



Turning a Problem into a Cure for Increased Ethanol Yield

The beneficial microbes that convert milk into yogurt and act in our guts to promote digestive health cause big problems in ethanol fermentation tanks. These lactic acid bacteria (LAB) proliferate in ethanol feedstock and inhibit growth of ethanol-producing yeast — which slows down fermentation, reduces biofuel yield by as much as 20% per pound of input material, and results in production shutdowns due to contamination. The most common control measures, chemical antimicrobials such as antibiotics, do not eliminate LAB. Additionally, the potential for antibiotic residue limits the marketability of dried distillers grains, a byproduct of the fermentation process used as animal feed.

Ecolyse Inc., based in College Station, TX, is working to address this issue by developing products to treat bacterial contamination. Ecolyse made the connection that the key to solving ethanol fermentation slowdown could be found in another industry's problem. Over the years, yogurt manufacturers have experienced episodes of widespread LAB culture death due to the activity of LAB phage. These phage are natural viral predators that specifically target LAB. Phage work by injecting their DNA into bacterial cells, replicating inside the cells, and then causing bacterial cell lysis (*i.e.*, bursting) to release the progeny phage.

"Ecolyse is seeking to harness the power of lactic acid bacteria phage in order to control unwanted LAB during ethanol fermentation," says Elizabeth Summer, co-founder and vice president of research at the company.

Each type of phage kills only one type of bacteria. This limited specificity makes phage safe enough for human

consumption; indeed, phage are present in many foods and natural environments. But this specificity also makes finding a suitable phage a challenge.

The development of phage-based products begins with gaining a full understanding of the species of bacteria that causes a particular problem. To identify the bacteria of concern, DNA is isolated from the raw material of interest (*e.g.*, corn mash), and then a molecular screening technique, such as polymerase chain reaction (PCR) or DNA sequencing, is used to identify the bacteria. The bacteria are grown in the lab, and then used as bait in what are known as phage hunts. In a phage hunt, a culture of the bacteria is spiked with an environmental sample thought to contain phage. If any phage specific for that host bacteria are present, they will grow and multiply in the sample — increasing in concentration from about one particle per milliliter to over 10,000 particles/mL after a day or so. Once enough different kinds of phage are isolated, they can be grown in liquid cultures of the host bacteria to make the large volumes needed for industrial treatment.

Ecolyse produces the phage as liquid lysates. They are best applied at the beginning of the fermentation cycle; like antibiotics, there is a window of opportunity for treatment. A 10- or 100-fold reduction in LAB increases ethanol yields. An additional plus is that replacing antibiotics during the fermentation process increases the marketability of the dried distillers grain byproduct, which can be labeled as antibiotic free.

Developing phage products to control LAB during ethanol fermentation represents an attractive phage applica-

THE COST IMPACT

Consider the U.S. corn ethanol industry, which consists of about 211 corn-ethanol biofuel fermentation plants, each with an average annual production capacity of 50–100 million gal/yr. Assuming LAB contamination reduces ethanol yield by 1% (a conservative estimate, as it may be as high as 20% in severe cases), the annual yield loss will be about 137 million gal/yr of ethanol, based on 2012 U.S. industry production rates. This yield loss translates to a \$308 million/yr monetary loss for the industry, assuming an ethanol value of \$2.25 per gallon. Furthermore, some plants voluntarily avoid antibiotic use, and experience even larger yield losses due to contamination.

tion in the larger picture of industrial microbiology. Until now, no company had developed phage products other than for medical and agricultural applications. This is partly due to a general lack of awareness of industrial contamination.

Certain industrial sectors, however, experience chronic detrimental effects of bacterial contamination. For example, the oil and gas industry faces staggering revenue losses due to product degradation by hydrogen sulfide-generating bacteria, and spends an estimated \$7 billion/yr for chemical biocide treatments. Furthermore, bacteria-related corrosion of metal and concrete structures is conservatively estimated to be about \$82 billion/yr.

Because LAB is a relatively well-defined target, ethanol fermentation is a promising application for phage control. To date, Ecolyse has developed formulations capable of controlling the dominant LAB strains. It is now optimizing production to reduce costs in anticipation of launching a commercial product in the near future.

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