SOCIETY FOR NATURAL SCIENCES STUDENT JOURNAL

Society for Natural Sciences Student Conference
University of Bath 8-9 April 2026

The Effects of pH On The Growth Of Bacillus Mycoides and Bacillus Subtilis Using Chemical Components In Fertilisers and Acid Rain On Soil

Aryan Hyder*, Harry Goodlock, Tamsyn Wilson, Remy Chait

Introduction

- Bacillus Mycoides is a gram-positive, rod shaped bacterium that thrives aerobically in soil and has a specialised root-like growth morphology, such that the species is known to grow distinct, clockwise colonies on agar plates[1].
- Beyond its applicability of being an aerobe, Bacillus Mycoides behaves as a plant growth-promoting bacterium, by acting as an endophyte that protects plants from pathogenic species [2].
- The microflora of soil, which is beneficial to arable agriculture in particular, is coming under increasing threat from abiotic factors concerning large pH changes through the applications of agrochemicals, fertilisers and increasing levels or acid rain [3].
- To combat this , Bacillus subtilis strains have been previously inoculated into soil as they are classed as plant growth promoting rhizobacteria, while regulating biogeochemical processes and reducing nitrogen leaching [4] [5].
- This study investigated the effects of acid rain constituents and soil acidification agents on both bacterial species, measuring growth responses over four days.

Method

- Bacterial cultures were grown on 1% agar LB plates with pH monitored using a standardised pH probe (pH 7 and 9.2 buffers). Three acids were tested: acetic acid (induces soil acidification) [6], nitric acid (used for nitrate production for plant growth) [7], and sulfuric acid (key for sulfur production promoting plant growth) [8].
- B. mycoides was inoculated centrally with B. subtilis at plate edges to enable distinct growth monitoring. Plates were incubated at 25°C for optimal microbial activity in temperature-controlled freezers [9]. Acid volumes (measured in µL) were adjusted to achieve known pH ranges found in both natural and artifical conditions.
- Aseptic technique prevented contamination during pipetting, and growth was documented using a Raspberry Pi microscopic camera (as shown in Figure 1). OD_{600} values provided non-destructive quantification without interfering with standard culture media. Experimental setup is detailed in Figure 5.

Further Refinements, Conclusions and Context

- In conclusion, acetic acid demonstrated limited nutritional gain for both bacterial species, achieving minimal peak growth (OD_{600} = 0.45). Conversely, sulfuric and nitric acids supported substantial growth (OD_{600} >2.0) until critical toxicity thresholds were reached and induced rapid inhibition.
- These dose-response profiles provide quantitative guidelines for: optimizing sulfuric and nitric acid concentrations in fertilizers while minimizing toxicity to beneficial soil bacteria; understanding acid rain impact thresholds on plant growth-promoting bacteria [10], and determining effective acetic acid concentrations for agricultural herbicides that preserve B. mycoides and B. subtilis populations essential for UK crop production systems.

Funded by the #ExeterForever ALUMNI ANNUAL FUND

Figure Panel

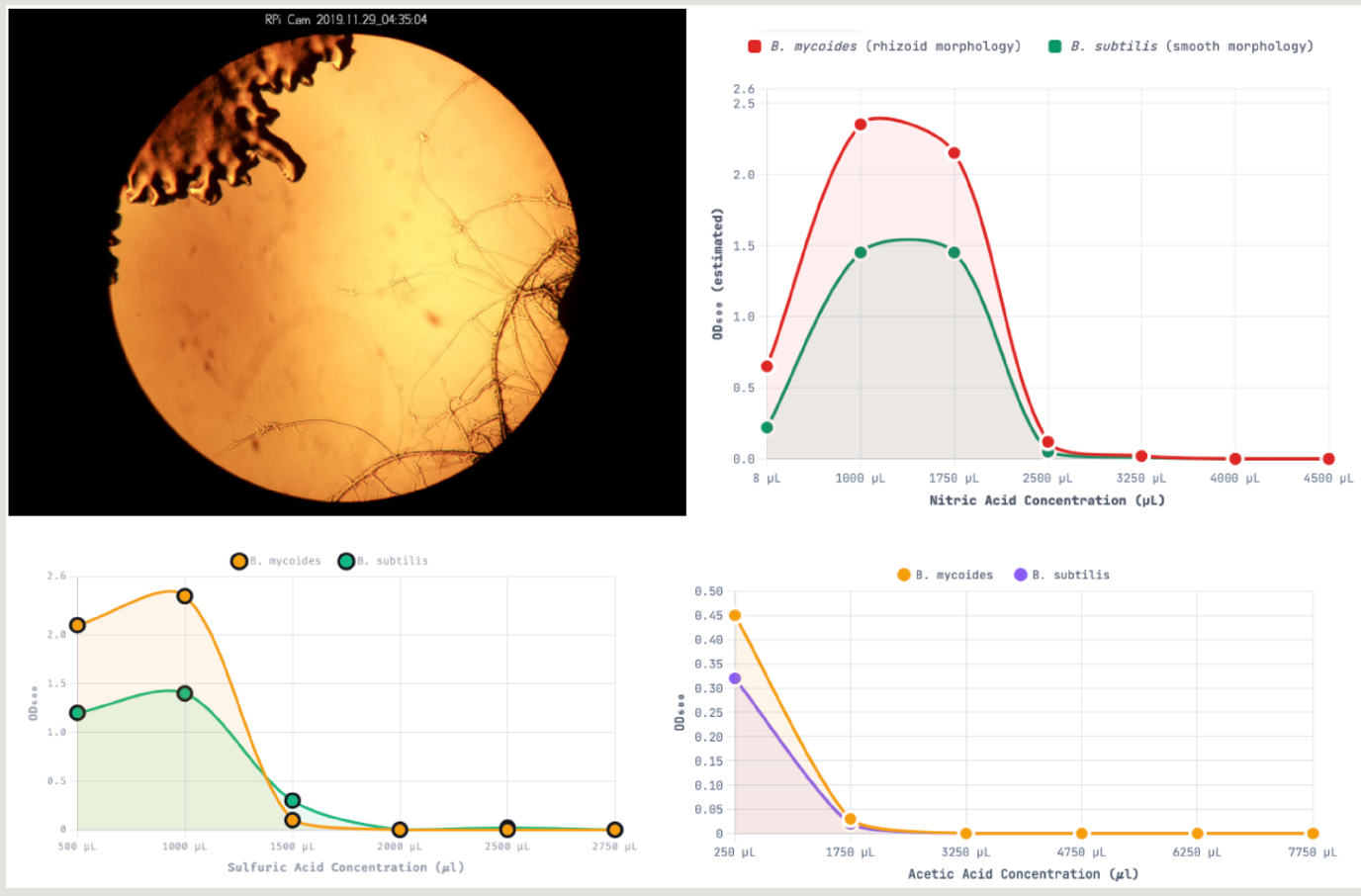


Figure 1 (Top Left): A microscopic Raspberry Pi camera image of B.mycoides (bottom right of the photo) and B.subtilis (top left of the photo) under 1000 µl of nitric acid; Figure 2 (Top Right): Line graph representing the OD600 values achieved with ascending nitric acid concentrations between B.mycoides (in red) and B.subtilis (in green); Figure 3 (Bottom left): Line graph representing the OD600 values achieved with ascending sulfuric acid concentrations between B.mycoides (in circled orange) and B.subtilis (in circled green); Figure 4 (Bottom Right): Line graph representing the OD600 values achieved with ascending acetic acid concentrations between B.mycoides (in orange) and B.subtilis (in purple)

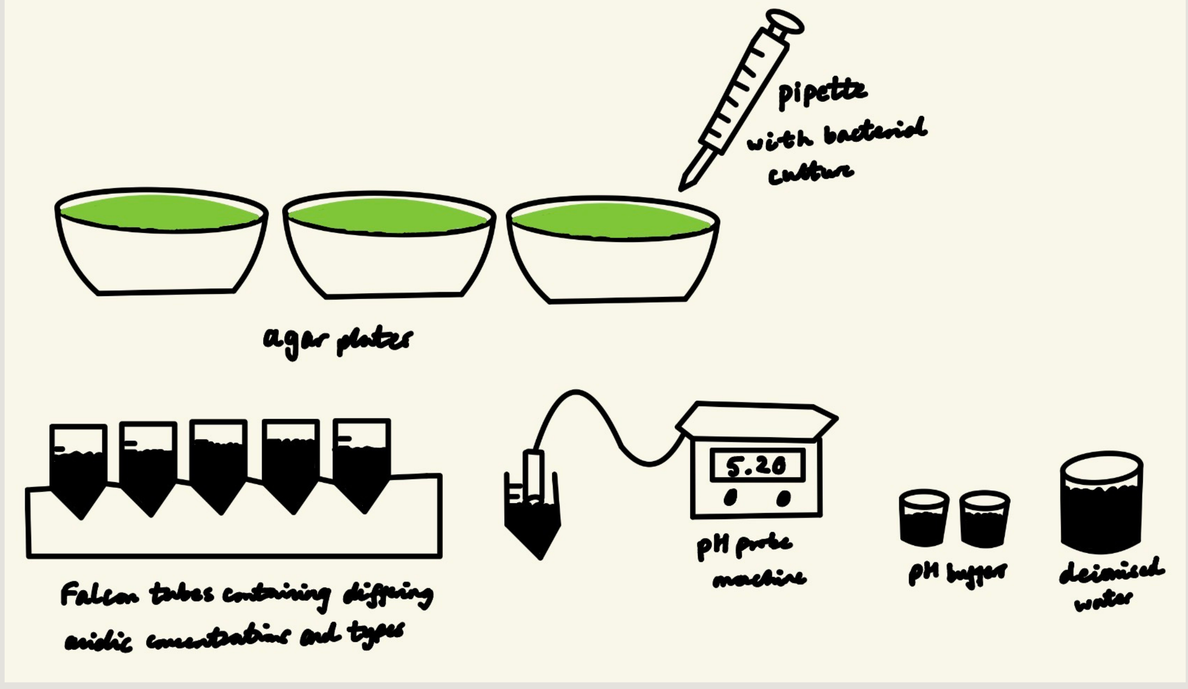


Figure 5: A schematic setup of the method used, where different pipette tips were used for each chemical concentration (0.1M acetic acid, 0.1M sulfuric acid and 0.1M nitric acid) and for each bacterial species placed. These plates were left to dry after this inoculation process before being placed in a freezer at 25°C

Results and Discussions

- Figure 2 shows peak growth at 1000-1750µL for both species, with B. mycoides achieving higher OD_{600} (2.35 vs 1.45) than B.subtilis, indicating potential nitrogen availability and pH buffering benefits for B.mycoides. Sharp inhibition occurs between 1750-2500µL (750µL range), with B. mycoides maintaining ~2× higher growth across all concentrations, suggesting species-specific acid resistance. Complete inhibition occurs >4000µL.
- Figure 3 demonstrates maximal growth potential (OD_{600} = 2.40) at 1000µL, followed by the sharpest toxicity drop observed (96% reduction between 1000-1500µL). This binary response pattern for B.mycoides and B.subtilis —optimal growth or lethal toxicity— reflects rapid protein denaturation and membrane destruction with minimal intermediate survival zones.
- Figure 4 exhibits reduced peak growth (OD_{600} = 0.45 at 250µL), suggesting inherent cell density constraints. Despite being a weak acid, complete inhibition occurs by 1750µL through multiple mechanisms: membrane lipid disruption, metabolic pathway inhibition, and osmotic stress. The progressive inhibition pattern contrasts with sulfuric acid's catastrophic drop, indicating concurrent stress pathways rather than single-point failure.

References: Variation in the Morphology of Bacillus mycoides due to applied force and substrate structure', James P Stratford et al., Available at the doi: 10.1371/journal.pone.0081549 [1], 'Combined effect of endophytic Bacillus mycoides and rock phosphate on growth and metal uptake in wheat' (Triticum aestivum L.), , p.4143, Saira A et al. Available at the doi: 10.1186/s12870-024-04812-3 [2], Hirshfield, I.N,, Terzulli, S. and O'Byrne,'Weak organic acids: a panoply of effects on bacteria'. Available on https://doi.org/10.3184/003685003783238626 [3], Subhasmita Mahapatra et al.,'Bacillus subtilis impact on plant growth, soil health and environment: Dr. Jekyll And Mr Hyde. Journal of Applied Microbiology, Volume 132, Issue 5, Pages 3543-3562, Available at https://doi.org/10.1111/jam.15480 [4], Bo Sun et al. 'Bacillus subtilis biofertilizer mitigating agricultural ammonia emission and shifting soil nitrogen cycling microbiomes', Available at https://doi.org/10.1016/j.envint.2020.105989 [5], 'The impact of ecological restoration on soil quality in humid region forest habitats: a systematic review', Wendy Jiba, Alen Manyevere, Chuene Victor Mashamaite, MDPI, Volume 15, Issue 11, Available on the doi: https://doi.org/10.3390/f15111941 [Accessed 22/02/2026] [6], Goulding KW. Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom'. Available at doi: 10.1111/sum.12270. Epub 2016 Jun 24. PMID: 27708478; PMCID: PMC5032897 [7], Lee SY, Kim EG, Park JR, Ryu YH, Moon W, Park GH, Ubaidillah M, Ryu SN, Kim KM, 'Effect on Chemical and Physical Properties of Soil Each Peat Moss, Elemental Sulfur, and Sulfur-Oxidizing Bacteria' Available at doi: 10.3390/plants10091901. PMID: 34579434; PMCID: PMC8469801 [8]M.E. Guntiñas, M.C. Leirós, C. Trasar-Cepeda, F. Gil-Sotres, 'Effects of moisture and temperature on net soil nitrogen mineralization: A laboratory study', European Journal of Soil Biology, Volume 48, 2012, Pages 73-80, ISSN 1164-5563, Available at https://doi.org/10.1016/j.ejsobi.2011.07.015.[9], Alejandro Moure Abelenda, Kirk T. Semple, Alfonso Jose Lag-Brotons, Ben M.J. Herbert, George Aggidis, Farid Aiouache, 'Impact of sulphuric, hydrochloric, nitric, and lactic acids in the preparation of a blend of agro-industrial digestate and wood ash to produce a novel fertiliser', Journal of Environmental Chemical Engineering, Volume 9, Issue 1,Accessed on https://doi.org/10.1016/j.jece.2020.105021 [10]