# Ethylene induces endophyte bacteria to control early and late stage development in several plant species.

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**ABSTRACT:**

Plant growth promoting bacteria (PGPB) can modify plant growth and increase nutrient uptake. This study focuses on additional applications for PGPB in pre-harvest and post-harvest biotechnology.In this study a Bacillus sp. were exposed to urea, cobalt, and iron cofactors and induced with ethylene gas. The induced bacteria enhanced early stage development in cucumber plants. The bacetria increased seed germination by 25% and increased the number of blossoms per plant increased by over 50%.

The induced Bacillus sp. controlled late stage development inseveral plants species. The bacteria delayed the effects of climacteric ripening in bananas and peaches. The PGPB may biosynthesize a compound that is released into the surrounding environment that affects early stage development and late stage development in several species of plants.

**Keywords:** Ethylene, Cyanide, Plant Growth Promoting Bacteria, PGPB, Climacteric Ripening

**INTRODUCTION:**

The agricultural industry is demanding smart alternative products to chemical fertilizers and spray preservatives to meet new consumer demands for healthy organic produce. Farmers are searching for cost effective products that are ecofriendly, organic, and versatile to enhance crop yields and protect organic crops from seed to harvest, (Trostle and Seeley, 2013).

Plant growth promoting bacteria (PGPB) are bacteria known to use several mechanisms to affect various stages of plant development including increasing nitrogen, phosphorous, and potassium uptake by plant root systems, (Vacheron et *al.*, 2013). Some species such as PGPB including Rhodococcus spp., Norcardia spp., and Xanthobacter spp. have been shown to degrade plant hormones like ethylene. Ethylene is commonly used by plants to regulate the plant aging process and climacteric ripening, (Ahemad and Kibret, 2014; Perry 2011; Trobacher, 2009; Elsgaard, 1998; Yang and Oetiker, 1998; DeBont, 1976; Burg, 1973).

PGPB have been used for centuries as bacterial inoculants to modify plant development. Recent studies suggest that PGPB may have additional applications as biological fertilizers, biological pesticides, or biocatalyst to prevent post-harvest loss, (Egamberdieva, 2012; Glick, 2012). Azobacter, Bacillus, Azosprillum, Acetobacter, Pseudomonas, and Rhodococcus species are some of the more studied species of PGPB, (Ahemad and Kibret, 2014; Perry, 2011; Binder et *al.*, 2004; Kloepper et. *al* 1991). Papers have shown PGPB are capable of biosynthesizing cyanohydrins and/or plant auxins, such as Indole-3-Acetic Acid (IAA) or Indole-3-Aceotonitirle (IAN) by utilizing multi-functioning enzymes like aldoxime dehydratase, nitrile hydratase, nitrilase, and amidase enzymes (Hayat et *al.*, 2010; Nomura et *al.*, 2012; Kato et *al.*, 2004; Nagasawa et *al.*, 2000). Several papers questioned the benefits of this symbiotic relationship, particularly the usage of byproducts of the ethylene degradation in bacteria (Perry, 2014; Ensign and Allen, 2003; Elsgaard, 2000; Allen and Ensign, 1998; Elsgaard and Allen 1998).

This paper focuses on the ability to initiate and perpetuate IAA and IAN biosynthesis in PGPB by exposure to short chained hydrocarbon inducers and heavy metal cofactors. The bacteria were induced, by products from the induction were exposed to seeds and fruits to determine if the byproducts would modify the plant development process.



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## *MATERIALS AND METHODS:*

### 2.1 Chemicals/ Media

Chemical Materials

Ethylene; Cyanide; Cobalt Chloride; Urea; Potassium Phosphate; Ammonium Chloride; Ferric Chloride Iron; Yeast Extract

Biological Materials

Soil samples 2.3kg of soil from the rooting systems of a *Vitis rotundifolia* (muscadine) vine and *pyrus communis* (pear) tree. Bacillus species (Hardy Diagnostic)

### 2.2 Ethylene and Cyanide Induction

Bacillus species were suspended in media that contained cobalt, urea, iron, and yeast extract. The bacteria was placed in a closed seal container with ethylene and cyanide for 3 days at 25˚C, (Ethylene 10-15% by volume). Soil samples were collected and tested for ethylene, cyanide, and cyanohydrin concentrations.

### 2.3 Early Stage Plant Development Trials

Germination Studies: The study used organic cucumber seeds and induced Bacillus sp. The bacteria was sprayed directly onto the soil and mixed thoroughly. These samples were labeled induced soil. The cucumber seeds were grown in a organic and degradeable 36 cube container. The first 6 cubes of the tray were labeled negative controls. No seeds were placed into the empty trays. Using sterile forceps three cucumber seeds were planted in each cube. of a 36 cube seed starter container. Tray one contained induced bacteria and seeds, tray two the control.

Crop Yield Studies: The study was designed to see if induced seeds would continue to show enhanced growth in an outside environment. Six cubes were removed from the both organic tray containers and planted into a small organic garden by a local gardner. The plants were placed throughout the garden in late August. Seedlings were watered during the initial transfer, but were not irrigated. Data was collected to observe plant growth.



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### 2.4 Late Stage Plant Development Trials

To observe the effects of the induced bacteria on late stage plant development. Induced bacillus species were placed into sealed containers with organic bananas. The Bacillus sp. were stored in petri tray lids placed in close proximity, but no direct contact to the fruit for 5 days, at 24-26°C. Bananas (Green Tomato Organic Market in Tallahassee, Fl) and digital images (Sony "Cyber Shot', 7.2 mega pixels).

## RESULTS/ DISCUSSION

### 3.1 Early Stage Plant Development

Previous studies show PGPB are capable of metabolizing compounds that can affect late stage plant development and delay fruit ripening, (Perry, 2011; Perry 2014). Data shows induced bacteria increased seed germination and reduced fungal rotting un organic seedlings, See Table 1 & Figure 1. Induced bacteria enhanced plant growth and increased cucumber plants potential for crop yield. The plants grown with induced bacteria produced significantly higher number of blossoms per plant, Table 2 & Figure 2.

| | **No. Cucumber Seeds** | **No. Cucumber Seedlings** | **Germination Percentage** | **Avg. Seeds Grown** |
|---|---|---|---|---|
| Induced Soil | 90 | 69 | 76.67% | 2.23 |
| Non-Induced Soil | 90 | 41 | 45.65% | 1.37 |

**Table 1:** Data to observe germination rates of control seeds compared to seeds grown with induced Bacillus sp. The data shows the induced bacteria increased seed gerimination by over 25%.

| | **Total Blossoms** | **Average Blossoms** | **Standard Deviation** |
|---|---|---|---|
| Induced Soil | 131 | 21.83 | ± 5.34 |
| Non-Induced Soil | 60 | 10.00 | ± 2.83 |

**Table 2:** Data to observe crop yield of control seeds compared to seeds grown with induced Bacillus sp. The data shows the induced bacteria increased seed gerimination by almost 50%.



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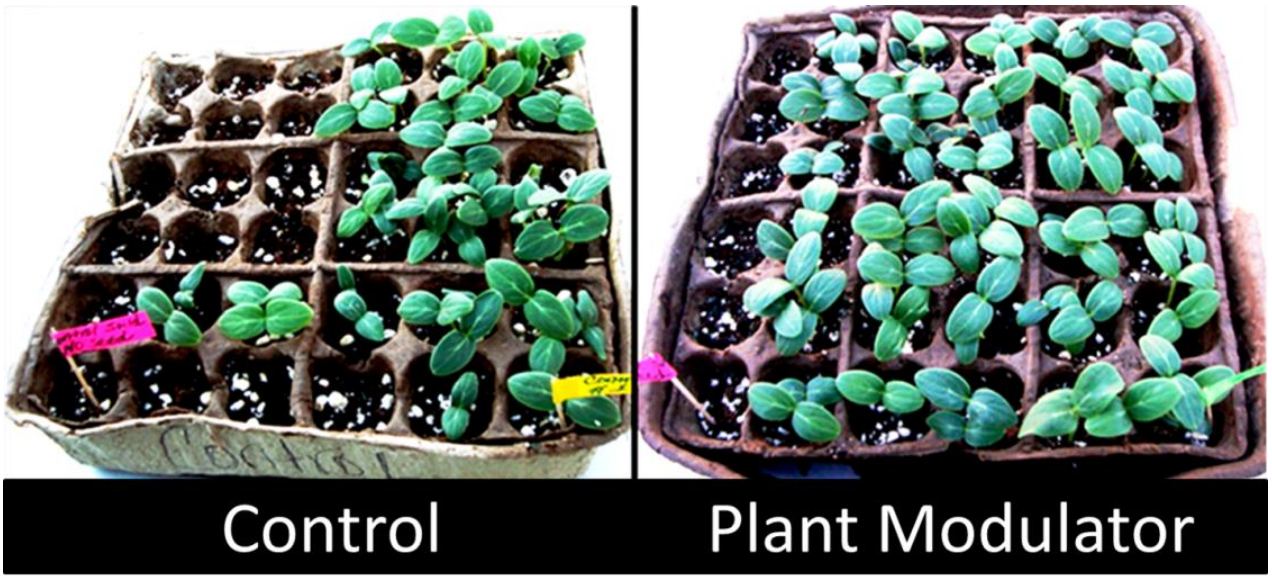


**Figure 1:** Control Tray: non-induced soil and organic cucumber seeds. Plant Modulator Tray: induced Bacillus sp. and organic cucumber seeds. Negative control row (first row on left) included no cucumber seeds.


**Figure 2:** Control: non-induced soil and organic cucumber plants. Sample: Cucumber plants grown with induced Bacillus sp. Person shows scale and difference in length.



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### 3.2 **Late Stage Plant Development**

Induced Bacillus sp. also affected late stage plant development in organic bananas. The bananas stored without bacteria were soft, spotted, and smelled sweet. Bananas stored with induced bacteria remained firm, no visible spotting, and no sweet odor or ripened smell, See Figures 3 & Figure 4. The experiment was carried out in triplicates and repeated twice.

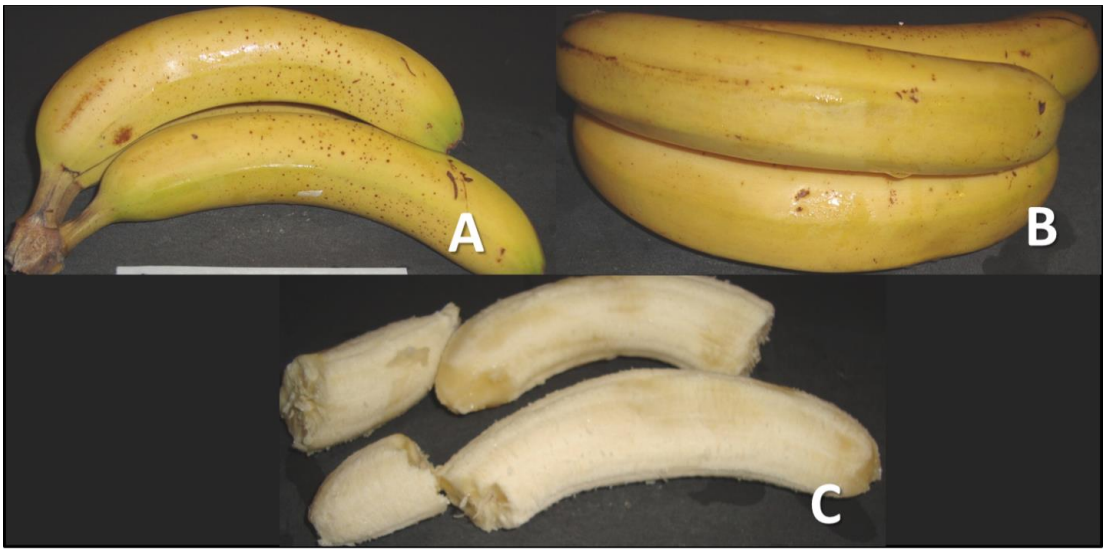


**Figure 3:** Negative Control: No bacteria were placed into the container with organic bananas. Bananas were removed and imaged after 5 days. Bananas appear over ripened and soft.


**Figure 4:** Induced Bacteria: Induced bacteria were placed into the container with organic bananas. Bananas were removed and imaged after 5 days. Bananas appear fresh, firm, and no visible spotting.



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## CONCLUSION:

There is a growing movement in the agricultural industry to focus on using bacteria to and microbial biosynthesized to grow organic crops (Kloepper et *al.* 1991). This paper focused on a preliminary understanding of the symbiotic relationship between PGPB and plants that enables the bacteria to delay the effects of fruit ripening and enhance plant growth. In a previous paper induced soil samples were analyzed to identify potential gaseous compounds released after ethylene induction. GC analysis of the induced samples showed acetonitrile, 2-methyl,1-propanol, 2 Pentanol, & 2-Pentanone were released from induced soil samples but not the non-induced soil samples, (Perry 2014). These compounds play a significant role in a proposed pathway that benefits bacteria and plants.

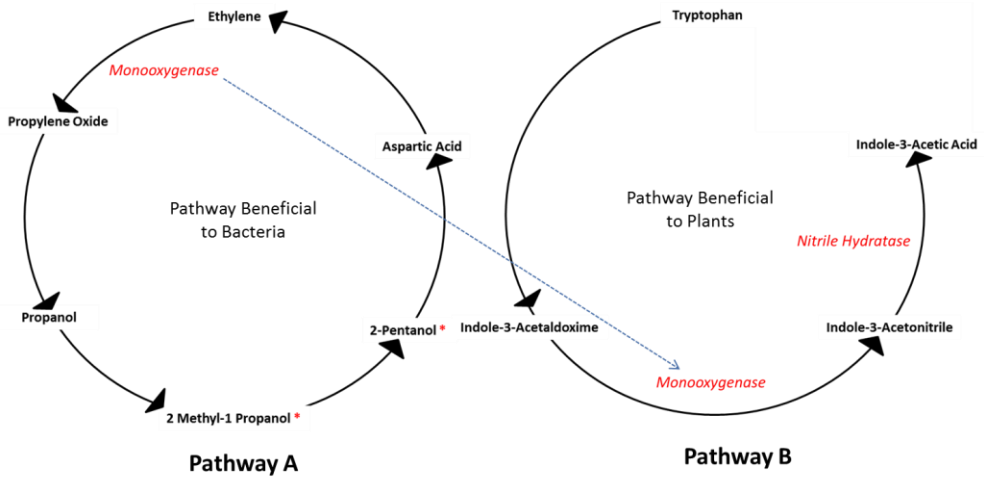


**Figure 5:** Hypothesized Pathway: Induced bacteria that release compounds that affect plant development.

Bacteria may have evolved to develop a symbiotic relationship with plants where the bacteria uptake and metabolize ethylene to  into an amino acid compound, and in exchange plants receive growth hormones such as indole-3-acetonitrile and indole-3-acetic acid that enhanceplant growth and development.



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