**LABIP Expert Workshop**

***“Major changes in the taxonomy of Bifidobacterium and Lactobacillus; consequences for industry”***

**4 – 5 October 2018**

**Verona**



**Summary**

In June this year, Salvetti and coworkers (\*) published the result of a whole genome sequence analysis of 269 species, primarily belonging to the 2 families of the  Lactobacillaceae and the Leuconostocaceae. Not surprisingly, the results showed a tree in which phylogeny and taxonomy were in serious disagreement. In order to solve this confusing situation, a considerable renaming at genus level might be necessary. The current genus *Lactobacillus* (containing over 200 species) is therefore in risk of being renamed and split into at least 10 new genera.

While very defendable from a scientific point of view, practical implications are far reaching, given the fact that many lactobacilli are involved in food fermentations and therefore have a legal status in relation to their use, functionality and safety (labelling, ingredient status, QPS status, GRAS notification, etc…). Name changes could also have consequences for the medical community, as new names may only slowly penetrate into medical laboratories.

In order to make an inventory of the possible consequences and discuss the different options to deal with this extensive renaming, LABIP has composed an impressive international  panel of experts, representing different fields in science, law and economics. During 2 days in October these stakeholders will discuss the pros, cons and possible consequences and make some recommendations for a smooth ‘taxonomic transition’.

While taxonomic renaming’s have been quite frequent in the past, the scale at which this is going to happen for microorganisms with such a great importance in food and health, is unprecedented.

We like to thank Prof. Giovanna Felis and Prof. Paola Mattarelli for offering to organize this meeting in the beautiful city of Verona, Italy and LABIP for logistical and financial support.

Bruno Pot

Member of the Taxonomic Subcommittee for lactobacilli, bifidobacteria and related organisms

*\* Elisa Salvetti, Hugh M. B. Harris, Giovanna E. Felis, Paul W. O’Toole. 2018. Comparative genomics reveals robust phylogroups in the genus Lactobacillus as the basis for reclassification. Appl. Environ. Microbiol. doi:10.1128/AEM.00993-18*

**Program**

**October 4**

12.00 Arrival and registration

13.00-13.10 Welcome and house rules

 Giovanna Felis, University Verona

13.10-13.30 Introduction to the Workshop

 Esben Laulund, Chairman LABIP

13.30-14.00 Introducing the Subcommittee on the taxonomy of “*Bifidobacterium*, *Lactobacillus* and related genera” and the procedure for publishing taxonomic names

 Charles Franz, Max Rubner-Institut

14.00-14.30 Disentanglement of the biogeography of the genus *Bifidobacterium* and its impact on bifidobacterial taxonomy

 Marco Ventura, University Parma

14.30-15.00 Isolation, cultivation and characterization of new bifidobacterial species

 Paola Mattarelli, University Bologna

15.00-15.30 Coffee/tea break

15.30-16.00 Phylogenomic analysis of the genus *Lactobacillus*

 Paul W. O’Toole, University College Cork

16.00-16.30 The expected taxonomic changes in the genus *Lactobacillus*

 Giovanna Felis, University Verona

16.30-18.00 Introduction of and questions to the experts

 Moderator: Esben Laulund

19.30 Dinner

**October 5**

9.00-9.30 “To name is to own”

Lorenzo Morelli, University Cattolica del Sacro Cuore – Presented by Pier Sandro Cocconcelli

9.30-10.00 QPS approach and WGS characterisation for risk assessment of microorganisms intended for deliberate introduction in the food chain

 Pier Sandro Cocconcelli, University Cattolica del Sacro Cuore

10.00-10.30 The legal implications for patents, regulations and ingredient lists

 Svend Laulund, Chr. Hansen

10.30-11.00 Coffee/tea break

11.00-11.30 How to deal with an evolving taxonomy from an industrial perspective

 Stephane Duboux, Nestlé

11.30-12.00 A paradigm shift in species description and its implication for data availability

 Erko Stackebrandt, DSMZ

12.00-13.00 Discussion around the timelines for proposed changes: impact for produces, legal institutions, consumers (discussion structured around a selection of questions proposed by LABIP members in advance)

13.00-14.00 Lunch (sandwich) and meeting adjourn

**Introducing the Subcommittee on the taxonomy of “*Bifidobacterium*, *Lactobacillus* and related genera” and the procedure for publishing taxonomic names**

Charles M.A:P. Franz and W.H. Holzapfel

Max Rubner-Institut

Taxonomy and systematics involve classifying organisms according to natural and evolutionary relationships. The current system used by all scientists dates back to Swedish botanist Carl Linnaeus who in 1735 established a way of naming, ranking, and classifying organisms. In the scientific classification established by Linnaeus, each species has to be assigned to a genus (binary nomenclature), which in turn is a lower level of a hierarchy of ranks (family, suborder, order, subclass, class, division/phyla, kingdom and domain). To avoid the confusion that would be caused by several taxonomists giving different names to the same organism at the same time, the taxonomic communities have long-established codes of practice for nomenclature of bacteria. Thus the International Committee on Systematics of Prokaryotes (ICSP) and the International Code of Nomenclature of Bacteria (Bacteriological Code) are responsible for the naming of prokaryotes. The ICSP is the body that oversees the nomenclature of prokaryotes, determines the rules by which prokaryotes are named and whose Judicial Commission issues Opinions concerning taxonomic matters, revisions to the Bacteriological Code, etc. The precepts on which this system of bacteriological nomenclature is based are divided into principles, rules, and recommendations. The principles (Chapter 2) form the basis of the rules and recommendations. The rules (Chapter 3) are designed (a) to make effective the principles given in Chapter 2, (b) to put the nomenclature of the past into order, and (3) to provide for that of the future. Thus naming of organisms is tightly governed by the Code. The ICSP relies on various subcommittees for taxonomic matters which are constituted by experts on the relevant bacterial groups, such as the Subcommittee on the Taxonomy of *Bifidobacterium, Lactobacilllus* and related organisms. These subcomittees can suggest minimal standards for identification of new taxa of the organisms in question and the last recommended minimal standards description of new taxa of the genera *Bifidobacterium, Lactobacillus* and related genera were published by Mattarelli et al. in 2014. However, taxonomy, like bacterial diversity is evolving. New methods such as multilocus-sequence analyses or genomics-driven taxonomy are now state of the art and a thorough revision of the previously published minimal recommended standards is required. Nevertheless, minimal standards for description of new taxa are to be seen separately from naming of the taxa which proceeds according to the rules of the Bacteriological Code.

**Disentanglement of the biogeography of the genus *Bifidobacterium* and its impact on bifidobacterial taxonomy**

Marco Ventura

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Members of thegenus *Bifidobacterium* are high G+C Gram positive bacteria belonging to the phylumActinobacteria, and represent common inhabitants of the gastro-intestinal tract (GIT) of mammals, birds and certain cold-blooded animals. Despite their prevalence and abundance, as well as their perceived positive impact on human health, it may come as a surprise that a large part of bifidobacterial genetic diversity is still unknown. Until relatively recently, bacterial taxonomy was heavily reliant on *in vitro* molecular biology approaches and in particular the comparative analysis of specific molecular marker sequences to measure the degree of genetic similarity and deduce phylogenetic relatedness of novel bacterial species to reference microbial taxa. Thanks to the advent of the genomic era, access to complete bacterial genome contents has now become a routine procedure, thereby offering the opportunity to investigate the overall genetic diversity of microorganisms. Here, we will provide a general overview of how the use of phylogenomic and metagenomic approaches allow us to access the dark matter of bifidobacterial communities and to discover heretofore unknown, i.e. novel, bifidobacterial species.

**Isolation, cultivation and characterization of new bifidobacterial species**

Paola Mattarelli and Monica Modesto

Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy

Bifidobacteria are important probiotic bacteria and the number of identified species of the genus *Bifidobacterium* is greatly increased in recent years mainly due to the study on poor investigated animal gastrointestinal niches. Thanks to the modern omics tools used for phylogenomic and genomic approaches, understanding host-bifidobacteria interactions (antibiotic resistance, adherence and biofilm formation as well as fitness, survival, and immunological functions) is becoming easier, allowing for more thorough molecular characterization. In this scenario on the other hand conventional microbial culture methods and identification processes for its accurate identification and characterization are not surpassed. Classical bacteriological studies targeted to one genus should be encouraged in order to accumulate isolates and their ecological, phenotypical and genotypical details: this is an important opportunity for useful, publishable, contributions to knowledge and to taxonomy and as support to molecular data and vice versa. For example, molecular data about the presence of bifidobacterial species in host microbiota where until now these species have not yet been isolated is a stimulus to unravel new findings. The addition of new species description should be considered not simply for academic validity, but also for their impact on applied microbiology especially for those species considered probiotics. The finding of recently new 23 bifidobacterial species in non-human primates, the most evolutionary closed species to humans, could be of great interest to obtain useful information also for human beings. Proposals for new species should include a suggested scheme for routine identification, and some reasonable basis for prediction of ecological, functional and technological properties as described in the Minimal Standards for new species description of *Bifidobacterium*, *Lactobacillus* and related genera. These requirements would provide a practical basis for the work in the routine laboratory. Academic taxonomists play an important role supplying expertise for probiotic applications because taxonomy should be the “working technical language of microbiology”.

**Phylogenomic analysis of the genus *Lactobacillus***

Paul W. O'Toole

School of Microbiology and APC Microbiome Institute, University College Cork, Ireland

The genome sequence provides the definitive molecular catalogue of the traits that a bacterium may express to produce its characteristic phenotype. The value of comparative genomics, if approached agnostically, is unaffected by factors that affect gene expression such as epigenetics or regulatory point mutations that cannot be separated from strain polymorphisms. It also provides access to features whose analysis illuminates simple relatedness, evolutionary history, radial adaption and selective pressure that have acted on taxa.

Our lab in the APC has an enduring interest in applying functional genomics to understand how commensal lactobacilli interact with the host. Driven in part by a desire to understand clade-specific function and its role in niche adaptation by the lactobacilli, we have employed phylogenomics to clarify their relatedness. Commencing in 2015 (Sun *et al*., Nature Communications), and in collaboration with several other labs, we sequenced the genomes of 213 *Lactobacillus* species, primarily type strains, and compared them to 12 genomes of strains from related genera. Analysis of ANI and TNI values for *Lactobacillus* species differed substantially from the distribution of values for genus and family in other bacteria, overlapping with values for order and class, or order and family, respectively. When we broadened the comparison, five other genera, *Pediococcus*, *Weissella*, *Leuconostoc*, *Oenococcus* and *Fructobacillus*, grouped within the lactobacilli as sub-clades. A maximum-likelihood tree based on 73 core genes confirmed the paraphyletic nature of the genus, and identified distinct clades, many of which were well recognizable from historical analyses.

We recently extended this analysis to 269 species belonging primarily to the families Lactobacillaceae and Leuconostocaceae (Salvetti *et al*., AEM 2018). Both distance-based and sequence-based metrics showed that the *Lactobacillus* genus was paraphyletic and revealed the presence of 10 methodologically consistent subclades. 15 orthologs were selected as putative clade-specific genes on the basis of their pattern of presence/absence among the phylogroups. One of the key genes was the glycolytic phosphofructokinase (Pfk) gene. Ten historically recognized *Lactobacillus* subgroups could also be identified from our analysis which updates the phylogroupings which we described in Sun et al, and which now comprise (i) *L. delbrueckii* (named after the type species of *Lactobacillus*), which also comprises the peripheral species *Lactobacillus amylophilus*, *Lactobacillus amylotrophicus*, and *Lactobacillus floricola*; (ii) *L. alimentarius*; (iii) *L. perolens*; (iv) *L. casei*; (v) *L. sakei* (without *L. selangorensis*); (vi) *L. coryniformis*; (vii) *L. salivarius*; (viii) *L. plantarum*; (ix) *L. reuteri*, which also includes *L. vaccinostercus*-related species; and (x) *L. buchneri*, which encompasses members of the *L. brevis*, *L. fructivorans*, and *L. collinoides* groups.

These data present two obvious ways to reclassify the *Lactobacillus* genus, with multiple possible nuances and refinements, that will be proposed in the following talk by Prof. Giovanna Felis.

**The expected taxonomic changes in the genus *Lactobacillus***

Giovanna E. Felis and Elisa Salvetti

Dept. of Biotechnology, University of Verona, Italy

The scientific and economic value of lactobacilli is well recognised as well as their extraordinary diversity, given the presence of over 200 species in the genus *Lactobacillus*. Systematics, which includes subdisciplines like identification, classification and nomenclature, is challenging in this genus, due to its high level of phenotypic and genotypic diversity.

Phylogenetic/phylogenomic analyses based on different sets of genes/species combined with distance-based metrics (average aminoacid identity, AAI, and average nucleotide identity, ANI, as well as percentage of conserved proteins, POCP,), reported by us and other groups, showed i) the genus *Lactobacillus* was paraphyletic and ii) the presence of at least 10 methodologically consistent subclades, which were also characterized by a distinct distribution of conserved signature orthologues.

Data obtained indicate that the description of novel more homogenous genera is feasible; however, different branching patterns and not-complete coherence in some parameters suggest several different possibilities of species grouping; furthermore, desirable reclassification must include the taxonomic level of family besides the genus rank, since *Lactobacillaceae* and *Leuconostocaceae* appear to be intermixed.

Comparative analyses are ongoing following a methodological scheme based on phylogenomic analyses to define the precise composition of all possible novel genera and their nomenclature, in the perspective of obtaining an updated classification scheme as much stable as possible.

After the initial resettlement, the use of novel names resulted from taxonomic analysis will facilitate the scientific communication and prevent misidentification issues, which are one of the major causes of mislabelling of probiotic and food products reported worldwide.

**“To name is to own!”**

Lorenzo Morelli

Director of Distas- Dept of Sustainable Food Process

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Identification, classification, taxonomic groupings and nomenclature are terms that, even scientists, are often using in a non-appropriate way

The presentation will provides definitions of these terms and some hints about the relevance of nomenclature for the applications to food industry and the regulatory bodies

While scientists are mainly focused on developing tools to identify and taxonomically classify living organism, regulatory agencies have more interest in the nomenclature, in order to establish “the long history of safe use” or the amount of the “body of knowledge” without the need of complex translation and conversion from one system of naming to another.

This need obviously does not match the willingness of scientists to evolve their identification tools in order to be more reliable and reflecting the evolutionary paths of the history of life on earth.

This is way bacterial identification moved from phenotypes to biochemical arriving to genomes, and phylogenetic trees.

However, while identification has solid, scientifically sound basis (even if changing), grouping is more arbitrary.

The levels of similarity used to establish the species, genera etc could be always debatable and they always are more or arbitrary.

Grouping is made on the basis of these similarities and then nomenclature is applied.

But, as stated above, nomenclature has a deep impact on the industrial use of bacteria but also on consumers’ perception and recognition.

Nowadays we, as scientists, have to face a big challenge: how to evolve our bacterial identification /grouping systems, taking in consideration new available data but also the fact the science is a never ending evolution, without hampering the industrial exploitation of our favorite bugs, alarming regulatory bodies and confusing consumers.

**QPS approach and WGS characterisation for risk assessment of microorganisms intended for deliberate introduction in the food chain**

Pier Sandro Cocconcelli

DiSTAS, Università Cattolica del Sacro Cuore

Scientific Expert EFSA. Member of CEP Panel and QPS working group

Microbial strains, when intentionally introduced in the food chain as feed additive, novel food, plant protection product, enzymes, amino acid and vitamin producers, are subjected to regulation and safety assessment by the European Food Safety Authority (EFSA). To avoid unnecessary testing and to specifically focus on microbiological risks, EFSA has developed in 2003 the qualified presumption of safety (QPS) concept. The aim was to provide a harmonized generic pre-market safety assessment of the microbial species seeking for an authorization in the EU and to define a fast track evaluation when risks are not foreseen. The pillars of the QPS approach are (i) the unambiguous definition of biological taxonomic units (TUs), in most of cases the species, (ii) the body of knowledge, including the history of use in the food chain, (iii) the available information on pathogenicity and (iv) the end use. Identified safety concerns for a certain TU can be, where reasonable in number and not universally present, reflected as ‘qualifications’. Examples are the toxigenic potential in *Bacillus* and the acquired antimicrobial resistance in bacterial species. The QPS concept is also applicable to genetically modified microorganisms used for production purposes. The QPS list is updated and published twice a year as EGSA statement assess all the species notified to the European Authority.

More recently, EFSA has developed a new guidance for the characterization and the safety assessment of microorganisms primarily based on whole genome sequence (WGS). This approach uses genomic data to achieve accurate information on the taxonomy, on the unique recognition of the strain and on safety, e.g. by detecting genes coding for known virulence factors or antimicrobial resistances. Phenotypic tests, such as the antimicrobial susceptibility and toxigenic potential are still required in the safety assessment of a microbial strain notified to EFSA.

The impact of QPS and WGS-based risk assessment will be discussed.

**The legal implications for patents, regulations and ingredient lists**

Svend Laulund MSc Pharm. External Affairs Manager, Chr. Hansen, Denmark

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Changes in taxonomy with new names to microorganisms have always been an evolutionary challenge to society, and history shows that it can take decades to implement. The changes that are foreseen with 12 new genera names for *Lactobacillus* and several new for *Bifidobacterium* can be regarded as a revolution. Several countries in the world have regulatory approval systems for products containing microorganisms. Many approved products with microorganisms will be subject to a change to the genera name – a name that is different from what is written in the approval documents. Positive lists for safe species of food cultures and probiotics are included in regulation in a number of countries; list in e.g. Canada, China, Denmark, India, Thailand, Malaysia must be updated. We also have international inventories documenting historical safe use of food cultures. European Food Safety Authorities have their Qualified Presumption of Safety (QPS) list of microorganisms. It is foreseen that 27 species of the present 34 species belonging to the genera *Lactobacillus* on the QPS list will no longer be *Lactobacillus* species. In the EU approvals the 27 *Lactobacillus* will keep their old name, but science driven companies will soon label with the new genus. Product labels with ingredients should be changed by the millions. But will company hesitate to do it? Coming patents with new taxonomy names are at risk of undermining exciting patents due to: “no prior use” as a false finding in an examination by patent authorities. The taxonomic changes will be a big challenge for food and feed authorities, patent systems, the industry and consumers all over the world. The history of the change must be well documented for us not to lose “Body of knowledge”. We do have “List of Prokaryotic names with Standing in Nomenclature” (LPSN), but for the general population an electronic and easy-to-use tool that can document: This species was “*Lactobacillus xx*” but is now “*Laulund xx*” 😊 and vice versa should be created.

**How to deal with an evolving taxonomy from an industrial perspective**

Stéphane Duboux 1

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With the recent advances in bacterial genomics and high throughput sequencing, major changes in the taxonomy of the *Lactobacillus* and *Bifidobacterium* genera are being proposed by the scientific community. Those two genera are of key importance for the industry, as they contain many starter strains used today in fermentation processes as well as many probiotic strains. Several challenges are therefore ahead for the industry. The first challenge is to keep up to date with those changes and to adapt the identification and nomenclature of strains stored within internal culture collections. Any nomenclature change will have then to be implemented throughout the company, having wide implications on ingredient lists, communication strategies and product dossier submitted to regulatory authorities. Many of these changes require proper internal change management, especially in big companies. If those aspects are dependent on the company internal organization, other challenges might be more difficult to handle as they are out of their control. Many country regulations do include either positive lists of strains or list of species and strains with qualified presumption of safety which will have to be updated. Through concrete examples, we will illustrate how most of those challenges can be handled and will depict which of those are today still representing a major challenge for the industry.

**A paradigm shift in species description and its implication for data availability**

Erko Stackebrandt1 and David Smith2

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The formative period of almost 140 years of describing species is a reflection of scientific progress; it harnessed properties which were apt to improve the characterization of isolates and their differentiation from related species. Starting with cultural properties, physiological and environmental traits, later extending to genomic, chemotaxonomic and phylogenetic (gene sequences) properties were added and most recently requests to include genome sequences are high on the agenda. Rarely were properties of doubtful genomic stability excluded. The mode of describing species is highly standardized and for many taxa, accompanied with the request to follow a set of properties outlined in ‘minimal standards’. As a result, publications containing species description resemble each other and are written with a high degree of textual overlap (up to 60%) as estimated by the Springer Nature publisher. Moreover, even within the body of a species description, a high level of redundancy occurs in the Abstract, Results, Tables and the species description parts. In many examples the Introduction just reflects the properties of the genus to which the new isolate is affiliated, as well as a list of species names of that genus. The main problem, however, is the lack of a machine-readable format (each journal has its own style), making it necessary to transfer properties by hand to external databases, error prone and delayed and not harmonized for search engines.

A solution to this problem would result in a paradigm shift. Abolishing the present format and replacing it with a single standardized tabular format would exclude redundant information and allow a direct transfer of data into external databases. The format for such a tabular format needs to be supported by journals as they have to agree to the mobilization of data. The basis has already been outlined by datasets such as those proposed in previous projects such as CABRI (http://www.cabri.org/ and https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4912550/), Digital Protologue Database, DPD (<http://imedea.uib-csic.es/dprotologue/>), BacDive (https://bacdive.dsmz.de/), or the ISO/ TC 276 global biobank database (https://www.iso.org/committee/4514241.html). The main challenge is to include, within a single table, options for all properties, allowing space for new, including genomic properties, as well as a format for Methods. While referenced methods do not pose a problem a new format needs to be found to accommodate their modifications as well as for comparative tables.

The new format will not diminish the authors’ scientific merits as it will make their data more visible and accessible, increasing the number of citations because of higher visibility of data following the rapid entry of data into search databases, accompanied with deep analysis of functional, ecological and evolutionary interactions, and, following the articles of the Nagoya protocol, of potential industrial exploitation.

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