proteinspotlight
> ONE MONTH, ONE PROTEIN <

Issue 164, September 2014

www.proteinspotlight.org

moving forward

Vivienne Baillie Gerritsen

Nature's imagination seems endless, and so is Man's. For as long as humans have existed, they have twisted Nature to meet their own needs. Wood has been used to keep them warm. Whale oil has been used to make light. Water has been harnessed to make electricity. And when the era of bio-engineering developed, it was not long before scientists found ways to tinker with an organism's genome for the benefits of mankind. Nowadays, the realm of medication depends heavily on biotechnology. And so, undoubtedly, will novel biofuels. With fossil fuels slowly trickling away, it is becoming paramount to find alternative sources of fuel. And ethanol, though not a new idea, is one. Ethanol is synthesized quite naturally by microorganisms – usually as a waste product – and is the result of the degradation of sugars, by an enzyme known as inulinase, and their subsequent fermentation.

John Rogers Cox, Gray and Gold

Wikipedia

Ethanol is not a particularly novel biofuel since it was already being used for lamps in the 1800s, and as one of the first fuels for engines in the 1900s. The German inventor Nikolaus Otto (1832-1891) is credited with having invented the first practical automobile engine that ran on alcohol – which was both plentiful and untaxed (as fuel) in Europe – while Rudolph Diesel (1858-1913) ran his engine on peanut oil. The Ford Model T which came out in 1908 was also designed to run on ethanol. At about the same time, however, crude oil was gushing out of the earth and, soon, it became cheaper than its biofuel counterpart, ethanol. In fact, the tango between using biofuels or fossil fuels has been going on for over a century in developed countries, all depending on which is cheaper, and available, at the time.

Ethanol is one of the fermentation products of a polysaccharide known as inulin. Inulin is a polyfructan, a linear polymer chain of ß-2,1-linked fructose monomers with a glucose monomer at each end, and is used as a carbohydrate reserve by over 30'000 plant species. There are 3 to 60 fructose monomers per inulin molecule, although most inulins are about 30 monomers long. The attention of scientists was drawn to this particular polysaccharide because of its abundance in nature but also because it is not expensive, making it of particular industrial interest. Starch is also commonly used to produce biofuel but the process is more costly because of the high energy input it requires. Converting inulin to ethanol, however, can be done in straightforward steps demanding the intervention of only a few enzymes – amongst which inulinase – and a little bit of bio-engineering.

The most effective fructan inulinase is endo-inulinase, a member of the glycosidase hydrolase family 32. Endo-inulinase cleaves inulin internally at the ß-2,1-link to produce several fructooligo-saccharides. These are subsequently converted to multiple short fructose molecules which are then easy to ferment for the production of ethanol. Endo-inulinases have hence become an important class of industrial enzyme, not only used for ethanol production but also for the manufacture of high-fructose syrups, butanediol, and lactic and citric acids to name a few.

The best sources for the commercial production of inulinase are microorganisms. The reason is twofold: microorganisms are easy to cultivate, and they are able to produce a lot of the enzyme. The thing is, the microorganisms that produce heaps of endo-inulinases – such as the mold *Aspergillus* spp. – are not the microorganisms that convert heaps of inulin-type sugars to ethanol, such as the yeast *Saccaromyces cerevisiae*. The solution to this was to create an organism which carries both features. Since *S.cerevisiae* multiples very fast, scientists chose to insert an *A.niger* endo-inulinase gene – inuA – into the yeast, so that the enzyme could break down inulin into short sugars that can then be fermented by one of the yeast's indigenous enzymes, an invertase.

Thus: providing *S.cerevisiae* with endo-inulinase creates a chimeric microorganism that will make full use of its inulin to produce ethanol by way of a saccharification process and a simultaneous fermentation process. These coinciding reactions help to prevent feedback inhibition of inulinase activity because the rapid fermentation of the sugars to ethanol keeps the sugar concentration low, which allows the two reactions to occur unhindered. Yet another reason for *S.cerevisiae* to be used extensively in the industry for large-scale ethanol fermentations.

Resolving the crystal structure of inulinase could help to design enzymes whose kinetics are even more effective and would improve the production – and hence the commercialization – not only of fructans but subsequently of ethanol, following their fermentation. The one drawback – although it might be a natural way of keeping human energy consumption down – is that biofuels are not as energy dense as fossil fuels. A gallon of ethanol, which can be made from corn or sugar cane for instance, only has 70% of the energy a gallon of gasoline contains. On the other hand, it doesn't take millions of years to grow a field of corn…

Cross-references to UniProt

Extracellular endo-inulinase inuA, *Aspergillus niger* : A5ABL2

References

1. Yuan B., Wang S.-A.
   Improved ethanol fermentation by heterologous endoinulinase and inherent invertase from inulin by *Saccharomyces cerevisiae*
   Bioresource Technology 139:402-405(2013)
   PMID: 23683966

2. Li Y., Liu G.-L., Chi Z.-M.
   Ethanol production from inulin and unsterilized meal of Jerusalem artichoke tubers by *Saccharmoyces sp*. W0 expressing the endo-inulinase gene from *Arthrobacter* sp.
   Bioresource Technology 147:254-259(2013)
   PMID: 23999259

3. Ohta K., Hamada S., Nakamura T.
   Production of high concentrations of ethanol from inulin by simultaneous saccharification and fermentation using *Aspergillus niger* and *Saccharomyces cerevisiae*
   Applied and Environmental Microbiology 59 :729-733(1993)
   PMID: 8481000