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Genome sequence of a fungus for the biofuel industry

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Much hope is pinned on the production of ethanol from plant biomass – the only naturally renewable raw material – to stave-off the fuel crisis due to fast-depleting reserves of fossil fuel. The spotlight has been on a fungus, *Trichoderma reesei* as the source of enzymes that are required to breakdown cellulose – the principal component of biomass – into fermentable sugars for the production of ethanol as a biofuel. This multicellular, multinucleate filamentous fungus had been awaiting its turn for genome sequencing 'to better understand this fungus and expand its extraordinary biotechnological potential'.

T. reesei belongs to a class of fungi which commonly reproduce by asexual spores (conidia), but under certain conditions produce sexual stage known as *Hypocrea jecorina*. Although rules of nomenclature recommend that the fungus be called by its sexual stage *Hypocrea*, since the sexual stage is infrequent and the fungus is commonly recognized by its asexual (conidia-forming) stage (image: wikimedia.org/wikipedia/commons/thumb/4/4d/Trichoderma_harzianum.jpg/250px), the name *T. reesei* is in common usage. Actually, *T. reesei* is a mutant strain derived from *T. viride* QM6a isolated during the Second World War from rotting military tents and clothing in the warm and humid jungles in the Solomon Islands in the South Pacific. Microbiologists in the US Army Research and Development Laboratories in Natick, Massachusetts, selected this fungus from over thousand strains of microbes because this strain secreted higher levels of cellulase – a group of enzymes that split the β, 1-4 glycosidic linkages in cellulose – the principal constituent of biomass. After a series of exposure to high energy electrons and ultraviolet irradiation that improved its cellulase productivity with concomitant alteration in its morphology, the high cellulase-producing mutant strain was renamed *T. reesei* in honour of Elwyn T. Reese (a biochemist in the Natick laboratories), who had envisaged exploiting this organism for enzymatic conversion of cellulose in plant biomass into ethanol using cellulases in a two-step process (Figure 1).

Certain strains of *T. reesei* secrete cellulase extracellularly in excess of 100 g/l, indicating that even unconcentrated culture broth containing cellulases could suffice for bioconversion of cellulose into glucose. It was hoped that genome sequencing will throw light on genes of *T. reesei* which encode components of the cellulase enzyme system and determine why this fungus is able to secrete an exceptional quantity of cellulases. This knowledge could help improve the productivity of other enzymes in other species of fungi. The genome sequence[1] was determined through shotgun cloning and sequencing and is now published with the customary long list (45 in this instance) of authors. Among the sequences identified were the genes whose predicted gene products had similarity to known proteins.

The *T. reesei* genome (33.9 Mb) contains 9129 genes compared to 10,620 in the model filamentous fungus, *Neurospora crassa* (38.7 Mb). Surprisingly, *T. reesei* has only seven genes encoding endo- and exoglucanases (cellobiohydrolase) – crucial components of the cellulase system! This suggests that rather than the number of copies of the genes, it is their transcription[2], innate characteristics of the gene products (folding), post-translational modifications (disulphide bond formation, glycosylation) of their gene products that are important in high enzyme productivity and stability. Orthologs (regions of DNA encoding genes with similar functions) of the yeast-secretory pathway are represented as single copies in the *T. reesei* genome. Thus, contrary to expectation,

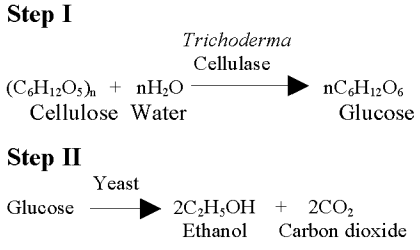

Figure 1. A scheme for the use of *Trichoderma reesei* in the production of fuel ethanol.

genome sequencing has not provided an insight into how this extraordinary capacity for protein synthesis and secretion comes about. It is assumed that the filamentous hyphae, though only 2–4 μm in diameter, are packed with endoplasmic reticulum, ribosomes, Golgi (even if they do not have a stacked arrangement typical of animal and plant cells), with Golgi budding-off ultramicroscopic vesicles filled with cellulase at astonishing speed and the vesicles fusing with the plasma membrane and the cell wall being rendered leaky to allow the exit of cellulase molecules into the surrounding aqueous medium.

T. reesei has the smallest set of enzymes (approximately half of the number in other species) discovered thus far among the plant cell wall-degrading fungi. The ten genes encoding enzymes involved in cellulose degradation, and the 16 genes encoding enzymes involved in hemicellulose degradation occur as different sets of genes in separate regions of the genome, suggesting their induction by different inducers (sophorose, cellulose, lactose) that were identified by the scientists in Natick. As complete hydrolysis of cellulosic and hemicellulosic substrates requires multiple enzymes acting synergistically, it is clear that *T. reesei* is a specialized fungus. It alone cannot provide the mixture of different classes of enzymes that would be required for conversion of plant biomass to fermentable sugars. For example, molecular data confirm that *T. reesei* lacks the ability to degrade lignin – the refractory compound enwrapping cellulose in biomass and thereby limiting the access of cellulases to β, 1-4 glycosidic bonds for hydrolysis of cellulose and release of glucose.

The genomic data, however, allow a prediction of the lifestyle of *T. reesei* in nature. Since *T. reesei* is a specialist decomposer of cellulose, it cannot be a primary colonizer of biomass that contains a mix of polymers – hemicellulose, lignin and cellulose. Rather, *T. reesei* or its ancestral related strains can exist in nature primarily as a secondary colonizer of dead organic matter (comprised mostly of the plant cell walls). In view of the genomic data having revealed 'handicaps' in the fungus for usage in the biofuel industry, and particularly in light of a transformation system for *T. reesei* which has already been developed, it seems likely that the genetic engineers will now 'counsel' *T. reesei* to have its genome supplemented by foreign genes from other species of microorganisms to enhance its capabilities for use in bioethanol biorefineries. Filamentous fungi have evolved with small genomes (~34 megabase). It remains to be seen how much new capabilities can be given through 'prosthetic surgery'. An option is to design mixtures of polysaccharide and lignin-degrading enzymes from different microbial sources for the lignocellulose–ethanol biorefineries.

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