Rapid growth and development of probiotics biology essay

Science, Biology

" as organisms and substances which contribute to intestinal microbial balance" by Parker 1974Fuller 1989 redefined probiotics as " a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance." [Azizpour K et al, 2009]Past research has found probiotics capable of delivering a number of health benefits, which include:

treat various forms of gastroenteritis

help lactose intolerant individuals

anti-mutagenic effects

lower serum cholesterol levels

reductions in blood pressure

may affect pathogens by means of competitive inhibition

may improve immune function

improved some symptoms of irritable bowel syndromeProbiotics are currently available in 3 main forms; capsule form – powder encased in gelatine capsule, powder form – powder is bottled or placed in plastic container and liquid form – centrifuged live bacteria is mixed with S. thermophilus and L. delbrueckii to achieve the desired flavour and texture [Saarela M et al, 2000] and other bacteria used to make yoghurt all undergo fermentation together following normal yogurt production procedures. The concept of probiotics has been around for more than a century as in the early 20th century scientists begun focusing their attention on the gut and the begun studying the benefits lactic acid bacteria could have on the human gut through various gut ecology studies. In 1908 Metchnikoff a respected Russian biologist, zoologist and protozoologist was one of the first

scientists to publicly support the health benefits of lactic acid bacteria in fermentated milk and fermentated milk products, this came after Metchnikoff had observed Bulgarian peasants and noticed that they had longer lives due which Metchnikoff proposed was due to the peasants high intake of fermentated milk & milk products [Elavarasu S et al, 2012]. Metchnikoff however thought that microbes in the gut were harmful rather than helpful to human health and considered the substitution of the microbes with lactic acid bacteria to be beneficial and because of this idea is why Metchnikoff is now considered by most as the first scientist to have formulated the idea of probiotics [Azizpour K et al, 2009]. In the present day there is a wide range of probiotics which mainly contain bacteria from the following families: Lactobaccillacaeae familyBifidobacteriaceae familyEnterobacteriaceae familyThe lactobaccillacaeae family has the generas Lactobacillus, Paralactobacillus and Pediococcus. Lactobacillus bacteria are used in probiotics as they belong to a family of lactic acid bacteria which have the ability to make ATP using oxygen (aerobic respiration) or fermentation anaerobic disgestion) depending on the environment, these bacteria are known as facultative anaerobic bacteria generating ATP by non-oxidative substrate-level phosphorylation. Lactobacillus has a rod shape and is gram positive. The bacteria is most commonly found in the gut and vagina, although it has been found in other regions [Sonenshein A et al 1993]. The Bifidobacteriaceae family contains the generas Aeriscardovia, Bifidobacterium, Alloscardovia, Gardnerella, Metascardovia, Parascardovia, Scardovia but the Bifidobacterium bacteria is of interest in probiotics. The Bifidobacterium bacteria is gram positive, anaerobic and non

motile bacteria, they are found in gut flora as one of the major genera that makes up the flora and are also found in the mouth and vagina [Sonenshein A et al 1993]. The Enterobacteriaceae family has an extensive genera of over 20 species, the Escherichia coli bacteria are used in probiotics but only a few strains as most E. coli strains are pathogenic. E. coli has a rod shape and is gram negative most commmonly found in the lower intestines [Sonenshein A et al 1993][World of enzymes & probiotics, 2013]. Other bacteria used in probiotics include: Streptococcus thermophilus is gram positive, non motile and a facultative anaerobe from the Streptococcaceae family. Enterococcus faecium is gram positive and alpha hemolytic or non hemolytic often appearing in pairs or short chains easily confused with streptococci. Enterococcus faecium is from the Enterococcus family of bacteria found in the gut. All the bacteria mentioned above are all of human origin and can be found in the body at anytime, this is crucial for the effectiveness of probiotics because for the bacteria to benefit the carrier it must initially survive the harsh conditions of the gut. There are many more traits/characteristics required from a bacterium for it to be used in probiotics; these traits include functional traits and safety traits, which are summarised in the table below:

Trait/Characteristic

Reason

Good sensory propertiesThe finished product i. e. yoghurt for example must still have a nice texture, smell, flavour even though it contains bacteria [Saarela M et al, 2000]. Phage resistanceThe bacteria must be able to withstand macrophages which might attack the bacteria. Viability during

processingThat the bacteria can survive the processing stage as the conditions maybe unfavourable depending once again on the desired delivery method of the bacteria into the carrier [Saarela M et al, 2000]. Stability in the product and during storageThe bacteria does not produce any toxic substances and can actually survive during storage and can that it does not produce any harmful substances in the product [Saarela M et al, 2000]. Acid toleranceThe bacteria can survive in the gut as mentioned beforeBile toleranceThe probiotic bacteria can survive in the small intestinesAdherence to epithelial surfaces in the gutAdherent strains of probiotic bacteria have a greater chance of staying in the gut for longer so increasing the chance of the bacteria showing its beneficial effects. However it could also be argued that strong adhesion increases the chance of infection and it should be noted that some probiotics have poor adhesive properties but still have positive effects in carriers. Immunostimulation without a proinflammatory effectThis increases the presence of cells involved in immune responses in the body at any time. Antagonistic activity against pathogens such as Helicobacter pyloriFor probiotics to exert their benefits on the carrier the bacteria must be antagonistic activity against pathogenic bacteria in the gut by either an excretory substance from the probiotic bacteria with anti microbial activity or competitive exclusion [Saarela M et al, 2000]. Non pathogenic history or association with any diseasesThe probiotic bacteria may mutate and then become pathogenic. Not deconjugate bile saltsBile salts are harmful in the small intestines (Marteau et al, 1995). Not carry transmissible antibiotic resistance genesAs these genes can be incorporated in the hosts genome with harmful effects [Saarela M et al, 2000]. However to date there has been

little agreement on the health benefits probiotics confer on the consumer between Food Standard Agencies (FSA) and probiotic manufacturers as numerous claims have been rejected by FSAs worldwide http://www. nutrafoods. eu/Detail. aspx? id= 79 in the past so limiting further growth of probiotics. The aim of this review will be to highlight the interactions between the host, gut flora and probiotic bacteria and the resulting health benefits from the interactions, to allow consumers to make their own judgement whether or not probiotics are beneficial. This paper will first start by describing the gastrointestinal tract, followed by a description of the roles gut flora have in the body, then followed by details of how the composition and quantity of the gut flora is determined, probiotic benefits and mechanisms of action and then end by discussing the potential benefits of probiotics. Probiotics main target area after ingestion is the gastrointestinal tract where they exert most of their health benefits from the gut. The gastrointestinal tract is composed of the stomach and the intestines, large and small. http://pepticulcersociety. files. wordpress. com/2010/12/gastrointestinal_tract. jpgImage taken from http://pepticulcersociety. files. wordpress. com/2010/12/gastrointestinal_tract. jpgThe stomach is a harsh environment as it is lined with gastric glands which secrete ~ 400–800 ml of acidic gastric juice (composed of mucus, pepsin, hydrochloric acid, potassium chloride and sodium chloride) at each meal. The glands are composed of parietal cells which secrete hydrochloric acid, chief cells which secrete pepsinogen an enzyme that breaks down proteins, mucus secreting cells and hormone secreting cells [Kimball J. W, 1994]. However the acidic conditions are

necessary for the activation of the digestive enzymes in the stomach which break down food for further digestion in the small intestines. The small intestine is more alkaline than the stomach; it is also the site of further digestion of food coming in the form of chyme from the stomach. The small intestine is lined with villi which are each covered with microvilli, which increase the intestine's surface area and facilitate in the absorption of disgested food. Microvilli also contain enzymes in their plasma membrane which carry out further digestion before absorption [Kimball J. W, 1994]. At the base of the villi are crypts that contain stem cells that are continuously dividing by mitosis producing: more stem cellsPaneth cells - secrete antimicrobial peptides that sterilize the contents of the small intestine. Whilst some of the stem cells migrate up the surface of the villus and differentiate into: goblet cells- secrete mucusendocrine cells- secrete a variety of hormonescolumnar epithelial cells - responsible for digestion and absorption [Kimball J. W, 1994]http://www. umb. no/img_cache/full/2729. jpgImage taken from http://www. umb. no/ikbm/artikkel/projectsThe large intestine receives the liquid residue after digestion and absorption in the small intestine. The residue is mostly composed of water as well as any compounds that were not digested in the small intestine. The large intestines main role is to reabsorb water to prevent dehydration [Kimball J. W, 1994]. The gastrointestinal tract is also home to millions of microorganisms such as bacteria, archaea & eukarya, separated from the internal environment by a single layer of epithelial cells [O'Hara AM & Shanahan F, 2006], collectively known as gut flora. The gut flora is composed of harmless microbes as most of the species that inhabit the gut are non pathogenic. The gut flora

population outnumbers the total number of cells in our body, bacteria are predominant in the population of microorganisms in the gastrointestinal tract and the ratio of anaerobes to aerobes is lower at the mucosal surfaces than in the lumen [O'Hara AM & Shanahan F, 2006]. The colon has the largest density of bacteria as conditions are not as harsh in comparison to the stomach and the small intestine. Bacteria flourish to such an extent that as much as 60% of the dry weight of the faeces may consist of bacterial cells [Kimball J. W, 1994]. Gut flora have various beneficial roles in the hosts' gastrointestinal tract displaying a form of mutualism as the flora and the host have a resource-resource relationship where the host supple the flora with food and the flora assist in the breaking down of certain foods that the host is incapable of breaking down on its own & provide compounds such as vitamins that the host cannot synthesize without the flora. For example vitamin K involved in bone metabolism M. J. Shearer, PhD was found in the gut in its storage forms, known as menaquinones (MK-n, M stands for menaquinone, K stands for vitamin K, and the n represents the number of isoprenoid side chain residues), after quantitative reverse phase high performance liquid chromatographic (HPLC) analysis of the distal colonic contents of 10 male volunteers Conly JM, Stein K.. The menaquinones were synthesised by the gut flora; for example Bacteroides produce MK-10 and MK-11, and MK-6 is produced by Eubacterium lentum http://www. fao. org/docrep/004/Y2809E/y2809e0g. htm#bm16. 5. Further studies then found menaquinones in the liver in high concentrations suggesting that they originated from the gut flora as they were in similar relative proportions and inter-individual variations to those found in the intestine. Conly JM, Stein K. In

another study vitamin-B12 production by bacteria in the small intestines was measured in people in India using a Euglena gracilis Z assay [Albert et al, 1980]. The results showed that some active B12 was indeed produced by bacteria, Klebsiella and Pseudomonas genera, and the results were confirmed using an Ochromonas malhamensis assay which is specific for B12 [Albert et al, 1980]. The flora is involved in:

Development of cell and tissue

Colonic flora secretes butyrate, a fatty acid, which has a regulatory role in cell growth and differentiation as it inhibits the transformation of cell growth and helps stop uncontrolled cell division which would result in neoplasia [Salminen S et al, 1998]. The development of the new blood vessels from older vessels in the intestinal villi is dependent on the gut flora, demonstrated using germ-free mice and ex germ free mice colonized by Bacteroides thetaiotaomicron & lacking Paneth cells which normally produce anti microbial peptides [Stappenbeck TS et al, 2002]. The villi from the mice were then compared 7 days after the ex germ mice were colonized by the bacteria (shown below) and clearly the villi from the mice colonized by Bacteroides thetaiotaomicron had better developedhealthier vessels in comparison to villi from germ free mice [Stappenbeck TS et al, 2002]. Image taken from Stappenbeck TS et al, 2002An external file that holds a picture, illustration, etc. Object name is pq2426042003. jpg Object name is pq2426042003. jpg An external file that holds a picture, illustration, etc. Object name is pq2426042003. jpg Object name is pq2426042003. jpgImages show villi where the green colour represents the vessels, Image A

shows villi from germ free mice without paneth cells, Image C shows villi from mice colonized by B. thetaiotaomicron also without paneth cells.

Maintanance of the intestinal structure

The intestinal mucus layer is a result of the balance between mucin secretion and degradation, the layer creates a barrier for proinflammatory compounds and antigens [Kleessen B & Blaut M, 2005]. It has been shown that butyrate induces secretion of antimicrobial peptides, mucin and other factors which together create a reinforced barrier in the colon but more in vivo human studies are necessary to fully understand the effects of butyrate [Hamer HM et al, 2008]

Metabolic Functions

Flora in the gut produce a number of products which humans are incapable of synthesizing and breakdown compounds which we cannot [Vyas U & Ranganathan N, 2012]. The gut bacteria are known to synthesize a large number vitamins i. e. vitamin B12, and carry out the biotransformation of bile which is important for the metabolism of glucose and cholesterol [Vyas U & Ranganathan N, 2012]. The bacterial species also provide pathways for the fermentation of non digestible substrates like fibres and endogenous mucus producing short chain fatty acids, absorption of the short chain fatty acids stimulates the absorption of water and other salts. The principal short chain fatty acid produced in the colon is acetate and is used by the body as a substrate for the biosynthesis of cholesterol. The flora is also known to hydrolyse glycosides & glucuronides and metabolise ammonia [Vyas U & Ranganathan N, 2012].

Protective Functions

The microorganisms that make up the gut flora produce antimicrobial compounds i. e. bacteriocins, and provide competition for pathogens attempting to colonize the gastrointestinal tract as they compete for nutrients and attachment sites in the lining so reducing the production of lipopolysaccharides and peptidoglycans which can be harmful to the host [Tlaskova-Hogenova H et al, 2004]. In an experiment mesenteric lymph node dendritic cells were from germ free mice were compared to mesenteric lymph node dendritic cells from conventional mice and were found to be less stimulatory for T cells in comparison to conventional mice which had gut flora. This data implied that the presence of gut flora enhanced stimulatory powers of the dendritic cells [Stagg AJ et al, 2007]. Gastrointestinal tract bacteria have been implicated in the development of regulatory T cells i. e. T helper type 1 and 2 cells, which are dependent on the signals given by the bacteria experiments with mice showed colonization by Clostridium strains resulted in the increased expression of Foxp3 transcription factor, TGF-β and T cells [Atarashi k et al, 2011]. Administration of Clostriduim in early life was shown to resulted in resistance to colitis and systemic immunoglobin E responses in adult mice [Atarashi k et al, 2011]. Although this has no direct link to how the same strain would react in humans it is a promising development as it proves that bacteria are capable of delivering benefits [Atarashi k et al, 2011]. The short-chain fatty acids produced by the bacteria have been shown to inhibit NF-kB activation in patients with ulcerative colitis thus the bacteria have immunomodulatory effects [Vyas U & Ranganathan N, 2012] in an experiment where 11 patients with distal ulcerative colitis were

treated for up to 8 weeks with butyrate at 100mM or placebo enaemas. At four and eight weeks the status of disease was noted, specifically grading inflammation and detecting the presence of NF-kappaB (p65) and NF-kappaB activation by double staining with antibodies against them [Lührs H et al, 2002]. In patients that received the placebo NF-kappaB was found to be activated in most of the macrophages whilst patients that underwent butyrate treatment had a significant decrease in activated NF-kappaB in macrophages. These results were concurrent with a major reduction in the Disease Activity Index [Lührs H et al, 2002]. The gut flora does not spontaneously appear in the gastrointestinal tract as babies are germ free whilst inside the womb but acquire their intestistinal bacteria during delivery from the mother air and skin surface [Dominguez-Bello MG et al, 2010]. It was also discovered that babies born by C-section have a different bacterial composition to vaginally delivered babies using multiplexed 16S rRNA gene pyrosequencing to characterize bacterial communities [Dominguez-Bello MG et al, 2010]. Bacterial communities of C-section babies were found to be mainly dominated by Staphylococcus, Corynebacterium,
and Propionibacterium species which are similar to bacterial communities found on the skin whilst vaginally delivered babies were found to be dominated by Lactobacillus, Prevotella or Sneathia species which resembled their mother vaginal flora [Dominguez-Bello MG et al, 2010]. Adults have a microorganism population that outnumbers the cells in the body [Vyas U & Ranganathan N, 2012]. Gut flora begins as a simple system and then grows with the host to become a complex ecosystem that needs to be maintained. The composition and quantity of the gut flora is determined by:

Age

The composition of the flora changes with age, noted after various studies on newborns, infants, juveniles and adults where bacterial 16S rRNAs from fecal samples were analyzed after amplification using PCR [Lagier JC et al, 2012]It was found that: The proportion of Bacteroides fragilis increased from 1 month to 1 year of life in newborns [Vael et al, 2011]. A statistically significantly higher abundance of Bifidobacterium and Clostridium genera was found to be in present in adolescents suggesting that the gut floral composition of adolescents is different from that of adults [Agans et al, 2011]. Finally elderly people were found to have a distinctly different gut flora composition from young adults as they had a greater proportion of Bacteroides species and the Clostridium group had distinct abundance patterns [Claesson et al, 2011]. In general the biodiversity which incorporates phylogenetic difference between species of the microbiome increases gradually over time with progressive temporal changes but the major phyla, genera, and species composition showed shifts in abundance corresponding to life events [Koenig et al, 2011].

Geography and environment

Numerous studies have found differences in the composition of gut flora depending on the subjects' geography;[Drasar et al, 1973] did a study on the aetiology of colon cancer by studying the fecal samples from subjects from India, Japan and Uganda which were compared to fecal samples from subjects from England, USA and Scotland, the samples from the western countries were primarily composed of Bacteroidetes and the gut flora had

different composition in comparison to the flora from subjects from India, Japan and Uganda.[Lee et al, 2011] did a comparison on the composition of fecal flora taken from 2 sets of adult twins from Korea and the United States and found that the gut flora shows some signature of biogeography most likely due to different diets and/or environmental factors; however these regional differences can be masked by other phenotypic variations. More recently it was shown that children from Malawi and Finland had different bacterial species as Finnish children had certain species absent in Malawian children characterized using FISH (Fluorescence in situ hybridization) combined with flow cytometry and qPCR. [Grzeskowiak et al, 2012]

Dietary habits

Dietary habits are thought to be a major contributing factor to the diversity of the human gut flora [Backhed et al, 2005]as the food eaten will also be used by the gut flora and so meaning only bacteria that can use the food the host eats will thrive in the gut. Diet can be used to explain part of the geographic diversity seen in gut flora in people from different regions [Lagier JC et al, 2012]. For example, African children from a rural area in Burkina Faso showed a specific abundance of Prevotella and Xylanibacter, known to contain a set of bacterial genes for cellulose and xylan hydrolysis, completely lacking in European children [De Filippo et al., 2010]. It was hypothesized that the abundance of these genera could be a consequence of the high intake of fibre, similar to the diet of early human settlements at the time of the birth of agriculture, maximizing the extraction of metabolic energy from the polysaccharides of ingested plants [De Filippo et al., 2010].

Vegetarian diets have been found to harbour a significant amount of Clostridium rRNA clusters XIVa and XVIII in the gut using T-RLFP (terminal restriction fragment length polymorphism) analysis. Vegetarian diets have been found to also decrease the amount, and change the diversity of the Clostridium cluster IV [Liszt et al, 2009]. Recently the bacterial composition of overweight people was studied, they were either given a control diet, a diet high in resistance starch, non starch polysaccharides and a reduced carbohydrate weight loss diet for 10 weeks and then their fecal matter was studied using large scale sequencing and quantitive PC. It was found that changes were only observed at a fine taxonomic level,
Eubacterium rectale and Ruminococcus bromii had a fourfold increase in overweight subjects on the diet high in resistant starch and Collinsella aerofaciens-related species decreased significantly in overweight subjects on the reduced carbohydrate diet. [Walker et al, 2011]. As reproducible changes were found only at the phylotype level, and not on a broader taxonomic level (the phylum or family Ruminococcaceae level), the results suggested the differences observed were mainly due to individual lifestyle rather than diet [Walker et al, 2011].

Obesity

Obesity has been found to affect the gut flora composition through various experiments. For example studies of the bacterial communities of the gut flora in obese patients revealed a decrease in the ratio of
Firmicutes and Bacteroidetes species [Armougom F et al, 2009]after developing a real-time PCR tool that included a plasmid-based internal

control which allowed for quantification of

Bacteroidetes, Firmicutes, Lactobacillus species and Methanobrevibacter smithii in the faeces of 20 obese subjects [Armougom F et al, 2009]. It was also found that Lactobacillus species were present in higher concentrations [Armougom F et al, 2009]. In another experiment the stools of 68 obese people and 47 controls were analysed by quantitative PCR (qPCR) and culture on a Lactobacillus-selective medium, targeting Firmicutes, Bacteroidetes, M. Smithii, Lactococcus lactis, Bifidobacterium animalis and seven species of Lactobacillus[Million M et al, 2012]. It was found that M. Smithii was depleted in obese people while L. Reuteri and B. Animalis were in higher concentrations in obese people [Million M et al, 2012]. More experiments found links between obesity and then gut flora and so led to the suggestion of the various mechanisms to connect gut flora and disease. One of the mechanisms suggested was that the gut microbiota may be involved in weight regulation as the flora hydrolyzes polysaccharides, which would otherwise be indigestible, to easily digestablable monosaccharides and also activate an enzyme lipoprotein lipase [Lagier JC et al, 2012]. This then causes glucose to be rapidly absorbed elevating the concentrations of glucose and insulin in serum which triggers lipogenesis[Lagier JC et al, 2012]. Fatty acids are also excessively stored and together the phenomena cause weight gain [Backhed et al, 2007].

Antibiotics

Antibiotics were a revolutionary breakthrough in terms of fighting infectious diseases but have raised concerns have been raised on the effects they

might have on the gut flora of the host when taken as in the process of fighting disease they noticeably impact the flora population. Different types have antibiotics have been revealed to have different effects on the composition of flora i. e. Clindamycin was found to strongly affect the flora composition whereas cephalosporins has weaker effects on gut flora composition. This was which was thought to be because of the low activity of this molecule on intestinal anaerobes [Donskey et al, 2003]. It was uncovered that antibiotic courses affect the diversity of the gut flora without any permanent effects on the total biomass. For example the broad-spectrum antibiotic cefoperazone was administered in mice models and was found to cause significant disturbances in the flora which continued to show long after (6 weeks) the drug was withdrawn from treatment although the biomass had recovered at six weeks of drug withdrawal [Robinson CJ & Young VB, 2010]. Other antibiotics like amoxicillin, metronidazole and bismuth for example do not have long lasting effects on the composition of the flora as the flora population recovers soon after the withdrawal of the drug [Robinson CJ & Young VB, 2010]. Another antibiotic clamoxyl was given to mice to investigate which bacterial generas would be affected by the administration of the antiobiotic, it was found that Lactobacillus species were nearly eradicated from the flora population and that Enterobacteriaceae and Enterococcus specie numbers were also greatly affected by the antibiotic after analysis of the small intestines [Schumann A et al, 2005]. The same study found that the antibiotic downregulated the expression the genes coding for Paneth cell products and the major histocompatibility complex class Ib and II [Schumann A et al, 2005]. Antibiotics have also been found to

have a stronger impact on the flora of infants aged 1 year or less thought to be because of the instability of the flora during early life as the flora is still be developing [Palmer C et al 2007]. Previous studies uncovered that the diversity of the flora population was greatly affected with Bifidobacteria and Bacteroides species being significantly lowered [Penders J et al, 2006], treatment with antibiotics in early life was furthermore implicated in the development of wheezing infants in another study [Alm B et al, 2008]. Given the importance of the roles microorganisms play in the digestive tract; this has resulted in the boom of probiotics as people are now in the opinion that is it necessary to keep gut flora healthy and balanced to protect the gut and prevent things an imbalance of the gut flora population, known as dysobosis which is when the harmful bacteria outnumber beneficial bacteria in the gut and this may bring about a number of possible consequences some of which include; [online, http://www. epidemicanswers. org/epidemic/biological-dysfunction/gut-dysbiosis/, accessed January 16th]Localized gut inflammationSystemic inflammationIncreased oxidative stressChronic infectionsImpaired energy metabolismImpaired nutrient synthesisImpaired enzyme activityAn external file that holds a picture, illustration, etc. Object name is nihms199923f2. jpg Object name is nihms199923f2. jpghttp://www. ncbi. nlm. nih. gov/pmc/articles/PMC2881665Probiotics can maintain the gut flora population and enhance the functionality of gut flora. Probiotic bacteria and the existing flora population compete for resources and so this will affect the composition and functioning of the existing flora population as some of the microorganisms will starve and only particular strains will thrive [O’Toole and Cooney, 2008]. Probiotic microorganisms will produce certain products

that will act as growth substrates or inhibitors to the existing flora depending on the composition [O'Toole and Cooney, 2008], for example exopolysaccharide produced by probiotics could act as a growth substrate for selected species of the microorganism present [O'Toole and Cooney, 2008]. This is supported by past research as past results from random controlled clinical trials where probiotics were used for the treatment of gastrointestinal diseases [O'Toole and Cooney, 2008]. Probiotics also:

Maintain and enhance the epithelial barrier

The intestinal epithelium is covered by a mucous layer which contains antimicrobial compounds, secretory IgA and the epithelial junction adhesion complex which together form a defensive barrier [Ohland CL et al, 2012]. The barrier stops antigens from coming into contact with the submucosa where they would normally induce inflammatory responses [Hooper LV et al, 2001]. Probiotics have been studied extensively for their involvement in the maintenance of this barrier but the mechanisms by which probiotics enhance the barrier are not yet fully understood [Bermudez-Brito M et al, 2012]. The mucous secreted by the epithelial cells also contains mucins, a family of complex glycoproteins and the principal component of mucous, which have been implicated in health and disease by past research, certain probiotics promote mucous secretion which in turn further improves the defensive barrier. Probiotics containing Lactobacillus species have been found to increase mucin secretion [Mattar AF et al, 2002] and another study found that Lactobacillus acidophilus increases MUC2 expression in HT29 cells independent of attachment [Kim Y, et al, 2008]. More recent studies have

indicated that probiotics are involved in the repair of the barrier function after it has been damaged. Escherichia coli Nissle 1917 has been found to prevent the disruption of the mucosal barrier pathogenic E. coli and even restoring the intergrity of the mucuosal layer in T84 [Bermudez-Brito M et al, 2012] and Caco-2 cells which are part of the epithelial cells [Stetinova Vetal, 2010]. Another probiotic VSL3 a mixture of pre and probiotcs has been found to protect the epithelial barrier aswell as increase tight junction protein expression by activating the p38 and extracellular regulated signalling pathways [Dai C et al, 2012]

Increase Adhesion to Intestinal Mucosa

It is essential for probiotics to be able to adhere to the intestinal mucosa as this is required for the colonization of the gut and is also important for: Interactions with the hostModulation of the immune systemAntagonism against pathogen/sLactobacillus species have been found to produce proteins that promote mucous adhesion [Van Tassell ML & Miller MJ, 2011] Lactobacillus reuteri in particular has been found to a produce an mucous targeting adhesion protein known as mucus-binding protein (MUB) [Bermudez-Brito M et al, 2012]. The majority of proteins that promote mucous adhesion are secreted and normally found on the surface of the bacteria, either anchored to the membrane or embedded in the cell wall. Another Lactobacillus species that was found to promote the production of mucins by goblet cells, in particular MUC2 and MUC3 mucins, and to inhibiting the adhesion of pathogenic E. coli to the intestinal mucous which normally causes haemorrhaging of the intestines [Hirano J et al,

2003]Certain strains of probiotics have been found to provoke the release of definsins from epithelial cells. Defensins are small cysteine rich proteins which reinforce the defensive barrier and are active against; bacteria, enveloped & non enveloped viruses and fungi. Defensins can inhibit the growth or virulence of the microorganisms directly or can do so indirectly by inducing the host's immune system and are one of the antimicrobial proteins (AMP) which are proteins that act as the first line of chemical defence against pathogens [Bermudez-Brito M et al, 2012].

Out compete pathogens

Probiotics out compete other pathogenic bacteria by creating a hostile environment for the other bacterial species present which probiotics achieve by outcompeting pathogens for nutrients and mucosal binding sites [Bermudez-Brito M et al, 2012]. Other methods of exclusion used by probiotics include inhibiting pathogen adhesion which occurs when specific surface proteins on probiotic bacteria and mucins in the mucous barrier interact [Bermudez-Brito M et al, 2012]. Lactobacilli and bifidobacteria are two species of probiotic bacteria that have been successfully proven to inhibit the colonization of the gut by the pathogens such as E. coli, Salmonella and Helicobacter pylori [Bermudez-Brito M et al, 2012].

Production of anti microbial substances

Probiotics produce a variety of compounds that have some sort of activity against pathogens, these compounds include: Low molecular weight compounds (less than 1000Da) such as organic acids i. e. lactic acid [Bermudez-Brito M et al, 2012]. These organic acids have strong inhibitory

effects against Gram-negative bacteria which were found by observing the effects of lactic acid on the outer membranes of pathogens, utilizing a fluorescent-probe uptake assays and sensitization to bacteriolysis [Alakomi H. L et al, 2000] where the non disassociated form of the organic acid enters the bacterium and disassociates, the splitting off of a proton, in the bacterium’s cytoplasm leading to the lowering of the bacterium’s intracellular pH or the accumulation of the ionized form of the organic acid which both result in the death of the pathogen [Bermudez-Brito M et al, 2012]. Higher molecular weightcompunds (more than 1000Da) such as antimicrobial compounds i. e. bacteriocins and antimicrobial proteins. Bacteriocins are small proteineous toxins produced by almost every bacteria and are usually active against related bacteria, the common mechanisms of action include inhibiting cell wall synthesis, permeabilizing the target cell membrane or by inhibiting RNase or DNase activity [Cleveland J et al, 2001]. For example bifidocin B a unique bacteriocin produced by Bifidobacterium bifidum NCFB 1454 is active against Gram positive bacteria [Yildirim Z & Johnson MG, 1998] peptides (AMPs) are also small proteins, with broad spectrum of antimicrobial activity against viruses, bacteria and fungi and are evolutionarily conserved peptides. AMPs usually carry a positive charge and have a hydrophobic and hydrophilic side so enabling the peptide to be soluble in aqueous environments also allowing it to penetrate lipid-rich membranes [Izadpanah A & Gallo RL, 2005]Derivatives of bile salts known as de-conjugated bile acids, which have stronger anti microbial activity in comparison to bile salts produced by the host[Bermudez-Brito M et al, 2012]. Metabolites with anti fungal and bacterial activity for example species of

lactobacillus coryniformis can produce proteinaceous compounds which exhibit antifungal properties [Bermudez-Brito M et al, 2012].

Interact with receptor

Probiotics have been found to be interact with Toll like receptors (TLRs) which are transmembrane innate immune recognition receptors expressed on of immune cells for example B cells and normal cells i. e. epithelial cells, [Means TK et al, 2000] TLRs are a type of pattern recognition receptors which is a class of innate immune response-expressed proteins that respond to pathogen-associated molecular patterns (PAMP) and endogenous stress signals known as danger-associated molecular patterns (DAMP). Lactobacillli species of bacteria are known to signal through the binding to TLR2 in combination with TLR6, the di-acylated membrane anchors of lipoproteins and lipoteichoic acids specifically bind to TLR2 and 6. TLR2 activation results in the increased production of cytokines and enhances the epithelial barrier [Bermudez-Brito M et al, 2012]. TLR2 can also recognize peptidoglycan a major component of the outer shell of Gram positive bacteria. Another important TLR is TLR9 which has the ability to recognize CpG DNA. Probiotics tend to release unmethylated DNA fragments containing CpG motifs in vivo which use TLR 9 activation to mediate anti inflammatory effects [Bermudez-Brito M et al, 2012]. It has also been shown that purified genomic DNA from L. plantarum (p-gDNA) inhibits LPS induced TNF-α production by THP-1 cells and also down regulates the expression of TLR2, TLR4 and TLR9 which bring on the activation of transcription factor nuclear factor NF-κB which controls host inflammatory responses to pathogenic bacteria [O'Hara A. M &

Shanahan F, 2006] the through the LPS signaling pathway results in the increased expression of inflammatory cytokines [Kim CH et al, 2012]. Probiotics are known to suppress intestinal inflammation via regulatory T-cell activation by the intestinal dendritic cells and low activation of the T-helper 1 and 2 cells after interacting with TLRs [Gómez-Llorente C et al, 2010]. Below is a table with some examples of proven probiotics and evidence of their functionality;

Probiotic Product

Bacteria Strain

Evidence/trials

Activa yogurtBifidobacterium animalis DN-173010, Streptococcus thermophilus and Lactobacillus bulgaricusA double-blind, parallel, placebo-controlled study was carried on 72 volunteers, (36 males and 36 females) (ages 21–42 years) (range46–88kg), to study the effect of B. lactis DN-173 010 fermented milk in a 125g container on colonic transit times in healthy humans. The control was identical to the B. lactis DN-173 010 fermented milk with the exception that the bacterium was killed using heat treatment. The volunteers consumed 3 containers daily of the control and the B. lactis DN-173 010 fermented milk and it was found that consumption of B. lactis DN-173 010 fermented milk significantly reduced total colonic transit time by 20. 6% and reduced the sigmoid transit time by 38. 9% compared with the control with any live bacteria. The improvement in total colonic transit time was statistically significant in both men and in women but worked better in women. Radiopaque pellets were used to measure colonic transit times.

http://www. activia. us. com/App_Master/pdf/approved-activa-summary-95837. pdfVSL#3 packetsB. breve, B. infantis, B. longum, L. acidophilus, L. bulgaricus, L. casei, L. plantarum and Streptococcus thermophilusThirty six patients who had pouchitis at least twice in the previous year or requiring continuous antibiotics, in whom remission was induced by four weeks of combined metronidazole and ciprofloxacin treatment, were randomly selected to for the study [Mimura T et al, 2004]. 20 patients were given VSL#3 and 16 were given a placebo. Remission was maintained at one year in 17 patients (85%) on VSL#3 and in one patient (6%) from the placebo group. It was then concluded that a daily high dose of VSL#3 is effective in maintaining antibiotic introduced remission for at least a year in patients with recurrent or refractory pouchitis [Mimura T et al, 2004]. DanActive cultured milkS. thermophilus, L. Bulgaricus and L. casei DN-114 001A double-blinded, randomized, placebo-controlled clinical trial was done638 children 3–6 years old in daycare or school [Merenstein D et al, 2010]. The participants were split into 2 groups the active group and the control group, the active group was given a fermented milk drink containing live cultures of Lactobacillus casei DN-114 001 whilst the control group were given a fermented milk drink with no live cultures for 90 consecutive days [Merenstein D et al, 2010]. Two primary outcomes were assessed (assessed by parental report): incidence of common infectious diseases and change of behaviour because of illness. The rate of change of behaviour because of illness was similar among active and control groups. However, the rate incidence of common infectious diseases in the active was 19% lower than that of the control group [Merenstein D et al, 2010].

Future of Probiotics

The future of probiotics looks bright as more and more potential uses for probiotics are being found as more viable strains of probiotics bacteria are being found for example Bifidobacterium vercorsense sp. nov a strain patented by the University of Liège and recently the human microbome project which was completed last year after 5 years http://www. nutraingredients. com/Research/138m-5-year-Human-Microbiome-Project-completion-excites-probiotic-community. The project characterized and analyzed the role of microbial communities in human health and disease found at several different sites in the human body such as the gastrointestinal tract and urogenital tract using metagenomic approach which allows for the analysis of genetic material derived from complete microbial communities harvested from natural environments http://commonfund. nih. gov/hmp/. The new knowledge and tools have the probiotic community excited as these new developments can only help improve current research. Below are some of the potential benefits which probiotics will be able to deliver to the consumer in the next decade. These benefits are not just aimed at the gut, some go beyond. The specific strains of the probiotic bacteria, the benefits and a brief summary of the mechanism of action will be listed. It should be noted that most but not all the strains have undergone clinical trials and for some, of the mechanisms of action of the bacteria are not yet fully understood. To fight surgical infectionStrain-L. plantarum 299Patients undergoing abdominal surgery for example stomach resection were given L. plantarum 299 and fibre, the patients had fewer infections, did not need as many antibiotics prescribed, had shorter hospital

stay and less complications compared to the controls [Rayes et al, 2009]. Mechanism of action is unknown. Treatment & prevention of diarrhoeaPrevention-Lactobacillus rhamnosus GG, Bifidobacterium lactis BB-12Treatment-Lactobacillus reuteri SD 2222, Bifidobacterium animalis Bb12 , Lactobacillus casei Shirota, Lactobacillus reuteriA randomized trial was conducted at 14 child care centres in Israel, on infants 4 to 10 months old. Infants were assigned randomly to formula supplemented with Bifidobacterium lactis (BB-12), Lactobacillus reuteri (American Type Culture Collection 55730), or no probiotics for the control [Weizman et al, 2005]. All infants were fed only the assigned formula and were not breastfed due to parental decision. Probiotic or prebiotic food products or supplements were not allowed, and the number of days and number of episodes with fever (> 38°C) and number of days and number of episodes with diarrhea or respiratory illness were measured [Weizman et al, 2005]. The infants from control group had significantly more febrile episodes, more and longer diarrhea. The L. reuteri group had a significant decrease of number of days with fever, clinic visits, child care absences, and antibiotic prescriptions [Weizman et al, 2005]. Mechanism of actionStructural lipopolysaccharides, glycopeptides and CpG DNA from probiotic bacteria interact with dendritic cells and toll-like receptors activating so regulating the production of peptides involved in innate immunity which then either exert anti microbial activity or modulate adaptive immunity. For example Lactobacillus rhamnosus GG increases the mucosal production of specific antirotavirus antibody sIgA, stimulates the production of anti inflammatory cytokines IL-10 and IL-4 and stops the production of proinflammatory cytokines, which

results in decreased fluid loss in children with inflammatory diarrhoea [Guarino A, 2009]. There are another 2 possible mechanisms as the main mechanism is unknown. Allergy treatmentStrains -Lactobacillus reuteri & Bifidobacterium lactisIn a recent study breast fed infants suffering from atopic eczema B were given probiotics containing B. lactis and L. rhamnosus GG which were effective in decreasing the eczema severity, L. rhamnosus GG was also found to be useful in preventing the atopic eczema in high risk infants when the mothers, who had at least one first degree relative with atopic eczema, allergic rhinitis or asthma, were given probiotics prenatally [Kechagia M et al, 2013]. Mechanism of action is unknown. Helicobacter pylori Infections and ComplicationsStrain-Lactobacillus acidophilusA clinical trial where 120 H. pylori positive patients were given a randomly given a 7 day triple therapy course of either antibiotics, inactive culture Lactobacillus acidophilus and an active culture of Lactobacillus acidophilus. Patients that underwent the antibiotic therapy had 72% of H. pylori pathogen eradicated, patients that underwent the probiotic therapy had 88% of the H. pylori pathogen eradicated and finally patients treated inactive cultures of with Lactobacillus acidophilus 87% of the H. pylori pathogen eradicated [Canducci, F et al, 2000]. Mechanism of action is unknown. CancerStrains-Lactobacillus rhamnosus GG, Lactobacillus rhamnosus LC-705, Propionibacterium freudenreichii ssp, Propionibacterium shermanii JSA randomized study was conducted on patients with superficial bladder cancer to investigate the safety of Lactobacillus preparation biolactis powder (BLP), 3 g/day, its effect on the recurrence after transurethral resection of the bladder tumour. 23 patients in the BLP group and 25 cases in the control

group completed the study. There were no significant differences in the patient characteristics for the two groups however comparison of the disease-free duration by the Kaplan-Meier method showed that the 50% recurrence-free interval after the transurethral resection was short lived in the control group at 195 days in comparison to the BLP group were the 50% recurrence-free interval was at 350 days. The long-rank test was carried out and detected a significant difference between the groups [Aso Y & Akazan H, 1992]. Mechanism of actionThe probiotic bacteria decrease the levels of carcinogenetic enzymes produced by gut flora by normalizing of the intestinal permeability and balancing the microflora. The bacteria also produce antimutagenic organic acids such as butyrate and lactate [Kumar M, 2013]. Glutathione transferase activity and colonic NADPH-cytochrome P450 reductase activity are increased, enhance removal of O6-methylguanine from colonic mucosa. [Kumar M, 2010]. Oral healthcareas more pathogens are gaining antibiotic resistance so pushing researchers to find new alternatives to deal with the resistance and the use of probiotics in oral health care is now one of the strongest emerging fields in this respect [Teughels W et al, 2008]. For example in the treatment of acute streptococcal pharyngitis infections as the current treatment, penicillin, does not completely eradicate pharyngotonsillitis caused by group A β-hemolytic streptococci , an issue as it could lead to streptococcal toxic shock syndrome [Teughels W et al, 2008]. Studies revealed that ~35% of patients treated with oral penicillin V and in 37% of patients treated with benzathine penicillin G failed from a microbiology perspective at either 10–14 or 29–31 days after therapy [Kaplan EL et al, 2001]. Scientists have hypothesised that bacterial

replacement therapy would be a much better form of treatment in comparison to antibiotic treatment as it would have a less harmful effect on the flora and so be a safer option for patients suffering from such infections but many more clinical trial are needed to confirm this hypothesis using human and animal models [Teughels W et al, 2008]. Gut -CNS linkLactobacillus farciminis is a bacterium that has been hypothesised to have effects that go beyond the gut. A study using Lactobacillus farciminis a probiotic strain of bacteria found that partial restraint stress enhances Fos expression, expression of Fos is an indirect marker of neuronal activity as Fos is expressed after some sort of neuronal activity , induced by in colorectal distension the spinal cord and that Lactobacillus farciminis treatment inhibited this the cascade of effects suggesting that the reduced sensitization to pain was due to a decrease of stressed induced activation of sensory neurons in the entric nervous system and spinal cord [Ait-belgnaoui A et al, 2009]It has long been known that there is a link between the gut and the central nervous system [Lyte et al, 1998] [Bercik P et al, 2012]. Recent animal studies have implicated that the gut flora plays a role in gut brain communication. Mice had altered anxious-like behaviour within several hours of early acute infection by Campylobacter jejuni before any significant immune response was mounted so suggesting this was not due the effect cytokines [Lyte et al, 1998]. Follow up studies showed that the pathogen triggered an activation pattern in multiple brain regions and activated vagal ascending pathways all previously shown in anxious behaviour [Bercik P et al, 2012]. Conclusion