POTENTIAL EFFECT OF THE HERBICIDE GLYPHOSATE ON THE HUMAN GUT MICROBIOME

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INTRODUCTION

- Glyphosate is the world's most widely used herbicide. The herbicide targets the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (**EPSPS**), present in plants, fungi and prokaryotes.
- Human nutrition has shown to have residues of glyphosate. Humans do not have the glyphosate target enzyme in their metabolism.
- However, glyphosate may affect human health by producing an imbalance in the composition of the human gut microbiota, for majority of the common gut bacteria species carry the sensitive EPSP synthase.
- **In this research we assess for the first time the potential sensitivity of the human gut microbiome to glyphosate.** (1)

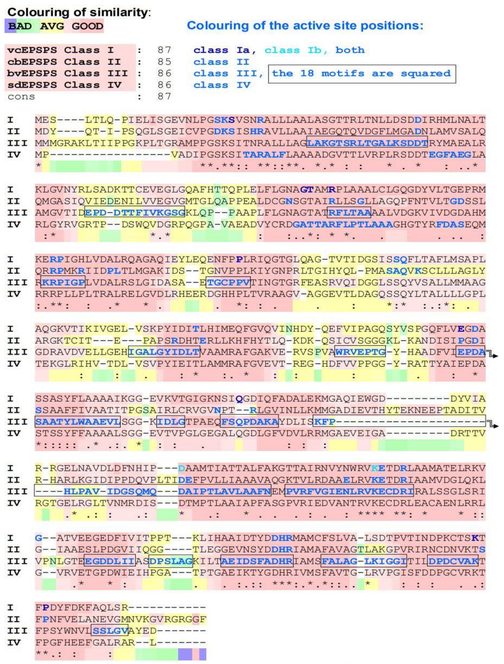


Figure 1. Multiple sequence alignment of the four EPSPS reference sequences of different classes. Color in the background describes the similarity of the sequences. Amino acid markers determining the class are highlighted in blue. (2,3)

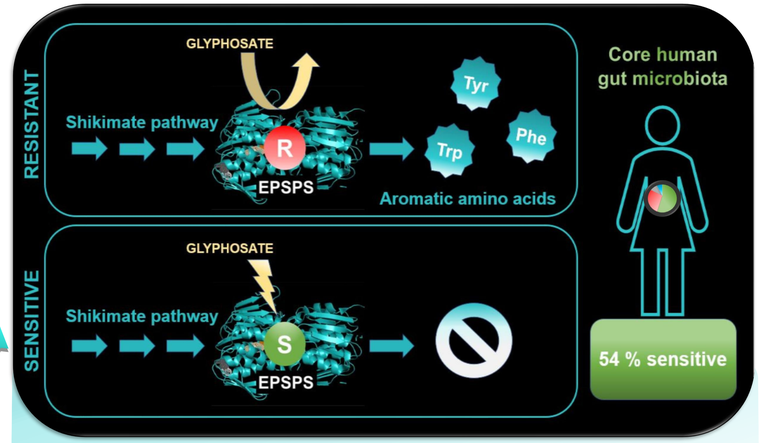


Figure 2. The potential toxic effects of the herbicide on the microbiome are based on the disruption of the shikimate pathway. Glyphosate blocks the **EPSPS class I (sensitive to the herbicide)** and inhibits the production of three essential amino acids (phenylalanine, tyrosine, tryptophan). Some bacteria, however, are potentially **resistant to the herbicide (EPSPS class II, III and IV).**

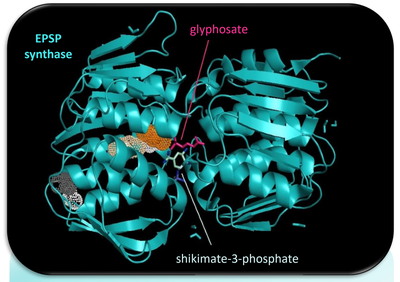


Figure 3. EPSP synthase of E. coli liganded with shikimate-3-phosphate and glyphosate. Some amino acids that can provide diverse levels of resistance when mutated are highlighted in colors.

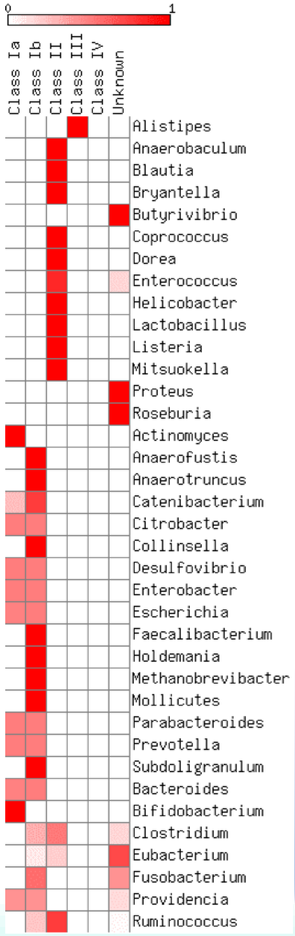
MATERIALS & METHODS

890 EPSPS → 101 species → Classification

- 890 EPSPS protein sequences belonging to 101 common human gut bacterial species were classified into potentially **sensitive** (class I) and **resistant** (class II, III and IV) based on known amino acid markers (highlighted in **figure 1**).
- EPSPS sequences were collected from the database *proteins* of the National Center for Biotechnology Information (NCBI). The web-server EPSPSClass was used to determine the type of EPSPS. (https://ppuigbo.me/programs/EPSPSClass)

RESULTS

Majority of the bacteria in the human core gut microbiome may be affected by glyphosate



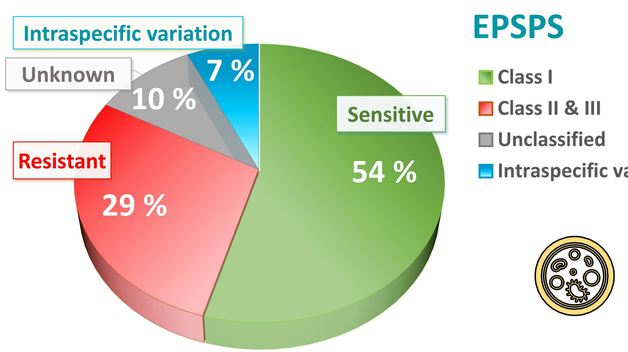


Figure 4. Glyphosate susceptibility among 101 human core gut bacteria species. EPSPS sequences belonging to different strains of the species were either all sensitive, all resistant, unclassified or varying within the species.

Thus, the intake of glyphosate residues from the diet may affect the composition of the gut microbiome. However, EPSPS sequences from several bacteria are yet to be classified and their susceptibility to glyphosate is unknown.

Figure 5. Proportion of bacteria strains that are sensitive (Class Ia & Ib) to glyphosate, resistant (Class II, III, IV) or the susceptibility is unknown.

CONCLUSIONS

- **Residues of glyphosate in human nutrition may alter the gut microbiota by promoting an over-time dominance of resistant bacteria.**
- **This research provides a solid base for further in-vitro and population-based studies that are necessary to determine the effects of glyphosate on the human gut microbiome and human health.**

References

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FINALIST

Lyydia Leino University of Turku, Turku, Finland Glyphosate is the world's most widely used herbicide for agriculture and home gardening purposes. The herbicide targets the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), present in plants, fungi and prokaryotes. Several studies have shown that human nutrition has residues of glyphosate. Humans do not have the glyphosate target enzyme in their metabolism, thus are presumably safe from the effects of the herbicide. However, glyphosate may affect human health by producing an imbalance in the composition of the human gut microbiota. The potential toxic effects of the herbicide on the microbiome are based on the disruption of the shikimate pathway. Glyphosate blocks the EPSPS class I (sensitive to the herbicide) and inhibits the production of three essential amino acids (phenylalanine, tyrosine, tryptophan). Some bacteria, however, are potentially resistant to the herbicide (EPSPS class II, III and IV). In this study, I determine the amount of bacteria putatively sensitive/resistant to glyphosate and assess the potential effects of the herbicide on gut microbial communities and human wellbeing. I classified EPSPS protein sequences from 890 strains belonging to 101 common human gut bacterial species. EPSPS sequences were collected from the database proteins of the National Center for Biotechnology Information (NCBI). The web-server EPSPSClass (https://ppuigbo.me/programs/EPSPSClass/), that classifies EPSPSproteins into potentially sensitive (class I) and resistant (class II, III and IV) based on known amino acids markers, was used to determine the type of EPSPS. The results indicate that the majority of bacteria in the human gut microbiome may be affected by the herbicide (54% of the species are putatively sensitive, 29% are putatively resistant and 17% are unclassified or varying intraspecifically). Thus, the intake of glyphosate residues from the diet may affect the composition of the gut microbiome — e.g., the genera Escherichia, Faecalibacterium and Collinsella are potentially sensitive to glyphosate, whereas the genera Helicobacter, Lactobacillus and Dorea are potentially resistant. However, EPSPS sequences from several bacteria are yet to be classified and their susceptibility to glyphosate is unknown. In conclusion, residues of glyphosate in human nutrition may alter the gut microbiota by promoting an over-time dominance of resistant bacteria. This research provides a solid base for further in-vitro and population-based studies that are necessary to determine the effects of glyphosate on the human gut microbiome and human health.