Review Article

MICROBIAL POLYSACCHARIDASES IN COLON SPECIFIC DRUG DELIVERY

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ABSTRACT

Targeting drugs to the colon is one of the contemporary research areas in pharmaceutical sciences. Use of polysaccharide excipients has been the most acceptable approach. Successful delivery of drugs to colon from oral dosage forms based on polysaccharide components and / or coatings is dependent on a battery of polysaccharidases secreted by colonic microflora. Most of the reviews in the subject focus on the available polymers for colon specific drug delivery. We present a systematic account of the bacterial enzymes responsible for colonic release that shall contribute in developing novel drug delivery systems for targeting drugs to the colon. The important polysaccharidases of human colon and their mechanisms of action are dealt with in this review. The use of molecular biology tools in study of colonic microflora and genetic aspects of bacteria responsible for secretion of polysaccharidases have been compiled. The importance of use of microbial polysaccharidases in in-vitro evaluation of colon specific formulations has been discussed.

Keywords: Colon specific drug delivery, Colon targeting, Polysaccharide excipients, Bacterial polysaccharidases.

INTRODUCTION

Delivery of drugs directly to the colon is one of the areas of interest to present day researchers in pharmaceutical sciences. The colon targeted delivery of pharmaceutical drugs is important to achieve localized effect for the treatment of colonic diseases and/or systemic drug delivery for drugs absorbed from colon, for e.g. peptides and proteins. The approach is significant in the delivery of oral insulin and other peptide hormones. The various options available include use of pH sensitive polymers, timed release systems, use of prodrugs and above all use of naturally occurring polysaccharides as carriers of drugs or components of prodrugs[1]. One of the well studied aspects is use of polysaccharides as excipients and components of prodrugs in order to achieve colon specificity [2].

Importance of microbial polysaccharidases in colon specific drug delivery of formulations containing polysaccharide excipients is beyond doubt. Present review presents a compilation of the important polysaccharide excipients, along with their metabolism in human colon, the mechanism of action of the degrading enzymes and genetic aspects of polysaccharide degrading colonic microflora shall help in the improvement in the field of colon targeting. Further, polysaccharidase based in-vitro evaluation systems are few and there is a need of substantial research to develop an ideal and economically feasible system for in-vitro evaluation of orally administered formulations containing polysaccharide excipients to achieve colon specificity.

ACHIEVING COLON SPECIFICITY IN ORAL FORMULATIONS

Colon targeted drug delivery is aimed at delivering bioactive agents for the treatment of colonic diseases and to deliver proteins and peptides to the colon for systemic absorption. The subject has been reviewed [1]. The various strategies presently available to target the release of drugs to colon are namely, formation of prodrug, coating of pH-sensitive polymers, use of colon-specific biodegradable polymers, timed released systems, osmotic systems and pressure controlled delivery systems. The most promising choice is use of polymers that are degradable by colonic bacteria that include inulin, dextran, pectin, guar gum, amylose, chitosan, chondroitin sulphate etc. These polymers are characteristic in having large numbers of derivatizable groups, wide range of molecular weights, varying chemical compositions, low toxicity, biodegradability and high stability. They are also approved as pharmaceutical excipients.

The feasibility of natural polysaccharides as targeting tools of drugs to the colon has been well documented [3]. As compared to the pH-dependent polymers that are used as coating materials to protect the drug from release in gastric environment, exploitation of metabolic activity of the colon is a better alternative to improve the drug carriers' specificity. Azo-reduction, glycosidic bond hydrolysis is common approach and chemically modified polysaccharides can be used as perspectives drug carriers.

The use of herbal excipients in novel drug delivery systems has been supported by [4]. They proposed that natural excipients are non-toxic, less expensive and freely available. The present day excipients are not just inert and cheap vehicles for the drug, they have become essential constituent of the formulation. They have

presented an overview of herbal excipients which are used in conventional dosage forms as well as novel drug delivery systems.

The importance of gums and mucilages as pharmaceutical excipients, widely used natural materials, for conventional and novel dosage forms has been reviewed [5]. These natural materials have advantages over synthetic ones since they are chemically inert, nontoxic, less expensive, biodegradable and widely available. The gums can be modified in different ways to obtain tailor-made materials for drug delivery systems and thus can compete with the available synthetic excipients.

POLYSACCHARIDE EXCIPIENTS

The extensive use of polysaccharides in colon specific drug delivery has been compiled by [6]. The rationale of using polysaccharide based delivery system for colon in the light of presence of large amounts of polysaccharidases in the human colon by the colonic microflora has been stressed. The microbial enzymes, β-D-glucosidase, β-D-galactosidase, amylase, pectinase, xylanase, β-D-xylosidase, dextranase and others ferment the coatings of drug core, embedding of the drug in biodegradable matrix and drug-sachharide conjugates (prodrugs). The polysaccharides used for the purpose include chitosan, pectin, chondroitin sulphate, cyclodextrin, dextrans, guar gum, inulin, amylase and locust bean gum.

The perspectives of biodegradable natural polysaccharides for site specific drug delivery to colon have been discussed [6]. The compounds have to pass to the lower part of the gastrointestinal tract without being degraded in the upper parts as stomach, upper part of small intestine. The role of polysaccharidases and the intestinal enzymatic flora in colon targeting has been extensively dealt with. The article presents overview of various approaches to target drugs to the colon using naturally available polymers, limitations and future developments in this field.

The advantages, limitations and future developments of pectin in formulations for colon specific drug delivery have been dealt by [7]. Pectin can be extracted from low value agricultural wastes as pulp of sugar beets and citrus fruits. It was attempted to shift the focus of pectin application from food industry to one of the excipients of pharmaceutical industry. It was suggested that since pectin is efficiently degraded by colonic microflora, pectin derived matrix can be utilized for controlled as well as colon specific drug delivery. The article elaborates various aspects of pectin based delivery systems covering glation of pectin, calcium pectinate, composites of pectin and other polymers, techniques to use pectin in different drug delivery vehicles and methods for in vitro evaluation of drug release.

The properties of synthesized polysaccharide prodrugs of 5-aminosalicylic acid (ASA) for colon specific delivery have been studied by [8]. Drug release was dependent on both on the property of the polymer and solubility of the prodrugs. The amide prodrug, chitosan-5-ASA did not release ASA in the cecal and colonic simulated fluids. The ester prodrugs, hydroxypropyl cellulose-5-ASA did not release the drug in any of the gastrointestinal contents of rats. Release of 5-ASA from cyclodextrins was higher in cecal and colonic contents as compared to stomach and small intestine. Thus, ester prodrugs with certain solubility could release 5-ASA in the cecal and colonic contents of rat.

The properties and suitability as a colon specific drug delivery excipient of a galactomannan polysaccharide, gaur gum, has been reviewed [9]. The suitability of gaur gum is due to its high viscosity resulting from its high molecular weight (1,000,000) and long polysachharide chain. Each 100 gm gaurgum contains galactomanan 80, water 12, protein 5, acid soluble ash 2 and fat 0.7 g. It consists of high molecular weight hydrocolloidal polysaccharide, composed of galactan and mannan units, combined through glycosidic linkages and is degraded in the large intestine due the presence of microbial enzymes.

A new natural angelica polysaccharide based colon-specific drug delivery system has been reported [10]. The study emphasizes the clinical necessity of colon specific drug delivery systems to treat colon diseases locally to reduce systemic side effects. A dexamethasone-angelina polysaccharide conjugate was synthesized that greatly reduced systemic absorption of the drug thereby overcoming the systemic immuno-suppression caused by dexamethasone. The conjugate was effective in treating ulcerative colitis in rats by gavage and proved to be a promising colon specific drug carrier.

The advancements in drug delivery technology, has redefined excipients currently included in novel dosage forms to fulfill specific functions[2]. These excipients, directly or indirectly influence the extent and/or rate of drug release and absorption. Plant polysaccharides fulfill many requirements expected of pharmaceutical excipients; they are non-toxic, stable, easily available and renewable and hence are extensively investigated for use in the development of solid oral dosage forms. Polysaccharides can be extracted from plants at relatively low cost and can be chemically modified to suit specific needs and are of variable physicochemical properties. Modified release dosage forms can be prepared in which many polysaccharide-rich plant materials are successfully used as matrix formers. Control drug release can be achieved with the help of some natural polysaccharides that show environmental-responsive gelation.

METABOLISM OF POLYSACCHARIDES IN COLON

The profiling of anaerobic bacterial inhabitants of human colon for polysaccharide degradation has been reported[11]. The strains fermented dietary fiber, structures resembling them and glycoprotein mucins. *Bacteroids* and *Bifidiobacterium* were the two genera found capable of

fermenting widest range of polysaccharides. The enzyme activity was cell bound and inducible for several *Bacteroids* species. It was concluded that the metabolic activity of the flora could be altered effectively by the amount and type of fiber in the diet, even though the composition of the flora itself remained unchanged. The fibres were degraded to yield monsaccharides and oligosaccharides of varying chain lengths.

Polysaccharide breakdown by mixed populations of human faecal bacteria has been compiled [12]. Presence of polysaccharide degrading activity in the washed bacterial load has been reported. Various polysaccharidases detected include amylase, pectinase and xylanase as major enzymes and arabinofuranosidase, xylosidase, galaxtosidase, glucosidase in minor quantities. They reported fermentation of starch, pectin, xyalan and arabinogalactan by mixed populations of bacteria found in human feces. They concluded that rate of degradation of polysaccharides was dependent on the solubility of the polymer. Polysaccharidases were mainly associated with cells during the initial phases of fermentation, however, extracellular enzymes accumulated towards the end of fermentation. The bacteria showed complete degradation of polysaccharide and the released sugars. Acetate, propionate and butyrate were produced during degradation of all tested polysaccharides with variation in molar ratios depending on the starting material. Starch did not inhibit the degradation of non starch polysaccharides. The glycosidases, which have the highest activity, are α-L-arabinofuranosidase, β-D-xylosidase, β-D-galactosidase, and β-D-glucosidases.

Fermentation of non digestible oligosaccarides in human colon have been discussed extensively [13]. It has been highlighted by researchers that human colon is a complex microbial ecosystem. The pH of colon is favorable for bacterial growth and the numbers may range in 10^{11} to 10^{12} per gram of gut contents. The members of endogenous flora of large intestine include *Bacteroides, Bifidobacteria, Clostridia, Eubacteria, Lactobacilli, Fusobacteria, Ruminococci, Peptococci, Peptostreptococci, Streptococci*, coliforms, methanogens and dissimilatory sulphate-reducing bacteria. The bacterial growth depends on the undigested carbohydrate and protein remains that reach the intestine. In-vitro studies with faeces have demonstrated that colonic bacteria can ferment starch to form various end products, e.g. short chain fatty acids (SFCA), with butyrate being suggested as clinically significant, and gases. The major starch degraders in the colon are the *Bacteroides*, *Bifidobacteria* and *Eubacteria*.

The degradation of cross-linked and non-cross-linked arabinoxylans (AX) by the intestinal microbiota in children has been reported [14]. The crosslinking of AX was done with ferulic acid to produce AXF and its breakdown by the children's intestinal microbiotas was compared with non-cross-linked AX and easily fermentable starch. The AXF showed slowest fermentation followed by AX and starch which showed fastest rate of fermentation. Starch digestion released acetate and butyrate while AX digestion released propionate. The presence of fermentable carbohydrate significantly increased the total anaerobe counts and eubacterial rRNA concentrations, while non-cross-linked AX digestion increased viable counts of *Bacteroides fragilis* group organisms, which was supported by increases in *Bacteroides-Porphyromonas-Prevotella* group rRNA. Starch was considerably more bifidogenic than AX in these fermentations. In conclusion, in this study we found that the effects of AX and AXF on the microbial ecology and metabolism of intestinal microbiotas are similar in children and adults.

The fermentation pattern in culture of *Bacteroides caccae* supplied with pectin and glucose were studied using a strain KWN isolated from rabbit caecum with an aim to identify enzymes involved in metabolism of pectin [15]. The fermentation patterns, changes in viscosity and enzyme reaction products were studied. Acetate, formate, lactate, fumarate and succinate were the products of pectin metabolism and the enzymes involved were extracellular exopectate, hydrolase, endopectate lyase and cell associated aldolase. Pectinolytic activity was inducible in the cultures.

Studies on cellulytic microflora of the human colon were done by [16] who found microcrystalline cellulose degrading bacteria in methane excreting individuals. Fecal samples of 34 individuals were collected and microcrystalline-cellulose-degrading methanogenc microbial communities quantified in both methane and non-methane excreting subjects. Non methane excretor fecal samples did not show presence of cellulose degraders. The cellulytic isolates were classified to *Ruminococcus* species and *Enterococcus* species closely related to *Enterococcus faecalis*.

Human fecal microorganisms were grown on mixtures of chemically diverse polymerised Carbon sources such as starch, pectin, xylan, mucin, arabinogalactan, inulin, guar gum in a three-stage continuous culture model of the colon [17]. The investigation included the effects of retention time on bacterial populations, their production of polysaccharide degrading enzymes, carbohydrate utilization and short chain fatty acid formation. The communities of bacteria were studied in fermenters that revealed predominance of *Bacteroides*, *Bifidobacteria*, *Clostridia* and other anaerobic Gram positive cocci. The population increase was correspondent to presence of degradable polysaccharide in the growth medium indicating inducible nature of the enzymes.

FACTORS AFFECTING THE BREAKDOWN OF NON-STARCH POLYSACCHARIDES (NSP) IN HUMAN COLON

In man, luminal or mucosal enzymes capable of catalyzing the hydrolysis of non starch polysaccharides (NSP) have not been detected, and it is obvious that the breakdown of NSP is accomplished anaerobically by intestinal microflora (predominantly colonic). Available bacteriological data indicate that a number of NSP-degrading bacteria are present in the human colon [18]. Some of the polysaccharidases produced by colonic

bacteria in humans are extra-cellular; most of the enzymes studied seem to be bound to the bacterial cell wall. Most of the microbial polysaccharide degrading enzymes are inducible.

NSP digestion is more efficient than cellulose digestion in man, some components of non cellulose polysaccharides (NCP) being very extensively degraded in the human gut. An average of more than three fourth of non starch polysaccharides from various sources were degraded by human colonic microflora. The cellulose component of the same fibre sources appears to be less well degraded; only half of the cellulose fraction from various fibre sources was degraded. This is comparable to 42-48% digestibility of cellulose found in man. A number of important physical factors have now been identified that explain the observed differences in the extent of colonic degradation of the different components of fibre. The solubility and pattern of absorption of digestible dietary carbohydrate is dependent on the accessibility of substrate to luminal enzymes. Cellulose being a relatively insoluble material, its rate of breakdown is related to the form and particle size which determines the surface area that is accessible to colonic bacterial polysaccharidases. This is the reason why pure cellulose isolated from wood is more poorly digested than the forms of cellulose in fruit and vegetables. Particle size is dependent on the source and method of processing and hence decides the degree of digestibility of cellulose. Presence of Lignin influences the extent of NSP degradation unfavorably, with high lignification of cell wall leads to poor degradation in human gut. Obviously, NSP in wheat bran is degraded much less than those present in the cell walls of cabbage and apples. Cellulose digestion is a slow process and contact time between substrate and enzyme also affect digestibility. Thus cellulose digestion is more complete the slower the transit time through the colon. Factors that affect colonic transit for example treatment with codeine phosphate and senokot therefore influences digestibility of polysaccharides. The majority of NSP in plant cell walls are in fact NCP. These are chemically very different from cellulose. They have an open chemical structure, are water soluble at the pH of human colon and hence metabolized to a greater extent by colonic bacterial polysaccharides than cellulose.

Some of the water soluble NCP such as pectin are completely degraded in the normal human colon. One of the important factors that affect the degradability of NSP is the activity of the colonic bacterial polysaccaridases. Many of the polysaccharide degrading systems are complex, and involve more than one enzyme. The polysaccharide degrading organisms are capable of producing more than one category of degrading systems and depend on proper induction of the enzyme cascades. The degree of digestibility of a single fibre source increases with time, leading to improved rate of degradation following long term administration of a fibre in diet. Further, antibiotic therapy reduces the activity of colonic bacterial polysaccharidases, hence digestibility of orally administered NSP.

MECHANISM OF POLYSACCHARIDE UTILIZATION BY COLONIC BACTERIA

Most intestinal bacteria are saccharolytic, obtaining carbon and energy by hydrolysis of host and dietary carbohydrate molecules. Polysaccharides from plant fibers, such as cellulose, xylan, arabinogalactan, and pectin, and vegetable starches such as amylose and amylopectin contain glycosidic bonds. *Bacteroides* have been shown to have a variety of glucosidase activities, including α,β-1,3-glucosidase activity responsible for laminarin degradation and a variety of a and β-1,4 and -1,6 xylosidase and glucosidase activities induced by the presence of hemicellulose[13]. It was believed that these enzymes were extracellularly, and the short oligosaccharides and monosaccharides produced by hydrolysis were taken up into the cell for fermentation. Analysis of the *B. thetaiotaomicron* starch utilization system has revealed that the polysaccharides initially bind to an outer membrane receptor system, and pulled into the periplasm for degradation into monosaccharides [19]. The *Bacteroides* use a similar approach for uptake and degradation of chondroitin sulfate. This mechanism helps *Bacteroides* to competitively utilize polysaccharides and sequester them in the periplasm thus protecting them from withdrawal by other intestinal organisms or loss by diffusion. Utilization of chondroitin sulfate by *Bacteroides thetaiotaomicron* is repressed in the presence of glucose, while utilization of other sugars in *B. thetaiotaomicron* is supressed in the presence of mannose. This suggests that *Bacteroides* may have a catabolite repression mechanism to allow for the utilization of some carbon sources in preference to others. However, this system is probably not similar to the catabolite repression systems of enteric bacteria, as the *Bacteroides* do not possess cyclic AMP. It is likely that most polysaccharide utilization systems of *Bacteroides* are controlled by repressor/inducer mechanisms, as *B. ovatus* and *B. thetaiotaomicron* are able to utilize several sugars simultaneously, and several polysaccharide utilization genes have been shown to be activated in the presence of their substrate. Carbohydrate fermentation by the *Bacteroides* and other intestinal bacteria results in the production of a variety of volatile fatty acids, specifically, acetate, propionate (from succinate), and butyrate. These short chain fatty acids are reabsorbed through the large intestine, and utilized by the host as an energy source.

Two new species of *Bacteroides* capable of pectin hydrolysis from the human intestinal tract were isolated[20]. Both the species showed de-esterification of pectin; the pectinesterase being secreted extracellularly by both the species. Both species secreted extracellular polygalactouronate depolymerizing enzymes dependent on calcium for their activity and required alkaline pH for optimal de-polymerization. The exopectate lyase activity had an unusual action pattern that resulted in terminal cleavage of unsaturated trigalacturonic acid units from

polygalacturonate. The major product accumulated in cell free reaction mixtures was the unsaturated trimer.

A mucin fermenting anaerobic bacteria from human feces has been characterized [21]. They found that the organisms isolated by enrichment from human feces were able to grow on arabiongalactan, pectin, xylan, wheat bran, guar, apple cell walls and mucin. The types of bacteria isolated from feces sample exhibiting capacity of fermenting polysaccharides and their properties varied from sample to sample. The isolates showed utilization of more than one type of polysaccharide, though, the capacity to utilize one polymer was not related to the capacity to utilize the other. Some organisms utilized mucin as well, however mucin fermenters showed poor utilization of complex polysaccharides, hence proving the unablility of polysacchrides in the colon in preventing mucin utilization by bacteria.

Herbal excipients in novel drug delivery systems are extensively reviewed [4]. The evolution of excipients from mere inert and cheap vehicles to the active drug in the formulation to an essential constituent of the dosage form ensuring the targeted and time dependent drug delivery has been explained.

The enzymic degradation of plant cell wall by a *Bacteroides* of human fecal origin was studied [22]. The sample of human feces was inoculated on isolated peanut cell walls. Eight isolates were capable of degradation and one of them, identified as *Bacteroides*, was selected for studying the mechanism of degradation. The enzyme was intacellular and greatly repressed by presence of glucose and xylose. There was a six fold increase in the enzyme activity following growth on the said medium which led to 11% release of sugars, predominantly uronic acid and xylose after 18 hr incubation.

GENETIC ANALYSIS OF POLYSACCHARIDASE PRODUCING COLONIC BACTERIA

The molecular characterization of human colonic bacterial species capable of polysaccharide utilization has been area of recent interest. Genomic analysis of gut bacteria exhibiting utilization of polysaccharides was successfully performed [23]. Though the focus of study was to understand the mechanisms of micorbiota involved in polysaccharide degradation so that biotechnological tools are used to convert lignocellulosic biomass into monosaccharides, the study can prove helpful in exploiting the hitherto unexplored area of using lignocelllosic materials as drug carriers.

Genomic analysis of a predominant polysaccharide degrading colonic bacteria *Bacteroides fragalis* and *Bacteroides thetaiotamicron* was done[24]. It was found that there are corresponding differences in the genomes of the two species, based on determination of the genome sequence comparative analysis. The two species are dominant among the colonic microbiota since they have an exceptional capability to use a wide range of dietary polysaccharides by gene amplification and the capacity to create variable surface antigenicities by multiple DNA inversion systems. However, the gene amplification for polysaccharide assimilation is more developed in *Bacteroides thetaiotamicron* as it is found internal in the colon. Both *Bacteroides* species have expanded similar paralogous groups in the genome which include those open reading frames involved in synthesis of proteins for utilization of dietary polysaccharide, mainly the glycosidase enzymes, the binding proteins and transport proteins, environmental sensing and signal transduction (extracytoplasmic function-type sigma factors and their cognate antisigma factors, and one- or two-component signal transduction systems), and capsular polysaccharide biosynthesis. Genes for polysaccharide utilization and environmental sensing are found at adjacent locations in the genome in both the species most probably to favor regulatory coordination of gene expression and nutrient availability.

Gene duplication for polysaccharide utilization is a feature commonly observed in colonic inhabitants. Compared with other sequenced colonic microorganisms such as *Bifidobacterium longum* and *Clostridium perfringens*, the *Bacteroides* species described above contain much larger numbers of polysaccharide-degrading enzymes with a wide range of substrate specificities. A complete assembly of receptor proteins involved in polysaccharide utilization (the Sus family) has been identified in the two *Bacteroides* species. The SusC family of outer membrane proteins constitutes the largest paralogous family in both *Bacteroides* (54 members in *Bacteroides fragalis* and and 79 in *Bacteroides thetaiotamicron*). As seen in *Bacteroides thetaiotamicron*, about half the genes for SusC family members are paired with genes encoding the SusD family of outer membrane proteins. These SusC- and SusD-family proteins are likely to be large group of polysaccharide receptors that help in the binding of a wide range of polysaccharides to the bacterial cell surface followed by their degradation in the periplasmic space. The presence of such polysaccharide utilization system minimizes the diffusion of digested products and makes them less available to the surrounding competitors thereby highly favoring *Bacteroides* in competition for growth in the colon.

Germ-free mice were maintained on polysaccharide-rich or simple-sugar diets and colonized for 10 days with, *Bacteroides thetaiotaomicron*, the normal resident of human colon[25]. After 10 days, whole-genome transcriptional profiling of bacteria and mass spectrometry of cecal glycans was done. These bacteria assembled on food particles and mucus, induced outer-membrane polysaccharide-binding proteins and glycoside hydrolases, consumed liberated hexose sugars on priority basis, and shifted to utilization of host mucus glycans when polysaccharides were absent from the diet. This flexible nature of the colonizing bacteria was supposed to contribute to ecosystem stability and functional diversity in the colon.

The intestine of adult humans is colonized hundreds of species and thousands of subspecies. The population is dominatd by Bacteroidetes and Firmicutes divisions. Genomes of *Bacteroides vulgatus* and *Bacteroides distasonis*, the organisms found normally in the human colon have been sequenced [26]. The niche and habitat adaptations of the bacteria were analyzed after comparison of the sequences with those of other gut and non gut Bacteroidetes. A large scale lateral gene transfer, mobile elements and gene amplification was found to play major role in affecting the ability of the gut Bacteroidetes to colonize the habitat, change their cell surface molecules, sense their environment and utilize the polysaccharide nutrients present in the colon.

B. thetaiotaomicron, a model symbiont and abundant colonizer of human gut has been discussed [27]. A comparison of the glycobiome of *B. thetaiotaomicron* with that of humans shows the bacterium has 226 predicted glycoside hydrolases as against the 98 known or putative glycoside hydrolases in the 2.85 Gb human genome. The starch utilization system (Sus) of the bacterium is a group of eight genes and is a well known example of polysaccharide utilization.

The genome sequences of some abundant human gut *Bacteroides* species has been analyzed and shown that there are some differences in the metabolic capacities of different species [28]. For example, *B. thetaiotaomicron* VPI-8254 could utilize glycans heparin, chondroitin, and hyaluronan derived from the host. Other *Bacteroides* species lacked the necessary enzymes to utilize these molecules. Other *Bacteroides* species, however, have glycolytic capabilities that are not found in *B. thetaiotaomicron*, for example, *B. vulgatus* possesses large complement of enzymes that target pectin. Since human intestine is colonized with many different *Bacteroides* species at the same time, the polysaccharide degradative abilities of the collective *Bacteroides* population within human being are enormous.

The impact of diet on the microbial composition of human colon has been well studied[29]. The study emphasizes the effect of intestinal microflora on human health and association with human diseases. The proliferation of *Bacteroides* in response to the dietary polysaccharide fructan was elucidated. The structural and genetic analysis indicated fructan utilization locus in *Bacteroides thetaiotaomicron* was under control of fructose-binding, hybrid two-component signaling sensor. The genome sequence of the locus varied from species to species in *Bacteroides* genera and is responsible for the specificity of range of fructans utilized by the species. *B. thetaiotaomicron* possess BT 1760, an extracellular β-2-6-endofrutanase responsible for utilization of β-2,6-linked fructan levan. The predominance of species was a result of in vivo competition in presence of dietary fructans. Gene sequences were used to distinguish metabolic capacities of the species and used as potential biomarker in microbiomic datasets and can be utilized to manipulate colonic microflora via human diet.

The genome sequence of *Bifidobacterium longum* was determined[30]. The organism is a key commensal and promotes healthy gastrointestinal tract. The genome of the bacteria is 2.26 Mb circular chromosome with 60% GC content and 1,730 possible coding sequences. Bioinformatic analysis revealed several physiological traits that could partially explain the successful adaptation of these bacteria to the colon. The organism showed large number of the proteins specialized for catabolism of a variety of oligosaccharides, some possibly released by rare or novel glycosyl hydrolases acting on nondigestible plant polymers or host-derived glycoproteins and glycoconjugates. This ability to scavenge from a large variety of nutrients likely contributes to the competitiveness and persistence of bifidobacteria in the colon. Many genes for oligosaccharide metabolism were found in self-regulated modules that appear to have arisen in part from gene duplication or horizontal acquisition.

A multi-step functionally based approach to guide the in-depth pyrosequencing of specific regions of human gut metagenome involved in encoding carbohydrate active enzymes involved in dietary fibre breakdown has been proposed [31]. The human gut microbiome is described as a complex ecosystem consisting of mainly non-culturable bacteria. Clones (310) showing beta-glucanase, hemicellulase, galactanase, amylase, or pectinase activities were isolated after high throughput functional screening to a library covering 5.4×10^9 bp of metagenomic DNA. In the final stages of screening procedure, 0.84 Mb nonredundant metagenomic DNA was sequenced that correspond to 26 clones with efficient degradation of raw plant polysaccharides. Seventy three carbohydrate active enzymes from thirty five different families were discovered which corresponds to five fold enrichment of target gene as compared to random sequencing of human gut metagenome. Comparison of 33 gene sequences with prevalent genes found in the gut microbiome of 20 individuals showed great homology. Eighteen multigenic clusters encoding complementary enzyme activities for plant cell wall degradation were also identified. The results indicated horizontal gene transfer among the dominant gut species and providing new insights into the human gut functional trophic chain.

Characterization of Firmicutes and Bacteroides, the two dominant bacterial phyla present in human gut has been carried out[32]. Finished genome sequences were generated from *Eubacterium rectale* and *E. eligens,* belonging to Clostridium Cluster XIVa of Firmicutes clad, and compared with 25 other gut Firmicutes and Bacteroidetes. The results revealed smaller genomes of Firmicutes and lesser number of glycan hydrolyzing enzymes. Whole-genome transcriptional profiling, high resolution proteomic analysis and biochemical assays of microbial–microbial and microbial-host interactions were performed after successful colonization of germ free mice with *E. rectale* and *Bacteroides thetaiotaomicron. B.*

thetaiotaomicron was found to adapt to *E. rectale* by increasing expression of different polysaccharide utilization loci encoding glycoside hydrolases and by signaling to the host to produce mucosal glycans which can be accessed by *it* but not *E. rectale*. *E. rectal,* in turn, adapts to *B. thetaiotaomicron* by decreasing production of its glycan-degrading enzymes, increasing expression of selected amino acid and sugar transporters, and facilitating glycolysis by reducing levels of NADH, in part via generation of butyrate from acetate, which in turn is used by the gut epithelium.

DRUG RELEASE STUDIES IN PRESENCE OF POLYSACCHARIDASES FROM COLON SPECIFIC ORAL FORMULATIONS

A well known method for in vitro evaluation of guar gum as a carrier for colon specific tablet formulations has been developed [33]. The results proved that guar gum protected the drug from being released completely in the physiological environment of stomach and small intestine. Phosphate buffered saline at pH 6.8 supplemented with rat cecal contents was used for studying the in vitro release of the drug indomethacin from the tablet and presence of caecal contents helped the release of drug. Furthermore, inducing the rats for 7 days with doses of guar gum before collection of caecal contents increased the rate of drug release from the tablets; proving thereby the importance of microbial enzymes in drug delivery. The study also revealed that the use of 4% w/v of rat caecal contents in phosphate buffered saline, obtained after 7 days of enzyme induction provide the best conditions for in vitro evaluation of guar gum containing formulations.

Attempts were made to develop a simulating fluid for in vitro dissolution of colon specific tablets[34]. They used Alternative thioglycollate medium supplemented with rat fecal matter as the dissolution medium with resazurin as an indicator for anaerobiosis. Comparisons were made for tablet dissolution in sterile un-supplemented medium and the developed dissolution medium. The results clearly indicated remarkable increase (from 10% to 80%) in the release of drug in presence of colonic microflora. The organisms were characterized to be *Bacteroides fragilis,* often reported for gaurgum and pectin degradation, the important components of colon specific oral dosage forms.

The biodegradability of gellan gum in presence of galactomannanase was studied to evaluate the suitability of the gum for development of colon specific controlled delivery system[35]. Calcium gelated gellan beads containing azathioprine were prepared and coated with eudragit S-100 to save the beads from gastric release. The release of drug from the uncoated and enteric coated beads was studied in simulated colonic fluid in presence of 15 mg /ml galactomannanase. Scanning electron micrograph was used to study the morphological changes in the structure of uncoated beads. Changes in viscosity of 1% deacetylated gellan gum by increasing concentration and time allowed for action of galactomannanase were studied. The results indicated 10% increase in the release of drug in presence of the enzyme. Enzymic degradation of gellan gum was dependent on enzyme concentration rather than time allowed for enzyme action.

The permeability and swelling properties of casted films of polysaccharide excipients for colon specific coatings has been studied[36]. Inulin oligosaccharide, galactomannan polysaccharides were combined with polymethacrylates on isolated films or film coatings for colonic delivery. The compositions were susceptible to fermentation by colonic microflora. The swelling and permeability of coatings depended on polymethacrylate-polysaccharide concentration ration and on the composition of the dissolution media.

CONCLUSION

Bacterial polysaccharidases secreted in human colon have a definitive role to play in the release of drugs form oral dosage forms containing polysaccharide as excipients and components of pro-drugs. Abundant literature available on the relationship of various polysaccharide degrading bacterial species in the colon along with their genome analysis can be utilized to give new direction to research regarding colon specific delivery of drugs. The data gives insight into interdependence of the colonic bacterial species and their enzyme secretion. Further, the composition of gut microflora can be modified by manipulation of the dietary polysaccharides, thus, opening new horizons for the field of colon targeting of the drugs. Bioinformatics tools have revealed the presence of novel degradative enzymes in the naturally occurring colonic bacteria. The results can be utilized to develop a whole new category of prodrugs and modification of naturally available polysaccharide resources to develop novel and tailor made drug delivery systems to suit the needs of specific subjects or population of subjects.

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