Probiotics, prebiotics, and synbiotics—approaching a definition[1–3]

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ABSTRACT Definitions of different pro-, pre-, and synbiotics suggested by different investigators are critically discussed. On the basis of this analysis, the probiotic concept is confined to effects exerted by viable microorganisms but is applicable independent of the site of action and route of administration. It therefore may include sites such as the oral cavity, the intestine, the vagina, and the skin. *Am J Clin Nutr* 2001;73(suppl):361S–4S.

KEY WORDS Probiotic, prebiotic, synbiotic, health claims, definitions, history

HISTORY OF HEALTH CLAIMS

There is a long history of health claims concerning living microorganisms in food, particularly lactic acid bacteria. In a Persian version of the Old Testament (Genesis 18:8) it states that "Abraham owed his longevity to the consumption of sour milk." In 76 BC the Roman historian Plinius recommended the administration of fermented milk products for treating gastroenteritis (1). Since the advent of the microbiology era, some investigators [eg, Carre (2), Tissier (3), and Metchnikoff (4)] attributed such health effects to shifts of the intestinal microbial balance. Metchnikoff (4) claimed that the intake of yogurt containing lactobacilli results in a reduction of toxin-producing bacteria in the gut and that this increases the longevity of the host. Tissier (3) recommended the administration of bifidobacteria to infants suffering from diarrhea, claiming that bifidobacteria supersede the putrefactive bacteria that cause the disease (3). He showed that bifidobacteria were predominant in the gut flora of breast-fed infants.

Indeed Rettger et al (5, 6) and Kopeloff (7) showed that *Lactobacillus acidophilus* may survive in the human gut but the "Bulgarian bacillus" did not. Attempts to implant non–lactic acid bacteria such as *Escherichia coli* for "causal fighting against pathological intestinal flora" were undertaken by Nissle (8) in 1916.

The significant role of the intestinal microflora for resistance to disease was shown by Bohnhoff et al (9), Freter (10–12), and Collins and Carter (13). Oral administration of antibiotics to mice rendered the animals more susceptible to infection with *Salmonella typhimurium*, *Shigella flexneri*, and *Vibrio cholerae*. Thus, $\leq 1\times10^{1}$ *Salmonellae enteritidis* were sufficient to kill germ-free guinea pigs, whereas 1×10^{9} bacteria were required to kill animals with complete intestinal microflora.

HISTORY OF THE TERM *PROBIOTIC*

The term *probiotic*, meaning "for life," is derived from the Greek language. It was first used by Lilly and Stillwell (14) in 1965 to describe "substances secreted by one microorganism which stimulates the growth of another" and thus was contrasted with the term *antibiotic*. It may be because of this positive and general claim of definition that the term *probiotic* was subsequently applied to other subjects and gained a more general meaning. In 1971 Sperti (15) applied the term to tissue extracts that stimulate microbial growth. Parker (16) was the first to use the term *probiotic* in the sense that it is used today. He defined probiotics as "organisms and substances which contribute to intestinal microbial balance." Retaining the word *substances* in Parker's definition of probiotics resulted in a wide connotation that included antibiotics. In 1989 Fuller (17) attempted to improve Parker's definition of probiotic with the following distinction: "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance." This revised definition emphasizes the requirement of viability for probiotics and introduces the aspect of a beneficial effect on the host, which was, according to his definition, an animal. In 1992 Havenaar et al (18) broadened the definition of probiotics with respect to host and habitat of the microflora as follows: "A viable mono- or mixed culture of microorganisms which applied to animal or man, beneficially affects the host by improving the properties of the indigenous microflora." Salminen (19) and Schaafsma (20) broadened the definition of probiotics even further by no longer limiting the proposed health effects to influences on the indigenous microflora. According to Salminen, a probiotic is "a live microbial culture or cultured dairy product which beneficially influences the health and nutrition of the host." According to Schaafsma, "Oral probiotics are living microorganisms which upon ingestion in certain numbers, exert health effects beyond inherent basic nutrition."

There are 2 aspects in Salminen's definition that in our opinion need revision. First, the definition given by Salminen includes beneficial influences on "nutrition of the host" in addition to health effects. It is not clear what the term *nutrition* should imply in this context, which would not be covered by the term *health*. In our opinion, major effects on nutrition also imply effects on health, whereas minor alterations are of no relevance to the definition "for life." Therefore, the term *nutrition* might be best omitted from the definition.

In contrast with previous definitions, Salminen's definition (19) considers cultured dairy products and microbial cultures to be

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probiotic. Indeed the matrix of a product may affect the activity of microbes and therefore the survival and effect of the microbes, and thus deserves consideration. However, because nondairy products, (eg, sauerkraut, fermented cereals and other plant-based foods, and salami) may contain viable probiotic microorganisms [eg, *Lactobacillus plantarum* (21)], the limitation of the definition to dairy products is not justified. Furthermore, cultured dairy products include products that are cultured and then pasteurized or sterilized, which results in the loss of viable microorganisms. In fact there is evidence for health effects beyond nutritional value of such products, eg, anticarcinogenic and immunomodulating effects have been exerted by yogurt fractions and cell-wall components of lactobacilli and bifidobacteria (22–25).

Abandoning the viability of microorganisms or omitting the survival of the microbes and their effects on the indigenous microflora as prerequisites for the claim probiotic has consequences for what may be called probiotic. The definitions given by Salminen (19) and Schaafsma (20) would include yogurt containing usual cultures (*Streptococcus thermophilus* and *Lactobacillus delbrüecki*, subsp. *bulgaricus*) because these cultures may compensate for lactase insufficiency in lactose maldigestion (26). This substitution may be even more pronounced when bacteria that do not survive in the small bowel are ingested and release their β-galactosidase into the upper intestine. This substitution may as well be achieved by bacteria that have been killed by irradiation, which leaves their cell walls intact and therefore enables protection during gastric transit (26).

REVISION OF THE DEFINITION *PROBIOTIC*

Considering the various arguments, particularly the discrimination of usual yogurt cultures and products derived from probiotic cultures and products, we propose the following definition as the one that is closest to the definition of the term *probiotic* given by Havenaar and Huis In't Veld (18): "A preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host." Reasons for the revision of Havenaar and Huis In't Veld definition are as follows:

1) the need to include products in addition to microorganisms, or preparations of microorganisms;
2) the requirement of sufficient microbial numbers to exert health effects;
3) preference for the phrase "alteration of the microflora" over "improving the properties of the…microflora," because the optimal properties of the indigenous microflora were not defined until now and the evidence of benefit can be shown only by health effects; and
4) definition of the term *indigenous microflora* refers to "the usually complex mixture of bacterial population that colonizes a given area in the host that has not been affected by medical or experimental intervention, or by disease" (27) and use of *to colonize* to describe a bacterial population that establishes in size over time without the need for periodic reintroduction of the bacteria by repeated oral doses or other means.

Transplantation is considered to have occurred when the administration of microorganisms results in colonization. *Transient invasion* is defined as the administration of microorganisms in large numbers such that the microorganisms can be cultured regularly from various regions. If these definitions were used, "improving the properties of the indigenous microflora" would unnecessarily confine the definition of probiotics. The positive effect of lactobacilli on the infection outcome by pathogenic bacteria (28–32) could be called probiotic only if the effect is achieved beyond implantation of the administrated bacteria or due to a change in the colonizing indigenous microflora. A direct inhibitory effect exerted by bacteria transiently passing through the gastrointestinal tract would fail to meet the definition. Because the transient state is the most common condition under which probiotics are used, we prefer the expression "microflora in a compartment of the host" to "indigenous microflora."

The above definition confines the probiotic concept to effects produced by viable microorganisms but is applicable independent of the probiotic site of action and the route of administration. Therefore, this definition may include such sites as the oral cavity, the intestine, the vagina, and the skin. In the case of probiotic foods, the health effect is usually based on alteration of the gastrointestinal microflora and, therefore, based on survival during gastrointestinal transit.

A UNIFYING HYPOTHESIS FOR HEALTH EFFECTS?

The health effects attributed to the use of probiotics are numerous. The following outcomes are well documented: *1*) lower frequency and duration of diarrhea associated with antibiotics (*Clostridium difficile*), rotavirus infection, chemotherapy, and, to a lesser extent, traveler's diarrhea; *2*) stimulation of humoral and cellular immunity; and *3*) decrease in unfavorable metabolites, eg, amonium and procancerogenic enzymes in the colon. There is some evidence of health effects through the use of probiotics for the following:

1) reduction of *Helicobacter pylori* infection;
2) reduction of allergic symptoms;
3) relief from constipation;
4) relief from irritable bowel syndrome;
5) beneficial effects on mineral metabolism, particularly bone density and stability;
6) cancer prevention; and
7) reduction of cholesterol and triacylglycerol plasma concentrations (weak evidence).

These numerous effects can hardly be explained by a unifying hypothesis that is based on a single quality or mechanism and remains valid for all microorganisms exerting one or the other effect mentioned above.

STRAIN CHARACTERISTICS AND HABITAT SPECIFICITIES

Different strains of probiotic bacteria may exert different effects based on specific capabilities and enzymatic activities, even within one species (33, 34).

Different microorganisms express habitat preferences that may differ in various host species (27). Lactobacilli are among the indigenous flora colonizing the chicken's crop, the stomach of mice and rats, and the lower ileum in man. Bacteria colonizing such high-transit-rate sites must adhere firmly to the mucosal epithelium (35–37) and must adapt to the milieu of this adhesion site. The competition for adhesion receptors between

probiotic and pathogenic microorganisms, therefore, is dependent on such habitat specifics.

On the other hand, bacteria are found in much higher numbers in the colon, particularly in the feces, than are lactobacilli. It is self-evident that effects bound to this luminal site of action may be exerted even more efficiently by such microorganisms, which do not necessarily need to adhere to the mucosa. Moreover, preferences for microhabitats have to be considered. Four microhabitats in the gastrointestinal tract were outlined by Freter (27) as follows: *1*) the surface of epitheliums cells; *2*) the crypts of the ileum, cecum, and colon; *3*) the mucus gel that overlays the epithelium; and *4*) the lumen of the intestine.

As mentioned above, several indigenous, pathogenic, or probiotic microorganisms target the surface of the epithelium by specific adhesion, often mediated by special organelles, eg, fimbriae (37, 38). The crypts are typically colonized by motile, spiral-shaped bacteria of the genera *Borellia*, *Treponema*, *Spirillium* (39, 40), and others, eg, *H. pylori* (41). The mucus layer can form a microbial habitat and can protect the host against colonization in some circumstances. As a result of its complex and varying composition and for technical reasons, its function in this context is least clarified.

The luminal content of bacteria depends greatly on bowel transit. Therefore, the microbial density in the small bowel is low, whereas it is abundant in the lumen of the colon, which gives space to microorganisms without adhesion molecules.

When the great variety of species, strain characteristics, and the habitat specifics are considered, it becomes clear that a proven probiotic effect on a one strain or species can not be transferred to other strains or species.

DEFINITION OF *PREBIOTIC*

The term *prebiotic* was introduced by Gibson and Roberfroid (42) who exchanged "pro" for "pre," which means "before" or "for." They defined prebiotics as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon." This definition more or less overlaps with the definition of dietary fiber, with the exception of its selectivity for certain species. This selectivity was shown for bifidobacteria, which may be promoted by the ingestion of substances such as fructooligosaccharides and inulin (42–44), transgalactosylated oligosaccharides (45–47), and soybean oligosaccharides (48, 49).

DEFINITION OF *SYNBIOTIC*

The term *synbiotic* is used when a product contains both probiotics and prebiotics. Because the word alludes to synergism, this term should be reserved for products in which the prebiotic compound selectively favors the probiotic compound. In this strict sense, a product containing oligofructose and probiotic bifidobacteria would fulfill the definition, whereas a product containing oligofructose and a probiotic *Lactobacillus casei* strain would not. However, one might argue that synergism is attained in vivo by ingestion of lactobacilli on the one hand and promotion of indigenous bifidobacteria on the other hand.

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