Your guide for safety assessment on microorganisms used as production strains, probiotics, live biotherapeutics, novel foods, feed additives and fermentation products.

# **Decoding the EFSA and FDA requirements** for microbial strain safety assessment

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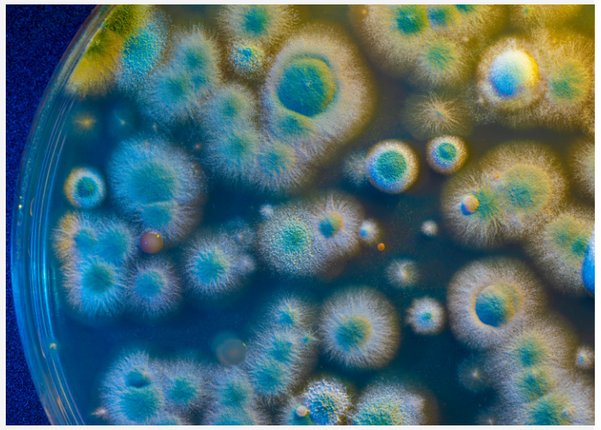
# Approval of feed and food additives

**Obtaining approval to use microbes as feed or food additive or as a production strain can be challenging. The European (EU / EFSA) and US (US-FDA) regulatory agencies now have strict guidelines for submitting dossiers. Applying the regulatory guidelines and rules to generated genomic and other data often presents both technical and interpretative challenges. In this article, we provide an overview of these regulatory requirements and the challenges that you can encounter in the process, and summarize the differences between the EFSA and FDA approaches to this subject.**



## Common issues with microbial genome sequence data and its interpretation

Both the EFSA and FDA nowadays require that the complete genome of the organisms you work with are sequenced and analysed. However, a number of technical challenges is encountered. Especially when analysing larger genomes such as those of fungi or yeast. In addition, it is often extremely difficult to extract and purify genomic and plasmid DNA of sufficient quality to allow long read sequencing. This is particularly important when using the PacBio or Oxford Nanopore sequencing platforms. The presence of plasmids in a strain and determining the correct sequence of these plasmids can be very challenging, because in many cases multiple plasmids are present that carry homologous genes and genetic elements.

In addition, in cases where a potential trait of concern is found, such an episomal plasmid or a partial antimicrobial resistance gene, the interpretation of this information is crucial to the final outcome. Are these traits a safety issue for the use of the microbe as a feed or food additive or as a production strain? Or are they normal biological characteristics of the genus or species which don't cause safety concerns? And how will this information be evaluated by the regulatory authorities? To address these issues effectively, it is important to have a good understanding of the current rules and guidelines from the regulatory authorities.

A critical requirement for the European legislation is that any new product must not contribute to the spread of anti-microbial resistance in the food chain and the environment.

## Safety assessment for feed additive applications: EU vs. USA

Not surprisingly, different countries apply different approaches when it comes to the safety assessment of food and feed products and ingredients. Consequently, their regulatory procedures and requirements are also different, despite some overlap. Here, we discuss the systems in place in the European Union and the United States specifically for microorganisms used as feed additives or as production organisms for additives like enzymes or vitamins.

The EU and USA have different starting points, resulting in quite different procedures. First of all, in the EU, unlike the USA, the risk assessment (science) and risk management (policy) are strictly separated. EFSA (European Food Safety Authority) is an independent agency responsible for the science-based risk assessment of any new feed additive. Following this assessment, the decision if an additive is approved for the EU market is taken by the risk managers, namely the European Commission, Member States and European Parliament, who are also responsible for defining the overall food and feed policy. In the USA, however, both tasks of risk assessment and risk management are performed by the FDA (U.S. Food and Drug Administration).

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In the EU, a feed additive must always undergo formal review and authorisation before it is placed on the market and is used, and there is one clear procedure for this purpose. To pursue authorisation, the notifier (i.e. the producer of the substance) submits a dossier to the EU Commission including data to support safety (for animals, humans and the environment), as well as efficacy (i.e. improvement of the characteristics of the feed, improvement of animal production, performance or welfare, or meeting of a nutritional need). EFSA reviews the data and publishes the conclusions of their risk assessment in a public "Scientific opinion", based on which the risk managers decide on a final approval, which is published in the form of an "Implementing regulation".

In the USA, all feed additives are in principle also subject to premarket review and approval by the FDA. Importantly, only safety is considered in the USA, because any claimed positive effect on animal production or welfare would classify the substance as a veterinary drug according to the US regulations. In which case, a very different and stricter regulatory system applies.

There is one important exception to the general rule of premarket approval in the USA, which is more and more often used for microorganisms with no safety concerns: the self-determined and notified GRAS, also known as "GRAS notice" or "GRAS notification". GRAS stands for "Generally Regarded As Safe". If sufficient data about the safety of the microorganism is available – for at least 6 months – in the public domain, the notifier can prepare a dossier and self-determine that the microorganism is safe for the specific intended use. The FDA will review the data and provide a response by means of a letter to the notifier. Importantly, in the case of a GRAS notice, the notifier holds the final responsibility for the safety of the product.



## EFSA requirements for feed applications

EU's regulatory framework includes a number of very detailed guidance documents developed by EFSA. An important tool worth mentioning is EFSA's QPS (Qualified Presumption of Safety) system. Often, the QPS system is misinterpreted as the counterpart of the American GRAS notice. However, this is incorrect. The QPS system is a tool for safety assessment that EFSA established in order to increase the efficiency of their safety assessment for microorganisms. Based on solid scientific data, EFSA established a QPS list which includes microbial taxonomic units for which sufficient evidence is available to conclude that they pose no safety risks. If a species of interest is on the QPS list, the notifier does not need to provide detailed safety studies, like tolerance studies in target animals or toxicological studies, which are not only expensive, but require many test animals. The QPS list is regularly updated by EFSA, based on research in the scientific literature, as well as information provided by notifiers in their applications.

For the characterisation and approval of microorganisms for feed applications, EFSA makes a distinction between additives containing viable microorganisms (i.e. probiotics, feed sileage agents) and additives produced by microorganisms and further purified (i.e. enzymes, vitamins), as shown in the table below [1].

A critical requirement for the European legislation is that any new product must not contribute to the spread of antimicrobial resistance in the food chain and the environment. Therefore, phenotypic and genotypic assessment of antimicrobial resistance is always required.

| | Food additives containing viable microorganisms | | Food additives produced by production organisms | |
|---|---|---|---|---|
| | Bacteria | Fungi-Yeast | Bacteria | Fungi-Yeast |
| Identification | x | x | x | x |
| Antimicrobial Susceptibility | x | | x | |
| Antimicrobial production | x | x | x | x |
| Toxigenicity and pathogenicity | x | x | x | x |
| Genetic modification | | | For GMMs only | For GMMs only |
| Absence of the production strain | | | x | x |
| Presence of DNA from the production strain | | | Where relevant | Where relevant |
| Compatibility with other authorised additives | Where relevant | Where relevant | | |



## Whole genome sequencing

Whole genome sequencing (WGS) plays an important role in many of the requirements mentioned above. First of all, whole genome sequencing (WGS) provides the information required to unequivocally identify the strain that is used. EFSA requires WGS for bacteria and yeasts; for filamentous fungi this is also recommended. Only when WGS is not available for filamentous fungi can the notifier perform identification by comparing 18S rRNA genes, ITS regions and other characteristic genes. Moreover, WGS has the advantage that it also provides information which enables further characterisation of the strains, specifically regarding potential functional traits, such as:

**ANTIMICROBIAL SUSCEPTIBILITY**
Microbial food additives should not add to the pool of antimicrobial resistance (AMR) genes that are already present in the gut bacterial population or otherwise increase the spread of antimicrobial resistance. This can be checked by searching the genome for the presence of known AMR genes.

**PLASMIDS AND MOBILE ELEMENTS**
The presence of AMR genes is not an issue if they are present on the chromosomal genome, and therefore cannot be easily transferred horizontally. However, AMR genes on episomal plasmids are a clear hazard according to European legislation. Specific data analysis and literature research can make a difference in these cases.

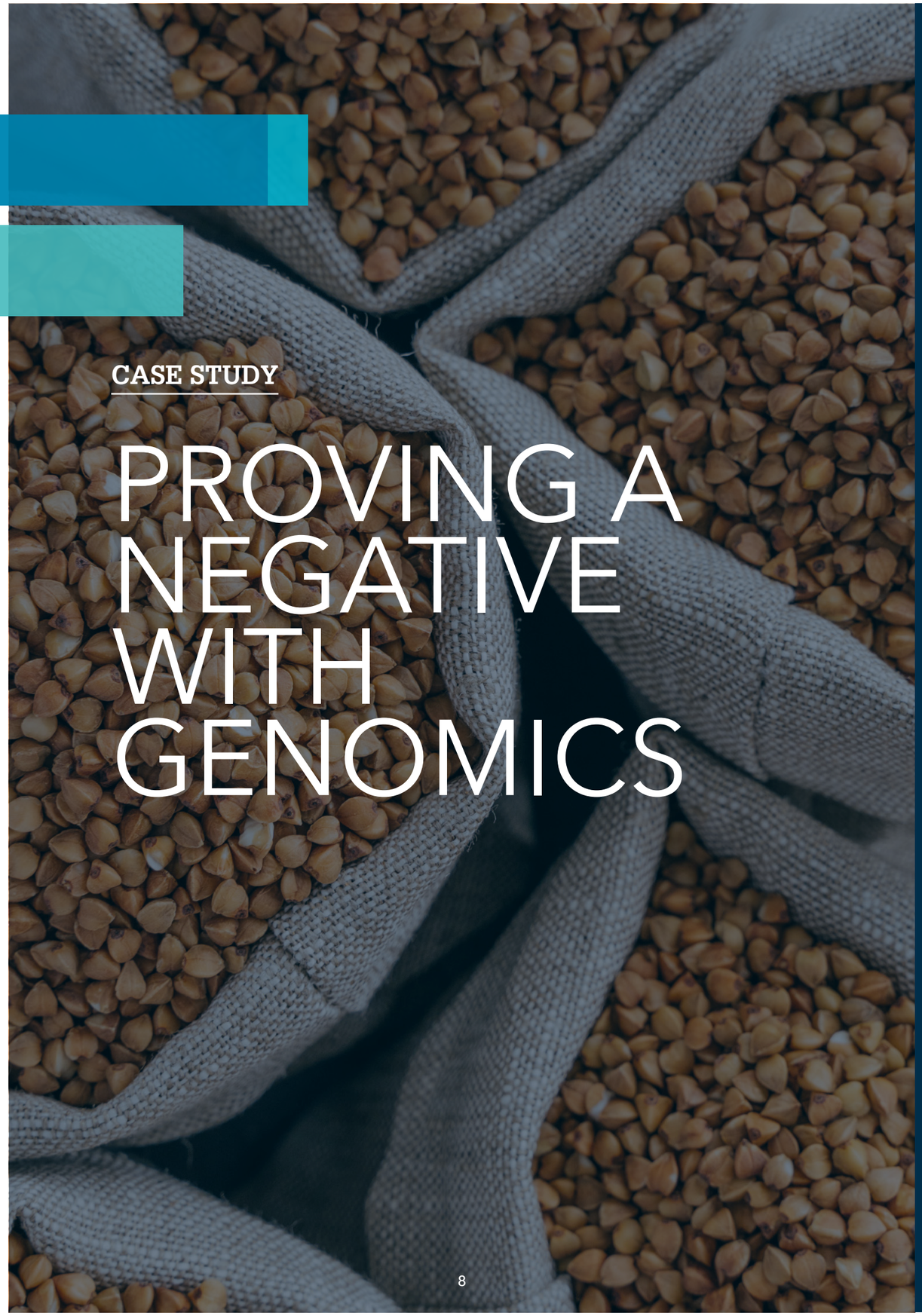
**THE PRODUCTION OF ANTIMICROBIALS**
The microorganisms should not produce antimicrobials that are clinically relevant. This must be assessed with laboratory assays on culture supernatants.

**TOXIGENICITY AND PATHOGENICITY**
The microorganisms should not cause diseases or produce toxic components. The genome can be searched for example for the presence of genes involved in the production of known toxic metabolites and the presence of virulence factors.

**GENETIC MODIFICATIONS**
For genetically modified production strains, the genome sequence must be analysed to assess if the modifications introduced are correct and stable over time.

## Dossier filing for EFSA and FDA applications

For final approval of a product, a safety assessment dossier with all the necessary evidence needs to be filed on the microorganisms used as production strains, probiotics, live biotherapeutics, novel foods, feed additive production and fermentation among other food & feed applications. For this purpose, it is essential that the appropriate whole genome sequencing and other data is generated that is compliant with regulatory requirements. Based on this data, a solid dossier is built with rigorous scientific justification to support the safety of strains introduced intentionally in the food chain.



CASE STUDY

# PROVING A NEGATIVE WITH GENOMICS

**Getting a new animal feed product approved for sale in the European Union can be a minefield. In particular, organic feed products require proof they have not been produced by a genetically modified organism (GMO) for approval. It is not so difficult to show that a production strain contains specific DNA fragments in its genome, but troublesome to prove their absence. This is why Trouw Nutrition approached BaseClear: they needed specific guidance on proving the non-GMO status of a strain used to make a vitamin for the organic feed market.**

Trouw Nutrition is a leading global supplier of animal nutrition products and services. They have been collaborating with BaseClear on the submission of a dossier to EU regulatory body EFSA for several vitamins produced by microorganisms. BaseClear's state-of-the-art analyses and expertise on genomics have been essential in preparing the safety dossier to gain approval to market Trouw's new feed additives in European markets.

There has been a gradual move over the past decades to use white biotechnology to produce substances such as vitamins, amino acids and enzymes used in feed and food.

## GUARANTEEING QUALITY ANIMAL FEED

Animal nutrition plays a pivotal role in the provision of protein foods and dairy to our kitchens. The addition of vitamins, minerals, amino acids and enzymes to animal feed delivers a balanced diet that maximises the health of the animals and their productivity, and protects the environment. Trouw Nutrition is committed to providing high quality and cost-effective animal feed products to livestock farmers worldwide. "Customers expect a safe product. It's extremely important to guarantee that is really the case" according to Reinder Sijtsma, Director Government Relations and Regulatory Affairs for Nutreco, the parent company of Trouw Nutrition.

All additives for animal feed must undergo a rigorous evaluation process by the European Food Safety Authority (EFSA) as part of the approval process to be marketed and sold in the European Union. This process guarantees the safety and efficacy of the products for humans, animals and the environment. EFSA approval is also a gateway to global markets: once the stringent EFSA approval is gained, other countries can be assured that it is of high quality and is effective.

White biotechnology and animal feed additives
Feed additives can be produced in different ways: mining for minerals, by using chemical synthesis, or biosynthesis by fermentation. There has been a gradual move over the past decades to use white biotechnology – when microorganisms such as fungi, yeasts and bacteria are used on industrial scale – to produce substances such as vitamins, amino acids and enzymes used in feed and food. Significant cost and environmental benefits have resulted from this gradual shift away from chemical processes. Recently in 2018, EFSA adopted additional guidance for feed additive production using microorganisms. The new guidance requires producers of feed additives to obtain a full genomic sequence of the production strain, test the final product for DNA fragments from the production strain, and prove that it does not contain genes for antibiotic resistance or known biological toxins. In addition, products for organic farming must not be produced by a GMO.



EFSA approval is also a gateway to global markets

## THE RIGHT PARTNERSHIP FOR GENOMICS EXPERTISE

When Trouw Nutrition recently needed genomic analysis and interpretation to get a new vitamin product approved for the European market, BaseClear was considered. "We needed both excellent analytical capabilities and knowledge of how genomics interacts with the complex regulatory environment in the EU. When a colleague suggested BaseClear, it soon became clear they could work within our team to actively support dossier preparation" says Hans van den Heuvel, QA manager for Trouw Nutrition.

Trouw Nutrition was looking for evidence that the vitamin product, produced by a microorganism, did not contain residual DNA fragments from the production process. In addition, they wanted to prove that it was not produced by a GMO, did not contain antibiotic resistance genes and was not capable of producing toxins. The non-GMO status was especially important because the product was intended for use in organic farming. Not only was BaseClear able to perform the required analyses, they could interpret the results as well as provide excellent documentation that formed an important annex to the regulatory dossier. BaseClear was an integral part of the Regulatory Affairs team that was fully invested in the end result. They could think along with Trouw Nutrition and challenge their approach.

Dennis Kap, Product Manager Regulatory Affairs for BaseClear, sees as this collaborative approach as a key strength of projects with BaseClear. "We are seeing an increase in demand for this kind of analysis and interpretation. BaseClear has built up an increasingly strong expertise in microbial genomics, and partners with customers from the initial phase of their project to help decide the best approach to use. We are proud we can support our customers in providing a complete solution for all their genomics needs." Reacting to challenges – now and in the future The field of genomics research is in motion. New breakthroughs are increasing the applicability of genomics every day. The regulatory environment is also changing. Customers demand more transparency about how their food is produced or new products are made, and this is reflected in new regulations about production processes. "Innovation is one of our key values, but we also expect it from our service providers. The world is changing very fast. Stay innovative, and stay a leader in the genomics segment" advises Reinder Sijtsma to BaseClear.

**MORE INFORMATION?**
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