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Biochemical characterization of the *Fibrobacter succinogenes* endoglucanase EGC, analysis of its gene and protein domains

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Fibrobacter succinogenes, a major cellulolytic rumen bacterium, possesses many different cellulases and hemicellulases. In order to elucidate the organization of the cellulasic system of this bacterium, many cellulase and xylanase genes have been cloned from the strain S85 in *Escherichia coli*, and their sequence determined (Forsberg et al, 1994, in: Genetics, Biochemistry and Ecology of lignocellulose degradation, Shimada et al, eds, Uni publishers Co, LTD, Tokyo, Japan, 125-136). An endoglucanase gene was previously cloned from the strain BL2 in *E. coli* (Harris, Gilmour and McConville, unpublished). The cloned DNA fragment was sequenced: an open reading frame encoding a protein of 620 amino acids named EGC was found. The protein sequence shows homology with the sequences of family E cellulases (glycosyl-hydrolases family 9), and particularly family E1. The best homology is found with endoglucanase EGB from the strain S85 of *F. succinogenes* (Forano et al, 1994, Current Microbiol, 28, 7-14; Broussolle et al, 1994, FEMS Microbiol Lett, 124, 439-448). Alignment of the amino acid sequence of EGC with the sequences of EGB and the other family E1 cellulases revealed that, like EGB, EGC is composed of a large catalytic domain of 453 amino acids, plus an adjoining domain of unknown function located in the N-terminal part of the protein. Furthermore, EGC contains another domain of 60 amino acids at its C-terminal part that is not present in EGB. The function of this domain, composed essentially of charged amino acids, is not known. Secondary structure prediction and Hydrophobic Cluster Analysis of the sequences of EGB and EGC suggest that the 2 proteins have a very similar 2D structure. The two proteins synthesized by recombinant *E. coli* cells, show the same substrate specificity. However, the optimal pH and temperature for their activity are different : respectively 7.0 and 37°C for EGC and 6.3 and 30°C for EGB. We are presently looking for the presence of a gene homologous to EGC in other strains of *F. succinogenes*.Study of the location of EGC in *F. succinogenes* cells and of the conditions of expression of its gene, compared with that of EGB, will be necessary to assess the role of this endoglucanase in *F. succinogenes*.