

Characterization of pectinases produced in the fungus *Chrysosporium lucknowense* C1.

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Dyadic International, Inc. owns and develops the fungus *Chrysosporium lucknowense* C1 as a platform for the hyper production of a broad variety of enzymes. These include versatile enzymes for the production of biofuels from non-starch renewable feed stocks.

Chrysosporium lucknowense C1

Production strains of *Chrysosporium lucknowense* (C1) have been developed that produce a large amounts of protein, routinely up to 60 g/l. These strains display a compact mycelium morphology during fermentation, referred to as propagules (Fig. 1), which allows for robust controlled fermentation with high protein yields, low energy input, short cycle times (Fig. 2, Table 1). C1 can be applied in large scale industrial fermentations up to 150,000 litres using relatively inexpensive and simple media.

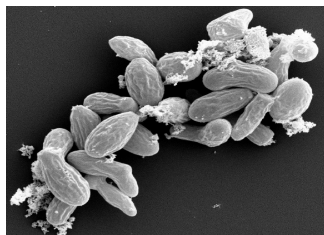


Fig. 1. C1 propagules by scanning microscopy.

A full genetic toolbox is now available for C1, which includes a set of gene expression systems and several secretion signals. Furthermore, C1 is characterized by a high transformation frequency and the possibility of self cloning (no foreign DNA). Besides the genetic toolbox, also a range of versatile production hosts have been developed, like low protease strains. The C1 genome has been sequenced and annotation of the C1-genome is performed. C1-genome mining revealed an impressive hydrolytic potential. For example a large set of putative (hemi-) cellulase and pectinase encoding genes have been discovered. An example of a subset of these genes is given in Table 1.

Table 1. Putative (hemi-) cellulases and pectinases in C1 in comparison with the same putative enzymes in *Aspergillus niger* and *Trichoderma reesei*. * from the CAZy database, ** from the JGI database.

Putative enzyme	C1	<i>A.niger</i> *	<i>T.reesei</i> **
Cellulases + GH61 enzymes	55	41	27
Xylanases	11	5	5
Arabinofuranosidases/arabinases	16	13	3
Galactanases/ β -galactosidases	6	13	2
Polygalacturonases	2	21	4
Pectate/Pectin lyases	6	6	n.d.
Esterases (Fae, Rga, Pme)	18	22	n.d.

Characterization of individual C1-pectinases.

Many of the (hemi-) cellulases and pectinases have been selectively produced in C1, purified and characterized in detail. It was shown that several of these enzymes were active towards the backbone or the hairy regions of pectin. Examples are shown in Fig. 2, Fig. 3 and Fig. 4.

Hydrolysis by commercial and experimental enzyme mixtures.

A commercial mixture of enzymes, Rocksoft MPL, produced by a C1 strain which was not optimized for pectin degradation yet, was already able to degrade pectin-rich biomass to a large extend. Based on this information and the characterization of single enzymes, tailor-made C1-enzymes will be developed that can be used for the complete degradation of pectin-rich biomass, like for example sugar beet pulp.

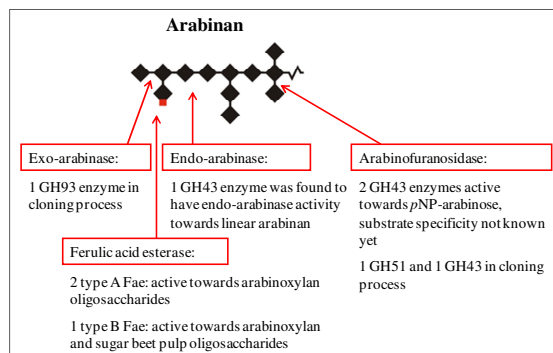


Fig. 2. Selectively produced enzymes from C1 which were found to be active towards arabinan side chains of pectin. ♦: arabinose; ■: ferulic acid.

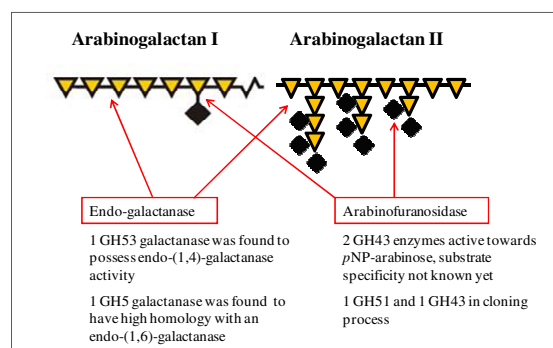


Fig.3. Selectively produced enzymes from C1 which were found to be active towards arabinogalactan side chains of pectin. ▼: galactose; ♦: arabinose.

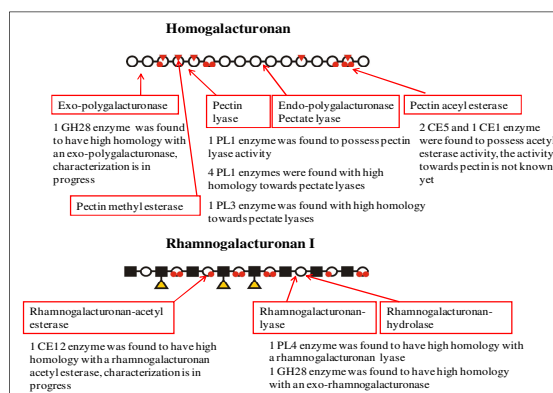


Fig.3. Selectively produced enzymes from C1 which were found to be active towards the pectin backbone. ○: galacturonic acid; ■: rhamnose; ▼: galactose; ▼: methyl ester; ●: acetyl ester.

Conclusion

C. lucknowense C1 is a source of numerous (new) enzymatic activities, including enzymes for the conversion of pectic materials. C1 has been developed into a mature, robust and versatile production platform for these enzymes.

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