



Health benefits of symbiotics: A review

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ABSTRACT

Prebiotic and probiotics when used simultaneously are termed as symbiotic. Various prebiotics like fructooligosaccharide, inulin, polysaccharides are there in the market also they are obtained from the natural origin like leeks, onions, garlic, aloe-vera, wheat, asparagus, jerusalem artichoke and chicory. Probiotics includes various lactobacillus and bifidobacterium strains and certain yeast like saccharomyces boluradi. These products confer health benefits to the hosts when taken in adequate amounts. Normal dose for probiotics depends upon number of colony formed by the microorganism in the region where it is required to be used. There are certain beneficial effects which includes treatment of various types of diarrhea, hyperlipidemia, irritable bowel syndrome, colon cancers etc.

Keywords: Probiotics, Prebiotics, Lactobacillus, Fructooligosaccharide, Halitosis, Atopic dermatitis.

INTRODUCTION

In the field of medicine various studies were conducted by the scientists in order to improve the health of human beings. Gut micro biota balance is one the approaches over which various scientists are working, since their occur a relationship between the gut micro biota and the health of the human, healthy the gut the person will be automatically healthy, manipulation in the micro biota lead to altered health.

As per WHO, probiotic are defined as the live microorganisms which when taken in adequate amounts confer health benefits to the host. However in certain countries like Japan 10⁷ CFU/g are considered as adequate amounts whereas in USA 10⁸ CFU/g are considered as adequate amounts. But the viability of the probiotic products should be more than 10⁶ -10⁸ CFU/g and in the range of 10⁸-10⁹ CFU/g.

Various species are their which are known to be as probiotics like

- Lactic acid producing bacterium includes *lactobacillus*, *bifidobacterium* and *streptococcus*
- Non-lactic acid-producing bacterial species *Bacillus*, *propionibacterium*
- Nonpathogenic yeasts *Saccharomyces*

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- Nonspore-forming and nonflagellated rod or *coccobacilli*.

There are certain criteria which are required to be fulfilled by the microorganisms to be called as probiotic which includes survival in the host's body, sufficient shelf life upon production and storage should exert health benefits and should be able to be produced in the industrial scale. Probiotics have been shown to be effective against a number of disorders. Some renowned effects are relieving diarrhea, improving lactose intolerance and its immunomodulatory, anticarcinogenic, antidiabetic, hypocholesterolemic, and hypotensive properties etc.

Prebiotics are the substances which are indigestible in the human intestinal tract and they selectively stimulate the growth and activity of probiotics in the gastrointestinal tract (GIT). Prebiotics include fructooligosaccharides (FOS), inulin, galactooligosaccharides (GOS), isomaltooligosaccharides, lactosucrose, glucooligosaccharides, sugar alcohols, and polysaccharides (e.g. resistant and modified starches) that escape digestion in small intestine and are fermented in the caecum and colon by the beneficial intestinal bacteria to further stimulate their numbers and activity. The by-products of such fermentation processes are short-chain fatty acids (SCFA), mainly acetate, propionate and butyrate, which may lead to inhibition of pathogenic bacteria, stimulation of the immune system, prevention of colon cancer, improving mineral absorption, reducing incidence of gastrointestinal diseases and improving blood lipid profiles.¹

2. VARIOUS EFFECTS OF PROBIOTICS

2.1 ANTIMICROBIAL EFFECTS

Introduction of a new microbial strain in the existing bionetwork is difficult, since existing intestinal microbial community is very complex, due to this the newly ingested strains competes with the

existing micro biota, and kills the other pathogenic bacteria. Probiotics upon fermentation produces acids like lactic acid, butyric acid, hippuric acid, and other substances like hydrogen peroxide, bacteriocin which can have antimicrobial properties and thus food borne pathogens and other pathogenic organisms can be killed.²

2.2 ANTIATHEROGENIC AND CHOLESTEROL-LOWERING ATTRIBUTES OF PROBIOTICS

Coronary heart diseases and cardiovascular diseases (CVD), major causes of most death in adults, are conditions in which the main coronary arteries supplying the heart are no longer able to supply sufficient blood and oxygen to the heart muscle (myocardium). Probiotics have the properties to reduce the cholesterol levels which are beneficial in cardiovascular disorders. Probiotics can lower the cholesterol by basically four mechanisms which are as follows

DE-CONJUGATION OF BILE SALTS

Probiotics are capable of producing bile salt hydrolase (BSH) an enzyme which is responsible for the de-conjugation of bile salts. Glycin and taurin are amino acids which are in conjugation with the bile acids to form the bile salts. BSH enzyme hydrolyses glycin or taurin conjugated bile salts and converts them into free amino acid residues and free bile acids. Deconjugated bile salts are less efficiently reabsorbed than its conjugated form. Also free bile acids are less efficient in solubilization and absorption of lipids in the gut, therefore deconjugation of bile salts lead to reduced serum cholesterol either by increasing demand for the synthesis of fresh bile acids or by reducing cholesterol solubility and thereby blocking absorption of cholesterol through intestinal lumen. The excreted bile acid is replaced by the new bile salts formed from cholesterol in the liver.³

CHOLESTEROL ASSIMILATION

It has been reported that the membranes of the growing cells of some probiotic strains can attach to the cholesterol, and some degree of attachment has been observed even on dead cell membranes. Since the probiotics are regularly shed in the faeces this effect results in mopping up of cholesterol from the GIT; however, this effect is highly strain-dependent.³

CONVERSION OF CHOLESTEROL TO 5 β - COPROSTANOL

A new mechanism for cholesterol reduction is conversion of cholesterol to 5 β -coprostanol (5 β -cholestan-3 β -ol) in the intestine. 5 β -Coprostanol is a 27 carbon stanol formed from the bio-hydrogenation of cholesterol in the intestine. It is less soluble than cholesterol and is associated with the solid phase in the gastrointestinal system, thus being directly excreted in the faeces. This eventually leads to a reduced concentration in the physiological cholesterol pool. Possible mechanism of cholesterol conversion into 5 β - coprostanol by intestinal bacteria has been evaluated in different studies and it was found that some bacterial strains such as *sterolibacterium denitrificans* were able to produce cholesterol dehydrogenase, responsible for catalyzing the transformation of cholesterol to cholest-4-en-3-one, an intermediate cofactor in the conversion of cholesterol to 5 β -coprostanol.³

TRANSPORT OF CHOLESTERYL ESTERS

In this mechanism hypocholesterolemic effect is due to the transport of cholesteryl esters via the interrelated pathways of lipid transporters (VLDL, LDL, and HDL), so that the concentration of cholesteryl esters is reduced in the LDL molecules and increased in the HDL molecules, thus HDL cholesterol plays a beneficial role in transporting the cholesterol to the liver for further hydrolysis.⁴

2.3 IMMUNOLOGIC ENHANCEMENT

Probiotics activates the nuclear factor kappa beta (NF- κ B) and thus neutralizes the T-helper cell response and thus stimulates the production of IgA and controls the inflammatory responses and increases the activity of macrophages. These processes are important in the management of irritable bowel syndrome.⁵ Probiotic bacterium attaches to the Toll-like receptors (TLRs) expressed on macrophages, dendritic cells, B cells and epithelial cells this leads to the stimulation of the intestinal lymphoid tissues.⁶ This attachment leads to the productions of cytokines by the immune cells and secretion of the IgA by plasma cells.⁷

2.4 INFECTIOUS DIARRHEA

Infectious diarrhea is a challenging aspect right now is in its treatment and prevention probiotic use is widely accepted. Rotavirus is the main cause of infantile diarrhea and ultimately leads to mortality.⁸ This virus replicates in the small intestinal epithelial cells and thus the normal micro-biota plays an important role in the prevention of this disease. Certain probiotic bacterium like *L. rhamnous GG*, *L. reteri*, *L. casie* etc. have the property to compete with rotavirus and thus can prevent or shortens the duration of infectious diarrhea. The proposed mechanism include competitive blockage of receptor site signals regulating secretory and motility defenses, enhancement of the immune system, and production of substances that can inactivate the virus particles.⁹

2.5 LACTOSE INTOLERANCE

Lactose intolerance occurs due to the deficiency of the enzyme beta-galactosidase resulting in inability to hydrolyse lactose to glucose and galactose. As the lactose reaches into the intestine the undigested lactose is degraded by the bacterial enzymes leading to osmotic diarrhea. Lactose intolerant individuals develops diarrhea, abdominal discomfort and flatulence upon ingestion of the milk or related products. Using certain yoghurt preparations using *S.theromophilus* and *L. delbrueckii* etc. can treat this problem since these have high beta-galactosidase activity.¹⁰

2.6 PROBIOTICS IN CARIES MANAGEMENT

Streptococcus mutans is the main causative organism for the caries and this bacterium has the ability to produce water insoluble glucan and fructans and these leads to the formation of oral bio-films.¹¹ It metabolizes sucrose, fructose and glucose to certain acids which leads to the lowering of the pH of the oral cavity and disturbs the normal bacterial population of the mouth. Certain studies proved that administration of the probiotic can maintain the normal flora of the mouth, probiotic milk containing *L. rhamnous GG* and *L. rhamnous LC* has the ability to reduce the counts of *S. mutans* in the saliva. Those probiotics can be administered in the ice-cream, yoghurt, cheese etc.¹²

2.7 PROBIOTICS AND HALITOSIS

Halitosis or bad breath, is mainly occurs due to the production of volatile sulfur compounds (VSC), predominantly by gram negative anaerobes residing in the periodontal pockets and on the tongue dorsum.¹³ *W. cibaria* is the causative organism for the malodor, use of probiotics can act as a beneficial tool, in certain studies it was found that *S. salivarius* K12 when taken in the lozenges form can reduce the VSC by 85%.¹⁴

2.8 PROBIOTICS AND ORAL YEAST INFECTION

Certain probiotics like *Lactobacillus rhamnosus GG* have the ability to reduce the fungal activity for certain yeasts. *Candida albicans* is the normal inhabitant of the oral cavity and it is the common cause of oral fungal infections. Certain studies on rats proved that this pathogenic potential of *Candida albicans* can be reduced. Hyposalivation was also normalized after the intake of *L. rhamnosus GG* in adequate amounts.¹⁵

2.9 PROBIOTICS FOR ARTHRITIS AND INFLAMMATION

Probiotics can act in both the ways, directly as well indirectly. In case of direct effects they acts on the gastro intestinal tract where they causes modulation of the resident bacterial species and vitamin production, as far indirect effects are concern they acts on joints, lungs and skin and thus they have an effect on immunity thus altering the effect of the inflammatory mediators like cytokines.¹⁶

2.10 PROBIOTICS AND DIABETES

Failure in the secretion of the insulin by pancreas leads to diabetes, diabetes mellitus is a fatal disease ranked 9th in terms of death causing diseases. It is a silent killer. Diabetes mellitus is of two types first is type 1 in which there occurs the destruction of beta cells of pancreas also known as juvenile diabetes and the type 2 occurs due to decreased insulin production and insulin resistance and these are linked with obesity. There are certain medications in the market for the treatment of diabetes but none of them can cure it completely. Certain researches are going on in order to treat diabetes and one of the approaches is the use of symbiotics. Certain research by the Danish people showed the there occurs a relation between the gut micro biota and the metabolic disorders. They used 36 subjects of different ages and different body weights, 18 were diabetic and rest were non diabetic and they came to know that there is a difference in the gut flora of the normal patient and the diabetic patient and they also found that mainly three phyla of bacteria are present in the human gut. These are *firmicutes* (which includes *Lactobacillus* which is a beneficial) others are *proteobacteria* and *bacteroidetes* which are opportunistic and pathogenic. Lower the levels of *firmicutes* and higher the levels of *proteobacteria* and *bacteroidetes*.¹⁷

2.11 UROGENITAL HEALTH CARE

Urogenital infections include urinary tract infections and yeast vaginitis. Nearly about 50 different species of microorganism resides in the vagina of the premenopausal women. *Lactobacillus* is the dominating species in the vaginal of the premenopausal women; however hormonal changes, pH changes and glycogen can alter the flora of the vagina. As the menopause is achieved the *Lactobacillus* count is gradually decreased, however estrogen

therapy can regain its original counts. Abnormal vaginal flora can lead to increased risks of urinary tract infections, lower the levels of *Lactobacillus* lead to UTIs. UTIs generally occurs due to sex with multiple partners and due to use of spermicides, these causes rise in pH of the vagina which ultimately leads to increase in the counts of pathological organism and decreased counts of *Lactobacillus* organism. UTIs occur mainly due to organisms like *Escherichia coli*, *Staphylococcus saprophyticus*, and *Enterococci*. Use of certain *Lactobacilli* strains like *Lactobacillus rhamnosus* and *Lactobacillus fermentum* can treat this problem. A dose of billion live bacteria once in week can treat this problem or either placing a capsule in the vagina can treat this problem.¹⁸

2.12 UPPER RESPIRATORY TRACT INFECTIONS

An upper respiratory tract infection includes inflammation of the larynx and trachea and common cold with symptoms like fever, cough and headaches. Symptomatic relief can be achieved by antipyretics and analgesics.¹⁹ Treatment involves the use of antibiotics; however intake of probiotics can also reduce the incidence of URTs. URTs caused by viruses can last up to 7 days. Instead of using antibiotics for pediatrics we can use probiotics for URTs, several fermented foods containing probiotics can be used like cheese, yoghurt which can reduce the incidence of URIs. Certain lactic acid bacteria and *bifidobacteria* are found to be beneficial.²⁰

2.13 ATOPIC DERMATITIS

Atopic disease consists of asthma, allergic rhinitis and atopic dermatitis and it is generally IgE mediated. However the incidences of atopic disease in developing countries are lower compared to the developed countries. This is because of reduced microbial exposure to the people and improved hygienic conditions in developed countries. Early exposure to microbes is thus essential to counteract the skewed Th2 immune phenotypes of the newborn and the maturation of the immune system to a non atopic state. Imbalance between *bifidobacteria* and *clostridia* is a key factor which triggers allergies, infants on breast feed are having lower incidences of allergies due to higher counts of *bifidobacteria* in their flora.²¹

2.14 TREATMENT OF *HELICOBACTER PYLORI* INFECTION

Helicobacter pylori is spiral shaped bacteria and is a gram negative bacteria which causes the degradation of the mucus lining, which protects the epithelial cells. Certain showed that as probiotics are used against the pathogenic bacteria in the similar way they can be used against the *Helicobacter pylori* induced peptic ulcers, certain in vitro studies proved that the use of certain probiotic strains like *Lactobacilli* and *bifidobacteria* can treat *H.pylori* infection, for example *Lactobacillus salivarius* is capable of producing high amount of lactic acid, which can inhibit the growth of *H. pylori*, however studies also revealed that alone probiotics cannot deal with these infections, so simultaneous use of antibiotics is advised. Probiotics in this case acts by producing antimicrobial substances, stimulating, increasing mucus formation and secretion, strengthening gut barrier function, competing for adhesion sites, stimulating specific and non-specific immune responses etc.²²

2.15 PROBIOTICS AND CANCER PREVENTION

Cancers especially, colon cancer occurs due to consumption of high fat diet, greater consumption of fats causes' excessive secretion of bile acids which in turn imbalances the microbiota of the GIT, bile acids like sodium cholate and sodium chenodeoxycholate are cancer promoters.²³ Studies on rats gives the incidences of chemically induced tumors were reduced upon intake on *L. acidophilus* containing food stuff. A possible mechanism for these anticancer effects relies on inhibiting intestinal bacterial enzymes that convert procarcinogens to more proximal carcinogens.²⁴

Table 1 Different type of Probiotic microbial strains, and their usage.²⁷

Diseases	Probiotic strain
Eczema	<i>Escherichia coli</i> <i>Bifidobacterium bifidum</i> <i>Bifidobacterium lactis</i> <i>Lactococcus lactis</i>
Food allergies Immunity	<i>Escherichia coli</i> <i>Bacillus circulans</i> PB7 <i>Lactobacillus plantarum</i> DSMZ 12028
Antibiotic effect removal	<i>Enterococcus mundtii</i> ST4SA <i>Lactobacillus plantarum</i> 423 <i>Lactobacillus brevis</i> KB290 <i>Lactobacillus strains</i>
Gastroenteritis Therapeutics	<i>Lactobacillus casei</i>
Intestinal Hyperpermeability	<i>Lactobacillus plantarum</i> species 299 (LP299)
Vaginal candidiasis (thrush)	<i>Lactobacillus rhamnosus</i> GR-1 <i>Lactobacillus reuteri</i> RC-14
Urinary tract infection	<i>Lactobacillus rhamnosus</i> GR-1
Lactose intolerance	<i>Lactobacillus acidophilus</i>
Irritable bowel syndrome	<i>Bifidobacterium infantis</i> 35624 <i>Escherichia coli</i> DSM17252 <i>Bifidobacterium infantis</i> 35624
Traveler's diarrhea	<i>Traveler's</i> <i>diarrhea</i>
Lactobacillus GG Lactobacillus plantarum	<i>Lactobacillus GG</i> <i>Lactobacillus plantarum</i>
Radiation-induced diarrhea	<i>Lactobacillus casei</i> DN-114 00
Prevention of colon cancer	<i>Enterococcus faecium</i> M-74 <i>lactic acid bacteria</i>
Ulcerative colitis	<i>Lactobacillus acidophilus</i> <i>Escherichia coli</i> Nissle 1917 <i>Bifidobacterium</i>
Peptic ulcer disease	<i>Lactobacillus acidophilus</i>
Prevention of atopy	<i>Lactobacillus rhamnosus</i> GG
Hypercholesterolemia and cardiovascular diseases	<i>Enterococcus faecium</i> M-74 <i>Lactobacillus plantarum</i> <i>Propionibacterium</i> <i>freudenreichii</i> <i>Lactobacillus plantarum</i> PH04

Certain studies on rats suggested that pathogenic bacterium in the intestine have certain enzymes like glucuronidase, nitroreductase and azoreductase which can convert pro-carcinogens to carcinogens and thus pathogenic bacterium are cancer promoter. But consumption of certain probiotic supplements can act prophylactically. *Lactobacilli gesseri* was found to be effective,

regular consumption lead to reduced levels of *staphylococcus* strain and p-cresol levels.²⁵

Several mechanisms by which probiotics act are preventing the growth of bacteria which can convert the pro-carcinogens to carcinogens, production of tumor necrosis factor by macrophages, inhibition of tumor growth by metabolites of *lactobacilli* stimulating the immune system.²⁶

3. PREBIOTICS

Prebiotics are the substances which are not digested in the human gastrointestinal tract and thus the probiotics feed on them and fermentation of the prebiotic occurs which leads to the formation of SCFAs (short chain fatty acids), they promote the growth of both the existing microflora and the ingested probiotics. Following are the examples of prebiotics.

3.1 FRUCTANS

A fructan is composed of fructose polymers which are generally linked to the moiety of a terminal glucose. Inulin and fructooligosaccharide are the examples.

INULIN

Inulin is a naturally occurring storage carbohydrate commonly found in leeks, onions, wheat, asparagus, garlic, Jerusalem artichoke and chicory. Commercially it is obtained from Jerusalem artichoke and chicory.²⁸ Regular intake of inulin proves to be beneficial by promoting the digestive health, improving lipid metabolism, increases the calcium absorption and thus treating the osteoporosis and reduces the chances of certain tumors of colon, breast cancers and other tumors.²⁹

FRUCTO-OLIGOSACCHARIDES

Fructo-oligosaccharides (FOS) are composed of a mixture of oligosaccharides consisting of glucose linked to fructose units by β -(1,2) links with a degree of polymerization (DP) between 1 and 5. FOS is obtained from several plants like asparagus, wheat, Jerusalem artichokes, and rye, onion contains around 40 % of FOS. FOS is produced enzymatically by the fungal enzyme fructosyltransferase.³⁰ In colon FOS is fermented to lactate, SCFA (acetate, propionate and butyrate), and gas. FOSs has the property to stimulate the growth of *bifidobacterium* and reduce the populations of pathogenic bacteria. Due to production of butyrate they also reduce the development of colon tumor cells and enhance the immune system. FOSs also aids in the absorption of magnesium ion. Production of SCFAs leads to decrease in fecal pH, thus decrease in the populations of colonic bacterium.³¹

3.2 GALACTO-OLIGOSACCHARIDES

Galacto-oligosaccharide (GOS) is classified under complex carbohydrates which consists of oligo-galactose with some lactose and glucose and it is produced from lactose by β -galactosidase. GOS is present in human milk and thus it protects the infants from pathogenic bacteria and also they promote the growth of *bifidobacteria* and other anaerobic bacteria.³² GOS is stable at high temperature and in acidic conditions and it also has many food applications like in the dairy products, sauces, soups, ice creams, bakery products etc. Also it is used as a sugar supplement in various food products.³³

3.3 LACTULOSE

Lactulose is a non-absorbable sugar which is used to treat constipation and hepatic encephalopathy, lactulose is a synthetic disaccharide which upon fermentation produces SCFAs which decrease the pH of the colon and thus the hepatic failure can be treated in which blood urea level is high.³⁴ Intake of lactulose increases the count of bifidobacteria and decreases the counts of *Clostridium perfringens* and *Bacteroidaceae* decreased and thus fecal indole, skatol and phenols, and fecal P-glucuronidase, nitroreductase and azoreductase activities were also decreased significantly.³⁵

3.4 XYLO-OLIGOSACCHARIDES

Xylo-oligosaccharides (XOS) are chains of xylose molecules linked by β 1-4 bonds which are produced enzymatically by hydrolysis of xylan from birch wood, oats, or corn cobs.³⁶ They selectively stimulate the growth of *Bifidobacteria* as evidenced in animal studies. Several studies in humans increase the *bifidobacterium* and thus butyrate concentration also increases α -glucosidase and β -glucuronidase activities and decrease the concentrations of acetate and *p*-cresol, showing a clear prebiotic effect.³⁷

4. FORMULATION OF PROBIOTICS

Probiotic delivery to the targeted organ is the matter of concern; probiotics can be administered in the form of yoghurt, cheese, in certain dosage form like powder, tablet, capsule, etc. In tablets probiotics can be given by using suitable polymers like sodium alginate, hyperomellose phthalate, hyperomellose acetyl succinate. These polymers can protect the bacterium from the harsh conditions of the stomach. Sodium alginate swells when it comes in contact with the acidic pH of the stomach due to the formation of alginic acid due to this it does not allow the water to come in contact with the formulation. Use of HPMC-AS or HPMC-P, increases the acid tolerance capacity of the formulation, since these are matrix forming enteric coating agents thus it is suitable in the formulation. Use of hydrophilic excipients should be avoided it decreases the viability for example use of primojel as disintegrant decreases the viability. Sodium alginate beads can also be prepared in which probiotics can be encapsulated but they are less stable upon storage, milk powder can be used which shows the highest viability after tableting but still when it comes in contact with the acidic medium the viability reduces. Tableting pressure is also an important parameter in the determination of the viability, higher the pressure lesser will be the viability.

Excipients used in probiotic formulation

Polymers obtained from biological sources are used for the encapsulation of the probiotics, polymers like alginates and pectin are used for encapsulation.³⁸

4.1 ALGINATES

Chemically they are linear un-branched polysaccharides containing assorted proportions of b- D mannuronic acid (M) and a-L-guluronic acid (G) residues. The M and G monomers are linked by

1-4 glycosidic bonds. The viscosity of gel formed by the alginates depends over the G/M ratio, G residues have higher affinity for the ions compared to the M residues. The alginates

having higher content of a-L-guluronic acid residues can tolerate the harsh conditions of the stomach better compared to the alginates having higher content of b- D mannuronic acid. Alginates can also be used as a binder and disintegrant in the tablet formulations. In presence of aqueous medium, it rapidly forms gel which can act as a diffusion barrier for probiotics. Alginates are also used as food and beverage industries as thickening, gel-forming, and colloidal stabilizing agents.³⁸

4.2 PECTIN

Pectin is also an important biopolymer which is used in probiotic formulations, chemically it is linear 1, 4-a-linked D-galactopyranosyl uronic acid chain, it is partially esterified polygalacturonide, and also contains arabinose, galactose, or sugars. It is soluble in water thus used in food industry as a stabilizer and gelling agent in various food products like jams, fruit jellies, yogurt drinks, etc.³⁸

4.3 HYPEROMELLOSE PHTHALATE

An enteric coating material is used to prevent the core formulation from the degradation by the gastric acid or to prevent the gastric irritation which is caused by certain drugs. HPMC-P is a cellulose derivative and chemically it is phthalic half ester of hydroxypropyl methylcellulose and the release of probiotics is controlled by varying the phthalyl content. There are two types of HPMC-P available according to their solubility characteristics which are HP-55 and HP-50, moreover HP-55S, which is a type of HPMC-55 is also available which is having higher molecular weight, higher film strength and higher resistance to simulated gastric fluid. HPMC-P is also used in sustain release formulations. Solvents used to dissolve the HPMC-P are methylene chloride/ethanol, acetone/ethanol (1:1 by weight), ethanol/water (8:2 for HP-55, 8.5:1.5 for HP-55S, 7:3 for HP-50 by weight). Pigments such as titanium dioxide and lakes are usually used. Plasticizers like triethyl citrate is effective, but other plasticizers including polyethylene glycol, cetanol, fats and oils such as olive oil, castor oil and monoglycerides of fatty acids can also be used, alone or in combination.³⁹

4.4 HYPEROMELLOSE ACETATE SUCCINATE

Pure cellulose when treated with chloromethane and propylene oxide gives HPMC, and when this HPMC is treated with acetic anhydride and succinic anhydride it gives hyperomellose acetyl succinate. Its chemical name is cellulose, 2-hydroxypropyl methyl ether, acetate, hydrogen butanedioate and it is soluble in various organic solvents like acetone, ethanol, methanol, diethyl ether and also soluble in water ethanol mixtures. It is having pH dependent solubility in the gastrointestinal tract it is soluble at the pH range of (5- 5.5). Hyperomellose have certain applications like conventional aqueous dispersion coating, aqueous dispersion coating using "Concentric Dual-Feed Spray Nozzle", Solvent-Based Coating, Ammonia-Neutralized Coating, dry coating and also in the preparation of solid dispersion.³⁹

5. EVALUATION PARAMETERS OF PROBIOTIC FORMULATIONS

WATER ACTIVITY

Water activity or a_w is the partial vapor pressure of water in a substance divided by the standard state partial vapor pressure of water. In the field of food science, the standard state is most often defined as the partial vapor pressure of pure water at the same temperature. Using this particular definition, pure distilled water has a water activity of exactly one. For a stable formulation the water activity should be low, ingredients which are hydrophilic in nature causes incorporation of water in the formulation and thus increases the water activity and the stability is decreased.⁴⁰

5.2 MOISTURE DETERMINATION

Moisture determination is an important parameter, viability of bacterium decreases if moisture is present in the formulation moreover excipients which can gain upon storage are required to be avoided in the formulation. For the determination of moisture initially weighed quantity of tablet (5g) (W_1) was taken and crushed to get powder and then it is taken on a petri dish (W_2). The petri dish is kept in hot air oven for 5 hours at 110°C and then cooled and reweighed.

Percentage moisture was calculated as

$$\text{Moisture (\%)} = (W_1 - W_3) / (W_1 - W_2) \cdot 41$$

WATER HOLDING CAPACITY

In this study 1g of sample is added to the centrifuge tube of 15 ml and then 10 ml of water is added to it then it is vortexed for 2 minutes. After the complete wetting the samples were allowed to stand for 30 minutes at room temperature and then centrifuged for 3000g for 20 minutes. The supernatant was decanted and the centrifuged and weighed. Water holding capacity was calculated by the formula:

$$\text{WHC} = (W_2 - W_1) / W_0$$

W_0 = weight of dry sample

W_1 = weight of dry sample and tube

W_2 = weight of tube and sediment.⁴¹

VIABILITY DETERMINATION FOR *L. PLANTARUM* AFTER TABLETING

General requirement that the bacterial should colonize is that it should be present in viable form; if a tablet contains 10⁹ CFU then it can show its beneficial effect in the gut. In order to check whether the bacterium can survive the tableting pressure or not viability studies are carried out. For this after the compression of the tablet the tablet is broken and is dispersed in 100ml of phosphate buffer pH 6.8. Serial dilution of this solution is carried out in 0.9 % sodium chloride until a suitable cell density is obtained and then it is spread over MRS agar plates in triplicates. These plates are then incubated for 48 hours at 30° C supplemented with thioglycolic acid to maintain anaerobic conditions.

Survival = number of cells after exposure to the medium / number of cells before exposure.⁴⁰

VIABILITY IN ACIDIC MEDIUM

In order to check that the bacterium can survive the harsh conditions of the stomach the viability is determined after the tablet is exposed to the acidic pH. For this the tablet is placed in the acidic

medium at pH 1 for 2 hours and then it is taken and dissolved in the phosphate buffer having pH 6.8, from this solution serial dilutions were prepared in 0.9% sodium chloride which can give 30-300 CFU/ tablet when spread over MRS agar media.⁴⁰

6. CONCLUSION

There is enough evidences available through which we can predict that the probiotics are multifunctional they can be used for many disorders like cancer, irritable bowel syndrome, hypercholesterolemia etc, also use of prebiotics also increase the efficacy of probiotics and prebiotics alone exerts their own beneficial affects to the host. But the stability is an important issue, moisture penetration; high tableting pressure and acidic pH are the reasons for the decreased viability.

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