Marine medaka's two-dimensional gel electrophoresis profile.

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Abstract

This dinoflagellate is now a severe threat to fish, shellfish, and zooplankton populations, and its blooms are typically accompanied by widespread fish mortality. Despite the identification of various toxins in K. mikimotoi, including gymnocins and gymnodimines, the processes underlying this species' ichthyotoxicity are still unknown, and molecular research on this subject has never been reported. Through comp arative proteomic analysis, the current study examines K. mikimotoi's fish-eating mechanisms. A model fish organism called marine medaka was exposed to K. mikimotoi over the course of three time periods. Two-dimensional gel electrophoresis was used to separate the fish's extracted proteins, and proteins with differential expression were found in comparison to an untreated control. The time-course of exposure led to changes in fish proteomes that were analyzed. 35 difference protein spots encompassing 19 distinct proteins were found in total, and the majority of these spots began to exhibit notable changes in expression levels at the earliest stage of intoxication. Some of the 19 proteins that were shown to exist have a close connection to energy metabolism, muscular contraction, and oxidative stress responses. We suggest that the symptoms that appeared during the ichthyotoxicity test, such as gasping for air, losing balance, and twitching of the body, may have been caused by oxidative stress-mediated muscle injury. Our findings establish the groundwork for in-depth investigations into the processes behind the ichthyotoxicity of K. mikimotoi.

Keywords: Fish Proteome, Harmful Algal Bloom, Ichthyotoxicity, Karenia Mikimotoi, Proteomics, Two-Dimensional Gel Electrophoresis.

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

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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, 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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