Bioprospecting: Screening Large Collections for Cellulase Activity Using Metabolic Profiles

Author(s) John Bissett[1], Parivash Shoukouhi[1], Philippe Dufresne[2], Loreta Gudynaite-Savitch[2]

Institution(s) 1. ECORC, Agriculture and Agri-Food Canada, Ottawa, Canada, K1A 0C6 2. Iogen, Iogen Corp., Ottawa, Canada

Abstract:

Metabolic profiles based on growth and respiration on 95 carbon substrates, assessed on Biolog FF MicroPlates™, were developed for 236 Trichoderma isolates (Fungi: Hypocreales). Cellulase activity (resorufin cellobioside fluorometric assay) was assessed, and multiple regressions performed to calculate a predictive model for cellulase activity based on metabolic data. This model was applied to an additional 1628 Trichoderma isolates, and separately to 3750 isolates of 'food- and airborne' fungi (1189 Penicillium isolates, 769 Aspergillus, 428 Fusarium, 1364 other genera) for which metabolic data were available. Finally, cellulase activity was determined using the resorufin assay for 306 isolates with highest predicted activity (151 Trichoderma isolates, 156 other genera). Trichoderma isolates with highest cellulase activities were all from the Viride-Koningii and Lixii clades - 6 had higher cellulase activity (389-1033 pmol/min) than four industrial isolates of Trichoderma reesei used for commercial production of ethanol from cellulosic feedstocks. Average cellulase production increased more than 5-fold in the predicted group (172 pmol/min) compared to the survey group (31 pmol/min). For Trichoderma, cellulase activity in the resorufin assay correlated reasonably with qualitative assays on acid swollen cellulose (ASC). The overall highest cellulase activities were found in the second predicted group ('FABF'), represented especially by Fusarium isolates. 28 of these strains had higher cellulase activities than industrial strains of T. reesei, exceeding 2000 pmol/min in Alternaria. However, these results were not clearly correlated with ASC assays, or in preliminary MUC (4-methylumbelliferyl-â-D-cellobiose) or EGDA assays (esculin gel diffusion assay) indicating that predictions from the Trichoderma survey may be less transferable extrapolated beyond the Hypocreales. This study demonstrates the applicability of metabolic profiles from Biolog microplates to select isolates with enhanced cellulase activity, and equally adaptable to survey collections for other enzymes with industrial applications. We are studying strains selected from metabolic profiles for assimilation of glycosides, pentoses, amines, organic acids, polyols, etc. that accumulate in the fermentation stream and degrade performance of the cellulosic ethanol bioreactor process.

Key words: Biolog, cellulase, metabolic profiles, Trichoderma