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Genetic Variability of the 5-Enolpyruvylshikimate-3-Phosphate (EPSP) Synthase Gene in Round-Up™ Resistant Soil Bacteria

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Abstract

Global use of Round-up™ has had a profound effect on agricultural systems. While there is a clear challenge with the emergence of resistant weeds, less emphasis has been placed on its impacts regarding the soil ecosystem. In this study, we sought to identify glyphosate resistance in two specific soil bacteria, Bacillus subtilis and Pseudomonas syringae pv. glycinea. To accomplish this purpose, selective media and colony PCR were used to isolate and identify colonies with glyphosate resistance. The presence of glyphosate resistance in soil bacteria was verified, and continuing work is being done to analyze sequence data that may lead to a better biochemical understanding of how resistance to glyphosate is achieved in various soil microorganisms.

Keywords

glyphosate, chemical resistance, Round-Up™, bacteria, genes, organisms, microbial mutation, enzymes

Disciplines

Soil Science

Comments

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July 31, 2015
University of Iowa, Better Futures for Iowans

ABSTRACT

Global use of Round-up™ has had a profound effect on agricultural systems. While there is a clear challenge with the emergence of resistant weeds, less emphasis has been placed on its impacts regarding the soil ecosystem. In this study, we sought to identify glyphosate resistance in two specific soil bacteria, *Bacillus subtilis* and *Pseudomonas syringae pv. glycinea*. To accomplish this purpose, selective media and colony PCR were used to isolate and identify colonies with glyphosate resistance. The presence of glyphosate resistance in soil bacteria was verified, and continuing work is being done to analyze sequence data that may lead to a better biochemical understanding of how resistance to glyphosate is achieved in various soil microorganisms.

BACKGROUND

Round-up™ ready technology has been highly successful. As such, application of Round-up™ is increasing. Over 1.4 billion pounds are applied annually in 160 countries around the world.[†] Genetically modified Round-up™ Ready crops are protected from the herbicide while the susceptible weeds are killed.

However, glyphosate is also an antibiotic. Research has already looked into Round-up™ resistant weeds, but less research has been conducted on how glyphosate application affects soil microbe populations.

Glyphosate, the active ingredient, targets the EPSP synthase enzyme in the Shikimate pathway. Because animals and humans do not utilize this metabolic route, Round-up™ has a low toxicity for them. However, this pathway is common to plants, bacteria, and fungi.

Soil microbes are an essential part of all ecosystems. The implications of alterations in the soil microbial communities in row crops is poorly understood. This research represents an opportunity to broaden the scope of assessment for the impact of agricultural interventions on overall ecosystem quality.

[†]Grossman, 2015: http://news.nationalgeographic.com/2015/04/150422-glyphosate-roundup-herbicide-weeds/

OBJECTIVES

- **Better understand Round-up™ resistance in beneficial and pathogenic microbes**
  - **Isolate and identify target soil organisms based on morphology and resistance to glyphosate**
  - **Create "tool kit" for assessing the EPSP synthase gene**
  - **Identify changes in the EPSP synthase gene**
  - **Relating genetic changes to soil history and glyphosate resistance**

METHODS

Sequence alignments of specific soil bacteria
↓
Design Primers for EPSP gene in each bacteria
↓
Isolation of specific soil bacteria
↓
Colony PCR on resistant colonies
↓
Gel electrophoresis
↓
Sequence the EPSP synthase gene in resistant bacteria
↓
Examine areas of mutations in the enzyme

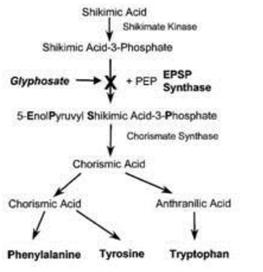

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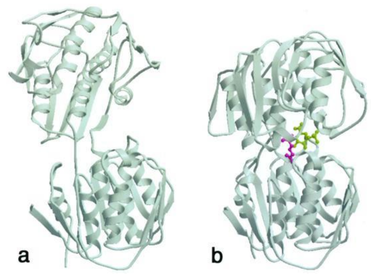
Figure 1: Shikimate pathway with the EPSP synthase inhibited by glyphosate.

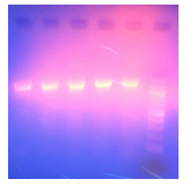


http://www.ncbi.nlm.nih.gov/pmc/articles/PMC29264/

Figure 2: Shows the EPSP gene without ligands (a) and with ligands glyphosate and S3P (b).

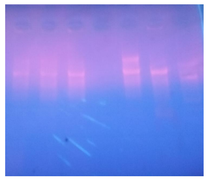
RESULTS

Bacillus subtilis

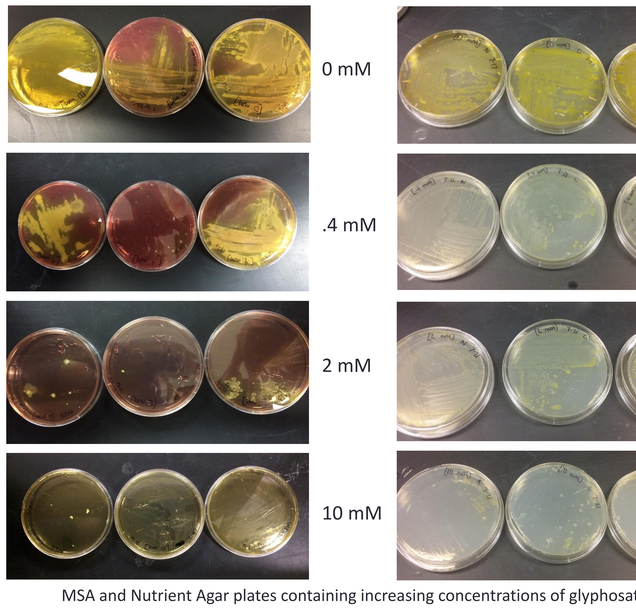


Confirmation of effective B. subtilis primers

Pseudomonas syringae pv. glycinea



Confirmation of effective P. syringae primers



MSA and Nutrient Agar plates containing increasing concentrations of glyphosate

RESULTS

Nucleotide Mutations

T219G	A258G	G264T	A297G	C471T	C474T	C510A	T789A

Amino Acid Alignment with Synonymous Mutations Highlighted

```
B_sub     MLNNIIENNDGPFENSTIQHIFKEIFKAGLELQEEDHSKALLVSRKKKPEDTIVDIKGEK 60
G13N      MLNNIIENNDGPFENSTIQHIFKEIFKAGLELQEEDHSKALLVSRKKKPEDTIVDIKGEK 60
          ************************************************************

B_sub     IGDGQQRFIVGPCAVESYEQVAEVAAAKKQGIKILRGGAFKPRTSPYDFQGLGVEGLQI 120
G13N      IGDGQQRFIVGPCAVESYEQVAEVAAAKKQGIKILRGGAFKPRTSPYDFQGLGVEGLQI 120
          *************************************************************

B_sub     LKRVADEFDLAVISEIVTPAHIEEALDYIDVIQIGARNMQNFELLKAAGAVKKPVLLKRG 180
G13N      LKRVADEFDLAVISEIVTPAHIEEALDYIDVIQIGARNMQNFELLKAAGAVKKPVLLKRG 180
          ************************************************************

B_sub     LAATISEFINAAEYIMSQGNDQIILCERGIRTYETATRNTLDISAVPILKQETHLPVFVD 240
G13N      LAATISEFINAAEYIMSQGNDQIILCERGIRTYETATRNTLDISAVPILKQETHLPVFVD 240
          ************************************************************

B_sub     VTHSTGRRDLLLPTAKAALAIGADGVMAEVHPDPSVALSDSAQQMAIPEFEKWLNELKPM 300
G13N      VTHSTGRRDLLLPTAKAALAIGADGVMAEVHPDPSVALSDSAQQMAIPEFEKWLNELKPM 300
          ************************************************************

B_sub     VKVNA 305
G13N      VKVNA 305
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CONCLUSIONS & NEXT STEPS

- **Conclusions**
  - **Glyphosate resistance is prevalent among soil bacteria.**
  - **We have designed primers capable of amplifying the EPSP synthase gene**
  - **Sequence variations exist between resistant strains and organisms.**
- **Next Steps**
  - **Include more organisms in the study**
  - **Annotate areas of common mutations**
  - **Predict structural effects of mutations on the EPSP synthase enzyme**
  - **Look at differential gene expression of resistant isolates with and without glyphosate pressure**