Effects of Ethyl Alcohol on Microbial Survivorship

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Ethyl Alcohol

- Pure alcohol or drinking alcohol
- Oldest recreational drug
- Alcohol intoxication
- Inhibits microbial growth and reproduction
Chemistry of Ethyl Alcohol

• Alcohol -the hydroxyl functional group is bound to a saturated carbon atom.
  • Two main metabolites

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\text{H} \\
\end{array}
\]
Microbial Flora

• Little is know about the association between humans and microbial flora
• Supplements and other consumables taken by humans might have unintended effects on the flora populations and their functions.
• Provide nutritional and digestive benefits, secrete vitamins, stimulate antibody production, and protect against pathogenic molecules
Gram (+) vs. Gram (-) Bacteria

- Most pathogenic bacteria in humans are Gram-positive organisms.
- Simple cell wall
- Some antibiotics work against the formation of the cell wall.

- Cell wall contains an extra layer of lipopolysaccharides for extra protection.
- Outer membrane protects bacteria from several antibiotics.
Escherichia coli

- gram-negative bacteria
- One of the most common forms of bacteria
- Most strains are not dangerous
- Common prokaryotic cell model
Staphylococcus epidermidis

• Gram positive bacteria
• Common surface symbiont in many mammals (including humans)
• Most forms considered non-pathogenic
• Potentially pathogenic upon systemic entry
• Forms biofilms
Purpose
To determine if ethyl alcohol will have an effect on microbial survivorship
Hypothesis

**Null Hypothesis:**
- Ethyl alcohol will have no significant effect on microbial survivorship

**Alternate Hypothesis**
- Ethyl alcohol will negatively effect microbial survivorship
Materials

- 100% Ethyl Alcohol
- Micropipettes
- Sterile filters
- Test tubes
- Test tube racks
- Luria broth agar plates (1% Tryptone, 0.5% Yeast extract, 1% NaCl)
- Ethanol
- Bunsen burner
- Safety goggles
- Sterile dilution fluid (10mM KH2PO4, 10mM K2HPO4, 1mM MgSO4, .1mM CaCl2, 100mM NaCl)
- Spreader bars
- Incubator(37° C)
- Vortexer
- Escherichia coli
- Staphylococcus epidermidis
Procedure

1. Bacteria (*E.coli* and *staph*) were grown overnight in sterile LB media.

2. Samples of the overnight cultures were added to fresh media in a sterile sidearm flask.

3. The cultures were placed in an incubator (37°C) until a density of 50 Klett spectrophotometer units was reached. This represents a cell density of approximately $10^8$ cells/mL.

4. The cultures were diluted in sterile fluid to a concentration of approximately $10^5$ cells/mL.
Procedure liquid (exp)

5. Pipetted amounts indicated on table of ethyl alcohol and sterile dilution fluid into 4 tubes. Then made two tubes for each concentration. Vortexed solutions to mix thoroughly.

6. Added indicated amounts of *E.coli* and *Staph* to Vortexed final solutions. Incubated solutions at room temperature for 10 minutes.

7. Pipetted 0.1 mL of solution from tubes (3 per tube) onto LB agar plate. Used a new pipette for SDF, ethyl alcohol, *E. coli*, and *Staph*.

8. Spread solutions evenly over agar plates with sterile spreader bar. Used a new spreader for each plate. Made 6 replicate plates for each concentration (40 plates in all).

9. Incubated plates for 1 day at 37°C. Recorded number of *Staph* and *E. coli* colonies. Each colony is assumed to have arisen from one cell.
<table>
<thead>
<tr>
<th></th>
<th>0%</th>
<th>1%</th>
<th>5%</th>
<th>20%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microbe</strong></td>
<td>0.1mL</td>
<td>0.1mL</td>
<td>0.1mL</td>
<td>0.1mL</td>
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<tr>
<td><strong>Ethyl Alcohol</strong></td>
<td>0mL</td>
<td>0.1mL</td>
<td>0.5mL</td>
<td>2mL</td>
</tr>
<tr>
<td><strong>SDF</strong></td>
<td>9.9mL</td>
<td>9.8mL</td>
<td>9.4mL</td>
<td>7.9mL</td>
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<tr>
<td><strong>Total</strong></td>
<td>10mL</td>
<td>10mL</td>
<td>10mL</td>
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Number of E. coli Colonies

<table>
<thead>
<tr>
<th>Concentrations of Ethyl Alcohol</th>
<th>Average Number of Colonies</th>
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<tbody>
<tr>
<td>0%</td>
<td>283</td>
</tr>
<tr>
<td>1%</td>
<td>252</td>
</tr>
<tr>
<td>5%</td>
<td>163</td>
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<tr>
<td>20%</td>
<td>111</td>
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P-value = 0.00878
<table>
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<th>Concentration</th>
<th>T-value</th>
<th>T-crit</th>
<th>Significance</th>
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<tr>
<td>1%</td>
<td>3.875</td>
<td>3.1</td>
<td>Significant</td>
</tr>
<tr>
<td>5%</td>
<td>15</td>
<td>3.1</td>
<td>Significant</td>
</tr>
<tr>
<td>20%</td>
<td>21.56</td>
<td>3.1</td>
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The LD50 is approximately 11%.
Number of Staph Colonies

Concentrations of Ethyl Alcohol

P-value = 0.000975
<table>
<thead>
<tr>
<th>Concentration</th>
<th>T-value</th>
<th>T-crit</th>
<th>Significance</th>
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</thead>
<tbody>
<tr>
<td>1%</td>
<td>2.5</td>
<td>3.1</td>
<td>Not Significant</td>
</tr>
<tr>
<td>5%</td>
<td>4.6</td>
<td>3.1</td>
<td>Significant</td>
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<tr>
<td>20%</td>
<td>6.3</td>
<td>3.1</td>
<td>Significant</td>
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Dunnett's Test (Staph)
Conclusion
The null hypothesis was rejected for:
• All alcohol concentrations with E. coli
• The 5% and 20% alcohol concentrations with Staph.
Limitations

• Plating, vortexing, pipetting, exposure times slightly unsynchronized
• Only three concentrations of variable
• Only one exposure time
Extensions

• More concentrations
• More precise plating and incubating
• Compare the effectiveness of different types of alcohol
• More replicates
• Test synergetic effects of other chemicals
Works Cited

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<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
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<td>324.94</td>
<td>4.8651</td>
<td>0.0087</td>
<td>3.0087</td>
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<tr>
<td>Within Groups</td>
<td>160.29</td>
<td>24</td>
<td>6.6789</td>
<td>0.33188</td>
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<td>Total</td>
<td>257.77</td>
<td>27</td>
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Anova (E. coli)
### Anova (Staph)

#### SUMMARY

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<thead>
<tr>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
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<td>704</td>
<td>176</td>
<td>176</td>
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<td>0.01</td>
<td>3</td>
<td>467</td>
<td>155.666667</td>
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<td>0.05</td>
<td>4</td>
<td>550</td>
<td>137.5</td>
<td>155.333333</td>
</tr>
<tr>
<td>0.2</td>
<td>4</td>
<td>497</td>
<td>124.25</td>
<td>200.3333333</td>
</tr>
</tbody>
</table>

#### ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>6009.316667</td>
<td>3</td>
<td>2003.105556</td>
<td>11.63110603</td>
<td>0.00097567</td>
<td>3.587433702</td>
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<tr>
<td>Within Groups</td>
<td>1894.416667</td>
<td>11</td>
<td>172.219697</td>
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<td></td>
<td></td>
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<tr>
<td>Total</td>
<td>7903.733333</td>
<td>14</td>
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