

Controlling *Lactobacillus* in Face of Antibiotic Resistance

Sanitation, Use of Hops Play Roll in Reducing Contamination

This article is based on a presentation by Marc von Keitz with the Biotechnology Institute, University of Minnesota, Twin Cities Campus (www.bti.umn.edu | 612-624-6774). von Keitz spoke at the 2006 International Fuel Ethanol Workshop in Milwaukee, WI.

Fuel ethanol plants are not designed to operate under pure culture conditions, thus a wide range of bacterial contaminants are present in all plants. Gram-positive Lactic Acid Bacteria (LAB) are of primary concern because of:

- Optimal growth conditions for LAB in fermenters (high sugar and low oxygen).
- Diversion of sugar away from ethanol production.
- Competition for trace elements with yeast.
- Production of compounds (lactic and acetic acid) detrimental to yeast growth.

Sources of bacterial contamination include natural LAB population on corn kernels or other starch/sugar sources' contaminated yeast supply; and residual population in the ethanol plant.

Control strategies. Current control strategies being utilized in ethanol production facilities include:

- Sanitation.
- Mash cooking.
- Use of good, competitive yeast strains.
- Antibiotic treatment. For gram+ LABs the most common antibiotics are penicillin and virginiamycin individually or in a combination of products.

Antibiotic treatments can be expensive. Preventive use of virginiamycin in a 125-million-gallon-per-year ethanol plant in 2005 cost \$40,000. Some of the antibiotics may carry over into the plant's distillers grains. Another concern is resistance to the antibiotic.

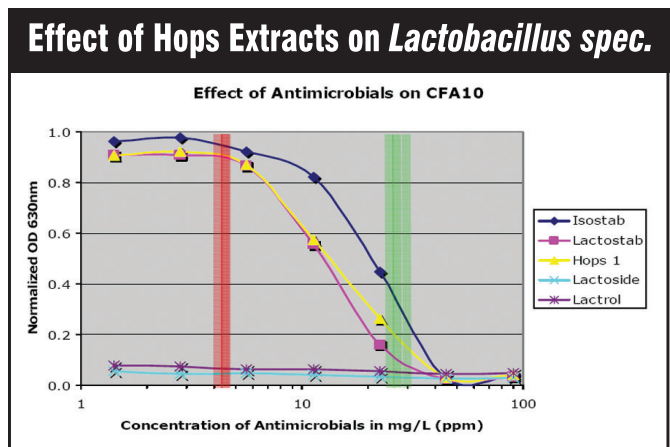
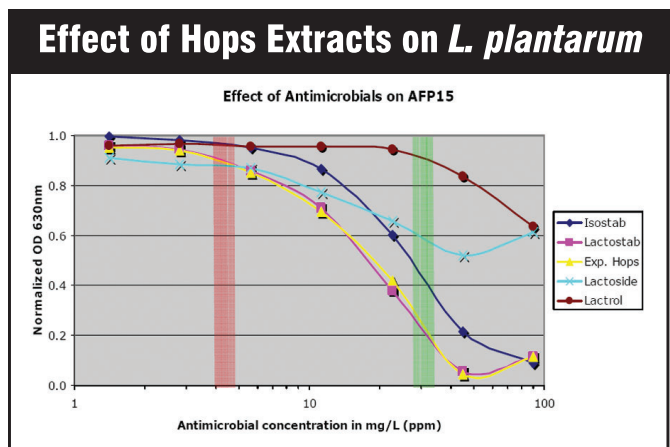
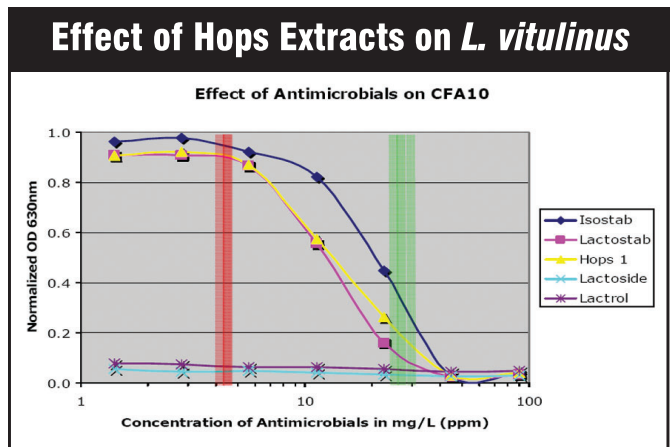
Antibiotic Resistant Strains

The Biotechnology Institute, an interdisciplinary institute founded in 1985 that focuses on biocatalysis, microbial physiology, and microbial evolution, collected samples from four dry mill ethanol plants with persistent contamination problems.

Researchers isolated and identified 11 strains of LAB by fatty acid methyl ester and gram stain analysis. They determined the minimum inhibitory concentration (MIC) for Penicillin G, Lactrol, and Lactoside for each strain.

MIC was defined as the first concentration to achieve greater than 80% reduction in the overall cell number compared to the antimicrobial-free control.

Study results. The 11 strains belonged to five different species of *Lactobacillus*—*L. plantarum*; *L. casei*; *L. vitulinus*; *L.*



buchneri; and *L. gasserii*.

Four strains had MICs for Lactrol of at least 11 mg/L with one having an MIC above 90 mg/L. Three strains displayed MICs for Penicillin G of at least 11 mg/L or higher. One strain had an MIC for Lactoside above 90 mg/L.

Hops Extracts

Hops has long been used as an antimicrobial and flavoring agent in the brewing industry. The 11 strains of LAB described above were retested for their sensitivity to two commercial and one experimental hops product.

For each of the strains at least one of the commercial products had MICs of 20 mg/L or below, which is within the recommended dosage range. See the charts on page 130 for the effects of hops extracts on three strains of *Lactobacillus*.

Conclusions

Sanitation is key. Rigorously clean facilities and design plants so that they can be cleaned easily.

Know what is contaminating the plant and the weaknesses of those contaminants.

The current combination of antibiotics do not offer complete protection against evolving resistance.

Hop products are an important new weapon in the antimicrobial tool kit.

Alternating between antimicrobial agents may add additional protection, since ethanol plants tend to be relatively isolated entities with endemic populations.

Use anti-microbials only on an as needed basis.

Governors' 2007 Ethanol Policy Recommendations

1. Expanded Renewable Fuels Standard.

The coalition recommends immediate expansion of the Renewable Fuel Standard (RFS) to include a short-term target of 12 billion gallons a year of ethanol and biodiesel utilization by 2010 and to establish longer-term BTU-based targets of 15% of total motor fuels consumption by 2015 and 25% by 2025, with equal incremental steps provided for each year in between. This goal includes 500 million gallons a year from cellulosic ethanol by 2012.

2. Provide a Financial Value to the RFS Trading Credit for Cellulosic Ethanol.

The coalition recommends that Congress assign a value to the trading credit by converting the \$0.51 Volumetric Ethanol Excise Tax Credit to a 10-year Cellulosic Ethanol Production Tax Credit.

3. Meeting the Vehicle and Infrastructure Challenge—A Regional E85 Market Strategy.

The coalition recommends that Congress adopt performance standards for major gas station owners and branders (e.g., owners of 100 or more fueling stations, high-volume stations) that would provide at least one E85 pump at 95% of their stations in at least one region over five years.

4. Research, Demonstration, and Incentives.

The coalition recommends providing \$213 million for the U.S. Department of Energy Biomass Program's research and demonstration activities in 2007.

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Reprinted from the January/February 2007 issue of *BioFuels Journal*.