Extraction of Mannanase from Bifidobacteria and its Effect On Starvation

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Abstract

Any members of the human gut micro biota including members of the gut micro biota that promote health like bifid bacterium *bifidbacterium spp*. Catabolize manna's for food. Few informationis available in the gut ecological niche regarding the enzymology of man nan deconstruction, below the biochemical properties if the first 5 subfamily 8 glycoside hydrolase (GH5 8) manna n as e derived from the biochemical properties of probiotic. The relationship between gut micro biota and biochemical metabolism to explore the relationship between gut micro biota. In traycomponents and some substances which are produced by the host, the primary intestinal microbe :bacteria and microbial species associated with dietary carbohydrates metabolism, strictly anaerobic and gram positive strain of specified bifid bacteria known as, *B. Lon gum* (60 percent of the sample)was the most commonly identified species followed by *B. bifida* (60 percent of the samples), while *B. Brief (*21 percent).

Keyword*: Mannanase, Bifidbacterium,* Starvation, probiotic, digestive tracts

Introduction

B-mannans are an excess of different structural plant and polysaccharide for storage. Some human man nans are abundant and complex polysaccharides of plant structure and storage. Some members of the human gut micro biota including health promoting members of the gut micro biota *bifid bacteriumspp*. catalyze man nans for food. Catabolize mannans for food, few information is available in the gut ecological niche regarding the enzymology of mannan deconstruction the is dependent on mannan deconstruction enzymology instomach. The biochemical features of probiotic biochemical mannanase of the first family 5 subfamily 8 glycoside hydrolase (GH5) mannanase *bifid bacterium animals bifid bacterium animals subs Lactic lactic*. Microbial manna n a se s are predominantly extracellular and can function in abroad range of industrial such as hydrogen number and temperature, pharmaceutical. Feed, oil and textile. Microbial mannanases have become biotechnologically essentialas they target the hydrolysis of complex plant tissue polysaccharides into pure molecules such as man no –oleo go saccharides and mannoses[1]. from plants and animals. Bacterial mannanases are often extracellular and can function in a wide range of hydrogen number and temperature, but more common are acidic and neutral mannanases. Complex manna structure and the complex of microbial enzymes involved in its complete breakdown, mannanase sources, conditions of growth. We characterized the β- GA lacto man Nan activity mechanism and the β-mannanase supplementation on the gut. The human colonic micro biota is a large and complex population of microbial[1,2].

This linked detaryportion metabolism, the human intestinal micro biota is a natural ecosystem that is now dynamic ecosystem that affects human death and wellbeing[1]. Morethan 400 species within the industrial tract canbe classified and can be classified within the intestinal micro flora as more than 400

species. In colon *Bifid bacterium bifid bacterium* were first discovered in infant feces by tissue who isolated a rare and tissue bacterium in infant feces, who isolated a unusual and distinctive Y-shaped bacterium and called it Y-shaped distinctive, and called it *Bacillus bifid us* [2]. This bacteria is a gram from 1900-1957 such bacteria are pleomorphic gram positive, non-motile, nonsporeform, pleomorphic. Speculate danaerobic bacteria with extremities[3] B*ifid bacterium bifid bacterium are* anaerobic, bacilli belonging to the dominant gut which belongs to the dominants gut micro biota. *Bifid bacterium bifid bacterium* have gained considerable attention in recent years because their association with various health promoting effects of *Bifid bacterium spp*. Isolation recently, bacteria play a crucial role in the digestive tract[4,5]. Probiotic are need to survive in the gastrointestinal microbial ecosystem adherenceto gut epithelium will enhance their ability to survive. Local isolation and detection of strains leads to specific growth potential. Specific *bifid bacterium* strains have a certain probiotic character in various biotopes[6, 7].

Materials and Methods

Isolation:

Bacteria were isolated from the digestive tracts of the bumblebee species Bombs Pascua rum, Bombs Pascua rum and Bombs lapidaries. Worker bumblebees were accumulated in central Bohemia at some point of summer 2006, and their intestinal tracts were weighed and transferred aseptically into tubes containing sterile MRS broth (Oxo id) supplemented with soybean peptone (5 g l−1) and cysteine hydrochloride (0.5 g l−1). The tubes have been flushed with O2-free CO2 and closed with rubber stoppers. The equal broth was used for serial dilutions of all samples. Aliquots (0.1 ml) were plated on TPY agar with mope Racine as described *Propionic bacterium acnes* and representatives of several novel species with incredibly low levels of 16S r R NA gene sequence similarity (92–95%) to the genus *Bifid bacterium* were present in the cultures[8].

Chemical Analysis:

The gross energy contents of feed, excreta, and digest samples were determined on a 0.5-g sample the use of an adiabatic bomb calorimeter with benzoic acid as standard.

The nitrogen contents of feed and digest samples were decided on a 0.25-g sample in a combustion analyzer the use of EDTA as a calibration standard, with crude protein being calculated through multiplying proportion N by acorrection element (6.25).

Proximateanalyses of the CM and eatingregimen samples have been conducted in accordance to through strategies 920.39 (for crude fat), 982.30 E (for total lysine), 975.44 (for reactive lysine), 978.10 (for crude fiber), 973.18 (for impartial detergent fiber and acid detergent fiber and 942.05 (for ash). Hemicellulose content material was calculated as the difference between NDF and ADF.

The impartial detergent insoluble nitrogen used tobe determined through measuring the nitrogen content material of the insoluble fraction received from the NDF assay. The Glico simulate content material of the meal was determined by way of colorimetric analyses the usage of a spectrophotometer according to the technique described via with the use of tetra chloral pall date as the quite particular regent for Glico simulates[9].

Results and Discussion

Mannanase and *bifid bacterium*

Mannans are known to be hydrolyzed by human digestive enzymes, and thus give manna no lytic gut bacteria a possible advantage. The fermentation of gum galactic man nan was demonstrated in the human gut and the ingestion of this polysaccharides partial hydro lysates induced the prolife ration of *bifid bacterium spp*[10]. but end β-1-4 mannanase which hydrolyze the internal β-1-4 linkages of backbone are central in man nan degradation. β- mannanase

are listed in the carbohydrate active enzymes (Casey) database of glycoside hydrolase (GH) families[11] many bacterial mannanase cluster according to a phylogeny based assignment in sub family 8 of GH5 8. GH5 mannanase use a double displacement mechanism with numeric structure retention. Many mannanase contain carbohydrate binding molecule (CBM) that have been assigned various molecules including targeting polysaccharides to enzymes, raising the concentration of local substrate or providing. *Bifid bacterium* animal is subs a probiotic bacterium B.lactis-*04*. In addition, the study proved that *Bifid bacterium* animal is subspp. is the GH5 mannanase retained within *bifid bacterium animal is subspplactic.* Displays optimum catalytic efficiency CBM members had been found to bind to insoluble microcrystalline cellulose. Insoluble mannan [11], the CBM 10 of the enzyme is the first low affinity man nan binding module described and to gather with close counterparts it forms a novel CBM 10 subfamily. The distinct differences in the biochemical properties of his enzyme compared to characterized gut micro biota β-mannanases illustrated the diversity of mannan utilization strategies which will be critical in adapting to a high competitive gut niche.. [12]

Table (1) *bifid bacterium species*. In the intestine of infants and adults (according to Reuter, 1971.[13]

	biotypes Reuter/Mitsuoka	infant	adult
B. bifidum (Tissier, 1900, Orla-Jensen, 1924)	a	•	+
	b	+^{T}	•
B. adolescentis (Reuter, 1963)	a$^{x)}$	•	+^{T}
	b$^{xx)}$	—	+
	c$^{xx)}$	•	+
	d$^{xx)}$	—	(+)
B. longum (Reuter, 1963)	a	•	+
	b	+^{T}	•
B. infantis (Reuter, 1963)	a	+^{T}	—
	b	+	—
B. breve (Reuter, 1963)	a	(+)^{T}	•*
	b	•	—
syn. *B. parvulorum* (Reuter, 1963)	c	•	—

frequency:
+ : frequently
(+) : moderately
• : occasionally
^{T} : including type strain ^{T} of species (Reuter, 1971)
* : also isolated from the human vagina
$^{x)}$: *B. adolescentis* sensu stricto
$^{xx)}$: *B. adolescentis* sensu lato, including *B. dentium* (b?), *B. catenulatum*, *pseudocatenulatum* (c), *B. angulatum* (d) (Scardovi, 1986; Gavini et al., 2001)

Mannanase and carbohydrate metabolism

Carbohydrate molecules are used as compounds in several different types, the outer structures on the cell surface of microorganism spreading the cellular inner and outer environment, the source of energy metabolism that is necessary for energy obtaining (TCA cycle), polymer structure for energy storage(glycogen), and inherited molecules as part of de oxy nucleic acids and ribonucleic acids. In addition , the polymer shape of the carbohydrate molecule modify the structure and stability of the protein molecule to maintain these biological processes, and many different types of modified sugar molecules are required. Nucleotide sugar molecules play one of the most important role for the construction of carbohydrate polymer structure among modified sugar molecules nucleotide sugar, an activated molecule for of sugar, is the sole substratum for polymer structure construction including a variety of sugar molecules. In

Achaea, the moiety GlcNAc is an essential component of the cell surface structure, while in eukaryote the activated molecule is necessary for the synthesis of chitin a component of fungal cell wall. The connector of glycosylate phosphatidylinositol a molecule that attaches a variety of cell surface proteins to the plasma membrane 14, 15.

Carbohydrate metabolism and starvation

Human diet is a complex mixture of cumulatively healthy, interacting components [16]. Mate holism macronutrient energies (carbohydrate, proteins, and lipids) are responsible for the bulk of the human dietary energy. Micronutrients (minerals and vitamins) play a central role in metabolism and tissue –function maintenance. Metabolism includes all the biochemical pathways that species use to synthesize and derive energy from structural and functional constituents. It is usually divide into anabolism, which involves macromolecules such as glycogen, proteins and lipids (triacylglycerol, TAG) and catabolism this involves macromolecular degradation to its simplest precursor: glucose, amino acids, glycerol and fatty acids. The free energy released via catabolic degradation through adenosine triphosphate and nicotine amide adenine diphosphate is used to drive anabolic biosynthesis endergonic processes. Anabolic hormones are pancreatic insulin and pituitary growth hormone (GH), other endocrine secretions (pituitary GH, adrenal cottice tropic hormone and prolactin) and weight gain. Androgens (testosterone) may have an anabolic synthesis of the proteins. Anabolism raises the requirements for all nutrients that (synthesis of glucose from non–glycosides substrates) and stimulation of adipose tissue to release free fatty acids (FFAs) and glycerol by lipolysis (TAG breakdown). Thyroid hormone (tri iodole thyroxin or T3) are catabolic and play a major in deciding the metabolism process over the long term. Acute injury catabolism (infection, surgery or trauma) leads to increased energy and protein breakdown, increasing vitamin and mineral requirements. The enzymes (and their substrates) of this bidirectional metabolism remain best studied in prokaryotic organisms (bacteria) such as *Coli*Importantly both gluconeogenesis and glycerol neo genesis are kata plea roti pathways as they convert cycle anions of citric acid into phosphor Enola pyruvate which is then used to produce either glucose or glucose 3 phosphate. Glucose and Fats are the most relevant energy substrates for most species (including humans) and the primacy of these fuels reflects the intermediate metabolism. Fasting metabolism also involves high levels of lipolysis and Fats through circulating to enable energy utilization. In fact, malnutrition is longer, potentially dangerous, and may result in a let hat outcome. Hunger is an adaptive response to food deprivation involving changes in the senses, cognitions and neuron endocrines. As carbohydrate reserves are rapidly depleted and protein supplies are low, the survival period of hungry people depends more on fat reserves than on muscle mass (obese people can live for many months without eating in clinically supervised weight loss programs). Breakfast, lunch and dinner corresponds to nocturnal fasting (approx. eight hours). Clearly adapted to fastening metabolism ensuring that endogenous substrate and resources are adequately utilized to sustain critical activity. It is characterized by low levels of insulin, high levels of glucagon, hepatic glycol gene lyeis, and gluconeogenesis to control serum glucose levels and cerebral function[17].

Conclusion

Polysaccharide-degrading enzymes are very important in many industrial processes, the study of these enzymes is an important field of research. These enzymes include those which degrade cellulose and hemicellulose two of the main components in plant cell walls. Such enzymes are often composed of two or several separated modules which perform different functions. Carbohydrate- binding modules (CBMs) are frequently present and are known to be important for efficient hydrolysis of cellulose. The binding of the CBM is not directed to the mannan-substrate. However, these results may be a reflection of the tight and complex organization of cellulose

and hemicellulose in the plant cell wall. Such enzyme systems are not only of academic interest but also they have potential biotechnological applications in a wide range of industrial enzyme markets, including food and feed technology, coffee extraction, bioethanol production, slime control agents, pharmaceutical field, pulp and paper industry. Exploitation of biodiversity to provide microorganisms that produce mannanases well suited for their diverse applications is considered to be one of the most promising future alternatives. The presence atypical low affinity CBM, which increases binding to enzyme to soluble mannan while causing minimal decrease in catalytic efficiency as opposed to enzymes with canonical mannan binding modules. These features highlight of catalytic and binding properties to support man Nan binding modules. However, microbes are most potent producers of mannanases and represent the preferred source of enzymes in view of their rapid growth, limited space required for cultivation, and ready accessibility to genetic manipulation. Microbial man Nan as e s have been used recently in the food, feed and detergent industries. Due to the complex nature of the plant cell wall and man nan-based substrates, several issues need to be addressed in order to achieve a better understanding of man nan-degradation. Firstly, the enzyme-polysaccharide interaction of manno side- hydrolases in the degradation of the more complex hetero man nans, like O-acetyl-galactic glycol manan, should be studied in more detail. In particular, the influence of different substituents on the rate of hydrolysis needs to be investigated further. Secondly, a larger comparative study of man nan-degrading enzymes from different enzyme families would possibly reveal any differences or similarities in substrate specificity. In general, improved methods for the separation and detection of polysaccharides and oligosaccharides would be very useful in these types of investigations. A recent trend has involved conducting industrial reactions with enzymes reaped from exotic microorganisms that inhabit hot waters, freezing Arctic waters, saline waters, or extremely acidic or alkaline habitats. The mannanases isolated from extremophiles organisms are likely to mimic some of the unnatural properties of the enzymes that are desirable for their commercial applications. Exploitation of biodiversity to provide microorganisms that produce mannanases well suited for their diverse applications is considered to be one of the most promising future alternatives. The existing knowledge about the structure-function relationship of man nan as e s, in evolving mannanases that were never made in nature and that would meet the requirements of the multitude of mannanase applications.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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