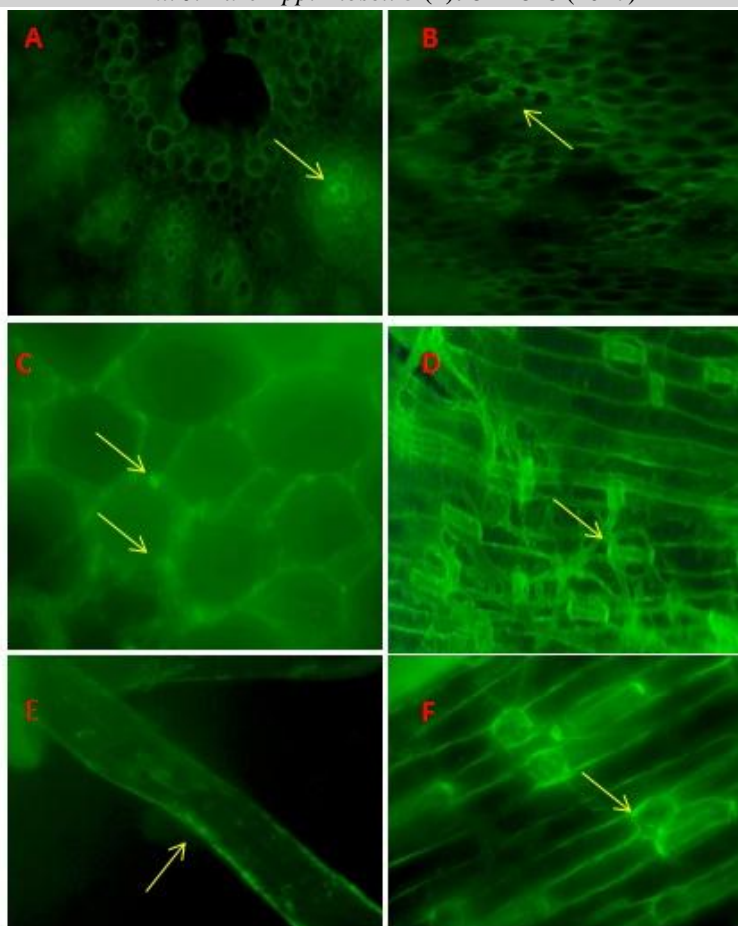


Fig 2 :Colonization of wheat seedlings by *Pseudomonas cedrina* strain CFML96-198 strain tagged with gfp. A, B, C, D, E and F – Transverse and longitudinal sections of root ,stem and leave.

### Acknowledgement

This Research work was funded by Deptt. Of Biotechnology, Junagadh Agricultural University, Junagadh, Gujarat.

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