Hormesis of *Bacillus velezensis* in response to low doses of polystyrene

Melanie R. Cham, Fatai A. Olabemiwo, Frederick M. Cohan

Department of Biology, Wesleyan University

Introduction

Within recent decades, microplastics have been an ongoing threat to ecosystems around the world. The plastics are a commonly identified as a threat to our aquatic ecosystems. However, they are also known to pollute the soil and its wildlife. This is due to its introduction into the ground through the agricultural sector. Many farmers use sludge fertilizers and plastic mulch as a cheaper way to maintain their crops. Microplastics have been categorized as primary and secondary. Primary microplastics are introduced through the use of synthetic fibers commonly found in most clothing which break down and go into the water treatment systems. The systems filter out the plastics that are then accumulated into the sludge fertilizers used to maintain crops. Essentially, even if microplastics are thought to be removed from the aquatic environment, they return to the soil. An additional cause of soil pollution is due to the use of plastic mulch. This is a type of sheet that is utilized for improving temperature control, regulating moisture, and preventing unwanted weed growth. However, after being exposed to UV and the environment− with a life of about one year− these sheets break down into secondary microplastics. In the long term, the plastic particles attach to the surrounding sediment creating clogs that retain water and prevent adequate drainage. Additionally, these plastics are absorbed by the plant cells through endocytosis which then inhibits plant growth.

The goal of this study is to examine bacterial diversity using *Bacillus* as a model organism. In doing so, we are aiming to find a strain that can be used to bioremediate plastics while also improving the crop yield and preventing microplastics from continuing to negatively impact the ecosphere. Bioremediation is to remove pollutants (in this case: microplastics) using living organisms such as bacteria. To accomplish this purpose, we need to find a strain that best grows in the presence of plastics.

References and Acknowledgements

1. Rabbee, M. F., Ali, M. S., Choi, J., Hwang, B. S., Jeong, S. C., & Baek, K.-H. *Bacillus Velezensis: a valuable member of BIOACTIVE molecules Within Plant Microbiomes*, 2019.
2. Blyth, A., & Evans, D. *Microplastics: In our soils too?* 2018.
3. Reva, O. N., Swanevelder, D. Z. H., Mwita, L. A., Mwakilili, A. D., Muzondiwa, D., Joubert, M., Chan, W. Y., Lutz, S., Ahrens, C. H., Avdeeva, L. V., Kharkhota, M. A., Tibuhwa, D., Lyantagaye, S., Vater, J., Borriss, R., & Meijer, J. *Genetic, epigenetic and phenotypic diversity of four bacillus velezensis strains used for plant protection or as probiotics*, 2019.

We would like to thank Dr. Cohan for being a wonderful, optimistic resource of support and guidance. Your understanding has allowed us to grow in knowledge and application. We also enthusiastically acknowledge the generous support of the John and Rosemarie Dooley Foundation and Wesleyan University.

Materials and Methods

We determined the bacterium's growth in the presence or absence of nanoplastics through a growth assay under a given concentration of polystyrene. This experiment was conducted under aerobic conditions.

Study organism

We used *Bacillus velezensis*, an aerobic, gram-positive, endospore-forming bacterium, as our study model. *B. velenzensis* is typically used to encourage plant growth by promoting rhizobacteria as well as being used as an ecologically safe biopesticide. This bacterium is the ideal model for also studying diversity in that they are found in varying environments with adaptive plasticity. In this study, three strains are examined: FZB24, GB03, and QST713.

Polystyrene (PS)

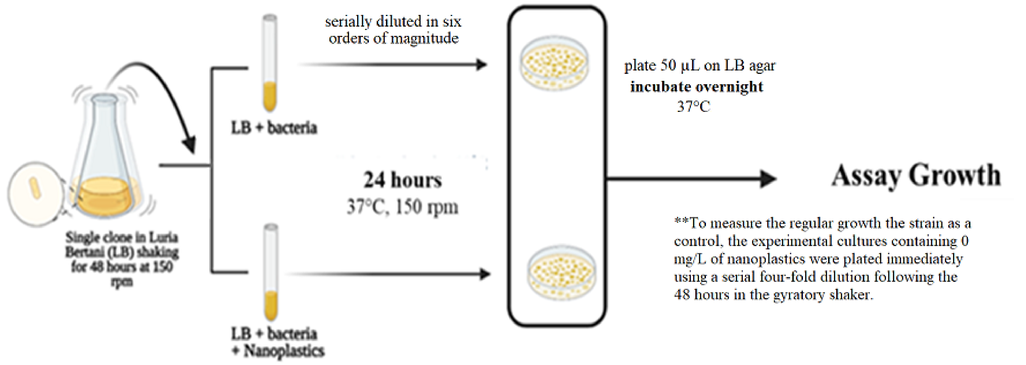
The PS is pre-sterilized through filtering to limit microbial contamination. The 25nm polystyrene nanospheres are placed in suspension requiring each sample to be vortexed for 10-20 seconds before use. Then, 19.67 mL was added to sterile Luria-Bertani broth (a nutritionally rich medium for growing bacteria) to create a treatment media of 50 mg/L. The control medium (LB) was prepared without PS nanoplastics addition.

Bacterium culture and inoculation

We inoculated a single colony of strains FZB42, GB03, and QST713 into 10 mL of sterile LB broth. The sample was then incubated under aerobic conditions for 48 hours in a gyratory shaker incubator (150 rpm, 37°C). The bacterium stops growing but remains metabolically active.

Growth assay

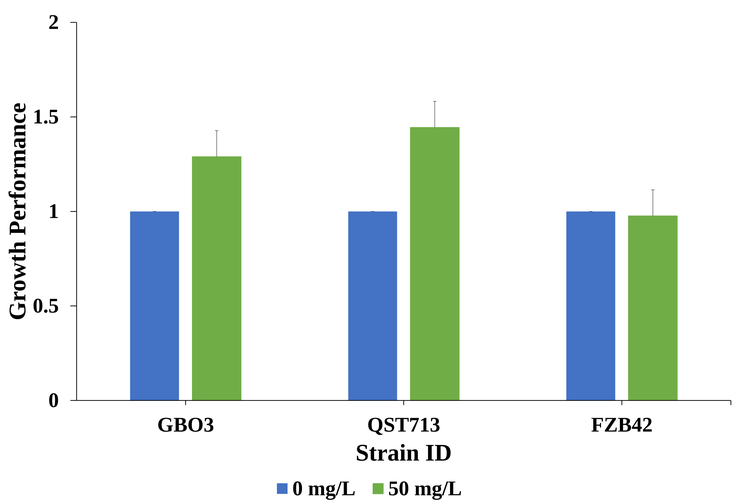
We conducted a growth assay using the plate count method.



Cell density = No. of colonies$^{\text{dilution of plate}}$/ volume of plated culture

The experiment was replicated twelve times to observe numerical consistencies.

Growth of *B. Velenzensis* in 0 mg/L and 50 mg/L PS



Results

It is found that the plastics can be beneficial to this bacterium, more so in strains QST713 than FZB42. Growth performance is determined using the equation below:

GP= Mean net growth $_{\text{control}}$ / mean net growth $_{\text{treatment}}$

With this information, these agricultural strains will be implemented into crop growth. The abundant growth of these strains has the potential to degrade the plastics and aid in plant growth. It is possible that these strains will eventually become better adapted to plastics which will allow it to better degrade plastic or become more tolerant of its environment.