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Probiotics: A Closer Look

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PROBIOTICS: A CLOSER LOOK

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Introduction

In the modern era of science, it is possible to get caught up with the chemical rush. Remedies that might have once worked for our ancestors are now ignored for the flashy medicines available over the counter. Amidst the excitement created by these chemicals, emerges an alternative to old age and new age medicines in the form of probiotics. Probiotics are bacteria which are non-pathogenic and have a positive effect on the physiology and overall health of the host they live in (Kleta *et al.*). Much research has been done and many experiments have been performed to find out exactly which bacteria are most beneficial, and in what ways. The main bacteria that have been examined in this study are various strains of the *Lactobacillus* and *Bifidobacterium* genus. It can be said that there is variation in effectiveness not only between genii, but also between species within the same genus (Nikoskelainen et al.).

Probiotics are bacteria, and are thought to be part of the natural flora in most animals. However, variation does exist between how much bacteria is between animals, and through this, arise problems (Kleta *et al*.). Pathogens which attack primarily the gastrointestinal tract have been examined in this study and it has been shown that probiotics provide prevention and relief against said pathogenic bacteria and associated symptoms (Olivares *et al*.) Aside from being a preventative measure as well as a somewhat “medicine”, probiotics are also given to non-breastfed infants as a supplement (Olivares *et al*.).

There are several mechanisms through which probiotics aid the body in fighting off pathogens. These range from fighting for adhesion on the tract itself, to displaying some sort of antibacterial properties (Bouzaine *et al*.) Methods have also been developed to trace the progress of the probiotics after they have been ingested, and also to verify the effects of the probiotics as they travel through the host body (Schultz *et al.*). Some people assume that as with modern medicine, probiotics have negative effects on the body. However, this has been disproven through laboratory tests, thereby proving several probiotic bacterial species completely nontoxic and usable for humans (Hong *et al.*).

A. Probiotics: A Closer Look

Probiotic bacteria have shown to positively affect the composition of and activity of the intestinal microbiota, and have demonstrated that they have a beneficial effect in the host. They do this by preventing or reducing effects of certain gastrointestinal infections (Bouzaine *et al*.). Especially in the last few years, much interest has accumulated for probiotic bacteria due to their potential benefits in the fields of normal intestinal microflora, stimulation of immune systems, relief in diarrhea, and other gastrointestinal afflictions (Morelli *et al.*). There are two main genii of interest where probiotics are concerned: Lactobacillus and Bifidobacterium. In one study, experiments were performed with four strains of Lactobacillus: *L. salivarius* CECT5713, *L. gasseri* CECT5714, *L. gasseri* CECT5715 and *L. fermentum* CECT5716. Researchers found that *L. salivarius* was the most beneficial to humans (Olivares *et al.*). Agar diffusion tests, competitive adhesion assays, and mucin expression assays were carried out in order to evaluate the antibacterial properties against pathogenic bacteria. They were able to determine that not only was *L. salivarius* the best in terms of *in-vitro* antibacterial activity, but also provided the highest protective effect against a Salmonella strain in a murine infection model (Olivares *et al*.)

Research has also shown that probiotic bacteria occur naturally in the gut micro flora of animals. For example, another species of probiotics, *Escherichia coli Nissle 1917*, has been proven to occur naturally in swine micro flora. However, further results of this experiment indicated that the colonization of individual animals can vary. A study was conducted on the intestinal microflora of pigs, and the *E. coli* Nissle 1917 strain was isolated and identified, from the given population. A feeding experiment was also conducted with four piglets, and it was found that they all showed viable *E. coli* Nissle 1917 in their intestines. Thus, Kleta and his colleagues concluded that this particular strain is pre-established in swine herds. They did see, however, that the colonization in individual animals can be variable. Colon and jejunum (area of the small intestine) sections from three piglets were examined and the number of *E. coli* Nissle 1917 isolates differed in all three, but was present at some level. (Kleta *et al.*)

Studies have also shown that some infections are more prevalent in organisms and people without a certain baseline level of probiotic bacteria. In addition to this, research has demonstrated that having the probiotic bacteria in one's system before being infected leads to better recovery. For example, *Helicobacter pylori* infections are seen more heavily in people that we know already have a lower baseline level of *Lactobacillus johnsonii La1*. The clinical studies that were conducted show that the Lactobacillus family of bacteria has positive effects on patients with *Helicobacter pylori* infections. Results show that when the mouse models that were being tested were fed *L. salivarius* prior to the challenge with *H. pylori*, no colonization was achieved. Furthermore, when the animals were already infected with *H. pylori*, and were then fed *L. salivarius*, the numbers of the pathogens were reduced to less than 1% of the control group. (Hamilton-Miller *et al.*)

In another study, the scientists involved studied in vivo the effects of probiotics in infants with atopic eczema dermatitis syndrome (AEDS) and those infants with cow's milk allergy (CMA). They had a total of 230 in vivo subjects and found that in atopic children, there were more *coliforms* and *clostridia* and fewer *bifidobacteria* and *lactobacillus* bacteria than nonatopic children (Viljanen *et al*.). Studies were conducted on the specific effect of probiotic bacteria on the specific effect of probiotic bacteria on the post treatment inflammation marker, C-reactive protein (CRP). The data presented in this particular study shows that there is a significant increase in the CRP levels in the Lactobacillus-treated infants with AEDS, than those infants in the control group. This is beneficial since the production of Human CRP leads to inhibition of production of different inflammatory cytokines and chemokines. Human CRP also enhances the production of IL-10, which displays potent abilities to suppress the antigen presentation capacity of antigen presenting cells. It is also stimulatory towards certain T cells, mast cells and B cells (Viljanen *et al*.).

Of course, there are many factors that affect probiotics, since they are living organisms. Many properties which could have an effect on the *Lactobacillus* strains or their mechanisms were examined in a study. The characteristics studied were acid and bile tolerance, adhesion and cell surface hydrophobicity, antimicrobial effect on common pathogens and cholesterol reduction. In vivo, all seven strains tested could survive at a pH of 3, which is significant since ingestion with food or dairy products raises the pH of the stomach to 3 or higher. It was also seen that the tolerance of the tested strains to acid was highly strain-specific, since only 3 out of the seven exhibited good tolerance to simulated bile (Mishra *et al*.)

C. Uses

Probiotic bacteria have a variety of uses in the present society. Primarily, they are used as a preventative measure against a variety of infections. In the study performed by Frick et al, the scientists tested whether strains modulating inflammatory reponses in vivo could prevent DSS-induced acute colitis. Key results were that they found that the probiotic bacteria increased the resistance of mice to the infection. They also saw that the probiotic bacteria inhibited inflammatory processes in vivo. The mouse model of oral Yersiniosis showed that the administration of viable *B. adolescentis* (the probiotic bacteria in question) protected mice from the generalization of *Y. enterocolitica* infection. In fact, in mice models with the dextran sodium sulfate-induced- colitis, *B. adolescentis* attenuated the development of colitis, indicating anti-inflammatory properties. (Frick et al.)

Studies also show that certain probiotic bacteria can protect against various fish pathogens, *Vibrio anguillarum, Aeromonas salmonicida*, and *Flavobacterium psychrophilum*. These are of special importance to humans because these are common pathogens that infect fish used for human consumption. It has been deduced that *L. rhamnosus* ATCC 53103 and *L. bulgaricus* can be considered for safe treatment in aquaculture (Nikoskelainen *et al*.) Adhesion, as will be discussed later, is one of the mechanisms by which probiotics are thought to benefit the host organism. In a particular study conducted by Nikoskelainen and his colleagues, it was deduced that *L. rhamnosus* and *L. bulgaricus* were the most adhesive to the gastrointestinal tract. This ability is thought to be the way by which probiotics suppress pathogen growth.

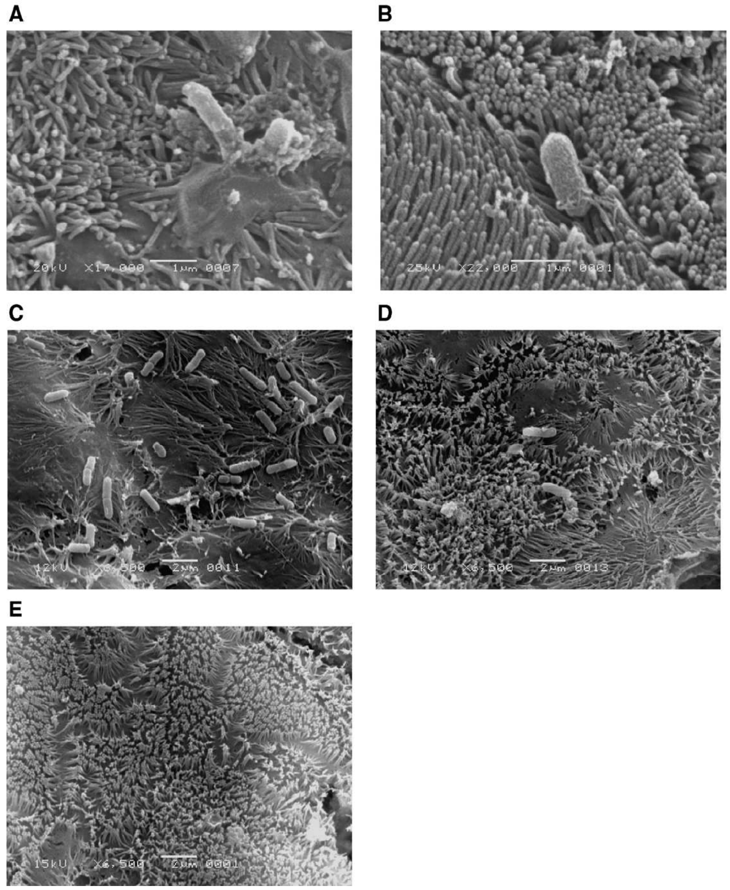
Other uses for probiotics involve the commensal bacteria to be used as a neonatal nutrition source. For example, research has shown that probiotic bacteria are transferred through breast milk from mother to infant (Olivares *et al.*) As mentioned previously, having proper intestinal flora is very important to gastrointestinal health. Thus, for infants that are not breast-fed, proper supplements must be given with the bacteria added to it, to ensure gut health.

D. Mechanisms

Studies have been performed to evaluate the adherence and colonization properties of the *Lactobacillus rhamnosus* TB1 strain, which is important since they know that the probiotic bacteria can localize and adhere to the GI tract, and thus provide competition to the pathogenic bacteria. This is one of the mechanisms by which probiotic bacteria provide resistance to pathogens (Bouzaine *et al.*)

Different strains of Lactobacillus have shown to have varying degrees of adherence. Researchers tested the adhesive capacity of selected enterococci to human, canine, and porcine intestinal music was investigated in order to select for potential probiotic strains with good adhesive properties for human or animal use. The key results were that a strong correlation was observed for the adhesion to human and canine intestinal mucus and also between porcine and canine or human mucus. Two surface proteins have been identified that are associated with association and may be involved in adhesion of *enteroccoci*: the aggregation substance and enterococcal surface proteins (Laukova *et al*.) The method by which probiotic adhesion blocks other bacteria can be seen in Figure 1, panels A-E.

Figure 1.



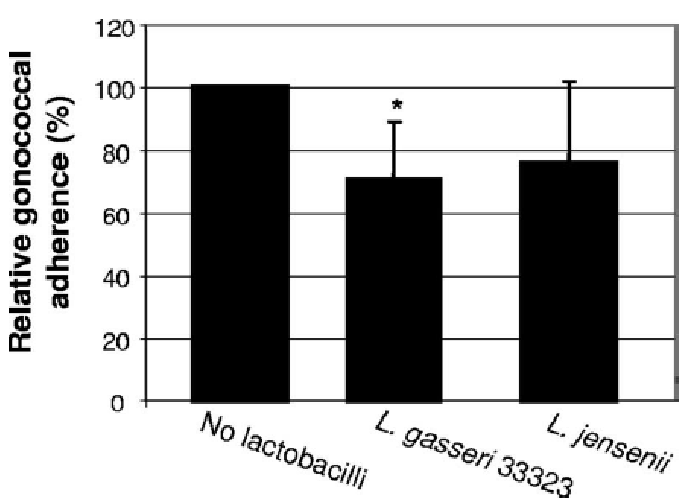
We can see in Panels A and C, cells that have been infected by *Salmonella enteridis* for 40 (A) and 120 (B) minutes. The pictures in panels (C) and (D) depict cells that hhave been infected by *Salmonella enteridis*, but have been incubated with cultures of *Lactobacillus kefir*. Panel (E) depicts uninfected cells, for comparison. It can be seen that with the presence of the *Lactobacillus* bacteria, the adherence of the *Salmonella* is greatly reduced, therefore leading to healthier patients (*Golowczyc et al.*)

While it has been established that adherence is key to Lactobacillus resistance of various gastrointestinal pathogens, studies have been done to examine where exactly the lactobacillus adheres to, to provide said resistance. The key results were that there were four sites of recovery for the probiotic bacteria: the cecum, the transverse, the descending colon, the sigmoid colon (Morelli *et al*.).

While competition is a strong mechanism of resistance, it is not the only one used by probiotics. Evidence has been given to show that probiotic bacteria Lactobacilli exert bacteriostatic or bactericidal effects against bacterial pathogens, namely, *Helicobacter pylori* (Cruchet *et al.*) Further research with this pathogen/probiotic shows that *Lactobacillus* not only kills it, but also prevents IL-8 release (Hamilton-Miller *et al.*) The authors speculate that the mechanism by which *Lactobacillus* blocks *Helicobacter pylori* is combination of the following: competition for nutrients, competition for exclusion, production of inhibitory compounds, and immunomodulatory stimuli (Hamilton-Miller *et al.*).

Another species of bacteria that is affected by probiotics is *N. gonorrhoeae*. Studies show that when probiotics are given to individuals who are infected with *N. gonorrhoeae,* symptoms are alleviated. The mechanism by which this is achieved, is again, when the *Lactobacillus* administered binds to the cells and therefore preventing the adhesion of the pathogenic bacteria. The adhesion results from the studies of Spurbeck and his colleagues can be seen in the figure below.

Figure 2.



Gonococcal adherence is reduced significantly when there is lactobacillus present due to the fact that the two bacterial strains fight for competition. (Spurbeck et al.)

The direct effect of probiotics on infants' intestinal systems by measuring changes in systemic levels of cytokines and inflammatory markers has also been evaluated. The researchers found that there was a significant increase in the levels of CRP (C-reactive protein) in infants treated with LGG, who had AEDS. CRP is an indication of inflammation. IL-6 induces gene activation of CRP in hepatocytes and it stimulates CRP secretion (Viljanen *et al*.)

Post inoculation with probiotic bacteria, the protein composition of the host organism also changes. A study was done to evaluate exactly what and how changes, and the result this causes on the organism. studies were performed in-vivo by inoculation of *Lactobacillus fermentum* into the rabbit jejunum for 4 hours. After inoculation, the bacteria were analyzed for proteome. The scientists performing this study found changes in protein biomarkers that are beneficial for *Lactobacillus* and intestinal epithelial cells in response to interactions. The key results were that there were undetectable values for Lactate dehydrogenase when *L. fermentum* is incubated inside rabbit jejunum. This indicates that lactate utilization is limited as a fuel in intestinal bacterium. There is also a decrease in dihydrolipoamide dehydrogenase, which is a component of the pyruvate dehydrogenase complex. This suggests that oxidation of pyruvate via the krebs cycle is low post incubation. And since substrate oxidation is associated with the production of oxygen free radicals, which are potentially toxic to bacteria, these changes in energy metabolism would favor the survival of *L. fermentum* in the intestine. Lastly, there is an increase in Glycoside hydrolase, which plays a role in mucin degradation on the surface of the intestine. The release of glycoside hydrolase from the bacterium may participate in the regulation of mucin turnover and thus integrity of the intestinal epithelium. Another important finding from this study is that the levels of some regulatory proteins were elevated in response to incubation with the probiotic, and some of these proteins would help protect against the inflammatory injury that occurs in response to bacteria. They summarized that proteins can serve as biomarkers for the metabolic changes that are beneficial for the lactobacillus and intestinal epithelial cells (Yang *et al.*)

E. Proof of Activity

There is a lot that can be said about the mechanisms and methods by which probiotics aid the human body, but where is the proof that this is actually happening? Scientists have thus come up with techniques to follow and track the progress of the probiotic bacteria through the gastrointestinal tract. In a study mentioned previously, a tests were performed in vivo and the scientists stained the bacteria with cFDA-SE and under observation, realized that the bacteria was localized in the rectum and also in the jejunum and ileum. This is important since they know that the probiotic bacteria can localize and adhere to the GI tract, and thus provide competition to the pathogenic bacteria (Bouzaine et al.)

Another method of tracking the progress of the probiotic through the GI tract is through GFP staining. Scientists realized that bacteria can be difficult to follow through the gastrointestinal tract, so they decided to label it with a plasmid containing GFP. They used rats for the in-vivo models, and then took samples from the stomach, jejunum, ileum, caecum, colon and rectum to test for presence of EcN. They found that the EcN-GFP was detectable in high numbers in the stomach and in lower numbers in the caecum but not in the rectum at the beginning of the experiment. They also found out that the organisms were localized close to the epithelial surface of the stomach, cecum, and rectum and also in the lumen of the intestine. The transformation of EcN to obtain EcN-GFP in this study had no detectable influence on the probiotic microorganism regarding adhesion on and induction of IL-8 secretion of HT-29 cells and allowed the detection in mixed microbial environments (Schultz *et al.*)

Figure 3.

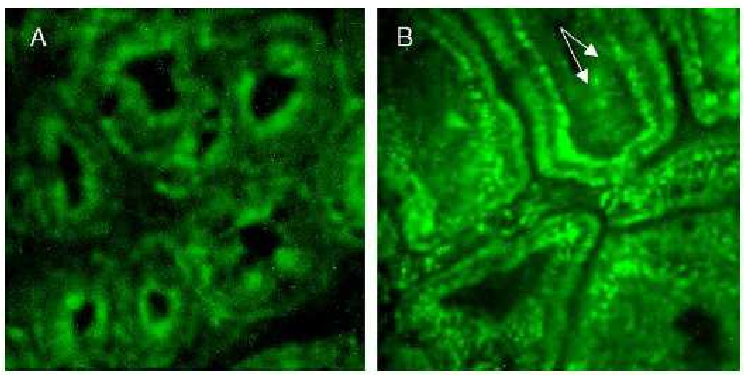


Figure 3 on depicts images of fluorescence microscopy of histological sections of the caecum from untreated controls (A, autofluorescence of intestinal tissues) and from mice challenged with EcN-GFP (B). EcN-GFP appears as a bright band close to the epithelial surface, and the treated animals display greater amounts of GFP, meaning greater amounts of resistance (Schultz *et al.*).

F. Safety

Probiotics as treatment are still a novel concept in the medical world, and leads to questions and doubts in the minds of many. However, one can rest assured that studies are heavily underway to test for the safety and reliability of probiotics. Research has been done to determine any toxicity present through the usage of probiotic bacteria, Bacillus (subtilis and indicus). Researchers tested guinea pigs and rabbits since they are considered the most sensitive lab animals. No noticeable differences could be seen in the animals tested, thus they can assume that the probiotic bacteria are harmless (Hong *et al.*).

G. Conclusions and Implications for the Future

Probiotics are a wave of the current era that are not only already useful, but also have great scope in the years to come. Already being used as supplements, probiotic bacteria are still under heavy research to be understood better and improved for further human use. Currently, we use largely the *Lactobacillus* and *Bifidobacterium* species, but we must keep in mind that more research should be performed on other commensal bacteria that could potentially improve human health. These bacteria, already naturally present in many organisms naturally, can be used as "medicine" to prevent infections such as those caused by pathogens, and other benefits such as stimulation of immune systems and relief in various gastrointestinal afflictions.

As mentioned, Lactobacillus bacteria are particularly important for humans, in particular *Lactobacillus salivarius* (Nikoskelainen et al.). In addition, another species of bacteria - *Escherichia coli* have been proven to occur naturally in swine micro flora and that they are particularly beneficial for intestinal health (Kleta *et al.*).

Research suggets several mechanisms by which probiotics accomplish the beneficial results in their host organisms. Largely, evidence shows that competitive adherence is the key to the probiotic success. Four major sites of adherence have been established: the cecum, the transverse, the descending colon and the sigmoid colon (Morelli et al.). Another mechanism suggested is the bactericidal affects of probiotics - and release of Interleukins (Hamilton-Miller et al.)

Probiotic bacteria have also been tracked through the body to verify their adherence and effects, through cFDA-SE staining. It has been established that probiotic bacteria can localize and adhere to the GI tract, and thus compete with pathogenic bacteria (Bouzaine et al.). GFP staining has also been used to verify the presence and adherence of probiotic bacteria throughout organisms (Schultz et al.).

Safety is an aspect of probiotic research that, though already tested and proven positive, needs to be further researched. Positive results have been obtained in rabbit and guinea pig models (Hong et al.), but not enough studies have truly examined all the effects of bacterial supplements to humans. This is particularly important, since we must be sure of the effects before administering probiotic bacteria to certain members of the population- such as infants, elderly and other high-risk individuals.

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