Roadmap for the assessment of bacterial candidates according to safety aspects and the Novel Food Regulation

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Abbreviations

ADH Alcohol Dehydrogenase

BAuA Federal Institute for Occupational Safety in Health

BIOHAZ Panel for Biological Hazards

CCA Consumers Affairs Agency

EC European Commission

EFSA European Food Safety Authority

EFTA European Free Trade Association

EU European Union

DG SANCO Directorate General for Health and Consumers

FAO Food and Agriculture Organization

FBO Food Business Operator

FDA Food and Drug Administration

FEEDAP Panel on Additives and Products or Substances used in Animal Feed

FFC Food with Functional Claims

FLAB Fructophilic Lactic Acid Bacteria

FMT Faecal Microbiota Transplants

FNFC Food with Nutrient Function Claims

FOSDU Food for Special Dietary Uses

FOSHU Foods for Specified Health Use

GMO Genetically Modified Organism

GRAS Generally Recognized as Safe

ISAPP International Scientific Association for Probiotics and Prebiotics

LAB Lactic Acid Bacteria

MAM Microbial Anti-inflammatory Molecule

MALDI-TOF MS Matrix Assisted Light Desorption Ionization- Time of Flight Mass Spectrometry

QPS Qualified Presumption of Safety

RCT Randomized Control Trial

RAPD Random Amplification of Polymorphic DNA

SCFA Short Chain Fatty Acids

TER Transepithelial Electrical Resistance

USA United States of America

WHO World Health Organization

1 Abstract

Bacteria are part of the human nutrition since ancient times and they fulfil a variety of essential steps during food production processes. Generally, the bacteria used in foods have a long history of safe use. The admission of novel bacteria as part of novel foods or as helping tool during food production is challenging on the European level. This thesis reviews the relevant safety and legal requirements for a successful application for novel bacteria. The crucial aspects relating to the QPS evaluation are as well discussed as the current situation and the upcoming changes in the Novel Food Regulation. For a better understanding of the different issues, three case studies are presented in this thesis. First, the case of *Bacteroides xylanisolvens* and its recent admission as a part of novel food is discussed by focusing on the EFSA assessment procedure. The promising novel bacterium for food and/or drug use *Akkermansia muciniphila* is then portrayed and evaluated according to the relevant QPS criteria. The third case study is about the newly detected fructophilic lactic acid bacteria (FLAB) and their possible future potential in the food industry. Finally, an overview about all the necessary steps for a successful admission of a bacterial candidate is presented.

Zusammenfassung

Bakterien sind seit jeher Teil der menschlichen Nahrung und sie erfüllen vielfältige Dienste im Zuge der Lebensmittelproduktion. Auf europäischer Ebene ist die Zulassung neuer Bakterienstämme als probiotische Lebensmittel oder als Teil der Nahrungsmittelherstellung besonders herausfordernd. In dieser Masterarbeit werden die aktuellen Anforderungen und die rechtlichen Rahmenbedingungen für neue Mikroorganismen und deren erfolgreichen Zulassung dargestellt. Es werden einerseits die wesentlichen Aspekte des QPS-Ansatzes betrachtet und andererseits ein genauer Überblick über die aktuelle und voraussichtliche Gesetzgebung präsentiert. Zur besseren Veranschaulichung werden außerdem drei Fallbeispiele ihm Rahmen dieser Arbeit näher erläutert. Zuerst wird das kürzlich zugelassene Bakterium *Bacteroides xylanisolvens* vorgestellt und die diesbezügliche Beurteilung der Lebensmittelsicherheitsbehörde EFSA genau betrachtet. Danach wird die vielversprechende Spezies *Akkermansia muciniphila* anhand der relevanten QPS-Kriterien beurteilt. Das dritte Fallbeispiel widmet sich der relativ neuen Bakteriengruppe der fructophilen Milchsäurebakterien und deren möglichen Potential in der Lebensmittelindustrie. Abschließend wird eine graphische Übersicht aller notwendigen Schritte für eine erfolgreiche Zulassung dargestellt.

2 Purpose of this study

Microorganisms constitute an essential part of human nutrition since ancient times. Mankind has used bacteria to help to initiate many food production processes without even knowing of their existence. After the invention of the microscope in the end of the seventeenth century and the detection of microorganisms, humans gradually started to intentionally use bacteria to influence shelf-life and taste of food. In the past years, increasing importance of consumer protection and the ongoing technological progress made a legal regulation of microorganisms in food necessary.

This study will provide an overview on the current safety assessment concepts for microorganisms, including the qualified presumption of safety (QPS) approach in the European Union. Further, the requirements for newly detected, identified and characterized bacteria to be accepted as novel food on the European market, will be discussed by a closer look at the Novel Food Regulation. Although this thesis has a clear focus on the European food sector, safety assessment procedures from the United States (GRAS concept) and Japan (FOSHU concept) will be also considered and discussed regarding their potential advantages and disadvantages.

To deepen the knowledge of the presented concepts, three specific case studies of bacterial species with description of regulatory assessment are part of this thesis too.

Bacteroides xylanisolvens was recently approved as novel food and the assessment process by EFSA will be demonstrated within the case study. The two other bacterial example candidates have no novel food authorization so far. The QPS concept will be exemplified with the promising species *Akkermansia muciniphila*, while the group of fructophilic lactic acid bacteria (FLAB) will be discussed regarding to their overall potential as a novel food candidate. The current knowledge available for the species will be presented and the chance for future application will be discussed.

In the end of the thesis, after capturing all available information, an overview of the necessary steps in the application chain from the bacterial strain to the novel food will be presented.

3 Introduction

3.1 General Considerations

3.1.1 The versatility of microorganisms

Microorganisms are capable to colonize nearly every available habitat on earth. They have managed to adapt to a wide range of environmental conditions: psychrophilic bacteria can handle temperatures down to -5°C, while thermophilic ones can multiply up to 90°C and halophilic bacteria even tolerate the high salt concentrations in saline lakes.

Microbes were also involved in essential evolutionary steps. Photosynthetic cyanobacteria changed the atmosphere due to oxygen production dramatically and ensured the development of higher life forms. Methane producing bacteria today may be involved with the climate change.

In general, microorganisms also have a great impact in global biogeochemical and transformation processes. They play a crucial role in degradation of organic matter in soil and other ecosystems and thereby for nutrient recycling (Sundh *et al.*, 2012).

Humans are constantly confronted with microbes in their daily life: not only as circulating microorganisms in the air, as residents of the soil or as part of our food, they also play a crucial role in our body. The human gut represents a reservoir for many different bacteria. Gut microbiota has gained a lot of attention in research for the last years and a balanced individual and functional microbiota is strongly connected with our well-being, though the exact definition is still missing (Eckburg *et al.,* 2005; Kataoka, 2016; Zmora *et al.*, 2016). The role of the microbes in the gastrointestinal tract and their interaction with the immune system will be a part of this thesis.

3.1.2 Microorganism for human use

Antonie van Leeuwenhoek provided the basics in discovering microbes after his invention of the microscope. The ongoing progress in understanding the biology of microorganisms during the 18th and 19th century lead to the study, isolation, identification and cultivation of single strains (Sundh *et al*., 2012).

Long before the discovery of the microbes in the 17th century mankind unintentionally used the help of microorganisms in fermenting and preserving different kinds of food and beverages. It is suggested that cheese-making was developed in Iraq about 8000 years ago, while alcoholic fermentation involved in winemaking and brewing was established 2000-4000 years ago by the Sumerians and Egyptians. The Egyptians were also responsible for the discovery of dough fermentation in the production of leavened bread. Nevertheless, it lasted

until the development of the pasteurization process to realize the crucial role of microorganism in the fermentation procedure (Ross *et al*., 2002).

Besides the food production area there are many different fields of application where microbes facilitate the work of humans:

- Biotechnical use to produce certain metabolites, e.g. citric acid production by *Aspergillus*
- Biofuel production by degradation of specific waste substrates, e.g. ethanol production by *Saccharomyces cerevisiae*
- Microbes as pest control agents, e.g. Cedomon® that contains the soil bacterium *Pseudomonas chloroaphis* to protect barley seeds against fungal diseases (Sundh *et al.,* 2012)
- Medical application of microorganisms, e.g. faecal transplantation to cure severe intestinal diseases (Landy *et al.,* 2011)

3.1.3 The gastro-intestinal tract and the role of probiotics

Bacteria are unevenly distributed along the gastro-intestinal tract: the very low pH value in the stomach leads to the destruction of many bacteria and a very low overall count. Gastric emptying helps the bacteria to survive and to reach the duodenum. In this neutral environment (pH 7) they are confronted with bile salts and pancreatic secretions. After a short stay in the small intestine, connected with low bacterial growth, the viable bacteria reach the colon (Bourlioux *et al.,*2002). The large intestine is colonized by a huge amount of different bacterial species, leading to a number of 10^{10}-10^{12} bacterial cells per gram of intestinal content. Although a quantity of core species is found in all human intestines, each individual has a unique “bacterial fingerprint”. Two bacterial phyla, Bacteroidetes and Firmicutes, represent more than 90 % of all bacterial species in the human colon (Aureli *et al*., 2011).

The intestinal mucosa represents the 2nd largest surface of the human body after the respiratory tract. Between 250 and 400 m^2 of epithelial interface are available for diverse interaction between the host and the microbiota (Aureli *et al.*, 2011). The mucus that covers the epithelium can be divided in two different layers: an insoluble gel which shows a strong adherence to the cells and a water-soluble viscous film covering the gel. Mucins, native glycoproteins, are the main components of the mucus. They are capable to bind bacterial adhesins and are so responsible for the direct contact with bacteria. The intestinal lumen is lined with carbohydrates thereby offering multiple adhesion sites for bacteria and their extra surface structures (Bourlioux *et al.,*2002). The binding to the mucus represents a crucial part for providing probiotic properties: bacteria need to stay at the mucus site to constantly ensure the stimulation of the local immune system, to provide the environment with SCFA or to fulfill other probiotic qualities.

In the beginning of the new millennium, scientists from the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) agreed to the following definition

for probiotics: “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO and WHO, 2001). Although this definition is still widely accepted, the misuse of the term probiotic can be observed in different areas: shampoos, aftershaves, disinfectants or even mattresses offer probiotic properties. Unfortunately, such products mostly do not meet the minimal criteria for probiotics, meaning a defined microbial content, an appropriate viable count at the end of shelf-life and a scientific proof for the claimed health-promoting effects (Hill *et al*., 2014). The International Scientific Association for Probiotics and Prebiotics (ISAPP) came together in 2013 to publish an expert consensus document to discuss and clarify the most relevant questions regarding the term probiotic (Hill *et al*., 2014). The ISAPP consensus panel corrected the grammatical error in the definition and divided the underlying mechanisms responsible for the beneficial probiotic functions in widespread, frequent and rare ones. Widespread mechanisms are found in a vast majority of the investigated probiotics (mostly lactic acid bacteria and bifidobacteria) including general characteristics like regulation of the intestinal transit, competitive exclusion of pathogens, production of specific short chain fatty acids (SCFA) or an increased turnover of enterocytes. Frequently observed mechanisms are mostly found on a species level and include vitamin synthesis, enzymatic activity, neutralization of carcinogens or gut barrier reinforcement. The rare mechanisms are described on the individual strain-level and they include immunological, neurological and endocrinological effects. The outlined mechanisms are responsible for a broad range of probiotic benefits. Such benefits are the support of a healthy digestive tract and a healthy immune system. The establishment or the maintenance of a healthy digestive tract is confirmed by various meta-analyses, demonstrating positive results in different clinical end points. Such clinical end points include the decrease or prevention of abdominal pain, infectious diarrhea, antibiotic-associated diarrhea (Allen *et al*., 2010; Ritchie and Romanuk, 2012) or necrotizing enterocolitis (Alfaleh and Anabrees, 2014). Other health-promoting benefits such as the support of the microbiota in the oral cavity, lungs, the gut-brain axis and skin are reported in several studies but their number is still too small to be considered as a general probiotic effect.

Among the food supply today, there is a high quantity of products containing microorganisms. But does this automatically mean that all of such products should be considered as probiotics? Traditionally fermented foods, especially fermented dairy products, also contain viable bacteria. For advertising purposes, the consumption of such food is often connected with the reduction of certain disease types. Fermented dairy products were able to reduce the risk of Type-2-diabetes (Tong *et al*., 2011), to decrease the weight-gain over a given time period (Mozaffarian *et al.*, 2011) or even to reduce the overall mortality (Soedhama-Muthu *et al.*, 2013). The ISAPP panel acknowledged the convincing study results but also stated the difficulty to verify if the beneficial results originate from the food matrix or the viable microorganisms. Additionally, the lack of an exact number and distribution of viable bacteria does not make traditionally fermented food suitable for the term probiotic. They suggested the term ‘containing live and active bacterial cultures’ instead of probiotic. Among the human gut microbiota there are several microbial candidates that may play a role in future probiotic products. One of them, *Akkermansia muciniphila*, will be discussed later in this thesis. Although commensal gut microbes are capable to provide beneficial probiotic effects, the ISAPP panel suggested a strain-by-strain

assessment until sufficient research data are available to concede the probiotic status on the species level. Besides such well-defined beneficial commensal microbes, there are un-defined commensals that may be used for faecal microbiota transplants (FMT). FMT is a suitable tool to fight *Clostridium difficile* related diarrhea, resolving the recurrence in nine out of ten cases (Smits *et al*., 2013). The identification and the composition of the different bacterial species is not known in such FMTs. The term probiotic is not suitable for this method: FMT is not yet a defined microbial consortium.

3.1.4 Handling and regulation of microorganisms in a historical context

Currently the European Union (EU) is a single-market system consisting of 28 member states together with the four members of the European Free Trade Association (EFTA: Iceland, Liechtenstein, Norway and Switzerland). Those EFTA states must follow European legislation to be part of the EU single market (Wessels, 2012).

In contrast to the United States of America (USA), a union since 1776, the basics of the EU were legally founded with the Treaty of Maastricht in 1992. Since then Europe is trying to establish and harmonize laws that are legally binding among all member states. Therefore, it is necessary to distinguish between the legal terms Regulation and Directive. A regulation is directly valid in all member states with no possibility of interpretation. On the contrary, directives can be interpreted and changed to a certain extent by the national governments. Such changes may lead to enormous hesitations until the EU Directive has passed all 28 parliaments. It is not surprising that the EU had a strong focus on developing regulations in the past years to accelerate uniform laws among the EU.

The USA represents the other big single-market system in the world. Due to its much longer tradition as a union it was able to establish an authority responsible for food and drug safety already in 1927: The Food and Drug Administration (FDA). The EU with its different history and development established the European Food Safety Authority (EFSA) in the beginning of the new millennia in 2002. In the United States, the FDA is a real decision maker whilst in the European Union the European Food Safety Authority is an expert organisation providing guidance to the decision maker, the European Commission.

During the 1990s some food scandals shocked the Europeans and increased the awareness regarding safe food. The Belgian dioxin case gained tremendous attention in 1999. Animal fat for the production of animal feed was stored in improperly cleaned tanks. This tanks were filled with mineral and industrial oil before and so the carcinogenic dioxin could enter the feed production chain. The contaminated animal feed was sold in Belgium and also exported to Germany, the Netherlands and France. The EU had to react and ordered a complete ban on Belgian agricultural exports of chickens, eggs, pork and beef (Tyler, 1999).

The European consumers were scared and the policy makers understood the need for reforms and new standards. This led to the publication of the White Paper of Food Safety (European Commission, 2000). The main purpose of this document was to retrieve the consumer confidence with a common European food safety policy. In 2001 the Treaty of

Nice increased the power of the European parliament in the legislative process which lead to a faster deployment of the food safety strategies. The General Food Law (OJEC, 2002) as the basis of a common European food safety policy passed the European parliament in 2002 and led to the founding of the European Food Safety Authority.

EFSA has the mandate to assess and to communicate all risks associated with the food chain in the EU. The Scientific Committees and Panels are responsible for providing scientific opinions. Ten different panels consisting of twenty-one independent European experts exist, each focusing on a different area of the food chain (f. e Feed/Food Additives, Genetically Modified Organisms or Plant Health). The Scientific Committee supports the work of the panels when subjects of different areas are involved. The main purpose of EFSA is the risk assessment and to provide scientific advice to the European Commission. The issue of risk management falls completely to the European Commission.

Before EFSA was established, risk assessment was performed by Scientific Committees assisting the Directorate General for Health and Consumers (DG SANCO). DG SANCO, now re-named to DG SANTE, has a crucial regulatory role regarding microorganisms in food and feed. Standing Committees representing member states and their interests have strong influence on the work of this Directorate General (von Wright, 2012).

When it comes to the handling of microbes, there are both national (Germany: Berufsgenossenschaft der chemischen Industrie, 2002) and international guidelines (WHO, 2004) which have similar requirements. Such guidelines have the main purpose to protect the people working with the microorganisms. According to pathogenicity, virulence and treatment possibilities the WHO (and the German "Work Safety Classification") classifies microbes in four different categories:

- Risk group 1 (no or low individual community risk)
  A microorganism that is unlikely to cause human or animal disease.
  Examples: *Lactobacillus reuteri*, *Lactococcus lactis*

- Risk group 2 (moderate individual risk, low community risk)
  A pathogen that can cause human or animal disease, but is unlikely to be a serious hazard to laboratory workers, community, livestock or the environment. Laboratory exposures may cause serious infections, but effective treatment and preventive measures are available and the risk of spread of infection is limited.
  Examples: *Streptococcus mutans* (crucial in caries development), *Clostridium tetani*

- Risk group 3 (high individual risk, low community risk)
  A pathogen that usually causes serious human or animal disease, but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
  Examples: *Mycobacterium tuberculosis*, *Yersinia pestis*

- Risk group 4 (high individual risk and community risk)
  A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.
  There is currently no microorganism listed in risk group 4.

During the evaluation certain criteria have to be considered to allocate the microorganism to the corresponding risk group (TRBA 450, 2000):

- Systemized nomenclature**:** Order/Family/Genus/Species/Subspecies
- Metabolic characteristics**:** autotroph or heterotroph
- Natural habitat and life style**:** free-living/host/host species (plant, animal)
- Pathogenicity and virulence**:** pathogenicity factors (adhesins, toxins, immune modulators)/ Virulence (obligate or facultative pathogen, opportunistic)/ Symptoms (incubation time, severe or acute disease)/ infective dose/ persistence/ ways of treatment (specific, symptomatic)/ preventive measures (vaccination, antibiotics)/ diagnosis (clinical, laboratory diagnosis)
- Interaction with other microorganisms**:** synergistic infections
- Ways and mechanisms of transmission**:** entry mode (oral, airborne, sexual, mucosal, bite or stitch)/ excretion (body fluids, droplets)/ spread (endospores)
- Epidemiology**:** disease (prevalence, incidence, morbidity, mortality)/ source of infection/ spread of disease
- Resilience**:** endospores/ resistance to antibiotics or chemotherapeutics

The above listed criteria contain on the one hand general information about the assessed microorganism, and on the other hand very crucial safety information concerning human health. These criteria also build the basis for further safety assessment concepts (GRAS: Generally Recognized as Safe, QPS: Qualified Presumption of Safety), which will be discussed in the next section of this chapter.

3.2 The Novel Food Regulation: an overview about the origin and the necessary changes over the time

During the past decades the number of members in the European Union increased consistently thereby leading to a variety of new challenges. Accompanied by globalization and an ongoing technical progress the number of new food products within the EU raised significantly. Due to the discussed food scandals in the introduction chapter, food safety became a topic of emerging importance. To ensure both the protection of the European consumer and the market, the need for a uniform regulation for novel food was obvious.

3.2.1 Establishment of the Novel Food Regulation

Article 14 in the General Food Law (OJEC, 2002) represents an essential mission statement that should reflect the general European policy: "Food shall not be placed on the market if it is unsafe". Novel food that enters the European market should not cause harm to human health, it should not mislead the consumer and it should not be nutritionally disadvantageous. There is no common consistent definition for novel food, leading to a variety of terms like functional food, not traditional food, nutraceuticals and many more. The current novel food legislation consists of the Regulation (EC) No 258/97, dealing with the placing of food and food ingredients on the market within the community and the Commission Regulation (EC) No 1852/2001 that is dealing with general public information management (European Commission, 1997 and 2001). Foods, food ingredients and production processes that have not been introduced to the EU to a "significant degree" before May 15th 1997 are covered by the current legislation. The regulation is not applicable to food enzymes, flavorings, extraction solvents and genetically modified organisms (GMOs) which are all covered in separate regulations. The novel foods and food ingredients are grouped in the following categories:

- Foods and food ingredients with a new or intentionally modified primary molecular structure
- Foods and food ingredients consisting of or isolated from microorganisms, fungi or algae
- Foods and food ingredients which consist of or are isolated from plants and ingredients isolated from animals
- Foods and food ingredients whose nutritional value, metabolism or level of undesirable substances has been significantly changed by a new production process

Considering the scope of this thesis, only the second food category is relevant. Originally, two additional food categories dealing with GMO sources were part of the regulation. Since GMOs are one of the most challenging topics in the European Union legislation, they were transferred into separate Regulation (EC) No 1823/2003 in 2004.

When a food business operator (FBO) wants to put a novel food on the European market, two different ways of applying are possible. The principle procedure including an application

and the simplified procedure including the established substantially equivalence to an already existing food or food ingredient (DG SANCO, 2002).

In the first step of the common application process, the FBO has to provide relevant information to the Commission and to the member state where the novel food is first launched. This relevant information includes any material that states that the novel food complies with the criteria of Regulation No (EC) 257/98 and a proposal for the labelling and presentation of the novel food. After accepting the provided material, the national competent authority has to carry out an initial assessment in accordance with the published Commission Recommendations (OJEC, 1997). Meanwhile, the Commission is transmitting the available information to the other member states. At the end of the initial assessment, the competent authority has to include the decision whether an additional assessment is necessary or not. If the assessing body comes to a positive result and no further additional assessment is recommended, the Commission and the member states now have the opportunity to check the report and to raise comments and objections. Such objections are triggering a decision of the Community whether the novel food is accepted or an additional assessment has to be performed. The community decision is developed under the advice of the Novel Food Working Group and the potential additional assessment is conducted by the Standing Committee for Foodstuffs (later replaced by EFSA in most cases).

The simplified procedure for placing food or food ingredients on the European market is by far faster. This process requires no initial assessment when the FBO is capable to demonstrate substantial equivalence of the novel food with already existing food or food ingredients on the market. The term substantially equivalent applies to the composition, nutritional value, intended use and the level of undesirable substances compared to currently available foodstuff. This equivalency can either be proven on the basis of generally recognized scientific evidence or based on the opinion of a competent authority. The FBO only notifies the Commission that the novel food is going to be placed on the market and provides the Commission with sufficient material to verify the substantial equivalence. The way of the common application procedure is shown in Figure 1.

The Commission Recommendation 97/618/EC serves as a guidebook for FBO to ensure that all necessary scientific and safety assessment data are included in the application.

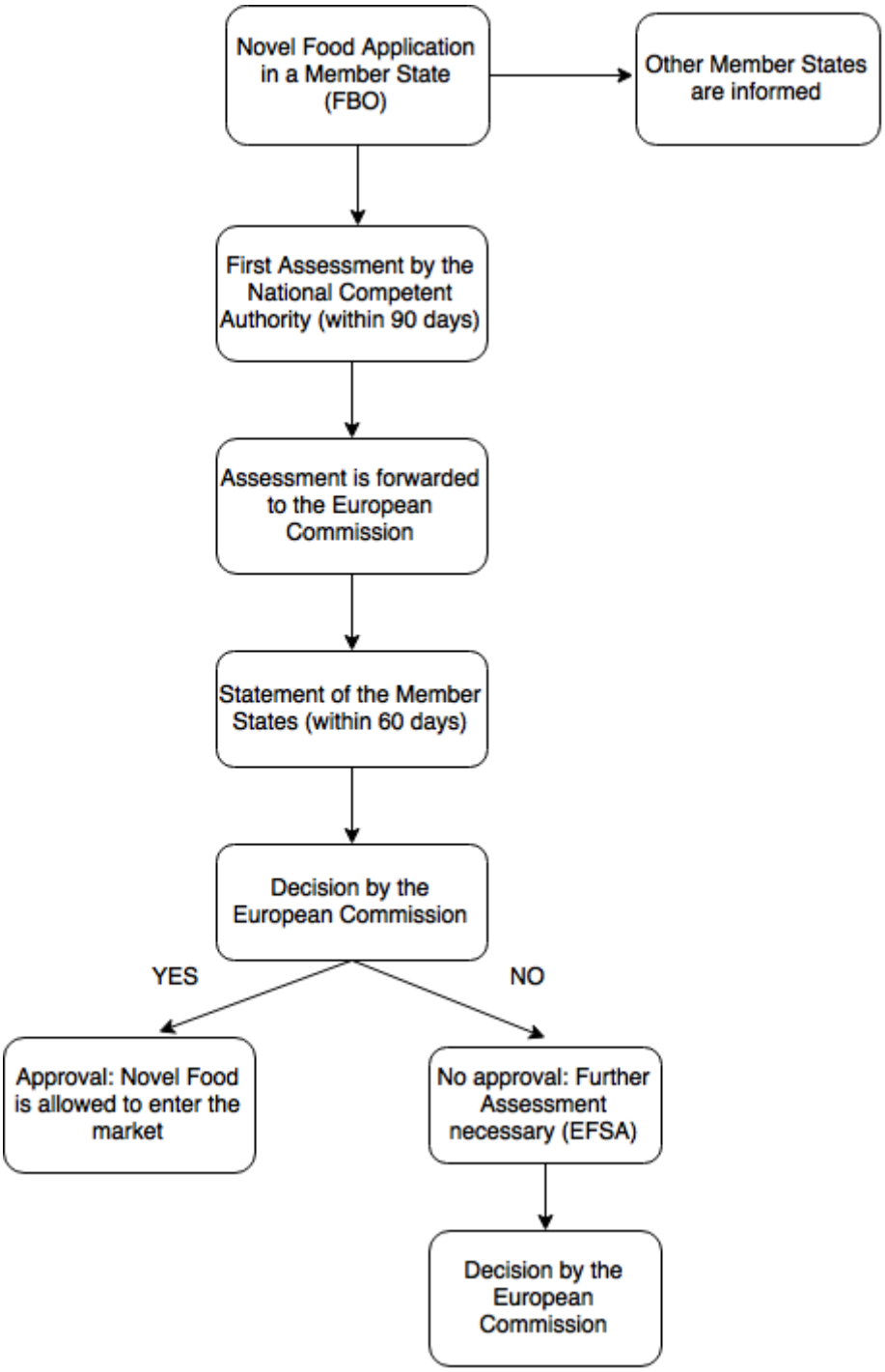

Figure 1: Common procedure for novel food application according to Regulation No (EC) 258/97 (adapted from the National Competent Authority Austria, AGES)

The recommendation further mentions key issues, such as allergenic potential, toxicological requirements, intake patterns in target populations or implications on the human nutrition, that have to be considered for the assessment of a novel food. Microorganisms with no safe history of use in the food industry cannot have a substantially equivalent counterpart, and a full safety assessment has to be applied. Several aspects are relevant for microorganisms:

the containment (if they are limited to the fermenter, killed in any part of the production process or stay alive in the final product), the toxic and pathological potential or the capability of colonizing the mammalian gut. Besides the already mentioned food categories in the Novel Food Regulation, the recommendation introduces a scientific classification of novel food:

- Pure chemicals or simple mixture from non-GM sources
- Complex novel foods from non-GM source
- Foods produced using a novel process

Three more scientific classes are mentioned in the official text, since all of them are related to GMO, they have no significance in the current Novel Food Regulation.

Finally, the recommendation provides decision trees for every key issue to help the applicant to verify if the presented information is sufficient. The decision tree for the necessary provided microbial information is shown in Figure 2.

3.2.2 Criticisms and revision of the first Novel Food Regulation

Continuing technological developments, like the application of nanoparticles in food or the cloning of animals are enduring driving forces for the renewal of the Novel Food Regulation. Besides the obvious technical progress, practical problems in handling the regulation appeared. Most stakeholders complain about the very long and extensive application procedure. A concrete example for the long-lasting authorization process represents the Unilever product 'yellow fat spreads with added phytosterol esters'. While South Africa approved the product within one day, the European Union needed 31 months for a successful authorization. Other relevant countries like the United States of America or Japan needed 3 months and 18 months for approving this specific Unilever product (Wendelin, 2009). By comparing the different time-spans of authorization it is evident that the European system is more extensive than the other ones. Especially FBOs have a high interest in the revising and shortening of the whole application approach. A further relevant criticism deals with the authorization of so-called 'traditional food from a third country'. Such food is defined as a novel food with a history of food use in a third country and therefore being part of the regular diet for at least one generation in a large part of the population.

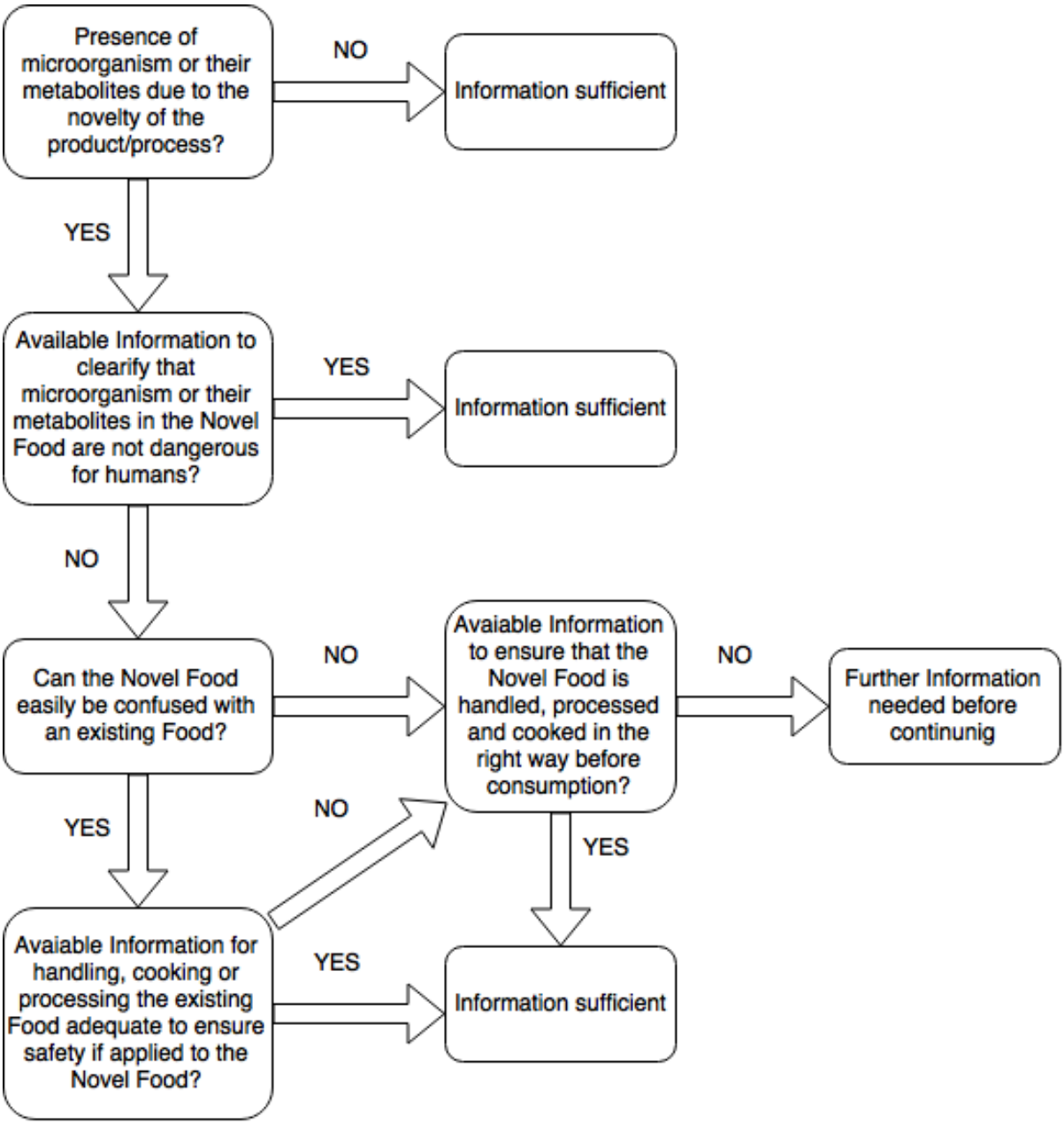

Figure 2: Scheme to check the available microbiological information of the potential microbes as novel food (adapted from OJEC, 1997)

The application process for traditional food is also very time-consuming. Thus, a shortening and simplification is desired by all relevant stakeholders. A further point of criticism was the wording 'significant degree' when it comes to the decision if a food was introduced at a sufficient level to the European market before the cut-off date May 15th, 1997. The European Commission suggested in a discussion paper that the term 'significant degree' is fulfilled if the food is generally available in food shops in at least one Member State of the European Union (European Commission, 2002). Other major concerns are the missing transparency and the lack of innovation due to the extensive authorization procedure.

The listed problems were soon realized by the relevant players and a long-lasting process of improving the Regulation (EC) No 258/97 began. This way to a revised regulation was characterized by failed negotiations between the European Council and the European Parliament. In March of 2011, both parties were unable to agree to a new proposal for the regulation. In some relevant points (nanomaterials, centralized authorization process and specific measures for traditional food) they came to a common solution but the subject of meat from cloned animals was still unsolved (Dalli, 2011). The Parliament wanted a complete ban of meat from cloned animals and their descendants, while the Council showed only support in banning meat from cloned animals but not from their offspring. Commissioner John Dalli proposed in his statement a temporary suspension of the cloning technique for food production in the EU, a ban on import of clones and traceability of reproductive material from cloned animals. The failure to revise the Novel Food Regulation prolonged the legal uncertainties in handling such hot topics like nanotechnology and meat from cloned animals.

In December of 2013, after further negotiations, the Commission published a new proposal for a new Novel Food Regulation (New European Union Commission's proposal, 2013). The debate about food from cloned animals was resolved by separating the subject from the novel food framework. The new proposal emphasized among other things the centralized procedure for novel food assessment and authorization. EFSA should adopt the risk assessment from the national competent authorities and the process should be reduced from three years to 18 months. A simplified procedure for marketing of traditional foods should be guaranteed if the food has a history of safe use in a non-EU country for over 25 years. The authorization should only last 4 months after notification to the Commission if no reasonable safety objections are received. For stimulating the EU food industry, the proposal also contained the introduction of a data protection regime. After a successful authorization as a novel food, the covered data may not be used for another application for 5 years.

In March of 2014, the European Parliament appointed James Nicholson as rapporteur on the novel food review. His agenda was to meet and consult with local producers, industry experts and FBOs to draft a legislative resolution for the European Parliament. After final negotiations, mainly about defining novel food and creating new novel food categories, the European Parliament and the Council agreed to the Regulation (EU) 2015/2283.

3.2.3 Overview of the major changes in the new Novel Food Regulation 2015/2283

- Centralized authorization system: for the simplification and acceleration of the authorization process. EFSA will perform the scientific risk assessment instead of the national competent authorities. A system of individual authorization is thereby replaced by a system of generic authorization. The Commission will manage the files of each applicant and prepare the proposal for the authorization of a novel food which is found to be safe (European Commission, 2015). To speed up decision-

making, EFSA is responsible to adopt its scientific opinion within 9 months from the date of receipt of a valid application (OJEC, 2015).

- New novel food categories: while the old regulation contained 4 different novel food categories, the new one was extended with 6 additional categories. Food of mineral origin or for instance food consisting of engineered nanomaterials are included in the new regulation. Relevant for this thesis is mainly one of the new food categories: food consisting of, isolated from or produced from cell culture or tissue culture derived from animals, plants, micro-organisms, fungi or algae (OJEC, 2015).
- Facilitating the trade of traditional food from non-EU countries: the target is the introduction of a more eligible assessment procedure for food new to the EU. The traditional food has to demonstrate a history of safe use in the origin country. If there are no objections raised by EFSA or the EU Member States, the FBO is allowed to put the traditional product on the European market on a basis of a notification (European Commission, 2015).
- Insects are part of the new Novel Food Regulation: due to gaining public interest in consumption of insects, this topic also made it in the new regulation. Insects fall within the definition of novel food as food ingredients isolated from animals (European Commission, 2015). Although insects are widely consumed in the whole world (except Europe), they are interestingly not treated as traditional food.
- Data protection: Companies now have the assurance that after a successful authorization, the newly developed scientific knowledge is not allowed to be used for another application for 5 years.
- Nanomaterials: they are part of an own novel food category. Engineered nanomaterials need an authorization as novel food before being used in food.

In November 2016, EFSA published two guidance documents on novel food and traditional food to simplify the application procedure for the FBOs (EFSA, 2016a; EFSA, 2016b). Between February and April 2016, before the guidance documents were finalized, the stakeholders were invited to raise objections and concerns during a public consultation period. EFSA obtained 193 comments from 25 interested parties, which confirms the need and the broad acceptance of such public interaction. The guidance document for the presentation and preparation for a novel food application provides scientific and technical counseling, emphasizing on a common format, a well-structured application and an outlining of the needed data (EFSA, 2016a). The guidance points out a list of general requirements that need to be covered in every application:

- Description
- Compositional data
- Production process
- Specification
- Proposed uses and use levels
- Anticipated intake

of the novel food. The applicant of course also has to provide information about absorption, distribution, metabolism, excretion, nutritional information, toxicological information, allergenicity and the history of use of the novel food or its source.

Since bacteria are the core of this thesis, the additional requirements for this group are discussed in the upcoming section. All novel foods that belong to the group 'foods consisting of, isolated from or produced from micro-organisms, fungi or algae' need to provide the following information (EFSA 2016a, Section 2.2.3):

- Scientific (Latin) name (family, genus, species, strain) according to the international codes of nomenclature
- Synonyms that may be used interchangeably with the preferred scientific name
- For bacteria and yeasts (unicellular organisms), verification of the species and strain identity according to internationally accepted methods; information on applicable methods for the characterization of bacteria and yeasts are provided in the EFSA Health Claim guidance (EFSA, 2016c). Molecular methods allow predictions of genes encoding for toxins, antimicrobial resistance and other pathogenic factors
- Origin of the organism
- If available, deposition in an officially recognized culture collection with access number

A further section in the guidance document is entirely dedicated to microorganisms. Bacteria and fungi with a history of safe use possess the qualified presumption of safety status. This QPS status is assessed by EFSA and declares that the assigned microorganism shows either no safety concerns or minor concerns that are defined and addressed with 'qualification' as expressed in the QPS list. Therefore microorganisms that are part of the QPS list need no exhaustive safety assessment apart from the need to evaluate the risk of antimicrobial resistance. Microorganisms with not fully understood safety properties should be exposed to a safety assessment. Such assessment needs to contain a distinct taxonomic classification on the species or strain level and a comprehensive strain characterization including whole-genome sequence analysis to identify potential virulence related genes and antibiotic resistances together with their horizontal transfer capabilities. For accurate safety evaluation the number and viability of microorganisms in the final product should be included by the FBO.

3.2.4 Currently authorized microorganism as novel food

The following section overviews the hitherto authorizations of bacteria as novel food after the Regulation (EC) No 258/97 came into force.

Leuconostoc mesenteroides:

In January 2001, the European Commission authorized the placing on the market of a dextran preparation produced by *L. mesenteroides* as a novel food ingredient in bakery products (OJEC, 2001). The application was filed by the company Puracor and the Belgian

competent authority performed the initial assessment. The Scientific Committee for food stated that the dextran preparation by *L. mesenteroides* is safe for human consumption up to 5 % in bakery products. Dextran was identified as a highly digestible bakery ingredient with similar nutritional properties like starch.

L. mesenteroides bacteria are Gram-positive, non-sporulating coccoid shaped members of the order Lactobacillales. They are often found on surfaces of different plant parts and they are responsible for the fermentation of white cabbage to sauerkraut. They are capable to convert a broad range of sugars, especially sucrose is used to build dextran.

Bacillus subtilis natto

In April 2009, the European Commission allowed the placing on the market of Vitamin K2 (menaquinone), produced by *Bacillus subtilis natto*, as a novel food ingredient according to Regulation (EC) No 258/97. NattoPharma, an Irish company, made the request to the competent authorities of Ireland to place *B. subtilis natto* derived Vitamin K2 on the market as a novel food ingredient to be used in foods for particular nutritional uses and for foods to which vitamins and minerals are added (OJEC, 2009). The Irish competent authority stated in their initial report that an additional assessment is acquired and so EFSA was requested to carry out the extended assessment. The Scientific Panel on Dietetic Products, Nutrition and Allergies came to the conclusion that *B. subtilis natto* is a safe source of Vitamin K2.

Bacillus subtilis natto is a Gram-positive, aerobic member of the spore-forming genus *Bacillus*. This species was discovered in Japan in 1906 and is responsible for the Natto dish, a type of fermented soy beans. Natto, produced by *B. subtilis natto,* has a long tradition and research in Japan and therefore it acquired FOSHU (Foods for Specified Health Use) approval for its health benefits by the Japanese Ministry of Health, Labour and Welfare. *B. subtilis* has a recognized history of safe use and so EFSA granted a QPS status for this microorganism (EFSA, 2010).

Clostridium butyricum

In December 2014, the European Commission authorized the placing on the market of *Clostridium butyricum* (CBM 588) as a novel food ingredient according to Regulation (EC) No 258/97 (OJEC, 2014). The British competent authority performed the initial assessment after the request by Miyarisan Pharmaceutical Co. Ltd. to place *Clostridium butyricum* on the market as a novel food ingredient to be used in food supplements. No additional assessment was necessary because the raised objections by the member states could be smoothed out after further explanations provided by the applicant. *C. butyricum* is authorized as novel food ingredient in food supplements at a maximum dose of $1{,}35 \times 10^8$ CFU per day.

The authorized strain is described as gram-positive, spore-forming, obligate aerobic, non-pathogenic, non-genetically modified bacterium. The product is characterized as white or pale grey tablet with a specific odor and sweet taste (OJEC, 2014).

Bacteroides xylanisolvens

The most recent authorization for bacteria according to Regulation (EC) No 258/97 took place in 2015. The European Commission approved the placing on the market of 'pasteurized milk products fermented with *Bacteroides xylanisolvens* DSM 23964' as a novel food. The detailed situation of *B. xylanisolvens* is discussed in chapter 4 (The case of *B. xylanisolvens*) of this thesis. Of note is, that *B. xylanisolvens* was approved in a heat-treated non-viable form.

3.2.5 Qualified Presumption of Safety (QPS) – A new approach

The concept of QPS was introduced by EFSA to establish a generic risk assessment approach for biological agents. The goal was to simplify and harmonize the assessment of notified biological agents across EFSA´s different Scientific Panels and Units. Furthermore, the QPS approach should allow a more focused use of available resources on agents with higher risk potential (Leuschner *et al.*, 2010).

The QPS concept was developed to establish a safety assessment for microorganisms used in feed and food production. During 2002 and 2003, a Working Group consisting of members from the former Scientific Committee on Plants, the Scientific Committee on Food and the Scientific Committee on Animal Nutrition made the first proposal for a suitable assessment tool (European Commission, 2003). This proposal was published online and was open for comments, ensuring a public debate. In 2004, EFSA organized a Scientific Colloquium to involve all relevant stakeholders and to give the opportunity to discuss the remaining open questions (EFSA, 2005). Since its introduction in 2007, QPS has been tested successfully within EFSA and the main user has been the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). FEEDAP is responsible for the authorization of additives for use in animal nutrition (EFSA, 2007). Since 2008, the Panel for Biological Hazards (BIOHAZ) is responsible for the annually revision und publication of the updated QPS list (recent update: EFSA BIOHAZ Panel, 2017). The updating process covers two different activities. On the one hand new information about already assessed taxonomic units is reviewed and on the other hand new taxonomic units are identified and assessed according to the QPS guidelines (Leuschner *et al*., 2010).

A safety evaluation generally can lead to three different consequences:

- No safety concerns are raised during the evaluation, the analyzed taxonomic unit will be recommended for the QPS list
- Existing safety concerns may be defined and excluded via a qualification and the taxonomic unit can also be recommended for the QPS list

- Microorganisms that fail the QPS assessment or are in general not suitable for the list; a full safety assessment is required

If a specific strain, that is notified for market authorization, can unambiguously be connected to a taxonomic unit on the QPS list, no further assessment steps apply. The assessment for QPS suitability is well-structured and relies on four pillars (EFSA, 2007):

- Taxonomic unit: represents the most crucial part of the assessment procedure. The genus and all known species, including a type species, are listed and described according to their main characteristics. The evaluated taxonomic unit must be unambiguously defined. If the assessed bacterial agent cannot be connected to any known species, there will be no recommendation for the QPS list. Furthermore, if the taxonomic identification is insufficient, the QPS status is also denied.

- Body of knowledge: in this part of the assessment many essential questions need to be answered: Is there a history of use for the assessed bacterial agent? What is the exact field of application? Is there sufficient scientific literature about the microorganism? Is it possible to exclude potential adverse effects for human, livestock and the wider environment?

- Possible pathogenicity: this section of the QPS evaluation clarifies the absence of pathogenic and virulence properties of the bacterial agent. The findings need to be supported by clinical data and scientific literature.

- Description of end use: Is the microorganism only used as a starter culture? Are the bacteria part of the final product? If so, are they inactivated or viable?

After the introduction of the QPS approach, a comprehensive number of bacterial species were assessed according to the new evaluation concept. A long history of safe use did not automatically mean the lacking of any safety concerns. Most bacteria that were recommended for the QPS list belonged to the group of Gram-positive non-sporulating bacteria. These group harbors many inhabitants of the digestive tract and they can exhibit a long history in food and feed production. EFSA´s Scientific Committee applied for this group a generic qualification for all taxonomic units on the list, requesting that all strains shall not carry any transferable antimicrobial resistance, unless viable cells are not in the final product (Leuschner *et al.,* 2010). The number of recommended Gram-negative bacteria is very low, only one representative, *Gluconobacter oxydans,* is part of the QPS list. Many members show a long history of safe use but safety concerns cannot be excluded. *E. coli*, for example, is used as a probiotic for a long time but it is also capable to cause a variety of diseases and show versatile virulence mechanisms. Nevertheless, opportunistic bacteria may be placed on the QPS list with an additional qualification, while pathogenic and toxin-producing bacteria are not suitable for the list at all.

Although the QPS approach was inspired and influenced by the American GRAS concept, some differences are obvious. QPS is defined as an 'assumption based on reasonable evidence' (European Commission, 2003), providing a helpful assessment tool for the European Safety Authority. In contrast to GRAS, it offers no legal status at all. EFSA is responsible for the burden of proof, while the GRAS concept refers the responsibility to the FBO. GRAS on the other hand, requires safety assessment by independent experts to the degree that another panel of independent experts would reach the same conclusion. The strong focus during the QPS assessment on the absence of acquired antibiotic resistances and virulence factors is a further difference between the two concepts.

3.2.6 Novel Food Regulations in other countries

United States of America

The American legislation does not differentiate between food and feed, therefore animal feed is covered by the same federal regulations as food intended for human use. Microorganisms that are added to food need to be declared as food additives or they are considered as GRAS under the condition of the intended use. Food additives have a very broad definition and this term covers nearly everything that can come in contact with food. The requested documentation for safety is considerable and food additives need an approval before they are allowed to enter the market (Wessels, 2012). The whole application process is very time-consuming and comprehensive, including the submission of toxicology and efficacy studies. In the end of the application chain, the FDA has to decide if the substance/microorganism is suitable for approval. Three dried microorganisms are listed as food additives: *Saccharomyces cerevisiae*, *Candida utilis* and *Saccharomyces fragilis* (Wessels, 2012).

FBOs also have the possibility to claim that their used bacteria are GRAS. The eligibility for this food substance classification can be demonstrated in two different ways: either by clearance via scientific procedures or through experience based on common use in food. The so-called "grandfather clause" enables the use of substances for food that demonstrated a substantial history of consumption by a significant number of consumers before January 1st 1958 (FDA, 2016). The scientific clearance should be demonstrated by scientific documents, where experts in the field confirm the safety of the substance. Manufacturers need to be capable to provide all necessary material in case of control from FDA (Wessels, 2012). It is important to point out that the manufacturers do not have to inform the FDA before they put their product on the market. The liability lies completely on the side of the responsible company. Knowing the American legal system and the high motivation on filing law suits, companies must be aware that safety problems can lead to high financial and reputational losses.

The FDA introduced another way for the manufacturer to ensure that the used substance or microorganism is safe and earns the GRAS status. The GRAS notification procedure is a voluntary act which can be submitted by any person that concludes that the used substance

is GRAS for the intended use in human or animal food. Although the notification is voluntary, the FDA highly recommends this step for every company that plans to place a food product on the market on the basis of the GRAS provision. The marketing of the food item is of course possible before the FDA has reviewed the GRAS notification (FDA, 2016).

The GRAS notification consists of seven parts (FDA, 2016):

- Signed statement and certification
- Identity, method of manufacture, specification and physical or technical effect
- Dietary exposure
- Self-limiting levels of use
- Experience based on common use in food before 1958
- Narrative
- List of supporting data and information in the GRAS notice

The FDA also provides a list of GRAS accepted substances for a better orientation for the manufacturer. The list itself does not claim to be all inclusive since the use of GRAS substances is not subject to pre-market review and approval by FDA, and so it is not possible to list all substances used in food based on a GRAS notification (FDA, 2016).

Japan

Japan has no comparable Novel Food Regulation that covers food or food ingredients that have never been introduced to the food market before. Instead they offer different certificates for FBO that want to put health foods on the market. Like other Western countries, Japan has to face an aging population and a rising number of life-style related diseases. The Japanese people are very health-conscious, making their country to the third largest health food market in the world (USDA, 2014). Since 2009, the Consumer Affairs Agency (CAA), with its Labeling Division, is responsible for the approval of the diverse health food certificates. There are two primary health food categories: Food for Specified Health Uses (FOSHU, established 1991) and so-called ´Health Foods´ (Non-FOSHU). A brief overview of the different health food categories is given in Figure 3.

FOSHU covers foods that include functional health ingredients with physiological functions and biological activity in the human body. This group of food is targeted to healthy people to maintain and improve their health and they have to be distinguished from pharmaceuticals. The CAA is responsible for the authorization of the food item and scientific data, including clinical trials, are needed for a successful application (CAA, 2011). Health foods are generally divided in 20 categories and 7 classifications. The highest market sales in the past years were obtained among the categories “Analeptics” (support of the nervous system, heightening mental and physical function), “Skin care” and “Prevent life-style diseases” (USDA, 2014). Currently, four different ways are available for FBO to receive the FOSHU claim (CAA, 2011):

- FOSHU: requires detailed review process with scientific evidence for each application.
- Standardized FOSHU: No requirement of detailed review process for food products meeting the established standards and specifications; Must be accompanied by

sufficient accumulation of scientific evidence; For efficiency: short cut process for products whose safety of use already approved.

- Reduction of disease risk FOSHU: Requires detailed review process with scientific evidence for each application; Permitted for products whose ingredients clinically and nutritionally established to reduce a risk of certain disease (i.e., Calcium for Osteoporosis and Folic acid for neural tube defects).
- Qualified FOSHU: Requires detailed review process with scientific evidence for each application; Permitted for products with ingredients showing certain health effects but not reaching the established standards for FOSHU approval; Labelled as "Qualified Food for Specified Health Uses."

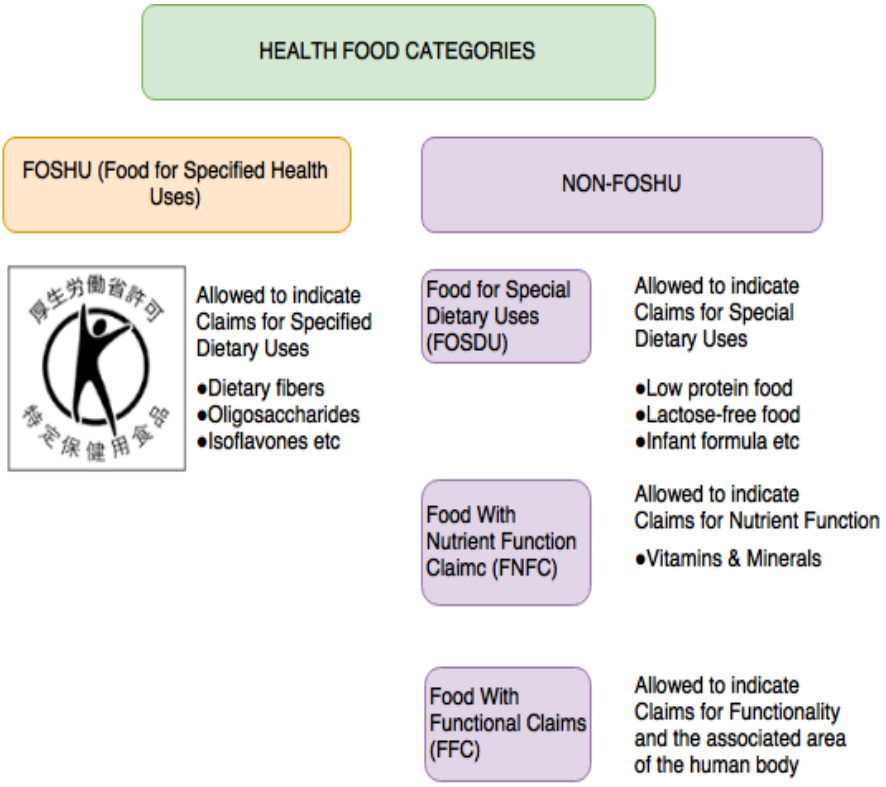

Figure 3. Overview of the different Japanese health food categories

The FOSHU authorization process is very time-consuming, between 6 months and 3 years, and expensive and therefore often unaffordable for small companies. Currently (July 2015), the number of FOSHU registered products is 1,173 (USDA, 2015). The procedure for a general FOSHU approval process is outlined in Figure 4. A further health food category that needs CAA approval is targeted to people with specific dietary needs: Food for Special Dietary Uses (FOSDU). This claim especially directed to pregnant or lactating women, infants and patients (CAA, 2011).

Among the NON-FOSHU categories, two different labels were introduced to the Japanese market to facilitate the access to health claims: Food with Nutrient Function Claims (FNFC, established 2001) and Food with Functional Claims (FFC, established 2015). Food that is

labeled with FNFC contains vitamins and minerals. The claims provide general information about the nutrient functions. The last revision of this claim also allowed the labeling of fresh food. Both labels need no registration procedure or individual approval from the Consumer Affairs Agency (USDA, 2015).

Figure 4. General procedure for FOSHU approval

4 Case studies

For a better understanding of the different safety assessment aspects, three case studies are presented in this chapter. First, *Bacteroides xylanisolvens* and its recent admission as a part of novel food is discussed by a closer look at the EFSA assessment procedure. The promising novel bacterium *Akkermansia muciniphila* is then portrayed and evaluated relating to the relevant QPS criteria. The third case study is about fructophilic lactic acid bacteria (FLAB) and their possible future potential in the food industry.

4.1 *Bacteroides xylanisolvens*

In 2015, 'pasteurized milk products fermented with *Bacteroides xylanisolvens* DSM 23964' were approved as a novel food according to the Novel Food Regulation No 258/97. The usage of this specific strain was restricted to the starter culture in the fermentation of pasteurized milk products. Only heat treated and therefore inactivated cells of *B. xylanisolvens* were allowed in the final product. Besides the novel food evaluation, EFSA automatically performed an assessment for *B. xylanisolvens* relating to the admission to the QPS list. Interestingly, EFSA revealed that the present information is not sufficient to put *B. xylanisolvens* on the QPS list.

In this chapter the case of *B. xylanisolvens* DSM 23964 and the rationale for the acceptance of this specific strain as a novel food are going to be discussed. The possible probiotic potential of *B. xylanisolvens,* although only dead cells are allowed in the final product, will also be an important part of this chapter. Finally, the admission of *B. xylanisolvens* may have an impact to other future candidates, such as *A. muciniphila*.

4.1.2 *Bacteroides*: Genus, species, type strain

The genus *Bacteroides* is one of the main inhabitants of the human colon, therefore accounting for approximately 30 % of the intestinal microbiota (Sears, 2005). *Bacteroides* are Gram-negative, obligate anaerobic, bile resistant and non-spore forming rods. This genus can have commensal attributes within the human body but can also contribute to the development of different diseases. *Bacteroides* spp play a major part in the fermentation and degradation of xylan and other plant fibers (Chassard *et al.*, 2008). Approximately 10 days after birth *Bacteroides* starts with the colonization of the gut in the newborn child, thereby being one of the first species after the genus *Bifidobacterium* to populate the gastro-intestinal tract (Simon and Gorbach, 1984). Polysaccharides from the human diet represent a highly available food source for *Bacteroides,* which can easily be fermented to volatile fatty acids that are reabsorbed through the large intestine and provide an essential part of the daily required energy of the host (Hooper *et al.*, 2002; Wexler, 2007). Besides the supply of nutrients to the host, *Bacteroides* may also play an important part in the

establishment of diseases. Once the bacterium escapes the gut, mainly due to ruptures in the colon or during surgery, it may be capable of initiating opportunistic infections. Such action may include abscess formation in multiple body sites (e.g., the brain, liver, abdomen, lungs and pelvis) as well as bacteremia. *B. fragilis* is mostly involved in such infections and is regarded as the most virulent *Bacteroides* species (Wexler, 2007). *B. fragilis* is able to adhere to the host tissue with its agglutinins and fimbriae. The polysaccharide capsule helps *B. fragilis* to evade the host´s immune defense mechanisms. This capsule with its three variants (PS-A, PS-B and PS-C) plays the key role in the above mentioned abscess formation (Wexler, 2007).

Interestingly, the levels of *Bacteroides* and *Firmicutes* seem to be connected to obesity in human and germ-free mice (Ley *et al*., 2005; Turnbaugh *et al*., 2006). Obese individuals show a lower number of *Bacteroides* and a higher number of *Firmicutes* in their gastro-intestinal tract. Turnbaugh and his colleagues suggested that this bacterial composition is capable to extract more energy from a given diet compared to the opposite bacterial profile of lean individuals. Turnbaugh also observed and reported that an enrichment of *Bacteroides* led to the loss of weight in obese people. Thus, the bacterial balance may be one factor influencing overweight development, the most important health risk in Europe and globally today.

During the search for xylan-degrading microorganisms in the human gastro-intestinal tract, Chassard and co-workers were able to isolate 6 xylanolytic, gram-negative and anaerobic rods (Chassard *et al*., 2008). Subsequent 16S rRNA analysis revealed that the obtained strains belonged to the genus *Bacteroides*. A sequence similarity between 99-100 % within these strains demonstrated that they all belonged to one species. The representative strain XBA1^T only showed a similarity of 41.9 % at DNA/DNA-Hybridisation analysis with its closest relative, *B. ovatus* (at least 70 % similarity would be necessary). Further differences to *B. ovatus* were established: the disability to degrade starch, no usage of cellulose as an energy source, the lacking of indole production and XBA1^T was catalase negative to the corresponding test. According to the obtained data they considered the 6 strains as a novel species and proposed to its xylan-degrading properties the name *Bacteroides xylanisolvens*. The main characteristics of the designated type strain XBA1^T = DSM 18836^T are shown in Table 1.

The German BAuA (Federal Institute for Occupational Safety in Health) assessed the safety of *B. Xylanisolvens* as working material (BAuA, 2011). Due to the lacking pathogenicity and diseases they classified the microorganism in the lowest Risk group 1. People that are dealing with members of the Risk group 1 during their work, need to obey general hygienic measures with no special additional requirements (BioStoffV, 1999).

However, one should note that *B. xylanisolvens* was first isolated in 2008 and developed to a potential probiotic thereafter. Thus, the role of *B. xylanisolvens* in any infections would require a strain-specific determination, which was reported later. As the identification became available, the potential to cause infection in the viable state became apparently, and some reports have been published recently (Pedersen *et al.*, 2013).

Table 1. General characteristics of *Bacteroides xylanisolvens*						
Phylum	**Class**	**Order**	**Family**	**Genus**	**Species**	
Bacteroidetes	Bacteroidetes	Bacteroidales	Bacteroidaceae	*Bacteroides*	*Bacteroides xylanisolvens*	
Type strain	DSM 18836T (XB1AT)					
Biosafety level	1*					
Origin	Feacal sample from a healthy adult volunteer					
Chararacteristics						
Morphology	pleomorph, Gram-negative, rod-shaped bacterium with rounded ends,					
	1.8-2.5 µm (length), 0.2-0.3 µm (width), no endospores, single or paired cells, no filaments					
Genome	Size: 6059 kb, ORF`s: 4922, GC content: 41 % (42.8 mol %)					
Physiology	Chemoorganoheterotroph, mesophilic: growth at 25-42°C (0ptimu: 38°C), pH optimum 6.8, obligate anaerob					
	Indole negative, catalase negative, xylan positive, D-mannito positve					
	No starch utilization					
*BioStoffV						

The recently approved strain *Bacteroides xylanisolvens* DSM 23964 was first analysed by Ulsemer and colleagues in 2011 (Ulsemer *et al*., 2011). They isolated this new strain from human faeces and performed several analytical techniques to verify if the strain is actually new. Results from biochemical analysis (no catalase activity, no indole production, incapable to degrade starch) revealed a closer relationship to *B. xylanisolvens* than to *B. ovatus*. 16S rRNA gene sequencing showed a 100 % match with *B. xylanisolvens*, while other species had a far lower consensus. To confirm the relation of the new strain to *B. xylanisolvens,* DNA/DNA-hybridisation was performed with the type strain DSM 18836, resulting in 98.65 % similarity. After the so far performed analyses no difference between the new strain and the type strain was established. Finally, the RAPD (Random Amplification of Polymorphic DNA) profile revealed significant differences and the existence of the new strain *B. xylanisolvens* DSM 23964 was confirmed.

4.1.3 Scientific Opinion of the EFSA panel to *Bacteroides xylanisolvens* DSM 23964 (EFSA, 2015)

Avitop GmbH, a German company, submitted a request according to the Novel Food Regulation No 258/97 to put their pasteurized milk products fermented with *B. xylanisolvens* DSM 23964 on the market. The initial assessment was performed by the competent Irish Food Safety Authority in 2013. They came to the conclusion that all necessary criteria for acceptance as a novel food were fulfilled and forwarded the result to the European Commission, which transmitted it to the member states. Several member states had concerns regarding the admission of the novel food and so EFSA was asked to perform an additional assessment. The most relevant concerns are listed below:

- A detailed product specification is missing and the proposed strain is not fully genetically analyzed
- There is no publication about the origin of *B. xylanisolvens* DSM 23964 and the information about it is not as comprehensive as of the type strain *B. xylanisolvens*

DSM 18836. Further there are worries about the close relationship to *B. ovatus*, that is potentially pathogenic in intra-abdominal infections

- Data about the comparability to traditional products are missing
- Concerns about the remaining hydrophilic enzyme activities after the pasteurization process. This may enhance further hydrolysis of fibers which may lead to flatulence and intestinal discomfort. The increased colonic fermentation may also lead to an increased energy value of the given diet, which may be disadvantageous for specific consumers

The novel food is classified as a complex food from a non-GM source, where the source of the novel food does not have a history of food use in the Community (class 2.2). The most crucial requirements for a submission in this specific food category are briefly described in the following sections:

1) Specification of the novel food

Following the application by Avitop GmbH, the novel food status is related to low-fat and skimmed milk products which were fermented with *B. xylanisolvens* DSM 23964 as the starter culture. After the fermentation, the product is heat treated for one hour and following the process no viable cells of *B. xylanisolvens* are found in the final product.

The applicant stated that the certain *Bacteroides* strain was isolated from the faeces of a healthy adult human subject. The German Resource Centre for Biological Material assigned the reference number DSM 23964 to the strain of the applicant (DSMZ, 2010).

The relevant phenotypic and genotypic information were provided by the applicant (see the previous section and Table 1) and the EFSA Panel considered the information for the characterization of *B. xylanisolvens* DSM 23964 as sufficient.

2) Production process applied to the novel food

The production process is described as followed:

Low-fat milk (< 1.8 % fat) or skimmed milk (< 0.3 % fat) is pasteurized or ultra-heat treated before the fermentation with *B. xylanisolvens* starts. The fermentation process lasts between 14 to 16 hours without stirring at 38.5 °C under constant gassing with CO_2. Finally, the product gets homogenized and heat treated at 75°C for one hour to ensure that no viable cells of *B. xylanisolvens* are contained in the final product. The obtained product is packaged like a regular liquid fermented milk product or spray dried to produce fermented milk powder.

The applicant supplied a study to guarantee the effectiveness of the heat treatment (Toutounian, 2008). In this study no viable *B. xylanisolvens* cells were detected after heat treatment at 75°C for various times (15, 30, 60, 120 and 180 seconds).

The EFSA Panel stated that the applied techniques are standardized among the dairy industry and considered that it is sufficiently described and no safety concerns were recognized.

3) History of the organism used as a source

The strain of the applicant has no history of use in the food industry. There is no strain among the genus *Bacteroides* with a proven history of use in food production.

4) Anticipated intake/extent of the use of the novel food

The novel food would be marketed in liquid and semi-liquid forms in fermented low-fat milk and skimmed milk products (fermented milks, buttermilks, yogurts and yogurt drinks) or as spray-dried powder (fillings and coatings of cereals, cereal bars fruits and nuts). Due to lacking European data, the applicant used US consumption data. A conservative scenario was chosen, where all the currently existing products (yogurts, buttermilks and many more) would be replaced from the products of the applicant. Relating to the US National Health and Nutrition Examination Survey food intake data and the associated Food Commodity Intake Database of raw agricultural composition data, the estimated mean daily intake of non-fat solids (from yogurt, buttermilk and acidophilus milks) was between 5.5 g and 15.3 g for the 90^{th} percentile among consumers.

5) Nutritional information on the novel food

The applicant provided compositional data for spray-dried skimmed milk cultured with *B. xylanisolvens* DSM 23964, including an overview of relevant macronutrients. He also supplied an overview of the vitamin B_2, vitamin B_{12}, free lysine and furosine (as marker for Maillard reaction) content to ensure that the heat treatment has no impact on the mentioned vitamins. The results showed no reduction or loss of the vitamins after the heat treatment.

Although the lactose content was missing in the analysis (they assumed a comparable amount to traditional products), the EFSA Panel considered that the consumption of the novel food is not nutritionally disadvantageous.

6) Microbiological information on the novel food

After EFSA was asked to perform an additional assessment, the EFSA Panel on Biological Hazards automatically performed a QPS assessment for *B. xylanisolvens* (EFSA BIOHAZ Panel, 2014). The EFSA BIOHAZ Panel noted that the available published studies about *B. xylanisolvens* are not sufficient to include the organism in the QPS list, although no relevant safety concerns could be established.

B. xylanisolvens DSM 23964 is harboring the *cepA* gene in its genomic DNA which provides the strain with a resistance to β-lactam antibiotics (Ulsemer *et al*., 2011). This resistance is very common among the genus *Bacteroides* (Wexler, 2007). No mobile elements like conjugative transposons or plasmids had been found. The strain was also screened for 8 different potential virulence genes that are common in other *Bacteroides* species but none of them could be detected. *B. xylanisolvens* DSM 23964 showed no adhesion to Caco-2 cells in cell culture model. (Ulsemer *et al*., 2011). The attachment to epithelial cells is an important factor in the progression of infections.

The panel considered that due to the heat inactivation and the lacking of plasmids a transfer of genes is not expected to take place.

7) Toxicological information on the novel food

The applicant provided a study about an intraperitoneal abscess formation model in mice (Ulsemer *et al.,* 2011). Abscess formation is a relevant pathology because some species among the genus *Bacteroides*, f. e *B. fragilis*, are able to induce abscesses on multiple sites in the body. *B. xylanisolvens* DSM 23964 was administered in varying doses but none of the mice developed an abscess.

Human data were also supplied to EFSA, including one pilot study and one Randomized Control Trial (RCT):

The pilot study (Ulsemer *et al*., 2012) lasted for three weeks and 2 groups of volunteers (10 males and 10 females in each group, 20-60 years old) consumed daily portions of 100 ml heat-treated low-fat milk cultured with *B. xylanisolvens* (5.5×10^{11} or 8.5×10^{11} inactivated cells/portion). The blood of the volunteers was analysed before the study started and after the three-week intervention. The product was well-tolerated by all participants and the assessment of the different parameters (haematological analyses, phagocytic activity, serum immunoglobulins levels, cytokine and chemokine analyses) revealed no significant effects.

The RCT (Ulsemer *et al*., 2012) was conducted over six weeks, 140 volunteers (18-65 years old) were separated in four different groups. The participants received a spray-dried pasteurized fermented milk product produced with *B. xylanisolvens* DSM 23964 as sole starter culture. The first three groups obtained a daily amount of inactivated cells in a range of 10^{10} to 10^{12} per portion while the last group received a milk powder placebo. In total four blood samples were taken from the volunteers: before the start, at the end and twice during the intervention period. Again, the product was well-tolerated by the volunteers and the assessed parameters (determination of liver enzyme and T-cell subpopulation; other measurements were identical to the pilot study) showed no significant differences.

8) Allergenicity

Although the applicant did not provide material regarding the allergenicity of the product, the EFSA Panel considered that it is unlikely that the allergenic potential should differ from

other fermented dairy products. The effect of heat treatment of milk on its allergenicity had been considered previously by EFSA itself (EFSA NDA Panel, 2014).

4.1.3 QPS evaluation of *B. xylanisolvens*

As briefly mentioned above, there has been no QPS assessment for *B. xylanisolvens* before EFSA was asked to perform an additional assessment for the specific strain. When EFSA receives an application for a novel bacteria strain or a food product that is containing a novel strain, an evaluation relating to the QPS scheme automatically takes place. It is necessary to consider that during a QPS evaluation only the specific strain is assessed and not the food product that is potentially put on the market.

In 2014, the BIOHAZ Panel performed the assessment for *B. xylanisolvens* (EFSA BIOHAZ, 2014). The panel criticized that the body of knowledge is insufficient. No history of *B. xylanisolvens* in fermentation processes is available and the very few existing studies are limited to fermented milk products. Although safety concerns seem not very likely, the panel emphasized that the number of published studies is too low to definitely exclude safety issues. The pilot studies (Ulsemer *et al*., 2012) only worked with small and healthy cohorts and the administered bacterial cells were inactivated. Taken together all the arguments, the panel did not recommend to put *B. xylanisolvens* on the QPS list.

4.1.4 *Bacteroides xylanisolvens* and its probiotic potential

Most bacteria found in the human intestine are anaerobic. Among those, the genus *Bacteroides* represents approximately 30 % of all bacteria in the human colon (Sears, 2005). There are many requirements for a bacterial strain to be referred as probiotic. An essential characteristic is the survival of the gastro-intestinal tract, to finally provide beneficial effects on the host. *B. xylanisolvens* DSM 23964 showed a survival rate of 90 % after spending three hours in gastric juice and a survival rate of 96 % spending four hours in intestinal juices (Ulsemer *et al*., 2011). The genus *Bacteroides* showed a higher induction of mucosal IgA production than *Lactobacillus* when co-cultured with Peyer´s patches lymphocytes (Yanagibashi *et al*., 2009). Such immunomodulatory effects are essential attributes of probiotics. Other data (Gerard *et al.*, 2007) indicate that specific *Bacteroides* strains are able to reduce cholesterol. Elevated levels of blood cholesterol are still a major public health concern in the Western world. The fermentation of diet induced polysaccharides to SCFA by *B. xylanisolvens* is also connected with health-promoting effects. SCFA may be involved in the establishing of satiety stimulation (Hosseini *et al*., 2011) or even provide anti-carcinogenic properties (Zhou *et al*., 2008).

Cell attachment tests with epithelial Caco-2 cells revealed negative results for *B. xylanisolvens* DSM 23964 (Ulsemer *et al*., 2011). The capability to bind the intestinal epithelial cells in the host is seen as a key role to provide probiotic properties. The available

studies published with fermented milk products containing *B. xylanisolvens* DSM 23964 are limited to inactivated bacterial cells. Studies with dead bacteria are useless because probiotics per definition need to be alive when administered to the consumer.

There are several potential probiotic properties connected to *B. xylanisolves*. However, the fact that *B. xylanisolvens* does not bind epithelial cells *in vitro* is a major disadvantage when it comes to the assessment of probiotic properties. Furthermore, studies are needed with living bacteria to increase the chance to be accepted as a probiotic.

4.1.5 Admission of *B. xylanisolvens* as novel food – impact for other candidates?

Bacteroides xylanisolvens has only been the fourth bacterium after *Leuconostoc mesenteroides*, *Bacillus subtilis natto* and *Clostridium butyricum* to be accepted as a novel food according to the Novel Food Regulation No 258/97. The positive outcome of the novel food assessment is independent from the results of the QPS evaluation. *B. xylanisolvens* and *C. butyricum* did not make it on the QPS list yet. It is necessary to point out that during the QPS assessment only the species itself is analysed and that the potential food products containing the bacteria are not of interest. Interestingly, only one Gram-negative bacterium, *Gluconobacter oxydans*, made it on the QPS list so far (EFSA, 2013).

4.2 *Akkermansia muciniphila*

The relatively new described species *Akkermansia muciniphila* is currently the only member within the genus *Akkermansia*. By February 2017, there has been no evaluation according to the QPS status, which would be performed and published by EFSA. Relating to its recent detection this microorganism has no history of use in the food industry. If there are proposals for use in the future, *Akkermansia* must be treated according to the Novel Food Regulation (OJEC, 1997). If the potential evaluation is not finished until the January 1ST 2018, the assessment will be performed according to the new and revised Novel Food Regulation (OJEC, 2015).

In this part, the genus *Akkermansia* and its type-strain *A. muciniphila* are discussed and analysed relating to the four pillars of the QPS evaluation (establishing identity, body of knowledge, possible pathogenicity and end use). This section focuses on the analysis of the microorganism itself and not on the potential use of *Akkermansia* in food.

4.2.1 QPS assessment for *Akkermansia muciniphila*:

Taxonomic unit defined

Akkermansia muciniphila was discovered in 2004, during the search for new mucus degrading bacteria in human feces samples. The newly detected organism was named after Antoon Akkermans, a well-respected Dutch microbiologist with significant contributions in the field of microbial ecology (Belzer and de Vos, 2012). *Akkermansia muciniphila* represents currently the only isolated and identified species of the genus *Akkermansia*, therefore acting as the type strain of the genus. This oval-shaped gram-negative microorganism belongs to the phylum Verrucomicrobia which was named after the first described species within this phylum, *Verrucomicrobium spinosum*. It further belongs to the class Verrucomicrobiae, the order Verrucomicrobiales and the family Verrucomicrobiaceae.

Metagenome data suggest at least 8 further *A. muciniphila* related species in the human intestine (van Passel *et al.*, 2011). Belzer and de Vos suggested according to 16S rRNA analyses of mammalian derived intestinal samples that the genus *Akkermansia* consists of 5 distinct clades. 4 of the 5 clades are associated with *A. muciniphila* and the range of sequence similarity lasts from 80-100 %.

Nearly all investigated mammalian members show *Akkermansia* related sequences in their intestinal samples. But *Akkermansia* derived sequences are not restricted to mammals, they were also found in other vertebrates: similar sequences were detected in zebrafish, in the Burmese python or for instance in the grenadier fish, which represents an inhabitant of the deep ocean (Roeselers *et al.,* 2011; Costello *et al.*, 2010). These ubiquitous findings suggest that *Akkermansia* already played a crucial part in the early steps of vertebrata evolution and that its occurrence has to be connected with important functions in the intestine.

Genomic analyses revealed that the single chromosome of *Akkermansia muciniphila* harbors 2,176 genes with a GC content of 55,8 % (van Passel *et al.,* 2011). The secretome which is responsible for the degradation of mucin, involves 61 different proteins, representing 11 % of the total protein content (Belzer and de Vos, 2012).

Body of knowledge

Since *Akkermansia* is a relatively new genus, it is expected that further species close to *A. muciniphila* will be detected and cultivated in the upcoming years. There is obviously no history of use in the food sector and the potential areas of application are discussed in the section dealing with *A. muciniphila* as a candidate for novel foods. However, it is a strict anaerobe and therefore difficult to produce in industrial quantities.

Despite the short time since the discovery of *Akkermansia* the basic characteristics of the microorganism are well-known. This gram-negative, oval-shaped bacterium is non-motile and specialized in degrading mucin. *A. muciniphila* is anaerob, chemo-organotroph and capable to use mucin as sole nitrogen, carbon and energy source (Derrien *et al.*, 2004). The habitat of *Akkermansia* spp. is also well-documented due to the ubiquitous occurrence

among many vertebrate intestines (Belzer and de Vos, 2012). The genome sequence of *A. muciniphila* has been fully determined (van Passel *et al.*, 2011). The general properties of *A. muciniphila* are listed in Table 2.

Table 2. General characteristics of *Akkermansia muciniphila* (adapted from Gomez-Gallego et al., 2016)

Phylum	**Class**	**Order**	**Family**	**Genus**	**Species**
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	*Akkermansia*	*Akkermansia muciniphila*
Type strain	MucT (ATCC BAA-835)				
Biosafety level	1 (U.S Public Health Service Guidelines)				
Origin	Feacal sample from a healthy adult volunteer				
Chararacteristics					
Morphology	Gram-negative, oval-shaped, non-motile				
Genome	Size: 2664102 bp, ORF`s: 2176, GC content: 55,8 %				
Physiology	Chemo-organotrophic; anaerobic; mesophilic: growth at 20-40°C (0ptimum: 37°C)				
	Capable of using mucin as energy, nitrogen and carbon source; mucolytic in pure culture				
	Sulphate release in free form from mucin fermentation				

Safety concerns

A. muciniphila is a regular inhabitant of the human intestine where it represents approximately about 1 to 4 % of the overall colon microbiota (Derrien *et al.*, 2008). Currently there are no published clinical human trials where viable cells were administered to people. Such studies only exist for animal models (Everard *et al*., 2013). Interestingly, Lagier and his colleagues reported two cases where *A. muciniphila* represented up to 80 % of the total intestinal microbiota without any noticeable decline in health (Lagier *et al*., 2015).

Currently, there is no clear evidence that *A. muciniphila* is connected to a specific disease (Derrien *et al.*, 2010). There are general concerns because *Akkermansia* shows pathogen-like behaviour: the adhesion to the mucus is considered as a crucial step during an infection. In contrast to pathogens, *A. muciniphila* as a mucin degrader stays in the outer layer of the mucus and never reaches the inner layer which would be fundamental for a successful infection (Gomez-Gallego *et al*., 2016). In addition, the mucin degradation itself resembles pathogen-like behavior (Donohue and Salminen, 1996). But mucin degradation is regarded as a regular process that is part of a balanced and self-renewing intestine (Derrien *et al.,* 2004).

Colorectal cancer patients showed a 4-fold increase of *A. muciniphila* in their stool samples compared to healthy subjects (Weir *et al.*, 2013). However, patients suffering from colorectal cancer have a reduced food intake and studies demonstrated that fasting is correlated with elevated levels of *A. muciniphila* (Remely *et al.*, 2015). In addition, colorectal cancer is also related to increased cell proliferation and mucus production and so it is evident that the main mucus-degrading bacterium is present in a higher number (Gomez-Gallego *et al.,* 2016).

There are apparently no specific safety concerns regarding the genus *Akkermansia* and its type strain *Akkermansia muciniphila*. Possible future concerns can be perhaps removed individually on a case-by-case review.

Units proposed for QPS status

Since there are no applications so far for *A. muciniphila* and therefore no history of safe use, the QPS status is apparently not available.

4.2.2 *Akkermansia muciniphila* – a candidate for novel food?

Currently there are no pending applications for products containing *Akkermansia muciniphila* according to the Regulation (EC) 258/97. Due to the fact that *A. muciniphila* was only discovered and isolated in 2004, there is still comprehensive research needed for a better understanding of this microorganism.

A. muciniphila was not used for human consumption to any ´significant degree` within the European Union before 15 May 1997. If a Food Business Operator is interested to put a product containing *A. muciniphila* on the market, an application according to the Novel Food Regulation would be necessary. The European Commission published a Recommendation (OJEC, 1997a) to help Food Business Operators which information they have to provide for a successful application procedure. Among those needed information there are still many unknown aspects about *A. muciniphila* (Gomez-Gallego *et al.,* 2016):

- Specification of the novel food: there is substantial knowledge about the species and taxon but currently there is no product containing *A. muciniphila* on the market
- Production process: no recipes and no process steps are known so far, but *A. muciniphila* has to be alive to fulfil its potential probiotic properties
- History of use: no history of use available
- Anticipated human intake: no intake patterns are known
- Nutritional information: nutritional assessment only in animal models
- Toxicological information: no toxicological assessment in humans
- Microbiological information: *A. muciniphila* is a commensal bacterium with no pathogenic nature
- Allergenic potential: not known so far
- Genetic engineering: no genetic modification so far

But how is the potential of *A. muciniphila* as a novel food? Regarding the collected knowledge so far this microorganism could be very interesting for Food Business Operators and consumers. *A. muciniphila* is part of the gut microbiota in (nearly) all humans and due its contributions to a functional gastro-intestinal tract it is providing probiotic properties.

Current data indicate that a decreased number of *A. muciniphila* in the colon is connected with a series of different relevant diseases.

Among those illnesses are lifestyle diseases like obesity and type 2 diabetes, but also specific colon-related diseases like Morbus Crohn, ulcerative colitis (Png *et al*., 2010; Rajilic-Stojanovic *et al.*, 2013) and severe acute appendicitis (Swidsinski *et al.*, 2011). Especially the lifestyle diseases, obesity and type 2 diabetes, represent a growing concern in the industrial nations but also in newly industrializing countries like India or China. Many countries have to deal with exploding health care costs and so every potentially helping probiotic product has a high sales market due to the growing need.

Everard and colleagues were able to show a decrease in abundance of *A. muciniphila* in obese and type 2 diabetic mice. They connected obesity with a decreasing mucus thickness and therefore leading to an enhanced gut permeability. After administration of *A. muciniphila* a reduction in fat mass gain, adipose tissue inflammation and a reversing of insulin resistance was observed. After treatment with prebiotics the abundance of *A. muciniphila* increased up to 100 times and improved the gut barrier function of genetic obese mice (Everard *et al.*, 2013). Recently, researchers revealed that *A. muciniphila* is able to provide beneficial effects even after pasteurization for 30 minutes at 70°C. The pasteurized bacteria were capable to reduce the fat mass development, the insulin resistance and dyslipidemia in obese and diabetic mice. Amuc_1100, an outer membrane protein, showed interaction with Toll-like receptor 2 and stability even during the pasteurization process. This membrane protein also improved the gut barrier and enabled ongoing beneficial effects after bacterial death (Plovier *et. al*, 2016).

The link between obesity and a decreased abundance of *A. muciniphila* is not restricted to rodents. A Swedish investigation showed that obese pre-school children have significant lower numbers of *A. muciniphila* compared to other normal weighted pre-school children (Karlsson *et al.*, 2012). Furthermore, the obese children showed a lower diversity of commensal bacteria in the gut.

In vitro studies revealed that the mucin degrader *A. muciniphila* does not bind to the mucus but it shows a strong binding to the human colon epithelial cell lines Caco-2 and HT-29 (Reuanen *et al.*, 2015). This is in contrast to other probiotic (non-mucolytic) bacteria like *Lactobacillus rhamnosus* or *Bifidobacterium bifidium*, which are able to bind to the mucus. In the same study the scientists also tested the adhesion properties of *A. muciniphila* to different extracellular matrix proteins. *A. muciniphila* was only capable to attach to laminin while other proteins like collagen, fibronectin and fetuin remain unbound.

Interleukin-8 (IL-8) is a chemokine that plays a crucial role in the first steps of the inflammation process by recruiting neutrophils and other granulocytes to the site of infection. Reuanen and co-workers demonstrated that *A. muciniphila* is needed in a 100-fold higher dose than *E. coli* to induce IL-8 production in colon epithelial cells. Such a number illustrates that *A. muciniphila* is not able to provoke a strong inflammation under normal conditions. This finding is also relevant regarding the potential use of *A. muciniphila* in food.

A. muciniphila can improve enterocyte monolayer integrity. This finding was established by determining a significant increase of the transepithelial electrical resistance (TER). The epithelial barrier function is measured by evaluating the TER and therefore concluding to the ion transfer through monolayers (Blikslager *et al.*, 2007). Reuanen and colleagues co-cultured *A. muciniphila* with a Caco-2 monolayer. The increase of the TER was significant higher with *A. muciniphila* compared to the sole monolayer or co-culturing with *E. coli* as a negative control. This strengthen of the epithelial integrity, although only shown *in vitro*, can be very useful in disease prevention. Impaired gut barrier functions are linked to a wide range of illnesses, especially the previous mentioned obesity and type 2 diabetes.

Despite the growing attention for *A. muciniphila*, there is still a lot of research needed to gain more specific and age restricted information. There are no randomized double-blind placebo-controlled clinical trials available so far. The clear majority of studies were performed with animals, and long-term studies on the human level are the next step that has to be taken. Although there is still a lack of information regarding the recommendation of the European Commission on novel food, but prospects for a future novel food application are quite promising. Especially the potential probiotic health benefits, like the maintenance or restoring of the epithelial barrier functions, could ease the application process.

Gómez-Gallego and his colleagues pointed out the fact that *A. muciniphila* is already consumed by humans via breast milk. Therefore, the question arises if it is necessary to prove the safety of an organism that is already part of the nutrition in the first stages of life. Further discussions are needed but the strict European legislation could be changed in future for such specific cases.

Placement of *A. muciniphila* on the QPS list is rather unlikely, since history of safe use cannot be provided and due to the fact that only one Gram-negative bacterium hitherto has made it on this list so far (*Gluconobacter oxydans;* EFSA, 2013).

4.3 Fructophilic lactic acid bacteria: a new member of the LAB family

Lactic acid bacteria (LAB) represent one of the most relevant groups of microorganisms in the food industry. Long before this group of bacteria was described by Orla-Jensen in 1919, mankind benefited from their positive attributions in various food production processes (Von Wright, 2012). As the name of the group indicates, LAB produce lactic acid as their main metabolic end product. The acidification during the food fermentation process leads to a strong reduction of pathogenic and spoilage organisms that are not as acid-tolerant as LAB. Further are some LAB capable to produce bacteriocins which hinder potential dangerous microorganisms to multiply in the food. LAB also contribute to the specific organoleptic and texture profile of the final product. LAB are involved in different relevant industrial fermentation processes: the huge dairy industry with their versatile yogurt products and the growing sector of probiotics represents the biggest operational area for LAB.

LAB build the order *Lactobacillales* and comprise a variety of families, where *Lactobacillus*, *Leuconostoc*, *Lactococcus* and *Streptococcus* are one of them. This order shares a variety of characteristics: they are coccus- or rod-shaped, acid-tolerant, Gram-positive, non-sporulating, non-respiratory bacteria. The main feature for LAB classification is their way to ferment glucose molecules. Homo-fermentative LAB are capable to convert one glucose molecule into two pyruvate molecules and finally two lactate molecules. This process leads to a net yield of two ATP molecules out of one glucose molecule. The genera *Streptococcus* and *Lactococcus* are for instance using this way to generate energy. Hetero-fermentative LAB represent the other way of metabolizing glucose. This group is able to process hexose and pentose molecules. During the catabolism of hexose molecules ethanol is produced at an equimolar amount with lactate. Pentoses lead to acetate production, instead of ethanol. The net ATP yield is lower compared to the homo-fermentative way, leading to only one ATP molecule from one glucose molecule.

Fructophilic lactic acid bacteria (FLAB) are building a subgroup among the diverse order of LAB. FLAB prefer fructose as substrate and they show only poor growth in glucose containing media. Therefore, FLAB inhabit different fructose-rich niches: Flowers, fruits, fermented foods like wine and cocoa beans and even the gastro-intestinal tracts of insects are harboring these microorganisms (Endo and Salminen, 2013). The FLAB group is divided into the genera *Fructobacillus*, with its type species *F. fructosus*, and *Lactobacillus*. This group of bacteria was characterized only a couple of years ago, minor or even major changes within its taxonomy will certainly arise in near future.

Four *Fructobacillus* species were former part of the *Leuconostoc* spp. but recent analyses suggested that the unique characteristics legitimate a renaming and a new taxonomic classification for this bacterial group. *Fructobacillus* shows a clear preference for fructose over glucose. Niche-specific evolution made it impossible for *Fructobacillus* to metabolize glucose without external electron acceptors like oxygen or pyruvate. *Fructobacillus* shows a significant smaller genome size than *Leuconostoc* which is mainly explained by the lack of a carbohydrate metabolic system and the lower gene number for energy production and energy conversion. The higher number of conserved genes among the coding sequences (62 % for *Fructobacillus* and 52 % for *Leuconostoc*) is maybe caused by a less complex and more consistent habitat with specific sugars only. A further difference between the two bacterial groups can be found in the missing of the *adhE* gene in *Fructobacillus.* This gene codes for an acetaldehyde/alcohol-dehydrogenase (AdhE) while *Fructobacillus* is harboring the *adh* gene coding for an alcohol-dehydrogenase only. Those findings provide strong support for the reclassification and renaming of *Fructobacillus* (Endo *et*. al, 2015).

Due to the recent identification of FLAB, the number of species is growing among both genera *Fructobacillus* and *Lactobacilllus*. *Fructobacillus* currently consists of *F. fructosus, F. pseudoficulneus, F. ficulneus, F. durionis* and *F. tropaeoli* (Endo *et* al., 2011). *F. pseudoficulneus* is the highest detected species among natural sources. This microorganism was isolated from figs, bananas, flowers and even taberna, a traditional beverage of Southern Mexico (Endo, 2012; Alcantar-Hernandez *et al*., 2010). The FLAB members among the genus *Lactobacillus* are *L. kunkeii* and *L. florum* (Neveling *et al.,* 2012).

Lactobacillus kunkeei was first isolated from wine and is classified as the sole obligate FLAB species among the genus *Lactobacillus* (Endo *et al.*, 2012). Interestingly, this microorganism represents the major species in the gut of honeybees (Endo and Salminen, 2013). Maeno and his co-workers analyzed 16 different *L. kunkeei* strains, in comparison to 57 strains from other members of *Lactobacillus* spp., to reveal a potential niche-specific evolution. The results showed a significantly smaller genome size and a lower number of coding sequences for *L. kunkeei*. The number of genes for carbohydrate transport and metabolism was decreased, resulting in a poor overall carbohydrate metabolic activity for the species. The *adhE* gene usually expresses a bifunctional ADH/aldehyde dehydrogenase (ADH/ALDH) protein (AdhE). In the case of *L. kunkeei*, the AdhE protein possesses only the ALDH domain while the ADH domain is missing. This gene reduction explains the need of electron acceptors for glucose metabolism. The obtained results indicate a specific reductive evolution for the adoption of fructose-rich environments, similar to the genus *Fructobacillus* spp. (Maeno *et al.*, 2016).

FLAB are separated in facultative and obligate heterofermentative lactic acid bacteria according to their ability to metabolize glucose. Facultative FLAB, with its only member *L. florum*, are capable to utilize glucose without an external electron acceptor at a delayed ratio. Further, facultative FLAB produce ethanol instead of acetate while metabolizing glucose (Endo *et al*., 2012). The obligate heterofermentative group, which consists of all other FLAB, do not show the above mentioned characteristics. In Table 3 the main features of FLAB are summarized.

In 2009, Endo *et al.* stated five main characteristics that provides a good overview about FLAB:

- FLAB prefer D-fructose over D-glucose
- FLAB need electron acceptors for the metabolism of D-glucose (with the exception of *L. florum*)
- FLAB use oxygen and pyruvate as external electron acceptors to stimulate growth with glucose
- FLAB prefer aerobic condition than anaerobic conditions of growth
- FLAB show poor general sugar fermentation abilities

Table 3. General characteristics of different FLAB (adapted from Endo *et al*., 2009)

Phylum	**Class**	**Order**		
Firmicutes	Bacilli	Lactobacillales		
Family	**Genus**			
Lactobacillacaea	*Fructobacillus*			
Leuconostocaceae	*Lactobacillus*			
Species	**Gram**	**Glucose End Products (Molar ratio)**	**Acid Production**	**Isolated from**
F. fructosus	positive	1:0.008:0.7 (L:E:A)	F, G, M	Azalea
F. pseudoficulneus	positive	1:0.005:0.8	F, G, M	Banana, Fig
L. kunkeii	positive	1:0.02:0.6	F, G, M, S, T	Narcissus, Cosmos
Lactobacillus sp.	positive	1:0.8:0.2	F, G, M	Peony, Bietou

L: Lactic acid, E: Ethanol, A: Acetic acid, F: Fructose, S: Sucrose; M: Mannitol, G: Glucose,T: Trehalose

Although FLAB were just recently discovered, this group of microorganism may have great potential for the food industry. A strong argument for future application in the industry is the relatedness to LAB. LAB are generally considered as safe and they are part of the human food production and nutrition since ancient times (Von Wright, 2012). Recent investigations revealed the occurrence of FLAB in many different food items. *F. pseudoficulneus* was detected during spontaneous cocoa bean fermentation process. Raw cocoa beans have an unpleasant and astringent flavor, therefore further preparation steps like fermentation, drying and roasting are necessary to obtain the desired taste. Yeasts, LAB and acetic acid bacteria are the main microorganisms that are responsible for the fermentation process. *F. pseudoficulneus* mainly occurs in the beginning of the coca bean fermentation process when enough fructose is available (Lefeber *et al.*, 2011). Another, *F. tropaeoli-like,* FLAB was detected during traditional Ecuadorian spontaneous coca bean fermentation (Papalexandratou *et al*., 2011). *F. durionis* was identified as one of the dominant bacteria during tempoyak production. Tempoyak is a very popular condiment in South-East Asia that is made by fermenting durian fruit. Durian is an expensive and exotic fruit that is appreciated for its unique flavor. Unfortunately, durian has a very short shelf-life between 2 and 3 days and so overripe and old fruits are used for tempoyak production. Dorian fermentation is a spontaneous, uncontrolled process with varying microbial composition that may last for months. The role of *F. durionis* in this natural fermentation is not well-understood but the dominance in the beginning is explained by the high fructose content (Chuah *et al*., 2016). *L. florum*, the facultative FLAB among *Lactobacillus*, was recently detected in grapes and wine, possibly contributing important properties in an oenological perspective (Mtshali *et al.*, 2012).

The occurrence of FLAB in the gastro-intestinal tracts of bees, giant ants, tropical fruit flies or bumblebees are an indication for possible probiotic characteristics (Endo, 2012) although there has been no detection of FLAB in vertebrate´s intestines so far (Endo and Salminen, 2012). FLAB may also play a crucial role in bee health, since *Fructobacillus* can promote growth of bee commensal lactobacilli (Rokop *et al.*, 2015). FLAB are also considered as tools of paratransgenesis for promotion of bee health (Maddaloni *et al.*, 2014; Rangberg *et al*., 2012). Recent studies suggested that administration of heat-killed *L. kunkeei* has potential beneficial properties on human health, including increased bowel movement and enhanced immunoglobulin A production (Asama *et al.,* 2015 and 2016).

5 Discussion

5.1 General and case study related issues

The new Novel Food Regulation, which will come into force on January 1st 2018, facilitates the authorization of future novel food. EFSA will take on sole responsibility for the scientific risk assessment, thereby ensuring a change from an individual (by the national competent authorities) to a generic assessment. The new regulation also clarifies that EFSA delivers the scientific opinion within nine months after receiving all valid application material. This renewal means an enormous time reduction and therefore a significant lowering of the financial expenses of the applying FBO. The newly introduced data protection for approved novel food authorizations also helps the companies to successfully compete on the food market.

EFSA introduced the QPS procedure in 2007, mainly to establish and assess the generic risk for microorganisms. The QPS concept should beyond that lead to a concentration of the available resources on bacteria with higher risk potential. The annually revised QPS list provides a helpful orientation for FBOs and the assessing authority itself. All the listed microorganisms, even those that are described with a so-called qualification, do not need an exhaustive safety assessment. However, the need to check potentially developed antimicrobial resistances, has to be considered for every used microorganism. Companies can check out the QPS list any time and find out if the bacteria they want to use during their food production process are eligible and safe for later marketing. The necessary steps for a successful admission of a new strain are summarized in Figure 5.

The revision of the Novel Food Regulation and the introduction of the QPS concept have improved and accelerated novel food authorization and biological agent safety assessment. Both actions have facilitated the work of EFSA and the access for FBOs to the food market without forgetting the safety of the European consumer. Although those improvements are remarkable, the authorization of novel food still seems more comprehensive compared to the GRAS concept in the United States. The precautionary principle is one of the fundamental pillars within the environment, food and health policy in the European Union. The prevention of any possible harm or danger for the consumer is the most important issue. This is especially true when it comes to the marketing of food containing bacteria which may be harmful. The exclusion of any possible health threat is therefore essential before a product is allowed to enter the European market. The European consumer is the most relevant player in the European legislation and companies understandably complain about such circumstances. Furthermore, the long revision process of the Novel Food Regulation demonstrates how difficult the change of the legislation on the European level can be. Since most decisions need to be unanimous, it is challenging to please all member states. Among the 28 members (soon only 27), it is obvious that some country at some point is not satisfied with the current progress and demands further changes.

How great is the potential for the reviewed bacteria to be acceptable in the future as a novel food or novel food ingredient? A brief evaluation of benefits and drawbacks is given in Table 4 (see section 5.2). *Akkermansia muciniphila* is a very promising candidate. The multitude of probiotic properties provides good chances for future food authorizations. As a part of the regular human gut microbiota, *A. muciniphila* is essential for a well-functioning gastro-intestinal tract. Lower numbers of this mucin-degrader were associated with obesity and type 2 diabetes in mice and human. Furthermore, *A. muciniphila* contributes to the maintenance and restoring of epithelial barrier functions. Some probiotic features are currently only shown in cell-cultures or animal studies and so the number of human studies, especially long-term interventions, need to be increased in future. The very important capacity of surviving the gastro-intestinal passage still has to be proved. A further interesting point was revealed by Plovier and co-workers: even the dead *Akkermansia* cells were capable to provide beneficial effects. A decrease in fat mass development, dyslipidemia and insulin resistance was demonstrated in obese and diabetic mice (Plovier *et al.*, 2016). This insight may facilitate future authorizations because non-viable cells may rise fewer concerns compared to living bacteria. Non-viable bacteria may more easily receive a safe status in novel food evaluation, though it may be a case-to-case decision.

In comparison to *B. xylanisolvens*, some information about *A. muciniphila* are still missing. Due to the lacking application so far, a specification of the novel food, its production process and nutritional information are naturally not known yet. On the other side, there is already information available regarding the taxonomy, culturing methods, the pathogenic and toxicological nature and nutritional assessment data in animal models (Gomez-Gallego *et al*., 2016). One of the major keys for approval of novel food is the supply of sufficient human data. Regarding *A. muciniphila* there is still a huge lack of appropriate studies. Randomized double-blind placebo-controlled clinical trials, dose-response studies, toxicological studies are just missing like studies dealing with the right amount of bacteria administered and studies examining the right matrix to provide probiotic properties. If the missing data will be available in future, *A. muciniphila* has similar chances to be accepted as a novel food compared to the also relatively new species *B. xylanisolvens*. Interestingly, EFSA came to a positive assessment for *B. xylanisolvens* although only two human studies were supplied by the applicant.

FLAB were just discovered recently and so it is challenging to predict in which way they may be part of future food products. Further research is needed to understand the exact contributions of this bacterial group in cocoa bean fermentation or for instance during wine production. Recent studies revealed that administration of heat-killed *L. kunkeei* provide probiotic properties, including increased bowel movement and enhanced immunoglobulin A production (Asama *et al.,* 2015 and 2016). The relatedness to LAB, which represent a very widely used group in the food industry, may facilitate a place on the QPS list and future novel food authorizations.

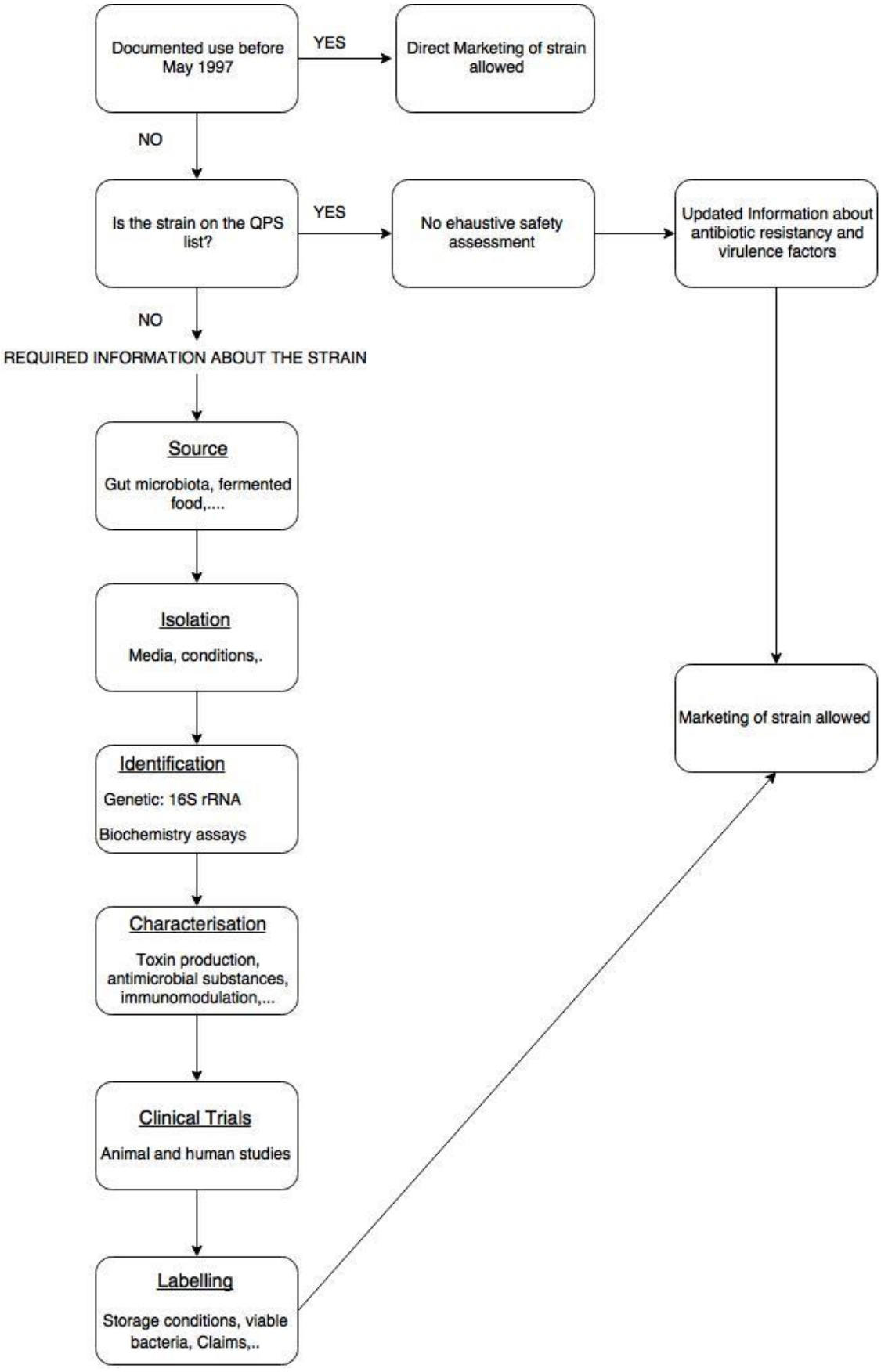

Figure 5. Checkpoints for a successful strain admission

5.2 Other potential bacterial candidates

The three case studies discussed in this thesis represent only a small fraction out of the potential bacteria that may be part of novel foods in future. Among the huge number of species in the gut microbiota, many microorganisms fulfil essential functions for the maintenance of a healthy gastro-intestinal tract. The ongoing identification and characterization of new bacteria even enlarges the reservoir of future probiotics. Further investigations of known food fermentation processes will reveal new bacterial species and may improve certain food production processes in future. In this chapter, three additional microbes of interest will be briefly presented, not without mentioning that many other bacteria also could have been chosen to be discussed.

Faecalibacterium prausnitzii is the sole member of the genus *Faecalibacterium* and is a commensal bacterium of the human gut microbiota. It was named after the German bacteriologist Otto Prausnitz, who was the co-inventor of an early immunological test, the so-called Prausnitz-Küstner test. This Gram-positive and obligate anaerobe microorganism is part of the class Clostridia. *F. prausnitzii* accounts for 3 - 5 % of total fecal bacteria and is therefore one of the predominant species in human faeces (Breyner *et al.,* 2017). *F. prausnitzii* is capable to digest dietary fibers and to produce butyrate among many other SCFA. This microbe may play a crucial role for the human state of health since lower numbers of *F. prausnitzii* were associated with Morbus Crohn or Major Depressive Disorder (Sokol *et al.,* 2008; Jiang *et al.*, 2015). Recent investigations revealed possible contributions to protective mechanisms, like self-defense against inflammatory reactions: *F. prausnitzii* was involved in the inhibition of pro-inflammatory cytokines and in the secretion of bioactive molecules which lead to the blockage of the NF- κB pathway. One of the bioactive molecules was identified in the supernatant of *F. prausnitzii* as Microbial Anti-inflammatory Molecule (MAM). Both supernatant and the bacterium itself showed protective effects in chemically-induced colitis mouse models (Breyner *et al.*, 2017). Further research with *Faecalibacterium* may increase the knowledge about its probiotic effects and may lead to the future use of this microorganism in the food industry.

Eubacterium hallii is another interesting member of the class Clostridia. It is a common inhabitant of the human gut microbiota and known for its versatile utilization of different carbon sources. *E. hallii* can produce butyrate from lactate, acetate and glucose. Butyrate is an important energy source for the ambient colonocytes and may impact cell differentiation and proliferation processes (Schwab *et al.,* 2017). Propionate is an additional SCFA that is produced by *E. hallii* from 1,2-propanediol. This essential SCFA is not only a precursor for gluconeogenesis in liver, it also impacts cell differentiation with potential health-promoting effects on cancer development and intestinal inflammation (Reichardt *et al.,* 2014). Both propionate and butyrate are fundamental for host/gut microbiota homeostasis as they interact with the host epithelium and affect the local immune system (Schwab *et al.,* 2017). A Swiss cohort study revealed that *E. hallii* is one of the first butyrate producers in the infant gut (Pham et al., 2016). A screening of Venezuelan, Malawian and American databases uncovered the existence of *E. hallii* in all available samples of 1 year old or younger children. Moreover, a low abundance of *E. hallii* was demonstrated in the first months of life, while

the adult level of this microorganism is reached in the age between 5 and 10 (Schwab *et al.*, 2017). These findings imply that *E. hallii* is one of the very first bacteria to colonize the infant gut and its utilization of metabolic bifidobacterial products, lactate and acetate, indicate a close collaboration between the different species. The early appearance in the human intestine and the production of essential SCFA may enable future novel food applications for *E. hallii* in the food industry.

The genus *Roseburia* currently consists of five species. These Gram-positive, obligately anaerobic bacteria are motile due to their many subterminal flagella (Tamani-Shacoori *et al.,* 2017). *Roseburia* spp. are one of the predominant intestinal bacterial species, thereby accounting for 2 to 15 % of the total human gut microbiota (Dostal *et al*., 2015). *Roseburia intestinalis* shows amylolytic and xylanolytic properties. The fermentation of xylan-rich substances produces many essential SCFA, including butyrate, propionate and lactate (Mirande *et al.,* 2009). The undersupply of iron is one of the major challenges among malnutrition and the performance of *R. intestinalis* is also affected by it: lower iron availability reduces the production of butyrate and hydrogen, while high iron availability shows opposite effects (Dostal *et al.*, 2015). Lower numbers of *R. intestinalis* are associated with Crohn`s disease and the pathogenesis of the inflammatory bowel disease, indicating how important a stable number of these bacteria may be for human health (Hoffman *et al*., 2015). Considering the production of essential SCFA and the very high number of *Roseburia* spp. among the human microbiota, members of this genus may have great potential as future probiotics in the food industry.

Table 4. Overview about chances for future novel food admissions

Species	Benefits	Drawbacks	Chance for future admission
A. muciniphila	Commensal, diverse probiotic properties, treatment of obesity and type 2 diabetes, non-viable cells also show probiotic effects	Gram-negative (QPS list) strict anaerobe (difficult working conditions) More human studies needed	**HIGH**
FLAB	Part of LAB (one of the most used bacterial group in food industry), already part of various food fermentation processes	Generally less knowledge on properties and functions More research needed	**HIGH**
F. prausnitzii	Commensal, producer of essential SCFA, Gram-positive	More research needed	**HIGH**
E. hallii	Commensal, producer of essential SCFA, Gram-positive, early gut colonization	More research needed	**HIGH**
R. intestinalis	Commensal, producer of essential SCFA, Gram-positive, amylolytic and xylanolytic properties	More research needed	**HIGH**

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