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Glyphosate Inhibits Keratinolytic Activity of *Bacillus* spp. Isolated from Wild Songbirds

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Abstract

Glyphosate is a frequently-used herbicide both domestically and in the agricultural industry worldwide. While the effect of glyphosate ingestion on bird health has been studied, less is known about the potential impact of glyphosate on bacteria found on avian plumage. This study investigated the effect of glyphosate on the degradation of bird feathers by keratinolytic plumage bacteria. To determine the effect of glyphosate on bacterial feather degradation, two strains of *Bacillus* spp. isolated from the plumage of wild songbirds captured in mist nets were tested. A disk-diffusion assay was used to determine that 8.44 g/L of glyphosate inhibited the growth of these two isolates. Feather degradation assays were subsequently performed on each of the *Bacillus* isolates to determine whether the keratinolytic activity of the bacteria was affected. For each isolate, four tubes were prepared that contained a basal salts medium and a white goose feather as the sole carbon and nitrogen source. To two of those tubes, 8.44 g/L of glyphosate was added, then one tube with glyphosate and one tube without glyphosate were inoculated with a *Bacillus* isolate. The other tubes with and without glyphosate were left uninoculated as controls. All experiments were done in replicates of 10, and all tubes were incubated at 37°C at 125 rpm for seven days. Aliquots from each tube were removed once every 24 hours. Feather degradation was determined by measuring the absorbance of the aliquots at 230 nm. At this wavelength, an increase in the absorbance indicated an increase in oligopeptides resulting from the degradation of keratin in the feathers. The results from uninoculated tubes indicated that glyphosate alone did not damage feathers. However, glyphosate inhibited bacterial feather degradation from 86% to 98% depending upon the isolate. These data suggest that exposure to glyphosate disrupts feather degradation by *Bacillus* spp.

Introduction

- Glyphosate is the most commonly used herbicide in the United States
- Glyphosate inactivates 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase, which is responsible for amino acid biosynthesis primarily in prokaryotes and higher plants (Quinn 1988)
- Some *Bacillus* spp. produce keratinase enzymes, which degrade the keratin found in bird feathers
- How does glyphosate affect feather degradation by *Bacillus* spp.?

Materials and Methods

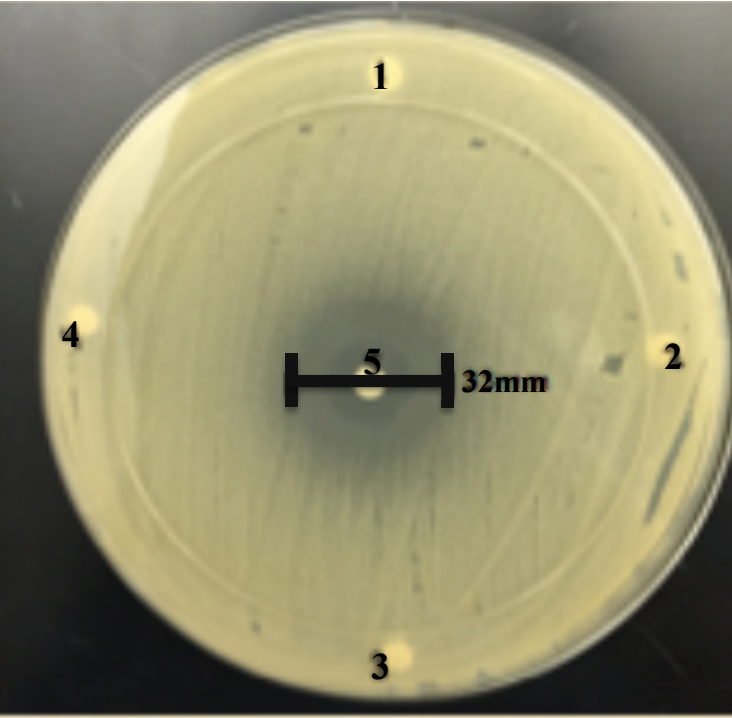


Figure 1. Kirby Bauer Test
Mueller-Hinton agar plate inoculated with a 0.5 McFarland standard of *Bacillus* isolate 3972 TB. Each disc corresponds to a different concentration of glyphosate.
1 = 8.44 µg/L 4 = 0.084 g/L
2 = 84.4 µg/L 5 = 8.44 g/L
3 = 844 µg/L

Conditions	
No Bacteria, No Glyphosate	Sterile feather + basal salts medium
No Bacteria, Glyphosate	Sterile feather + basal salts medium + glyphosate
Bacteria, No Glyphosate	Sterile feather + basal salts medium + <i>Bacillus</i> sp.
Bacteria, Glyphosate	Sterile feather + basal salts medium + glyphosate + <i>Bacillus</i> sp.

Experiments were performed using three *Bacillus* spp. in replicates of 10. Aliquots were taken every 24 hours for seven consecutive days (day 0 to day 6). Absorbance was measured by microplate reader at 230 nm to determine the amount of oligopeptides released from feather degradation.

Results

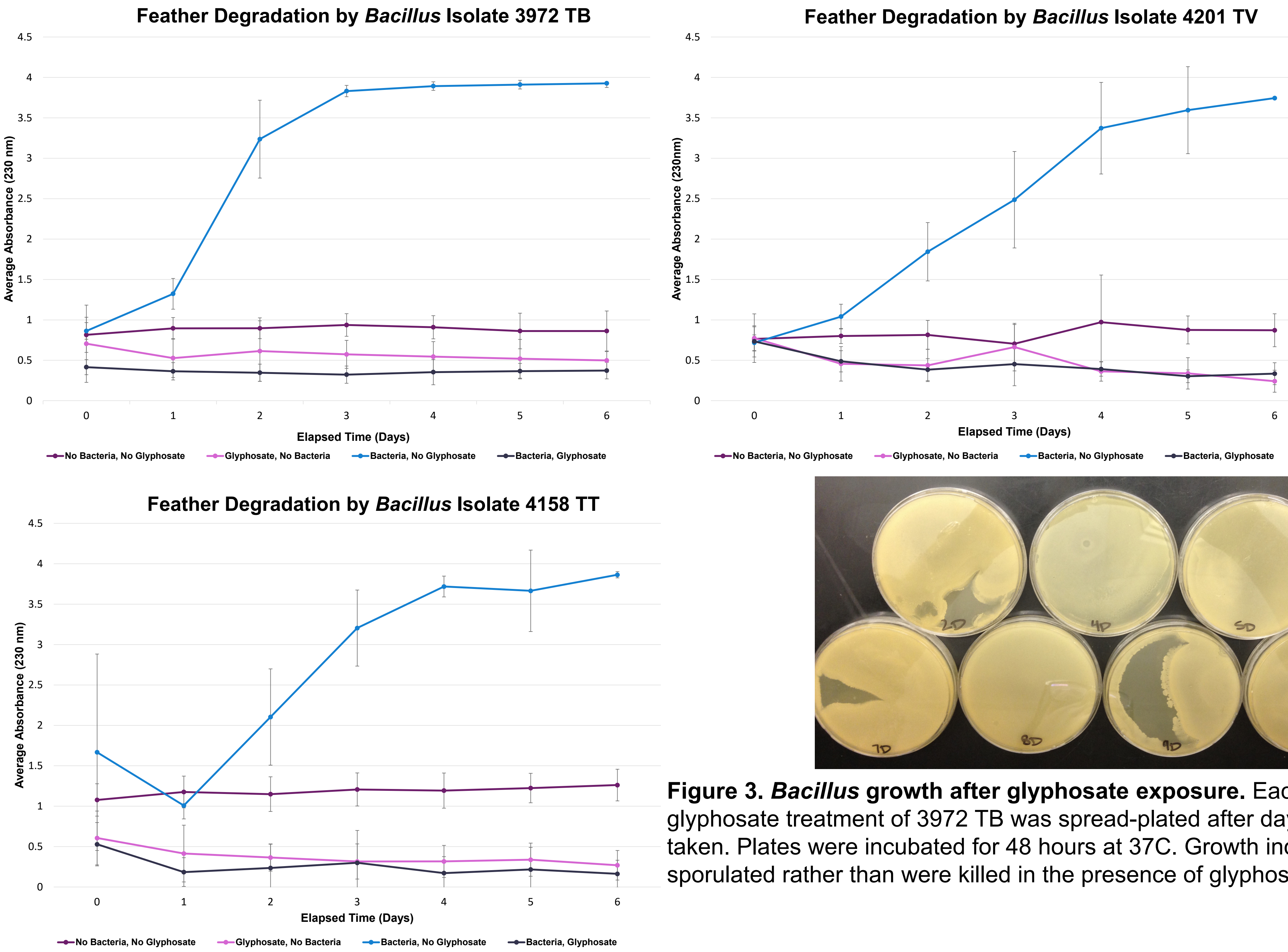


Figure 2. Feather degradation measured as absorbance
Absorbance of each replicate for all three isolates was measured by microplate reader at 230 nm. Absorbance corresponds to the amount of oligopeptides present from feather degradation.

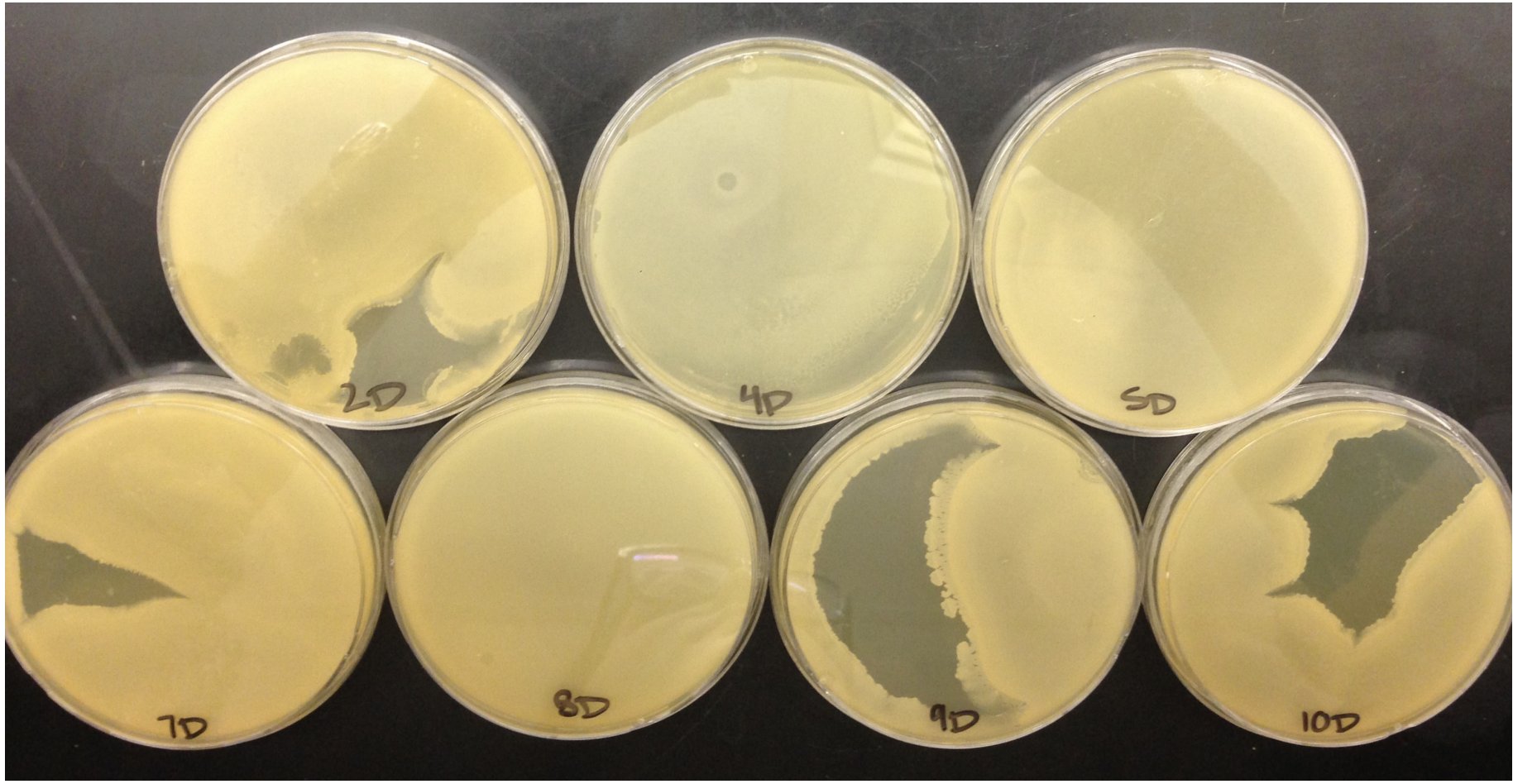


Figure 3. *Bacillus* growth after glyphosate exposure. Each replicate for the glyphosate treatment of 3972 TB was spread-plated after day six samples were taken. Plates were incubated for 48 hours at 37°C. Growth indicates *Bacillus* sporulated rather than were killed in the presence of glyphosate.

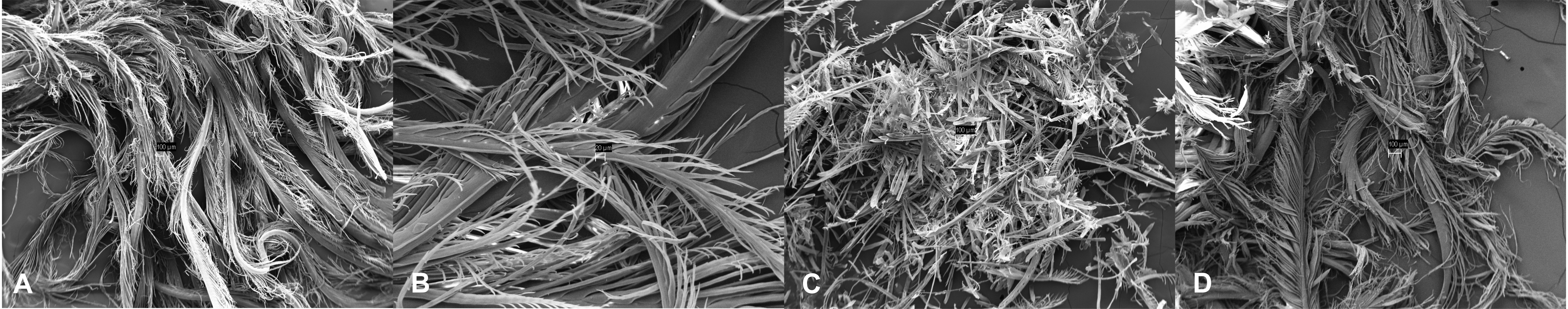


Figure 4. SEM Images of Feather Samples. Feathers used in degradation experiments were imaged by scanning electron microscopy. The no bacteria, no glyphosate feather (Panel A), the glyphosate, no bacteria feather (Panel B), and the bacteria, glyphosate feather (Panel D) show no signs of damage or degradation. Degradation by *Bacillus* is evident in the bacteria, no glyphosate feather (Panel C). Feathers were imaged after day six samples were taken.

Discussion and Conclusions

- Glyphosate inhibits keratinolytic activity of *Bacillus* spp.
- Disruption of the shikimic acid pathway in *Bacillus* may account for inhibition of keratinolytic activity
- Bird feathers are not damaged by glyphosate
- *Bacillus* spp. sporulate when exposed to glyphosate in artificial, soil-free media

Future Directions

- Repeat degradation experiments in media with soil to determine if decreased bioavailability of glyphosate affects the rate of feather degradation

References

Quinn JP, Peden JMM, Dick RE. 1988. Glyphosate tolerance and utilization by the microflora of soils treated with herbicide. Appl Microbiol Biotechnol. **29**:511-516.

Acknowledgments

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