Aggregation and Surface Response of Wild Strains

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Bridge UnderGrad Science (BUGS) ***Summer Research Program***

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Abstract

In this study, we investigated the migratory behavior of six different wild types across agar gels with varying agar concentrations (0.3%, 0.5%, and 0.7%). The movement distances of the strains were observed over 23 hours. Surprisingly, for these specific wild types, agar gel concentration did not significantly impact migration behavior. Despite this, understanding how agar concentration affects migration is essential for interpreting microorganism movement in natural environments, as agar gels mimic substrates like soil and sediment. The findings suggest that the studied wild types (Priestia aryabhattai, Priestia megaterium, Peribacillus frigoritolerans, Cytobacillus firmus, and Pseudomonas laurylsulfativorans) exhibit consistent migration regardless of agar concentration. Further research should explore the mechanisms underlying agar concentration's influence on migration across a broader range of microorganisms and environmental conditions, contributing to a comprehensive understanding of microbial movement in natural habitats.

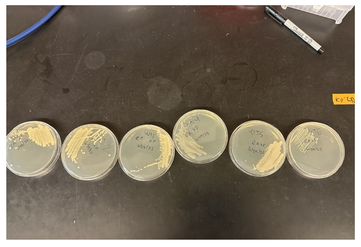
Objectives

- How much do wild strains migrate across agar gels at different gel stiffnesses?
- How can we identify the species of the isolated wild-type strains from the soil?
- What is the quantity of cell aggregation for wild strains?

Overview

Isolating Samples

To set up the experiment, we took a sample of soil and we made a 1-10 dilution 6x with LB. We streaked out the 6 plates 3 times for full isolation. We were able to make frozen stock of all six for future experiments, and we could also make overnight cultures with LB to use for the next day.



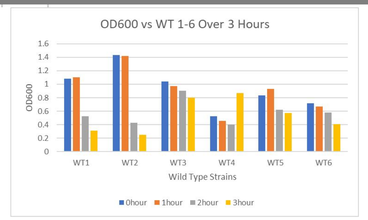
Aggregation

To measure aggregation, we made overnight cultures with 5 mL LB and we used a pipette tip to take frozen stock. We measure OD600 off the top of the culture for 3 hours and we were able to calculate an aggregation index for all 6 strains from it.

Migration

To measure migration across different surfaces, we made agar with concentration of 0.3%, 0.5%, and 0.7%. We made 6 plates for each concentration(18 in total). We put 5 µL of the sample onto each plate and placed them into 30.0C incubator with a camera that pictured every thirty minutes.

Aggregation



*Cell Aggregation Index = (1 - (Average of 0 hour / Average of 3 hours)) * 100*

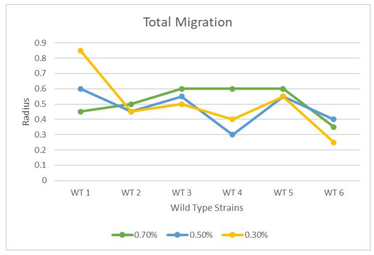
WT1: Average at 0 hours = 0.98125 Average at 3 hours = 0.311
Cell Aggregation Index = **215.44304**
WT2: Average at 0 hours = 1.1315 Average at 3 hours = 0.25
Cell Aggregation Index = **77.94**
WT3: Average at 0 hours = 0.931 Average at 3 hours = 0.803
Cell Aggregation Index = **13.56**
WT4: Average at 0 hours = 0.56425 Average at 3 hours = 0.871
Cell Aggregation Index = **35.201**
WT5: Average at 0 hours = 0.73925 Average at 3 hours = 0.576
Cell Aggregation Index = **-28.348**
WT6: Average at 0 hours = 0.595 Average at 3 hours = 0.411
Cell Aggregation Index = **44.496**
WT5(REDO): Average at 0 hours = 1.1225 Average a 3 hours = 1.07
Cell Aggregation Index = **95.09**

5 of the 6 wild strains had aggregation indexes of greater than zero. This indicates that cells are aggregating or clustering in some way. Cell aggregation refers to the process by which individual cells come together to form clusters or groups. This phenomenon can occur for various reasons, such as in cellular development, immune responses, or disease conditions. Scientists and researchers may use different methods to quantify and characterize cell aggregation, and the cell aggregation index is one such metric.

We were able to find this through measuring the OD600 off the top of our culture for the span of three hours. We made a 4x dilution with 800 microliters of LB and 200 microliters of the sample.

WT5 ended up with a negative aggregation index. This was puzzling but it probably resulted from improper settling assays. The culture tube may have been shaken before testing and it did not allow cells the clump and settle at the bottom of the tube. We were able to redo this strain and we ended up getting an aggregation index of 95.09.

Migration



The data shown here highlights the strain-specific responses with each concentration of LB-based agar gels. For WT 1, the growth radius generally decreases as the agar gel concentration decreases. Notably, at 0.3% agar gel, the growth radius reaches its highest value, suggesting the strain's preference for lower agar concentrations. WT strains 2,3,5 show only slight variations in growth radius, indicating their resilience to changes in agar concentrations. WT 4 has a distinct decline in growth as agar gel concentration decreases showing that the strain has a preference for higher agar concentrations. WT 6 on the other hand shows a noticeable increase in growth radius at 0.30% agar gel, suggesting a strong preference for lower agar concentrations compared to the other strains.

Each chart shows the migration level over time for each Wild-Type Strain.

WT 1: At 18:47 we see a sharp growth in almost all the agar concentrations. After that, the 0.7% and 0.5% seem to stabilize between 9mm and 11mm whereas the 0.3% continues to grow showing a preference for lower agar concentrations.

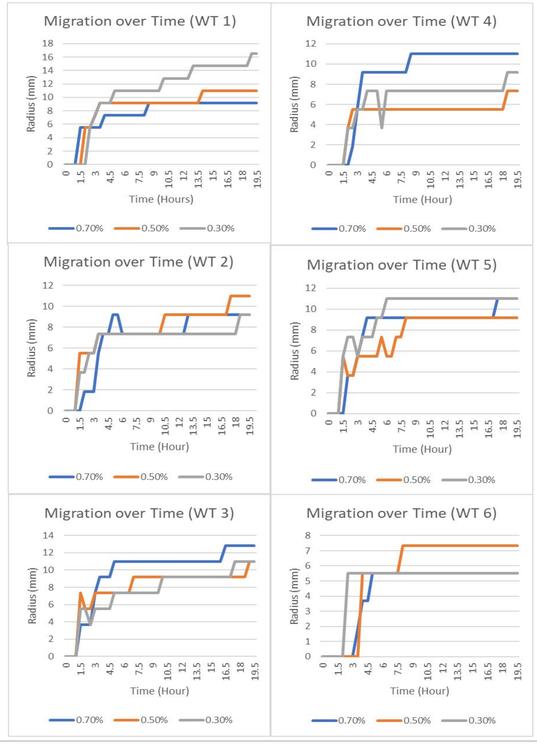
WT 2: Similar to WT 1, there is a sharp growth from 18:47 to 21:47 across all agar concentrations but then all of them slowly stabilize between 9 and 11mm. However, unlike WT 1, this strain shows a preference for higher agar concentrations.

WT 3: Again, we see a steep growth, however 0.7% levels off first followed by 0.3% and then 0.5%. This strain also seems to stabilize between 9 and 11mm and shows a preference for the 0.5% agar concentration.

WT 4: This strain has a very steep growth rate, especially for the 0.5% concentration as it goes from 0 to 9.17 mm within 2 hours. The other concentrations follow to an extent but this strain also shows a preference for the 0.5% agar concentration.

WT 5: All three concentrations grow together and level off relatively close to one another, however this strain shows a preference for lower agar concentrations.

WT 6: This strain has a mildly steep growth at the beginning but stays pretty close to the original values after the original spike in the growth. This strain shows a preference for high agar concentrations.



Identification

Wild Type 1: **Priestia aryabhattai** B8W22 16S ribosomal RNA, partial sequence AND **Priestia megaterium** NBRC 15308 = ATCC 14581 16S ribosomal RNA, partial sequence **(99.93%)**

Wild Type 2: **Priestia megaterium** NBRC 15308 = ATCC 14581 16S ribosomal RNA, partial sequence **(100%)**

Wild Type 3: **Peribacillus frigoritolerans** strain DSM 8801 16S ribosomal RNA, partial sequence **(100%)**

Wild Type 4: **Cytobacillus firmus** strain NBRC 15306 16S ribosomal RNA, partial sequence **(99.25%)**

Wild Type 5: **Peribacillus frigoritolerans** strain DSM 8801 16S ribosomal RNA, partial sequence **(99.93%)**

Wild Type 6: **Pseudomonas laurylsulfativorans** strain AP3_22 16S ribosomal RNA, partial sequence **(99.19%)**

Summary

This study explores the migration behavior of six different wild types across agar gels with varying concentrations (0.3%, 0.5%, and 0.7%). The results indicate that agar gel concentration may not significantly impact migration for these specific wild types. However, understanding the role of agar concentration is crucial for interpreting microorganism movement in natural environments, as agar gels mimic soil and sediment substrates. Further research should delve into the mechanisms underlying agar concentration's influence on migration across diverse microorganisms and environmental conditions to enhance our understanding of microbial movement in natural habitats.

CONTACT US

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