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Mini-Review: Probiotics and Disease Prevention in Different Host Systems

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Authors' contributions

This mini-review was carried out in collaboration among all authors. Author EFB designed the review and wrote all the drafts of the manuscript. Authors RDS and WT reviewed the drafts and provided suggestions. All authors contributed to the literature searches and approved the final manuscript.

Review Article

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ABSTRACT

This review details the success of different probiotic agents to provide protection in the host from infection by pathogenic microbial agents. Probiotics are bacteria that interfere and kill pathogens but the mechanisms employed by these agents in preventing infection and disease vary from host to host. In this review the use of probiotics in evolutionary distinct hosts are discussed. The early discovery of antibiotics (such as penicillin and streptomycin) and newer generation drugs have played and continue to play vital roles in controlling infections by pathogenic agents. The extensive and indiscriminate uses of antibiotics have contributed to the survival of resistant microbial agents that cannot be controlled by conventional antibiotics. The resistant strains damage cells, tissues and organs resulting in injury and or death to the host. Probiotic agents block sites pathogenic agents need for adherence to surfaces and simultaneously activates innate and adaptive components of the immune system. The multipronged attack by probiotics are more efficient than just relying on antibiotics to disrupt cell wall structures and or poison metabolic pathways in pathogenic agents.

Keywords: Probiotics; pathogens; antibiotics; resistant Strains.

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1. INTRODUCTION

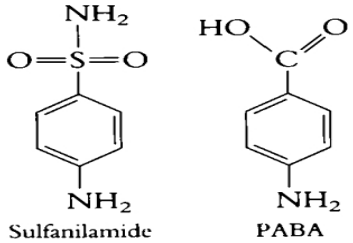
The World Health Organization defines probiotics as “live microorganisms” which when administered in adequate amounts confers a health benefit on the host [1]. Probiotics are bacteria and the successful growth of these organisms depends on indigestible carbohydrates that are available in thousands of different plants and fruits. Collectively the nutrients that probiotics need for “survival” are called prebiotics. The term synbiotic is used when probiotics and prebiotics are presented together [2]. A major role of probiotics is to protect the host by displacing and preventing colonization of pathogenic agents. That is, infection is reduced and disease prevented when pathogenic agents are not successful in adhering to sites in the gastrointestinal tract [GI], upper and lower respiratory tracts, colon, genitals, and urinary tract [3].

Other equally important mechanisms employed by probiotic in disease prevention include the production of bacteriocins and other products that result in a drastic change in pH with the creation of an acidic environment to hinder the survival of pathogenic agents [4]. It is well documented that probiotics enhances immune activation and the subsequent production of antibodies of IgM, IgG and IgA classes [5,6,7]. Antibody isotypes such as IgM and IgG target the pathogenic agents for destruction by complement proteins and phagocytic cells [6,7,8]. Cellular immune mechanisms are enhanced by probiotic agents with a subsequent recruitment of macrophages to damage and destroy pathogenic agents. In this review the common modes of action of probiotics in phylogenetically distinct hosts are discussed.

1.1 History of Probiotics

Our initial understanding of the importance of probiotics as immune modulators were advanced by Elie Metchnikoff [9]. Dr. Metchnikoff was awarded the Nobel prize in 1908 for his discovery of phagocytosis and the importance of macrophages and microphages (neutrophils) in killing pathogenic bacteria. Dr. Metchnikoff proposed that useful microbes (probiotics) would modify the gut flora and replace harmful microbes by beneficial bacteria. Metchnikoff saw the aging process as a disease that results from microbes producing toxic substances in the large bowel. The toxic substances were responsible for what he called "intestinal auto-intoxication" which caused the physical changes associated with old age. The following were the basis for his thesis: (a) milk fermented with lactic-acid bacteria inhibits the growth of proteolytic bacteria because of the low pH produced by the fermentation of lactose and (b) rural populations in Bulgaria and Russian incorporated fermented milk in their diets were exceptionally long lived in those countries. Based on these observations Metchnikoff proposed that consumption of fermented milk would repopulate the intestine with harmless lactic-acid bacteria and the products from the probiotic organisms would decrease the intestinal pH and subsequently suppress the growth of harmful bacteria.

Antibacterial Compounds in Disease Prevention



Sulfanilamide PABA

For over 75 years, antibiotics have played and continue to play prominent roles in limiting growth and in killing both pathogenic and nonpathogenic microbial agents [10]. In 1939 Dr. Gerhard Domagk received the Nobel Prize in medicine for his discovery of a sulfonamide, a synthetic antibacterial compound (called prontosilrubrum), which was effective against pathogenic strains of Gram-positive bacteria [including staphylococci and streptococci], Gram-negative bacteria and some protozoans as well. Sulfonamides were the first systemic antibacterial drugs used in humans. The drug kills by poisoning metabolic pathways in pathogenic and nonpathogenic bacteria [11,12]. All cells require folic acid for growth. Folic acid diffuses or is transported into human cells. However, folic acid cannot cross bacterial cell walls by diffusion or active transport [13]. For this reason bacteria must synthesize folic acid from para-aminobenzoic acid [PABA]. Sulfonamides interfere with folic acid synthesis and inhibits incorporation of para- aminobenzoic acid into dihydrofolic acid preventing the formation of folate that is vital for growth of microorganisms. Resistance to sulfonamides may develop when bacterial mutations results in excessive production of PABA, low affinity of sulfonamides for folic acid synthesizing enzyme and the loss of cell permeability to sulfonamides. Nonetheless, sulfonamides are useful in treating urinary tract infections, but the single use of the drug contributes to the development of resistant bacterial strains. Therefore, fixed drug combination of trimethoprim-sulfamethoxazole [Bactrim] has supplanted many previous clinical cases involving the strict use of sulfonamides [14].

1.2 Antibiotics and Prebiotics

Dr. Selman Waksman [1964] coined the term antibiotic which he defined as any substance produced by a microorganism that kills or inhibits the growth of other microorganisms. This definition excludes substances that kill bacteria that are not produced by microorganisms such as synthetic antibacterial compounds (sulfonamides). At the other end of the spectrum prebiotics describes a "substance(s)" secreted by one microorganism which stimulates the growth of "another" microorganism [15]. Collectively the nutrients that probiotics need for "survival" are called prebiotics (Table below).

Classification and Sources of Prebiotics

Classification	Origin/manufacturing procedure
Disaccharides	
Lactulose	Lactose synthetic
Lactitol	Lactose synthetic
Oligosaccharides	
Fructo-oligosaccharides	Legumes, vegetables, extracts/ hydrolysis of cereals
Soybean oligosaccharides	Extraction/hydrolysis of soy bean
Xylo-oligosaccharides	Plant sources
Trans galacto-oligosaccharides	Lactose synthetic
Polysaccharides	
Inulin	Extracts obtained from legumes, vegetables, and cereals
Resistant starches	Extracts obtained from legumes, vegetables, and cereals

Permission to copy Table obtained from Editor of JNTRUHS, November 2012

1.3 Indiscriminate Use of Antibiotics

The indiscriminate use of antibiotics has resulted in the emergence of many drug resistant strains. The data show that the wide spread use of antibiotics in the United States from 1999 to 2000 lead to 292,000 hospitalizations for staphylococcal infections and of that 126,000 or 43.15% were drug resistant [16]. Eighty million prescriptions for antibiotics for human use were filled in 1998 [17]. Actually, the 80 million prescriptions that were filled underestimates the full human exposure to antibiotics. To better approximate total exposure one must also consider the presence of antibiotics in livestock and agricultural foods as well. For example, agricultural practices account for over 60% of antibiotic usage in the United States. In terms of antibiotic resistance approximately 70% of bacteria that cause infections (staphylococci and pneumococci) in hospitals do not respond to common drug treatment and suggests the need to develop strain specific probiotics to contain infections and prevent diseases [17]. Antibiotic resistance can be associated with significant morbidity, longer hospitalization, and more expensive antibiotic therapy. The economics of antibiotic resistance has been investigated and the results are in the figures below [18].

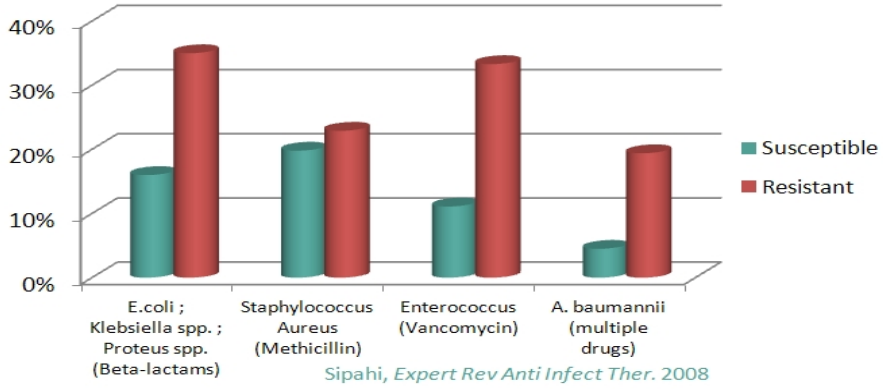


Fig. Mortality rates of infections by antibiotic resistant/susceptible bacteria

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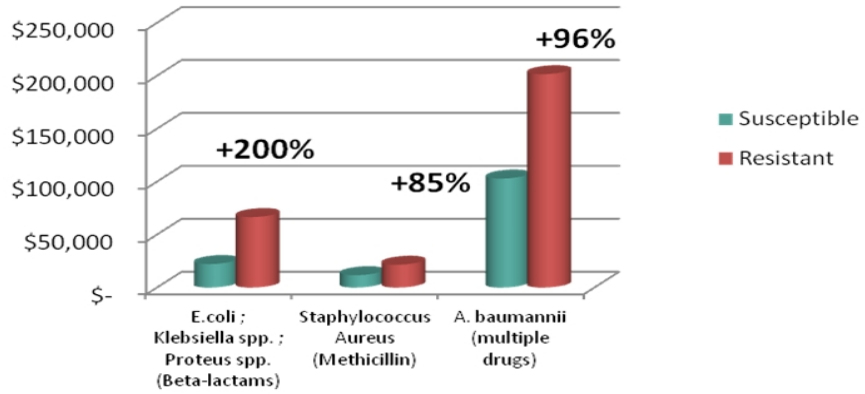

Fig. In-hospital costs for treating antibiotic resistant/susceptible infections

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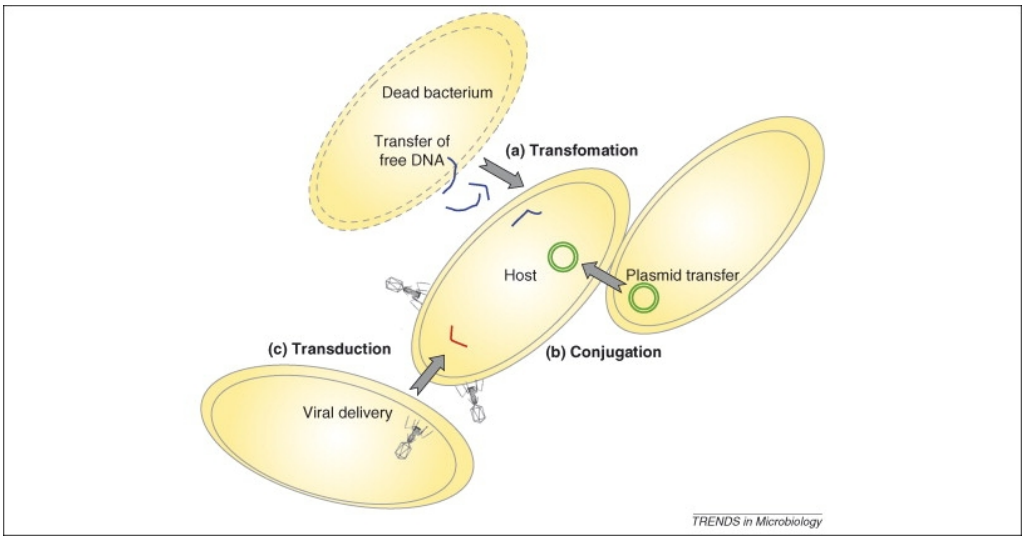
1.4 Mechanisms of Antibiotic Resistance

There are at least three mechanisms that bacteria employ to acquire antibiotic resistance and those are conjugation, transduction and transformation [19,20,21]. Conjugation involves transfer of genetic material between bacteria through direct cell to cell contact. Transduction is the process by which bacteria acquire DNA from their environment and transformation describes the uptake of extra-chromosomal elements from one bacterial cell to another via bacteriophages or plasmids (diagram below).



Trends in Microbiology, Volume 16, Issue 7, July 2008, Pages 303-308

It should be clear that a multipronged attack by probiotic organisms and their products (bacteriocins etc.) are attractive alternatives to kill pathogenic agents because killing involves displacing and preventing pathogenic agents to adhere to specific sites in the GI tract. Additionally, products from probiotics can kill pathogenic agents because the acidic environment produced by these products will drastically alter the pH and make it difficult for the pathogenic strains to survive. Conceivably the acidic conditions contribute to improper protein folding on the agent, the creation of neoepitopes and the subsequent activation of antibody classes and phagocytic cells to kill pathogenic agents. In 2006 the European Union [E.U.] banned the use of antibiotic growth promoters in animal feed supplementation but allowed the use of probiotics as a viable alternative to antibiotics [22]. Probiotic feed supplementation benefits the animal host directly, by preventing infection and augmenting the host's immune responses as described above.

1.5 Probiotics and Dendritic Cells

Dendritic cells are the quintessential antigen presenting cells that regulate the adaptive arm of the immune system. These cells have the ability to take up antigens directly by extending their dendrites into the lumen or indirectly after transport of the antigens by M cells overlying Peyer's patch. In a recent article in the World Journal of Gastroenterology investigators described the activities of probiotic modulation of dendritic cells co-cultured with intestinal epithelial cells [23]. In that study the researchers demonstrated that probiotics augmented

the surface expression of the co-stimulatory molecules CD80 [B7-1], CD86 [B7-2], CD40 and the major histocompatibility complex [MHC] class II on dendritic cells to maximize their interaction with T and B cells. The traffic of dendritic cells through the lymphatics to the mesenteric lymph nodes, mediates the homing of activated effector/memory T cells, lgA-secreting B cells and stimulates regulatory T cells to produce interleukin IL-10 and transforming growth factor [TGF-β]. Hence, probiotics are very important in the stimulation of dendritic cells to better recognize pathogenic agents via Toll like receptors and to present immunogenic peptides to the adaptive immune system to control infection and prevent disease in the host.

1.6 Probiotics and Viral Activity

Viruses are a major cause of farm animal diarrheal disease, including transmissible gastroenteritis virus [TGEV] and rotavirus [RV]. Viruses do not have their own metabolic machinery but rely upon the host cell metabolic pathways for replication. Therefore, antibiotics do not have effects on viral activities. Probiotics are needed to stimulate macrophage activation with the subsequent release of inhibitory cytokines to control viral infection [24]. Investigators examined the potential antiviral activity of probiotic lactic acid bacteria [LAB] employing animal and human intestinal and macrophage cell line models of non tumor origin [24]. Various probiotic strains were found to exhibit moderate to complete monolayer protection against rotavirus or transmissible gastroenteritis virus disruption. However, the highest protective effects were recorded with *Lactobacillus rhamnosus GG* and *Lactobacillus Casei Shirota* strains against both rotavirus [RV] and transmissible gastroenteritis virus [TGEV]. Antiviral activities were also attributed to following probiotic strains: *Enterococcus faecium* PCK38, *Lactobacillus fermentum* ACA-DC179, *Lactobacillus pentosus* PCA227 and *Lactobacillus plantarum* PCA236 and PCS22 [24]. The study demonstrated that probiotic bacteria can activate macrophages to release nitric oxide, hydrogen peroxide and other reactive oxygen species [ROS] to damage viruses and help protect the host from infections (Table below).

Reactive Oxygen Species [ROS]

Superoxide	$O_2^{\cdot}$
Hydroxyl	$OH^{\cdot}$
Hydroperoxyl	$HOO^{\cdot}$
Singlet oxygen	$^1O_2$
Ozone	O_3
Hypochlorous acid	HOCl
Hydrogen peroxide	H_2O_2
Nitric oxide	NO

Battino et al., 1999 [25]

1.7 Probiotics and Bacterial Sepsis

Bacterial sepsis is life threatening and results from the excessive production of inflammatory cytokines [26]. The cytokines are produced as a consequence of the interaction of cells in the

immune system with bacteria and its cell wall constituents. Some bacterial products enhance disease in the host by functioning as superantigens. Superantigens [Sags] are microbial products that have the ability to promote massive activation of immune cells, leading to the release of inflammatory mediators that can ultimately result in hypotension, shock, organ failure and death. Superantigens achieve this by simultaneously binding and activating major histocompatibility complex class II molecules on antigen-presenting cells and on T-cell receptors bearing susceptible V regions. Sepsis is a serious clinical condition that represents a patient's response to a severe infection and has a very high mortality rate [27]. Normal immune and physiologic responses eradicate pathogens, and the pathophysiology of sepsis is due to the inappropriate regulation of these normal reactions. In an ideal scenario, the first pathogen contact with the inflammatory system should eliminate the microbe and quickly return the host to homeostasis. The septic response may accelerate due to continued activation of neutrophils and macrophages/monocytes. Up regulation of lymphocyte co-stimulatory molecules, rapid lymphocyte apoptosis, delayed apoptosis of neutrophils, enhanced necrosis of cells/tissues also contribute to the pathogenesis of sepsis. Probiotics do have roles in controlling the overproduction of mediators from immune cells that lead to bacterial sepsis. The use of probiotics to kill pathogenic bacteria in the host is needed to remove sources of “superantigens” to prevent uncontrolled activation of T cells in bacterial sepsis.

1.8 Components of Probiotics in Disease Prevention

Investigators employed components rather than the intact probiotic bacterium to determine if the components can activate proinflammatory cytokines from immune cells in their studies [28]. The researchers produced bacteria-free, lysozyme-modified probiotic components [Lz MPC)] by treating the probiotic bacteria, *Lactobacillus* sp., with lysozyme. The study demonstrated that oral delivery of Lz MPC effectively protected rats against lethality from poly microbial sepsis induced by cecal ligation and puncture. Orally administrated Lz MPC was engulfed by macrophages in the liver and protection was associated with an increase in bacterial clearance in that same organ. *In vitro* studies demonstrated that Lz MPC up-regulated the expression of cathelicidin-related antimicrobial peptide (CRAMP) in macrophages and enhanced bactericidal activity of these cells. Cathelicidins are the precursors of potent antimicrobial peptides and have been identified in several mammalian species. Functional studies demonstrated CRAMP to be a potent antibiotic against Gram-negative bacteria by inhibiting growth of a variety of bacterial strains [29]. Macrophages from LzMPC-treated rats had an enhanced capacity of cytokine production in response to LPS or LzMPC stimulation. Therefore, it was concluded that LzMPC, a novel probiotic product, is a potent immunomodulator for macrophages and may be beneficial for the treatment of sepsis.

Components of probiotics have been used with success to augment innate and adaptive responses in fish. Immunization with extracellular, cell wall and whole cell proteins from probiotic Kocuria SM1 and Rhodococcu in rainbow trout provided significant protection in fish that were challenged with Vibrio Anguillarum. The increased protection ranged from a significant increase in respiratory burst activity with heighten production of reactive oxygen intermediates and elevated leucocyte and antibody levels [30]. The ability of probiotic components to activate innate and adaptive responses in the host is an added layer of protection in preventing disease by pathogenic agents.

1.9 Probiotics and Fish Aqua Farming

Approximately a fourth of the world's populations obtain their protein from fish [31]. To cultivate freshwater and saltwater populations of fish under controlled conditions the aqua farming industry has utilized growth promoters, antibiotics, and probiotics to prevent disease and to increase fish production. Studies have shown that probiotics are important for weight gain in fish. Probiotics protect fish by: (a) blocking adhesion sites for pathogens, (b) production of organic acids (formic acid, acetic acid, lactic acid) to lower pH and alter protein structure (c) production of hydrogen peroxide and reactive oxygen species to damage enzyme systems in pathogens and (d) the activation of innate and adaptive immune responses to amplify killing of pathogenic agents (Table below).

Probiotics have been used with success in controlling pathogenic agents that effect the shrimp aqua farming industry in New Caledonia (New Caledonia is a French-administered territory that is located in the southwest Pacific Ocean, 750 miles east of Australia and 10,026 miles east of France). In New Caledonia, domesticated shrimp is resistant to Infectious hypodermal and hematopoietic necrosis virus (IHHNV) a widely distributed single-stranded DNA parvovirus that is responsible for major losses in wild and farmed shrimp populations. However two bacterial diseases Winter Syndrome caused by *Vibrio peneicida* and Summer Syndrome caused by *Vibrio nigripulchritudo* have affected the production of farmed shrimp. The probiotic Bacillus strains have been used with success in lowering the abundance of the pathogenic vibrios by colonizing the shrimp intestinal tract and displacing vibrios in the gut [32].

In fish the IgM antibody class is the predominant isotype of the adaptive humoral immune system with four not five monomeric units as exists in vertebrates [33]. In man and fish, IgM antibodies are potent activators of the complement system and both the complement system and the immune system communicate with each other to control pathogenic infections. In fish cellular immune response are equally important in terms of the roles of macrophages and dendritic cells to present immunogenic peptides to activate T cells to further orchestrate the killing of intracellular pathogens.

Components of the Fish Immune System

Non specific Immunity	Specific Immunity / Anamnestic responses
Skin and Mucus Lysozyme lectins Lytic enzymes Transferrin Complement system Monocytes Tissue macrophages Granulocytes (neutrophils) Cytotoxic cells Macrophage activation via cytokines increases the killing ability of these cells	**T cell mediated responses** Intra cellular pathogens controlled by T cell mediated immunity Macrophage activation and presentation of immunogenic peptides to T cells **B cell responses** Restricted to tetrameric IgM Antibody is not transduced from the serum but is produced locally (i.e., by lymphocytes in the skin). Tetrameric IgM Needed for Opsonization of bacteria Needed for Neutralization of toxins Needed for Neutralization of viruses

1.10 Probiotics and Apiculture (Beekeeping)

The Bee immune system recognizes specific proteins present on a wide variety of pathogens. Bees have “constitutive” or “innate” defenses such as roaming hemocyte cells and enzymes in the hemolymph. The hemocytes recognize invaders via pattern recognition receptors and signals the enzyme phenoloxidase to initiate a cascade of chemical responses involving the production of reactive oxygen species to kill the invader. This system consists of four non-autonomous pathways implicated in inducible host defense, Toll, Imd, Janus kinase (JAK)/STAT and JNK [34]. The system responds quickly to bacterial infections. It has been demonstrated that a mealworm beetle can nearly clear its system of an injection of 4 million bacteria in about 30 minutes [35]! In Honey Bees hemocytes release chemicals that penetrate the cell nuclei in bees, to up regulate immune response genes for the production of primary RNA transcripts and to have those transcripts translated to mRNA for the synthesis of antimicrobial peptides to kill the invading pathogens.

Probiotic research has been used with success with apiculture (beekeeping) and social insects [36]. Scientists have investigated bacterial probiotics to induce an immune response in the Honey Bee [*Apismellifera*] [37]. A primary goal of honey bee research remains to breed bees that resist or tolerate pests and pathogens. Investigators have focused on the abilities of bees to inhibit pathogens through their internal “immune” defense systems. The researchers are particularly interested in immune responses toward *Paenbacillus larvae larvae*, a Gram-positive bacterium responsible for the widespread Honey Bee disease known as American foulbrood (AFB) [38,39]. Evans and Lopez proposed the use of probiotics to enhance honey Bee immunity to help bee larvae, and other life stages known as instars [developmental stage of the larvae)] survive attacks from pathogenic mites in the field. Common disease agents in bees are restricted to several pathogens, two of which are the Gram-positive bacterium Paenibacillus and the *fungus Ascosphaera apis*. Evans et al. have demonstrated that probiotics do enhance immune responses in the bee by stimulating the production of antimicrobial peptides to protect against Paenibacillus and Ascosphaeraapis infections [37].

Pathways that are components of the immune system in insects

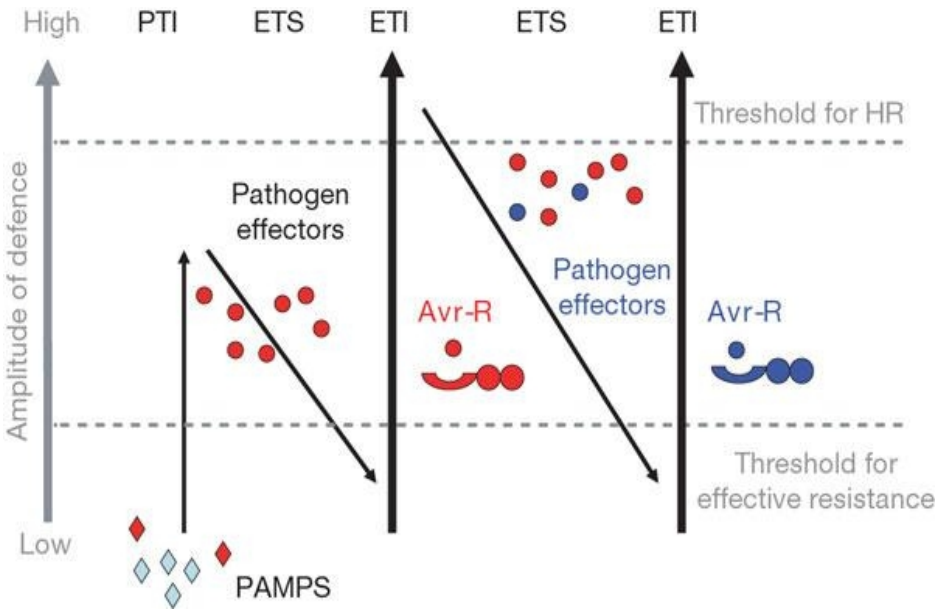
Toll Pathway	Receptors recognize components of Gram-positive bacteria
IMD Pathway	Receptors recognize components of Gram-negative bacteria
JAK Pathway	**JAK activation occurs upon ligand-mediated receptor multimerization. This occurs when two JAKs are brought into close proximity allowing trans-phosphorylation.**
STAT Pathway	STATs are latent transcription factors that reside in the cytoplasm until activated.

1.11 Probiotics and Plant Growth

Plants lack mobile defender cells and a somatic adaptive immune system. Instead, they rely on the innate immunity of each cell and on systemic signals emanating from infection sites. The immune system in plants consists of two branches [40]. One branch employs transmembrane pattern recognition receptors (PRRs) that respond to slowly evolving microbial- or pathogen-associated molecular patterns (MAMPS or PAMPs), such as flagellin. The second branch is very active inside the cell, using the polymorphic NB-LRR protein products encoded by most R genes. Additionally, many of the innate immune receptors or

disease resistance (R) proteins contain a NB-LRR (Nucleotide-binding site, Leucine-rich repeat) structure.

A biochemical basis for disease in plants involves a direct interaction between a pathogen-derived avirulence (Avr) gene product and a receptor protein, which is encoded by the matching resistance (R) gene of the host plant. Investigators in Dr. Mark Holland's laboratory are studying a type of *Methylobacterium* described as pink-pigmented facultative methylotrophic or PPFM bacteria that are found in plants and can be called the probiotics of plants [41]. These bacteria are found in relatively large numbers on all kinds of plants and in seeds. Research in his laboratory demonstrated that seeds cured of their PPFMs no longer germinate well or develop normally, but reinoculating the cured seeds with a population of the bacteria restores growth, higher crop yields and increased nutritional quality of plants. The four phase diagram below illustrates events for susceptibility and infection in plants by pathogens. Presumably probiotic agents specifically PPFM bacteria enhances plant immunity and interferes with the steps needed by pathogens to cause infection and disease.



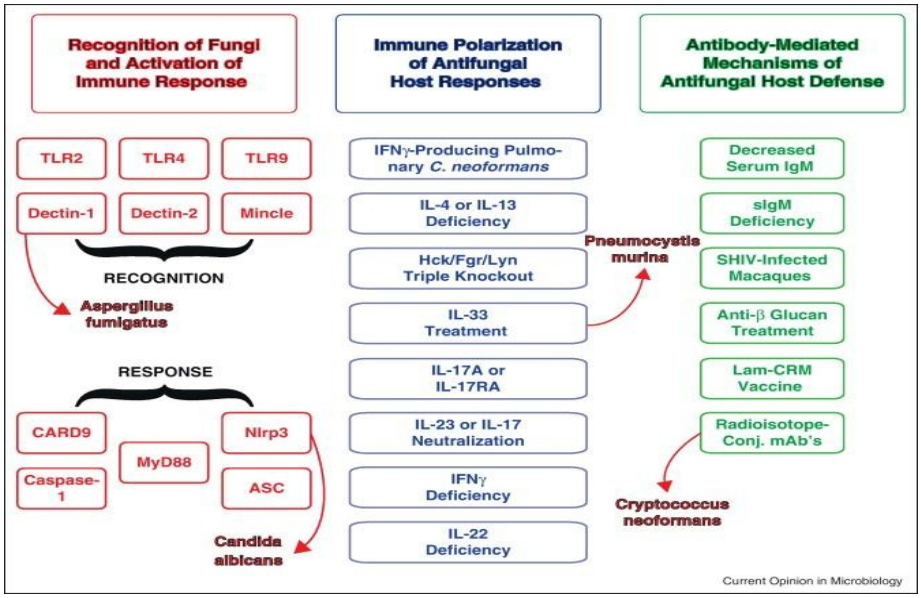
Jones, Nature 2006. Vol 444. A zigzag model illustrates the quantitative output of the plant immune system

In this scheme, the ultimate amplitude of disease resistance or susceptibility is proportional to [PTI – ETS + ETI]. In phase 1, plants detect microbial/pathogen-associated molecular patterns (MAMPs/PAMPs, red diamonds) via pattern-recognition receptors (PRRs) to trigger PAMP-triggered immunity (PTI). In phase 2, successful pathogens deliver effectors that interfere with PTI, or otherwise enable pathogen nutrition and dispersal, resulting in effector-triggered susceptibility (ETS). In plants, many of the innate immune receptors or disease resistance (R) proteins contain a NB-LRR (Nucleotide- binding site, Leucine-rich repeat) structure. In plants a biochemical basis for disease involves a direct interaction between a pathogen-derived avirulence (Avr) gene product and a receptor protein, which is encoded by the matching resistance (R) gene of the host plant. In phase 3, one effector (indicated in red) is recognized by an NB-LRR protein activating effector-triggered immunity (ETI), an amplified

version of PTI that often passes a threshold for induction of hypersensitive cell death (HR). Finally, in phase 4, pathogen isolates are selected that have lost the red effector, and perhaps gained new effectors through horizontal gene flow (in blue)—these can help pathogens to suppress ETI. Presumably PPFM bacteria stimulates new plant NB-LRR alleles that can recognize one of the newly acquired effectors, resulting again in ETI.

1.12 Probiotics and Fungal Infections

Phytopathogenic fungi cause a decrease in quantity and quality of crops in plants and acute toxicity in humans and livestock. Fungi produce mycotoxins in a wide variety of grains and foods. Mycotoxins are metabolic products produced by fungi that prevent bacteria or other fungi from growing in the same area. When those metabolic products cause health problems in animals or humans, they are called mycotoxins. Mycotoxins are natural products that are highly stable and cannot be destroyed by boiling and is believed to play important roles in some types of cancers, immunosuppression, and nervous disorders. Mycotoxins can be metabolized by livestock fed contaminated grains and it can be found in milk, eggs, and other organs of the domesticated animals [42]. Probiotics have been shown to have a beneficial effect on preventing the growth of fungi in animals and plants with a significant reduction in the production of mycotoxins [43]. The diagram below summarizes the importance of Innate and adaptive immune responses in the control of fungal infections. It also shows how cytokine and immunoglobulin deficiencies contribute to inability to remove the pathogen and enhances the disease process in vertebrate hosts [44].



Steele, Current Opinion in Microbiology Nov 16, 2012

1.13 Probiotics and the Neonate

Probiotics are often targeted for neonatal applications. Specifically, necrotizing enterocolitis remains an enigmatic and potentially devastating condition with high morbidity and mortality. Recent studies have demonstrated that prophylactic administration of probiotics to preterm

neonates decreases both the incidence and severity of subsequent necrotizing enterocolitis [45]. Probiotics play a vital role in neonatal immune responses to environmental antigens. For example in allergic disease in the neonates probiotics amplifies a TH2-type cytokine profile [IL-3, IL-4, IL-5 and IL-13] to help control the disease [46,47]. It is of interest that allergic diseases have increased substantially in developed countries because of a reduced exposure of neonates to microbial stimuli with a subsequent increase in TH2 versus a TH1 cytokine profile [48].

Probiotics have been shown to have several effects that might be of benefit to the neonate, including: modulating the establishment of intestinal microbiota, degrading antigens, promoting mucosal barrier functions, inhibiting mucosal pathogen adherence, and enhancing the maturation of the innate and adaptive immune systems. Results from clinical trials suggest that specific probiotics might be useful in reducing the risk of necrotizing enterocolitis and infectious disease in infancy. In addition, probiotic supplementation commenced in the neonatal period might reduce the risk of atopic disease in later life [49].

1.14 Specificity of Probiotics

Clinical or laboratory effects of one probiotic cannot be assumed for another probiotic species or for different strains of the same species [50]. In this regard *Bifidobacterium* species isolated from human feces were found to be genetically heterogeneous. Different strains *Bifidobacterium* varied significantly in terms of acid and oxygen tolerance and growth conditions. Such variations were confirmed in murine investigations with a wide range of clinical effects among the probiotic strains of *Bifidobacterium*. The effects of four different probiotic species *[L. reuteri, L. acidophilus*, LGG and *B. animalis*] in preventing colonization and sepsis with Candida albicans in both athymic and euthymic mice were studied [51]. The four probiotic species were protective, but there were significant differences in efficacy and a great diversity of immune effects in terms of antibody and proliferative responses to *C. albicans* and intestinal inflammatory cell infiltration. *In vitro* studies supported the diversity of actions of different probiotics and demonstrated that antagonistic effects were present among one of the strains. Studies of the effects of *Bifidobacterium* species on dendritic cell function have shown marked variation among the species. In terms of variation among the species LGG has specific effects in enhancing immunoglobulin A [IgA] responses against rotavirus that are completely absent with other *Lactobacillus* species. In 1985, Gorbach and Goldin isolated a probiotic strain of *Lactobacillus* which was designated *Lactobacillus* GG [LGG] [52]. *Lactobacillus* GG is named after co-discoverers, Sherwood Gorbach and Barry Goldin. LGG is the best-studied and most extensively documented probiotic lactic- acid bacteria strain. The strain stabilizes human intestinal micro flora and hastens the removal of pathogenic microorganisms. Its beneficial effects in treating gastrointestinal disorders and bacterial and viral infections are well documented.

Probiotic strains in combination vary in their levels of protection. In the treatment of infective diarrhea, S. thermophilus and *L. bulgaricus* were ineffective, whereas *L. acidophilus* and *L. bifidus* were effective [53]. Therefore, one cannot generalize the effects of one probiotic strain to another, even within the same species. However, in some clinical scenarios, a range of different probiotics appear to be effective—presumably by acting through a mechanism common to a range of nonpathogenic microbes. Additional work is needed to clarify the relative importance of strain-specific effects in different scenarios and the nature of probiotic-probiotic interactions. Probiotic bacteria provide a variety of health benefits to immunocompetent hosts; however, their use in immunodeficient patients may pose problems. Some probiotics are closely related to bacteria that are opportunistic pathogens

and can transfer antibiotic resistance genes. Intestinal bacteria have recently been associated with inflammatory and autoimmune diseases in immune deficient hosts; similar problems may arise if probiotics are fed to immunodeficient patients. The safety, efficacy, benefits and costs of feeding probiotic bacteria to immunodeficient patients must be considered and fully researched. This will ensure that probiotics will not cause infectious, inflammatory or autoimmune diseases in susceptible hosts.

2. DISCUSSION

Probiotics are effective because they orchestrate killing of pathogens by the innate and adaptive arms of the immune system [modified Table [54]. Probiotics work by blocking sites on host tissue that are necessary for the adherence of pathogenic agents. They produce bacteriocins and activate macrophages to release reactive oxygen species to limit infection by pathogens. The reduction in the pH by products from probiotics creates an acidic environment that will make it difficult for pathogenic agents to survive.

Normal flora consists predominately of harmless and beneficial bacteria. However, the indiscriminate use of antibiotics will select for resistant strains that have the potential to become pathogenic. Pathogenic strains that gain entry via breaks in the skin, inhalation or in contaminated foods may lead to disease and the death of the vertebrate host. Distinct hosts such as veterbrates, fish, plants, insects etc. have developed unique innate and “adaptive” systems to kill pathogens and prevent disease. Extra cellular fluids in those hosts have lysozyme like substances and other hydrolytic enzymes to control infection. The presence of Toll like and IMD like receptors on immune and non-immune cells recognizes broad categories of microbial agents. Probiotics produce antimicrobial products and augments killing of microbial pathogens by host defense mechanisms to prevent disease.

Innate Immune System	**Acquired immune system**
Physical barriers Skin and Mucous membranes prevents the entry of antigens into systemic circulation Cell-mediated barriers Phagocytic cells, e.g. neutrophils, macrophages, engulf foreign antigens Basophils, mast cells release inflammatory mediators, such as histamine, prostaglandins Natural killer cells destroys infected or malignant cells Dendritic cells present antigens to lymphocytes Soluble factors Cytokines activate/recruit other cells Complement proteins enhance phagocytosis Acute-phase proteins promote repair of damaged tissue	B-lymphocytes are transformed into plasma cells which secretes antibodies T-lymphocytes CD4+ T-cells induce activation of lymphocytes T_{H1} cells promote cell-mediated responses T_{H2} cells promote humoral (antibody) responses CD8+ T-cells Cytotoxic T-cells Destroy infected or malignant cells Suppressor T-cells Suppress activity of lymphocytes

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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