Trends in Food Science & Technology 21 (2010) 425–435

TRENDS IN FOOD SCIENCE & TECHNOLOGY

Review

Qualified presumption of safety (QPS): a generic risk assessment approach for biological agents notified to the European Food Safety Authority (EFSA)

Renata G.K. Leuschner[a], Tobin P. Robinson[b], Marta Hugas[a,*], Pier Sandro Cocconcelli[c], Florence Richard-Forget[d], Günter Klein[e], Tine R. Licht[f], Christophe Nguyen-The[g], Amparo Querol[h], Malcolm Richardson[i], Juan E. Suarez[j], Ulf Thrane[k], Just M. Vlak[l] and Atte von Wright[m]

[a]Biological Hazards (BIOHAZ) Unit, European Food Safety Authority (EFSA), Largo N. Palli 5/A, I-43121 Parma, Italy (Tel.: +39 0521036216; fax: +39 05210360398; e-mail: marta.hugas@efsa.europa.eu)

[b]Emerging Risks (EMRISK) Unit, European Food Safety Authority (EFSA), Largo N. Palli 5/A, I-43121 Parma, Italy

[c]Istituto di Microbiologia, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy

[d]INRA, Institut National de la Recherche Agronomique, UR 1264, MycSA, 71 av Edouard Bourleaux BP 81, 33 883 Villenave d'Ornon cedex, France

[e]Institute for Food Quality and Food Safety, University of Veterinary Medicine, Bischhofsholer Damm 15, 30173 Hannover, Germany

[f]National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark

[g]INRA, Institut National de la Recherche Agronomique, UMR 408, Site Agroparc, 84914 Avignon cedex 9, France

[h]Departamento de Biotecnología, Instituto de Agroquímica y Tecnología de Alimentos (C.S.I.C.), Paterna P.O. Box 73, 46100 Burjasot (Valencia), Spain

[i]Regional Mycology Laboratory, Education and Research Centre, University Hospital of South Manchester, Southmoor Road, Manchester M23 9LT, UK

[j]Area of Microbiology, Faculty of Medicine, University of Oviedo, Julián Clavería st., 33006 Oviedo, Spain

[k]Center for Microbial Biotechnology, DTU Systems Biology, Technical University of Denmark, Søltofts Plads 221, DK-2800 Kgs. Lyngby, Denmark

[l]Laboratory of Virology, Department of Plant Sciences, Wageningen University, Droevendaalsesteeg 1, 6708 PB, The Netherlands

[m]University of Kuopio, Applied Biotechnology Unit, Department of Biosciences P.O. Box 1627, FI-70211 Kuopio, Finland

Qualified Presumption of Safety (QPS) is a generic risk assessment approach applied by the European Food Safety Authority (EFSA) to notified biological agents aiming at simplifying risk

* Corresponding author.

doi:10.1016/j.tifs.2010.07.003

assessments across different scientific Panels and Units. The aim of this review is to outline the implementation and value of the QPS assessment for EFSA and to explain its principles such as the unambiguous identity of a taxonomic unit, the body of knowledge including potential safety concerns and how these considerations lead to a list of biological agents recommended for QPS which EFSA keeps updated through an annual scientific review and assessment.

Introduction

Value of the QPS approach to European food safety authority (EFSA)

EFSA is responsible for risk assessments of a broad range of notified biological agents intended to be introduced into the food chain in the context of market authorisations. Qualified Presumption of Safety (QPS) was introduced as a generic risk assessment approach for harmonising the assessment of notified biological agents across different EFSA's Scientific Panels and Units. In addition, the QPS approach allows for a prioritisation of risk assessment resources proportionate to the potential risk. Following assessment by a dedicated standing EFSA Working Group, biological agents recommended for the QPS list would subsequently benefit from a simplified risk assessment by the Scientific Panel responsible for the respective notification.

QPS has been successfully applied *within* EFSA and is appreciated as a common sense approach by both assessors and applicants. Since its introduction at the end of 2007, EFSA's Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has been the principal user, and has increasingly benefitted from the QPS assessment when carrying out risk assessments in the context of authorisation request for additives for use in animal nutrition (OJEU, 2003). Since its introduction at the end of 2007 until end of 2008, the QPS approach has been applied by FEEDAP, in the assessment of four animal dossiers concerning *Saccharomyces cerevisiae*, *Lactobacillus rhamnosus*, *Lactobacillus farciminis* and *Bacillus amyloliquefaciens* (EFSA, 2008a–e). In 2009, a reduced risk assessment was applied to six out of fourteen published microbiological feed additive Opinions concerned with safety (EFSA, 2009a). Six applications requested safety assessments for products containing QPS recommended microorganisms belonging to *Pediococcus acidilactici*, *S. cerevisiae*, and *Bacillus subtilis* (EFSA, 2009b–g). A seventh dossier concerned a mixture of microorganisms of five QPS recommended taxonomic units (*Lactobacillus acidophilus*, *Lactobacillus helveticus*, *Lactobacillus bulgaricus*, *Lactobacillus lactis*, *Streptococcus thermophilus*) and *Enterococcus faecium* which is not recommended for QPS. As a consequence a full safety assessment was carried out (EFSA, 2009h).

QPS currently aids the consistency and transparency of risk assessments of biological agents notified to EFSA, and allows a more focused use of available resources for those agents with the greatest risks or uncertainties without compromising consumer safety.

Development of the QPS concept

The Qualified Presumption of Safety (QPS) being defined as 'an assumption based on reasonable evidence' and qualified to allow certain restrictions to apply, was suggested for the safety assessment of microorganisms used in food and feed production, by a Working Group consisting of members of the former Scientific Committee on Animal Nutrition (SCAN), Scientific Committee on Food (SCF) and the Scientific Committee on Plants (SCP) of the European Commission in 2002/2003 (European Commission, 2003). It was proposed that a safety assessment of defined taxonomic units would be made independently of any particular pre-market authorisation process. If the taxonomic unit raised no safety concerns or, if safety concerns existed, but could be defined and excluded *via* a qualification, the taxonomic unit would be recommended for the QPS list. A specific strain, notified in the context of market authorisation, whose identity could be unambiguously established and assigned to a QPS taxonomic unit, would subsequently be freed from the need for further safety assessment other than meeting any qualifications specified in its QPS assessment. Each qualification for a QPS recommended taxonomic unit would have to be assessed at strain level on a case-by-case basis. Microorganisms not considered suitable for QPS would remain subject to a full risk assessment, as would those failing a QPS qualification.

Views of stakeholders on the QPS proposal were sought by both the three Commission Scientific Committees in 2002/3 and by EFSA at a Scientific Colloquium (EFSA, 2005a). The main principles of the QPS assessment are the taxonomic level, the body of knowledge including history of use, scientific literature, clinical aspects, industrial applications and ecology and the identification of safety concerns which may be addressed as one or several qualifications. EFSA's Scientific Committee recommended to apply QPS to the risk assessment of microorganisms intended to be deliberately introduced into the food chain notified to the various EFSA Scientific Panels (EFSA, 2005b). Taxonomic units of microorganisms likely to be referred to EFSA were reviewed, and where appropriate, recommended for the QPS list (EFSA, 2007). Since 2008, the Biological Hazards (BIOHAZ) Panel reviews and publishes annual updates to the list of biological agents recommended for QPS (EFSA, 2008a, 2009a).

Perception of the QPS approach in the scientific literature

Since QPS has been adopted in EFSA as a tool to undertake risk assessment of biological agents (including microorganisms and recently also viruses used for plant protection purposes) notified to EFSA, several scientific publications have referred to it. These were discussed in detail in the EFSA Opinions (EFSA, 2008a, 2009a). Some of the reviewed publications reflect the QPS approach as it is adopted by EFSA and support its fundamental aspects (Plumed-Ferrer & von Wright, 2008; Sohier, Berthier, & Reitz, 2008; Wassenaar & Klein, 2008), while others outline that the application of some of its elements proved to be useful in the frame of safety assessments for applications outside the intended use of QPS such as for probiotics for human use and for undefined starter cultures (Fukao *et al*., 2009; Rossetti, Carminati, Zago, & Giraffa, 2009; Tompkins, Hagen, Wallace & Fillion-Forté, 2008). However, it should be noted that the list of QPS recommended biological agents should not be applied to uses outside of its assessment remit such as for biological agents proposed, for example, for direct human prophylactic or therapeutic treatments (Besselink *et al*., 2008; Sorokulova *et al*., 2008). Occasionally, reference is made to GRAS status and QPS as if they would be similar or equivalent, which is not the case (Chamba & Jamet, 2008; European Commission, 2003; Sanz-Penella, Tamayo-Ramos, Sanz, & Haros, 2009; Vankerckhoven *et al*., 2008).

QPS assessment of biological agents

Overview and methodology

The review of the list of biological agents recommended for QPS is carried out annually by EFSA's BIOHAZ Panel. The process consists of two activities. Firstly, the review of new information concerning taxonomic units already assessed through the QPS assessment, and secondly, the identification and assessment of taxonomic units that have not been previously considered. The determination of the appropriate level of taxonomic unit considered requires that the unit must be unambiguously defined, and that the number of qualifications necessary to accommodate all strains within that unit remains reasonable. In practical terms, this usually means QPS at the species level. With this in mind, each year notifications that were already assessed are reviewed taking into account the available body of knowledge in relation to the field of application an authorisation is sought for, and new notifications as received by EFSA are considered. A long history of use is not necessarily equal to safety and is subject to in depth consideration for a potential presence of safety concerns. Data and knowledge are usually shared within the scientific community- and web-supported databases of *e.g*. risk data and pathogen sequences have also revolutionized access to, and use of knowledge (Hogg, Couto, Teixeira, & Malcata, 2008). Identified safety concerns or gaps in the body of knowledge could be reflected as one or several 'qualifications' of a biological agent recommended for the QPS list as an alternative to an exclusion from it. The qualifications are equally subject to an annual review. While the QPS assessment concentrates on the characteristics of the biological agent, it is recognised that certain aspects related to safety are strongly influenced by the specific conditions of preparation, formulation and application of the final product. This is currently out of scope of the generic QPS assessment. An example would be the potential formation of biogenic amines. Neither safety of users handling the product nor genetic modifications are taken into account. These aspects are assessed, where applicable, separately by the EFSA Panel responsible for assessing the notification.

Gram-positive non-sporulating bacteria

Many genera and species of Gram-positive non-sporulating (GPNS) bacteria are normal inhabitants of the digestive tract of mammals and are commonly used in the preparation of foods and feed (Axelsson, 2004). There has been a long safe history of human exposure with only occasional reports of negative health effects, for example, through opportunistic infections. The following genera have been evaluated: *Bifidobacterium*, *Corynebacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Propionibacterium* and *Streptococcus*. The taxonomy of the genera, species and the possibility to unambiguously identify strains within these taxonomic units was described and evaluated in a previous EFSA Opinion (EFSA, 2007). Hence, this review focuses on the body of knowledge and potential safety concerns. The present list of taxonomic units recommended for QPS is presented in Table 1 (EFSA, 2009a). As is indicated, for GPNS bacteria a generic qualification applies to all taxonomic units on the list, that strains shall not carry any transferable antimicrobial resistance, unless viable cells are not present in the final product. This was initially recommended by EFSA's Scientific Committee and continues to be reviewed and maintained (EFSA, 2005b, 2007, 2008a, 2009a).

Bifidobacterium

Bifidobacteria are part of the normal gut microbiota of mammals. They are mainly exploited in dairy products. In Europe only a few species are used (*Bifidobacterium animalis*, *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium bifidum* and *Bifidobacterium adolescentis*) and are often applied in combination with lactic acid bacteria (Reuter, Klein, & Goldberg, 2002). None of the bifidobacteria used for industrial purposes or as food or feed supplements have been associated with human clinical disease (EFSA, 2007). Although there are few studies on the antibiotic resistance of bifidobacteria strains, presence of the acquired tetracycline resistance gene tet (W) has been reported in strains of *B. animalis* subsp. *lactis* and *B. bifidum* (Meile, Le Blay, & Thierry, 2008). Both species are recommended for the QPS list subject to a corresponding qualification.

Corynebacterium

Corynebacterium glutamicum is a soil bacterium and widely used for industrial biotechnical fermentations such as amino acid production. There are no known reports raising safety concerns for mammals, however very limited information regarding potentially acquired antibiotic resistance is available. Relating to its intended use, limited direct exposure of consumers to this species is expected

Table 1. Gram-positive non-sporulating (GPNS) bacteria recommended for the QPS list (EFSA, 2009a).

Species[a]
Bifidobacterium adolescentis Bifidobacterium animalis, Bifidobacterium bifidu,Bifidobacterium breve, Bifidobacterium longum
Corynebacterium glutamicum[b]
Lactobacillus acidophilus, Lactobacillus amylolyticus, Lactobacillus amylovorus, Lactobacillus alimentarius, Lactobacillus aviaries, Lactobacillus brevis, Lactobacillus buchneri, Lactobacillus casei[c]*, Lactobacillus cellobiosus, Lactobacillus coryniformis, Lactobacillus crispatus, Lactobacillus curvatus, Lactobacillus delbrueckii, Lactobacillus farciminis, Lactobacillus fermentum, Lactobacillus gallinarum, Lactobacillus gasseri, Lactobacillus helveticus, Lactobacillus hilgardii, Lactobacillus johnsonii, Lactobacillus kefiranofaciens, Lactobacillus kefiri, Lactobacillus mucosae, Lactobacillus panis, Lactobacillus collinoides, Lactobacillus paracasei, Lactobacillus paraplantarum, Lactobacillus pentosus, Lactobacillus plantarum, Lactobacillus pontis, Lactobacillus reuteri, Lactobacillus rhamnosus, Lactobacillus sakei, Lactobacillus salivarius, Lactobacillus sanfranciscensis*
Pediococcus acidilactici, Pediococcus dextrinicus, Pediococcus pentosaceus
Leuconostoc citreum, Leuconostoc lactis, Leuconostoc mesenteroides
Oenococcus oeni
Propionibacterium freudenreichii, Propiobacterium acidopropionici
Streptococcus thermophilus

[a] Generic qualification for all QPS bacterial taxonomic units: the strains should not harbour any acquired antimicrobial resistance genes to clinically relevant antibiotics.
[b] Qualification: QPS status applies only when the species is used for production purposes.
[c] The species '*Lacobacillus zeae*' has been included in the species *Lactobacillus casei.*

which was substantiated by a qualification 'usage for production purposes only' with a recommendation for the QPS list.

Enterococcus

Previous EFSA Opinions concluded that species of the *Enterococcus* genus could not be recommended for the QPS list (EFSA, 2005b, 2007, 2008a, 2009a). Some strains of *E. faecium* are authorised for use as feed additives. However, the risk assessment was carried out on a case-by-case basis because it is not possible to distinguish between virulent and non-virulent strains without resorting to the level of investigation used in a strain level risk assessment (Pimentel *et al*., 2007). Enteroccoci, in particular the multidrug-resistant strains, are amongst the leading causes of community- and hospital-acquired (nosocomial) infections (Woodford & Livermore, 2009). Infections often result from strains of *Enterococcus faecalis*, but increasingly from virulent strains within *E. faecium*, the species most commonly deliberately introduced into the food chain (Willems & van Schaik, 2009). A continuous review of increasing available information about the virulence determinants in enterococci has so far not resulted in a recommendation for the QPS list.

Lactobacillus

The characteristics and habitat of most *Lactobacillus* species are well-known. Some of the species of this genus have a long history of apparent safe use in industrial and agricultural applications. Members of the *Lactobacillus* genus are daily consumed in large quantities in a variety of fermented foods by people of all ages, ethnic groups and health status with apparently no ill-effects. Apart from their possible involvement in the development of dental caries, lactobacilli have generally been considered to be non-pathogenic. However, there is an increasing number of reports that they might occasionally be involved in human disease and many *Lactobacillus* species have been occasionally encountered in clinical specimens, the clinical significance of which was not always clear (EFSA, 2007). Certain strains of *L. rhamnosus* are occasionally isolated from severely immunocompromised patients, but also immunologically healthy individuals with a history of rheumatic endocarditis or heart valve replacement (EFSA, 2008a). As such, these infections can be considered opportunistic. The QPS recommendation for *L. rhamnosus* is subjected to a regular review.

Several examples of antibiotic resistant lactobacilli isolated from food or from the gut of animals exist. Acquired genes for antibiotic resistance have been detected in *Lactobacillus* species. Moreover, several of these genetic determinants in *Lactobacillus* are harboured by extrachromosomal elements (Mathur & Singh, 2005). The recent detailed studies on the antibiotic resistance profiles of lactic acid bacteria have demonstrated the occasional presence of acquired resistance genes in *Lactobacillus* species (Morelli, 2008). Moreover, several of these genetic determinants in *Lactobacillus* are harboured by extrachromosomal elements. Recent examples of the prevalence of acquired antibiotic resistance genes include the frequent occurrence of *tetM* and *ermB* genes (conveying resistance to tetracycline and erythromycin, respectively) in lactobacilli isolated from different fermented foods (Comunian *et al*., 2010; Zonenschain, Rebecchi, & Morelli, 2009). Obligate and facultative heterofermentative lactobacilli, and *Lactobacillus salivarius*, are intrinsically resistant to vancomycin and other glycopeptide antibiotics. Since this resistance is due to the lack of the target site for these antimicrobials in all members of these *Lactobacillus* subgroups, this type of resistance is poorly, if at all, transferable. However, transferable glycopeptides resistance elements, have been specifically demonstrated to be absent in some probiotic *Lactobacillus reuteri* and *L. rhamnosus* strains (Klein *et al*., 2000).

Two new *Lactobacillus* species, *Lactobacillus cellobiosus* and *Lactobacillus collinoides* were recently recommended for the QPS list. In both cases the body of knowledge was limited however *L. cellobiosus* is closely related to *Lactobacillus fermentum* and *Lactobacillus colloides* is naturally present in some foods and its recommendation is supported by the overall extensive body of knowledge for the genus *Lactobacillus* (EFSA, 2009a). In conclusion, most of the *Lactobacillus* species described to date can rightly be considered to be non-pathogenic to humans (Bernadeau, Vernoux, Henri-Dubernet, & Guéguen, 2008).

Pediococcus

Pediococci are consumed in large quantities in cheese and fermented sausages by people of all ages, ethnic groups and health status with apparently no ill-effects. Pediococci have generally been considered to be non-pathogenic. They are rarely isolated from clinical specimens (Heinz *et al*., 2000). Acquired genes for antibiotic resistance have been detected in the *Pediococcus* genus and determinants for tetracycline and erythromycin resistance have been found (Gevers, Danielsen, Huys, & Swings, 2003; Tankovic, Leclercq, & Duval, 1993). A bifunctional aminoglycoside-modifying enzyme was further described (Tenorio, Zarazaga, Martinez, & Torres, 2001). In summary, *Pediococcus acidilactici*, *Pediococcus dextrinicus* and *Pediococcus pentosaceus* are recommended for the QPS list based on the absence of specific safety concerns, whereby their susceptibility to antibiotics should be assessed (EFSA, 2007).

Lactococcus

The dairy species *Lactococcus lactis* is thoroughly described regarding its characteristics and habitat. It is extensively used as a starter for the production of cheese and fermented milks and consumed in large quantities in dairy products by consumers around the world. The relatively low growth temperature optima make even opportunistic infections unlikely. An occasional association with extremely rare individual cases of infections such as endocarditis, septicaemia, necrotising pneumonitis and liver abscess should not be regarded as an indication of human pathogenicity taking into account the extent of exposure to these microorganisms. Dairy lactococci are in general sensitive to most clinical antibiotics and therefore can antibiotic residues in milk cause starter failures. They are however known to contain plasmids and to exchange genetic material by intra- and intergeneric conjugation and therefore the potential for the spread of transferable antibiotic resistances exists (Morelli, Vogensen, & von Wright, 2004). The dairy species of *Lactococcus lactis* (*Lactococcus lactis* subsp. *lactis*, its biovariant *diacetylactis*, and *Lactococcus lactis* subsp. *cremoris*) have a long history as dairy starters and an excellent safety record. The occasional and extremely rare infections, in which these organisms have been associated, do not warrant specific safety concerns. Thus, these subspecies can be recommended for the QPS list (EFSA, 2007, 2008a, 2009a).

Leuconostoc and Oenococcus oeni

The genus *Leuconostoc* contains obligate heterofermentative lactic acid cocci and several species are recommended for the QPS list (EFSA, 2007). *Leuconostoc pseudomesenteroides* strains are found in fermentations of different foods of plant origin, although there is only a limited body of knowledge based on reports of application of *Leuconostoc pseudomesenteroides*. There are reports linking *L. pseudomesenteroides* to opportunistic infections in human clinical cases. Particularly, because of the currently limited body of knowledge, it was not recommended to include *L. pseudomesenteroides* on the QPS list unlike *Leuconostoc mesenteroides*, *Leuconostoc lactis* and *Leuconostoc citreum* and *Oenococcus oeni* (*Leuconostoc oenus*) which are included based on their long history of apparent safe use in food production and absence of safety concerns (EFSA, 2009a).

Propionibacteria

Propionic acid bacteria (PAB) can be divided into dairy species (DPAB) and mucocutaneous PAB. Regarding the mucocutaneous PAB, an association of some species (*Propionibacterium acnes*, *Propionibacterium propionicum*) with acne and other human infections excludes them from the QPS list (EFSA, 2007). DPAB are traditionally associated with certain types of cheese (Emmenthaler or Swiss cheese). *Propionibacterium freudenreichii* and its subspecies have been extensively intentionally used in cheese making. Due to the antifungal properties of propionic acid, this species has been included in certain protective cultures as well as in silage starters. The other DPAB, although commonly found in dairy products, have been considered as naturally occurring microorganisms with more limited associated safety data regarding human exposure. However, *Propionibacterium acidipropionici* is a well-known silage starter, particularly for cereal based silages and its engineered mutants have been proposed for industrial propionic acid production (Zhang & Yang, 2009). No human or animal infections associated with this bacterium have been reported. Thus, while *Propionibacterium freudenreichii* and *Propionibacterium acidipropionici* are recommended for the QPS list based on a history of apparent safe use and absence of safety concerns, the present gaps in the body of knowledge on other DPAB require more research on their safety aspects before this can be decided (EFSA, 2009a).

Streptococcus

Streptococcus thermophilus represents an exception in the genus *Streptococcus* being the only species of this genus to have a relevant role in food production. It is consumed in large quantities in dairy products and there are no safety concerns beside few reports of antibiotic resistance and detected acquired resistance genes in this species. Hence the species is recommended for the QPS list subject to the qualification of absence of antibiotic resistance (EFSA, 2007, 2008a, 2009a).

Gram-positive sporulating bacteria

The majority of Gram-positive sporulating bacteria (GPSB) currently notified to EFSA belong to the genus *Bacillus*, and the remaining few to closely related genera such as *Brevibacillus* and *Paenibacillus*. Recently a strain of *Clostridium butyricum* has been assessed as feed additive by EFSA. Specific members of the *Bacillus* genus are subject to safety concerns such as *Bacillus anthracis*, the cause of anthrax, and *Bacillus cereus*, which causes foodborne illness (EFSA, 2005c; Stenfors Arnesen, Fagerlund, & Granum, 2008). The assessment for a recommendation for the QPS list was consequently carried out on a species level at the appropriate taxonomic unit. For GPSB bacteria a similar generic qualification as for GPNS bacteria applies to all taxonomic units on the list, that strains shall not carry any transferable antimicrobial resistance, unless viable cells are not present in the final product. (EFSA, 2007, 2008a, 2009a).

For decades, strains belonging to several *Bacillus* species have been deliberately introduced into the food chain either as plant protection products or as animal feed supplements. There is an extensive body of knowledge of apparent safe use. Because the vast majority of strains belonging to the *Bacillus cereus* group are toxin producers, it was not considered for the QPS list. In contrast, foodborne intoxication caused by *Bacillus* species other than *Bacillus cereus* is uncommon, but does occur. *Bacillus* strains associated with foodborne illness produce either toxic peptides which cause emetic disease, and/or enterotoxins causing diarrhea. Production of enterotoxins and toxic peptides represent a qualification in the QPS approach which thereby excludes strains of *Bacillus* affiliated with safety concerns. In spite of their diversity, potentially emetic peptides from *Bacillus* species can be rapidly detected due to their ability to inhibit sperm motility (EFSA, 2007, 2008a; Häggblom, Apetroaie, Andersson, & Salkinoja-Salonen, 2002). Likewise, enterotoxic potential can be revealed by PCR or cytotoxicity in cell-lines. *Bacillus* species with a sufficient body of knowledge and history of apparent safe use in the feed or food chain were therefore included in the QPS list, subject to a qualification of 'absence of toxigenic potential' (Table 2).

The species *Paenibacillus macerans* previously notified for b-cyclodextrin production as food additive was not recommended for the QPS list despite a large body of knowledge for enzyme production and usage as food additive. Since even though safety concerns to mammals are very rare, the body of knowledge concerning the presence of this species in the food chain and its safety implications are limited (EFSA, 2009a).

Gram-negative bacteria

The species *Escherichia coli*, *Serratia rubidae*, *Pseudomonas chlororaphis* and *Rhodospeudomonas palustris* were following an assessment not recommended for the QPS list, mainly because of safety concerns posed by representative strains of these species to human health (EFSA, 2009a).

Table 2. Gram-positive sporulating bacteria (GPSB) recommended for the QPS list (EFSA, 2009a).

Species	Qualification[a]
Bacillus amyloliquefaciens, Bacillus atrophaeus, Bacillus clausii, Bacillus coagulans, Bacillus fusiformis, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus mojavensis, Bacillus pumilus, Bacillus subtilis, Bacillus vallismortis, Geobacillus stearothermophilus	Absence of toxigenic potential

[a] Generic qualification for all QPS bacterial taxonomic units: the strains should not harbour any acquired antimicrobial resistance genes to clinically relevant antibiotics.

In the case of *Escherichia coli*, despite a long history of use of several strains as probiotics for human use such as *E. coli* Nissle 1917 and an extensive body of knowledge concerning this species, there are indications of a large diversity of diseases in mammals caused by representatives of this species and of the presence of complex virulence mechanisms (Kaper, Nataro, & Mobley, 2004; Schultz, 2008).

Serratia rhubidaea has, despite its use as antifungal agent for preserving animal feedingstuffs, a limited body of knowledge of use in the food chain and some representatives of the species have been associated with human infections.

Pseudomonas chlororaphis strains used for plant protection purposes were assessed by the Environmental Protection Agency in the United States and the Standing Committee on the Food Chain and Animal Health Scientific Committee on Plant in Europe as posing no health concerns for humans (Anonymous, 2001, 2002, 2004; OJEU, 2004). Nevertheless, there remains the potential for the production of secondary metabolites described for representatives of this species, in particular because the conditions under which this may occur are not defined (EFSA, 2009a).

Rhodopseudomonas palustris has a comprehensive body of knowledge for use as biomass feed in aquaculture. However, this is only one aspect of the whole food chain and while there appears to be no safety concerns reported to humans the overall information was not considered as sufficient.

Yeasts

Notifications for yeasts were received for use as feed additives and as plant protection products. Currently, the yeast species *Debaryomyces hansenii*, *Hanseniaspora uvarum*, *Kluyveromyces lactis* and *Kluyveromyces marxianus*, *Saccharomyces bayanus*, *S. cerevisiae*, *Saccharomyces pastorianus*, *Schizosaccharomyces pombe*, *Xanthophyllomyces dendrorhous*, and for enzyme production purposes only, *Pichia angusta*, *Pichia anomala*, *Pichia jardinii* and *Pichia pastoris* are recommended for the QPS list (EFSA, 2009a).

The importance of yeast in food production, in particular the bakers yeast *Saccharomyces cerevisiae* was realized in the last century and an extensive body of knowledge and history of apparent safe use has build-up since. Compared with other microbial groups, yeasts are not seen as aggressive pathogens, but they are capable of causing human disease in opportunistic circumstances. *Candida albicans* and *Cryptococcus neoformans* are well-known in this regard, and are responsible for causing a range of mucocutaneous, cutaneous, respiratory, central nervous and systemic infections. However, some species included in the QPS list are associated with opportunistic infections and also a subtype of *Saccharomyces cerevisiae*, commonly referred to as *Saccharomyces boulardii* was previously highlighted in this context (EFSA, 2007, 2008a). Of some concern were mainly species of *Rhodotorula*, *Pichia anomala* (formerly *Hansenula anomala*), *Issatchenkia orientalis* (anamorph *Candida krusei*) and *Kluyveromyces marxianus* (formerly *Kluyveromyces fragilis*). Recently, few cases were also attributed through re-identification by molecular techniques to *Debaryomyces hansenii* (Hazen, 1995; Murphy, & Kavanagh, 1999). However, the knowledge concerning these infections is currently limited and further work is required to better characterize the virulence factors in *S. cerevisiae* clinical strains and in other currently recommended QPS yeast species where isolates have been described in human infections *e.g. Pichia anomala*, *Kluyveromyces marxianus* and *Debaryomyces hansenii* (EFSA, 2008a).

Another important point is the potential resistance to antifungals of QPS recommended yeast species, which was carefully considered in the recent review of the list of QPS recommended biological agents (EFSA, 2009a). The rapid development of drug resistance has prompted the search for new broad-spectrum antifungal agents that are minimally toxic and unlikely to result in the development of resistance. The increasing threat of fungal infections, mostly caused by yeasts not recommended for the QPS list, has stimulated the search for better antifungals with a distinct mode of action. Resistance to antifungal agents is not transmissible among yeasts and therefore solely the presence of resistance as a 'direct hazard' is to be considered. However, little information is available about resistance to antifungals of yeast species recommended for the QPS list, with *in vitro* data on antifungal resistance only being available for *Saccharomyces cerevisiae* (Barchiese *et al*., 2003; Zerza, Hollis, & Pfaller, 1996). It was nevertheless concluded that a qualification with regards to an absence of resistance to therapeutical antimycotics for yeast species recommended for the QPS list was justified (EFSA, 2009a).

Filamentous fungi

The risk assessment of notified filamentous fungi intended to be deliberately added to food production systems is one of the objectives of the QPS approach developed by EFSA. In 2007, EFSA concluded that filamentous fungi were not to be recommended for the QPS list due to the lack of knowledge concerning the potential risk of production of toxic metabolites (EFSA, 2007). In 2009, the general body of knowledge on filamentous fungi has been updated, considering in particular the progress and limitations in the taxonomy, in the identification of the production of toxic compounds and the increased knowledge of metabolic pathways. New issues were considered, such as the resistance of fungi to therapeutic antifungal agents and the risks linked to the use of fungi as plant protection products. The body of knowledge was also specifically updated for each species and genus addressed in the 2007 Opinion and new species were investigated for which safety assessment was required in the meantime (EFSA, 2009a).

In line with the 2007 conclusion, the 2009 EFSA Opinion confirmed that filamentous fungi would not be recommended for the QPS list. The rationale for this is that the methods for identification of fungal cultures to genus/species level remain very difficult and need in depth mycological expertise, even though molecular diagnostics are under development. There is an ongoing debate on species concepts amongst mycologists, which results in a lack of a universally accepted fungal taxonomy. Moreover, the body of knowledge concerning production of toxic compounds needs to be clarified, as far too little is known about the factors controlling their production. We can however reasonably assume that the recent availability of fungal genomic data will allow tremendous progress in the near future (Stadler & Keller, 2008).

In most cases, the information concerning a lack or occurrence of toxic compounds under production conditions is not available. In addition, there are only few validated and certified analytical methods for the detection of a limited number of mycotoxins. Lastly, the body of knowledge concerning the toxicology of fungal secondary metabolites is insufficient. In general, mycotoxins, i.e. fungal secondary metabolites that in small concentrations are toxic to vertebrates when introduced *via* a natural route (ingestion, inhalation and skin penetration), have a non-acute effect which complicates the assessment of their toxicological potential in real cases. A long history of use is not equal to safety, as many fungal metabolites are known to affect the immune system, which could lead to secondary infections. Moreover, the toxicological knowledge is of little or no relevance to real life situations, *e.g.* lack of information on synergistic effects. In conclusion, all notified filamentous fungal species and strains should be evaluated on a case-by-case basis when subjected to a risk assessment.

Bacteriophages

Notifications were obtained previously for two bacteriophages infecting *Clostridium sporogenes* and *Clostridium tyrobutyricum*. EFSA recently provided technical assistance to the Directorate General on Health and Consumer Protection of the European Commission on the mode of action and persistence of phages when added to foods of animal origin, whether they acted as processing aids or as food additives. The main conclusion was that it depended on the particular food-matrix/phage combination, so that a general answer could not be provided (EFSA, 2009i). In this context, the possibility to recommend bacteriophages for the QPS list was considered in depth (EFSA, 2009a). Phages lyse their bacterial hosts, thus having a potential as biocontrol agents. They are innocuous and do not alter the sensory properties of foods.

However, phages cannot be recommended for the QPS list because their taxonomy is not settled. Furthermore, temperate phages integrate into the genomes of their hosts, mediating the acquisition of properties such as virulence and spoilage determinants. Finally, those that package their genomes through the headful mechanism may transduce bacterial genes, worsening the problem. Consequently, only virulent phages that package DNA by recognition of cohesive ends are to be used as food decontaminants. The only safe way to ascertain whether a phage is virulent, does not carry harmful genes and presents cohesive ends is genome sequencing, complemented with experiments to exclude the generation of lysogens and confirm the presence of sticky ends. The risk assessment of phages has therefore to be carried out on a case-by-case basis.

Viruses used for plant protection

Notifications have been received by the EFSA's PRAPeR Unit regarding viruses used for plant protection purposes including strains of baculoviruses and a weak strain of a zucchini yellow mosaic virus (ZYMV, Potyviridae). A QPS assessment of these notifications was subsequently carried out (EFSA, 2009a).

The above viruses can be unambiguously identified. Baculoviruses (containing double-stranded DNA) can be unequivocally characterized by sequencing three to five highly conserved core genes (polyhedrin, DNA polymerase, lef-8, lef-9 or pif-2) using universal primer sets and polymerase chain reaction (PCR) procedures (Herniou & Jehle, 2007; Van Oers & Vlak, 2007). ZYMV (containing single-stranded RNA of positive polarity) can be identified on the basis of sequencing nucleotide polymorphisms in isolates (Pfosser & Baumann, 2002). The sequences of baculoviruses and ZYMV can be compared with existing data bases and phylogenetic trees. The highest taxonomic units applied in the given context of the notifications were the family Baculoviridae and for the zucchini yellow mosaic virus the family Potyviridae.

Baculoviruses are lethally pathogenic for insects (Lepidopterans, Dipterans and Hymenopterans) and have been used as insect biocontrol agents for more than a hundred years. They were extensively tested for potential adverse health effects on mammalians in the 1960s and 1970s. However no detrimental effects were identified due to, among others, the host specificity of these viruses, limited to often one or a few related insect host species (Anonymous, 2006; Burges, Croizier, & Huber, 1980; EPA, 1996, pp6; Gröner, 1986). Mammalians as well as many other vertebrates have been naturally exposed to baculoviruses for as long as the latter existed without apparent negative health effect.

Weak strains of zucchini yellow mosaic viruses (ZYMV) are applied for plant protection to 'immunise' the plant against infection with a virulent strain of this virus by applying the principle of cross-protection, used as strategy for about fifty years to control plant viruses (Fulton, 1986; Lecoq, 1998; Lecoq *et al*., 2009; Lecoq, Lemaire, & Wipf-Scheibel, 1991; McKinney, 1929). Weak strains of ZYMV generate RNA and/or protein signals in the plants that prevent subsequent severe variants of ZYMV when entering a plant to replicate and cause disease. Negative effects on mammalians attributed to plant viruses have never been found or reported despite continuous exposure to plant viruses through food and feed (Zhang *et al*., 2006).

The body of knowledge for both virus families (Baculoviridae and Potyviridae) was extensive in the field of application for which the notification was sought for. Safety concerns have been addressed but none were reported valid despite extensive testing. Therefore Baculoviridae and Potyviridae were recommended for inclusion on the QPS list (EFSA, 2009a).

Future developments/applications

The QPS list is increasingly applied by EFSA's FEEDAP Panel and it is expected that it can be applied more widely across EFSA in the future. The 2009 QPS update recommended for the first time, viruses used for plant protection (Baculoviridae and Potyviridae) to be included on the QPS list (EFSA, 2009a). This is of relevance for EFSA's Pesticide Risk Assessment Peer Review (PRAPeR) Unit responsible for the EU peer review of active substances used in plant protection products in line with procedures and deadlines set out in the European legislation (OJEU, 1991). As a consequence of recent regulatory initiatives adopted by the European Parliament and Council concerning a common procedure for evaluation and authorisation of food additives and enzymes the QPS risk assessment approach may gain increasing importance for the corresponding Scientific EFSA Panels for Food Additives and Nutrient Sources added to food (ANS) and for Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) (OJEU, 2008).

Conclusions

While QPS is already successfully applied within EFSA for harmonising and prioritising risk assessment of biological agents, it has the potential to be applied in an increasingly wider context in the future. Furthermore, QPS may also foster economic development as commercial parties can be more proactive in the development of their future product portfolio once a notified biological agent was recommended for an inclusion on the QPS list.

References

Anonymous. (2001). *Pseudomonas chlororaphis* strain 63-28 environmental protection agency (EPA), United States, (006478) Fact

Sheet, issued 4/01. (http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_006478.htm).

Anonymous (2002). Opinion of the scientific committee on plants on specific questions from commission regarding the evaluation of *Pseudomonas chlororaphis* in the context of council directive 91/414/EEC. Scientific Committee on Plants. http://ec.europa.eu/food/fs/sc/scp/outcome_ppp_en.html. CSP/PSEUDOM/002-Final.

Anonymous. (2004). Commission working document – Review report for the active substance *Pseudomonas chlororaphis*. Finalised in the standing committee on the food chain and animal health at its meeting on 30 March 2004 in view of the inclusion of *Pseudomonas chlororaphis* in Annex I of Directive 91/414/EEC. *Pseudomonas chlororaphis* 4204/VI/98-Final, 29 March 2004.

Anonymous. (2006). *Safety Assessment of Transgenic Organisms: OECD Consensus Documents,* Vol. 2. Organisation for Economic Co-operation and Development (OECD), Paris, France, pp. 238–311.

Axelsson, L. T. (2004). Lactic acid bacteria: classification and physiology. In S. Salminen, A. Ouwehand, & A. von Wright (Eds.), *Lactic acid bacteria. Microbiological and Functional aspects* (pp. 1–66). New York: Marcel Dekker Inc..

Barchiese, F., Caggiano, G., Maracci, M., Arzeni, D., Scalise, G., & Montagna, M. T. (2003). Antifungal susceptibility patterns of yeast isolates causing bloodstream infections. *Journal of Antimicrobial Chemotherapy*, *51*, 431–433.

Bernadeau, M., Vernoux, J. P., Henri-Dubernet, S., & Guéguen, M. (2008). Safety assessment of dairy microorganisms: *lactobacillus* genus. *International Journal of Food Microbiology*, *126*(3), 278–285.

Besselink, M. G. H., van Santvoort, H. C., Buskens, E., Boermeester, M. A., Van Goor, H., Timmerman, H. M., et al. (2008). Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. *The Lancet*, *371*, 651–659.

Burges, H. D., Croizier, G., & Huber, J. (1980). A review of safety tests on baculoviruses. *BioControl*, *25*, 329–339.

Chamba, J. F., & Jamet, E. (2008). Contribution to the safety assessment of technological microflora found in fermented dairy products. *International Journal of Food Microbiology*, *126*(3), 263–266.

Comunian, R., Daga, E., Dupré, I., Paba, A., Devirgiliis, C., Piccioni, V., et al. (2010). Susceptibility to tetracycline and erythromycin of *Lactobacillus paracasei* strains isolated from traditional Italian fermented foods. *International Journal of Food Microbiology*, *138*(1–2), 151–156.

EFSA. (2005a). *QPS – Qualified presumption of safety of microorganisms in food and feed*. EFSA Scientific Colloquium, 13–14 December 2005, Brussels, Belgium, Summary Report, EFSA, Pama, Italy.

EFSA (2005b). Opinion of the scientific committee on a request from EFSA related to a generic approach to the safety assessment by EFSA of microorganisms used in food/feed and the production of food/feed additives. *The EFSA Journal*, *226*, 1–12.

EFSA (2005c). Opinion of the scientific panel on biological hazards on *Bacillus cereus* and other *Bacillus* spp in foodstuff. *The EFSA Journal*, *175*, 1–48.

EFSA (2007). Opinion of the scientific committee on introduction of a qualified presumption of safety (QPS) approach for assessment of selected microorganisms referred to EFSA. *The EFSA Journal*, *587*, 1–16.

EFSA (2008a). Scientific opinion of the panel on biological hazards on the maintenance of the list of QPS microorganisms intentionally added to food or feed. *The EFSA Journal*, *923*, 1–48.

EFSA (2008b). Scientific opinion of the scientific panel on additives and products or substances used in animal feed on safety and efficacy of Levucell SC20/Levucell SC10ME, a preparation of *Saccharomyces cerevisiae*, as feed additive for lambs for fattening. *The EFSA Journal*, *772*, 1–11.

EFSA (2008c). Scientific opinion of the scientific panel on additives and products or substances used in animal feed on safety and efficacy of Biosaf Sc47 (*Saccharomyces cerevisiae*) as feed additive for dairy buffaloes. *The EFSA Journal*, *837*, 1–10.

EFSA (2008d). Scientific opinion of the scientific panel on additives and products or substances used in animal feed on the safety and efficacy of the product Sorbiflore, a preparation of *Lactobacillus rhamnosus* and *Lactobacillus farciminis*, as feed additives for piglets. *The EFSA Journal*, *771*, 1–13.

EFSA (2008e). Scientific opinion of the scientific panel on additives and products or substances used in animal feed on the safety and efficacy of Ecobiol® (*Bacillus amyloliquefaciens*), as feed additive for chickens for fattening. *The EFSA Journal*, *773*, 1–13.

EFSA (2009a). Scientific opinion of the panel on biological hazards on the maintenance of the list of QPS microorganisms intentionally added to food or feed. *EFSA Journal*, *7*(12), 1–93.

EFSA (2009b). Scientific opinion of the scientific panel on additives and products or substances used in animal feed on safety and efficacy of Bactocell PA (Pediococcus acidilactici) as feed additive for fish. Summary of Opinion. [http://www.efsa.europa.eu/en/scdocs/scdoc/1037.htm].

EFSA (2009c). Scientific opinion of the scientific panel on additives and products or substances used in animal feed on safety and efficacy of Bactocell PA (*Pediococcus acidilactici*) as feed additive for shrimps. Summary of Opinion. [http://www.efsa.europa.eu/en/scdocs/scdoc/1038.htm].

EFSA (2009d). Scientific opinion of the scientific panel on additives and products or substances used in animal feed (FEEDAP) on the safety and efficacy of MycoCell (*Saccharomyces cerevisiae*) for dairy cows. *EFSA Journal*, *7*(10), 1–12.

EFSA (2009e). Scientific opinion of the scientific panel on additives and products or substances used in animal feed on the safety and efficacy of Yea-Sacc1026® (*Saccharomyces cerevisiae*), as a feed additives for horses. *The EFSA Journal*, *991*, 1–14.

EFSA (2009f). Scientific opinion of the scientific panel on additives and products or substances used in animal feed on the safety and efficacy of Biosprint® (*Saccharomyces cerevisiae*), as a feed additives for sows. *The EFSA Journal*, *970*, 1–9.

EFSA (2009g). Scientific opinion of the scientific panel on additives and products or substances used in animal feed (FEEDAP) on the safety and efficacy of *Bacillus subtilis* PB6 as a feed additive for chickens for fattening. *EFSA Journal*, *7*(9), 1–14.

EFSA (2009h). Scientific opinion of the scientific panel on additives and products or substances used in animal feed (FEEDAP) on the safety and efficacy of Probiotic LACTINA® (*Lactobacillus acidophilus*, *Lactobacillus helveticus*, *Lactobacillus bulgaricus*, *Lactobacillus lactis*, *Streptococcus thermophilus*, *Enterococcus faecium*) for chickens for fattening, piglets and pigs. *The EFSA Journal*, *912*, 1–13.

EFSA (2009i). Scientific opinion of the panel on biological hazards on the use and mode of action of bacteriophages in food production. *The EFSA Journal*, *1079*, 1–26.

Environmental Protection Agency (EPA) (1996). Microbial pesticide test guidelines: acute oral toxicity/pathogenicity. *Office of Prevention, Pesticides and Toxic Substances*, *885*, 3050, (p.6).

European Commission (2003). *A working paper open for comment on a generic approach to the safety assessment of microorganisms used in feed/food and feed/food production*. http://ec.europa.eu/food/fs/sc/scf/out178_en.pdf

Fukao, M., Tomita, H., Yakabe, T., Nomura, T., Ike, Y., & Yajima, N. (2009). Assessment of antibiotic resistance in probiotic strain *Lactobacillus brevis* KB290. *Journal of Food Protection*, *72*(9), 1923–1929.

Fulton, R. F. (1986). Practices and precautions in the use of cross protection for plant virus disease control. *Annual Review of Phytopathology*, *24*, 67–81.

Gevers, D., Danielsen, M., Huys, G., & Swings, J. (2003). Molecular characterization of tet(M) genes in *Lactobacillus* isolates from different types of fermented dry sausage. *Applied Environmental Microbiology*, *69*(2), 1270–1275.

Gröner, A. (1986). Specificity and safety of baculoviruses. In R. R. Granados, & B. A. Federici (Eds.), *Biology of baculoviruses, vol. 1* (pp. 177–202). Boca Raton, Florida: CRC Press.

Häggblom, M. M., Apetroaie, C., Andersson, M. A., & Salkinoja-Salonen, M. S. (2002). Quantitative analysis of cereulide, the emetic toxin of Bacillus cereus, produced under various conditions. *Applied Environmental Microbiology*, *68*(5), 2479–2483.

Hazen, K. C. (1995). New and emerging yeast pathogens. *Clinical Microbiology Reviews*, *8*(4), 462–478.

Heinz, M., von Wintzingerode, F., Moter, A., Halle, E., Lohbrunner, H., Kaisers, U., et al. (2000). A case of septicemia with Pediococcus acidilactici after long-term antibiotic treatment. *European Journal of Clinical Microbiology and Infectious Diseases*, *19*(12), 946–948.

Herniou, E. A., & Jehle, J. A. (2007). Baculovirus phylogeny and evolution. *Current Drug Targets*, *8*(10), 1043–1050.

Hogg, T. A., Couto, J. A., Teixeira, P., & Malcata, F. X. (2008). Building on scientific excellence via sharing of scientific expertise – the case study of food safety. *Trends in Food Science and Technology*, *19*, S9–S13.

Kaper, J. B., Nataro, J. P., & Mobley, H. L. T. (2004). Pathogenic *Escherichia coli*. *Nature Reviews Microbiology*, *2*, 123–140.

Klein, G., Hallmann, C., Casas, I. A., Abad, J., Louwers, J., & Reuter, G. (2000). Exclusion of vanA, vanB and vanC type glycopeptide resistance in strains of *Lactobacillus reuteri* and *Lactobacillus rhamnosus* used as probiotics by polymerase chain reaction and hybridization methods. *Journal of Applied Microbiology*, *89* (5), 815–824.

Lecoq, H. (1998). Control of plant virus diseases by cross protection. In A. Hadidi, R. K. Khetarpal, & H. Koganezawa (Eds.), *Plant virus disease control* (pp. 33–40). St Paul, MN, USA: APS Press.

Lecoq, H., Lemaire, J. M., & Wipf-Scheibel, C. (1991). Control of zucchini yellow mosaic virus in squash by cross protection. *Plant Disease*, *75*, 208–211.

Lecoq, H., Wipf-Scheibel, C., Chandeysson, C., Le Van, A., Fabre, F., & Desbiez, C. (2009). Molecular epidemiology of Zucchini yellow mosaic virus in France: an historical overview. *Virus Research*, *141* (2), 190–200.

Mathur, S., & Singh, R. (2005). Antibiotic resistance in food lactic acid bacteria – a review. *International Journal of Food Microbiology*, *105*, 281–295.

McKinney, H. H. (1929). Mosaic diseases in the Canary Islands, West Africa and Gibraltar. *Journal of Agricultural Research*, *39*, 557–578.

Meile, L., Le Blay, G., & Thierry, A. (2008). Safety assessment of dairy microorganisms: Propionibacterium and Bifidobacterium. *International Journal of Food Microbiology*, *126*(3), 316–320.

Morelli, L. (2008). Introducing the ACE-ART project: from the expression of interest to 42 months of research. *International Journal of Probiotics and Prebiotics*, *3*(4), 241–246.

Morelli, L., Vogensen, F., & von Wright, A. (2004). Genetics of lactic acid bacteria. In S. Salminen, A. von Wright, & A. Ouwehand (Eds.), *Lactic acid bacteria. Microbiological and Functional aspects* (pp. 249–293). New York: Marcel Dekker Inc..

Murphy, A., & Kavanagh, K. (1999). Emergence of *Saccharomyces cereviasiae* as a human pathogen – mycological aspects. Implications for biotechnology. *Enzyme and Microbial Technology*, *25*(7), 551–557.

OJEU (1991). Council Directive 91/414/EEC concerning the placing of plant protection products on the market. *Official Journal of the European Union L*, *230*, 1–32, 19.8.1991.

OJEU (2003). Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on additives for use in animal nutrition. *Official Journal of the European Union L*, *268*, 29–43.

OJEU (2004). Commission directive 2004/71/EC amending council directive 91/414/EEC to include *Pseudomonas chlororaphis* as active substance. *Official Journal of the European Union L*, *127*, 104–106.

OJEU (2008). Regulation (EC) No 1331/2008 of the European Parliament and of the council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavorings. *Official Journal of the European Union L*, *354*, 1–6.

Pfosser, M. F., & Baumann, H. (2002). Phylogeny and geographical differentiation of zucchini yellow mosaic virus isolates (*Potyviridae*) based on molecular analysis of the coat protein and part of the cytoplasmic inclusion protein genes. *Archives of Virology*, *147*, 1599–1609.

Pimentel, L. L., Semedo, T., Tenreiro, R., Crespo, M. T. B., Pintado, M. M. E., & Malcata, F. X. (2007). Assessment of safety of enterococci isolated throughout traditional Terrincho cheesemaking: virulence factors and antibiotic susceptibility. *Journal of Food Protection*, *70*(9), 2161–2167.

Plumed-Ferrer, C., & von Wright, A. (2008). Fermented pig liquid feed: nutritional, safety and regulatory aspects. *Journal of Applied Microbiology*, *106*(2), 351–368.

Reuter, G., Klein, G., & Goldberg, M. (2002). Identification of probiotic cultures in food samples. *Food Research International*, *35*(2), 117–124.

Rossetti, L., Carminati, D., Zago, M., & Giraffa, G. (2009). A qualified presumption of safety approach for the safety assessment of Grana Padano whey starters. *International Journal of Food Microbiology*, *130*(1), 70–73.

Sanz-Penella, J. M., Tamayo-Ramos, J. A., Sanz, Y., & Haros, M. (2009). Phytate reduction in bran-enriched bread by phytase-producing bacteria. *Journal of Agriculture and Food Chemistry*, *57* (21), 10239–10244.

Schultz, M. (2008). Clinical use of *E. coli* Nissle 1917 in inflammatory bowel disease. *Inflammatory Bowel Diseases*, *14*(7), 1012–1018.

Sohier, D., Berthier, F., & Reitz, J. (2008). Safety assessment of dairy microorganisms: bacterial taxonomy. *International Journal of Food Microbiology*, *126*(3), 267–270.

Sorokulova, I. B., Pinchuk, I. V., Denayrolles, M., Osipova, I. G., Huang, J. M., Cutting, S. M., et al. (2008). The safety of two *Bacillus* probiotic strains for human use. *Digestive Diseases and Sciences*, *53*, 954–963.

Stadler, M., & Keller, N. (2008). Paradigm shifts in fungal secondary metabolite research. *Mycological Research*, *112*, 47–50.

Stenfors Arnesen, L. P., Fagerlund, A., & Granum, P. E. (2008). From soil to gut: *Bacillus cereus* and its food poisoning toxins. *FEMS Microbiology Reviews*, *32*(4), 579–606.

Tankovic, J., Leclercq, R., & Duval, J. (1993). Antimicrobial susceptibility of *Pediococcus* spp. and genetic basis of macrolide resistance in *Pediococcus acidilactici* HM3020. *Antimicrobial Agents and Chemotherapy*, *37*(4), 789–792.

Tenorio, C., Zarazaga, M., Martinez, C., & Torres, C. (2001). Bifunctional enzyme 6′-N-aminoglycoside acetyltransferase-2"-O- aminoglycoside phosphotransferase in *Lactobacillus* and *Pediococcus* isolates of animal origin. *Journal of Clinical Microbiology*, *39*, 824–825.

Tompkins, T. A., Hagen, K. E., Wallace, T. D., & Fillion-Forté, V. (2008). Safety evaluation of two bacterial strains used in Asian probiotic products. *Canadian Journal of Microbiology*, *54*(5), 391–400.

Van Oers, M. M., & Vlak, J. M. (2007). Baculovirus genomics. *Current Drug Targets*, *8*(10), 1051–1068.

Vankerckhoven, V., Huys, G., Vancanneyt, M., Vael, C., Klare, I., Romond, M.-B., et al. (2008). Biosafety assessment of probiotics used for human consumption: recommendations from the EU-PROSAFE project. *Trends in Food Science & Technology*, *19*(2), 102–114.

Wassenaar, T. M., & Klein, G. (2008). Safety aspects and implications of regulation of probiotic bacteria in food and feed supplements. *Journal of Food Protection*, *71*(8), 1734–1741.

Willems, R. J., & van Schaik, W. (2009). Transition of *Enterococcus faecium* from commensal organism to nosocomial pathogen. *Future Microbiology*, *4*(9), 1125−1135.

Woodford, N., & Livermore, D. M. (2009). Infections caused by Gram-positive bacteria: a review of the global challenge. *Journal of Infection*, *59*(Supplement 1), S4−S16.

Zerza, L., Hollis, R. J., & Pfaller, M. A. (1996). In vitro susceptibility testing and DNA typing of Saccharomyces cerevisiae clinical isolates. *Journal of Clinical Microbiology*, *34*(12), 3031−3034.

Zhang, A., & Yang, S.-T. (2009). Engineering *Propionibacterium acidipropionici* for enhanced propionic acid tolerance and fermentation. *Biotechnology and Bioengeering*, *104*(4), 766−773.

Zhang, T., Breitbart, M., Lee, W. H., Run, J. Q., Wei, C. L., Soh, S. W. L., et al. (2006). RNA viral community in human feces: prevalence of plant pathogenic viruses. *PloS Biology*, *4*(1), e3.

Zonenschain, D., Rebecchi, A., & Morelli, L. (2009). Erythromycin- and tetracycline-resistant lactobacilli in Italian fermented dry sausage. *Journal of Applied Microbiology*, *107*(5), 1559−1568.