Part A: Optimization of reaction conditions for C. thermocellum cellulases

The following combinations of buffer and pH were evaluated for use in assay of C. thermocellum cellulase activity on crystalline cellulose at a given total protein acting against 1% Avicel at 40°C, with 300 mM NaCl, 1 mM CaCl₂ as an assay buffer:

- 50 mM Tris, pH 7.0
- 50 mM Tris, pH 7.6, without calcium
- 50 mM MES, pH 6.0

20 mM calcium

The results of the assay indicated that C. thermocellum cellulases were active at pH 5.0 (50 mM MES) and at pH 5.0 (50 mM Tris) activity. In one buffer, native, cellulases and hemicellulases activities were assayed at 20 and 50 mM MES (NaCl and 1 mM CaCl₂ as an assay buffer), native hemicellulases activity was assayed at pH 5.0.

Native C. thermocellum cellulases were prepared from the total protein of cell-free broth of C. thermocellum, ATCC 27450, grown as 3% (v/v) inoculum.

Part B: Comparison of C. thermocellum and fungal cellulases

The following three enzymes were used for activity assay against 1% Avicel:

- Native cellulases (Cth, see the Part A)
- Total cell-free protein from culture broth of a new strain of Trichoderma reesei (Tne)

(Note: see reaction conditions as described in Part A)

The following conclusions could be drawn from Figures 2:

- Fungal cellulolytic activity (30% 1%, and 35% conversion) was stronger than that of C. thermocellum (30% conversion).
- After a lengthy (30 to 50 hours) digestion period, the major product of digestion by fungal enzymes was glucose, whereas products of C. thermocellum cellulases digestion contained slightly more cellobiose than glucose.

Part C: Library of cellulosomal enzymes for assembly of engineered minicellulosomes

- All fifty-four C. thermocellum cellulosomal genes assigned as being related to biomass degradation, including cellulases, hemicellulases, proteases and multienzymatic modules, have been cloned and overexpressed in E. coli (Table C). Ratios of these genes were soluble at equal molar loadings, as indicated by selecting 55 enzymes solubilizing.

- The activities of 25 purified cellulosomal enzymes have been assayed, and 10 of these have apparent activity as MUC (multiple unitLicensed) enzyme (Table 3).

Part D: Assembly of engineered cellulosomes

Our first goal is to construct active, engineered (“mini-”) cellulosomes for efficient degradation of crystalline cellulose. These minicellulosomes have been designed and employed for the assembly of engineered minicellulosomes.

(1) In order to do the activity-assaying of recombinant expression of cellulosomal enzymes from the bacterial cell wall, we chose to use the same type of expression units (type II) to express C. thermocellum and E. coli recombinant enzymes with type I or type II of dockerin, applied in equal molar loadings, used for induction (by the expression) assembly of engineered minicellulosomes. A study of their activity is in progress.

(2) In a more directed approach, a double recombinant with 5 cellulases taken from different species were used for the specifically targeted assembly of 4 C. thermocellum enzymes, each of which had been fused to dockerins corresponding to one of the enzymes.

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