Superenzymes to help turn the corner on biofuel costs

By Marcia Wood, Agricultural Research Service – USDA

Splat! A blob of bright-red tomato sauce from that juicy hamburger you're eating just landed on your favourite white shirt.

No worries. You know that a few dabs of the enzyme-powered spot remover that's on your laundry room shelf will make every trace of the wayward condiment vanish in the wash.

If only it were that easy to find equally strong, fast-acting enzymes for biofuel chores. What's needed are 'superenzymes' that would make quick work of disassembling the tight matrix of compounds – cellulose, hemicellulose, and lignin – in cell walls of plants.

Right now, these recalcitrant compounds push up the cost and complexity of making the next generation of cellulosic ethanol and co-products.

That's why researchers at the ARS Western Regional Research Center in Albany, California, are in hot pursuit of stable, highly active enzymes for biorefineries.

Chemist Charles Lee inspects a petri dish containing xylan, a component of the hemicellulose in plant cell walls. Bacteria that produce xylanase were streaked onto the dish in a wavy pattern, and the clear areas are where the xylanase is degrading the xylan. (PHOTO: Peggy Greb)

Enzyme explorers

The search for excellent enzymes has taken chemist Charles Lee, and co-workers with the Albany centre's Bioproduct Chemistry and Engineering Research Unit, to outdoor places where decomposers live and work. This team has, for example, probed dank soil beneath eight-metre-high piles of decaying rice straw. And they've carefully drawn samples of the murky liquid in dairy-waste lagoons.

Back at the lab, these and other environmental samples – a miscellany of anonymous microbes – surrender their genetic material for what's known as a 'metagenomic analysis' (see box article next page).

Metagenomics compresses the amount of time it would otherwise take to find genes that contain the blueprints for elusive enzymes. Charles and colleagues have, for instance, used metagenomics to find a xylanase gene from a microbe living in a dairy lagoon. As its name indicates, xylanase degrades xylan, the backbone of hemicellulose.

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"Xylanase genes aren't new," says Charles. "But this one, xyn8, interests scientists because the enzyme it codes for can perform well at temperatures considered cold in the world of biofuel production. Bioenergy-related enzymes that thrive at cool temperatures are prized because they don't need costly heating."

The scientists documented their discovery in a 2007 article in the journal *Extremophiles*.

A bag of compost starter mix from a nursery turned out to harbour an enzyme adept at dismantling one of the many different chemicals that branch out from hemicellulose's xylan backbone. The new enzyme is nicknamed 'AF' or 'AFase', short for alpha-L-arabinofuranosidase.

"It's one of the key enzymes needed to digest plants," says Kurt Wagschal, a chemist at Albany.

Kurt and coinvestigators reported the work in a 2007 issue of *Enzyme and Microbial Technology*.

"AFase isn't a star performer," says Kurt. "But once we know more about its properties, we might combine this enzyme with another that has a similar genetic sequence. From the two, we may get a better enzyme."

PCR also works

Another interesting way to create a talented new enzyme is to use PCR technology – short for polymerase chain reaction. Though perhaps best known for its ability to accurately copy scarce or rare bits of DNA in a sample, PCR's other, lesser known use is to randomly rearrange genetic material – somewhat like the evolutionary changes that occur over time in nature.

Molecular biologist Sarah Batt transfers a culture of yeast that has been engineered with genes for degrading biomass. (PHOTO: Peggy Greb)

The approach, known as "directed molecular evolution," yields improved enzymes and is a tactic the researchers have been using for about six years.

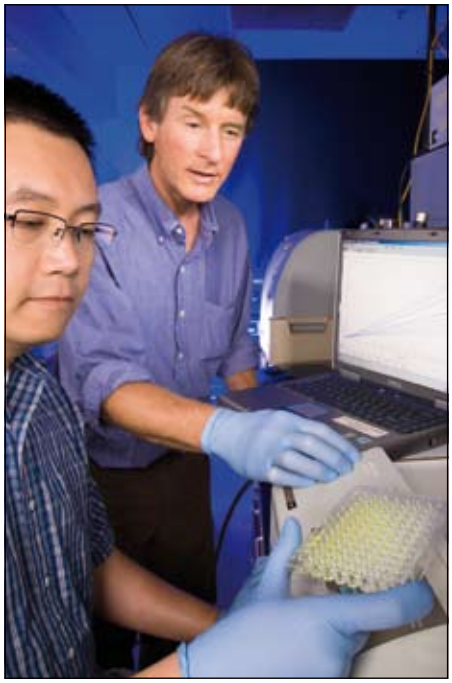
The quest for new enzymes to neatly excise branches from the xylan spine has been slowed somewhat by lack of assays that detect such enzymes. To remedy that, the scientists are developing rapid, reliable tests. Charles and colleagues have created an assay for pinpointing alpha-glucuronidase enzymes. These take apart one of the branches extending from the hemicellulose spine.

The new assay is unique because it can process samples more quickly than any other assay developed previously for this enzyme.

But wait, there's more. Chemist Dominic Wong's focus is enzymes that would cleave the branches that link hemicellulose to lignin. "This and other enzymes with similar actions would untangle the cell-wall structure and speed up its breakdown to sugars," says Dominic.

In addition, Dominic is engineering yeasts so that they will produce not just the usual fermentation enzymes – the ones that convert sugars into ethanol – but the cell-wall-digesting enzymes, as well.

"We'd have a more efficient bioconversion process if all the enzymes that are needed were in the yeasts," says Dominic.



Technician Chamroeun Heng, left, and chemist Kurt Wagschal use high-throughput enzyme library screening to discover improved enzymes. The yellow colour in the sample represents enzyme activity. (PHOTO: Peggy Greb)

Equipping yeasts to do both jobs may lower costs. In the struggle to make biofuels economical, potential savings from versatile yeasts would be welcome indeed.

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METAGENOMICS: A PROVEN SHORTCUT TO ENZYME GENES

Metagenomics may at first seem to be a long, involved route to finding genes essential for making an industrial enzyme.

Actually, it is much faster than many other approaches.

Here's how it works. First, microorganisms are collected from the outdoors and brought into the lab. The microbes' genes and other DNA are, for research purposes, transferred into other organisms – such as a harmless laboratory strain of *Escherichia coli*.

Each *E. coli* can take up and activate only a few of the borrowed genes. That makes close-up scrutiny of those genes much easier.

In petri-dish tests, scientists determine whether any of the borrowed genes endow any *E. coli* with the ability to produce an enzyme of interest. One that degrades xylan would be of interest because xylan forms the backbone of hemicellulose, a molecule that complicates production of cellulosic ethanol.

If any *E. coli* are now equipped with a xylan-degrading enzyme, or "xylanase," it takes only a few more steps to find which gene holds the blueprint for making that enzyme.

Once the new xylanase gene is found and its structure determined, the gene can be copied. After that, it can be shuttled into other microbes, such as yeasts. The yeasts follow the gene's instructions, churning out large quantities of the enzyme for further study.

If the enzyme proves to be a champion, it might be produced commercially.