



Background

One of mankind's earliest ventures into the world of biotechnology was the use of yeast to ferment juices into ethyl alcohol (ethanol). Ethyl alcohol has proved itself beyond the arena of pleasure as a disinfectant. With so many uses for one chemical, it isn't unimaginable that supply problems would develop. As recently as February 25th, 2015, American Reagent has been undergoing an ethanol production shortage "due to manufacturing delays" (AHSP).

The scientific community is currently trying to address this looming problem. Bioethanol, which accounts for 95% of all ethanol, requires two major processes, fermentation and distillation (Onuki). The greater scientific community has focused on improving distillation, but have turned a blind eye on improving the basic fermentation process.

Theory

Theoretically if a unicellular organism that rapidly reproduces, undergoes alcoholic fermentation under anaerobic conditions, and can be prevented from forming lactic acid, it would possibly stand to replace yeast.

The bacteria used in the experimentation, *Bacillus cereus* and *Escherichia coli* met those criteria. They will undergo alcoholic fermentation in an anaerobic environment (Dupont) and with the addition of a lactate dehydrogenase inhibitor, or LDHi, lactic acid production won't occur. The LDHi used will be Galloflavin (Manerba), Gossypol (Ikeda).

Procedure

This lab had common yet unique issue. The chemical galloflavin was to be made in a lab to reduce the cost of the procedure. However, galloflavin is an under-researched chemical, only appearing on two scientific journals in the last 100 years. Galloflavin was prepared in a 50% ethanol solution by the oxidation of Gallic acid in a basic environment followed by an acidification, then finally being vacuum filtered via a Buchner funnel.

After a slew of preliminary trials to establish the most effective procedure the experiment was scoped down to one bacteria and one LDHi. Gossypol, proved far superior to galloflavin in early testing, and was selected for final testing. *Bacillus cereus* and *Escherichia coli* tested comparably in ethanol production however, the *Escherichia coli* produced a pungent odor that was deeply concerning, and it was decided that only *Bacillus cereus* would be used in further testing.

The bacterial culture was nutrient broth with a 0.05 mM concentration of gossypol in multiple 20 mL test tubes containing Durham tubes to measure CO₂ production, an indication of ethanol production. This was tested against a similar set up using yeast and sabouraud dextrose broth. These were allowed to ferment for 7 days, and CO₂ production was recorded in terms of length of the gaseous part of the Durham tube.

Results

37C	Yeast 1 (cm)	B. 1a (cm)	B. 1b (cm)	B. 1c (cm)	B. 1d (cm)
1 Day	0	0	0	<0.5	0
2 Days	5	<0.5	<0.5	<0.5	<0.5
3 Days	5	<0.5	<0.5	<0.5	<0.5
7 Days	5	0.5	0.5	0.5	0.5

25C	Yeast 1 (cm)	B. 1a (cm)	B. 1b (cm)	B. 1c (cm)	B. 1d (cm)
1 Day	3.5	<0.5	<0.5	<0.5	<0.5
2 Days	5	<0.5	<0.5	<0.5	0.7
3 Days	5	<0.5	<0.5	<0.5	0.8
7 Days	5	0.5	0.5	0.5	0.8

Analysis

Under 0.5 cm, the CO would present as a gathering of miniature bubbles that made accurate measurement impossible. Though it is painfully obvious that yeast out competes bacteria in terms of ethanol fermentation, there are still discoveries that will have a lasting effect.

For one, in trying to inhibit LDH, and using CO₂ production as quantification, a biological LDHi assay, or a test that will tell if a chemical will inhibit LDH activity, has been stumbled upon. What is better, is the fact that the materials needed for this assay, nutrient broth, Durham tubes, *Bacillus cereus*, and test tubes, are all commonly found in microbiological laboratories. This compared to Sigma Aldrich's "Lactate Dehydrogenase Activity Assay Kit" which costs \$350 for 500 tests. This will lower the cost of entry for those who want to do research into LDH activity.

In addition, this has shed a light on how under researched galloflavin was and a research team run by professor Luzik in the University of New Haven Chemistry Department has been assembled to further investigate this chemical and its properties.

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