Comparison of Butyrivibrio's proteome and enzymatic profiles.

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Abstract

The rumen microbiota is one of the most complex consortia of anaerobes, involving archaea, bacteria, protozoa, fungi and phages. They are very effective at utilizing plant polysaccharides, especially cellulose and hemicelluloses. The most important hemicellulose decomposers are clustered with the genus Butyrivibrio. As the related species differ in their range of hydrolytic activities and substrate preferences, Butyrivibrio fibrisolvens was selected as one of the most effective isolates and thus suitable for proteomic studies on substrate comparisons in the extracellular fraction. The B. fibrisolvens genome is the biggest in the butyrivibria cluster and is focused on "environmental information processing" and "carbohydrate metabolism". Extracellular β-endoxylanase as well as xylan β-xylosidase activities were induced with xylan. The comparison of four carbon sources resulted in the main significant changes in B. fibrisolvens proteome occurring outside the fibrolytic cluster of proteins. The affected proteins mainly belonged to the glycolysis and protein synthesis cluster.

Keywords: Proteome, Genome, Hemicelluloses, Microbiota.

Introduction

B. fibrisolvens was isolated from rumen fluid in the mid-fifties by Bryant and Small. It is a strictly anaerobic, non-spore-forming, monoflagellated, butyrate producing bacterium, which is a member of the family Lachnospiraceae (order Clostridiales, class Clostridia, phylum Firmicutes). This genus belongs to the core or heritable rumen species, which represent nearly half of the rumen population. Together with the genera Ruminococcus, Butyrivibrio, and the Christensenellaceae family, all members of Firmicutes, they form an enriched rumen microbiota population in the prepartal period. Most strains can ferment various soluble sugars, disaccharides and oligosaccharides, producing butyrate as the major end product. This feature makes Butyrivibrio strains an important component of digestive tract microbiota influencing the healthy state of colonocytes for which butyrate is the main energy source [1].

The enzymes and genes involved in the decomposition of xylan by Butyrivibrio fibrisolvens strains have been thoroughly studied and characterized after xylanase cloning and expression in E. coli after its purification from the culture medium. Valuable data coming from the whole genome sequencing and proteome analysis has helped to supply the respective databases (UniProt, Cazy) with relevant information and create an integrated picture of the enzymatic map of this important bacterium for biomass conversion in ruminant animals [2].

The utilization of hydrolytic products from hemicelluloses usually occurs under carbon catabolic repression (CCR), resulting in a preference for hexose over pentoses in bacteria. The mechanism of simultaneous pentose and hexose utilization was observed in thermophilic anaerobes (TGPA). An isolate of Thermoanaerobacter sp. utilized both hexose and pentose simultaneously. It was found that its glycobiome is organized into 13 modules and these genes are functionally coherent, presumably based on positive co-expression. In Butyrivibrio species, both CCR and simultaneous metabolism were observed. The type strain of B. fibrisolvens is able to utilize xylose and glucose simultaneously. Therefore these substrates were chosen for this proteomic study [3].

Nowadays, molecular methods for fibre degradation including genomics and proteomics are preferred to obtain a deep understanding of the digestion process. Among ruminal xylanolytic bacteria, advanced proteomic and mass spectrometric methods for exploring xylan degradation have been used exclusively for B. proteoclasticus. Its complete genome sequence together with the studies of its extracellular polysaccharide-degrading proteome, its cytosolic oligosaccharide-degrading proteome and its carbohydrate transporting membrane proteins substantially expanded current knowledge about the hydrolytic capability of the B. proteoclasticus type strain [4].

Butyrivibria are considered to be an integral part of the bovine rumen bacteriome associated with the host genetic background,

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thus forming inheritable microbiota. The biggest advantage of these bacteria is in the relatively broad spectrum of utilizable substrates and especially important is their xylanolytic activity. The substrate flexibility of butyrivibria species was clearly documented by a study of the B. proteoclasticus glycobiome which covered a wide range of degrading and transporting proteins for different structural and storage polysaccharides, as well as a wide spectrum of oligosaccharides. Due to the mobility of Butyrivibria, mediated by flagella, these strains also represent the most rapid colonizers of solid substrates in the rumen [5].

Conclusion

This study however mainly aimed to examine changes in the extracellular protein expression of B. fibrisolvens when this xylanolytic organism was grown on a variety of bioenergy-relevant substrates in order to identify the proteins responsible for substrate-specific breakdown and/or utilization. The substrates chosen ranged from simple (monomeric) to complex (polymeric), and varied in their general composition. Proteomic analysis resulted in the highest number of spots when xylan was used as a substrate. Nearly all proteins were located in the central pI region. Comparison of proteome derived from simple sugars (glucose, xylose) exhibited a higher fold change in the proteins in the strain cultured on glucose, which indicates a tendency for a preferred sugar, which in the rumen is generally glucose, released by the hydrolysis of polysaccharides.

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