Analysis Tutorial Prospectus

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1. Title

Evaluating the control effect on Cyanobacteria Microcystis (MC) by Amoeba Vanella’s grazing behavior (Van Wichelen et al., 2016)

1. Research question(s)

Does Amoeba Vanella have a universal and significant control effect on different strains of Microcystis, including toxin producer and non-toxin producer?

1. Objective(s)

Visualizing the MC growth curve by measuring pigments, the control effect is defined as the gaps between test group lines and control group lines. Statistical analysis on the results.

1. Approach

Raw samples were diluted to stock culture, maintaining approximately same visual clarity under light. The total volume per replication is 150 ml, including 20 ml of Cyanobacteria and 130 ml of 1X BG-11 media for the control groups. For the testing group, 20 ml of Cyanobacteria, 15 ml of Amoeba, and 115 ml of 1X BG-11 were added together. The initial 20 ml of Amoeba stock culture was scaled up to 180 ml to fulfill the requirement (15 ml per replication \* 12 replications). The sample IDs are LE21 22.1 ISO1 MC+, LE21 22.1 ISO2 MC-, LE21 52.2 ISO3 MC+, LE21 52.2 ISO2 MC-, MC Amoeba Van A22.3-1, 1/7/25. Pigment measurements are performed every 2 days. Samples were incubated under 23 °C and 12/12 hours' light/dark conditions. The raw data in excel file is imported to R first by exp <- read\_excel("metadata.xlsx"). The daily measurement data will be processed with mean\_value <- mean (data$Pigment, na.rm = TRUE) function to get the average number from the replications and save using write\_xlsx(average\_values, "average\_results.xlsx"). Growth curves will be plotted by ggplot (Weger et al., 2024) and using facet\_grid (pigment ~ Host, scales = "free") to make matrix for line graphs. ANOVA/ Tukey’s test will be applied to test the significance of control effect among different strains (Van Wichelen et al., 2010), by anova\_model <- aov() and tukey\_result <- TukeyHSD(anova\_model). Packages could be used includes ggplot2, readxl, dplyr, tidyverse, ggthemes, scales, writexl, multcomp.

1. Selected References

Van Wichelen, J., D ’hondt, S., Claeys, M., Vyverman, W., Berney, C., Bass, D., & Vanormelingen, P. (2016). A Hotspot of Amoebae Diversity: 8 New Naked Amoebae Associated with the Planktonic Bloom-forming Cyanobacterium Microcystis A Hotspot of Amoebae Diversity: 8 New Naked Amoebae Associated with the Plank-tonic Bloom-forming Cyanobacterium Microcystis. *Acta Protozoologica*, *55*(2). https://doi.org/10.4467/16890027AP.16.007.4942ï

Van Wichelen, J., van Gremberghe, I., Vanormelingen, P., Debeer, A. E., Leporcq, B., Menzel, D., Codd, G. A., Descy, J. P., & Vyverman, W. (2010). Strong effects of amoebae grazing on the biomass and genetic structure of a Microcystis bloom (Cyanobacteria). *Environmental Microbiology*, *12*(10), 2797–2813. https://doi.org/10.1111/j.1462-2920.2010.02249.x

Weger, H. G., Polasek, A. K., Wright, D. M., Damodaran, A., & Stavrinides, J. (2024). Grazing preferences of three species of amoebae on cyanobacteria and green algae. *Journal of Eukaryotic Microbiology*, *71*(2). https://doi.org/10.1111/jeu.13018