Butyric Acid as a Postbiotic Nutraceutical

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Abstract:

Prebiotics, probiotics and symbiotics are nutraceutical supplements used for promoting human gut microbiome which indirectly supports health by combating free radicals, toxins, contaminants and others. The success of these supplements depends on the microbial colonization and duration of availability in the colon. Such uncertainty can be overcome by postbiotic molecules where the beneficial nutraceutical molecule is synthesized in the fermenter and beneficial molecules are directly delivered without depending on the live status of the microbes in the gut. One such postbiotic molecules like butyric acid is of great interest for researchers due its gastrointestinal tract impact in cancer and inflammatory bowel disease patients.

1 Introduction

The microbiota in human body is considered as a virtual organ (Gill et al, 2006).The presence of microbiota in stomach is very low (0-10%) and gradually increases in the small intestine and reaches very high concentration in the colon. The Gram positive anaerobes are the main constituents of the microbiota present in the colon and amongst it 1000 and 1150 have been identified. The major functions of this microbiota are essential amino acid and vitamins (K, B2, B1, folic acid) synthesis and extraction of energy from components of diet as some are not digestible polysaccharides of plan origin. Also it contributes in the maintenance of intestinal cell wall integrity modulating responses to pathogenic infection, and represents a key factor in the maturation of immune system. Change in the intestinal microbial composition and functions i.e. dysbiosis has been correlated with diabetes, cancer, asthma and others.

Postbiotic molecules are produced by the residential microbes in the gut and can be used directly to treat microbiota dysbiosis with a particular focus on alleviating acute dysbiosis. Supplementation of the molecule that is depleted in a disease (i.e. butyrate) is an emerging approach to strengthen the microbiota. The major three short chain fatty acids produced in colon by Gram-positive anaerobic bacteria belonging to the Firmicutes phylum (*Faecalibacterium prausnitzii, Eubacterium rectale*) are acetic acid, propionic acid, butyric acid in ratio of approximately 60:20:20. Although, butyrate is present in least amount, it is the most important for colonocyte metabolism as 70-90% of butyrate is metabolised by colonocytes as preferred energy source. Other well established roles of butyrate are reduction of inflammation associated with ulcerative colitis and risk of colorectal cancer as anti-cancer agent.

2 Postbiotic Molecules as a New Generation Nutraceuticals

2.1 Prebiotic, Probiotic and Postbiotic Nutraceuticals

Prebiotic is a selectively fermented ingredient that stimulates specific changes, both in the composition and/or activity of the gut microbiota that confers health benefits to the host (Gibson et al, 2004; Roberfroid, 2007). The beneficial prebiotic nutraceuticals available are inulin, FOS (fructo-oligosaccharides), GOS (galacto-oligosaccharides), XOS (xylo-oligosaccharide), IMO (isomalto-oligosaccharides), SOS (soybean-oligosaccharides), galactan raffinose and stachyose, arabinoxylans, lactulose, polydextrose, lactosucrose, lactilol, chitin, and polysaccharides from fungi (Anadón et al, 2016). Although prebiotics helps to improve the health by stimulating the growth of beneficial bacteria but alteration in intestinal miroflora could also result in adverse effects.

Probiotics on the other hand are bacteria with beneficial effect upon colonization in the host colon. It can be classified as inflammatory or anti-inflammatory depending on its capacity to stimulate immune or non-immune cells (Mileti et al, 2009). Development of T-REG cell and preservation of intestinal homeostasis by modulating immune cell is stimulated by probiotics (Mileti et al, 2009; Giacinto, 2005). Currently, 75 bacterial species are listed as 'Generally Recognized As Safe' (GRAS) to be used as probiotics. Amongst these *Lactobacilli* and *Bifidobacteria* are commonly used. Research is advancing in the direction of using genetically modified probiotic organisms to treat certain medical conditions. Thus strict bio-containment measures are required to ensure that the organism does not survive after leaving the body. Due to various constraints of pre- and probiotic nutraceuticals, a new approach has emerged for strengthening the microbiota by using postbiotic molecules.

Postbiotics refers to soluble factors (products or metabolic by-products), secreted by live bacteria, or released after bacterial lysis, such as enzymes, peptides, teichoic acids, peptidoglycan-derived muropeptides, polysaccharides, and organic acids. These postbiotics have drawn attention because of their clear chemical structure, safety dose parameters, long shelf life and the content of various signalling molecules which may have anti-inflammatory, immune modulatory, anti-obesogenic, antihypertensive, hypocholesterolemic, anti-proliferative, and antioxidant activities (Cavallari, et al., 2017). It can improve the host health by improving specific physiological functions without depending on the living status of the microbes (probiotics).

2.2 Butyric Acid for Human Health

Butyric acid is a 4-carbon chain compound of industrial significance. In 2002, 100–500 million pounds of butyric acid was produced in the USA from petroleum and is listed as a high production volume chemical (Jang et al., 2013). Butyric acid, currently is petroleum-derived but bio-based processes are in high demand for applications in food/feed industry as flavors, preservatives, as precursor molecule for biofuels, animal feed and beverages, as an anhydride in cosmetic, plastic and textile fiber industries, and as bioactive compound in pharmaceutical and nutraceutical manufacture (van Immerseel et al., 2005; Zhang et al., 2009).

It is well established the butyrate support barrier function in the gut (Ploger et al, 2012). The damaged colonic defence barrier due to disease can be reinforced by utilizing butyrate molecules that increases production of mucin and anti-microbial peptides in the colon. Thus,

butyrate decreases the intestinal mucosal permeability to toxins and pathogens which may contribute to inflammatory bowel disease (IBD) and increases the expression of tight junction proteins and seals the 'leaky gut'.

The gastrointestinal tract is exposed to wide range of genetoxicants in diet including plant toxins, contaminants or may be produced during cooking. The epithelial cells undergo apoptosis to eliminate the damaged cell by the exposure to dietary carcinogens. The colonic epithelium is a dynamic tissue as apoptosis remove the cell at the top of the crypt and the cell in the lower two third of the colonic crypt proliferate. A disruption of the cell gain through mitosis and cell loss through apoptosis may contribute in colo-rectal carcinogenesis. The examination of physiologically-obtainable butyrate on cell lines derived from colonic adenomas and carcinomas showed that butyrate can induce apoptosis (Hague et al, 1994).Three short chain fatty acids butyrate ,propionate, acetate can induce apoptosis in cell lines with no P53 protein or having mutant P53 (Hague et al 1994,1995). Bcl-2 is a apoptosis inhibitor protein largely confined to the base of the crypt (Hockenbery et al 1991; Hague et al 1994). It is found that in a apoptosis induced condition the level of Bcl-2 protein is decreased when the cells are exposed to butyrate. Butyrate could be targeted to carcinoma cell using tumour-specific monoclonal antibody to deliver a measured dose in a liposome capsule (Otaka et al 1989).

2.3 Production of Butyric Acid

Some *Clostridium* strains are known to produce butyric acid along with acetic acid, lactic acid, acetone, ethanol and butanol by using glycolytic pathway. However, butyric acid production from known strains suffers from low product titer, yield and productivity (Mitchell et al., 2009; Song et al., 2011). The best reported butyrate producing strain is *Clostridium tyrobutyricum* ATCC 25755, obligate anaerobic bacterium which grows at 37°C and pH 6.0. The butyrate yield is 0.33 g/g of glucose (67% of theoretical maximum) and other metabolic end products are acetate, hydrogen and n-butanol (Liu et al., 2006). Amongst thermophiles, *Thermoanaerobacterium thermosaccharolyticum* M18 produces low concentration of butyrate while major metabolic end product is hydrogen (Cao et al., 2014). The *Thermoanaerobacterium* strains are of great interest for many leading research groups due to its wide-range of substrate utilization capability; it utilizes both hexose and pentose sugars which make it a potential candidate for consolidated bioprocessing (CBP) (Taylor et al., 2009). Such a strain *Thermoanaerbacterium* sp RBIIT, ferments hexose and pentose sugars to butyrate, lactate, acetate and n-butanol. Carbon recovery of this strain RBIIT was ~90% where 55% carbon was converted to butyric acid, 21% to lactic acid and 14% to acetic acid (Biswas et al., 2018). This strain is interesting from industrial standpoint due to recovery of almost all carbon as useful industrial products.

Various genetic modifications of the central metabolic pathway for conversion of hexose and pentose to butyrate have improved the titre and productivity. Knockout mutants with pathway deleted for acetate production showed increased butyrate titre in *C. Acetobutylicum* (Liu et al,2006; Zhu et al, 2005). Fibrous-bed bioreactor with immobilized cells of *C. tyrobutyricum* improved butyric acid production compared to suspended cell fermentation on glucose (Najafpour et al, 2006). Though production of butyric acid from xylose is hampered by glucose-mediated catabolic repression, the inhibition of xylose uptake was not observed in fermentation by *C. tyrobutyricum*.

Refactoring redox cofactor regeneration in *Escherichia coli* for high yield biocatalysts to produce butyric acid from glucose is another advance approach to achieve the economic feasibility of butyric acid production. In *E.coli*, a synthetic metabolic pathway was introduced that generated NAD+ from NADH using butyrate as the only electron accepter and enabled high production of butyric acid from glucose. Three heterologous genes *hbd* (3-hydroxybutyryl- CoA dehydrogenase) and *crt* (crotonase) from *C. acetobutylicum*, and *ter* (trans-enoyl-CoA reductse) from *T. denticola* were introduced into *E.coli* to convert acetoacetyl CoA the native intermediate produced by atoB (acyl CoA acetyltrasferase) into butyryl CoA. Finally the butyryl CoA is converted into butyrate by *E. coli* native acyl-CoA thioesterase. The industrial potential of butyric acid production from non-native host is also high.

3 Conclusion

As there is a demand for butyric acid production by microbial fermentation, the major challenge will be to increase its titre and yield on lignocellulosic biomass. Such bio-based production of butyric acid can be cost effect nutraceuticals for maintaining healthy gut microbes for elderly and people suffering from colon cancer.

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