

Fitness of Potassium Benzoate Evolved *Escherichia coli* K-12 Under Various Permeant Acid Stresses

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Abstract

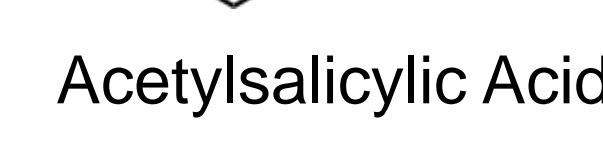
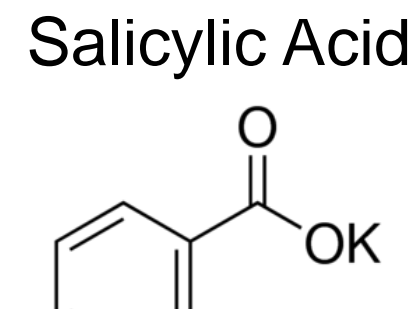
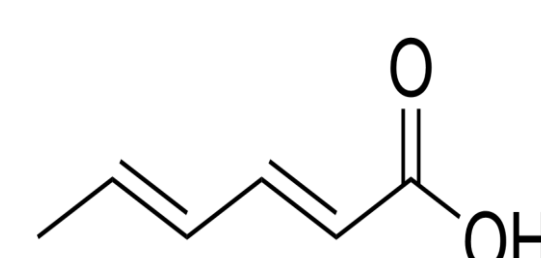
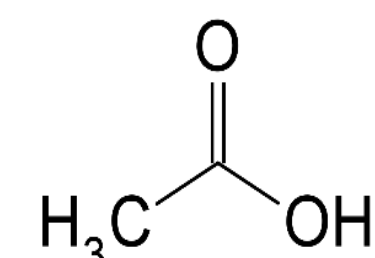
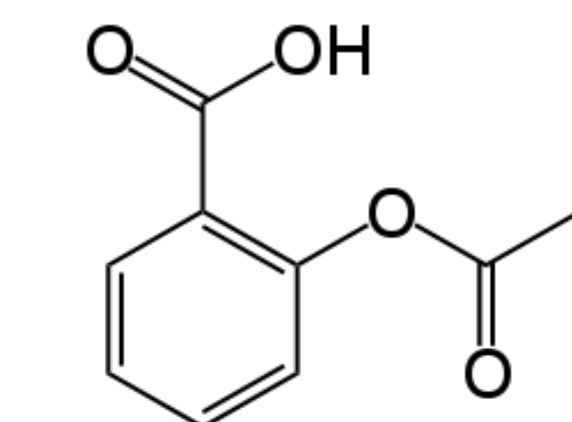
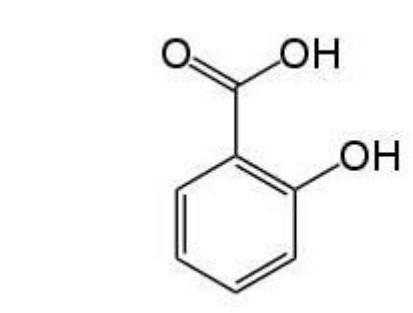
While *Escherichia coli* have the ability to adapt to various extreme changes of pH while traversing the human gut, permeant acids are used in food preservatives to inhibit the growth of such organisms. This study reports that *E. coli* can be adapted to have increased resistance to the permeant acid potassium benzoate. Strains that were evolved to have this resistance were tested to see if they also had an increase in resistance to two benzoate derivative permeant acids, salicylate and acetylsalicylate, and two non-benzoate derivative permeant acids, acetic acid and sorbic acid. The concentration of the acids used were 10mM for salicylate, 10mM for acetylsalicylate, 20mM for sorbic acid, and 60mM for acetic acid. Our results showed that all of the adapted strains had a significant increase in growth rate in salicylic acid, while significant increases in growth rate in other permeant acids varied from strain to strain. This suggests that all the adapted strains evolved with increased growth rate specific to benzoate derivative permeant acids, while some strains and not other developed increased resistance to all permeant acids. Future work with these evolved strains will include looking into what specific genes play a role in this type of resistance and testing the strains' resistance to other permeant acids, aromatics and non-aromatics, both benzoate derivatives and non-benzoate derivatives.

Background

- Escherichia coli* (*E. coli*) endures several different types of external pH stresses while traveling through the human intestinal tract, from basic conditions to extremely acidic conditions. (3)
- Despite this strong flux in acidity, the bacteria retains its ability to survive and thrive in the human gut. (3)
- Permeant acids such as potassium benzoate, salicylic acid, and sorbic acid are used as food preservatives to inhibit the growth of bacteria and other microorganisms. (2)
- E. coli* can be evolved over time to grow significantly better in high potassium benzoate permeant acid stress than its wild type (3)
- It is unknown whether this evolution allows the evolved bacteria strains to grow significantly better than their ancestral strain under any permeant acid stress or only with benzoate derivatives.
- It is predicted that the evolved bacteria strains will grow significantly better than their ancestral strain in both benzoate analog (salicylic acid, potassium benzoate, acetylsalicylic acid) and non-benzoate analog (acetic acid, sorbic acid) permeant acids.

Benzoate Derivative permeant Acids

Non-Benzoate Derivative permeant Acids



Potassium Benzoate

Methods

- Strain Selection.** The eight strains (A5-1, A5-2, C3-1, C3-2, E1-1, E1-2, G5-1, G5-2) that underwent experimentation were evolved for 2,000 generations under a maximum of 20mM potassium benzoate stress.
- Growth Curves.** The evolved strains and ancestral strain were grown over night at 37°C in LBK experimental growth media to ensure that ancestral strains could handle the stress. The next day the growth media was diluted 1:400 and grown for five hours at 37°C. Growth samples were taken after the first hour, then after the next 30 minutes, and then every 20 minutes. The log of the growth readings showed the growth rate of the strains.
- Stress Strengths.** Through trial and error the following experimental media strengths were determined.
 - Salicylic Acid:
 - Overnight: 10mM
 - Experimental: 10mM
 - Acetylsalicylic Acid
 - Overnight: 5mM
 - Experimental: 10mM
 - Sorbic Acid
 - Overnight: 10mM
 - Experimental: 20mM
 - Acetic Acid
 - Overnight: 30mM
 - Experimental: 60mM

Results

Potassium Benzoate Evolved Strain Responses to Various Permeant Acids

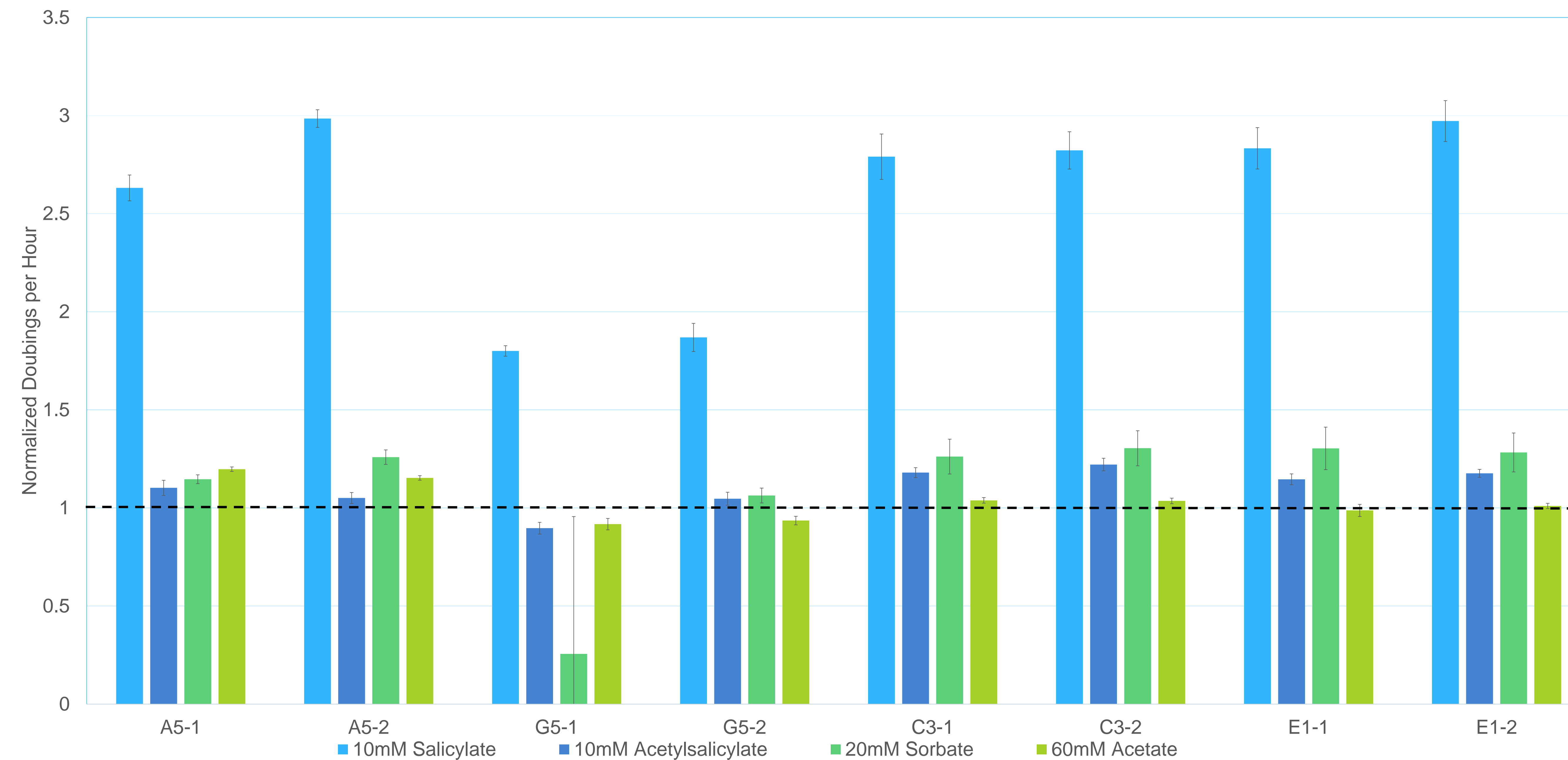


Figure 1: Logarithmic rate of growth of eight potassium benzoate evolved strains of *E. coli* normalized to growth rate of ancestral D13-W3110. Stresses used include 10mM salicylic acid, 10mM acetylsalicylic acid, 20mM sorbic acid, and 60mM acetic acid.

Sample Growth Curve Analysis of Strains A5-1, A5-2, G5-1, and G5-2 vs D13-W3110 in 10mM Salicylic Acid

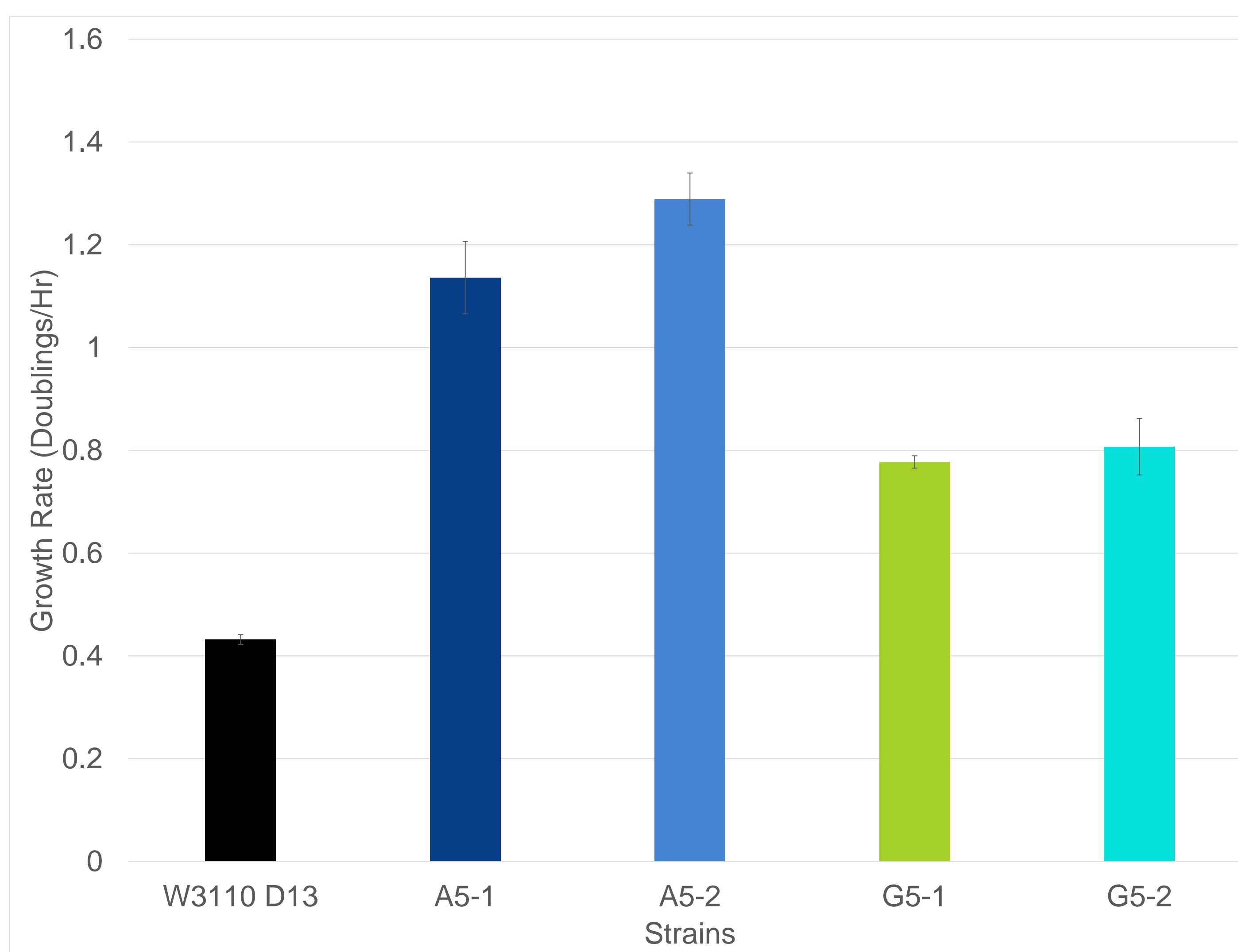


Figure 2: Comparison of logarithmic growth rates of strains A5-1, A5-2, G5-1, and G5-2 versus ancestral strain D13-W3110 in pH 6.5 LBK buffered media with 10mM salicylic acid. Growth curve ran over five hours with readings taken after an hour, then 30 minutes, then every 20 minutes.

Sample Growth Curve of Strains A5-1, A5-2, G5-1, and G5-2 vs D13-W3110 in 10mM Salicylic Acid

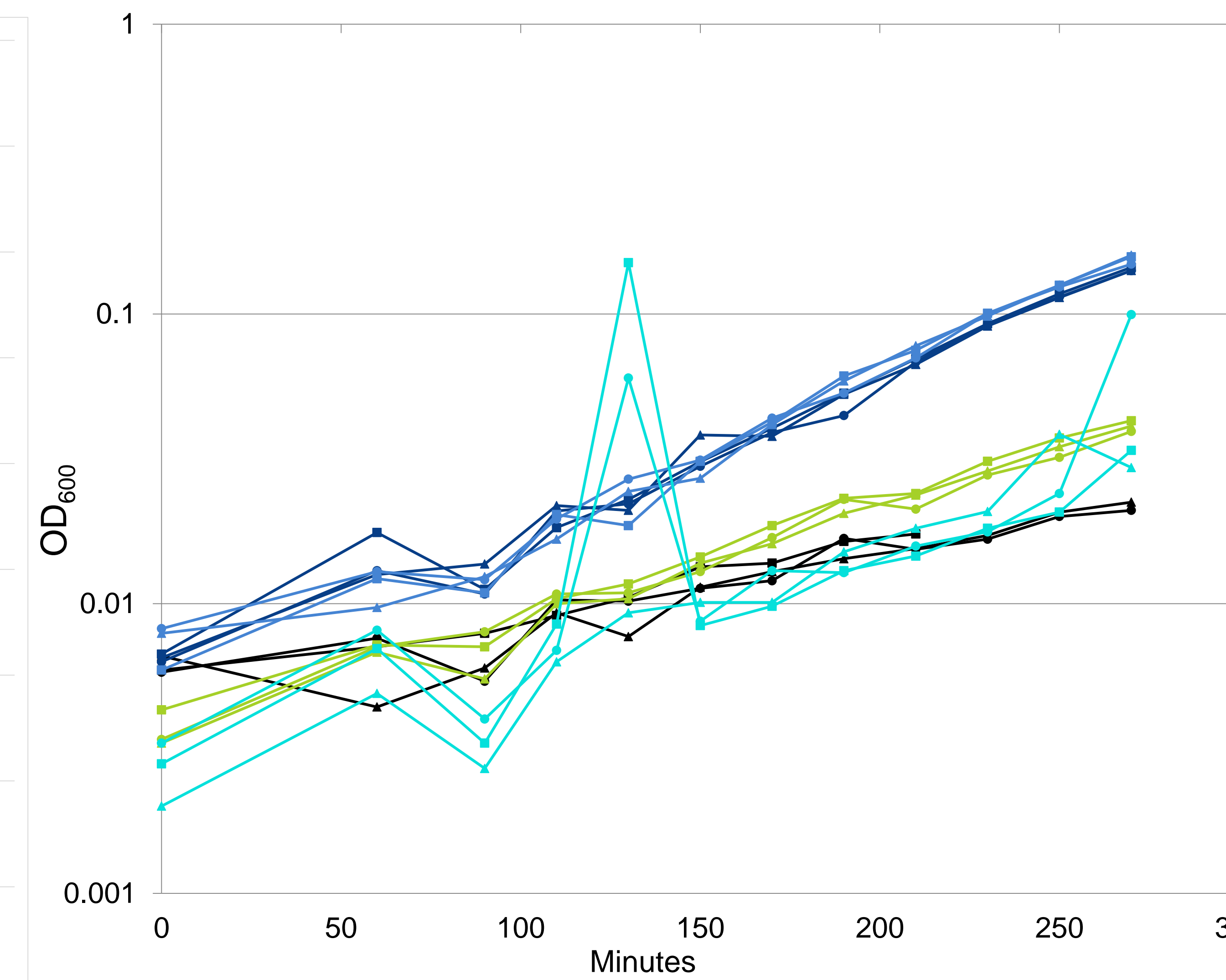


Figure 3: Growth curve of strains A5-1, A5-2, G5-1, and G5-2 versus D13-W3110 in pH 6.5 LBK buffered media with 10mM salicylic acid. Curve ran over 5 hours with OD₆₀₀ readings taken after an hour, then 30 minutes, then every 20 minutes.

Discussion

Reaction to Benzoate-Derivative Permeant Acids

- The stress condition that produced the most noticeably higher growth rate in experimental strains compared to wild type was salicylic acid.
- Acetylsalicylic acid produced significantly higher growth rates in strains A5-1, C3-1, C3-2, E1-1, and E1-2 compared to the wild type.

Reaction to Non-Benzoate-Derivative Permeant Acids

- Sorbic acid, at double the concentration of the benzoate derivative acids, produced growth that was significantly higher than the wild type in some strains (A5-1, A5-2, C3-1, C3-2, E1-1, E1-2).
- Acetic acid produced growth significantly higher than the wild type in two strains (A5-1, A5-2)

Conclusions

- The potassium benzoate evolved strains have evolved to better maintain homeostasis in the presence of specifically benzoate derivative permeant acids
- Some evolved strains that show significantly higher growth in benzoate derivative and non benzoate derivative permeant acids may have evolved, to a lesser extent, to be resistant to all permeant acids

Future Areas of Study

- Identification of which specific genes play roles in this resistance to benzoate derivative permeant acids
- More permeant acid antibacterial substances can be tested on these potassium benzoate evolved strains to see if there is any other substance that produces the same growth difference as salicylic acid has between the evolved strains and their ancestral strain
- An array of concentrations of the benzoate derivative substances can be tested on the evolved strains to determine at what point the stress inhibits cell growth completely.

Acknowledgements

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