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Probiotics for every body

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In this perspective we report for the first time the concept of 'generic probiotics', as a practical solution to create access to probiotics for people in the developing world. Analogous to generic drugs, we reason that patent-expired probiotics are free to be used by others. In this context we discuss the importance of probiotic genome stability for linking health claims of the mother strain to the generic strain.

To date, hundreds of studies on probiotic functionality and their impact on prevention and treatment of gastrointestinal diseases have been reported. One of the most widely described probiotic strains is *Lactobacillus rhamnosus* GG, for which the first publication on the efficacy against *Clostridium difficile* colitis appeared 25 years ago [1]. A recently conducted meta-analysis has concluded that probiotics, including *L. rhamnosus* GG, are generally beneficial in treatment and prevention of gastrointestinal disease, considering that different probiotic strains show different efficacy across these diseases [2].

At present, probiotic products are mainly available in the western world (including Japan and Oceania), where intestinal health is relatively good. However, in resource-disadvantaged countries, poor hygienic conditions, malnutrition, and acute and chronic enteric infections frequently lead to complex diarrheal disorders. Therefore, people in these countries might benefit the most from probiotics [3–5]. However, such products with well-documented health benefits are not readily available or affordable to the majority of people in these countries. The World Health Organization (WHO) has estimated that >800 000 children per year are dying because of diarrhea [6], and many more children and adults suffer from diarrhea and other gastrointestinal disorders, impacting social and economic development. One of the major challenges we are facing today is how to create access to probiotics for people in the developing world. This seems feasible, because they are relatively inexpensive to produce and can be included by relatively simple adjustments to the production process of locally fermented foods such as yogurt, which only requires milk, a source of heat, and bacterial starter cultures.

In this perspective we report for the first time the concept of generic probiotics. In analogy to generic drugs, this concept refers to probiotic bacteria introduced under a novel brand name, after: (i) intellectual property rights have expired; (ii) there is no patent coverage; or (iii) the use is restricted to certain countries, methods of use, or clinical applications. The approach that we are following with the Yoba for Life foundation includes the introduction of generic probiotics in Africa through existing local production facilities (http://www.yoba4life.com). Accordingly, the local product portfolio of fermented dairy is extended with addition of a probiotic product, whereas related production costs are kept to a minimum. The net effect is that both the health of local communities is improved and some economic benefits are accrued based upon a social business model.

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Generic drugs

A generic drug is a pharmaceutical product that is manufactured without a license and marketed after the expiry date of the related patents or other exclusive rights, according to a definition adapted from the WHO [http://www.who.int/trade/glossary/story034/en/index.html]. Generic drugs are frequently as effective as, but much cheaper than, brand-name drugs. Well-known examples are fluoxetine and acetaminophen of which patents expired in 2001 and 1963, respectively, and which are sold globally under many different brand names as an antidepressant and pain and fever reliever, respectively.

Unlike pharmaceuticals, bacteria cannot be synthesized *de novo*, even though recently the construction has been reported of a bacterial cell controlled by a synthetic genome [7]. We propose that a clone of the mother strain can be obtained from a culture collection or a probiotic product, provided that the intellectual property (IP) rights have been expired or do not exist, no specific material transfer agreement was signed, and the genetic stability of the genome has been verified. Analogous to generic drugs, patent-expired probiotics are free to be used by others, and claims from the expired patents can be linked to the generic probiotic strain. Please note, we do not state that in marketing strategies direct reference can be made to still active IPs around probiotics and related health claims. However, when a generic strain is clearly identical to the originally described mother strain, then it is obvious that upon administering, its entire claimed effectiveness can be assumed, independent from what is still bound to patent rights.

We have recently applied this concept of generic probiotics and isolated a *Lactobacillus* from a commercially available product, containing *L. rhamnosus* GG, which we purchased in a supermarket. The original patent for *L. rhamnosus* GG filed in Europe in 1985 has expired [8], and others subsequently filed in various territories have also expired, which is the most important requirement for a generic probiotic. The identity of the isolated strain was confirmed by 16S rRNA sequencing and the strain was deposited at the Belgian Co-ordinated Collections of

Microorganisms/Laboratorium voor Microbiologie Gent (BCCM/LMG) culture collection under the name of *L. rhamnosus* yoba. Our main interest in this strain relates to its claimed adhesion abilities to mucosal intestinal cells and the occurrence of beneficial or prophylactic effects in the gastrointestinal tract upon administration to humans or animals [8]. However, as required for the approval of generic drugs and related pharmacological efficacy, one needs to confirm that the generic probiotic strain is identical to the brand-name strain; meaning in this case that the genome sequence of *L. rhamnosus* yoba is identical to that of the *L. rhamnosus* GG strain.

Genome stability and probiotic functionality

This brings us to the point of the genome stability of probiotic bacteria. It should be noted that gene decay in lactobacilli is known to be associated with the transition from dynamic and nutritionally variable environments, such as the human gastrointestinal tract, to the relatively constant and nutrient-rich dairy niche [9]. Hence, propagation of strains isolated from a plant or from the gut, as is the case for many probiotics, including *L. rhamnosus* GG, could result in metabolic simplification and loss of functional genes. In addition, the *L. rhamnosus* GG genome contains 69 transposons [10], which enhances genome instability. This instability could affect the probiotic functionality of the strain, in particular in *L. rhamnosus* GG, where a gene cluster with pilin-encoding genes, which are essential for probiotic functionality, is flanked by these transposons [11]. In order to avoid loss of claimed functionality in probiotic strains, we propose to include validation steps in the production and release process of generic probiotics, confirming the presence and DNA sequence of genes associated with functional traits, in particular within unstable genetic regions. In this context it is important to set up innovative research programs for the development of cultivation conditions that keep a selective pressure on maintenance of probiotic functionality. In addition, it is desirable to confirm that strains, which are marketed today, have the same genetic make-up as the strains used in clinical studies on which their health claims have been based.

Generic probiotics

Finally, we would like to comment on the extent to which variability in genomes might affect strain ownership and related claims. Ownership of probiotic strains grants a return on investment for experiments and clinical trials underlying specific health claims, hence, we prefer a policy that permits intellectual property protection, which is not at risk when mutations in the genome occur during the process of cultivation. Therefore, we would like to propose a policy based on plant breeders' rights (http://www.upov.int). These rights provide the breeder of a new plant variety exclusive control over the propagating material for several years. In order to qualify for these exclusive rights, a variety must be new, distinct, uniform, and stable. Applying this concept to probiotic strains would suggest that the functional trait for a certain species has to be new and well-described, and the method of cultivation should assure stability on the level of the translated functional genes, ignoring silent or noncoding mutations. An exemption to the plant breeder's right is the principle of farmers' privilege, which would mean for probiotics the right to recultivate the strain for personal use.

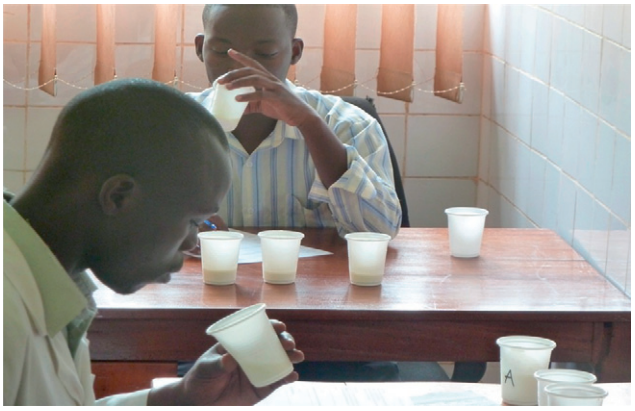

Figure 1. Sensory evaluation of the yogurt containing the *Lactobacillus rhamnosus* yoba bacterium, Uganda 2012.

Notwithstanding the above, when patent rights have expired or are not held in a country, the related strains are free to be used and further cultivated as generic probiotics. We have recently sequenced and analyzed the genome of *L. rhamnosus* yoba (manuscript in preparation) and brought this bacterium to a small dairy factory in the Mukono district in Uganda for the production of Yoba, a mixture of the traditional yoghurt and milk fermented with *L. rhamnosus* yoba (Figure 1). The Yoba product is now used in a school feeding program and studies are ongoing as to its benefits. At present, the Yoba for Life foundation is looking to expand its not-for-profit efforts through acquisition of effective generic probiotic strains, thereby enhancing access to probiotics for every body.

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Disclaimer statement

Remco Kort and Wilbert Sybesma are the founders of the Yoba for Life foundation, which is a nonprofit organization.

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