Commentary

When regulation challenges innovation: The case of the genus *Lactobacillus*

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A B S T R A C T

The majority of probiotic bacteria belong to the genus *Lactobacillus* which includes a large number of safe species integral to fermented food production.

In the European Union the conversion of ensuing data into successful claims that are compliant with regulatory requirements has proved difficult. Furthermore, the study of lactobacilli has been challenging because of their phenotypic and genomic diversity.

Here issues pertaining to the marketing authorization of novel foods and probiotics are outlined, taking *Lactobacillus* genus as reference.

We highlight the drawbacks regarding the taxonomic characterization and the safety assessment of these bacteria and the validation of their beneficial mechanisms.

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Background

In recent decades the Western diet has dramatically changed, being now characterized by high amounts of processed foods, refined sugars, refined fats and oils. This dietary shift has contributed to the increased incidence of chronic diseases such as type II diabetes, coronary heart disease and some cancers (Tilman & Clark, 2014). To tackle the scale of this social problem, the European Union has been promoting actions that aim to meet the consumers' need for safe, healthy, high quality and affordable food, and developing new dietary solutions and innovations focused on preventing chronic diseases and disorders (https://ec.europa.eu/programmes/horizon2020/en/h2020-section/societal-challenges). Although a number of novel functional foods have recently been introduced in the market, probiotics still remain the most popular. Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill et al., 2014; FAO/WHO, 2001). Many organisms now considered probiotic have traditionally been used as starter cultures in the manufacture of fermented foods. Probiotics available today comprise a much broader range of products including pharmaceuticals, a large variety of foods including juices, nutrition bars, infant formulas, relishes and condiments, sweeteners, waters, pizza crust, and other products such as gum, lozenges, dietary supplements, toothpaste, and cosmetics (Hoffman et al., 2014).

The health and wellness claims associated with probiotics have led consumer demand for these products to grow at a fast pace: the market for probiotic ingredients is projected to reach USD 46.55 billion by 2020, with Europe and the Asian-Pacific region estimated to be the largest and the fastest-growing markets, respectively (http://www.marketsandmarkets.com/PressReleases/probiotics.asp).

The lack of a well-established regulatory status of probiotic products at international level has led some manufacturers to market probiotic products in Europe without any pre-market approval (Caselli et al., 2013). This has led to the misuse of the term "probiotic", which have been used for some foods in Europe even in the absence of an approved health claim (Katan, 2012; Sanders, 2015).

Despite the fact that the European food industry has guidelines governing how to produce and market probiotic products, and the EU recognises probiotic bacteria as having the status of nutrients (EU regulation 1924/2006), substantial confusion reigns due to the application to probiotic foods of regulatory schemes initially designed to regulate pharmaceutical development (reviewed in Hill et al., 2014). Different policies are used in the Member states which

result in a lack of clear recommendations for the appropriate and accurate communication of probiotic statements to the different stakeholders including researchers, industries, legislators, consumers and health-care professionals, who are responsible for the different steps of bringing probiotic to the consumer (Van Buul & Brouns, 2015).

At the same time as probiotics proliferate in the market, policy makers and regulators are simultaneously, and usually on an *ad hoc* basis, trying to critically develop the most appropriate regulatory structure for probiotics, which needs on the one hand to be rigorous in defining the level of accuracy required in claim dossiers, but on the other hand needs to be flexible enough to stimulate research and innovation, and thus encourage the release of new health-promoting products (Hoffman et al., 2014). The second part of this paradigm is arguably not working.

The approval of health claims for probiotic-containing foods by the European Food Safety Authority (EFSA), which was appointed by the EU to provide scientific opinion on candidate claims and to protect the consumer from misleading information, has become very challenging due to the requirements for validating probiotic mechanisms in the target consumer, for proper strain characterization, and for conformity to required product characteristics (EFSA, 2016b; Miquel et al., 2015). Although a large volume of data about the beneficial effects of some probiotics has been obtained, precise mechanisms of probiotic action remain largely elusive except for a few examples, and thus the conversion into actual claims and compliance with the regulatory requirements in particular regions have proved difficult.

Probiotic properties of *Lactobacillus* species include competitive exclusion of medically significant pathogens (Kanmani et al., 2013); immune system modulation (Klaenhammer, Kleerebezem, Kopp, & Rescigno, 2012), and the reduction of antibiotic therapy side effects (Lönnermark et al., 2010).

From a regulatory point of view, the *Lactobacillus* genus includes 36 species that have been assigned Qualified Presumption of Safety (QPS) status by EFSA (EFSA, 2016a) and 12 species are Generally Recognised as Safe (GRAS) by the U.S. Food and Drug Administration (FDA) (http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices). This means that they are suitable to be used as food/feed additives and they do not need *a priori* risk assessment.

Furthermore, lactobacilli constitute 43% (84 species) of the total number of microorganisms with certified beneficial use (195 species representing 28 genera of phyla Actinobacteria, Firmicutes and Proteobacteria), (Bourdichon et al., 2012), with 22 of them represented by strains that are patented in Europe due to their potential probiotic properties (Table 1).

Despite their particular relevance, exploiting lactobacilli has always been very challenging due to their unusual phenotypic and genotypic diversity, unclear species identity and uncertain degree of relatedness between them and other commercially important lactic acid bacteria (Sun et al., 2015).

In 2015, the genome sequences of almost all *Lactobacillus* type strains and some historically associated genera were determined (Sun et al., 2015; Zheng, Ruan, Sun, & Gänzle, 2015), thus providing a definitive genomic resource for mining all relevant phylogenetic and functional information. This data repository should also prove useful for understanding the species-restricted distribution of probiotic traits, thus supporting probiotic claim substantiation.

Despite the unprecedented availability of genome sequences and increasing functional information about lactobacilli, the development of functional products containing these bacteria is challenged by the laborious nature of currently prescribed taxonomic characterization, the shortcomings regarding the validation of their beneficial mechanisms, and the drawbacks attached to determining their safety for consumption, issues that we will now expand upon.

Taxonomic characterization of *Lactobacillus* probiotics

Isolation and the full characterization of a candidate probiotic is the first essential requirement for a novel food marketing authorization and a health claim submission (EFSA, 2017; EFSA 2016b). The taxonomic determination of the genus, the species and the strain contained in a probiotic product provides useful preliminary information regarding the main physiological and metabolic properties of the organism, and allows its discrimination from other closely related but potentially non-beneficial strains (ILSI, 2013).

The ideal characterization of microorganisms should include both genotypic and phenotypic tests; the combination of these data strands allows identity of the microorganism at both the species and strain level (EFSA, 2015).

Taking account of the current state-of-the-art techniques for identification and molecular characterisation of microorganisms, EFSA recommends sequence analysis of at least two robust taxonomic markers (i.e. 16S rRNA gene sequence) or fully assembled and validated whole-genome sequence analysis for species identification. Genome sequencing is also suggested for strain typing, but this can also be achieved by other internationally accepted genetic typing molecular methods like whole genome mapping (WGM) or optical mapping analysis. The bacterium is considered to be sufficiently characterised only when these two criteria are fulfilled. In addition, the EFSA advocates that the strain is deposited in at least one recognised international culture collection and encourages naming of strains according to the International Code of Nomenclature (EFSA, 2016b).

The widespread use and characterization of lactobacilli are both hindered by the complex taxonomic structure of the genus, reflected in a poor correlation between the phylogenetic relationship and the physiological properties of *Lactobacillus* species (Zheng et al., 2015). Moreover, the ongoing description of novel species, whose number increased from 152 (Salvetti, Torriani, & Felis, 2012) to more than 190 in the last 3 years (http://www.bacterio.net/lactobacillus.html), has resulted in significant taxonomy changes within the genus, causing confusion and leading to the misidentification of lactobacilli.

Although 16S rRNA gene sequence analysis is the standard method for *Lactobacillus* species identification thanks in part to the availability of up-to-date and internationally recognised databases (ie. EzTaxon, http://www.ezbiocloud.net/eztaxon), there are still shortcomings to this approach, such as the low taxonomic resolution afforded by 16S rRNA gene comparison especially when trying to separate closely related species (i.e *Lb. plantarum/Lb. paraplantarum/Lb. pentosus* or Lb *casei/Lb. paracasei/Lb. rhamnosus*). To overcome this, housekeeping genes as *pheS*, *rpoA* (Naser et al., 2007) and *recA* (Torriani, Felis, & Dellaglio, 2001) have been used as alternative phylogenetic markers which provide a higher discrimination between lactobacilli. Although the application of these molecular markers offers useful potential in the probiotic field, data interpretations by taxonomic experts remains crucial to ensure reliability of the identification results (Sanders et al., 2010).

When the genomes of the type strains of around 175 *Lactobacillus* species were recently sequenced (Sun et al., 2015; Zheng et al., 2015), the ensuing analysis of the Average Nucleotide Identity (ANI) and the phylogenomics based on the core genes showed that the