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Selection criteria for probiotic microorganisms

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Probiotics are preparations of live microorganisms which beneficially affect the host by improving the properties of the indigenous microbes. Since the human intestinal flora plays an important role in health and disease of man, probiotics are used to improve intestinal health and to stimulate the immune system. The microbes commonly used as probiotics for humans are the lactic acid bacteria (LAB). In early studies the strains used for fermenting milk products for human consumption were frequently used as probiotics. Subsequently, it was realised that it would be more appropriate if the strains originated from the human intestinal tract and that in addition to LABs, other microorganisms could be used either singly or in combination. Today, strict selection criteria are employed to obtain functional probiotic strains. It is generally agreed that the strain should be of host origin, well characterised, able to survive the rigours of the digestive tract and possibly colonise, biologically active against the target as well as to be stable and amenable to commercial production and distribution. In addition, information on dosages and evidence of efficacy needs to be obtained. *In vitro* and *in vivo* studies are frequently combined to allow investigation of the various parameters, and ultimately clinical trials are required. Although lactic acid bacteria have been generally recognised as safe, the question of safety is discussed for LAB and non-LAB probiotic strains in terms of potential pathogenicity of the strains and risk to the individual and the community. Finally, even though the techniques for genetic manipulation of many probiotic strains are available, it is not envisaged that this issue will be addressed in the near future because of regulatory implications. It is proposed that when this type of selection criteria is employed, probiotics strains with demonstrable efficacy can be obtained.

Background

The concept of probiotics was in use in the early 1900s, however, the term was only coined in 1965 by Lilly and Stillwell and has subsequently evolved. Numerous definitions have been proposed. Initially it was used by Lilly and Stillwell to refer to the stimulation of the growth of one microbe by another, in other words, the opposite of antibiotic. Today it is generally agreed that a probiotic is a preparation "of live microorganisms which, applied to man or animal, beneficially affects the host by improving the properties of the indigenous microbiota"[17].

The indigenous microbiota of the gastrointestinal tract plays an important role in the health and well being of the host[27]. Some of the beneficial and harmful effects of the gastrointestinal microbiota are summarised in Table 1. It is envisaged that these parameters may be influenced by probiotic administration. Initially lactic acid bacteria (LAB), particularly lactobacilli, were orally administered to man with promising but often non-conclusive effects. In fact, there has been considerable controversy over the validity of statements about the beneficial effects of lactobacilli and probiotic preparations[15]. This is best exemplified by the titles of some reviews around 1990, namely, "Lactobacillus: fact and fiction"[4], "Probiotics--fact or fiction?"[24] and "Probiotic bacteria: myth or reality?"[28] The most striking common feature for all reviews of the time is the comment that not all lactobacilli strains behave the same, and a stringent criterium for strain selection is required in order to consistently achieve positive results using probiotics. The careful analysis in the early 1990s of probiotics lead to recommendations for the future and heralded the way for effective probiotic preparations of tomorrow by strict attention to strain selection. This is summarised in a recent review entitled "The coming of age of probiotics"[22].

Table 1. Influences of the human intestinal microbiota on the host

Beneficial effects	Harmful effects
Inhibition of pathogens	Constipation
Stimulation of immune system	Diarrhoea
Synthesis of vitamins	Infections
Aid in digestion	Liver damage
Produce metabolic fuel for enterocytes	Cancer
Maintain stability of ecosystem	Flatulence
Metabolise drugs	

In this paper, the range of microorganisms used as probiotics is presented and the mechanisms by which probiotics are beneficial to the host are discussed. The parameters for selecting and evaluating strains of microorganisms for use as probiotics are then discussed in

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terms of the beneficial effects that can be achieved.

Microorganisms used as probiotics

Although many of the early studies primarily used lactobacilli and in particular those used for production of fermented milks, preparations of microorganisms have not been limited to fermented milk products. In the 1920s, it was shown that the bacillus in bulgarian fermented milk did not survive in the human gut and consequently intestinal isolates of *Lactobacillus acidophilus* were used as dietary supplements and clinical trials gave encouraging results. Over the years, a number of microbes have been utilised as probiotics for humans and these are presented in Table 3.

The rational for using the LAB is historically based since Metchnikoff[26] originally proposed use of this type. In addition, this group of bacteria is very rarely pathogenic.

Today, there is increasing interest in the use of strains other than the traditional LAB (lactobacilli and bifidobacteria). For example, *Saccharomyces boulardii*, non-pathogenic *Escherichia coli*,[6] *Salmonella typhimurium*[1] and *Clostridium difficile*[3] and complex mixtures of intestinal microbes[35] have been used.

Beneficial effects of probiotics

The beneficial and harmful effects that the indigenous microbiota exert on the host have been summarised in Table 1. These are consistent with the reported or proposed beneficial effects of probiotics as presented in Table 2. While evidence is accumulating that probiotics may be beneficial to man, there is still a sparsity in the literature of well conducted clinical trials proving efficacy. The subject has been extensively reviewed[9,10,20,29,30,34].

Table 2. Areas of application of probiotics for humans

Enhancement of resistance against pathogens
Stimulation of the immune system
Lactose intolerance
Prevention or reduction of constipation
Prevention of diarrhoea
Reduction in the risk of colon cancer
Reduction in levels of faecal enzymes associated with cancer
Reduction of irritable bowel syndrome
Improved health
Reduction in cholesterol

Table 3. Microorganisms used as probiotics for humans

Lactobacillus acidophilus	*Bifidobacterium bifidum*
L. plantarum	*Bif. infantis*
L. casei	*Bif. adolescentis*
L. casei ssp. rhamnosus	*Bif. longum*
L. delbrueckii ssp. bulgaricus	*Bif. breve*
L. fermentum	*Strep. salivarius ssp. thermophilus*
L. reuteri	*Enterococcus faecalis*
Saccharomyces boulardii	*Enterococcus faecium*
Lactococcus lactis ssp lactis	*Lactococcus lactis ssp cremoris*

There is a positive trend that probiotics can function in a diverse range of applications. Unfortunately, studies are usually carried out on a limited number of subjects. Inherent with the use of human subjects, the researchers are plagued with variations (between individuals themselves and with their diets) which can only be reduced by using a large number of subjects. A very promising article presenting evidence of immuno-modulation in man following the ingestion of LAB has recently been published[31].

In some instances, it is the probiotic microbes themselves which function for the benefit of the host, such as antimicrobial mechanisms, while in other cases, the probiotic microbe may trigger the indigenous microbes or the host physiology to induce the action. It can be envisaged that any one probiotic preparation may be, but need not be, multi-functional. The beneficial effects presented in Table 2 can be grouped as follows:

- antimicrobial
- biochemical
- physiological and immunological

Antimicrobial mechanisms refer to the actions of the probiotic preparation on another microbe or group of microbes. These are directly applicable to the use of probiotics for enhanced resistance against intestinal pathogens and prevention of diarrhoea[7]. The types of interactions include competitive colonisation as well as adhesion and growth inhibition.

Competitive colonisation refers to the fact that the probiotic strain can successfully outcompete the pathogen for either nutrients or the site of colonisation. Since many gastrointestinal pathogens attach to the intestinal mucosa as the first step in infection, it would be beneficial to the host if this adhesion could be inhibited. There are reports that lactobacilli produce components which inhibit attachment of enterotoxigenic *Escherichia coli* to intestinal mucosa,[2] however, there is no evidence as yet that this occurs in the digestive tract. In addition, various compounds produced during growth of the probiotic have been shown to inhibit pathogen growth[7,21]. These include organic acids such as lactic and acetic acid, reuterin and bacteriocins[33]. The organic acids lower the pH and thereby can indirectly affect growth of the pathogen. In addition, the lactic and acetic acids can be toxic to microbes. Reuterin which inhibits the growth of a very broad range of cells[23], is produced by *Lactobacillus reuteri* when grown in the presence of glycerol. Numerous bacteriocins have been reported to be produced by lactobacilli, for example, Acidophilin, Acidolin, Lactocidin, Bacteriocin, Bulgarican, Lactolin, Lactobacillin and Lactobrevin[7,21]. They can either have a very broad range of activity or alternatively specifically inhibit the growth of a very limited range of closely related microbes. For example, *Lactobacillus sp* exhibited specific antagonistic effects towards *Clostridium ramosum.*[25]

Biochemical effects of the probiotic include:

a) the reduction of faecal enzymes which can convert co-carcinogens to carcinogens in the digestive tract
b) decrease of lactose intolerance
c) reducing of serum cholesterol.

The ingestion of lactobacilli has resulted in a reduction in faecal enzymes such as beta-glucuronidase, azoreductase and nitro-reductase in humans[14]. These enzymes can be produced by the bacteroides group of bacteria and it is probable that the presence of the probiotic strains influences either the production of the enzyme or the levels of the specific microbes which produce the enzyme. Lactose intolerance occurs in subjects who lack the enzyme, lactase (a beta-galactosidase). Symptoms include abdominal pain and osmotic diarrhoea after eating foods high in lactose since the lactose is not degraded and absorbed in the upper regions of the small intestine and hence can be used by the indigenous microbiota. This results in production of gases and organic acids which give rise to the symptoms in lactose intolerant patients. Ingestion of probiotic microbes which contain and produce beta-galactosidase results in degradation of the lactose before it reaches the indigenous microbes in the lower part of the small intestine.

It is reported that probiotics such as lactobacilli can assimilate cholesterol[12] and deconjugate bile acids[11] and that this will lead to a reduction in serum cholesterol levels. At present the evidence for this is based on the laboratory evidence of assimilation of cholesterol and *in vivo* action in one study, however, the findings have not been confirmed by other workers and it is proposed that the assimilation may in fact be co-precipitation of the cholesterol with bile acids at low pH. Since propionic acid can reduce *de novo* synthesis of cholesterol in the liver, it has been suggested that probiotics that produce propionic acid could reduce cholesterol synthesis. Unfortunately, it is unlikely that sufficient levels of propionic acid in the liver can be achieved for this mechanism to influence cholesterol levels.

Physiological mechanisms of probiotics refer to the influences of these microbes on the host responses and include the following:

a) stimulation of the immune system
b) reduction of the risk of colon cancer as measured by tumour suppression

There is accumulating evidence that lactobacillus cell components directly stimulate the immune response[18]. This has ramifications for both protecting the host from infection and for conditions which involve the immune response, such as irritable bowel syndrome and colon cancer. In some cases an adjuvant effect has been noted and this represents a general enhancement of the immune status of the host as a result of probiotic dosage. Such a general enhancement may also assist the host is suppressing tumours and there is evidence available from animal model studies that this can occur[13].

Parameters for evaluating probiotic strains

Non-conclusive and even contradictory studies on probiotic usage have been reported over the years. It is now generally agreed that more rigorous attention to strain selection would yield more conclusive results[4]. For example, it is now acknowledged that not all *Lactobacillus acidophilus* are the same, and that bacterial strains which produce desirable food products may not necessarily have a beneficial effect on the host. There is an increasing demand that strains used in probiotic preparations are stringently selected[5,8,16].

The parameters recommended to be included for selecting functional probiotic strains are presented in Table 4. This list of parameters for screening microorganisms for potentially valuable probiotic strains is based on the fact that we need strains which can be viable and metabolically active within the gastrointestinal tract and are biologically active against the identified target. In addition, it is imperative that viability of the strain and stability of the desirable characteristics of the strain can be maintained during commercial production and in the final product. Finally, it is crucial that the strain is safe. The parameters included in a strain selection criterium will be influenced by the intended target for use.

Table 4. Parameters used for selecting a functional probiotic strain.

Specified target	Host origin
Strain identified	Biological activity against target
Colonisation potential	Survival in situ
Stability of numbers	Stability of characteristics
Safe	Demonstrable efficacy
Dosage required	

Since it cannot be assumed that a probiotic strain will be effective for a broad range of applications, the target for the use of the probiotic needs to be identified. This would allow one to select strains with biological activity against the target. For example, the antibiotic associated diarrhoea has been one condition which could be treated by using probiotic microbes. The causative agent of antibiotic associated diarrhoea is often the bacterium, *Clostridium difficile*. Consequently, in order to select a probiotic strain for use in antibiotic associated diarrhoea conditions, one would select a strain which has an antagonistic effect against the *C. difficile* cells[32]. It is also necessary to ensure that antagonistic effects demonstrable *in vitro* may also be effective *in vivo*.

In order for the strain to be viable and metabolically active in the digestive tract, it is recommended that the strain be of host origin, have the potential to colonise the tract and be able to survive the rigours of the tract, such as low pH and bile acids. The rational for selecting strains of host origin is that there have been several studies reporting that strains isolated from the digestive tract of one animal, can not survive or colonise another animal. Consequently, strains originating from the human gastointestinal tract are screened for use as human probiotic microbes[19]. If the probiotic can colonise, it will ensure that the strain is maintained in the tract for a longer period of time.

Since it is technically difficult to test which of a number of strains can colonise the digestive tract of man, the colonisation potential is tested *in vitro* by studying the capacity of the strains to adhere to gastrointestinal mucosa and their capacity to grow in intestinal extracts. While this procedure is relatively straight forward, close attention to the controls in the assay is required. This aspect has been discussed in detail in a previous paper[5]. Briefly, although bacteria can adhere in an *in vitro* assay, non-specific adhesion may be involved. This can be examined by using control proteins as well as intestinal mucosa in the *in vitro* assay. Survival in conditions within the intestine can be relatively easily studied in the laboratory by using buffers of defined pH values and by the addition to buffers and growth media of bile acids or other components such as digestive enzymes, antibiotics and food additives to which the probiotic may be exposed.

Stability of viability during preparation and storage is easily monitored, and is a very important parameter. It has been shown that microbes can lose some characteristics when maintained in laboratory conditions. For example, some strains originating from the gastrointestinal tract rapidly loose the capacity to adhere to epithelial mucosa while others retain this capacity during extensive subculturing in the laboratory[4]. It is therefore important to ensure that the potential probiotic strain can retain desirable characteristics both in the laboratory and during commercial production and storage. Similarly, standard tests must be established to confirm that the strain is safe and identified taxonomically.

Clinical studies

Clinical studies are a pre-requisite for proving efficacy of a particular probiotic because of limitations with extrapolating from data obtained from *in vitro* and animals studies. Ideally, clinical studies should be conducted double blind in a cross-over fashion. The term double blind describes how the probiotic preparation must be evaluated against a placebo control and the identity of the preparations should not be know to either the medical staff or the subjects involved in the study until completion of the study. By cross-over, we refer to the fact that each subject is its own control and that all subjects are treated with both placebo and test preparations, separated by a washout period. This approach reduces the limitations of low numbers of subjects and large individual variations since each subject functions as its own control.

Strain improvement possibilities

Frequently today, microorganisms used for commercial purposes are improved by genetic manipulation. Techniques are now available to perform such manipulations on many of the strains used as probiotics as discussed by Tannock[34]. It is therefore reasonable to propose that desirable characteristics can be combined in a single strain by gene technology. While this is most probably the future for probiotics, it is not envisaged that such preparations will be introduced in the near future.

Summary

Evidence is accumulating that confirms that probiotics can benefit the host by improving intestinal well being. In order to have functional probiotic strains with predictable and measurable beneficial effects, strict attention to strain selection is required. A combination of *in vitro* and *in vivo* studies culminating in clinical trials are therefore required. It is envisaged that probiotics can be targeted for specific uses or be used to generally maintain stability of the indigenous microbes in the digestive tract.

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原生菌（Probiotic）的選擇標準

摘要

原生菌（Probiotic）是活微生物製劑，它改善原有的微生物的特性而有益宿主。人體腸道菌群在人的健康和疾病扮演重要的作用，所以原生菌（Probiotic）被用於增強腸道健康和刺激免疫系統，做為原生菌（Probiotic）常用於人體的細菌是乳酸杆菌，在早期研究，用於發酵奶製品供人體食用的菌株是常用的原生菌（Probiotic）。後來，認識到如果該菌株來自人體腸道將更合適，並且除乳酸杆菌之外，其他微生物也可以單獨或是聯合應用。今天，嚴格的選擇標準被用於獲得有效的原生菌（Probiotic）菌株。普遍認為，菌株必須來自宿主，有特性，能夠在消化道中存活和可能移生，對靶目標有生物活性，以及穩定並可改良為商品銷售。另外，需要獲得劑量和效應方面的資料，通常結合體外和體內研究，以研究不同的參數，最後需要臨床試驗，雖然乳酸杆菌總的被認為是安全。這裡從菌株的潛在致病性和對個體及社會的危險性，討論了乳酸杆菌和非乳酸杆菌原生菌菌株的安全性。最後，具備很多基因操縱技術，但此估計近期難以應用，因為涉及到論理學方面，可預料，當這選擇標準被採用時，獲得具有效應的原生菌菌株將不成問題。

References

1. Barrow PA, Tucker JF, Simpson JM. Inhibition of colonization of the chicken alimentary tract with *Salmonella typhimurium* by Gram negative facultatively anaerobic bacteria. J. Hygiene (Camb.). 1987; 98: 311-322.
2. Blomberg, L, Henriksson, A, Conway, PL. Inhibition of *Escherichia coli* K88 to piglet ileal mucus by *Lactobacillus spp*. Applied and Environmental Microbiology. 1993; 59: 34-39.

3. Borriello, SP, Barclay, FE. Protection of hamsters against *Clostridium difficile* ileocaecitis by prior colonisation with non-pathogenic strains. J. Med. Microbiol. 1985; 19: 339-350.
4. Conway PL. Lactobacilli: fact and fiction. In: Grubb, Midtvedt and Norin ed The regulatory and protective role of the normal microflora. London. The MacMillan Press LDT, 1989:263-282.
5. Conway PL, Henriksson A. Strategies for the isolation and characterization of functional probiotics. In: ed Human health: the contribution of microorganisms. IFAB Communications, 1994: 75-94.
6. Duval-Iflah Y, Ouriet MF, Moreau C, Daniel JC, Gabilan JC, Raibaud P. Implantation precoce d'une souche de *Escherichia coli* dans l'intestin de nouveau-nes humains: effect de barriere vis-a-visde souches de *E. coli* antibioresistantes. Ann. Microbiol. 1982; 133A: 393-408.
7. Fernandes CF, Shahani KM, Amer MA. Therapeutic role of dietary lactobacilli fermented dairy products. FEMS Microbiology Reviews. 1987; 46: 343-356.
8. Gibson SAW, Conway PL. Recovery of a probiotic organism from human faeces after oral dosing. In: Gibson ed Human Health: The contribution of microorganisms. London. Springer-Verlag, 1994: 119-144.
9. Gilliland SE. Acidophilus milk products: a review of potential benefits to consumers. J. Dairy. Sci. 1989; 72: 2483-2494.
10. Gilliland SE. Health and nutritional benefits from lactic acid bacteria. FEMS Microbiol. Rev. 1990; 87: 175-188.
11. Gilliland SE, Speck ML. Deconjugation of bile acids by intestinal lactobacilli. App. Environm. Microbiol. 1977; 33: 15-18.
12. Gilliland SE, Walker DK. Factors to consider when selecting a culture of *Lacobacillus acidophilus* as a dietary adjunct to produce a hypocholesterolemic effect in humans. J. Dairy Sci. 1990; 73: 905-911.
13. Goldin BR, Gorbach SL. Effect of *Lactobacillus acidophilus* dietary supplements on 1,2-dimethylhydrazine dihydrochloride induced intestinal cancer in rats. J. Natl. Cancer Inst. 1980; 64: 263-265.
14. Goldin BR, Gorbach SL. The effect of milk and lactobacillus feeding on human intestinal bacterial enzyme activity. American Journal of Clinical Nutrition. 1984; 39: 756-761.
15. Gorbach SL. Lactic acid bacteria and human health. Annals of Medicine. 1990; 22: 37-41.
16. Havenaar H, Ten Brink B, Huis Int Veld J. Selection of strainsm for probiotic use. In: Fuller ed Probiotics: The scientific basis. London. Chapman and Hall, 1992: 209-224.
17. Havenaar R, Huis in't Veld JHJ. Probiotics: A general view. In: Wood ed The lactic acid bacteria in health and disease. London. Elsevier Applied Science, 1992: 209-224.
18. Havenaar, R, Spanhaak, S. Probiotics from an immunological point of view. Current Biology. 1994; 5: 320-325.
19. Johansson M-L, Molin G, Jeppsson B, Nobaek S, Ahrné S, Bergmark S. Administration of different Lactobacillus strains in fermented oatmeal soup: *In vivo* colonisation of human intestinal mucosal and effect on the indigenous flora. Appl. Environ. Microbiol. 1993; 59: 15-20.
20. Juven BJ, Meinersmann RJ, Stern NJ. Antagonistic effects of lactobacilli and pediococci to control intestinal colonization by human enteropathogens in live poultry. Journal of Applied Bacteriology. 1991; 70: 95-103.
21. Klaenhammer TR. Bacteriocins of lactic acid bacteria. Biochemie. 1988; 70: 337-349.
22. Lee Y-K, Salminen S. The coming of age of probiotics. Trends in Food Science and Technology. 1995; 6: 219-251.
23. Lindgren SE, Dobrogosz WJ. Antagonistic activities of lactic acid bacteria in food and feed fermentations. FEMS microbiology reviews. 1990; 87: 149-164.
24. Marshall VM. Gut-derived organisms for milk fermentation. In Probiotics-fact or fiction? Journal of Chemical Technology and Biotechnology. 1991; 51: 548-553.
25. McCormick EL, Savage DC. Characterization of *Lactobacillus sp.* strain 100-37 from the murine gastrointestinal tract: ecology, plasmid content, and antagonistic activity toward Clostridum ramosum H1. Appl. Environ. Microbiol. 1983; 46: 1103.
26. Metchnikoff E. The prolongation of life. London: Heineman, 1907
27. Mitsuoka T. The human gastrointestinal tract. In: Wood ed The lactic acid bacteria in health and disease. London. Elsevier Applied Science, 1992: 69-114.
28. O'Sullivan MG, Thornton G, O'Sullivan GC, Collins JK. Probiotic bacteria: myth or reality? Trends Food Sci Technol. 1992; 3: 309-314.
29. Sanders, ME. Effect of consumption of lactic cultures on human health. Advances in food and nutrition research. 1993; 37: 67-130.
30. Sanders ME. Lactic acid bacteria as promoters of human health. In: Goldberg ed Funtional foods. New York. Chapman and Hall, 1994:294-322.
31. Schiffrin EJ, Rochat F, Link-Amster H, Aeschlimann JM, Donnet-Hughes A. Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. Journal of Dairy Science. 1995; 78: 491-497.
32. Silva M, Jacobus NY, Deneke C, Gorbach SL. Antimicrobial substance from a human *Lactobacillus* strain. Antimicrobial Agents and Chemotherapy. 1987; 31: 1231-1233.
33. Tagg JR, Dajani AS, Wannamaker LW. Bacteriocins of gram positive bacteria. Bacteriol. Rev. 1976; 40: 722-756.
34. Tannock GW. Role of probiotics. In: Gibson and Macfarlane ed Human colonic bacteria. Boca Raton. CRC, 1995:257-272.
35. Tvede M, Rask-Madsen J. Bacteriotherapy for chronic relapsing *Clostridum difficile* diarrhoea in six patients. The Lancet. 1989; I: 1156-1160.