

An Assay of Phytohormonal Activity in *Rhizobium Rubi* and *Rhizobium Radiobacter*

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Abstract:

Two rhizobacteria with potential for plant growth modulation through secretion of hormones were used in an assay to determine the degree to which these secretions affected the growth of Alaska peas. Each bacterial species was grown separately in a liquid medium and the bacterial secretions were extracted from this medium. Varied concentrations of these extracts were applied to subapical epicotyl sections of 7-day-old dark-grown Alaska pea seedlings. Difference in growth was measured between experimental groups and appropriate controls. In general, the experimental groups were different than their control groups. However, though general trends are promising, standard deviation is high within individual groups. ANOVA tests confirmed that the difference between groups is not statistically significant.

Key Terms:

- Rhizobacteria: A bacteria that inhabits the roots of plants
- Assay: A test of the biological activity of a substance
- Epicotyl: The stem of a seedling
- ANOVA: A statistical test termed “Analysis of Variance”
- Phytohormone: A chemical signal that has effects in plants

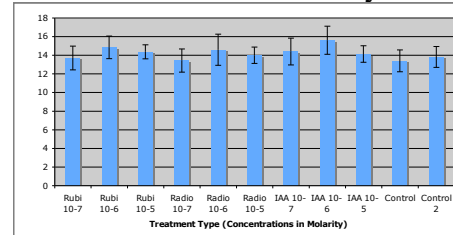
Presentation Outline:

1. Background and Significance
 - Bacteria and Plants: How Plants and Bacteria Interact
 - Negative interactions
 - Plant infections
 - Positive interactions
 - Nitrogen fixation
 - Other essential nutrients
 - Hormonal stimulation
 - Significance of Research
 - Rhizobium: Lives on the Roots of Legumes
 - Suggested phytohormonal activity in earlier research
 - Phytohormonal control as an alternative to environmentally damaging crop enhancement
2. Methods
 - Extraction
 - Bacteria removed from frozen state and grown on Petri plates
 - Bacteria grown in liquid medium (liquid culture)
 - Liquid culture tested for purity and bacterial component removed
 - Extraction performed for rough purification
 - Concentration of auxin determined by spectroscopy

- Plant Growth Assay
 - Varied concentrations of bacterial extract and IAA formulated in a 20 ml solution containing a 0.01 molar phosphate buffer and 3% sucrose to support plant growth
 - Alaska pea plants dark grown for 7-8 days
 - Subapical epicotyl sections excised and incubated for two days in hormone solutions
 - Sections measured with calipers

3. Results

4. Discussion



Selected Annotated Bibliography:

Patten C and Glick B (2002) Role of *Pseudomonas putida* Indoleacetic Acid in Development of the Host Plant Root System. Applied and Environmental Microbiology 3795-3801

In their research, Patten and Glick wished to answer the question as to whether IAA produced by plant-growth promoting rhizobacteria (PGPR) is involved in the process by which they increase plant growth. They state that IAA has been “implicated in the induction of plant tumors” in deleterious bacteria but the function of IAA in symbionts is unclear. In order to address their question, Patten and Glick manufactured a strain of *Pseudomonas putida* that lacked the GR12-2 gene and tested its ability to promote growth in canola seedlings against that of wild-type *P. putida*. Root growth on seedlings inoculated with the wild-type *P. putida* was significantly larger, pointing to IAA as a large factor in the promotion of plant growth. Similar results were obtained using mung bean cuttings.

Sergeeva E, Hirkala D and Nelson L (2007) Production of indole-3-acetic acid, aromatic amino acid aminotransferase activities and plant growth promotion by *Pantoea agglomerans* rhizosphere isolates. Plant Soil 297:1-13

Sergeeva et al studied the effect of six bacterial isolates, confirmed by 16S rRNA analysis to be *Pantoea agglomerans*, on canola, pea, and lentil growth. Five of these isolates were shown to “enhance root length, root weight or shoot weight by 15-37% in at least one of the plant species.” Using colony hybridization, Sergeeva et al showed that the bacterial isolates contained a gene that codes for an integral enzyme in the IAA synthetic pathway that is dependant on tryptophan (the ipdC gene). However, research data showed that the isolates were able to produce significant, though lower, amounts of IAA in the absence or near-absence of tryptophan. Researchers believe this may contribute to the bacteria’s ability to colonize the rhizosphere of these plants due to the fact that IAA production is somewhat independent from tryptophan concentration.

Sarwar M and Kremer R J (1995) Enhanced suppression of plant growth through production of L-tryptophan-derived compounds by deleterious rhizobacteria. Plant and Soil 172:261-269

In their article, Sarwar and Kremer state the goal of “[assessing] the ability of DRB (Deleterious Rhizobacteria) originating from weed seedlings to synthesize auxins from L-tryptophan (L-TRP), determine effects of DRB with or without L-TRP on seedling root growth, and characterize auxins produced from L-TRP.” Using Salkowski reagent, the auxin production of 70 rhizobacterial isolates was tested. Do to its high auxin production, *Enterobacter taylorae* was used to carry out the other portions of the research. HPLC chromatography of culture filtrate from *E. taylorae* revealed the presence of L-TRP, tryptamine, indole-3-lactic acid, indole-3-aldehyde, tryptophol, indole-3-acetonitrile, and IAA. Treatment of a variety of crop plants and weeds with an inoculum of *E. taylorae* in concert with L-TRP showed a significant degree of root growth inhibition, with weeds generally more affected than crop plants.

Tsavkelova E, Cherdyntseva T, Klimova S, Shestakov A, Botina S and Netrusov A (2007) Orchid-associate bacteria produce indole-3-acetic acid, promote see germination, and increase their microbial yield in response to exogenous auxin. Arch Microbiol 188:655-664

This article generated by Tsavkelova et al explores the attributes of four bacterial strains isolated from orchid roots: *Rhizobium*, *Microbacterium*, *Sphingomonas*, and *Mycobacterium*. After carrying out a 16S rRNA analysis of all bacterial strains used, Tssvkelova et al measured the amount of IAA produced by each strain in a variety of media using HPLC analysis and calorimetric assay techniques. IAA production was found in almost all media, though greatly enhanced in media containing tryptophan. Tsavkelova et al also found that exogenous IAA positively influenced bacterial growth and biomass accumulation. A concentration of 100 micrograms per milliliter was found to induce the most marked growth response. Orchid seeds were also inoculated with each of the above bacteria and germination (orchid seeds require fungal or bacterial promoters in order to germinate) was observed. All bacterial strains except *Rhizobium* supported germination.