RUBINUM
improves animal health, safety and welfare

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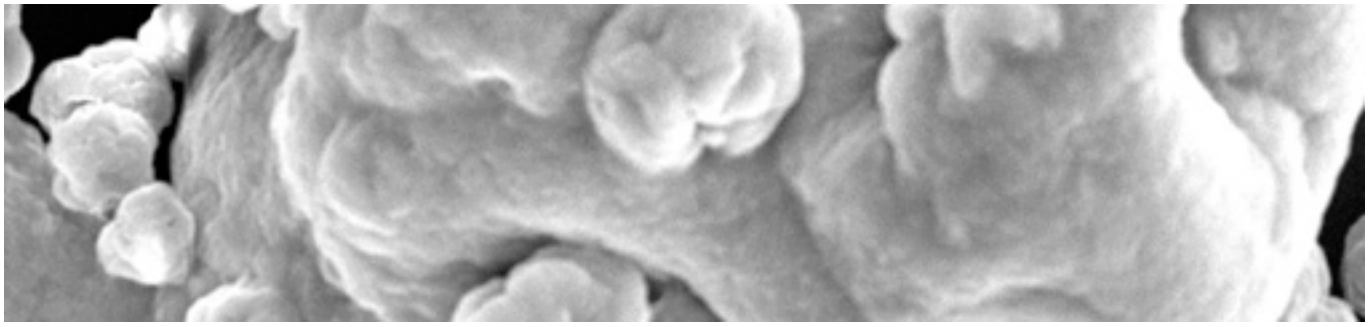
1. WHAT ARE PROBIOTICS



According to Roy Fuller's definition (1989 see P.3), probiotics are "live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance".

This definition poses the main conditions for a product to be qualified as probiotics. In other words, it must contain **microorganisms** (either bacteria or yeast) which are **alive** (probiotic quantification is expressed as **CFU***, which represents the number of LIVING microorganisms), and exert a **proven benefit** on the **target species.** These conditions form the basis of the European registration dossier (see more info P.5).

Probiotics commonly used in animal feed are mainly Gram-positive bacteria belonging to the types *Bacillus*, *Enterococcus, Lactobacillus or Pediococcus*, and certain yeast strains from the *Saccharomyces cerevisiae* species in particular (*S. boulardii*).

A probiotic is defined by its genus and species and a unique strain number. Registration dossier and scientific publications report to a given strain and it is admitted that the effects of probiotics are strain-specific. The strain number should be mentioned on the label of the product or compound feed containing the product.

THE MAIN BENEFITS OF PROBIOTICS ON ANIMAL PRODUCTION ARE:

- Improvement of gut microflora balance
- Prevention of diarrhoeas
- Improvement of feed efficiency
- Improvement of ADWG
- Improvement of microflora establishment in the young animal
- Reduction of food-borne pathogen carriage
- Improvement of the immune status
- Prevention of metabolic disorders (subacute acidosis in ruminant)
- Improvement of digestive health and comfort
- Optimisation of gut development
- Reduced impact of stresses

***CFU:** CFU stands for colony forming unit. It is the standard used by microbiologists to measure the number of viable microoganisms in a sample, using the plate count method. This technique is different from the direct microscopic counts that include both dead and living organisms.
The sample to assess is diluted and spread onto agar plate with the appropriate growth medium. After incubation, the number of microbial colonies which have formed on the plate provides a direct count of living organisms in the volume of dilution plated. Each colony is a visible aggregate of microorganisms derived from a single live cell. The number in the original sample can be calculated according to the dilution and volume used, and is usually expressed in CFU/mL or CFU/g.

2. THE HISTORY OF PROBIOTICS: THE PEOPLE AND THEIR CONTRIBUTIONS

Antoni Van Leeuwenhoek

Louis Pasteur

Elie Metchnikoff

Roy Fuller

Jules Tournut

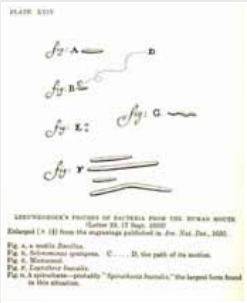
Existence of an invisible world

Definition of probiotic concept

European regulation/ Popularization in animal nutrition

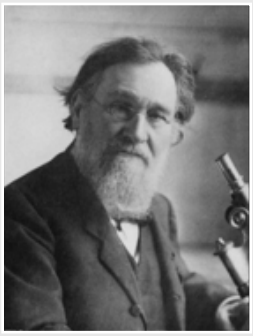
Identification of Microorganisms Microbes have a Biological activity

Health Benefits



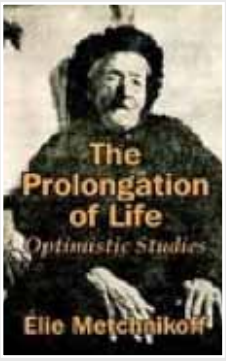
Antoni Van Leeuwenhoek (1632 –1723). Considered as the father of Microbiology, this Dutch tradesman is well-known for his work on the improvement of the microscope. Using his handcrafted microscopes (of which he kept the manufacturing secret to himself!), he was the first one to observe and describe single celled organisms, which he originally referred to as animalcules. His observations of single-cell organisms were first met with skepticism, and the existence of thousands of these living organisms in a single drop of water challenged the 'spontaneous generation' theory of his times. Van Leeuwenhoek was the first to observe and describe bacteria (1676), yeast cells and red blood cells.

Louis Pasteur (1822 –1895). Nearly two centuries after Van Leeuwenhoek's observation of microorganisms, Pasteur, a French chemist, was the first one to describe their biological activity, supporting the germ theory of disease. Pasteur is well-known for his remarkable breakthroughs in the causes and preventions of diseases. He created the first vaccine for rabies and anthrax and invented pasteurization. He is regarded as one of the three main founders of microbiology, together with Ferdinand Cohn and Robert Koch.



Elie Metchnikoff (1845-1916). Although the term probiotic was not used yet, this Russian Nobel Prize winner is the first to have described the beneficial effects of bacteria, which, since Pasteur, where undeniably linked to diseases and catastrophes. Metchnikoff can be considered as the father of the probiotic concept.

Metchnikoff's theories on human longevity, as described in his book called The prolongation of Life- Optimistic Studies (1910), were based on the postulate that human aging was mainly linked to the presence in our blood stream of toxic substances produced by the bacterial community residing in our large bowel. Metchnikoff's initial corrective measure for preventing this "bacterial decay" was to suggest removal of the large bowel! Fortunately, a less drastic suggestion was to try to lessen or replace the "putrefactive bacteria" in the intestine. Based on the observation that



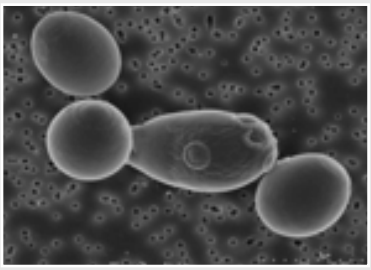
bacteria producing lactic acid prevented milk from putrefying, it was believed that these bacteria might have a similar effect on the digestive tract. A theory corroborated by Metchnikoff's observation of the exceptional longevity and good health of certain Eastern European populations who consumed fermented dairy products on a daily basis.

Metchnikoff thus attributed health benefits to lactic-acid producing bacteria, suggesting that "*oral administration of cultures of fermentative bacteria would implant the beneficial bacteria in the intestinal tract*."

Metchnikoff himself consumed daily sour milk fermented with the bacteria he called "Bulgarian Bacillus" (later described as *Lactobacillus delbrueckii subsp. Bulgaricus*) and physicians began prescribing the sour milk diet for their patients. **Probiotics were born**.

Around the same period, other contributors include **Henry Tissier** from Institut Pasteur, who isolated *Bifidobacteria* from a breast-fed infant in 1899. He found that *bifidobacteria* were dominant in the gut flora of breast-fed babies and he observed clinical benefits from treating diarrhea in infants with Bifidobacteria.

During an outbreak of shigellosis in 1917, **Alfred Nissle** isolated a strain of *Escherichia coli* from the feces of a soldier who was not affected by the disease, and he used this strain in acute gastrointestinal salmonellosis and shigellosis.



In 1923, Henri Boulard isolated the main probiotic yeast still in use today: S. boulardii (photo) in South-East Asia, after he observed local population chewing on the skin of lychee and mangosteen in an attempt to control the symptoms of cholera.

The term Probiotic itself was introduced by **Lilley and Stillwell** in 1965 to describe substances secreted by one microorganism which stimulates the growth of another: the opposite of an antibiotic. But it was not before 1974 that the term probiotic was actually used to describe a feed or food supplement by **Parker**, who defined it as *"organisms and substances which contribute to intestinal microbial balance".*

Roy Fuller, an expert in gut microecology, based in Reading, UK, has written several books about probiotics and the gut microflora, and we owe him the modern definition of the probiotic concept. In 1989, Fuller modified Parker's definition to: ***"live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance".***

This new definition removed the word "substances" which could have included antibiotics. Moreover, Fuller's definition emphasizes the requirement of viability for probiotics and introduces the aspect of a beneficial effect on the host.

Jules Tournut (1919-1998). A French Veterinarian and Professor at the Toulouse Veterinary School, Jules Tournut played an important part in the popularization of the probiotic concept for animal nutrition in Europe in the 1980s and 1990s. In the late 80s, Prof. Tournut was part of the French Committee in charge of products dossiers examination. He worked actively for the harmonization of the regulation at the European level and today we owe him the implementation of a European regulation for probiotic used in animal feed, a key to the official recognition of probiotics that lead the way for other countries.

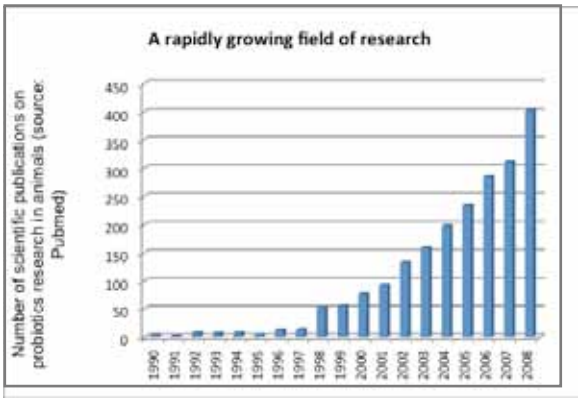
3. PROBIOTICS IN ANIMAL NUTRITION: FROM 'MAGICAL CURE' TO A SCIENCE-VALIDATED

If the benefits of probiotics have been described a century ago by Metchnikoff, their use in animal nutrition only really spread in the last 30 years, first in the US where the concept of Direct Fed Microbial was in use since the 1970s, then in Europe, where the first commercial strains arrived on the market in the mid 80s.
Much progress has been realized in only 20 years, on the scientific, technical and regulatory sides, coupled with a positive evolution of the probiotic image and increased credibility among the animal production industry and general public.

In the early days, the first companies and scientists who saw the potential of probiotics were met with scepticism: very limited data were available on the concept, the modes of action of probiotics were largely unknown and pharmacological solutions were the rule.

In the late 1980s, the early players present on the European market had to lay the ground work by **educating** the industry and they multiplied field trials to gain and share **technical expertis**e on probiotic and convince animal producers.

At the same time, much technical efforts were put on **product development** to ensure its stability and activity. Indeed, probiotics are not just like any other ingredients: they are living organisms. Due to its very definition, a probiotic must remain live and active, from the factory to the animal's digestive tract, its site of action. This implies a resistance to feed production processes and storage, but also to gastric acidity. To ensure probiotic viability, producers worked on selecting the most resistant probiotic strains, and developed optimal production processes as well as protection technologies such as microencapsulation or post-pelletting applications which enabled to broaden the applications of probiotic.



Industrials were backed by certain scientists and veterinarians who brought **scientific credibility** to probiotics and popularised the concept.

A rapid search on Pubmed shows the **exponential growth in the number of scientific publications about probiotics**, especially in the past ten years. In parallel, the same is true in human health, and no doubt that the increased credibility of probiotic in human medicine, supported more and more by the medical community, brings more credit for animal health.

4. REGULATORY SET-UP

In parallel to technical and scientific advances, an important market driver for probiotic in Europe has been the implementation of the regulation that recognised registered probiotics as zootechnical feed additives (see P.5 for more details about the current authorisation process).

In the late 80s, probiotic were submitted to local regulations and there was no European regulation. In 1993, probiotics became zootechnical feed additive, submitted to the feed additive regulation: Directive (EC) N° 70/524, under the newly created category "microorganisms". From there, the first authorisation dossiers were submitted to the authorities, which had to prove a product (strain) identity, safety and efficacy. A temporary authorisation was delivered for 3 years, allowing time to the manufacturer for constituting a complete dossier for a permanent authorisation.

The present regulation, Regulation 1831/2003, was implemented in October 2004. Under this new Regulation, authorisations are delivered for a ten year period (vs. "no time limit" under Directive 70/524). Moreover, Regulation 1831/2003 defines functional groups for feed additives, allowing to link a probiotic to a functional claim (see P. 5). The new Regulation also saw the creation of FEEDAP, the Panel on Additives and Products of Substances used in Animal Feed from EFSA (European Food Safety Authority). Indeed, until 2004, the authorisation dossiers were examined by each member state, while they are now submitted to FEEDAP experts who emit their scientific opinion.

The regulation helped shape the probiotic market in Europe: producers were forced to make important investments to build-up their registration dossiers, a time, energy and money consuming process. Only approved products, which prove their quality, safety and efficacy, can be marketed as probiotics, bearing defined and proven efficacy claims. This regulatory set-up participated to the credibility of probiotics and showed the way for other countries.

5. EVOLUTION OF THE MARKET'S EXPECTATIONS

As the market, the science and the technology have progressed, so have the animal producers' objectives and expectations. Over the years, as new challenges arise for the animal producer and the science brings additional benefits and potential applications, the reasons to use probiotics have evolved.

For example, in the early days, probiotics were used mainly as performance enhancers (increased ADWG), as a potential replacer for antibiotics as the 2006 deadline approached.

Later on, increased awareness about environmental issues and consumers and retailers demand for safe, natural food products, and animal welfare issues came into the picture: probiotics can also help answer some of these issues as a natural approach backed by scientific and technical data with a guarantee of traceability and food safety (no residues in food products, but also some strains have been proven to help limit pathogen carriage to food products). In terms of animal welfare, a promising and emerging field of research in human and animal is the effect of probiotics on stress management, an effect which had been observed by many farmers over the years, in particular in intensive rearing conditions.

Today, producers are faced with increased economical pressure and the potential of probiotics to improve feed efficiency remains a key criteria.

And tomorrow? the increased concern about environmental issues and food safety can only open new opportunities for probiotics with possible applications such as the reduction of greenhouse gases emissions, or effects on animal product quality, but also applications 'beyond the gut and the animal'...

6. THE EU REGULATION: A SEAL OF SAFETY, QUALITY AND EFFICACY

Since 1993, probiotics are classified as zootechnical feed additives and, as such, are bound to a very strict regulation which makes provision for scientific assessment by a committee of experts during which manufacturers must produce evidence of the identity, safety and efficacy of their product.
When this assessment is positive, the relevant product receives an authorization fixing very specific usage and labelling conditions.

7. THE REGISTRATION PROCESS (REGULATION (EC) N° 1831/2003)

This process, lengthy and costly for the manufacturer, provides a guarantee of the safety, traceability and efficacy of the probiotics entering the feed and food chain.

The dossier, compiled by the manufacturer, shall be composed of three main sections (see text frame). Dossier construction can take several years, to which more years for basic product development must be added (selection, characterization of one specific microorganism strain among thousands of natural strains, development of the production process...).

The European Commission passes the dossier to EFSA (European Food Safety Authority) which relies on a specialized experts panel (FEEDAP) for its scientific assessment. For each section of the dossier, methodology and validity of results are assessed. Proposed claims are verified against the presented results on efficacy. If questions are raised during the assessment, the manufacturer is required to answer them. EFSA, which has a strictly consultative role, releases a scientific opinion, after which the dossier is submitted for the vote by the 27 Member States.

Upon receiving the dossier from the Commission, EFSA has three months to verify its content, then the assessment period is limited to a maximum of six months if no question is raised, while each question can "stop" the clock.

THE THREE SECTIONS OF A EUROPEAN DOSSIER

1. IDENTITY AND QUALITY: strain characteristics (taxonomy, metabolism, properties…), manufacturing process, stability (on its own or in mixture), method of analysis.

2. SAFETY: for the target animal species (harmless at 10 times the recommended dose), the handler, the consumer (lack of antibioresisance, genotoxicity and mutagenicity) and the environment.

3. EFFICACY: to be demonstrated for the target species through at least three significant studies in two different locations. The efficacy section describes the target species, the conditions (age, physiological stage, type of production), the usage doses, the claimed performances as well as the possible action mechanisms.

8. FUNCTIONALITY CLAIMS

The classification of feed additives according to Regulation (EC) No 1831/2003 is based on their functionality and not their composition. Five categories have been defined for feed additives:

- TECHNOLOGICAL ADDITIVES,
- SENSORY ADDITIVES,
- NUTRITIONAL ADDITIVES,
- **ZOOTECHNICAL ADDITIVES**
- COCCIDIOSTATS/HISTOMONOSTATS.

These categories are further divided into functional groups. Probiotics, which come under the zootechnical additives category, can be classified under different functional groups:

- **DIGESTIBILITY ENHANCERS**
- **GUT FLORA STABILISERS, OR**
- **OTHER ZOOTECHNICAL ADDITIVES (FAVOURABLY AFFECT ANIMAL GROWTH)**

DID YOU KNOW?

A few facts about the fascinating world of microorganisms!

- Life on earth wouldn't exist without bacteria: four billion years ago bacteria were the first living form on earth.
- Microbes are everywhere: bacteria have been found in the most unexpected and extreme environments from clouds to the depth of the ocean, accounting for 'unexplained' phenomenon (recent discoveries have indentified bacteria thriving at 120°C!).
- We (human and animals) wouldn't live without bacteria: the bacteria in our digestive tract help us process some nutrients which our body is unable to process by itself (e.g. Vitamin K).
- Probiotics can affect the immune response: the intestine harbours the largest mass of immune cells in the human body: the gut-associated lymphoid tissue (GALT), which protects the body from invasion, and numerous studies have shown a direct effect of probiotics on several components of the immune response (innate and adaptive).
- Our gut hosts 100 000 billion bacteria, representing 10 times the number of cells in our body!
- Without the fibrolytic microorganisms present in their rumen, ruminants would not be capable of processing cellulose, the primary source of dietary carbon on earth.
- Fermentations (resulting from the activity of yeast or bacteria) have been part of human nutrition since the down of civilizations: the Egyptian were mastering the wine making and bread making processes... 8000 years ago!

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