Selection criteria for probiotics

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BACKGROUND AND INTRODUCTION

For centuries, lactic acid bacterial strains have been used for the preservation of food for the human consumption. Although the ancients wouldn’t have identified the many nutrients formed by the actions of beneficial bacteria on foods, we now know our optimal health depends upon nutrients formed by the actions of beneficial bacteria on foods. Over 1000 years ago, the ancient Egyptians used fermented milk as a food product. The Egyptians added yeast to the fermented milk, thereby increasing the beneficial properties of the lactobacillus strains added to the milk. Since those ancient days, probiotics have been used to maintain the health of animals, and to enhance the quality of food production. For centuries, lactic acid bacterial strains have been used for the preservation of food for the human consumption. Although the ancients wouldn’t have identified the many nutrients formed by the actions of beneficial bacteria on foods, we now know our optimal health depends upon nutrients formed by the actions of beneficial bacteria on foods. Over 1000 years ago, the ancient Egyptians used fermented milk as a food product. The Egyptians added yeast to the fermented milk, thereby increasing the beneficial properties of the lactobacillus strains added to the milk. Since those ancient days, probiotics have been used to maintain the health of animals, and to enhance the quality of food production.

S. K. Dash’s interest in probiotics came while he was working as the director of the Food & Drug Administration for South Dakota from 1973 to 1979. He learned that some time earlier – in the 1950s – a probiotic product had been licensed by the U.S. Department of Agriculture as a drug for treatment of the disease known as scour in pigs, which is caused by E. coli infection.

Dash’s further research revealed a probiotic was 97 percent effective in combating E. coli infection in pigs, a cure rate as effective as the antibiotic neomycin sulphate, the standard treatment. Unlike antibiotics, the natural probiotic left no harmful drug residues in the edible portions of the pork.

During this period, the pharmaceutical industry had already used millions of dollars in research, development, and promotions to dominate the market with antibiotics. There simply weren’t the funds available from probiotic firms to compete with this behemoth. Little interest was shown in this wonderful approach to a serious medical problem.

In 1970s the probiotic rich foods and supplements were sold in USA and other countries without proper labelling and quality control. Safety and efficacy of these products were questioned. Not surprisingly, Consumer Labs and others examining probiotic products have found that some 70 to 80 percent of samples tested did not measure up to their label claims. They have also found that about half of the tested samples did not have even 10 percent of the claimed number of live micro organisms listed on the label. Approved lists of bacterial names are available from:

- http://www.bacterio.cict.fr/

DNA-DNA hybridization is the reference method to specify that a strain belongs to a species. DNA Sequences Encoding 16S rRNA is a suggested suitable substitute. World Health Organization (WHO) recommends all strains should be deposited in an internationally recognized culture collection.

Probiotic genus, species and strain

It is necessary to know the genus and species of the probiotic strain. It is well recognized that probiotic effects are strain, condition and dose specific. Strain identification is done by phenotype and genotype methods. Nomenclature of the bacteria must conform to the current, scientific recognized name. Protracted use of older or misleading nomenclature is not acceptable on product label. In Vitro tests to screen potential probiotics

In vitro testing is critical to assess the safety of probiotic micro organisms. This testing is useful to gain knowledge of the strains and the mechanism of the probiotic effect. Probiotics for human use will require substantiation of efficacy with human trials. Appropriate target specific in vitro tests that correlate with in vitro results are recommended. For example – In vitro bile salts resistance was shown to correlate with gastric survived in vivo. Currently used in vitro tests for study of probiotic strains are:

- Resistance to gastric acidity
- Bile acid resistance
- Adherence to mucus
- Antimicrobial activity against potentially pathogenic bacteria
- Ability to reduce pathogen adhesion to surfaces
- Bile salt hydrolase activity
- Resistance to spermicides (applicable to probiotics for vaginal use)

SELECTION OF PROBIOTIC STRAIN(S)

For the selection of microbial strains to be used as probiotics, certain criteria must be used for safety, production/manufacturing, administration and application, survival and colonization in the host. In vitro experiments are available to investigate whether the microbial strains fulfill the above criteria. Based on these selection criteria validated in vitro experiments, it is possible to screen micro organisms on their potential as probiotic strains. Further validation can be done for these microbial strains with animal and human trials.

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Focus on Dietary fibres - Pre/Probiotics

Gene technology
The use of gene technology for the improvement of microorganisms probably offers great promise for future development of probiotic strains. It is possible to isolate the genes that are responsible for a specific factor such as adherence, and to transfer these genes to a probiotic strain without altering other beneficial properties.

Sources of probiotic strains
The sources can be from human origins like human large intestine, small intestine or breast milk; animal origins; food sources like raw milk or fermented foods. Probiotic strains isolated from human flora are more likely to adhere to the human intestinal wall than others and more likely to be safe. The bacterial strains used in the probiotic foods and supplements should play an important role in:
- Colonization in the intestinal, respiratory and urogenital tracts
- Cholesterol metabolism
- Inhibiting the carcinogenesis, directly or indirectly, by stimulating of immune system
- The metabolism of lactose, the absorption of calcium and the synthesis of vitamins
- The reduction of yeast and vaginal infection
- Gastritis and ulcers
- Acne and skin problems
- The production of natural antibiotics, lactic acid and hydrogen peroxide.

Probiotics must be safe
Probiotic strains such as Lactobacillus species, Bifidobacterium species and Streptococcus species have long history of safe use and are Generally Recognized As Safe (GRAS). Other microorganisms may be problematic, they need toxicological study prior to use as probiotic. Soil based organisms and spores claimed as probiotics must have safety data to be considered as probiotics. Safety studies include – taxonomy, in vitro tests, animal feeding trials, human trials, genome sequence.

Probiotic stability
Probiotics must stay viable in food, feed, and dietary supplements (powder, capsules and tablets). Poor stability discredits the entire probiotic category. Manufacturing has great impact on probiotic stability. Probiotic stability is affected by high temperature, oxygen, humidity and high water activity in culture and expipient. Stability is also strain specific. Nitrogen packaging enhances stability which is used by UAS Laboratories. Micro encapsulation improves stability by protecting the core probiotic material from oxygen and moisture. Patented stabilization technology used by UAS Laboratories and others significantly enhance stability. If the probiotic products do not contain the same microorganisms with same viability, they will not offer same consistent good result.

Probiotics must be resistant to in vivo conditions
Probiotics must be acid and bile resistant when given orally. DDS-1 L. acidophilus and some other probiotics are acid and bile resistant. Orally administered probiotics must be resistant to enzymes in the oral cavity. Depending on the administration site of probiotics, they should be resistant to the specific conditions occurring on or in that location of the body. Similarly, probiotic strains must be resistant to the specific conditions in milk, yogurt, cheese, chocolate, cereal and other foods and drinks.

Probiotics must adhere and colonize
In order to produce enzymes, lactic acid, vitamins and natural antibiotics, the probiotic strains must adhere to the intestinal wall, colonize and multiply. Probiotic strains isolated from human gut adhere and colonize better than probiotic strains with animal origins. Probiotic strains with the ability to compete with other microorganisms, for example by the production of antimicrobial substances, have the best chance to colonize. DDS-1 L. acidophilus is a good example.

Antimicrobial activities of probiotics
Some probiotic strains have antimicrobial properties such as DDS-1 L. acidophilus. They inhibit pathogenic microorganisms such as E. coli, Salmonella and others (US Patent Number 3,689,640 – In Vitro Antibacterial Activity of DDS-1 L. acidophilus). The inhibition of pathogenic microorganisms is done by the selected probiotic strains due to their:
- Production of antibiotic like substances – acidophilin
- Lowering of pH by producing lactic acid
- Production of hydrogen peroxide
- Decreasing the digest potential
- Consumption of available nutrients.
Selection of the probiotic strains capable of producing antimicrobial substances is most important in developing probiotic supplements and probiotic rich foods.

Probiotic formulation
Probiotic blends and supplements must have probiotic strains which are Generally Recognized As Safe (GRAS). Multi-strain and multi-species probiotics have improved functionality as compared to single strain. However, special attention should be given to avoid combination of probiotic strains showing inhibitory properties, i.e., through the production of Hydrogen Peroxide H2O2, bacteriocin or bacteriocin like substances. Undocumented high potency probiotics: multi-strain and/or multi-species, must not be included in probiotic formulations. Probiotics are strain specific, condition specific and dose specific. Excipient mixed with probiotic strains in the formulation must contain low moisture and low water activity.

GOOD MANUFACTURING PRACTICES (GMP)

Good manufacturing Practices must be applied in the manufacture of probiotic foods and supplements with quality assurance and shelf-life conditions established.
Storage, Handling and Shipping

Storage of probiotic supplement at 40°F is recommended to maintain the viability of the micro organisms and they should be used prior to the expiration date of the product (all probiotic supplements must bear expiration date). Probiotic supplements, if not kept refrigerated, may not have viable micro organisms. There are some probiotic products which may be shelf-stable according to their manufacturers and their storage and shipping requirements must be met.

Quality control
- The criteria and procedures for quality control must be determined and implemented
- Genes, species, strains must be identified and viable micro organisms must be expressed (CFU/g) for every batch of probiotics manufactured
- Test the viability of micro organisms at manufacturing and at expiration date
- Test for pathogens and heavy metals in the probiotic culture and the finished product(s)
- Contamination of probiotic products with undesirable micro organisms is possible in uncontrolled fermentation
- Other ingredients mixed with probiotic products may add contamination
- Blending and packaging equipments may contribute to probiotic product contamination (follow good manufacturing practice).

Probiotic Labelling Guidelines from IPA & WHO

The probiotic food and supplement label should contain the following information:
- Genus, species and strain designation
- Functionality of the strains(s)
- Minimum viable numbers of each probiotic strain (CFU/g) at the end of the shelf-life.
- The suggested serving size must deliver the effective dose of probiotic related to the health claim(s)
- Total servings per container
- Health claim(s) if any (substantiated with scientific research)
- Storage condition requirements
- Nutrition/Supplement facts
- Manufacturer’s name and address
- Manufacturer’s lot number and expiration date.

How and when to use probiotic supplements

Excessive use of antibiotics, chlorinated water, processed and junk foods, and polluted environment reduce the number of friendly bacteria in the gastrointestinal tract. Seventy percent of women and forty percent of men have yeast infection and lack probiotics in their gut. The studies at the VA Hospital of Minneapolis show apparently healthy individuals who have almost no probiotics in their gastrointestinal tracts. For this reason, it is advisable that one should take a proven probiotic supplement daily. Probiotic foods and supplements containing L. acidophilus, Bifidobacterium species and Fructooligosaccharides (FOS) with two to five billion live cells [2-5x 10^9 CFU/g] should be taken daily just before breakfast or between meals for maintenance. Health professionals recommend probiotic supplements for candidiasis (yeast infection), digestive disorders (including diarrhea and constipation), gastritis, lactose intolerance, gas, heartburn, irritable bowel, collitis, Crohn’s disease and immune dysfunctions and as a follow up to antibiotic therapy. Under these conditions higher amounts of the probiotic supplements are used.

Remember, if the probiotic supplement does not contain right probiotic strains, right potency, right formula and not viable and acid and bile resistant, it will offer no health benefits.

A reminder: Use of probiotic supplements or any other supplements should be taken with the advice of a health professional who has knowledge and expertise in probiotics and other supplements.

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REFERENCES AND NOTES