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# Evidence of Probiotic Strain Specificity Makes Extrapolation of Results Impossible From a Strain to Another, Even From the Same Species

*Philippe Marteau*

**Affiliation:** *Medicosurgical Department of Digestive Diseases, Lariboisière Hospital AP-HP & Paris 7 University, Paris, France*

## ABSTRACT

Clinical trials show that some probiotic effects are observed with specific strains but not in control strains. The strains compared were often members of different species or even genus. There is a tendency to believe that some extrapolation of results could be possible for microorganisms of the same species, and this is especially done by some companies selling products that were not studied and using studies performed with different strains. However, several studies compared the composition, characteristics, and physiological properties of probiotic strains in comparison to very closely related strains within the same species. These studies show strain or family-specific differences. This paper will provide examples showing that related bacterial strains can differ significantly in their genotype, phenotype, and properties. The implications of this strain-specificity are that (1) for commercial products, documentation of health effects must be conducted on the specific strain contained in the product; (2) one should avoid any extrapolation of positive or negative effects between probiotic strains; (3) meta-analysis of the effect of probiotics with different active molecules should be avoided; (4) one should not call a probiotic a strain that has not been studied just on the basis that it is taxonomically related to a well-proven probiotic strain. These rules are presently frequently violated.

Keywords: probiotics, strain specificity, *Lactobacillus*, *Bifidobacterium*

Correspondence: Philippe Marteau, MD, PhD, Department of Digestive Diseases, Lariboisière Hospital 2, rue Ambroise Paré, 75010 Paris, France. Tel: 33-1-49-95-25-79; Fax: 33-1-49-95-25-77; e-mail: philippe.marteau@lrb.aphp.fr

## INTRODUCTION: OBJECTIVES

According to the definition, probiotics are: ''Live microorganisms which when administered in adequate amounts confer a health benefit on the host.''[1,2] Only a limited number of microbial strains have been properly studied and have shown beneficial properties for humans. The European Food Safety Authority (EFSA) highlighted as critical factors for probiotic health claim submissions genetic typing of the strain(s), internationally recognized naming protocols and evidence of consistency in the final product. In this paper, I will summarize what is known on similarities and differences between probiotic microorganisms and properties. I will provide examples of strain specific properties, which demonstrate that extrapolation of results from a strain to another should be banned.

Organisms classified in any particular group have certain common characteristics but also display variations (for example in size, shape, colors). Taxonomy, which allows naming the organisms and classifying them into groups of various levels, is based on the fundamental concepts of unity and diversity among living organisms. The principle is that members of higher-level groups share fewer characteristics than those in lower-level groups. The usual classification of microorganisms follows the Linnaeus rules and uses a binomial system. The names (italicized in print) designate first the genus and second a specific epithet for the species. A strain is a subgroup of a species with original intraspecies characteristics; it is identified by a name, number, or letter that follows the specific epithet. An example of probiotic strains and their taxonomy are given in **Table 1**. Taxonomy may use phenotypic and/or genotypic characteristics (ie, DNA–DNA relatedness). Presently, probiotic properties have not been sufficiently linked to specific phenotypic properties or to specific genes; they cannot therefore be safely predicted from taxonomy.

## DATA SOURCE STUDY SELECTION

I searched the medline database using the search words ''probiotics,'' ''probiotic strains,'' and ''probiotics randomized controlled trials.'' I selected papers that mentioned strain comparisons in their title or abstracts. I read these papers fully as well as their relevant references. The majority of them compared strains of different species but some compared strains of the same species and I especially analyzed those.

## RESULTS: EXAMPLES OF STRAIN SPECIFIC PROBIOTIC PROPERTIES

Several properties that are thought to be important for the probiotic effect as they can (at least) modify the survival capacity of the strain in vivo clearly differ between strains of

**Table 1.** Examples of Probiotic Strain Taxonomy (Genus, Species, and Strain Details)

| Genus Species Strain | Genus Species Strain |
|---|---|
| *Lactobacillus casei Shirota* YIT9029 | *Bifidobacterium lactis* BB-12 |
| *L. casei* DN114-001 | *B. animalis* DN173-010 |
| *L. acidophilus* NCFM | *B. infantis* 35624 |
| *L. rhamnosus* GG | *B. longum* BB536 |
| *L. plantarum* 299V | |
| *L. johnsonii* La1 | |
| *L. reuteri* SD2112 | |

different or similar species. They include tolerance to acid, bile, and pancreatin;[3] adherence to mucus or to epithelial cells;[4–7] enzymatic activity;[8] and antibiotic resistance or production of antimicrobial compounds.[9,10]

In some instances, the genetic differences between probiotic and nonprobiotic strains have been established. For example, Coudeyras et al. compared the genome of two probiotic strains of *Lactobacillus rhamnosus*: LR35 and LGG and detected five LR35-specific sequences by subtractive hybridization using the LGG genome as a driver.[11] Klaenhammer et al.[12] performed a comparative genomic analysis of four completely sequenced *Lactobacillus* strains vs 25 lactic acid bacterial genomes and highlighted strain-specific genes coding for mucus-binding proteins involved in cell-adhesion. Tallon et al.[7] evaluated the adhesion properties of 31 strains of *L. plantarum* to mucins or epithelial cells and showed that these properties varied greatly depending on the strain. A similar phenomenon with relevant intraspecies differences in adhesion properties has been also reported with *L. plantarum*[4,5] *L. casei*, and bifidobacteria.[6]

Several studies have also shown differences in the immunomodulating properties of various probiotics between strains within the same species. For example, Medina et al.[13] showed that different strains of *Bifidobacterium longum* varied greatly in their capacity to induce cytokine production (IL-10, IFN-γ and TNFα) by peripheral blood mononuclear cells and could even drive the immune responses in different directions. López et al.[14] evaluated the specific immune activation properties of 12 different *Bifidobacterium* strains belonging to four species on the maturation pattern of human monocyte-derived dendritic cells, and on their ability to induce cytokine secretion. All bifidobacteria tested were able to induce dendritic cell maturation (common trait) but showed differences in the levels of cytokine production, especially IL-12, IL-10, TNFα, and IL-1β (intraspecies strain specificity). Lin et al.[15] compared the effects of various strains of *L. reuteri* (intraspecies specificities) and showed that they exhibited differential immunoregulatory capabilities including an original TNF and MCP-1 suppression.

Denou et al.[16] performed a series of experiments to identify genotypes associated with the different phenotypes of *L. johnsonii* including the probiotic strain NCC533. Their first observation was that *L. johnsonii* NCC533 and *L. johnsonii* ATCC 33200 had a significantly different pharmacokinetics (a phenotypic characteristic) after oral feeding to mice. They hybridized DNA of the ATCC strain against a microarray of the NCC533 strain, and identified 233 genes specific for the long-gut-persistence isolate. Whole-genome transcription analysis of the NCC533 strain identified 174 genes that were expressed in the jejunum of mice colonized with this strain. Fusion of the two microarray data sets identified three gene loci expressed in vivo and specific to the long-gut-persistence isolate. These genes include glycosyltransferase genes and a sugar phosphotransferase system; their deletion (in knockout mutants) decreased the gut residence time of the bacteria in the gut.

Head-to-head comparisons of different strains in human studies are rare. Several randomized and blinded trials compared strains belonging to different species and showed significant differences in clinical efficacy.[17–23] For example, in a randomized controlled trial, *B. infantis* 35624 reduced symptoms of irritable bowel syndrome while *L. salivarius* UCC4331 was not effective.[17] Other randomized controlled trials showed that different probiotic species had different effects in immune modulation, protection against infections, and diarrheal diseases.[18–23] These is also indication of the strain specificity of the safety of probiotics.[24–27] In my literature search, I did not find randomized controlled trials comparing two products with a single bacterial species and differing at the strain level. Such study exists, however, in an animal model and also shows strain specificity. Yoshimura et al.[28] examined the protective effect of nine bifidobacterial strains from six species against *Escherichia coli* O157:H7 infections using gnotobiotic mice. Seven days after oral administration of each *Bifidobacterium* strain, the mice were orally infected with the pathogen *E. coli* O157:H7 and were clinically followed. *B. longum subsp. infantis* 157F-4-1 and *B. longum subsp. longum* NCC2705 protected against the lethal infection, while mice associated with the other *Bifidobacterium* strains including type strains of *B. longum subsp. infantis* and *B. longum subsp. longum* died. There were no significant differences in the numbers of *E. coli* O157:H7 in the feces among the mouse groups but the Shiga toxin concentrations in the cecal contents and sera of the mice associated with the specific protective strains were significantly lower than those of the other groups.

## CONCLUSIONS

Although some properties may be shared by various microbial strains, others for sure do not. There is clear evidence that some gentotypic and phenotypic properties differ between strains of bacteria even when these strains belong to the same species, and there are examples of different pharmacokinetics and efficacy of probiotic strains from the same species. The implications of this strain-specificity are that (1) for commercial products, documentation of health effects must be conducted on the specific strain being sold; (2) one should avoid any extrapolation of positive or negative effects between probiotic strains or products; (3) meta-analysis of the effect of probiotics with different active molecules should also be avoided; (4) one should not call a

probiotic a strain that has not been studied just on the basis that it is taxonomically related to a well-proven probiotic strain. These rules are presently frequently violated.

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