

Letter to the Editor

Not all lactic acid bacteria are probiotics, . . .but some are

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The WHO⁽¹⁾ defines probiotics as: ‘live microorganisms, which when administered in adequate amounts confer a health benefit on the host’, a definition which implies that a beneficial health effect must be demonstrated in human subjects. In October 2009, the European Food Safety Agency (EFSA) issued scientific opinions concerning more than 500 health claim applications; all those concerning probiotics were unfavourable⁽²⁾. The EFSA has clearly explained that in nearly all cases, the rejection of a probiotic claim was due to a lack of characterisation of the micro-organism. Unfortunately, the media’s interpretation of the EFSA’s negative response, and subsequently the consumers’ understanding, has been that all probiotics are the same and all are equally ineffective.

We would like to correct this misinterpretation and re-emphasise that micro-organisms are defined by their genus (for example, *Lactobacillus*), their species (for example, *rhamnosus* or *johnsonii*) and finally their strain name (for example, *L. rhamnosus* GG (LGG) or NCC533). Inclusion of all lactobacilli in one ‘probiotic group’ is misleading in the same way that the *Homo sapiens* species should not be confused with other *Homo* species that have lived on earth; the assertion that the complete *Homo* genus is responsible for prehistoric drawings and having walked on the moon is, at best, erroneous. The same works for lactobacilli: the fact that a bacterium belongs to a lactobacilli (or bifidobacteria) genus does not alone confer the status of ‘probiotic’.

As well as taxonomic considerations, we believe the species and strain levels to be critical when studying the biological effects of probiotic bacteria, and probably the clinical effects that they may elicit. We list below the various arguments that support our position, illustrated by a selection of recent studies on this topic.

Different strains of a similar species possess different genomes and these differences correspond to different phenotypes

Lactic acid bacteria (LAB) have been used in the fermentation process for millennia. Recent applications such as the use of living cultures as probiotics have significantly increased industrial interest. Related bacterial strains can differ considerably in their genotype and phenotype. Features from one bacterial strain or species cannot necessarily be applied to a close relative. Differential blast comparative analysis of the complete published genomes of thirteen probiotic LAB highlighted strain-specific genes that were represented only in some LAB and identified group-specific genes shared within lactobacilli⁽³⁾. Whole-genome transcriptional profiling of *L. acidophilus*, and isogenic mutants thereof, has revealed

the impact of varying conditions (pH, bile, carbohydrates) and food matrices on the expression of genes involved in probiotic-linked mechanisms.

In another study⁽⁴⁾, seven reference strains from the *L. casei* group were compared at the molecular level. *L. casei* ATCC 334 gathered in a coherent cluster with *L. paracasei* type strains, unlike *L. casei* ATCC 393, which was closer to *L. zeae*. This confirms the lack of a relationship between the two *L. casei* strains. Further characterisation by pulsed-field gel electrophoresis or repetitive DNA element-based PCR identified distinct patterns for each strain. This clearly demonstrates that differences exist among the same lactobacilli species. Such differences have also been confirmed between *L. acidophilus* strains⁽⁵⁾. Multilocus sequence analysis, DNA typing, microarray analysis and *in silico* whole-genome alignments provided a remarkably consistent pattern of similarity within the *L. acidophilus* complex. On microarray analysis, 17 and 5% of the genes from *L. johnsonii* strain NCC533 represented variable and strain-specific genes, respectively, when tested against four independent isolates of *L. johnsonii*. The observation of a stepwise decrease in similarity between the members of the *L. acidophilus* group suggests a strong element of vertical evolution in a natural phylogenetic group.

These three publications (among others) clearly establish that each probiotic strain has unique genetic traits that may support different phenotypes. Consequently, no general probiotic statement on a genus or a species could be established, and a probiotic must always be fully characterised at the strain level.

Different probiotic strains or species have demonstrated different biological effects in relevant experimental models

The different genes carried and potentially expressed by different species of the same genus or by different strains of the same species lead these species or strains to exhibit different biological effects. This has been clearly demonstrated by numerous *in vitro* or animal studies. Adhesion capabilities of given species, such as *Bifidobacterium longum*⁽⁶⁾ or *L. casei*⁽⁷⁾, to mucin glycoproteins or to human colonic fragments show a high intra-species variability, which can be of importance when considering the role of adhesion in pathogen exclusion. Interactions with commensal microbiota metabolism have also been shown to be species – and strain – dependent⁽⁸⁾. Indeed, among three strains of *Propionibacterium freudenreichii*, two altered the faecal microbiota composition of human microbiota-associated rats, and one strain also increased the caecal concentration of acetate, propionate and butyrate, while the third strain did not have any of these effects.

More specific functions also vary – sometimes widely – according to bacterial species. For example, when studied in rodent bone-marrow-derived macrophages⁽⁹⁾, the presence of *L. fermentum* CECT5716 induced pro-inflammatory cytokines, in contrast to the activation of IL-10 induced by *L. salivarius* CECT5713, both species being isolated from human breast milk. *In vivo* assays in mice showed similar differences: *L. fermentum* enhanced the production of Th1 cytokines by spleen cells and increased the IgA concentration in faeces, whereas *L. salivarius* induced IL-10 production by spleen cells. Overall, *L. fermentum* CECT5716 stimulated immunity, in contrast to the anti-inflammatory effect of *L. salivarius* CECT5713. Such differences in the immunomodulatory properties of LAB was also demonstrated on inflamed mucosal explants of Crohn's disease patients: release of TNF- α was significantly reduced by co-culture with either *L. casei* or *L. bulgaricus*, whereas changes induced by *L. crispatus* were not significant⁽¹⁰⁾.

These different biological properties can lead to different clinical effects

Although there are no clinical trials comparing clinical effects of different species of the same genus or strains of a same species, some recent trials have shown that different probiotic genera exhibit different results in identical experimental settings. Alternatively, some clinical effects are elicited by certain probiotic species, sometimes belonging to different genera, but not by other ones.

Anti-inflammatory properties of three probiotics (LGG, *P. freudenreichii* and *B. animalis* ssp. *lactis* Bb12) were compared in healthy adults⁽¹¹⁾. Serum hs-C-reactive protein was affected differently by the three probiotics, as well as the production of IL-2, and the differences were significant, indicating specific anti-inflammatory effects. Such findings may support the hypothesis relating to the still-inconclusive anti-allergy effects of probiotics in atopic children. Although no comparison has been made in a unique trial, some studies have suggested a preventative effect, whereas others have not found any change after probiotic consumption: probiotic specificity might be part of the explanation of these differences⁽¹²⁾.

The extent to which a probiotic can be clinically efficient seems to depend also on the probiotic genus or species. Nursery school infants fed a formula supplemented with *L. reuteri* or *B. lactis* had fewer and shorter episodes of diarrhoea, with no effect on respiratory illnesses⁽¹³⁾. These effects were more prominent with *L. reuteri*, which was also the only probiotic to improve additional morbidity parameters.

The micro-organism is not the only one important factor: the final product in which it is contained and the target population should also be considered

Before the EFSA, national agencies, such as AFSSA (French Food Standard Agency) in France, delivered scientific evaluations about health claims. To illustrate the importance of the food matrix, or generally speaking of 'the final product', when assessing its claimed health properties, one can consider two AFSSA opinions on two different products, both containing the same probiotic strain: *B. lactis* strain Bb12.

In the first submission⁽¹⁴⁾, the product was an infant formula presented as a freeze-dried powder containing milk and the Bb12 strain. The applicant had performed several human trials, generally randomised, placebo-controlled and double-blinded. This dossier clearly demonstrated that the product had a beneficial effect on infant diarrhoea, rotavirus shedding and enhancement of immune response. The opinion was positive and the health claim was favourably evaluated by AFSSA. In the second submission⁽¹⁵⁾, the same Bb12 strain was included in a liquid yogurt (bottle of about 500 ml) and the claim referred also to the immune system, yet for a general population. However, no new human study was performed with this new product and AFSSA refused the claim arguing that: (i) the food matrices were different, (ii) the target populations (infants and adults) were different and (iii) new bacteria (*L. bulgaricus* and *Streptococcus thermophilus* classically used to prepare yogurt) were added. This is an example, among others, which illustrates the fact that the strain environment must be considered.

Conclusion: probiotics and probiotic products are different from each other

Some may not be efficacious at all, and, in this case, they do not comply with the probiotic definition and should not be called 'probiotics'. This does not mean that other probiotic and probiotic products are not beneficial and valuable. However, each probiotic product should be accompanied by scientific evidence proving that it is active; its activity cannot be extrapolated to other micro-organisms, even if they belong to the same species or genus. This will allow both the consumer and the clinician to distinguish among the (probably too) abundantly available products and the ones which deserve interest.

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