

# Prebiotics and Probiotics within the Framework of the Hologenome Concept

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## Abstract

Prebiotics and probiotics fall within the framework of the hologenome concept which posits that the holobiont (host plus all of its associated microorganisms) with its hologenome, acting in consortium affects all aspects of the holobiont's fitness (adaptation, survival, development, growth and reproduction) and evolution. Variation in the holobiont can be brought about by amplification of endogenous microbes and by acquisition of novel strains. Prebiotics and probiotics function by these mechanisms and can cause rapid changes in the diverse microbiota, which not only can benefit (or harm) the holobiont in the short term but also can be transmitted to offspring and lead to long lasting cooperations and changes which cannot as yet be predicted. The role of bacteriophages in moderating the effects of prebiotics and probiotics is also discussed.

**Keywords:** Prebiotic; Probiotic; Hologenome; Holobiont; Symbiosis; Bacteriophage

## Introduction

Interest in the natural microbiota and claims of its advantage to human health paralleled the development of microbiological research in the last half of the 19<sup>th</sup> century. The Nobel laureate Élie Metchnikoff is considered the first to suggest that it is possible to modify the gut microbiota and to replace harmful microbes with useful microbes [1]. Subsequently, Tissier recommended the administration of bifidobacteria to infants suffering from diarrhea, claiming that bifidobacteria were predominant in the gut microbiota of breast-fed infants [2]. Further evidence for the beneficial effects of intestinal microbiota came from studies in the 1950s showing that mice treated with antibiotics were much more sensitive to infection with pathogens [3,4]. It was not till 1965 that Lilly and Stillwell [5] first introduced a definition of *probiotics*, "substances secreted by one microorganism which stimulates the growth of another". As will be discussed later, this definition is far from the one currently accepted.

During the last two decades, the development of molecular tools for analyzing microbial communities without having to culture the microorganisms has inspired numerous studies dealing with the human microbiota. This has brought together many disciplines concerned with health and disease of animals and plants, including prebiotics and probiotics. The latter two fields have remained applied, essentially testing different formulations for their health benefits. The goal of this review is to indicate how consideration of the complex interactions that take place between microbes themselves and between them and their hosts can broaden the scope of researchers dealing with prebiotics and probiotics, thereby stimulating new and interesting research. The hologenome concept, described briefly below, provides a broad theoretical framework for understanding the role of symbiotic microorganisms in adaptation, development, health and evolution of animals and plants, and has special implications regarding prebiotics and probiotics.

## The hologenome concept

The hologenome theory of evolution considers the holobiont with its hologenome, acting in consortium, as a unit of selection in evolution [6-8]. The holobiont has been defined as the host organism and all of its

symbiotic microbiota [9]. and the hologenome is the sum of the genetic information of the host and its microbiota. The theory is based on four generalizations, each of which is supported by a large body of empirical data:

(i) All animals and plants harbor abundant and diverse microorganisms. The surfaces of animals and plants contain a great abundance and variety of microorganisms. The number of microbial cells and their combined genetic information often far exceeds that of their hosts. For example, using the powerful technique of metagenomics, it has recently been reported that the number of different bacterial genes present in the human gut exceeds 3.3 million [10]. By comparison, the human host genome contains about 20,500 genes [11]. This large number of microbial genes considerably increases the potential for adaptation of the holobiont. In spite of the large number of bacterial species in the gut, very few strains and even fewer species are used as probiotics [12]. This fact emphasizes how little we know about the functions of specific strains or species within the human microbiota. The unexplored potential is great. In addition, wherever microbes exist bacteriophages also occur and probably play an important role in microbiota dynamics, which will be discussed later.

(ii) Microbial symbionts affect the fitness of the holobiont. Considering the holobiont as a unit of selection in evolution, we argue that the cooperation between the microbiota and the host generally leads to improved fitness of the holobiont, by the host outsourcing [13] different kinds of functions to its microbiota and vice versa. The human/mouse gut symbiosis has provided a wealth of detailed

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information on how microbiota contributes to the health of the human holobiont [7]. There is at present also several well-documented health claims attributed to probiotics, the main ones being: (1) reduction in the frequency and duration of different types of diarrhea, (2) stimulation of the humoral and cellular immune systems, (3) decrease in unfavorable metabolites in the colon, and (4) possible reduction of *Helicobacter pylori* and respiratory tract infections.

(iii) Symbiotic microorganisms are transmitted between generations. The hologenome theory of evolution relies on ensuring the continuity of partnerships between holobiont generations. Accordingly, both host and symbiont genomes must be transmitted with accuracy from one generation to the next. The precise modes of vertical transmission of host genomes are well understood and need not be discussed here. However, in recent years, it has become clear that microbial symbionts can also be transmitted from parent to offspring by a variety of methods. In mammals including humans, the initial population of symbionts is derived during passage through the birth canal and subsequently by close physical contact with parents. A greater similarity of microbiota within family members was found than between families [14] in addition to similarity between microbiota of vaginally delivered infants and their mother's vaginal microbiota [15]. Furthermore, over evolutionary timescales, the composition of the gut microbiota among great ape species is phylogenetically conserved and has diverged in a manner consistent with vertical inheritance [16]. The fact that individuals can acquire and transfer symbionts throughout their lives, and not just during their reproductive phase, means that the parents, grandparents, friends, siblings, spouses or any organism that is in close contact with a fellow human and can transfer symbionts and thereby influence the holobiont of the next generation. As is discussed below, this clearly has implications with regard to probiotic therapy. Lombardo [17] has argued that that access to mutualistic endosymbiotic microbes is an underappreciated benefit of group living.

(iv) Variation in the hologenome can be brought about by changes in either the host or the microbiota genomes. According to the hologenome theory of evolution, genetic variation can arise from changes in either the host or the symbiotic microbiota genomes. Variation in host genomes occurs during sexual reproduction, chromosome rearrangements and ultimately by mutation. These same processes occur in microorganisms with the noteworthy difference that in haploid bacteria recombination occurs, within the same species, by conjugation, transduction and DNA transformation. In addition to recombination and mutation, changes in the genome of the microbiota of holobionts can occur by two other processes: microbial amplification and acquisition of novel strains. Let us also bear in mind that all modes of bacterial variation can be driven by random events and/or can occur as a result of deterministic effects such as change brought about by prebiotics and probiotics.

Microbial amplification is the most rapid and easy to understand mode of variation in holobionts. It involves changes in the relative numbers of the diverse types of associated microorganisms that can occur as a result of nutrient availability, exposure to antibiotics or prebiotics. The holobiont is a dynamic entity with certain microorganisms multiplying and others decreasing in number as a function of local conditions. Considering the large amount of genetic information encoded in the diverse microbial population of holobionts, an increase in the number of a particular microbe is equivalent to gene amplification- which may come into use during changing conditions such as diet or illness.

Another mechanism for introducing variation into holobionts is acquiring new symbionts from the environment. Animals and plants come in contact with billions of microorganisms during their lifetime, including probiotics, some of which may become rapidly established while others may need to be seeded again and again. Unlike microbial amplification, acquiring new symbionts can introduce entirely new genes into the holobiont.

These points taken together suggest that the genetic wealth of diverse microbial symbionts, including those acquired by probiotics, can play an important role both in health and adaptation of humans and other organisms. During periods of stress, this diverse microbial symbiont community can aid the holobiont in health maintenance, healing and survival. When the stress cannot be overcome by either the host or the microbial symbionts, and the life of the holobiont is threatened, the symbionts may separate from their host [18].

### Prebiotics and the principle of variation of holobionts by amplification

The classical definition of prebiotics was given by Gibson and Roberfroid [19] who defined prebiotics as non digestible food components, usually oligosaccharides, which evade digestion by mammalian enzymes, in the upper regions of the gastro intestinal tract, reach the colon in an intact state and are metabolized by the beneficial members of the indigenous microbiota. More recently the same group put forth a more general definition: "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" [20]. The implication of this definition is that prebiotics in the form of functional carbohydrates change the gut microbiota and its metabolic activity similarly to natural fiber present in the normal human diet, except that prebiotics are targeted at specific bacteria and are degraded in a specific form and therefore are more controlled. Both fiber and prebiotics have been shown to exert health benefits [21], the main ones being: improving or stabilizing microbiota, improvement of intestinal function, increase of mineral absorption, modulation of gastro-intestinal excretions, energy metabolism and satiety, modulation of intestinal immune functions, and reduction in the risk of intestinal infection.

During the last few years, there have been numerous studies demonstrating that changes in the diet results in change in the gut microbiota [e.g., 22,23]. Certain bacterial species are amplified, whereas other decrease in number. This leads to a change in the hologenome, which can either be beneficial, neutral or disadvantageous to the holobiont [24,25]. It is interesting to note that prebiotics can not only lead to a rapid effect on of the health of the holobiont, but can also affect its behavior and evolution. Recently, it was shown that the fruit fly *Drosophila melanogaster* fed a diet containing starch, instead of sucrose, amplified *Lactobacillus plantarum* and developed homogamic mating preference [8], i.e., "starch flies" preferred to mate with "starch flies" and "sucrose flies" preferred to mate with "sucrose flies". The significance of this finding is that mating preference is considered to be an early stage in sexual isolation and speciation [26,27]. Interestingly, when the flies were treated with antibiotic prior to changing their diet, they failed to develop mating preference [28]. These data suggest that, in general, potentially beneficial bacteria could be irreversibly eliminated by antibiotic treatment, as was suggested previously [29], and that their replacement via probiotics could provide a wealth of health benefit lost by antibiotic treatment [30].

## Probiotics and the hologenome concept

As mentioned above, animals are constantly being exposed to novel microorganisms in the food they eat, water they drink and air they breathe. A small group of these microbes are pathogens. For over 100 years, infectious disease has been a major area of microbiological research, including modes of infection, virulence and transmission. However, to the best of our knowledge, natural epidemics of beneficial bacteria have not been considered. Although not easy to detect, it is reasonable to assume that such beneficial epidemics do take place and contribute to the evolution of holobionts. Recently, we presented the “coral probiotic hypothesis” to explain how certain corals became resistant to a specific pathogen, *Vibrio shiloi*, even though corals do not produce antibodies [31].

Probiotics has been defined in different ways since 1965 when initially proposed. The following accepted definition of *probiotics* appears to capture our current knowledge of the subject: “Live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” [32]. Unlike variation of the hologenome by acquisition of microbes from the environment, probiotic technology involves the nonrandom introduction of specific bacteria to improve the health of the host. The effects of probiotics can even be transferred to the next generation as was shown in a study [33] where pregnant women treated with *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* had reduced frequency of gestational diabetes mellitus and diminished risk of larger birth size in affected cases. In view of the fact that large birth size is a risk factor for later obesity, the present results are of significance for public health in demonstrating that this risk is not only modifiable but also transferred from one generation to the next. Going one step further we speculate that, since probiotics can be transmitted to future generations, they could, in principle, be used to treat not only metabolic and alimentary tract diseases such as diabetes, coronary heart disease, and alimentary tract diseases, but also genetic and behavioral human diseases. In addition, probiotics could also be used more intensely in agriculture to combat diseases and improve yields and achieve specific characteristics in plants [34,35] and animals [36].

## The possible modulating effect of bacteriophage on prebiotics and probiotics

Animals and plants contain abundant and diverse viral populations that are part of the holobiont. Bacterial viruses (bacteriophages) are of particular interest because they regulate the numbers of specific bacteria by predator/prey relationships [37]. Bacteriophages are host-specific, often attacking only a specific bacterial strain within a species. Because bacteriophage/host dynamics predicts that the most abundant bacteria (the ones that would outcompete the others in the absence of predators) are attacked and lysed much more rapidly than less abundant bacteria, bacteriophage predation results in a change in the bacterial community, the so-called ‘killing the winner’ hypothesis [37].

With regard to the human gut, it has been reported, using metagenomic analysis, that fresh feces contain an abundant and diverse community of bacteriophage [38]. It follows that these bacteriophages must have been multiplying and lysing bacteria in the gut. Although it has not yet been studied, it is likely that bacteriophages influence the composition of bacterial populations in the intestine through specific predation on their hosts. As a particular bacterial strain becomes dominant, bacteriophage can infect and lyse their host, allowing another bacterial strain, either of endogenous or exogenous (e.g., probiotic)

origin, the opportunity to become abundant. We thus suggest that one of the contributing factors to the success of probiotics may be that the introduction of a novel bacterial strain could provide an advantage over endogenous strains because bacteriophage specific to the novel strain may be absent or in low numbers. The role of bacteriophage on modulating probiotics has recently been discussed by Ventura et al. [39]. Prebiotics affecting the abundance of different strains may also have an advantage since the prebiotic amplified bacteria were previously probably in a minority and therefore not affected by phages.

## Conclusions and Implications

The hologenome theory considers all of the diverse microbiota associated with the human, animal or the plant as part of the developing, growing, reproducing, surviving, adapting and evolving holobiont. Changing the microbial community by (i) amplification of some endogenous species and (ii) acquisition of novel microbes from the environment provide important mechanisms for adaptation and evolution. These two mechanisms are the basis of prebiotic and probiotic activity: amplification of preplanned specific microorganisms can be achieved by appropriate prebiotics and acquisition of novel bacteria can be achieved via probiotics. Probiotics and prebiotics may have great potential in aiding human, animal and plant health. Despite the very interesting possibilities, great caution should be applied to these technologies until more is known about the “healthy” natural microbiota and its metabolic effects and more is understood about the dynamics and health effects of probiotic bacteria and prebiotic ingredients. In light of this, we predict that in the future both prebiotics and probiotics will not remain solely within the realm of food industry but will move into the field of medicine and be treated as medicine. However, extensive large clinical trials are required before prebiotics and probiotics can be used as therapy or prophylaxes.

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