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REVIEW ARTICLE

# Probiotics - A Review

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**Abstract:** Probiotic bacteria are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. While this beneficial effect was originally thought to stem from improvements in the intestinal microbial balance, there is now substantial evidence that probiotics can also provide benefits by modulating immune functions. Scientists continue to work on elucidation of the mechanisms of the most common probiotic strains. The results that might arise from could be extremely important because the use of probiotics to maintain health must be considered promising, although much remains to be elucidated. The universal use of some strains seems less reasonable from an ecological point of view than selection of strains from their natural habitat were they are adapted to the ecological niche. It is important to understand that all probiotic strains are unique and different and their properties and characteristics should be well defined.

**Keywords:** *Culture based method, Structural target probed, Fluorescent in-situ hybridization, Immunomodulation.*

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## Introduction

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2001). Probiotics have a long history of safe consumption in fermented foods such as yogurts and pickled edibles and considerable interest exists in their use as food additives and supplements. *Lactobacillus* and *Bifidobacterium* constitute the bacterial genera most frequently employed in probiotic preparations for human use. Probiotic preparations must meet strict criteria related to quality, safety and functionality [1]. A key quality criterion is that they contain accurately defined numbers of viable cells as expressed on the product label.

Some investigators, however, have found that commercial products did not contain the stated cell numbers [2] but had significantly lower levels than reported [3, 4]. As probiotics are live organisms, it is critical to enumerate accurately the population of viable microbes in the preparation and express this information to the consumer on the product label. Several significant challenges exist. First, culture-based enumeration of specific organisms requires specialized and standardized methodologies, which will only detect bacteria that are able to replicate on synthetic media and under specific conditions. Use of culture-independent techniques, with a more holistic definition of viable probiotic bacteria, have the potential to provide direct, rapid enumeration methods for both researchers and industry-based scientists faced with the challenge of providing the dose available for the final product. Standardized methods are available for a limited number of species in certain dairy products, such as publications from the International Organization of Standardization (ISO) regarding enumeration standards for *Lactobacillus acidophilus* (ISO 20128/IDF 192:2006) and *Bifidobacterium* (ISO 29981/IDF 220:2010).

Secondly, a consensus on the operational definition of live, viable cells needs to be established. Most probiotic strains are well adapted to living in or on the mammalian host, but may be poorly adapted to other environments [5].

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When subjected to environmental stress during formulation and storage, constituent microbes may transition to a viable but non culturable state (VBNC), a protective response in which they are dormant yet metabolically active [6, 7]. Microbes in this state can reestablish broad functioning and replicate when they encounter a more hospitable environment [7].

Because standard culture-dependent method enumerate replicating cells only, culture techniques may underestimate the numbers of viable organisms that contribute to the functional capacity of the probiotic preparation once constituent microbes reach the anatomical niche in the host to which they are well-adapted.

## Evaluation of Culture-dependent Techniques for Enumerating Probiotic Organisms

### *Availability and Reliability of Selective Media for Strains of Probiotic Interest*

Probiotics were initially characterized by their phenotypic characteristics (such as colony morphology) microscopic details (such as Gram stain reaction and cell morphology), and physiologic characteristics (such as fermentation patterns and enzymatic activity) [8].The range of selective media available to identify and enumerate strains of probiotic interest is relatively limited and it should be noted that no one single medium and/or set of techniques for isolation of the strain is applicable to all probiotic strains [9] as shown in Table 1.

Selective media for specific species of *Lactobacillus* are available; by contrast, members of the genus, *Bifidobacterium*, can be identified, but no standard selective media are available to differentiate among *Bifidobacterium* species [10, 11]. To overcome this, selective differential media have been developed, but the subjectivity of identification requires skilled personnel for reliable results. No single culture-based methodology is applicable to all probiotic organisms, as there is considerable variability between species and even strains in their response to plating procedures.

### *Quantification of Culturable Probiotic Microbes by Heterotrophic Plate Counts*

Quantification of bacteria in a given sample is routinely achieved by counting the total number of colony-forming units (CFUs) grown on an agar plate from serial dilutions, expressed as CFU per gram or mL of the original sample. This yields an estimate of the number of cells present based on a skilled interpretation of the number of colonies on a plate. It is a skewed estimate, as only cells that can form colonies under the given experimental conditions (e.g. incubation media, temperature, time, and oxygen conditions) are counted.

Colonies may arise from individual cells or from cell clusters that happened to be sufficiently separated after plating to be distinguished following growth. Thus, depending on original concentration estimates prior to dilution, a colony could arise from one cell or several thousand. Hence, they are referred to colonies, not cells. Reliable quantitation requires an acceptable range of countable colonies on a plate.

These are based on historical ranges and have been refined by various authoritative bodies. Commonly used ranges for countable numbers of colonies on a plate are 25-250 and 30-300 (Table 2). The matter of selecting plates to be used in computing a count becomes a matter of considerable judgment" [12].The upper limit of the enumeration is reached when bacteria compete for space and nutrients.

This depends on bacterial swarming behavior as well as the plating surface area, a critical factor when using small membranes instead of standard plates. TNTC (too numerous to count) can be reported in several ways. ASTM (1998) recommends reporting this as greater than the upper limit (e.g. a 1:10 dilution with more than 200 CFU on a spread plate would be reported as N2000 CFU/mL).

FDA's BAM recommends counting the colonies from the dilution giving plate counts closest to 250 and estimating the total number and then using that number as the estimated aerobic count. The lower limit of enumeration can be based on the limit of quantification (LOQ) (25 CFU, from a countable range of 25–250) or the limit of detection (LOD) (i.e. 1 CFU). ASTM recommendations rely on the LOD and to report that answer if no colonies are recovered.

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**Table 1: Selected examples of culture-based methods for identification/enumeration of probiotic strains**

| Methods and media for selective enumeration of probiotic strains based on viable replicating technique | | | |
|---|---|---|---|
| **Medium** | **Base** | **Selectivity/supplement** | **Notes** |
| *Bifidobacterium* spp. *Bifidobacterium* selective medium (BSM) | MRS (deMan, Rogosa, Sharpe) | Cysteine HCl and Mupurocin | Incubated for 72 h @ 37 °C; Potential concerns regarding development of Mupurocin-resistant *Staphylococcus aureus* |
| NPNL (Neomycin sulfate, paromycin, nalidixic acid, and Lithium chloride) | MRS or BL (blood–liver–glucose) | Neomycin sulfate, paromycin sulfate, nalidixic acid and lithium chloride | When L-cysteine not present, *Bifidobacteria* do not grow or form pinpoint colonies; Time consuming to prepare |
| Raffinose *Bifidobacterium* medium | LCL(liver–cystein lactose) | Proprionate, lithium chloride, and raffinose | Antibiotic free-medium Some *B. bifidum* strains do not grow well on this agar |
| MRS-raffinose | MRS | Raffinose, lithium chloride (0.05%) | Incubation @ 45 °C specific for enumeration of *B. lactis* BB12 LiCl inhibits lactobacilli |
| *Lactobacillus acidophilus group (L. acidophilus, L. johnsonii, L. gasseri, L. crispatus)* | | | |
| MRS-clindamycin | MRS | Clindamycin | Anaerobic incubation @ 37 °C for 72 h Use of antibiotic for suppression |
| X-Glu | Rogosa agar | 5-Bromo-4-3-indoyl-ß-Dglucopyrananoside | Visualization of the ß-D glucosidase activity. More selective than MRS and Rogosa for yogurt and related products |
| *Lactobacillus casei* group *(L. casei, L. paracasei, L. rhamnosus*) MRS-salicin | MRS | Salicin | Conflicting reports; Cannot be used in products containing *L. acidophilus* |

Different regulatory bodies have suggested and/or identified acceptable ranges of colonies to count from spread plates over the years since the original 1916 [12] as shown in Table 2.In summary, culture-based techniques provide estimates of those microbes that are capable of replicating under experimental conditions. Selective media exist only for a limited subset of potential strains of interest. Reliable plate count enumeration is based on a relatively narrow countable range (generally considered to be 25-250 CFU bacteria on a standard Petri dish) and the lack of consensus on the use of a LOD (1 CFU) or LOQ for the lower limit of quantitation introduces a larger degree of variability than is necessary. It is also worth noting that although counts of CFU follow a Poisson distribution, mention is rarely made of the transformation used to approximate a normal distribution prior to the use of normal statistical analytical tools. Consequently, despite its common usage, the plate count method does not support precise, reproducible estimations of cell densities of probiotic strains, especially in mixed cultures [13]. Moreover, it estimates only the subset of viable organisms that replicated under the conditions of culture. It should also be noted that beyond the art and skill of the

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technician to culture the sample under the correct environmental conditions, that rapid turnaround is not possible as a minimum of 24-72 h of growth in an incubator is necessary before enumeration of colonies on agar plates is possible. The International Scientific Association for Probiotics and Prebiotics recognized that culture based analysis of strains can underestimate the number of viable cells and fails to account for the impact of bacterial growth modes [14].

**Table 2: Acceptable plate counts recommended by authoritative organizations and others.**

| CFU range acceptable | CFU range unsatisfactory | Notes |
|---|---|---|
| 50–200 CFU/mL of the average | <400> 30 | The number of colonies needed to be within 20% |
| 25–250 CFU/mL | - | - |
| 25–250 CFU/mL | - | - |
| 25–250 CFU/mL | - | - |
| 20–80 CFU/membrane, 20–200 CFU/spread plate, 30–300 CFU pour plate | - | - |

### Alternative Culture-independent Methods for Enumeration of Viable Microbes

In the recent years, alternative, culture-independent methods have been used to accurately enumerate probiotic strains based on viability and deliver results in a timely manner (Table 3). Enumeration techniques that lend themselves to quantifying viable cells either use dyes to differentiate live and dead cells by direct observation, measure the presence of an intact cell membrane (membrane integrity), or characterize some aspect of metabolic activity, such as the synthesis of nucleic acids, or respiration; these parameters indicate that the cells are alive even if they are unable to develop into colonies on culture media.

**Table 3: Published studies related to enumeration of probiotic strains by culture-independent techniques**

| Culture-independent methods for enumeration of probiotic bacteria | | |
|---|---|---|
| | Method | Structural target probed |
| Imaging | Fluorescent in situ hybridization (FISH) | Presence of nucleic acid |
| | Live–dead staining and microscopic counting | Cellular integrity |
| Molecular Biology | EMA or PMA-qPCR (vPCR) | Cellular integrity/Nucleic Acid |
| | Real-Time qPCR | Presence of nucleic acid |
| Cell Sorting | Quantification of 16S rRNA | Presence of nucleic acid |
| | MALDI-TOF mass spectrometry | Presence of nucleic acid |
| | Flow cytometry/FACS | Cell integrity or metabolic activity |

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*Direct Imaging and Visual Enumeration-Fluorescent in Situ Hybridization*

Bacteria in a sample can be directly visualized microscopically, but enumeration of viable microbes requires differentiating live and dead bacteria. Direct epifluorescent counting has been described as a suitable method for enumeration of total bacteria in environmental samples [15, 16].The optical sectioning capability of Confocal Scanning Laser Microscopy (CSLM) increases sensitivity and reduced out-of focus blur, enabling observation of subsurface structures of foods in situ [17].Digital acquisition of images by CSLM enables rapid enumeration of bacteria by digital image analysis [18].

This technique may be of value for the rapid estimation of viable bacteria in some dairy products, which could take over three days [19]. FISH consistently estimates higher yields than plate counts for dairy products but lower for cheese products and spray-dried cultures, highlighting the need for further work to establish the effect of the matrix. The use of this technique as well as the combination of species specific qPCR has allowed unequivocal methods for enumeration of probiotic strains into a variety of cheese products [19, 20].

**Nucleic Acid-based Enumeration Methods**

*Polymerase Chain Reaction (PCR)*

Detection of nucleic acid sequences (DNA, mRNA and r RNA) is a molecular technique that can be applied to bacterial enumeration. Most molecular analyses target amplification of nucleic acid to maximize analytical sensitivity. DNA amplification by PCR was investigated for enumeration of live bacteria based on the assumption that DNA would be degraded more rapidly after cell death than other cellular components and that intact DNA sequences would indicate cell viability [21].

Although most DNA detection is undertaken by PCR [22], hybridization-based detection methods also have been employed [23]. However the presence of DNA does not necessarily indicate viability, although the detection of longer intact DNA sequences correlates more closely with viability than shorter sequences.

*Reverse Transcriptase PCR (RT-PCR)*

RT-PCR is one of the many variants of PCR and allows multiple copies of a particular sequence through amplification. It should be noted that ribonucleic acid (RNA) is first transcribed in reverse into its DNA complement that utilizes the reverse transcriptase. Attention has turned to the use of mRNA as a marker of viability. This marker is a highly labile molecule with a very short half-life (seconds) in bacteria. Hence, detection of bacterial mRNA transcription should provide a more reliable indication of viability than DNA-based methods.

The most common amplification techniques for detecting mRNA are reverse transcriptase PCR (RT-PCR) and nucleic acid sequence based amplifications (NASBA) [24].Both have been applied to the determination of bacterial viability with variable success. More recently, reverse transcriptase-strand displacement amplification (RT-SDA) has been used as an indicator of bacterial viability [25]. Ribosomal RNA (rRNA) has also been investigated as an indicator of viability and can be positively correlated with viability under some bacterial regimes [22].

*Real Time-quantitative Polymerase Chain Reaction (RT-qPCT or qPCR)*

RT-qPCR is a DNA amplification technique that uses fluorescent reporter dyes to combine the amplification and detection steps of the PCR reaction in a single tube format whereas traditional PCR measures the accumulation of the PCR product at the end of all the PCR cycles, RT-qPCR quantifies PCR amplification as it occurs. RT-qPCR detection measures the increase in fluorescent signal, which is proportional to the amount of DNA produced during each PCR cycle.

A quantification cycle (Cq) value is determined by plotting fluorescence against the cycle number. Cq corresponds to the number of cycles for which the fluorescence is higher than the background fluorescence. RT-qPCR is a quantitative technique because data are collected during the exponential growth (log) phase of PCR when the quantity of the PCR product is directly proportional to the amount of template nucleic acid. Using this technique allows microbial populations to be quantified by measuring the abundance of a target sequence in DNA samples extracted from food products [26].

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Combined with reverse transcription (RT), this technique can also be used to estimate the amount of mRNA transcripts. An investigator could choose a particular transcript related to metabolic activity (such as production of lactic acid during fermentation) for a more direct indication of the activity of living cells.

*Ethidium Monoazide-PCR and Propidium Monoazide-PCR*

Ethidium monoazide-PCR (EMA-PCR) and propidium monoazide- PCR (PMA-PCR) are emerging techniques that limit enumeration to live cells [27] and can also be referred to as viability- PCR (vPCR) [28].Cells with intact membranes are assumed to be viable. Ethidium monoazide (EMA) is an azide-bearing, DNA-intercalating dye thought to enter only membrane-compromised cells. EMA covalently crosslinks DNA when the azide group converts to a highly reactive nitrene radical upon exposure to bright visible light.

Water simultaneously inactivates unbound EMA and the reaction product remains free in solution. EMA treatment is followed by genomic DNA extraction and qPCR analysis. Cross linking strongly inhibits PCR amplification of the modified DNA, such that only unmodified DNA (from presumptively intact cells) can be amplified.

EMA treatment in conjunction with qPCR led to signal reduction of up to four log10 units in the case of membrane-compromised cells [29].It was later shown that, in some bacterial species, EMA does penetrate cells with intact membranes [30].However, propidium monoazide (PMA), an analog of EMA that functions through similar chemistry, is efficiently excluded from cells with intact cell membranes, probably due to an increased positive charge. PMA-qPCR is applicable to a wide range of Gram-negative and Gram-positive bacteria.

This approach has been used successfully to assess the killing efficacy of disinfectants [30, 31] and to detect viable *Escherichia coli* and *Pseudomonas aeruginosa* for water quality assessments [32].PMA treatment also limits detection to intact microbial cells when used with end-point PCR in combination with denaturing gel electrophoresis [33]. Challenges have been encountered when applying PMA-PCR to samples that have insufficient light transparency [34].This limitation might be overcome by using a trigger other than light to induce DNA cross-linking or by manipulating pH or temperature to alter turbidity.

*Flow Cytometry (FC)/Fluorescent Activated Cell Sorting (FACS)*

Cell sorting methods, such as Coulter counters and flow cytometry (FC) were originally developed for counting red blood cells. Today, FC has been upgraded to analyze much smaller cells, such as bacteria, and to deliver high-throughput data. The technique allows simultaneous multi parametric analysis of physical and/or chemical characteristics of up to thousands of particles per second. The cell surface or its components must first be labeled with one or more fluorescent dyes.

A mono disperse suspension (single, unclumped cells) is made so that single, labeled cells are aligned to pass individually through a laser beam. Laser-excitation of the fluorescent molecules causes them to emit light at various wavelengths and the amount and type fluorescence indicates the percentage of various cell types or cell components present in the sample. FC allows the examination of a large number of cells at a time (200 to 2000 cells per second), recording, for each cell, several different parameters that can later be linked to a wide variety of cellular characteristics [35].

A variety of fluorescent probes can be applied to examine physiological characteristics of living cells, such cell membrane integrity, intracellular enzyme activity, cytoplasmic pH, and membrane potential, all of which provide a measure of viability [36].

Fluorescent Activated Cell Sorting (FACS) is a specialized form of flow cytometry that sorts a heterogeneous mixture of biological cells into two or more containers based on the fluorescent characteristics as well as light scattering. These powerful and rapid cell-sorting techniques could reduce the time needed to determine probiotic strain abundance, size, and metabolic activity.

Fluorescent DNA stains, nucleic acid probes, and immuno fluorescence probes directed at cell proteins, extend the capabilities of the technique, enabling cells to be discriminated based on amount and type of nucleic acids,

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amount of respiratory enzymes, or membrane integrity. The potential exists to measure cell size, cell granularity, and indicators of viability such as levels of newly synthesized DNA, specific gene expression from transcription of messenger RNA, and even transient signaling events in living cells. Such techniques offer significant promise for more robust enumeration of viable probiotic strains (whether replicating or VBNC).

## Mechanism of Probiotic Action

### Immune Modulation

The intestinal lymphoid tissue is the largest in size compared with other areas of the body. It is well known that bacteria are critical for the development and functioning of the immune system at this level, being actually the defense mechanism against infection by pathogens [37, 38].Intestinal lymphoid tissue makes contact with the food components, the antigens and with the beneficial or pathogenic bacteria. Antigens, substances that can trigger an immune response, enter the body through the intestinal mucosa that is essential in controlling immunity to invasion of pathogenic bacteria. The adaptability to various antigens is extremely important if we consider that the composition of intestinal mass change very frequently. Most of the antigen is released from first contact with the intestinal mucosa [39].

After crossing the epithelial barrier by transcytosis, they are restructured by a lysosomal degradation processes. A further screening is in the presence of M cells (cells of follicular epithelium associated with lymphoid tissue) followed by the T cells (lymphocyte cells belonging to the group of white blood cells) which are then differentiated as cells that mediate an immune response and promotes cell differentiation and secreting IgA (immunoglobulin A) [40].IgA is an antibody that plays a crucial role in mucosal immunity.

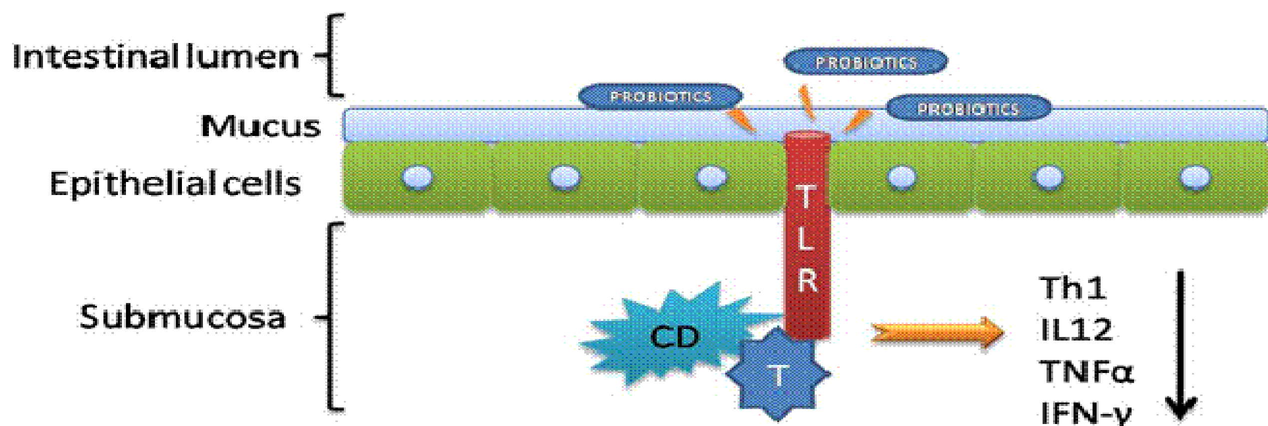


**Figure 1: The effect of probiotic bacteria on the immune system [41].**

In Figure 1 through TLR receptors (Toll Like Receptors), dendritic cells (DC) and T cells, probiotics, leads to reduced secretion of TH1 (lymphocyte involved in an enhanced immune response), IL12 (interleukin which is naturally produced by dendritic cells), TNFα (inflammatory cytokine) and IFN-γ (cytokine that is critical for innate and adaptive immunity) which are responsible for the onset of inflammatory processes in the intestines.

The mechanisms by which epithelial cells are making the difference between probiotic and pathogenic microorganisms appear to be different. Pathogenic bacteria induce a pro-inflammatory response in epithelial cells by activating transcription factor NF-kB [42]. Quality and dose of probiotic preparations influence the IL-8 secretion via the enterocites. IL- 8 is associated with the development of intestinal inflammation. IL-6 stimulation was achieved by administering *L. casei* CRL431 and *L.helveticus* R389 [43].

### Inhibition of Pathogenic Bacteria

The gastrointestinal environment contains a wide range of contents ranging from harmless beneficial dietary and microbial flora to harmful pathogenic bacteria. The mammalian organism fights against these pathogenic bacteria through various ways: blocking pathogenic bacteria effects by producing bactericidal substances and competing with pathogens and toxins for

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adherence to the intestinal epithelium; regulation of the immune responses by enhancing the innate immunity and modulating pathogen-induced inflammation via toll-like receptor-regulated signalling pathways; regulate intestinal epithelial homeostasis by promoting intestinal epithelial cell survival, enhancing barrier function, and stimulating protective responses (Figure 2).

The strategy is based on the ability of probiotic bacteria (B) to bind pathogens (C) in intestinal epithelial tissue (A). Anti-pathogenic action of probiotics consists in production of lactic acid (D) which decreases the pH, interacts with the toxins produced by pathogens (E), with the production of hydrogen peroxide (F) and synthesis bacteriocine (G).

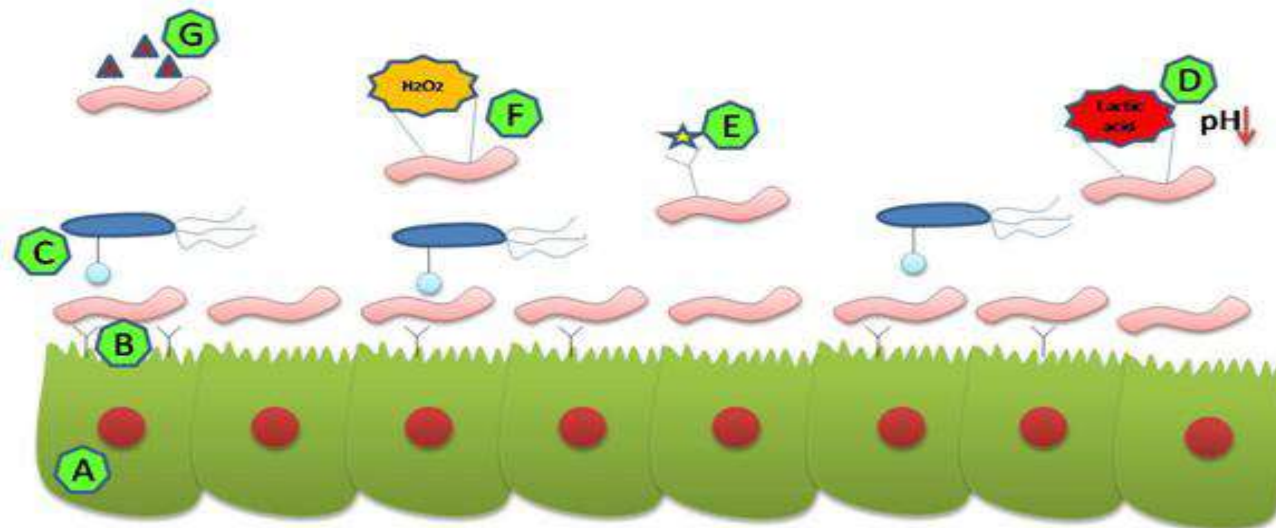


**Figure 2: Schematic representation of the mode of action of probiotics in the intestine**

Production of antimicrobial substances (bacteriocins), in situ in the intestine can be improved by increasing the ability of probiotic bacteria to adhere to the intestinal mucosa. Bovine colostrum contains substances that can triple the capacity of *Lactobacillus casei* species to adhere to intestinal cell line CaCO-2. However, in situ production of microbial substances adversely affect intestinal microflora beneficial to the host organism [39].

Ruminal bacteria can also produce such bacteriocins which by their presence are able to modify the ruminal ecosystem. Some studies even recommend using ruminal bacteriocins as an alternative to antibiotics in cattle [45].In vitro studies have shown that strains of lactic acid bacteria are effective in removing or stopping the activity of pathogenic bacteria. Administration of probiotics (*L. rhamnosus* HN001) in animals, under experimental conditions, resulted in an improved immune response following *Salmonella enterica* infestation [45].

It is also interesting that the animals who were artificially infected with *Salmonella* and which received probiotics have synthesized high levels of serum antibodies leading to increased survival to infection but also to a decrease in the presence of these pathogens in liver and spleen. The same effects have been identified when *L. salivarius* CTC2197 is administered to Leghorn birds [46]. Bacteriocins produced by *Enterococcus faecium* SH528, SH632, *Pediococcus pentosaceus, Enterococcus faecium* SH740 were proven to be effective in combating *Listeria monocytogenes* [47].

Efficacy of probiotics was also proven in urogenital infections and was tested by studies performed on healthy patients or female patients who were diagnosed with uro-vaginal infections. Results from these studies suggest beneficial effects of the use of probiotics in preventing urinary tract infections [48].

## Claimed Health Benefits of Individual Probiotic Microorganisms

Many publications using well-designed and well-conducted trials substantiate the health benefits of specific strains of probiotics on the risk reduction and management of a variety of diseases and conditions. Some of the documented health claims of probiotics proposed by their authors include: stimulation of various components of the

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immune system, gut immune response and intestinal homeostasis [49]; prevention and treatment of diarrhea [50, 51, 52, 53]; improvement of faecal properties and microbiota, treatment of irritable bowel syndrome, inflammatory bowel disease and constipation [54-58]; prevention and treatment of *Clostridium difficile*-associated diarrhea in adults and children [59, 60]; alleviation of symptoms of lactose intolerance and other food allergies [58]; prevention of necrotising enterocolitis in preterm infants [61, 62, 63]; decrease in plasma cholesterol level [49, 64, 65]; improvement of *Helicobacter pylori* eradication regimens [66, 67, 68]; therapeutic effects by supporting the immune response of HIV-infected children and adults [49], anti-proliferative activity on tumour cells [69,70]; reduction of viral-associated pulmonary damage through controlling immune-coagulative responses and clearing respiratory viruses [71]; immune-stimulatory properties of low molecular mass molecules produced by probiotic bacteria [72].

Although probiotics have even been proposed as treatment for eczema [73], randomized controlled trials to date do not have sufficient evidence to recommend probiotics as primary prevention [74, 75]. The most common probiotic microorganisms with claimed health benefits for humans from the most recent scientific literature are noted in Table 4.

**Table 4: Recently published claimed health benefits of probiotic microorganisms**

| Genus | Species | Recently published health claims |
|---|---|---|
| *Lactobacillus* | *L. rhamnosus* | Reduction of viral-associated pulmonary damage (*L. rhamnosus* CRL1505) ; prevention and reduction of severity of atopic dermatitis in children (*L. rhamnosus* GG) ; reduction of risk for developing allergic disease (*L. rhamnosus* GG) , (*L. rhamnosus* HN001 ; anti-diabetic potential (various strains from human infant faecal samples); prevention of necrotizing enterocolitis in newborns (*L. rhamnosus* GG) ; prevention or treatment of bacterial vaginosis (*L. rhamnosus* GR-1) ; aid in weight loss of obese women (*L. rhamnosus* CGMCC1.3724) ; treatment of acute gastroenteritis in children (*L. rhamnosus* GG) ; reduction of risk for rhinovirus infections in preterm infants (*L. rhamnosus* GG and *L. rhamnosus* ATCC 53103); protection of human colonic muscle from lipopolysaccharide-induced damage (*L. rhamnosus* GG) |
| | *L. acidophilus* | Treatment of travellers' diarrhoea; reduction of hospital stay of children with acute diarrhoea; antifungal activity (*L. acidophilus* ATCC-4495); prevention or treatment of bacterial vaginosis; treatment of *C. difficile*-associated diarrhoea; reduction of incidence of febrile urinary tract infections in children; reduction of irritable bowel syndrome symptoms. |
| | *L. plantarum* | Prevention of endotoxin production; antifungal activity (*L. plantarum* NRRL B-4496) reduction of irritable bowel syndrome symptoms. |
| | *L. casei* | Treatment of functional constipation in adults (*L. casei* Lcr35 and *L. casei* Shirota); treatment of *C. difficile*-associated diarrhoea; restoration of vaginal flora of patient with bacterial vaginosis (*L. casei* Lcr35); reduction of irritable bowel syndrome symptoms; reduction of diarrhea duration of antibiotic-associated diarrhoea in geriatric patients (*L. casei* Shirota); immunomodulatory mechanisms (*L. casei* Shirota); improvement of rheumatoid arthritis status (*L. casei* ); protection against *Salmonella* infection (*L. casei* CRL-431); prevention of *Salmonella*-induced synovitis; treatment of intravaginal staphylococcosis (*L. casei* IMV B-7280). |
| | *L. delbrueckii* subsp. | Antibiotic resistance of yogurt starter culture; enhancement of systemic immunity in elderly (*L. delbrueckii* subsp. *bulgaricus* 8481); antibacterial action against *E. coli*; |

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| | | |
|---|---|---|
| | *bulgaricus* | modulation of brain<br><br>activity. |
| | *L. brevis* | Protective role in bile salt tolerance (*L. brevis* KB290); reduction in plague acidogenicity<br><br>(*L. brevis* CD2). |
| | *L. johnsonii* | Impact on adaptive immunity for protection against respiratory insults; reduction of occurrence of gastritis and risk of *H. pylori* infection (*L. johnsonii* MH-68) ; inhibition of *S. sonnei* activity<br><br>(*L. johnsonii* F0421); treatment of perennial allergic rhinitis in children together with levocetirizine (*L. johnsonii* EM1). |
| | *L. fermentum* | Prevention or treatment of bacterial vaginosis (*L. fermentum* RC-14) ; blockage of adherence of<br><br>pathogenic microorganisms on vaginal epithelium ; antistaphylococcal action (*L. fermentum* ATCC 11739) ; potential for reduction of insulin resistance and hypercholesterolemia<br><br>(*L. fermentum* NCIMB 5221). |
| | *L. reuteri* | Reduction of low-density lipoprotein cholesterol (*L. reuteri* NCIMB 30242) ; treatment of acute<br><br>gastroenteritis in children; reduction of diarrhoea duration in children (*L. reuteri* ATCC 55730) management of infant colic (*L. reuteri* ATCC 55730 and *L. reuteri* DSM 17938); reduction of onset of gastrointestinal disorders in infants (*L. reuteri* DSM 17938); reduction of frequency of proven sepsis, feeding intolerance and duration of hospital stay in preterm infants (*L. reuteri* DSM 17938). |

| Genus | Species | Recently published health claims |
|---|---|---|
| *Bifidobacterium* | *B. infantis* | Reduction of irritable bowel syndrome symptoms ; reduction of necrotizing enterocolitis in preterm infants. |
| | *B. animalis* subsp. *lactis* | Treatment of functional constipation in adults (*B. animalis* subsp. *lactis* DN-173 010), reduction of incidence of febrile urinary tract infections in children; modulation of brain activity; reduction of necrotizing enterocolitis in preterm infants; reduction of total microbial counts in dental plaque (*B. animalis* subsp. *lactis* DN-173 010); reduction of total cholesterol (*B. animalis* subsp. *lactis* MB 202/DSMZ 23733); reduction of risk of upper respiratory illness (*B. animalis* subsp. *lactis* BI-04) . |
| | *B. bifidum* | Reduction of hospital stay of children with acute diarrhoea ; reduction of necrotizing enterocolitis in preterm infants; reduction of total cholesterol (*B. bifidum* MB 109/DSMZ 23731). |
| | *B. longum* | Prevention and treatment of necrotizing enterocolitis in newborns; reduction of radiation induced diarrhoe; reduction of necrotizing enterocolitis with Bifidobacteria cocktail (*B. breve*, *B. infantis*, *B. bifidum*, *B. longum*); reduction of irritable bowel syndrome symptoms; treatment of gastrointestinal diseases (*B. longum* CMCC P0001); perinatal intervention against onset of allergic sensitization (*B. longum* CCM 7952). |
| *Saccharomyces* | *S. boulardi* | Treatment of travellers' diarrhoea; treatment and reduction of diarrhoea duration regardless of cause; treatment of irritable bowel syndrome ; treatment of moderate ulcerative colitis; treatment and reduction of recurrent pseudomembrane colitis infection caused by *C. difficile*; treatment of acute gastroenteritis in children. |

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| *Lactococcus* | *L. lactis* subsp. *lactis* | Treatment of antibiotic-associated diarrhoea; adhesion to vaginal epithelial cells (*L. lactis* subsp. *lactis* KLDS4.0325); nisin production (*L. lactis* subsp. *lactis* CV56); modulation of brain activity; antimicrobial activity against *C. difficile*; antimicrobial and probiotic properties (*L. lactis* subsp. *lactis* ATCC 11454) |
| *Enterococcus* | *E. durans* | Antibiotic and antioxidant activity (*E. durans* LAB18s), adherence to colonic tissue and anti-inflammatory activity. |
| | *E. faecium* | Treatment of antibiotic-associated diarrhoea ; efficient animal probiotic . |
| *Streptococcus* | *S. thermophilus* | Reduction of irritable bowel syndrome symptoms; antibiotic resistance of yogurt starter culture; reduction of necrotizing enterocolitis in preterm infants. |
| *Pediococcus* | *P. acidilactici* | Pediocin production with antimicrobial and probiotic properties (*P. acidilactici* UL5); bacteriocin production ; elimination of *H. pylori* infections (*P. acidilactici* BA28) |
| *Leuconostoc* | *L.mesenteroides* | Leucoin production, probiotic profile (survival at low pH, in presence of bile salts, in presence of pepsin) (*L. mesenteroides* B7) |
| *Bacillus* | *B. coagulans* | Treatment of antibiotic-associated diarrhoea , treatment of bacterial vaginosis (*B. coagulans* ATCC PTA-11748); immunological support (*B. coagulans* GandenBC30); prevention of caries in children. |
| | *B. subtilis* | Efficient animal probiotic; treatment of diarrhoea and aiding in *H. pylori* eradication (*B. subtilis* R0179); production of nitric oxide. |
| | *B. cereus* | Efficient animal probiotic (*B. cereus* NVH75/95). |
| *Escherichia* | *E. coli* Nissle 1917 | Treatment of functional constipation in adults ; treatment of inflammatory bowel disease; treatment of gastrointestinal disorders; pro-inflammatory potential; prevention of surface ocular diseases; reduction of *Salmonella enterica* Typhimurium intestinal colonization by iron competition . |

## Conclusion

Both traditional cell culture methods, as well as the alternative techniques (direct imaging and visual enumeration, nucleic acid-based enumeration methods, and flow cytometry and cell sorting), offer advantages and limitations for enumerating probiotic microorganisms. The new methods and techniques show considerable promise for quantifying live microorganisms in different metabolic states.

But the probiotic efficacy cannot be predicted solely on the basis of viable cells. Very few microorganisms have been subjected to thorough *in vitro* studies confirming their specific health promoting activity, and even fewer have been subsequently subjected to and passed the appropriate human trials. Additionally, probiotics can be dangerous, as they have been linked to an increase in mortality rate if administered to severely immunocompromised patients. Subsequent studies are needed to evaluate the health-promoting activity of probiotic bacteria.

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## References

1. Vankerckhoven V, Huys G, Vancanneyt M, Vael C, Klare I, Romond M, Entenza, Moreillon, P, Wind R, Know J, Wiertz E,

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Pot B, Vaughn E, Kahlmeter G, Goossens H. Biosafety assessment of probiotics used for human consumption: recommendations from the eu-prosafe project. Trends Food Sci Technol. 2008; 19:102-14.

2. Lin WH, Hwang CF, Chen LW, Tsen H. Viable counts, characteristic evaluation for commercial lactic acid bacteria products. Food Microbiol. 2006; 23:74-81.
3. Carr J, Ibrahim SA. Viability of *bifidobacterium* in commercial yogurt products in North Carolina. Milchwissenschaft. 2005; 60: 414-16.
4. Al-Otaibi, MM. Evaluation of some probiotic fermented milk products from Al-Ahsa markets, Saudi Arabia. Am. J Food Technol. 2009; 4:1-8.
5. Mills S, Stanton C, Fitzgerald G, Ross RP. Enhancing the stress responses of probiotics for a lifestyle from gut to product and back again. Microbial Cell Factories. S19 2011. (http://www.microbialcellfactories.com/content/10/S1/S19).
6. Xu HS, Roberts N, Singleton FL, Attwell RW, Grimes DJ, Colwell RR. Survival and viability of non culturable *Escherichia coli* and *Vibrio cholera* in the estuarine and marine environment. Microb Ecol. 1982; 8: 313-23.
7. Lahtinen SJ, Ahokoski H, Reinikainen JP, Gueimonde M, Nurmi J, Ouwenhand AC, Salminen SJ. Degradation of 16S rRNA and attributes of viability of viable but non culturable probiotic bacteria. Lett Appl Microbiol. 2008; 46: 693-98.
8. Conway PL, Henriksson A. Strategies for the isolation and characterization of functional probiotics. In: Gibson, S.A.W. (Ed.), Human Health: The Contribution of Microorganisms. 1994; 1. Springer-Verlag, New York.
9. Vinderola CG, Reinheimer JA. Culture media for the enumeration of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in the presence of yoghurt bacteria. Int Dairy J. 1999; 9: 497-505.
10. Hartemink R, Rombouts FM. Comparison of media for the detection of bifidobacteria, lactobacilli and total anaerobes from faecal samples. J Microbiol Methods. 1999; 36 (3): 181-92.
11. Roy D. Media for the isolation and enumeration of bifidobacteria in dairy products. Int J Food Microbiol. 2001; 69:167-182.
12. Breed R, Dotterrer WD. The number of colonies allowable on satisfactory agar plates. J Bacteriol. 1916; 1: 321-31.
13. Sohrabvandi S, Mortazavian AM, Dolatkhnejad MR, Monfared AB. Suitability of MRS-bile agar for the selective enumeration of mixed probiotic bacteria in presence of mesophilic lactic acid cultures and yoghurt bacteria. Iran. J Biotechnol. 2012; 10: 16-21.
14. Champagne CP, Ross RP, Saarela M, Hansen KF, Charalampopulos D. Recommendations for the viability assessment of probiotics as concentrated cultures and in food matrices. Int J Food Microbiol. 2011; 149: 185-93.
15. Kepner RJ, Pratt JR. Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. Microbiol Rev. 1994; 58: 603-615.
16. Brooker BE. Imaging food systems by confocal scanning laser microscopy. In: Dickinson, E. (Ed.), New Physico-chemical techniques for the characterization of complex food systems. Blackie Academic and Profession, London, United Kingdom. 1995; 43-68.
17. Heertje I, van der Vlist P, Blonk JCG, Hendrickx HAC, Brackenhof GJ. Confocal scanning laser microscopy in food research: some observations. Food Microstruct. 1987; 6: 115-20.
18. Caldwell DE, Korber DR, Lawrence JR. Confocal laser microscopy and digital image analysis in microbial ecology. Adv Microb Ecol. 1992; 12: 1-67.
19. Auty MAE, Gardinere GC, Mc Breaty SJ, O'Sullivan EO, Muerill DM, Collins JK, Fitzgerald GJ, Stanton C, Ross RP. Direct in situ viability assessment of bacteria in probiotic dairy products using viability staining in conjunction with confocal scanning laser microscopy. Appl Environ Microbiol. 2001; 67: 420-25.
20. Villlarreal MLM, Padiha M, Vieira ADS, de Melo Franco BDG, Ruiz Martinez RC, Isay Saad SM. Advantageous direct quantification of viable closely related probiotics in Petit-Suisse cheeses under *in vitro* gastrointestinal conditions by

©2009-2021, JGPT. All Rights Reserved 

propidium monoazide-qPCR. PLoS One. 2013. http://dx.doi.org/10.137/journal.pone.009212 (December 17).

21. Jamil S, Keer J T, Dockrell HM, Stoker NG, Lucas SB, Chiang TJ, Hussain R. Use of polymerase chain reaction to assess efficacy of leprosy chemotherapy. The Lancet. 1993; 342: 264-68.
22. McKillip JL, Jaykus L, Drake MA. Nucleic acid persistence of *Escherichia coli* O157:H7 in artificially contaminated skim milk. J Food Prot. 1999; 62(904): 908.
23. Meijer A, roholl PJ, Gielis-Proper SK, Meulenberg YF, Osewaarde JM. Chlamydia pneumonia in vitro and in vivo: a critical evaluation of in situ detection methods. Journal of Clinical Pathology. 2000; 53: 904-10.
24. Chan AB, Fox JD. NASBA and other transcription-based amplification methods for research and diagnostic microbiology. Rev Med Microbiol. 1999; 10:185-96.
25. Hellyer TJ, Nadeau JG. Strand displacement amplification: a versatile tool for molecular diagnostics. Expert. Rev Mol Diagn. 2004; 4: 251-61.
26. Postellec F, Falentin H, Pavan S, Combrisson J, Sohier D. Recent advances in quantitative PCR (q PCR) applications in fold microbiology. Food Microbiol. 2011; 28: 848-61.
27. Nogva HK, Dromtorp SM, Nissen H, Rudi K. Ethidium monazide for DNA-based differentiation of viable and dead bacteria by 5′ nuclease. PCR Bio Tech. 2003; 810: 804-13.
28. Fittipaldi M, Nocker A, Codony F. Progress in understanding preferential detection of live cells using viability dyes in combination with DNA amplification. J Microbiol Methods. 2012; 91 (2):276-89.
29. Rudi K, Moen B, Dromtrop SM, Holck, AL. Use of ethidium monoazide and PCR in combination for quantification of viable and dead cells in complex samples. Appl Environ Microbiol. 2005; 71: 1018-24.
30. Nocker A, Cheung CY, Camper AK. Comparisons of propidiummonoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells. J Microbiol Methods. 2006; 70: 252-60.
31. Nocker A, Sossa K, Campber AK. Molecular monitoring of disinfection efficacy. J Microbiol Methods. 2007a; 70: 252-60.
32. Gensberger ET, Sessistsch A, Kostic T. Propidium monoazide-quantitative polymerase chain reaction for viable *Escherichia coli* and *Pseudomonas aeruginosa* detection from abundant background micro flora. Ann Biochem. 2013; 441: 69-72.
33. Nocker A, Sossa P, Burr M, Camper AK. Use of propidium monoazide for live dead distinction in microbial ecology. Appl Environ Microbiol. 2007b; 73: 5111-17.
34. Wagner AO, Malin C, Knapp BA, Illmer P. Removal of free extracellular DNA from environmental samples by ethidium monoazide and propidium monoazide. Appl Environ Microbiol. 2008; 74: 2537-39.
35. Tracy BP, Gaida SM, Papoutsakis ET. Flow cytometry for bacteria: enabling metabolic engineering, synthetic biology and the elucidation of complex phenotypes. Curr Opin Biotechnol. 2010; 21: 85-99.
36. Chen S, Cao Y, Ferguson LR, Shu Q, Garg S. Flow cytometric assessment of the protectants for enhanced in vitro survival of probiotic lactic acid bacteria through simulated human gastro-intestinal stresses. Appl Microbiol Biotechnol. 2012; 95: 345-56.
37. Cebra JJ. Influences of micro biota on intestinal immune system development. Am J Clin Nutr. 1999; 69: 1046S-51S.
38. Falk PG, Hooper LV, Midtvedt T, Gordon JI. Creating and maintaining the gastrointestinal ecosystem: what we know and need to know from gnotobiology. Microbiol Mol Biol Rev.1998; 62:1157-70.
39. Sanderson IR, Walker WA. Uptake and transport of macromolecules by the intestine: possible role in clinical disorders (an update). Gastroenterology. 1993; 104: 622-39.
40. Strober W, Kelsall B, Marth T. Oral tolerance. J Clin Immunol. 1998; 18:1-30.
41. Corcionivoschi N, Drinceanu D. Probioticele-la timpul prezent. Editura Mirton, Timisoara. 2009.
42. Neish AS, Gewirtz AT, Zeng H, Young AN, Hobert ME, Karmali V. Prokaryotic regulation of epithelial responses by

©2009-2021, JGPT. All Rights Reserved 

inhibition of IkappaB-alpha ubiquitination. Science. 2000; 289: 1560-63.

43. Vinderola G, Matar C, Perdigon G. Role of intestinal epithelial cells in immune effects mediated by gram-positive probiotic bacteria: involvement of toll-like receptors. Clin Diagn Lab Immunol. 2005; 12: 1075-84.
44. Russell JB, Mantovani HC. The bacteriocins of ruminal bacteria and their potential as an alternative to antibiotics. J Mol Microbiol Biotechnol. 2002; 4:347-55.
45. Gill HS, Shu Q, Lin H, Rutherfurd KJ, Cross ML. Protection against translocating *Salmonella typhimurium* infection in mice by feeding the immuno-enhancing probiotic *Lactobacillus rhamnosus* strain HN001. Med Microbiol Immunol. 2001c; 190: 97-104.
46. Pascual M, Hugas M, Badiola JI, Monfort JM, Garriga M. *Lactobacillus salivarius* CTC 2197 prevents *Salmonella enteritidis* colonization in chickens. Appl Environ Microbiol. 1999; 65: 4981-86.
47. Shin MS, Han SK, Ji AR, Kim KS, Lee WK. Isolation and characterization of bacteriocin-producing bacteria from the gastrointestinal tract of broiler chickens for probiotic use. J Appl Microbiol. 2008; 105: 2203-12.
48. Petricevic L, Witt A. The role of *Lactobacillus casei rhamnosus* Lcr35 in restoring the normal vaginal flora after antibiotic treatment of bacterial vaginosis. BJOG. 2008; 115: 1369-74.
49. Savard P, Lamarche B, Paradis ME, Thiboutot H, Laurin E, Roy D. Impact of *Bifidobacterium animalis* subsp. *lactis* BB-12 and, *Lactobacillus acidophilus* LA-5-containing yoghurt, on fecal bacteria counts of healthy adults. Int J Food Microbiol. 2011; 149: 50-7.
50. Johnston BC, Goldenberg JZ, Vandvik PO, Sun, X, Guyatt GH. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. Cochrane Database Syst Rev. 2011; 11:CD004827.
51. McFarland, LV. Meta-analysis of probiotics for the prevention of traveler's diarrhea. Travel Med Infect Dis. 2007; 5: 97-105.
52. Doron SI, Hibberd PL, Gorbach SL. Probiotics for prevention of antibiotic-associated diarrhea. J Clin Gastroenterol. 2008; 42: S58-63.
53. Gueimonde M, Salminen S. Microbiota of the Intestine: Probiotics. Encyclopedia of Human Nutrition, 3rd ed.; Elsevier: Amsterdam, the Netherlands. 2013;175-81.
54. Chmielewska A, Szajewska H. Systematic review of randomised controlled trials: Probiotics for functional constipation. World J Gastroenterol. 2010; 16: 69-75.
55. Choi CH, Jo SY, Park HJ, Chang SK, Byeon JS, Myung SJ. A randomized, double-blind, placebo-controlled multicenter trial of *Saccharomyces boulardii* in irritable bowel syndrome: Effect on quality of life. J Clin Gastroenterol. 2011; 45: 679-83.
56. Guslandi M, Mezzi G, Sorghi M, Testoni PA *Saccharomyces boulardii* in maintenance treatment of Crohn's disease. Dig Dis Sci. 2000; 45: 1462-64.
57. Guslandi M, Giollo P, Testoni PA. A pilot trial of *Saccharomyces boulardii* in ulcerative colitis. Eur J Gastroenterol Hepatol. 2003; 15: 697-98.
58. Gueimonde M, Salminen S. Microbiota of the Intestine: Probiotics. Encyclopedia of Human Nutrition, 3rd ed.; Elsevier: Amsterdam, the Netherlands. 2013; 175-81.
59. Fitzpatrick LR. Probiotics for the treatment of *Clostridium difficile* associated disease. World J Gastrointest Pathophysiol. 2013; 4: 47-52.
60. Goldenberg JZ, Ma SS, Saxton JD, Martzen MR, Vandvik PO, Thorlund K, Guyatt GH, Johnston BC. Probiotics for the prevention of *Clostridium difficile*-associated diarrhea in adults and children. Cochrane Database Syst Rev. 2013; 5: CD006095.
61. Guthmann F, Kluthe C, Buhrer C. Probiotics for prevention of necrotising enterocolitis: An updated meta-analysis. Klin Padiatr. 2010; 222: 284-90.
62. Bernardo WM, Aires FT, Carneiro RM, Sa FP, Rullo VE, Burns DA. Effectiveness of probiotics in the prophylaxis of necrotizing enterocolitis in preterm neonates: A

©2009-2021, JGPT. All Rights Reserved

systematic review and meta-analysis. J Pediatr (Rio J.) 2013; 89: 18-24.

63. AlFaleh K, Anabrees J. Efficacy and safety of probiotics in preterm infants. J Neonatal Perinat Med. 2013; 6: 1-9.
64. Lee SJ, Bose S, Seo JG,Chung WS, Lim CY, Kim H. The effects of co-administration of probiotics with herbal medicine on obesity, metabolic endotoxemia and dysbiosis: A randomized double-blind controlled clinical trial. Clin Nutr. 2013; doi:10.1016/j.clnu.2013.12.006
65. Wong JM, de Souza R, Kendall, CW, Emam A, Jenkins DJ. Colonic health: Fermentation and short chain fatty acids. J Clin Gastroent. 2006; 40: 235-43.
66. Wilhelm SM, Johnson JL, Kale-Pradhan PB. Treating bugs with bugs: The role of probiotics as adjunctive therapy for *Helicobacter pylori*. Ann Pharmacother. 2011; 45: 960-66.
67. Hemsworth JC, Hekmat S, Reid G. Micronutrient supplemented probiotic yogurt for HIV-infected adults taking HAART in London, Canada. Gut Microbes. 2012; 3: 414-19.
68. Enos MK, Burton JP, Dols J, Buhulata S, Changalucha J, Reid G. Probiotics and nutrients for the first 1000 days of life in the developing world. Benef Microbes. 2013;4:3-16.
69. Serban DE. Gastrointestinal cancers: Influence of gut microbiota, probiotics and prebiotics. Cancer Lett. 2013; 345: 258-70.
70. Orlando A, Russo F. Intestinal microbiota, probiotics and human gastrointestinal cancers. J Gastrointest Cancer. 2013; 44: 121-31.
71. Zelaya H, Tsukida K, Chiba E, Marranzino G, Alvarez S, Kitazawa H, Aguero G, Villena J. Immunobiotic *Lactobacilli* reduce viral-associated pulmonary damage through the modulation of inflammation-coagulation interactions. Int Immunopharmacol. 2014; 19: 161-73.
72. Kmonickova E, Kverka M, Tlaskalova-Hogenova H, Kostecka P, Zidek Z. Stimulation of nitric oxide, cytokine and prostaglandin production by low-molecular weight fractions of probiotic *Lactobacillus casei* lysate. Neuro Endocrinol Lett. 2012; 33: 166-72.
73. Boyle RJ, Bath Hextall FJ, Leonardi Bee J, Murell DF, Tang ML. Probiotics for the treatment of eczema: A systematic review. Clin Exp Alergy. 2009; 39: 1117-27.
74. Azad MB, Coneys JG, Kozyrskyj AL, Field CJ, Ramsey CD, Becker AB, Friesen C,Abou-Setta AM, Zarychanski R. Probiotic supplementation during pregnancy or infancy for the prevention of asthma and wheeze: Systematic review and meta-analysis. BMJ. 2013; 347.
75. Elazab N, Mendy A, Gasana J, Vieira ER, Quizon A, Forno E. Probiotic administration in early life, atopy, and asthma: A meta-analysis of clinical trials. Pediatrics. 2013; 132: e666-76.
76. Sabina Fijan. Microorganisms with claimed probiotic properties: An overview of recent literature. Int J Environ Res Public Health. 2014; 11: 4745-67.

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