SCIENTIFIC OPINION

Scientific Opinion on the maintenance of the list of QPS microorganisms intentionally added to food or feed (2009 update)[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2, 3]

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ABSTRACT

EFSA is requested to assess the safety of a broad range of biological agents in the context of notifications for market authorisation as sources of food and feed additives, enzymes and plant protection products. The qualified presumption of safety (QPS) concept was developed by EFSA for its own use to provide a generic food safety assessment approach applicable across EFSA's scientific Panels, for biological agents notified for intentional use in the whole food chain. The safety of unambiguously defined biological agents at the highest taxonomic unit that is appropriate for the purpose for which an application is intended are assessed, considering if the body of knowledge is sufficient. Identified safety concerns for a taxonomic unit could be reflected as 'qualifications' when considered appropriate for an inclusion on the QPS list. The list of QPS recommended biological agents is reviewed and updated annually. The 2009 update reviews the previously assessed microorganisms including bacteria, yeasts and filamentous fungi and assesses several additional notifications concerning gram-positive and gram-negative bacteria and yeasts. *Lactobacillus cellobiosus*, *L. collinoides*, *Propionibacterium acidopropionici* and *Oenococcus oeni* were included in the QPS list. No filamentous fungi were included because of potential production of toxic metabolites. For the first time viruses were assed. Insect viruses (*Baculovirideae*) and in the case of zucchini yellow mosaic viruses the *Potyvirideae* family as the highest possible taxonomic unit were added to the QPS list. Bacteriophages were considered as not appropriate for the QPS list. A potential presence of antimycotic resistance of yeasts referred to on the QPS list was considered. It was concluded that yeast strains resistant to antimycotics used for treatment of infections in humans might be of public health concern.

KEY WORDS

Qualified presumption of safety, QPS, food, feed

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SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on biological hazards (BIOHAZ) to deliver a scientific Opinion on the maintenance of the list of QPS microorganisms intentionally added to food or feed (2009 update).

The Opinion reviews the previous assessments of microorganisms in the context of a proposal for a qualified presumption of safety (QPS). The previous list of QPS microorganisms that was published in 2008 was reviewed and confirmed. An assessment of already notified and additional notifications resulted this year in an inclusion of two new *Lactobacillus* species, *Lactobacillus cellobiosus* and *L. collinoides*, *Oenococcus oeni* (*Leuconostoc oenus*) and *Propionibacterium acidopropionici* on the QPS list.

The information on filamentous fungi that was published in a previous EFSA Opinion was reviewed and updated this year. In line with the previous conclusions it was confirmed that filamentous fungi would not be included on the QPS list because of their potential to produce toxic metabolites. They will have to be assessed on a case by case basis.

Bacteriophages which were notified to EFSA were considered in this Opinion. It was concluded that bacteriophages cannot be included on the QPS list. The reason for this conclusion is that each phage should be assessed on a case-by-case basis for the nucleic acid sequence to demonstrate the impossibility of a lysogenic cycle and the absence of any potential virulence factors and/or antimicrobial resistance genes. Their genome packaging mechanism shall be assessed to minimise the risk of potential transduction of bacterial genes.

Viruses used for plant protection are assessed for the first time in this Opinion. The highest taxonomic units applied in the given context of notifications were *Baculoviridae* and *Potyviridae*. Both families were included on the QPS list.

Resistance of yeasts to antimycotics used in medical treatments was considered and while limited knowledge is available it was concluded that this aspect would justify a qualification with regards to an absence of resistance to therapeutic antimycotics for yeast species included on the QPS list.

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BACKGROUND AS PROVIDED BY EFSA

A wide variety of bacterial and fungal species are used in food and feed production, either directly or as a source of additives or food enzymes. Some of these have a long history of apparent safe use, while others are less well understood and may represent a risk for consumers. Experience has shown that there is a need for a tool for setting priorities within the risk assessment of those microorganisms used in the production of food/feed which are captured by present legislation and consequently the subject of a formal safety assessment.

In 2002/3 a working group consisting of members of the former Scientific Committees on Animal Nutrition, Food and Plants of the European Commission proposed the introduction for selected microorganisms of a Qualified Presumption of Safety (QPS)[4].

In April 2003, responsibility for the safety assessments of food/feed undertaken by the Scientific Committees of the Commission formally passed to the European Food Safety Authority (EFSA). Shortly after EFSA asked its own Scientific Committee to consider whether the approach to safety assessment of microorganisms proposed in the QPS document could be used to harmonise approaches to the safety assessment of microorganisms across the various EFSA scientific panels.

The Scientific Committee concluded that QPS as a concept could provide a generic approval system *for use within EFSA* that could be applied to all requests received for the safety assessments of microorganisms deliberately introduced into the food chain[5]. The benefits of the introduction of QPS would be a more transparent and consistent approach across the EFSA panels and the potential to make better use of resources by focussing on those organisms which presented the greatest risks or uncertainties.

On the basis of these conclusions the Scientific Committee recommended that EFSA should develop a strategy for the introduction of an assessment system based on the QPS concept. This should be limited to microorganisms introduced into the food chain or used as producer strains for food/feed additives until the robustness and value of such a system could be tested in practice.

EFSA accepted the recommendation of its Scientific Committee and proposed that the Committee should continue its assessment of the QPS system with a view to implementation[6]. Specifically, the Scientific Committee was asked first to establish that were the most commonly encountered microorganisms in notifications received by EFSA, including those used as a source of microbial products. Then, on the basis of this survey, to select relevant groups of microorganisms, examine the available data on safety and propose whether QPS status would be appropriate. If this proved possible in a significant number of cases then the Scientific Committee should consider how implementation of QPS across the various panels could be achieved.

The Scientific Committee reviewed the range and numbers of microorganisms likely to be the subject of an EFSA Opinion[7]. They found that a large majority of these species were found to fall within four broad groupings: i) Gram-positive non-sporulating bacteria; ii) *Bacillus* species, iii) yeasts and iv) filamentous fungi. Accordingly, bacteria, yeasts and fungi falling within these four groups were selected for an initial assessment of their suitability for the QPS list, and the resulting list of microorganisms recommended for QPS was published[7].

In reaching its conclusion on the value of QPS as an assessment tool, the Scientific Committee recognised that there would have to be continuing provision for reviewing and modifying the list of organism given QPS status. They recommended that the EFSA via its Science Department should take prime responsibility for this and should review the suitability for QPS status of the existing list and any additions at least annually. Reviews may occur more frequently as necessary but there should be a

4 See http://ec.europa.eu/food/fs/sc/scf/out178_en.pdf
5 See www.efsa.europa.eu/en/science/sc_commitee/sc_opinions/972.html
6 See www.efsa.europa.eu/en/science/sc_commitee/sc_documents/1368.html
7 See www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178667590178.htm

formal requirement that even when no changes are proposed, a statement should be made annually that QPS status is being maintained for the published list.

The Scientific Committee recommended[5] that a QPS system for microorganisms should be introduced and that it should be implemented across EFSA and applied equally to all safety considerations of microorganisms intentionally added to the food chain that EFSA is required to assess.

The Biological Hazards Panel was identified as being the most appropriate to take up the task of carrying out an annual review of the QPS list. In the first annual review[8], the existing list of QPS microorganisms was reviewed and EFSA's initial experience in applying the QPS approach was described. In addition, following the identification of antimicrobial resistance as a universal qualification of safety in the previous Opinion on QPS, the issue was addressed in line with the Opinion developed by the BIOHAZ Panel[9] on 'Foodborne antimicrobial resistance as a biological hazard', and related Opinions[10] and guidance documents[11] of other EFSA Panels. The potential application of the QPS approach to microbial plant protection products was also discussed.

TERMS OF REFERENCE AS PROVIDED BY EFSA

EFSA requests the BIOHAZ Panel to:

1. Carry out an annual review of the list of QPS status microorganisms. Where appropriate new taxonomic groups should be assessed for their suitability for inclusion on the QPS list, and taxonomic groups previously assessed should be reviewed where new information has become available. The review should include an update of the list of microbial species notified to EFSA, which should be a starting point for identifying new taxonomic groups for review under the QPS system. Only those taxonomic groups relevant to current legal requirements for notification to EFSA for feed/food use (principally as sources of food and feed additives, food enzymes and plant protection products) shall be considered.
2. Consider the introduction of a qualification for yeast regarding resistance to antimycotics.
3. Consider the application of QPS to viruses (used in plant protection) and bacteriophage (for example used as decontamination agents).
4. Update the information on mycelial fungi.

8 Opinion of the Scientific Panel on Biological Hazards on a request from EFSA on the maintenance of the list of QPS microorganisms intentionally added to food or feed. The EFSA Journal (2008) 923, 1-48.

9 Opinion of the Scientific Panel on Biological Hazards on a request from EFSA on foodborne antimicrobial resistance as a biological hazard. The EFSA Journal (2008) 765, 1-87.

10 Technical guidance prepared by the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on the update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance. The EFSA Journal (2008) 732, 1-15.

11 Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Microorganisms and their Derived Products Intended for Food and Feed Use. The EFSA Journal (2006) 374, 1-115. www.efsa.europa.eu/EFSA/Scientific_Document/comm_Guidance%20doc_GMM_en,0.pdf

ASSESSMENT

1. INTRODUCTION

A wide variety of microorganisms are intentionally added at different stages into the food chain, either directly or as a source of additives or enzymes. In this context, approximately 100 species of microorganisms have been expected to be referred to EFSA for a safety assessment. The majority are the result of notifications for market authorisation as sources of food and feed additives, food enzymes and plant protection products received by EFSA.

The purpose of the present Opinion is to review the list of previously Qualified Presumption of Safety (QPS) granted miroorganisms that was last established in 2008 (EFSA, 2008a). The QPS approach was developed by the Scientific Committee to provide a generic concept to prioritise and to harmonise risk assessment of microorganisms intentionally introduced into the food chain within EFSA in support of the respective Scientific Panels and Units in the frame of authorisations (EFSA, 2007a). The list, first established in 2007 (EFSA, 2007a) is to be reviewed annually.

In the QPS concept a safety assessment of a defined taxonomic unit is considered independently of any particular specific notification in the course of an authorisation process. If the taxonomic unit does not raise any safety concerns, or if existing safety concerns can be clearly defined as specific qualifications to ensure their absence (exclusion) in the context of a specific notification, a particular taxonomic unit could be recommended for the QPS list. Subsequently, any specific representative of a QPS proposed taxonomic unit, would not need to undergo a further safety assessment other than to satisfy any of the qualifications specified if applicable. Representatives of groups that fail to satisfy a qualification would be considered unfit for the QPS list and would remain subject to a full safety assessment, in the frame of a notification within the responsible EFSA Scientific Panel (EFSA, 2007a).

The QPS concept does not address hazards linked to the formulation or processing of the biological agents added into the food or feed chain.

Concerning microorganisms discussed in previous Opinions, the continuously evolving body of knowledge possibly reveals new information that could lead to a modification of the list of QPS recommended taxonomic units, for example to an ex- or inclusion of taxonomic units on the list. An assessment of taxonomic units, not previously considered for the QPS list, and for which representatives are notified to EFSA is also discussed. These include, beside microorganisms, viruses used in the context of plant protection and bacteriophages. Consequently, the QPS 2009 update will consider for the first time taxonomic units of biological agents other than microorganisms. Biological agents intended for usages outside the remit of EFSA, and biological agents which have not been notified to EFSA, are not considered in this Opinion.

In 2008 antimicrobial resistance was introduced as a possible safety concern for the assessment of the inclusion of bacterial species in the QPS list (EFSA, 2008a). In the present Opinion, the possibility to consider antimycotic resistance for yeast is reviewed and discussed.

In accordance with the recommendation by the Scientific Committee that the QPS concept should be implemented within EFSA where relevant, an impact assessment of the QPS system by EFSA Units in the frame of authorisations and its quotation in the scientific literature is provided.

1.1. Experience of using the QPS approach and reference to it in the scientific literature

The QPS approach has proved to be a useful tool to harmonise and prioritise safety assessment within EFSA and is appreciated by both assessors and applicants. It has been applied by EFSA's Panel on Additives and Products of Substances used in Animal Feed (FEEDAP) in 2009, in the assessment of

six dossiers out off a total of 14 dossier dealing with microorganisms feed additives (EFSA, 2008c-f; 2009a;b).

Since the publication of the EFSA 2008 Opinion (EFSA, 2008a) where references to the QPS approach in the scientific literature were discussed several additional publications make reference to the concept (Fukao et al., 2009; Plumb-Ferrer and von Wright, 2009; Rossetti et al., 2009; Sanz-Penella, 2009).

Plume-Ferrer and von Wright (2009) referred to QPS in the context of animal feeds, which is within EFSA remit. It highlights that the QPS approach reduces the need of extensive safety testing of microorganisms with a history of safe use, provided that certain qualifications are met and refers to the EFSA 2008 Opinion (EFSA, 2008a).

Rosetti et al. (2009) make an attempt to apply the QPS concept to dominant lactic acid bacteria in undefined starters associated with Grana Padano cheese way starters. The authors develop a QPS reasoning based on some characterisation and safety assessment of undefined starter cultures and conclude on a potential application of the QPS approach to other types of undefined-strain cultures such as natural milk starter or commercial mixed-strain cultures. Although used outside its normal context, the QPS approach proved useful for Rossetti et al. (2009) to assess the safety of *Lactobacillus* used as cheeses starters. As all strains were identified to species included in the QPS list, the authors only verified that they followed the qualification on antibiotic resistance.

In a recent study the QPS approach was applied to determine the resistance of the probiotic strain *Lactobacillus brevis* KB290. The authors concluded from their investigation that the antibiotic resistance observed in *L. brevis* KB290 was due not to a potentially acquired mechanisms but to intrinsic resistance. It was concluded that according to the QPS criteria, these results provided safety assurance for the ongoing use of *L. brevis* KB290 as a probiotic (Fukao et al., 2009).

Sanz-Penella et al. (2009) refer in the abstract of their article to QPS and GRAS as if they were equivalent concepts which is not the case. It has to be noted that the QPS approach is solely seen as an internal tool apllied within EFSA to harmnise risk assessment and to use resources effectively with a focus on areas that need attention concerning public health.

2. REVIEW OF THE LIST OF QPS ASSESSED MICROORGANISMS

2.1. Gram-positive non-sporulating bacteria

2.1.1. *Lactobacillus* species already included in the QPS list

In 2008, EFSA concluded that although they can be a rare cause of human infections, all the *Lactobacillus* species previously assessed suitable for the QPS could remain in the QPS list (EFSA, 2008a). However, EFSA stressed that these human infections should remain a topic for surveillance.

Since publication of the last Opinion on QPS (EFSA, 2008a) no scientific reports on clinical infections by *Lactobacillus* spp. have been published[12].

2.1.2. New *Lactobacillus species and Leuconostoc* species notified to EFSA

Some strains belonging to *Lactobacillus* and *Leuconostoc* species, not assessed for the list of QPS taxonomic units in the previous Opinions, have since been notified to EFSA, with the purpose of being used as feed additives.

12 According to a Pubmed search (*Lactobacillus* & infection; *Lactobacillus* & pathogen)

2.1.2.1. *Lactobacillus cellobiosus*

L. cellobiosus is considered very closely related to *L. fermentum*, both phenotypically as well as due to DNA homology (Dellaglio et al., 2004). However, it remains until today as a separate species. The body of knowledge attributed to *L. fermentum* therefore can be considered valid also for *L. cellobiosus*. Specific applications in food production include regional fermented food specialities, e.g. from Africa (Mugula et al., 2003). No specific references of *L. cellobiosus* as the causative agent of clinical human cases are reported. Despite a limited body of knowledge of this species and limited technological application, based on the phenotypic and genotypic similarity to *L. fermentum* it can be included in the QPS list.

2.1.2.2. *Lactobacillus collinoides*

The species *L. collinoides* is known since 1972 (Carr and Davies, 1972) and was isolated in fermenting apple juice. Only recently isolated as a component of natural microbiota of olive fermentation (Chamkh et al., 2008), but several reports refer also to spoilage of food, e.g. Fuji et al. (2005). No specific references of *L. collinoides* as the causative agent of clinical human cases were found. However, the production of biogenic amines was shown for strains of *L. collinoides* with the conclusion, that the production is rather strain specific than species specific (Garai et al., 2007). While there is limited knowledge of intentional use for this species in the food chain, it is naturally present in some foods. In addition the overall body of knowledge for the genus *Lactobacillus* is taken into consideration and it is proposed for the QPS list.

2.1.2.3. *Oenococcus oeni (Leuconostoc oenus)*

Oenococcus oeni is an important organism in the fermentation of wine (Dicks et al., 1995). As for *L. collinoides* the production of biogenic amines was shown for strains of *Oenococcus oeni* with the conclusion, that the production is rather strain specific than species specific (Garai et al., 2007) and in the case of *Oenococcus oeni*, can be lost after subcultivation under laboratory conditions (Lucas et al., 2008). *Oenococcus oeni* has a long history of safe use in food production and is therefore recommended for the QPS list.

2.1.2.4. *Leuconostoc pseudomesenteroides*

L. pseudomesenteroides strains are found in fermentations of different food of plant origin, e.g. in cocoa (Nielsen et al., 2007). There is only a limited body of knowledge based on reports of application of *L. pseudomesenteroides*. However there are reports linking *L. pseudomesenteroides* to opportunistic infections in human clinical cases (Rodriguez et al., 1999), (Capelli et al., 1999). Especially because of the limited body of knowledge, at the moment it is not recommended to include *L. pseudomesenteroides* on the QPS list.

2.1.3. Enterococci

In previous Opinions EFSA concluded that species belonging to the *Enterococcus* genus could not be included in the QPS list (EFSA, 2007a; 2008a). However, some strains of *Enterococcus* species are used as feed additives and are notified to EFSA. Because research on this genus is very active and could provide new information for safety assessment, it was decided that the body of knowledge on *Enterococcus* should be reviewed annually.

Taxonomic unit

Enterococcus faecium recently evolved from a generally avirulent commensal into a multidrug-resistant bacterium associated with nosocomial infections. Most of these isolates belong to specific

lineages, the ST78, ST18 and ST17. Hospital-adapted clones display a higher pathogenic potential than endogenous *E. faecium* strains (Willems and Schaik, 2009). *Enteroccocus gallinarum* is an uncommon enterococcal species and has been associated to a nosocomial outbreaks (Contreras et al., 2008)

Body of knowledge

The status of *Enterococcus* genus for QPS purpose was reviewed in 2008, reaching the conclusion that a strain specific evaluation is necessary to assess the risk associated with the intentional use of enterococci in the food chain.

A new bibliographic survey has been performed, to determine if any new scientific information could be used to define specific qualifications aimed to differentiate virulent from safe strains.

The survey in Pubmed using *Enterococcus* retrieved 993 publications in the last 12 months, most of them related to the pathogenicity and antimicrobial resistance of this microbial group. A search combining '*Enterococcus* and virulence' retrieved 93 records in the same period, reflecting the increase body of knowledge on enteroccocal virulence.

Virulence factors

The role of aggregation substance Asc10, encoded by sex pheromone plasmids, in increasing the virulence of *Enterococcus faecalis* in experimental pathogenesis models, including infectious endocarditis models, has been demonstrated (Chuang et al. 2009).

Experimental infection models (rat endocarditis model) and studies with isogenic mutants, demonstrated the *E. faecium* collagen adhesin AcmA is relevant for *E. faecium* pathogenesis (Nallapareddy et al. 2008a). The majority of strains harbouring this virulence factor are part of the CC17 (Nallapareddy et al. 2008b).

The *perA* gene encoding a putative AraC-type transcriptional regulator was identified on the pathogenicity island (PAI) found among virulent *Enterococcus faecalis* strains. Coburn et al. (2008) demonstrated PerA regulates determinants important to pathogenesis, such as biofilm formation and survival within macrophages.

Two pilus-like structures, designated PilA and PilB, have been identified on the surface of a hospital-adapted *Enterococcus faecium* bloodstream isolate. The gene cluster coding for these structures is widely diffused in hospital-aquired *E. faecium* isolates, suggesting that pili may contribute to *E. faecium* pathogenesis (Hendrickx et al. 2008)

The enterococcal surface protein Esp, identified as a potential virulence factor, is specifically linked to nosocomial clonal lineages. Heikens et al. (2009) demonstrated that Esp is not essential for Caco-2 cell adherence and intestinal colonization or translocation of *E. faecium* in mice.

A gene termed hyaluronidase (hyl Efm) was identified as a potential virulence gene, found to be more abundant in clinical isolates (Rice et al., 2003).

Conclusions on the QPS status

Despite of the increase infromation on *E. faecium* lineage involved in pathogenesis and on the role of virulence factors, there is not sufficiently known to allow a precise prediction of pathogenicity on the basis of the presence of an individual gene or gene product.

In summary, the worldwide increasing importance of enterococci as a cause of nosocomial infections makes the safety assessment of enterococcal strains a difficult task. While a more profound understanding of the mechanisms of pathogenicity and the emergence of novel techniques to characterize the strains might eventually change the situation, the need of a strain specific evaluation will remain. Thus there are no grounds to include enterococci on the QPS list.

2.1.4. Dairy propionic acid bacteria other than *Propionibacterium freudenreichii*

Of the dairy propionic acid bacteria (DPAB; *Propionibacterium acidopropionici, P. australiense, P. cyclohexanicum, P. freudenreichii* subsp. *freudenreichii*, *P. freudenreichii* subsp. *shermanii*, *P. jensenii, P. thoenii* and *P. microaerophilum*) only *P. freudenreichii* and its subspecies are included in the present QPS list. This bacterium has been extensively intentionally used in cheese making, and consequently the body of knowledge regarding its safe history of use was considered sufficient for the QPS status

Strains from other species of *Propionibacterium* have been notified to EFSA for applications in feed production.

The other DPAB, although commonly found in dairy products, have been considered as naturally occurring micro-organisms with more limited associated safety data regarding the human exposure. However, *P. acidipropionici* is a well known silage starter, particularly for cereal based silages (Filya et al., 2004, Bolsen et al., 1996) and its engineered mutants have been proposed for industrial propionic acid production (Suwannakham et al., 2005, Zhang and Yang, 2009). No human or animal infections associated with this bacterium have been reported.

Recently certain pigmented variants of *P. jensenii* have been shown to have very similar haemolytic properties as a known but totally unrelated pathogen, *Streptococcus agalactiae* (Vanberg et al., 2007). While apparently no cases of infections caused by *P. jensenii* have been reported, the presence of a potential virulence factor warrants certain prudence before making conclusions of the safety of the species.

Thus, while *P. acidipropionici* has a history of safe use and can be considered for QPS together with *P. freudenreichii*, the present gaps in the body of knowledge on other DPAB require more research on their safety aspects before this can be decided.

2.2. Gram-positive sporulating bacteria

2.2.1. *Gram-positive sporulating bacteria already on the QPS list*

Several species of *Bacillus* are on the QPS list, with a qualification concerning the absence of food poisoning toxins and enterotoxic activities. In 2008, EFSA outlined: "the possibility that new virulence factors, not detected by the qualification proposed, could be discovered should be kept under attention" (EFSA, 2008a). In addition, *Bacillus* spp. also cause rare local or systemic infections. In the present Opinion, these two topics are considered.

No new toxins which could be involved in foodborne poisoning have been identified since the previous maintenance of the QPS list (EFSA, 2008a)[13]. These toxins from *Bacillus* spp. are peptides and lipopeptides. Since the previous maintenance of the QPS list, according to a search in the Web of Knowledge[14], the toxicity of surfactin C, produced by some strains of *B. subtilis,* was assessed on a rat model (Hwang et al., 2009). The role of these lipopeptides in the biocontrol of plant diseases by

13 According to a search in the Web of Knowledge: *Bacillus* and Toxin; excluding *Bacillus cereus*, *Bacillus thuringiensis* and *Bacillus anthracis*

14 *Bacillus* and lipopeptid or *Bacillus* and peptid

Bacillus spp, was recently reviewed (Ongena and Jacques, 2008), thus confirming previous EFSA Opinions (EFSA 2007a AppendixB; 2008a).

One publication described a strain of *Bacillus pumilus* isolated from soil, carrying the gene for synthesis of cereulide, the emetic toxin of *B. cereus* (Parvathi et al., 2009). Even if cereulide production in some strains of *B. pumilus* were confirmed, it would be detected by the qualification proposed for the *Bacillus* species of the QPS list and *B. pumilus* would remain in the QPS list.

A search in the Web of Knowledge[15] retrieved the description of one case of bloodstream infection due to *Bacillus pumilus* (Farhat et al., 2008). The mode of infection was not identified but food was not suspected. The patient suffered from cancer and was immunodepressed after a recent chemotherapy. This report is in line with the conclusions of the previous EFSA Opinions (EFSA 2007a; 2008a) on the existence of rare infections by QPS *Bacillus* spp, presumably not linked with the presence of the bacteria in the food or feed chain and mostly concerning patients with severe underlying diseases.

In conclusion, the little information published since the previous EFSA Opinion on the QPS list (EFSA, 2008a) does not indicate that any modification of the QPS list for *Bacillus* species is needed.

2.2.2. New Gram-positive sporulating bacteria assessed for the QPS list

2.2.2.1. *Paenibacillus macerans*

In its previous Opinion, EFSA (2007a AppendixB) did not include *Paenibacillus* species in the QPS list. The use of *Paenibacillus macerans* to produce a food additive was approved by the Scientific Committee for Foods in 2000 (Anonymous, 2000). Whether this species could be included in the QPS list is assessed in the present Opinion.

Taxonomic unit

Paenibacillus macerans is a Gram-positive rod shaped bacterium producing endospores. Before 1994 *Paenibacillus macerans* was part of the genus *Bacillus* and named *Bacillus macerans* (Anonymous, 2009a; Claus and Berkeley, 1986). Guinebretière et al. (2001) found that strains isolated from foods and identified with phenotypic methods to *P. macerans* or *B. macerans* actually belonged to other *Paenibacillus* spp. Therefore, there could be some uncertainties on the identity of strains which were only identified by phenotypic methods.

Body of knowledge[16]

P. macerans was mostly studied for its ability to degrade plant biomass and for the production of enzymes used in carbohydrate biotechnology. In particular its cycloglycosyltransferase is used to produce Béta-cyclodextrines, which are food additives (Anonymous, 2004a). Production of other carbohydrates, *e.g.* fructooligosaccharides proposed as prebiotics (Maiorano et al. 2008) is also described. *P. macerans* has also been investigated as a potential plant growth promoter (Li et al., 2008a) and inhibitor of plant pathogens (Li et al., 2007).

A few works described its presence in some foods, such as in raw milk (Uraz et al., 2001), during the processing and ripening of some cheeses (Roman-Blanco et al., 1999), or during cheese spoilage (Quiberoni et al. 2008), although it was not described as a major component of the bacterial

[15] *Bacillus* and disease or infection; excluding *Bacillus cereus*, *Bacillus thuringiensis* and *Bacillus anthracis*

[16] Search on ISI WOS, *Bacillus macerans* or *Paenibacillus macerans* as Topic, gave 195 references since 1992. All were screened. Most concerned enzymes active on carbohydrates.

population of these foods. *P. macerans* is also described in the microflora of traditional fermented foods in Brasil (Almeida et al., 2007), Nigeria and in Asia (Isu and Njoku, 1997; Wang and Fung, 1996). It has also been described in silage of poor quality (Rossi and Dellaglio, 2007).

Safety concerns

Farhat et al. (2008) described a fatal case of bacteremia caused by a strain of *P. macerans,* following a lung infection. The patient was immunodepressed after a recent chemotherapy. A brain abscess due to *B. macerans* was also reported (Bert et al., 1995), following the intracranial penetration of a foreign body. In cheese, *P. macerans* was identified as an important producer of histamine (Rodriguez-Jerez et al., 1994).

Conclusions

P. macerans has an important history of use for enzyme production and processing of food additives. Reports of safety concerns to human or animal are very rare, but the body of knowledge on the presence of the bacterium in the food or feed chain is limited. In its evaluation of *P. macerans* for the production of cyclodextrines, the SCF (Anonymous, 2000) also noted that information on the toxicity of *P. macerans* is very limited, and gave a positive assessment only considering that the 'purification steps included in the production processes, constitute a dilution factor of several orders of magnitude for any *Bacillus* toxins possibly elaborated by the producer organism'. Therefore, the history of safe use of *P. macerans* for cyclodextrin production presumably reflects more the quality of the purification steps than the safety of the bacteria. It is therefore recommended to not include *P. macerans* on the QPS list.

2.3. Yeast

Several yeasts species are already present in the QPS list, and several have been assessed in the previous Opinions (EFSA, 2007a AppendixC; EFSA, 2008a) as being not suitable for the QPS list. With regards to the previously assessed yeast species, a current literature review has not revealed new information that would result in a change of the previously concluded species proposed for the QPS list. Two yeast species, not previously assessed for QPS, have been notified to EFSA as plant protection products and are considered in the present Opinion.

2.3.1. *Aureobasidium pullulans*

Taxonomic unit

The genus *Aureobasidium* comprises 14 species; among these, *Aureobasidium pullulans* is the only well-known species. Whether *Aureobasidium* species are filamentous fungi or yeasts (is also known as a black yeast) has been discussed however they are now included in the yeast taxonomic book (Kurztman and Fell, 2000). They are subsequently presented in the yeast section of this Opinion.

Body of knowledge

This species is ubiquitous and found mainly in soil, including Antarctic soils, water, the phylloplane, wood, and many other plant materials, rocks, monuments, and limestone.

It is not associated with any food processes and is commonly considered as a contaminant (Kurztman and Fell, 2000).

A. pullulans it is biotechnologically important yeast that can be used in different fields. Different strains of *A. pullulans* can produce amylase, proteinase, lipase, cellulose, xylanase, mannanase, transferase, pullulan and the genes encoding proteinase, lipase, cellulose and xylanase have been cloned and characterized (Chi et al., 2003; Leathers, 2003).

Safety concerns

A. pullulans is one of the causative agents of phaehyphomycosis. It may cause keratomycosis, pulmonary mycosis with sepsis and other opportunistic infections, as well as cutaneous mycoses such as eumycotic dermatitis. Was isolated in patients with peritonitis and may also colonize hair, skin, and nails in humans. The infections caused by *Aureobasidium* remain limited and are rare (Wilson et al., 2000; Bolignano and Criseo, 2003).

Conclusion

The body of knowledge is not sufficient to recommend *A. pullulans* for the QPS list.

2.3.2. *Pseudozyma flocculosa*

The genus *Pseudozyma* represents ustilaginomycetous anamorphic yeasts and *Pseudozyma flocculosa* is mainly isolated from plants. This species is known to produce an antifungal glycolipid and has been formulated into a fungicide (Konstantinidou-Doltsinis et al., 2007). However, fatty acids with antibiotic (antifungal) activity have also been reported (Avis et al., 2000). In addition, a few other *Pseudozyma* species have been isolated from human blood in Asia.

Considering the capacity of this species to produce biological active compounds it is concluded to not recommend *Pseudozyma flocculosa* for the QPS list.

2.4. Filamentous fungi

In 2007 EFSA concluded that filamentous fungi could not be included in the QPS list due to the potential risk of production of toxic metabolites (EFSA, 2007a). The body of knowledge on filamentous fungi was not reviewed in the 2008 EFSA Opinion (EFSA, 2008a). However, a large range of filamentous fungi have been notified for the purpose of plant protection, which was not considered in the previous assessments (EFSA, 2007a AppendixD).

Therefore, the general body of knowledge on filamentous fungi has been updated in the present Opinion, considering in particular the progress and limitation in the taxonomy, in the knowledge of metabolic pathways and in the identification of the production of toxic compounds. 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 for each species or genus considered in the previous Opinion (EFSA, 2007a Appendix D) was updated. In addition, fungal species, not considered in the previous Opinion and notified as plant protection product or feed additive, were assessed if they would qualify for the QPS list.

Due to the extensive amount of sections which needed updating in the previous Opinion, and in order to present a consistent document on filamentous fungi, the detailed assessment is presented in Appendix C of this Opinion, following the same format as the 2007 EFSA Opinion (EFSA, 2007a Appendix D).

Conclusion on the assessment detailed in Appendix C of this Opinion

No filamentous fungi can be proposed for inclusion on the QPS list. The rationale for this is that the methods for identification of fungal cultures to genus/species level are very difficult and often need in depth mycological expertise. There is an ongoing debate on species concepts in the mycological society which result in a lack of a universally accepted fungal taxonomy. This makes identification of fungal cultures intended for commercial use a difficult issue and often the result should be verified by one or more independent specialists. For the time being there is no universally accepted method for fungal identification.

The body of knowledge concerning production of toxic compounds is insufficient, as far too little is known about the factors controlling the production of these compounds. 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 makes it very difficult to assess their toxicological potential in real cases. In several cases it has been demonstrated that toxic compounds can be produced under production conditions, but often this information is not available. In addition, there are only few validated and certified analytical methods for the detection of a limited number of mycotoxins. For the majority of fungal secondary metabolites no validated method exists.

The body of knowledge concerning the toxicology of fungal secondary metabolites is insufficient. Bioassays are developed to address specific needs and are not validated. Often the toxicological knowledge is of little or no relevance to real life situations, *e.g.* lack of information on synergistic effects. The 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. Also the knowledge on long-term effects is insufficient. In conclusion, all notified fungal species and strains should be evaluated on a case-by-case basis.

2.5. Assessment of the QPS status for new taxonomic groups and specific issues

2.5.1. Gram-negative bacteria

Gram-negative bacteria have never been considered by EFSA for QPS. Some species of Gram-negative bacteria have been notified as feed additives or plant protection products, and are assessed in the present Opinion.

2.5.1.1. *Escherichia coli*

Taxonomic unit

Escherichia coli is a Gram-negative, rod-shaped bacterium, belonging to the family *Enterobacteriaceae*. It is very closely related to *Shigella* spp.

Body of knowledge

E. coli has been the model of choice for investigations into the physiology and genetics of Gram-negative bacteria. The genome of several strains of *E. coli* has been sequenced (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi). It is also extensively used in biotechnology and in laboratory experiments as a recipient for extra-chromosomal elements such as virulence and antimicrobial resistance determinants

E. coli is a common facultative anaerobe species in the gut of humans and other warm blood animals (McClure, 2005), where most organisms exist as harmless symbionts (Yan and Polk, 2004).

Several strains have been used as probiotics for humans and animals, some with a long history of safe use, such as *E. coli* Nissle 1917. For instance, in clinical trials on humans with probiotic *E. coli* strains, the duration of accute diarrhoea in infants was reduced (Henker et al., 2007), and permitted remission of ulcerative colitis (Kruis et al., 2004, Adler, 2006, Fitzpatrick et al., 2008). In all these trials, some with duration of up to 12 month and daily oral administration of high number of living *E. coli* (e.g. 10^8 cells per day), no safety problem was recorded. Probiotic *E. coli* are also used to treat diarrhoea in farm animals (Schroeder et al., 2006). An *E. coli* strains has also been tested to prevent urinary tract infections in patients at risk (Wiles et al., 2008). The full genome sequence of *E.coli* Nissle 1917 has been recently published (Grozdanov et al., 2004; Sun et al., 2005).

Safety concerns

E. coli is also the cause of a wide range of human and animal diseases. At least four types of diseases in humans caused by *E. coli* are predominantly foodborne (McClure, 2005, Kaper et al., 2004), and should be considered when assessed the safety of *E. coli* introduced in the food and feed chain. In these diseases, the site of infection is the gastro-intestinal tract (GI). The corresponding causative agents have been classified as: Entero-invasive *E. coli* (EIEC); Entero-toxic *E. coli* (ETEC), Entero-pathogenic *E. coli* (EPEC) and Entero-Haemorragic *E. coli* (EHEC). The EHEC group encompasses organisms such as Shiga-toxin producing *E. coli* / Verotoxin-producing *E. coli* (STEC/VTEC). Details of the symptoms and pathogenicity mechanisms of the above types can be found in Kaper et al. (2004). Some of these *E. coli* can also be carried and/or cause diseases in animals (McClure, 2005).

Other diseases caused by *E. coli* include bacteraemia resulting from extra-intestinal infections, mostly of the urinary tract, but also of the blood, the central nervous system, or other tissues. Although these infections may not be directly foodborne, carriage in the GI tract of a virulent *E. coli* is a major risk factor for urinary tract infection and systemic infection (Johnson and Russo, 2005). In addition, some animal pathogenic *E. coli* (*e.g.* pathotypes causing disease in poultry, cattle, pigs and sheep) could represent a reservoir of strains and virulence factors similar to those in human extra-intestinal pathogenic strains. Therefore, extra-intestinal pathogenic *E. coli* should also be considered when assessing the safety of *E. coli* introduced in the food or feed chain.

Resistance to therapeutic antimicrobials is a growing problem for the treatment of extra-intestinal infections (Johnson and Russo, 2005; Livermore and Woodford, 2006), and of some gastro-intestinal infections (Huang et al. 2006), caused by *E. coli.* Such resistance is frequently carried by mobile genetic elements, the transfer of which can lead to multi drug resistance (MDR). *E. coli* is also used as an indicator of poor hygiene in food processing and of faecal contamination in drinking and recreational waters, and these aspects have to be considered in the current regulatory hygiene framework of the European Commission (Anonymous, 2005). Although not a direct safety concern, the intentional introduction of *E. coli* in the food production chain might interfere with the implementation of these criteria.

Could safety concerns be excluded?

Diseases caused by *E. coli* are multi-factorial. They have been associated with several factors including, or virulence genes, some specific to one type of diseases, while others may be common to different types (Kaper et al., 2004). Assuming that identification of key virulence genes could be possible for each type of disease, the exclusion of potentially virulent strains would therefore represent a complex qualification and would presumably not bring any simplification, compared to a case by case assessment. For some pathogenic *E. coli*, (e.g. STEC/VTEC) a few virulence factors seems necessary for infection (*e.g.* Attachment Effacement Locus and Shiga toxins, EFSA, 2007b), although some exception exist with strains lacking the attachment effacement locus (Kaper et al., 2004). For other pathogenic *E. coli* (*e.g. E. coli* causing extra-intestinal infections) a large number of virulence genes have been epidemiologically associated with clinical symptoms (*e.g.* up to 42 genes

proposed by Johnson and Russo, 2005), although clinical strains causing the same type of disease do not always carry the same virulence genes (Wiles et al., 2008). In contrast the same infection mechanism can be achieved by a different set of virulence genes (Ron, 2006). Some genes associated with virulence are also present in commensal *E. coli* (Wiles et al., 2008). Even the sequence of the genome of three strains from urinary tract infection did not permit definitive identification of common genomic features (Wiles et al., 2008).

Many virulence genes in *E. coli* are on potentially transferable elements. New associations of virulence genes might appear, creating new infectious mechanisms and new types of diseases (Kaper et al., 2004). An association between phylogenetic groups, or serogroups, with the ability to cause some type of diseases in human has been observed (EFSA, 2007a, Wiles et al., 2008, Jaureguy et al., 2008). However this is not the case for all the types of diseases caused by *E. coli* (Jaureguy et al., 2008). The pathogenicity of *E. coli* is too complex, presumably resulting from multiple evolution mechanisms, to permit a qualification of QPS based on the identification of virulence factors.

Concerning the presence of antimicrobial resistance, EFSA has defined microbiological breakpoints for *E. coli* to identify strains potentially harbouring acquired microbial resistance (EFSA, 2008b).

Conclusion

Although some *E. coli* have a long history of safe use as probiotics, and in spite of the large body of knowledge acquired for this species, it cannot be recommended for the QPS list because of the large diversity of human and animal diseases caused by *E. coli* and the complexity of the virulence mechanisms.

2.5.1.2. *Serratia rubidaea*

Taxonomy

The genus *Serratia*, belongs to the family *Enterobacteriaceae* and contains 13 species and 4 subspecies. *S. rubidaea* contains no subspecies (Anonymous, 2009b; Ewin et al., 1973; Skerman et al., 1980).

Body of knowldege and history of use

The effects of salt stress on pigment production of a strain of *S. rubidaea* and its potential use as indicator strain for screening quorum sensing inhibitors from marine microbes was investigated (Yamazaki et al., 2006). An earlier study describes the investigation of two novel lipids from *S. rubidaea*. The importance of such surface-active exolipids in bacterial occupancy on surfaces was suggested (Matsuyama et al., 1990). The potential of *S. rubidaea* for a reduction of cyclohexanones was further investigated (De Conti et al., 2001).

An US patent presents as invention the application of anti-fungal bacterial strains, in particular bacterial strains of *Serratia*, for use in preserving animal feedstuff composition, including silages and hay. Anti-fungal and anti-bacterial strains of *S. rubidaea* are described to be useful for these applications, and permit hay to be baled at higher moisture contents (Anonymous, 1994).

Strains of *S. rubidaea* were described to be beneficial rhizobacteria of oilseed rape with antifungal properties (Kalbe et al., 1996).

Safety concerns

S. rubidaea was decribed in the literature as a cause for human disease. A case of *S. rubidaea* endophthalmitis of a boy following trauma to one eye was described (Joondeph and Nothnagel, 1983). *S. rubidaea* was reported to produce red pigments and was isolated from silastic foam used as a dressing for chronic crural ulcers (Parment et al., 1984; Hughes and Marks, 1985). *S. rubidaea* was isolated from the bile and blood of a patient (Ursua et al., 1996). Another study describes a rare case of *S. rubidaea* sepsis in a 48-year-old male (Okada et al., 2002). In a more recent study, *S. rubidaea* is described as an opportunistic pathogenic bacterium, which is rarely identified in man, and when so, generally found in the respiratory tract, wounds, feces, bile, but also in blood. The study reports the case of a 54-year-old carrying an arterial catheder for two weeks where two hemocultures were positive in the first 48 h with identification of *S. rubidaea* (Sekhsokh et al., 2007).

Conclusion

The history of use of *S. rubidaea* in the food or feed chain is limited, and the species has been isolated from human infections. It can therefore not be included in the QPS list.

2.5.1.3. *Pseudomonas chlororaphis*

Taxonomy

The genus *Pseudomonas* contains 185 species and 13 subspecies. *Pseudomonas chlororaphis* contains three subspecies: *P. chlororaphis* subsp. *chlororaphis*, *P. chlororaphis* subsp. *aureofaciens* and *P. chlororaphis* subsp. *aurantiaca* (Anonymous, 2009c; Peix et al., 2007).

Body of knowledge and history of use.

A *Pseudomonas chlororaphis* isolate, obtained from perch intestine, was evaluated with regards to its potential to control *Aeromonas sobria* disease in farmed perch. An infection of perch with labelled *P. chlororaphis* indicated the bacterium is able to transiently colonise juvenile fish and fingerlings and to reduce *A. sobria* associated mortalities (Gobelin et al., 2009).

An isolate from green pepper rhizospere that was identified as *P. chlororaphis* produced secondary metabolites that have shown broad-spectrum antifungal activity against various phytopathogens of agricultural importance in vitro (Liu et al., 2007).

A strain of *Pseudomonas chlororaphis* was described as an effective biocontrol agent against *Pythium aphanidermatum*, the causal agent of damping-off of hot pepper in greenhouse vegetable production systems. The strain induced development of plant defence-related enzymes and increased growth of hot pepper seedlings (Nakkeeran et al., 2006).

Root colonisation by a plant-beneficial rhizobacterium. *Pseudomonas chloroaphis* O6, induces disease resistance in tobacco against leaf pathogens *Erwinia carotovora* causing soft-rot and *Pseudomonas syringae* pv. *tabaci* causing wildfire (Han et al., 2006).

It was reported to produce an antifungal metabolite which is a crucial trait in its competition with the pytopathogenic fungus *Fusarium oxysporum* in the rhizosphere (Chin et al., 2005). Specifically, *P. chlororaphis* was described to control tomato foot and root rot caused by *Fusarium oxysporum* by root colonisation (Chin et al., 2000).

P. chlororaphis was used in some works as a representant of the epiphytic microflora of cilantro (Brandl, 2002) and green endive chicory (Carlin and Mandrell, 1996), indicating that it is presumably common on the surface of some fresh produce.

Safety concerns

DG Sanco of the European Commission finalised a review report for the active substance *Pseudomonas chlororaphis* 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 (Anonymous, 2004b). The overall conclusion from the evaluation report was that it may be expected that plant protection products containing *Pseudomonas chlororaphis* will fulfil the safety requirements laid down in Article 5(1)(a) and (b) of Directive 91/414/EEC (Anonymous, 2004c).

The Environmental Protection Agency in the United States evaluated *P. chlororaphis* strain 63-28 and prepared a Fact Sheet (Anonymous, 2001). In the summary it is stated that the strain is a naturally occurring bacterium that can be used in controlling various fungi that attack crop roots. The bacterium has shown no toxicity or pathogenicity to humans, wildlife, or the environment. Its use is limited to vegetables and ornamental crops in containers in greenhouses.

Pseudomonas clororaphis is further described in the literature for its potential to produce secondary metabolites. For example, a strain of the nonpathogenic bacterial species *P. chlororaphis* was reported to be capable of producing rhamnolipids (Gunther et al., 2005). *P. chlororaphis* produces several secondary metabolites, in particular phenazine compounds, which contribute to some of its biocontrol activity (Anonymous, 2002; van Rij et al., 2004). Phenazine compounds, from other sources than *P. chlororaphis*, were reported to have some toxic effects on animal and human cells (Gamage et al., 2002; Allen et al., 2005; Lavaggi et al., 2008) and have been identified as virulent factors of the human pathogenic *Pseudomonas aeruginosa* (Mahajan-Miklos et al., 1999). In its Opinion on 2002, the Scientific Committee on Plant (Anonymous, 2002) noted that the information on toxicity of *P. chlororaphis* is limited but concluded that 'noting absence of sustained colonisation, the number of *P. chlororaphis* associated with the harvested grain as well as the concentration of any metabolites produced would be very low'.

The safe use of *P. chlororaphis* as a plant protection product presumably relies in the absence of colonisation of the edible part of plants. No information permits to assess the risk if *P. chlororaphis* were used in a situation where it could produce secondary metabolites in food or feeds.

Conclusion

Pseudomonas chlororaphis cannot be recommended for the QPS list due to the production of potentially toxic secondary metabolites.

2.5.1.4. *Rhodopseudomonas palustris*

Taxonomy

Species of the genus *Rhodospeudomonas* constitute the majority of phototrophic purple non-sulfur bacteria, and are characterised as rod-shaped, motile cells that show polar growth and asymmetrical division or budding as the mode of reproduction (Mehrabi et al., 2001). The genus comprises photosynthetic bacteria found widely distributed in aquatic sediments and members of the genus catalyse hydrogen gas production, carbon dioxide sequestration, and biomass turnover (Oda et al., 2008). The genus comprises the species *R. acidophila*, *R. capsulata*, *R. gelatinosa*, *R. globiformis*, *R. palustris*, *R. spaeroides*, *R. sulfidophila*, *R. sulfoviridis*, *R. viridis* (Skerman et al., 1980). The genome

sequence of *Rhodospeudomonas palustris* revealed a richness of metabolic versality (Oda et al., 2008). Strains of *R. palustris* that are commonly found in soil and sediment have been shown to be able to degrade a wide variety of carbon compounds, including benzoate and 3-chlorobenzoate, which makes them interesting and potentially useful and has resulted in genome sequencing by the United States Department of Energy's Carbon Management Program (*Rhodopseudomonas palustris* Genome Project [http://www.jgi.doe.gov/JGI_microbial/html/rhodopseudomonas/] (Bent et al., 2003).

Body of knowledge and history of use

Rhodospeudomonas palustris is used in Chinese aquaculture and represents one of the earliest and most widely used probiotics in China since 1980. Nowadays, using photosynthetic bacteria as probiotics is common practice in many fish or shellfish hatcheries and farms in China. Many commercial photosynthetic bacterial products are labeled as either single or multiple species at concentrations higher than 10^9 cells ml^{-1} (Qi et al., 2009). In an earlier study, a vitro assessment of gastrointestinal viability of *R. palustris* was carried out (Zhou et al., 2007). The objective of the study was to test the viability of *R. palustris* isolated from ponds sludge in simulated gastric transit conditions (pH 8, with or without 0.3 % bile salts), in an attempt to conclude whether it has a potential use as a probiotic in aquaculture. The authors concluded from their study that the strain of *P. palustris* had a high capacity of upper gastrointestinal transit tolerance and was relatively safe for epithelial cells of tilapias, and could potentially be used as a probiotic. In a different study the nutritional value of a strain of *R. palustris* was investigated together with an assessment of its toxicity and acceptability as an aquaculture feed or supplement (Getha et al., 1998). The authors concluded from their study that the strain of *R. palustris* was acceptable to Artemia larvae as a feed. They suggested for future studies to determine the optimal concentration of the bacterial biomass in the feed formulation and to assess the suitability of the bacterial biomass in the culture of penaeid shrimps and fishes. An additional study dealt with biomass production and studies on *R. palustris* grown in an outdoor, temperature controlled, underwater tubular photobioreactor (Carlozzi and Sacchi, 2001).

Safety concerns

No relevant publications were found in a PubMed search with the following keywords: *R. palustris* & infections, *R. palustris* & antimicrobial resistance, *R. palustris* & disease.

Some information was obtained with a literature research with the keyword 'toxin'. It appears that the biological activities of lipopolysaccarides of *R. palustris* were virtually nontoxic for mice and nonpyrogenic for rabbits (Galanos et al., 1977).

Conclusion

The history of use is restricted to the use of biomass for fish feeds and does not permit to include this species in the QPS list.

2.5.2. Bacteriophages

Two bacteriophages active against *Clostridium* species and proposed to be used as feed additives have already been notified to EFSA. The present Opinion therefore assesses for the first time whether some bacteriophages could be included into the QPS list.

Bacteriophages (phages) are the viruses of bacteria. They share habitats with their hosts and, consequently, may be considered ubiquitous as a group. Infection depends on specific recognition between surface cell-receptors and phage anti-receptors. This means that, in general, they have narrow host ranges. Usually, upon infection phages follow a lytic cycle, at the end of which the cell is lysed

and a viral progeny is produced. In addition, some phages may follow a lysogenic cycle, where the phage DNA (the prophage) synchronizes its replication to that of the host to be inherited by its offspring. Phages that can only follow the lytic cycle are called virulent, while those that can choose between a lytic and a lysogenic cycle are called temperate. Comprehensive information on bacteriophages is described in the literature (Calendar, 2006; McGrath and van Sinderen, 2007; Waldor et al., 2005).

It is anticipated that phages will start to be proposed as antagonistic agents for bacteria that cause spoilage of feed and food and for pathogens that use these matrices as reservoirs and sources of infection. In this respect, phages may present some advantages over physical and chemical decontamination procedures. For example, they are completely innocuous for eukaryotic organisms, so that their use might allow a reduction in the concentration of potentially toxic preservatives. In addition, phages are not expected to alter the sensory properties of foods, which contrasts with the effects of many physical preservation treatments and, finally, phages would not affect the viability of fermented food starter bacteria, due to their narrow host specificity.

Taxonomic unit

The International Committee on Taxonomy of Viruses (ICTV) recognizes 14 families of phages which are mainly defined by the nature of their nucleic acid and virion morphology (Anonymous, 2002b). Eleven of these families are not grouped in a superior taxonomical category, while the other three are included in the order *Caudovirales*. This comprises the vast majority of phages (96 %) and its members have in common genomes located in a double stranded DNA molecule and a complex morphology with a capsid of regular symmetry (the head) and a DNA injection apparatus of helicoidal symmetry (the tail). The morphology of the tail defines the three families of the order: i) *Myoviridae*, with a long, contractile tail; ii) *Siphoviridae*, with a long, non-contractile tail, and iii) *Podoviridae*, which have a short tail.

The distribution of phages within families into lower ranks, genera and species, has been attempted but has been confronted with the lack of functional specific data because phages do not have their own metabolism and, in many cases, with the absence of a detailed knowledge of their biology. As a consequence, while genera and type phages were defined, most of the isolates described in the literature remain unclassified.

On the other hand, there is the problem of prophages. Frequently, it is not possible to propagate them, possibly because many are defective or simply because an appropriate host has not been found. This precludes their inclusion into a virion morphology based classification (Ackermann, 2006; Nelson, 2004).

This is why genomic and proteomic analyses are being applied to phage classification, taking advantage of their modest genome size. However, this task is resulting to be more difficult than expected; first of all, many phage genes do not have counterparts in the databases or cluster with other phage derived sequences of unknown function. Besides this, a universal phage gene does not exist, such as those encoding rRNAs in all cellular organisms. Finally, phages interexchange DNA segments with other phages, with prophages and even with their bacterial hosts.

As a consequence, many different algorithms have been applied to classify phages based on their deduced proteome (Liu et al, 2006; Rohwer and Edwards, 2002), location of functional modules with respect to the overall genome, number and position of individual genes in the genome (signature genes) and inside the modules, etc. (Brüssow and Desiere, 2006; Li et al, 2008b; Lima-Mendez et al, 2008). All these approximations present discrepancies but, in general, reveal an overall picture that resembles the ICTV official classification into families, although with some relevant exceptions; for example, some *Siphoviridae* phages (the D29 group) appear to be more related to one of the major divisions of the *Podoviridae* (the T7 group) than to other siphoviruses.

Curiously, most of the applied algorithms do not take into consideration the taxonomy of the bacterial host nor give a special relevance to the presence/absence of a genetic switch that allows the phage to follow a lysogenic cycle. These two considerations are of paramount importance to define whether phages or at least some of them might reach the QPS status, as will be reasoned below.

Given these considerations, it is clear that the classification of bacteriophages is far from being settled.

Body of knowledge

The history of phages used as biocontrol agents in food goes back as far as the third decade on the last century. *Vibrio cholerae* specific phages were poured into drinking water wells by I. Asheshov while working in India, and resulted in a tenfold reduction in the incidence of cholera with respect to people that used alternative water sources (Sulakvelidze and Kutter, 2005). The interest in phages as food-decontamination agents faded almost completely until the end of the 20th century, with some exceptions such as the attempts of Ellis et al. (1973) and Greer (1982) to reduce the counts of psychrotrophic *Pseudomonas* in dairy and meat products respectively. By the early 1990s, it was reasoned that phages specific against starters of dairy fermentations might accelerate ripening through release of intracellular bacterial enzymes which, in addition, might improve the flavor of the products (Crow et al., 1995). Other early attempts included those aimed at reducing the concentration of *Listeria* in the environment of food-production facilities (Roy et al., 1993).

The interest in the application of phages to reduce the concentration of food-borne pathogens and spoilage microorganisms exploded almost suddenly at the onset of this century. The work was mainly focused on *Listeria monocytogenes*, *Salmonella*, *Campylobacter* and *Escherichia coli* O157:H7 (Hudson et al, 2005; Hagens and Offerhaus, 2008) and also on *Staphylococcus* (Garcia et al, 2007). This was backed by a significant increase in the number of reports dealing mainly with the application of phages to alimentary matrices that had been artificially contaminated with relevant bacteria but also looking at their effect under production conditions. There have even been attempts to feed farm animals with phage cocktails with the aim of reducing the contamination of meat, during or after slaughter, by enteric bacteria (Fiorentin et al, 2005; Raya et al, 2006) without having observed any signs of toxicity associated to their ingestion (Berchieri, 1991). These studies have been complemented with some toxicity tests (Carlton et al, 2005).

As a consequence of this interest and taking into account the generally accepted assumption of lack of adverse effects associated with phage ingestion, several phage-based preparations have been licensed for use in the United States. Among them are commercial products aimed to be used against *Listeria monocytogenes* contamination, tomato and pepper spot and specific *E. coli* and *Salmonella* washes for treatment before slaughter.

The implementation of phages-based biocontrol food-systems may not be as simple in Europe, however, although this possibility is being addressed. The European Commission, through its Directorate General on Health and Consumer protection, has recently asked EFSA to provide technical assistance on an assessment on the mode of action of phages when added to foods of animal origin (animal carcasses, meat products, dairy products and others) and on their persistence i.e.: whether their antibacterial effect would be short-lived or whether they would protect against recontamination at subsequent stages of the processing or even in the final product. In the first case, phages would be considered as processing aids and then not a subject for European regulation, although they might fall under Regulation (EC) No 853/2004 (Anonymous, 2004d), when applied to remove surface contamination, while in the second, they would be considered as food additives, and so be affected by the Council Directive 89/107/EEC and Regulation (EC) No 1333/2008 (Anonymous, 1989; Anonymous 2008). The document delivered by EFSA (2009c) concludes that persistence of the antibacterial effect depends on the particular phage/bacteria combination, on the

food matrix being treated, on the conditions of application and on environmental factors, so that a general answer cannot be provided.

Safety concerns

Bacteriophages only infect bacteria and, consequently, they cannot be considered as direct pathogens for humans, animals or plants. Besides this, they are not spoilage agents since they do not have their own metabolism. On the other hand, phages may be the most abundant biological entities on earth and are ubiquitous (Abedon, 2009). Consequently, they are being continuously consumed, especially with fermented foods, where phages active on the bacterial starters are common (McGrath et al., 2007). They also parasitize the components of the normal microbiota, and then are produced in the body cavities, to the point that they are routinely being used as indicators of fecal contamination (Jofre, 2009; Letarov and Kulikov, 2009). Finally, they have been used as vaccine adjuvants and even as therapeutic agents against bacterial infections, without any signs of pathogenicity or undesirable side effects associated to their use (Hanlon, 2007; Matsuzaki et al., 2005).

However, phages may influence the phenotypes of their bacterial hosts. This is especially true for temperate phages which, frequently, harbor genes that are expressed during lysogeny (phage conversion). Of special relevance are determinants that encode virulence factors, such as the diphtheria and botulism toxins produced by lysogenic strains of *Corynebacterium diphteriae* and *Clostridium botulinum*, respectively and many others (Brüssow et al., 2004).

In addition, bacterial DNA can be transferred from cell to cell, inside viral capsids (transduction). By this mechanism bacteria may exchange genetic information located in their chromosomes (Miller, 2001), plasmids (Novick et al., 1986) and mobile elements (Chen and Novick, 2009) which includes antibiotic resistance (Zhang and LeJeune, 2008), virulence (Brabban et al., 2005) and spoilage determinants (Matthews and Novick, 2005). Transduction is much more frequent in phages that package their own genome by the so called headful mechanism, which present a more relaxed control of the DNA to be introduced to the capsid than phages that specifically recognize the end sequence of their genomes (Oliveira et al., 2005).

The conclusion is that phages do not cause any safety concerns by themselves but through the transmission and expression of viral and bacterial genes, which may enhance the virulence and spoilage abilities of their hosts.

Possibility to exclude safety concerns

Phage conversion of the bacterial host is dependent on the ability of the incoming virus to lysogenize and on the presence in its genome of harmful genes such as those encoding virulence determinants. Lysogenization requires a genetic switch that may repress the expression of the genes that lead phage development towards the lytic cycle and, in most cases, of a site-directed recombinase that catalyzes the incorporation of the phage genome to that of the bacterial host through a phage-bearing *attP* sequence. For the moment, the only safe way to ascertain whether these elements are part of the gene pool of a bacterial virus is the analysis of its genome sequence, complemented with physiological experiments to exclude the generation of lysogenic cells upon infection. Similarly, the DNA-packaging mechanism and transduction ability of any phage can only be assessed with *ad hoc* experiments. These characteristics could be used in a case by case safety assessment of phages.

Consideration of bacteriophage for the QPS list

Phages are inert particles until they infect a bacterial host and, even then, all metabolic functions encoded by their genomes have to be fulfilled by the host. From this perspective, and taking into consideration that only the genetic material of the phage enters the bacterium, phages might be

visualized as DNA replicons rather than microorganisms. Their main difference to plasmids is that most are able to follow a lytic cycle by which they become encapsulated into a protective shell that, in addition, has host-recognition and genome injection elements. These restrict the phage-susceptible bacteria to organisms closely related with its host, almost never going further than the species limits. Conversely, phages do not have conjugation machinery such as the one that allows plasmids and conjugative transposons to spread through bacterial communities. This last system, in contrast with general assumptions, may affect phylogenetically distant bacteria, even traversing the boundaries between Gram positive and Gram negative organisms (Mazodier et al., 1989).

On the other hand, bacteriophages do not exert any effect on eukaryotic organisms by themselves but through their bacterial hosts, which express the genetic determinants harbored in the genomes of prophages (as they do with plasmid traits, including those encoding virulence and antimicrobial resistance). This is a crucial difference to animal and plant viruses, whose pathogenicity is directly exerted, without any need of an intermediate biological entity.

With all these arguments in mind, it may be argued that the unit for QPS consideration should be the bacterial host rather than the phage. A bacterial species is considered to be QPS when it does not have a record of causing pathology after a long history of mutual interaction with humans, animals or plants. This means that its gene pool does not encode any virulence or other harm determinants that might represent a threat to eukaryotic organisms. The gene pool includes of course the chromosomally encoded traits, but also those harbored by its indigenous plasmids and phages.

For this reason it could be proposed that the consideration of bacteriophages to the QPS status should be linked to the qualification as such of their bacterial hosts. However EFSA will have mostly to deal with phages to fight against pathogenic bacteria. Therefore, notifications for phages from QPS bacteria are not expected.

Conclusion

Bacteriophages cannot be included on the QPS list for the following reasons:

- Impossibility to allocate them to precise taxonomical units (genera and species).
- The only way to discriminate between their temperate/virulent nature and to know whether they carry undesirable genes in their genomes is DNA sequencing and analysis.
- Absence of an *a priori* indication of their ability to transduce bacterial DNA which has to be deduced from their DNA-packaging characteristics.

These circumstances would allocate phages in a typical case by case analysis situation.

Assessment of two clostridial specific bacteriophages

Two phages were notified previously, which infect *Clostridium sporogenes* and *Clostridium tyrobutyricum*. It is impossible to ascribe these phages to a precise taxonomic unit, there is a lack of scientific information on the usage of phages in feed against clostridial development and a lack of data that demonstrate that these phages are not virulent and unable to transduce bacterial DNA.

Therefore, following the assessment presented in the former section, these two phages cannot be included on the QPS list and will have to be assessed on a case-by-case basis.

2.5.3. Viruses used in plant protection[17]

Baculoviruses (Bac V) and Zucchini Mosaic Virus (ZYMV) have been notified to EFSA as plant protection products. Therefore, at the request of EFSA the present Opinion assesses whether some taxonomic units corresponding to these two kinds of viruses could be included in the QPS list.

2.5.3.1. *Baculovirus*

Following a request from EFSA, the QPS Working Group of the Panel on Biological Hazards on the maintenance of the list of QPS microorganisms intentionally added to food or feed was asked in the terms of reference to consider the application of *Baculovirus* (BacV) used for control of insects that cause damage to plants for the QPS concept.

Taxonomic unit

Baculoviruses are a family of arthropod-specific, rod-shaped (baculum = rod), enveloped viruses with a circular double-stranded DNA genome. The family *Baculoviridae* was recently reclassified and is now divided into four genera (Jehle et al. 2006; ICTV, 2008). Genus *Alphabaculovirus* comprises the *Nucleopolyhedroviruses* (NPVs) (formerly nuclear polyhedrosis virus) of Lepidoptera. Genus *Betabaculovirus* encompasses the Granuloviruses (GV) (formerly granulosis virus) of Lepidopteran insects. Genus *Gammabaculovirus* contains the NPVs of Hymenopteran insects and the Genus *Deltabaculovirus* the NPVs from Dipteran insects. These genera form monophyletic groups within the *Baculoviridae* family (Jehle et al., 2006).

The International Committee on Taxonomy of Viruses (ICTV) now recognizes 42 baculovirus species based, among others, on the complete nucleotide sequence of these species (ICTV, 2008). A further 150 or so are recognized as potential baculovirus species based on partial DNA sequencing and phylogenetic analysis; about 450 remain tentative until they have been molecularly characterized (Martignoni and Iwai, 1986). Baculoviruses are distantly related to the Nudiviruses (insect-specific) (Wang and Jehle, 2009) and Hytrosaviruses (dipteran flies) (Abd-Alla et al., 2008).

Baculoviruses form a distinct and well characterized group of arthropod-specific viruses which can be distinguished from other viruses by a number of unique properties described in the following. The most prominent characteristic of baculoviruses is their occurrence as occlusion bodies (OB). The OBs are formed in the nuclei of infected cells of insect larvae and can be easily detected by light microscopy (phase-contrast or dark-field) as highly refractile particles or bodies ranging in size between 0.1 and 10 μm. These largely proteinaceous bodies contain rod-shaped virions (singly or as multiples), which in turn contain a large circular double-stranded DNA molecule. The OBs protect the virions against decay upon assembly and can survive in the environment for a long time (> 20 years). Baculoviruses contain a double-stranded circular DNA genome ranging in size between 80-180 kilobase pairs depending on the virus species. All baculovirus species share 30 so-called core genes and contain collectively more than 1000 other genes, which may be related to virulence, specificity and host range of the pertinent baculovirus species (Herniou and Jehle, 2007).

Body of knowledge

Baculoviruses occurred in ancient times in silkworm cultures and often ruined silk production. The first attempts to use baculoviruses for biological control can be dated back to the year 1892. During a dramatic population increase of nun moths (*Lymantria monocha* L.), a severe pine pest in Europe, the use of the infectious agent causing the so called 'Wipfelkrankheit' or 'tree top disease' was intended

[17] Section 2.5.3 of the opinion was shared with the PRAPeR Unit of EFSA for information and commenting. Received comments were taken into consideration.

to combat the insect pest (Huber, 1986). The successful biological control of insect pests in field crops, plantation, orchard crops, and forests was demonstrated by field experimentation and applications of widely different scales all over the world (Moscardi, 1999). These resulted in the development of fully registered baculovirus insecticides in several countries for insect pests in forestry, arable crops, horticulture and orchards (Anonymous, 2006). A detailed continent-by-continent survey on the developmental, experimental and commercial use of baculovirus insecticides was recently compiled (Hunter-Fujita et al., 1998; Moscardi, 1999; Erlandson, 2008). Some examples of several most important baculovirus insecticides tested and used in the field were summarized and include amongst others *Adoxophyes orana* GV, *Cydia pomonella* GV, *Helicoverpa armigera* NPV, *Anticarsia gemmatalis* NPV, *Neodiprion sertifer* NPV, *Lymantria dispar* NPV and *Spodoptera spp.* NPV (Anonymous, 2006).

Methods of characterization

Currently baculoviruses are characterized by sequencing three to five highly conserved core genes (*polyhedrin*, *DNA polymerase*, *lef-8*, *lef-9* and *pif-2*) using universal primer sets and polymerase chain reaction (PCR) procedures (Herniou and Jehle, 2007; Van Oers and Vlak, 2007). This gives unequivocal identification of baculovirus species. Registration as a biocontrol agent requires the entire sequence of the active component (baculovirus genome).

Human and animal health considerations

Baculoviruses are naturally occurring pathogens of arthropods. Their host range is exclusively restricted to terrestrial arthropods (Barber et al., 1993; Doyle et al., 1990; Cory and Hails, 1997). No member of this virus family is infectious to plants or vertebrates or microorganisms. Baculoviruses are ubiquitously present in the environment and have been used for biological insect control for more than 100 years. Circumstantial evidence for the safety of baculoviruses emerges from the history of contact between baculoviruses and humans for such a long time without any detrimental effects (Summers et al., 1975; Anonymous, 2006). In addition baculoviruses are also a natural control agents of insects by controlling the size of insect populations, e.g. in forestry (nun moth, gypsy moth), and animals and man have been exposed to baculovirus throughout history until the present day. For example, cabbage from the open market may contain a lot of baculoviruses as a result of a polyhedrosis in caterpillars foraging on cabbage (Heimpel et al., 1973). So, mankind has been in contact with these viruses for a very long time. Currently baculoviruses are used, among others, on large areas of cotton (100,000 ha), soybean (1,500,000 ha) and orchards (100,000 ha) annually for 5-20 years or more showing a perfect health card for workers and consumers. In Europe baculoviruses are mostly used in orchards, on vegetables and in flower culture (arable and protected crops).

Baculovirus products that are commercially available as insect biocontrol product and have been extensively tested for their effect on human and animals (EPA, 1996). These tests included exposure of mice and/or rabbits to high doses of occlusion bodies (OBs) by inhalation, epidermal application, subcutaneous, intravenous and intracerebral injections of OBs into mice, rats and rabbits, skin irritation tests, etc., but none of these showed any negative effects (Ignoffo, 1973; Rogoff, 1975; Burges et al., 1980). Furthermore, teratogenicity, carcinogenicity and replication potential in mammalian cells have been tested all negative (Miltenburger, 1978; Gröner, 1986). Baculoviruses do not produce toxins in their hosts.

Some baculoviruses can readily infect insect cells with the budded form (the form that is circulating in the larval body) of the virus after virus infection (Vlak et al., 1996). Infection of mammalian cells with OBs turned out to be negative. When mammalian cells were infected with (occlusion body) OB-derived virions (rod-shaped particles), viral DNA could be found inside the cell but no replication was recorded. Cytogenetic studies in mammalian cells indicated no chromosomal aberrations (Reimann and Miltenburger, 1983; Miltenburger, 1978). Because baculoviruses do not replicate in mammalians

or mammalian cells, have a large genomic capacity to include foreign DNA, are easy to scale up and are intrinsically safe for humans, they are attractive gene therapy vectors for mammals and man (Hu, 2006).

Conclusion

On the basis of the available literature and other available sources of information it can be concluded that baculoviruses are safe for animals and human consumption. Baculoviruses in the form of OBs are specific for (certain) insects and do not productively infect (cells of) non-target insects or other organisms including humans and animals. It is therefore recommended to include plant protection viruses, more specifically baculoviruses (*Baculoviridae*) as the highest taxonomic unit, on the QPS list.

2.5.3.2. *Zucchini yellow mosaic virus (ZYMV)*

Body of knowledge

ZYMV is a viral plant pathogen and member of the potyvirus family (Potyviridae). This family contains 111 recognized species and 86 tentative species, and probably encompasses - as a family - the largest number of recognized plant virus species. Five genera are established and ZYMV belongs to the Genus Potyvirus (ICTV, 2005).

ZYMV was isolated in 1973 from zucchini and mainly infects commercial cucurbit plants (Cucurbitacaea, i.e. cucumber, pumpkin, squash, melon and zucchini) (Lisa and Lecoq, 1984). It is transmitted (vectored) by the aphid *Aphis gossypii* in a non-persistent way (i.e. the virus does not replicate in the vector, which is thus a virus reservoir and a virus carrier). The disease is characterized by the appearance of mosaic or yellowing symptoms on the surface of the plant leaves and malformation and discoloration of the fruit. This is a very specific response to virus infection. The virus can reliably be identified by either DAS-ELISA or RT-PCR technologies (Desbiez et al., 2002).

Control of the disease is mainly achieved by using insecticides to eliminate the aphid vector. Natural resistance in plants against ZYMV is rare and therefore alternative strategies for control are sought including genetic engineering (not discussed here) and the use of weak strains of the virus to exploit the phenomenon known as premunition or cross-protection (Cho et al., 1992; Walkey et al., 1992).

Cross-protection is a phenomenon in plants where infection with a ‘mild’ strain of a certain plant virus induces tolerance or resistance in plants to a subsequent infection with a ‘severe’ or ‘virulent’ strain of the same virus or a related virus. This phenomenon was first described by McKinney (1929), the term ‘cross protection’ was coined first by Matthews (1949). The mechanism of cross protection is thought to be based on (i) coat protein or CP-mediated resistance and / or (ii) RNA-mediated resistance (Gal-On and Shiboleth, 2006). The first mechanism is thought to be based on the prevention of uncoating of the ‘severe’ virus upon entry into the plants and the inability to initiate replication because the viral RNA is recoated (Beachy, 1990). The second mechanism is based on gene-specific RNA-silencing (Ratcliff et al., 1999), a plant response to virus infection. A detailed account on the principles of cross protection is given by Gal-On and Shiboleth (2006), whereas practical applications have been described by Fulton (1986).

Cross-protection is most successful in annual crops, although examples in orchards have been reported. A few examples of successful implementation have been reported. The latter include control of Cucumber Mosaic Virus (CMV) in cucumber (Tien and Wu, 1991), Tomato Mosaic Virus (ToMV) in tomato (Rast, 1972) and Zucchini Yellow Mosaic Virus (ZYMV) in zucchini and sqash (Cho et al., 1992; Lecoq et al., 1991; Yarden et al., 2000).

Taxonomic unit

In the case of ZYMV the *Potyviridae* family is the highest possible taxonomic unit. The family is characterized by the presence of a 750 nm-long flexuous filamentous particle, which contains a single stranded RNA molecule as genetic entity of about 9500 base pairs and with positive polarity (RNA ready to be translated). The full-length viral RNA is translated into a large polyprotein, which is after synthesis proteolytically cleaved within the cell into 10 proteins. The entire sequence of many potyviruses, including ZYMV (Baker et al., 1992; Gal-on, 2007), is known and phylogenetic analysis using the coat protein as a marker showed that the potyviruses form a monophyletic clade (Pfosser and Bauman, 2002; Simmons et al., 2008). Many genetic variants or strains of ZYMV exist, but they are all very closely related and highly monophyletic as a group within the Potyvirus Genus. On the basis of nucleotide polymorphisms and phylogenetic analysis further subgroups of ZYMV can be identified (Lecoq et al., 2009).

History of use

The principle of cross protection against ZYMV was applied in the late 1980s when a natural variant of the virus was obtained, which gave mild symptoms in zucchini and which protected the zucchini against virulent strains of the virus. Such mild strains and the cross protection strategy have been tested for their agronomic potential fighting the disease (Perring et al., 1995) and a ZYMV-based product has been registered since 1997 in various parts of the world. However, there is limited use of this product and it expected that further development of other viral products along the same lines will be there but slow. In the USA products containing mild strains of ZYMV are considered pesticides and their registration is carried out accordingly (US-EPA, 2007).

Human and animal health assessment

Since large amounts of ZYMV will be used and on edible crops, the question is whether there is a safety problem for vertebrate animals, including mammals and man when the virus ends up in the food or feed. In general plants viruses are not thought to pose any risk for human or animal consumption. Although plant viruses usually have a very wide plant host range, infection of vertebrates, mammals or mammalian cells has never been reported. Even though some plant viruses relate to animal viruses, e.g. comoviridae and picornaviridae, cross infections between animals and plants and vice versa have not been recorded. In fact, humans and animals have been exposed to plant viruses for a very long time, as plants (food and feed) are often naturally infected with plant viruses. Negative effects on mammalians attributed to plant viruses have never been found or reported even though they are continuously exposed to plant viruses (Zhang et al., 2006). Plant viruses are also present in tobacco products. Plant viruses are also not known to contain or produce toxins. Rabbits are commonly used to produce antibodies against plant virus proteins, but no toxicology or pathology has been recorded. Because of this positive health profile the registration in Europe and the USA exempted applicants from carrying out toxicological and pathogenicity tests in mammals (waver requests granted). Due to the positive health card in general, plant viruses are considered as safe carriers for human and veterinary vaccines (Grill et al., 2005).

Conclusion

On the basis of the available literature and other available sources of information it can be concluded that plant viruses are safe for animal and human consumption. Plant viruses are specific for plants and do not infect other organisms including humans and animals. It is therefore recommended to include potyviruses (*Potyviridae*) on the QPS list.

2.5.4. Consideration of a qualification for yeast regarding resistance to antimycotics

Introduction

Invasive fungal infections have become one of the leading causes of death among patients with aggressive haematological malignancies, transplant recipients with aggressive haematological malignancies, transplant recipients, and other immunocompromised patients such as those with Acquired Immunodeficiency Syndrome (AIDS). The emergence of various opportunistic fungal infections and, for several human fungal pathogens, 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 systemic mycoses and especially those fungi that cause opportunistic infections, such as *Candida* species (mainly *C. albicans, C. glabrata, C. parapsilosis, C.tropicalis, and C. krusei*) and the filamentous fungi *Aspergillus fumigates.* The increasing threat of fungal infections has stimulated the search for better antifungals with a distinct mode of action. However, the effective use of these antifungals has often been minimized by their toxicity and their narrow spectrum. The azoles and the polyenes are the safest antifungal in use to fight systemic fungal infections.

Classes of Antifungal Drugs

Systemic antifungals currently in use (see Table 1) belong to one of four different classes of compounds (Zao and Calderone, 2002; Li and Calderone 2004; Gauwerky et al., 2009):

- The polyenes are inhibitors of plasma membrane function through binding to ergosterol.
- Azoles inhibit the conversion of lanosterol to its demethylated form during ergosterol synthesis. These two classes are the most commonly used types of antifungals.
- Fluoropyrimidines are used much less frequently in a clinical setting than the first two. The only example of a fluoropyrimidine in clinical usage is 5-fluorocytosine (5-FC), and although fungicidal, this compound has a rather limited spectrum of activity so it is used almost exclusively in combined therapy regimens with amphotericin-B.
- Allylamines/thiocarbamates are also inhibitors of ergosterol synthesis. However, their mode of action in inhibiting ergosterol synthesis is different than the azoles in that the allylamines/thiocarbamates inhibit the enzyme squalene epoxidase, which together with the enzyme (2,3)-oxidosqualene cyclase, catalyzes a cyclization of squalene to lanosterol. In general, the allylamines, such as terbinafine, have almost exclusively been used to treat superficial fungal infections, especially dermatophytosis.

Some of their general features are described and summarized in Table 1. The compounds listed in Table 1 under azoles represent examples of either imidazoles or triazoles but is not intended to include all known azoles.

Resistance to antifungals

The resistance to all the major groups of compounds has been described, but the extent of resistance to any particular drug is both organism and drug dependent (for a review see (Prasad et al., 2002; Prasad and López-Ribot, 2004). Thus, resistance among fungi to drugs that are more commonly used (fluconazole) is greater than resistance to drugs less commonly used (ketoconazole). On the other hand, clinical resistance to amphotericin-B (polynes group) is uncommon even though this drug is generally used for treating systemic fungal infections. Interestingly, of those mechanisms of resistance which have been described for pathogenic fungi, modification of a drug resulting in its inactivation

has not been described in fungi. This is in stark contrast to the case in bacteria where such mechanisms are common. In order to understand the mechanism of antifungal resistance, it is essential that the site and mechanisms of action of these drugs are elucidated. Table 2 lists most of the known antifungals with their proven targets and mechanisms of resistance.

Some of the major mechanisms resulting in MDR (Multi drug resistance) are:

- Decreased accumulation of drugs, which is the dominant feature of MDR.
- Changes in expression of some cellular proteins, e.g., P-glycoprotein, MRP (multidrug resistance-associated protein), catalase, and topoisomerase.
- Changes in cellular physiology affecting the structure of plasma membrane, cytosolic pH, and lysosomal structure and function.

The most predominant human pathogenic yeast, *C. albicans* is naturally more resistant to several drugs than *Saccharomyces cerevisiae,* which is interesting to food industry. In addition, the incidence of *C. albicans* cells resistant to antifungals like azoles has increased considerably in recent years, which has posed serious problems toward successful chemotherapy. The incidence of antifungal resistance has also increased in the non-*albicans* species, such as *C. glabrata, C. parapsilosis, C. tropicalis*, and *C. krusei*.

Candida infections are treated with antifungal agents, particularly with the triazole derivatives fluconazole, itraconazole, and ketoconazole. To combat the attack of antifungals, *Candida* has evolved a variety of mechanisms to acquire resistance to these drugs. The resistance to azoles in *C. albicans* was earlier thought to occur primarily through an alteration or an overexpression of the target enzyme 14-alfa-lanosterol demethylase (P45014DM) involved in sterol biosynthesis. However, the characterization of the ATP-Binding Cassette (ABC) proteins Cdr1p and Cdr2p and CaMdr1p, a transporter of the major facilitator superfamily (MFS), led to the suggestion that efflux mechanisms represent an important determinant of antifungal susceptibilities in *C. albicans*.

In spite of the use of polyene antibiotics for several years, there are limited instances of *Candida* resistant to amphotericin-B (AmB) and nystatin; however, resistance to AmB in some *Candida* spp., *C. lusitaniae, C. tropicalis* and *T. beigelii*, is common.

In particular, the strains resistant to AmB, which have been isolated from patients with candidiasis, belong mostly to non-*albicans* species such as *C. lusitaniae* and *C. tropicalis*. The resistance to AmB has been mainly associated with changes in sterol content of the cell. The clinical isolates of *C. albicans* resistant to AmB were shown to lack ergosterol and accumulated 3-beta-ergosta-7, 22-dienol and 3-beta-ergosta-8-enol due to a defect in delta^{5,6}-desaturase enzyme. The decreased ergosterol content resulted in reduced binding of polyenes as compared to susceptible cells. In certain instances of AmB resistance, an increased catalase activity has also been shown as a part of the mechanism of resistance to control oxidative damage caused by the drug. In another pathogenic isolate of *Cryptococococcus neoformans* which was isolated from AIDS patients who failed antifungal therapy, a similar correlation between polyene resistance and sterol contents was reported. There are also reports to suggest that polyene action could be affected by alteration in components other than sterols, cell wall component and membrane phospholipids.

It is noteworthy that in the case of yeast any, horizontal transfer of extrachromosomal material across fungal species and a longer fungal generation time, thereby slowing vertical transfer of mutant traits are known as a mechanisms of resistance to antifungal agents (Hof, 2008).

Resistance to antifungal agents in yeasts in not carried on mobile genetic elements, as in the case of bacteria, and cannot be transmitted among strains (Hof, 2008).

QPS yeast resistance to antifungal drugs

Although yeasts are well known for producing fermented foods and beverages, as sources of food ingredients and as spoilage yeasts, very few species present in foods included in the QPS list (EFSA, 2008a). For this reason the food yeast resistance to antifungal has largely been overlooked. Very few information are available about these resistance to antifungal of these species and only data about antifungal resistance of *S. cerevisiae* are available in vitro. Zerza et al. (1996) and Barchiese et al. (1998) tested in vitro the susceptibilities of *S. cerevisiae* isolates to fluconazole, itraconazole, ketoconazole, 5FC and amphotericin B. In general these authors observed observed a wide range of MICs with high values for some isolates in the case of fluconazole and itraconazole, ranging respectively from 0.12 to 16 mg/l and from 0.015 to 1 mg/l. In contrast, all isolates were inhibited by low concentrations of amphotericin B and 5 FC. In AIDS patients with oral thrush, colonization with S. *cerevisiae* increased after treatment with fluconazole or clotrimazole.

Conclusions

- QPS yeasts are generally not regarded as pathogenic or infectious however there have been reports that they are able to cause rare opportunistic infections in humans
- Human infections caused by QPS yeasts are rare and therefore there is a lack of data to assess whether resistance to antifungal agents in QPS yeasts impaired the efficacy of therapeutic treatments.
- However, because isolates from QPS yeasts species can cause infections in human, it is recommended that strains voluntarily introduced in the feed and food chain are not resistant to the relevant antifungal agents used in therapeutic treatments.
- Resistance to antifungal agents is an increasing concern for the treatment of yeast infections, but this mostly concerns species which are not on the QPS list.
- Resistance to antifungal agents is not transmissible among yeasts. Therefore 'indirect hazards' defined for bacteria (i.e. presence of resistance in a non pathogenic strain which could be transmitted to a pathogenic strain) do not apply for yeasts. Only 'direct hazards' (i.e. presence of antimicrobial resistance in a pathogenic strains which will reduce the efficacy of therapeutic treatments) are to be considered

Table 1. General characteristics of the major antifungals (Zhao and Calderone, 2002)

Classes of compounds examples	Trade name	Clinical use[a]	Cidal or static	Target	Other activities[b]	Resistance[b]
Azoles			Static broad-spectrum	$P450_{DM}$	Yes	Common
Imidazoles						
Ketoconazole	Nizoral	system/super				
Miconazole	Monistat	Super				
Clotrimazole	Lotrimin	super				
Triazoles						
Itraconazole	Sporonox	system/super				
Fluconazole	Diflucon	system/super				
Terconazole	Terazol	vulvovag				
Ticonazole	Vagistat	vulvovag				
Allylamines		super	Static	Squalene	No	Uncommon
Terbinafine				Epoxidase		
Polyenes	Fungizone	system	Broad-spectrum	Ergosterol	Yes	Uncommon
Amphotericin B[c]			Cidal			
Fluoropyrimidines		system	Cidal	Protein synthesis	No	Common
5-fluorocytosine[d]						

a System= systemic use; super= superficial; vulvovag =vulvovaginal.
b Except for fluconazole and the polyenes, few data are available.
c Primarily binds to ergosterol, causing membrane perturbations; also causes oxidative damage to susceptible cells.
d Limited usefulness, primarily in the treatment of cryptococcal meningitis and some types of candidiasis.

Table 2. Targets and mechanisms of resistance of some antifungals (Prasad et al., 2002)

Antifungal	Target	Mechanism of resistance
Pyrimidine 5-Flucytosine	Thymidylate synthetase	Failure to metabolize 5-FC to 5 FUTP and 5 FdUMP Loss of feedback control of pyrimidine biosynthesis Defect in cytosine permease
Polyenes Nystatin, amphotericin-B ergosterol	Membrane ergosterol	Alteration in membrane lipids, mainly (resistant clinical isolates lack ergosterol and accumulate 3-beta-ergosta-7,22-dienol and 3-beta-ergosta-8-enol, due to defect in delta-5,6-desaturase gene (*ERG3*) Enhanced catalase activity
Azoles Fluconazole, ketoconazole, itraconazole, voriconazole, clotrimazole	14 alfa-demethylase (*ERG11,* also designated *ERG16* earlier)	Mutations in the target enzyme cytochrome P450 14 alfa-demethylase which alters the affinity of this enzyme to the azoles Overexpression of 14 alfa-demethylase Failure to accumulate azoles due to rapid efflux mediated by ABC and MFS family of MDR transporters Alteration of sterol delta 5,6-desaturase (*ERG3*)
Allylamines Naftifine, terbinafine, tolnaftate	Squalene epoxidase (*ERG1*)	Overexpression of *CDR1, CDR2,* and *CaMDR1*
Morpholines Amorolfine	delta14-reductase (*ERG24*), delta 8,7- isomerase (*ERG2*)	Overexpression of delta 14-reductase (*ERG24*) or sterol C-24 (28) reductase (*ERG4*) genes Overexpression of *CDR1* and *CDR2*
Lipopeptides Echinocandins, cyclopeptamine pneumocandins, aculcacins	beta-1,3-glucan synthetase (encoded by *RHO1*)	Mutations in *FKS1* gene alters affinity of *FKS1* and the enzyme

2.5.5. The 2009 updated list of QPS recommended biological agents

Taking into account the previous list of QPS microorganisms (Appendix A), and the conclusions the assessments of notifications in this Opinion, the table is updated as follows:

Table 3. The 2009 updated list of biological agents recommended for QPS

Gram-Positive Non-Sporulating Bacteria			
Species			**Qualifications *****
Bifidobacterium adolescentis *Bifidobacterium animalis*	*Bifidobacterium bifidum* *Bifidobacterium breve*	*Bifidobacterium longum*	
Corynebacterium glutamicum			QPS status applies only when the species is used for production purposes.
Lactobacillus acidophilus *Lactobacillus amylolyticus* *Lactobacillus amylovorus* *Lactobacillus alimentarius* *Lactobacillus aviaries* *Lactobacillus brevis* *Lactobacillus buchneri* *Lactobacillus casei* ** *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*	
Lactococcus lactis			
Leuconostoc citreum	*Leuconostoc lactis*	*Leuconostoc mesenteroides*	
	Oenococcus oeni		
Pediococcus acidilactici	*Pediococcus dextrinicus*	*Pediococcus pentosaceus*	
Propionibacterium freudenreichii	*Propiobacterium acidopropionici*		
Streptococcus thermophilus			
Bacillus			
Species			**Qualifications**
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 food poisoning toxins*. Absence of surfactant activity.* Absence of enterotoxic activity.*

** When strains of these QPS units are to be used as seed coating agents, testing for toxic activity is not necessary, provided that the risk of transfer to the edible part of the crop is very low*

** The previously described species "*Lactobacillus zeae*" has been included in the species *Lactobacillus casei*

*** For all QPS bacterial Units, the strains should not carry any transferable antimicrobial resistance, unless cells are not present in the final product, as described in EFSA (2008b).

Table 3. Continued - The 2009 updated list of biological agents recommended for QPS

Yeasts [18]			
Species			**Qualifications ******
Debaryomyces hansenii			
Hanseniaspora uvarum			
Kluyveromyces lactis	*Kluyveromyces marxianus*		
Pichia angusta	*Pichia anomala*	*Pichia jadinii*	QPS status applies only when the species is used for production purposes.
Pichia pastoris			
Saccharomyces bayanus	*Saccharomyces cerevisiae*	*Saccharomyces pastorianus (synonym of Saccharomyces carlsbergensis)*	†
Schizosaccharomyces pombe			
Xanthophyllomyces dendrorhous			
Virus			
Family			
Potyviridae	*Baculoviridae*		

****Absence of resitance to antimycotics used for medical treatment of yeast infections
† *S. cerevisiae*, subtype *boulardii* is contraindicated for patients of fragile health, as well as for patients with a central venous catheter in place.

[18] Yeast Synonyms commonly used in the feed/food industry
Pichia anomola: synonym *Hansenula anomala*, *Saccharomyces anomalus*
Pichia jadinii: anamorph *Candida utilis*; synonyms *Hansenula jadinii*, *Torulopsis utilis*
Saccharomyces cerevisiae synonym *S. boulardii*

CONCLUSIONS

Answers to the terms of reference:

Carry out an annual review of the list of QPS microorganisms.

The list of QPS proposed microorganisms from the last year was reviewed and extended (Table 3). The addition of the following bacterial species to the QPS list is proposed:

Lactobacillus fermentum

Lactobacillus collinoides

Oenococcus oeni

Propionibacterium acidipropionici

For taxonomic units already present in the QPS list, no modification is felt necessary and the conclusions of the previous QPS Opinion (EFSA, 2008a) remain valid.

The present annual review noted that some strains belonging to QPS bacterial species, in particular strains of *Lactobacillus* species can produce biogenic amines.

Enterococci were previously assessed as not appropriate for the QPS list. The information published since the last update of the list (EFSA, 2008a) does not permit to modify this conclusion.

Several taxonomic units, not already present in the QPS list, have been notified to EFSA but were not included in the list because of an insufficient body of knowledge and/or the presence of safety concerns which cannot be excluded.

Consider the introduction of a qualification for yeast regarding resistance to antimycotics.

The range of therapeutic antimycotics is limited and potential resistance to these antimycotics among some yeasts causing clinical infections needs attention. This has to be seen in the context that some yeasts were reported to be the cause of rare opportunistic infections in humans where susceptibility to therapeutic antimycotics becomes an important aspect for a successful treatment and for human health and safety. A qualification regarding potential resistances of yeasts against antimycotics used for human treatments which are currently on the QPS list is therefore considered as important and a more in depth analysis will be carried out for the next year update.

Consider the application of QPS to bacteriophage (for example used as decontamination agents) and viruses (used in plant protection)

Bacteriophages cannot be included in the QPS list for the following reasons:

- Impossibility to allocate them to precise taxonomical units (genera and species).
- The only way to discriminate between their temperate/virulent nature and to know whether they carry undesirable genes in their genomes is DNA sequencing and analysis.
- Absence of an *a priori* indication of their ability to transduce bacterial DNA which has to be deduced from their DNA-packaging characteristics.

These circumstances would allocate phages in a typical case by case analysis situation.

Baculoviruses are specific for insects and do not productively infect (cells of) non-target other organisms including humans and animals. It is therefore recommended to include baculoviruses (*Baculoviridae*) as the highest taxonomic unit, in the QPS list.

Plant viruses are specific for plants and do not infect other organisms including humans and animals. It is therefore recommended to propose potyviruses (*Potyviridae*) for the QPS list.

Update the information on mycelial fungi.

Mycelial fungi were not included in the QPS list (EFSA, 2007a), because of the possible production of a wide diversity of potentially toxic secondary metabolites. Recent scientific information confirms this conclusion.

RECOMMENDATIONS

A review of current and a need for additional qualifications proposed to biological agents on the QPS list is recommended in the context of possible implication on human health.

Among the biological agents notified to EFSA, many were not included in the QPS list due to an insufficient body of knowledge. They will have to be assessed on a case by case basis by the relevant EFSA Panels or Units. More research is needed to obtain the knowledge necessary to design methods for a safety assessment of non QPS microorganisms.

REFERENCES

Abd-Alla AMM, Cousserans F, Parker AG, Jehle JA, Parker NJ, Vlak JM, Robinson AS and Bergoin M, 2008. Genome analysis of a Glossina pallidipes salivary gland hypertrophy virus reveals a novel, large, double-stranded circular DNA virus. J. Virol. 82, 4595-4611.

Abedon ST, 2009. Phage evolution and ecology. Adv. Appl. Microbiol. 67, 1-45.

Ackermann HW, 2006. Classification of bacteriophages. In R. Calendar (ed), The Bacteriophages, Plenum Publishing Corporation, New York, USA.

Adler SN, 2006. The probiotic agent *Escherichia coli* M-17 has a healing effect in patients with IBS with proximal inflammation of the small bowel. Digest. Liver Dis. 38, 9, 713-713.

Allen L, Dockrell DH, Pattery T, Lee DG, Cornelis P, Hellewell PG and Whyte MKB, 2005. Pyocyanin production by *Pseudomonas aeruginosa* induces neutrophil apoptosis and impairs neutrophil-mediated host defenses in vivo. J. Immunol. 174, 6, 3643-3649.

Almeida EG, Rachid C and Schwan RF, 2007. Microbial population present in fermented beverage 'cauim' produced by Brazilian Amerindians. Int. J. Food Microbiol. 120, 1-2, 146-151.

Anonymous, 1989. Council Directive of 21 December 1988 on the approximation of the laws of member States concerning food additives authorized for use in foodstuffs intended for human consumption (89/107/EEC) OJ L 40, 11.2.1989, p.27.

Anonymous, 1994. US Patent 5371011 - Mold control in forage. US Patent issued on December 6, 1994. (http://www.patentstorm.us/patents/5371011/fulltext.html)

Anonymous, 2000. Opinion of the Scientific Committee on Food on β-cyclodextrin produced using cycloglycosyltransferase obtained from *Paenibacillus macerans* (adopted by the SCF on 22/6/2000). European Commission SCF/CS/ADD/AMI 48 Final.

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, 2002a. Opinion of the Scientific Committee on Plants on specific questions from Commission regarding the evaluation of *Pseudomonas chlororaph*is in the context of Council Directive 91/414/EEC. Scientific Committee on Plants, CSP/PSEUDOM/002-Final.

Anonymous, 2002b. ICTVdB - The Universal Virus Database, version 4. (http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/index.htm)

Anonymous, 2004a. Arrêté du 21 juillet 2004 modifiant l'arrêté du 2 octobre 1997 relatif aux additifs pouvant être employés dans la fabrication des denrées destinées à l'alimentation humaine. Journal Officiel de la République Française 189. 15 août 2004, texte n°1. (http://www.legifrance.gouv.fr)

Anonymous, 2004b. Commission working document - Review report for the active substance *Pseudomonas chlororaphis*. European Commission 4204/VI/98-Final, 29 March 2004.

Anonymous, 2004c. Commission Directive 2004/71/EC amending Council Directive 91/414/EEC to include *Pseudomonas chlororaphis* as active substance. OJ L 127/1004, 29.4.2004.

Anonymous, 2004d. Corrigendum to Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004. OJ L 226/22 25.6.2004.

Anonymous, 2005. Commission Regulation (EC) 2073/2005, 15 November 2005, on microbiological criteria for foodstuffs. Official Journal of the European Union, 22.12.2005, L338, 1-26.

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

Anonymous, 2008. Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives OJ L 354/16, 13.12.2008.

Anonymous, 2009a. J.P. Euzéby: List of Prokaryotic names with Standing in Nomenclature - Genus *Paenibacillus*. LSPN (http://www.bacterio.cict.fr/p/paenibacillus.html#r)

Anonymous, 2009c. J.P. Euzéby: List of Prokaryotic names with Standing in Nomenclature - Genus *Serratia*. LPSN (http://www.bacterio.cict.fr/s/serratia.html)

Anonymous, 2009b. J.P. Euzéby: List of Prokaryotic names with Standing in Nomenclature - Genus *Pseudomonas*. (LPSN http://www.bacterio.cict.fr/p/pseudomonas.html)

Avis TJ, Boulanger RR and Bélanger RR, 2000. Synthesis and biological characterization of (Z)-9-heptadecenoic and (Z)-6-methyl-9-heptadecenoic acids: Fatty acids with antibiotic activity produced by *Pseudozyma flocculosa*. J. Chem. Ecol. 26, 987-1000.

Barber KN, Kaupp WJ and Holmes SB, 1993. Specificity testing of the nuclear polyhedrosis virus of the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae). Can. Entomologist 125: 1055-1066.

Baker CA, Hiebert E, Marlow GC and Wisler GC, 1992. Comparative sequence analysis of the Reunion isolate of zucchini yellow mosaic virus. Phytopathol. 82, 1176.

Barchiese F, Caggiano G, Maracci M, Arzeni D, Scalise G and Montagna MT, 1998. Antifungal susceptibility patterns of yeast isolates causing bloodstream infections. J. Antimicrob. Chemotherapy 51, 431-433.

Beachy RN, 1990. Coat-protein-mediated resistance to tobacco mosaic virus: discovery mechanisms and exploitation. Philosophical Transactions of the Royal Society London B. Biol. Sci. 354, 659-664.

Bent SJ, Gucker CL, Oda Y and Forney LJ, 2003. Spatial distribution of *Rhodopseudomonas palustris* ecotypes on a local scale. Appl Environ Microbiol 69, 9, 5192-7.

Berchieri A, Lovell MA and Barrow PA, 1991. The activity in the chicken alimentary tract of bacteriophages lytic for *Salmonella typhimurium*. Res. Microbiol. 142, 541-549.

Bert F, Ouahes O and Lambertzechovsky N, 1995. Brain-Abscess Due to Bacillus-Macerans Following a Penetrating Periorbital Injury. J. Clin. Microbiol. 33, 7, 1950-1953.

Bolignano G and Criseo G, 2003. Disseminated nosocomial fungal infection by *Aureobasidium pullulans* var. *melanigenum*: a case report. J Clin Microbiol. 41, 9, 4483-5.

Bolsen KK, Bonilla DR, Huck GL, Youung MA and Hart-Thakur RA, 1996. Effect on propionic acid bacterial inoculants on fermentation and aerobic stability of wheat and corn silage. In: Report of Progress of Kansas State University Agricultural Experiment Station. pp. 78 – 81. Manhattan, KS, Kansas State University, USA.

Brabban AD, Hite E and Callaway TR, 2005. Evolution of foodborne pathogens via temperate bacteriophage-mediated gene transfer. Foodborne Pathog. Dis. 2, 287-303.

Brandl MT and Mandrell RE, 2002. Fitness of *Salmonella enterica* serovar Thompson in the cilantro phyllosphere. Appl. Environ. Microbiol. 68, 3614–3621.

Brüssow H, Canchaya C, and Hardt WD, 2004. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. Microbiol. Mol. Biol. Rev. 68, 560-602.

Brüssow H and Desiere F, 2006. Evolution of tailed phages: insights from comparative phage genomics. In R. Calendar (ed), The Bacteriophages, Plenum Publishing Corporation, New York, USA.

Burges HD, Croizier G and Huber J, 1980. A review of safety tests on baculoviruses. *Entomophaga* 25, 329-339.

Calendar R, 2006. The Bacteriophages. New York, USA, Plenum Publishing Corporation.

Cappelli EA, Barros RR, Camello TC, Teixeira LM, Merquior VL, 1999. Leuconostoc pseudomesenteroides as a cause of nosocomial urinary tract infections. J. Clin. Microbiol. 37,12, 4124-6.

Carlin F, Nguyen-The C, Morris CE, 1996. The influence of the background microflora on the fate of *Listeria monocytogenes* on minimally processed fresh broad leaved endive (*Cichorium endivia* var. *latifolia*). J. Food Prot. 59, 698-703.

Carlozzi P and Sacchi A, 2001. Biomass production and studies on *Rhodopseudomonas palustris* grown in an outdoor, temperature controlled, underwater tubular photobioreactor. J. Biotechnol. 88, 3, 239-49.

Carlton RM, Noordman WH, Biswas B, de Meester ED and Loessner MJ, 2005. Bacteriophage P100 for control of *Listeria monocytogenes* in foods: genome sequence, bioinformatic analyses, oral toxicity study, and application. Regul. Toxicol. Pharmacol. 43, 301-312.

Carr JG and Davies PA, 1972. The ecology and classification of strains of *Lactobacillus collinoides* nov. spec.: a bacterium commonly found in fermenting apple juice. J. Appl. Bacteriol. 35, 3, 463-71.

Chamkh M, Sayadi S, Bru V, Godon JJ, 2008. Microbial diversity in Tunisian olive fermentation brine as evaluated by small subunit rRNA - Single strand conformation polymorphism analysis. Int. J. Food Microbiol. 122, 1-2, 211-5.

Chen J and Novick RP, 2009. Phage-mediated intergeneric transfer of toxin genes. Science 323, 139-141.

Chi Z, Wang F, Chi Z, Yue L, Liu G and Zhang T, 2009. Bioproducts from *Aureobasidium pullulans*, a biotechnologically important yeast. Appl. Microbiol. Biotechnol. 82, 5, 793-804.

Chin AWTF, Bloemberg GV, Mulders I H, Dekkers LC and Lugtenberg BJ, 2000. Root colonization by phenazine-1-carboxamide-producing bacterium *Pseudomonas chlororaphis* PCL1391 is essential for biocontrol of tomato foot and root rot. Mol. Plant Microbe Interact. 13, 12, 1340-5.

Chin AWTF, van den Broek D, Lugtenberg BJ and Bloemberg GV, 2005. The *Pseudomonas chlororaphis* PCL1391 sigma regulator psrA represses the production of the antifungal metabolite phenazine-1-carboxamide. Mol. Plant Microbe Interact. 18, 3, 244-53.

Cho JJ, Ullman DE, Wheatley E, Holly J and Gonsalves D, 1992. Commercialization of ZYMV cross protection for zucchini production in Hawaii. Phytopathol. 82, 1073

Chuang ON, Schlievert PM, Wells CL, Manias DA, Tripp TJ and Dunny GM, 2009. Multiple functional domains of *Enterococcus faecalis* aggregation substance Asc10 contribute to endocarditis virulence. Infect. Immun. 77, 1, 539-48.

Claus D and Berkeley RCW, 1986. Genus *Bacillus* Cohn 1872, 174AL, p. 1105–1139. In: Sneath P HA, Mair NS, Sharpe ME, and Holt JG, (eds.), Bergey's manual of systematic bacteriology, vol. 2. Williams and Wilkins, Baltimore, Md. Springer, New York.

Coburn PS, Baghdayan AS, Dolan GT and Shankar N, 2008. An AraC-type transcriptional regulator encoded on the *Enterococcus faecalis* pathogenicity island contributes to pathogenesis and intracellular macrophage survival. Infect. Immun. 76, 12, 5668-76.

Contreras GA, DiazGranados CA, Cortes L, Reyes J, Vanegas S, Panesso D, Rincón S, Díaz L, Prada G, Murray BE and Arias CA, 2008. Nosocomial outbreak of *Enteroccocus gallinarum*: untaming of rare species of enterococci. J. Hosp. Infect. 70, 4, 346-52.

Cory JS and Hails RS, 1997. The ecology and biosafety of baculoviruses. Curr. Opinion Biotechnol. 8, 323-327.

Crow VL, Martley FG, Collbear T and Roundhill SJ, 1995. The influence of phage assisted lysis of *Lactococcus lactis* subsp. *lactis* ML8 on Cheddar cheese ripening. Int. Dairy J. 5, 451-472.

De Conti RM, Porto ALM, Augusto J, Rodrigues R, Moran PJS, Manfio GP and Marsaioli AJ, 2001. Microbial reduction of cyclohexanones. J. Mol. Catalysis B: Enzymatic 11, 4-6, 233-236.

Dellaglio F, Torriani S and Felis GE, 2004. Reclassification of *Lactobacillus cellobiosus* Rogosa et al. 1953 as a later synonym of *Lactobacillus fermentum* Beijerinck 1901. Int. J. Syst. Evol. Microbiol. 54, 3, 809-12.

Desbiez C, Wipf-Scheibel C and Lecoq H, 2002. Biological and serological variability, evolution and molecular epidemiology of *Zucchini yellow mosaic virus* (ZYMV, *Potyvirus*) with special reference to Caribbean islands. Virus Res. 85, 5–16.

Dicks LMT, Dellaglio F and Collins MD, 1995. Proposal to reclassify *Leuconostoc oenos* as Oenococcus oeni [corrig.] gen. nov., comb. nov. Int. J. Syst. Bacteriol. 45, 395-397

Doyle CJ, Hirst ML, Cory JS and Entwistle PF, 1990. Risk assessment studies: detailed host range testing of wild-type cabbage moth, *Mamestra brassicae* (Lepidoptera: Noctuidae) nuclear polyhedrosis virus. Appl. Environ. Microbiol. 56, 2704-2710.

EFSA, 2007a. Scientific Opinion of the Scientific Committee on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. The EFSA Journal 578, 1-16.

EFSA, 2007b. Monitoring of verotoxigenic Escherichia coli (VTEC) and identification of human pathogenic VTEC types. Scientific Opinion of the Panel on Biological Hazards. The EFSA Journal 579, 1-61.

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. Technical guidance. Update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance. Prepared by the Panel on Additives and Products or Substances used in Animal Feeds. *The EFSA Jounal* 732. 1-15.

EFSA, 2008c. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the safety and efficacy of Bactocell PA (*Pediococcus acidilactici*), as feed additives for fish. EFSA-Q-2007-205. Summary of Opinion.

EFSA, 2008d. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the safety and efficacy of Yea-Sacc$^{1026®}$ (*Saccharomyces cerevisiae*), as feed additives for horses. The EFSA Journal 991, 1-14.

EFSA, 2008e. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the safety and efficacy of Bactocell PA (*Pediococcus acidilactici*) as feed additive for shrimps. EFSA-Q-2008-421. Summary of Opinion.

EFSA, 2008f. 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 feed additives for sows. The EFSA Journal 970, 1-9.

EFSA, 2009a. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Scientific Opinion on the safety and efficacy of *Bacillus subtilis* PB6 as a feed additive for chickens for fattening on request from the European Commission. EFSA Journal 7, 9, 1314.

EFSA, 2009b. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) Scientific Opinion on the safety and efficacy of MycoCell (*Saccharomyces cerevisiae*). EFSA Journal 7, 10, 1353.

EFSA, 2009c. Scientific Opinion of the Panel on Biological Hazards on a request from European Commission on The use and mode of action of bacteriophages in food production. The EFSA Journal 1076, 1-26.

Ellis DE, Whitman PA and Marshall RT, 1973. Effects of homologous bacteriophage on growth of *Pseudomonas fragi* WY in milk. Appl. Microbiol. 25, 24-25.

Environmental Protection Agency (EPA) 1996. Microbial pesticide test guidelines: Acute oral toxicity / pathogenicity. Office of Prevention, Pesticides and Toxic Substances 885.3050, pp6.

Environmental Protection Agency EPA, 2007. Biopesticide Registration Action Document for Zucchini Yellow Mosaic Virus – Weak Strain PV-593. Office of Pesticide Programs, pp31.

Erlandson M, 2008. Insect pest control by viruses. In: Encyclopedia of Virology, Mahy BWJ and van Regenmortel MHV (Eds.) Academic Press, Oxford, p. 125-133.

Ewing WH, Davis BR, Fife MA and Lessel EF, 1973. Biochemical characterization of *Serratia liquefaciens* (Grimes and Hennerty) Bascomb et al. (formerly *Enterobacter liquefaciens*) and *Serratia rubidaea* (Stapp) comb. nov. and designation of type and neotype strains. Int. J. System. Bacteriol. 23, 217-225.

Farhat H, Chachaty E, Antoun S, Nitenberg G and Zahar JR, 2008. Two cases of *Bacillus* infection and immunodepression. Medecine Maladies Infectieuses 38, 11, 612-614.

Filya I, Sucu E and Karabulut AA, 2004. The effect of *Propionibacterium acidipropionici*, with or without *Lactobacillus plantarum*, on the fermentation and aerobic stability of wheat, sorghum and maize silages. J.Appl. Microbiol. 97, 818-826.

Fitzpatrick LR, Small J, Hoerr RA, Bostwick EF, Maines L and Koltun WA, 2008. In vitro and in vivo effects of the probiotic *Escherichia coli* strain M-17: immunomodulation and attenuation of murine colitis. Brit. J. Nutrition 100, 3, 530-541.

Fiorentin L, Vieira ND and Barioni Jr. W, 2005. Oral treatment with bacteriophages reduces the concentration of *Salmonella enteritidis* PT4 in caecal contents of broilers. Avian Pathol. 34, 258-263.

Fujii T, Nakashima K and Hayashi N, 2005. Random amplified polymorphic DNA-PCR based cloning of markers to identify the beer-spoilage strains of *Lactobacillus brevis*, *Pediococcus*

damnosus, *Lactobacillus collinoides* and *Lactobacillus coryniformis*. J. Appl. Microbiol. 98, 5, 1209-20.

Fukao M, Tomita H, Yakabe T, Nomura T, Ike Y and Yajima N, 2009. Assessment of antibiotic resistance in probiotic strain *Lactobacillus brevis* KB290. J. Food Prot. 72, 9, 1923-9.

Fulton RW, 1986. Introduction to classical cross-protection for plant virus disease control. Annual Rev. Phytopathol. 24, 67-81.

Galanos C, Roppel J, Weckesser J, Rietschel ET and Mayer H, 1977. Biological activities of lipopolysaccharides and lipid A from *Rhodospirillaceae*. Infect. Immunity 16, 2, 407-12.

Gal-On A and Shiboleth YM, 2006. Cross-Protection. In: Natural Resistance Mechanisms of Plants to Viruses. Loebenstein G and Carr JP (Eds.) p. 261-288.

Gal-On A, 2007. Zucchini yellow mosaic virus: insect transmission and pathogenicity – the tails of two proteins. Mol. Plant Pathol. 8, 139–150.

Gamage SA, Spicer JA, Rewcastle GW, Milton J, Sohal S, Dangerfield W, Mistry P, Vicker N, Charlton PA and Denny WA, 2002. Structure-activity relationships for pyrido-, imidazo-, pyrazolo-, pyrazino-, and pyrrolophenazinecarboxamides as topoisomerase-targeted anticancer agents. J. Medicinal Chem. 45, 3, 740-743.

Garai G, Dueñas MT, Irastorza A and Moreno-Arribas MV, 2007. Biogenic amine production by lactic acid bacteria isolated from cider. Lett. Appl. Microbiol. 45, 5, 473-8.

Garcia P, Madera C, Martinez B and Rodriguez A, 2007. Biocontrol of *Staphylococcus aureus* in curd manufacturing processes using bacteriophages. Int. Dairy J. 17, 1232-1239.

Gauwerky K, Borelli C and Korting C, 2009. Targeting virulence: A new paradigm for antifungals. Drug Discovery Today 14, 214-222.

Getha K, Chong VC and Viineswary S, 1998. Potential use of the phototrophic bacterium *Rhodopseudomonas palustris*, as an aquaculture feed. Asian Fisheries Sci. 10. 223-232.

Gobeli S, Goldschmidt-Clermont E, Frey J and Burr SE, 2009. *Pseudomonas chlororaphis* strain JF3835 reduces mortality of juvenile perch, *Perca fluviatilis* L., caused by *Aeromonas sobria*. J. Fish Dis. 32, 7, 597-602.

Greer GG, 1982. Psychrotrophic bacteriophages for beef spoilage pseudomonads. J. Food Prot. 45, 1318-1325.

Grill LK, Palmer KE and Pogue GP, 2005. Use of plant viruses for production of plant-derived vaccines. Crit. Rev. Plant Sci. 24, 309-323.

Gröner A, 1986. Specificity and safety of baculoviruses. In: Biology of Baculoviruses, Volume I. (Granados RR and Federici BA, Eds.). CRC Press, Boca Raton, Florida. pp. 177−202.

Grozdanov L, Raasch C, Schulze E, Sonnenborn U, Gottschalk G, Hacker J and Dobrindt U, 2004. Analysis of the genome structure of the nonpathogenic probiotic *Escherichia coli* strain Nissle 1917. J. Bacteriol. 186, 16, 5432-5441.

Guinebretiere M-H, Berge O, Normand P, Morris C, Carlin F and Nguyen-The C, 2001. Identification of bacteria in pasteurized zucchini purees stored at different temperatures and comparison with those found in other pasteurized vegetable purees. Appl. Environ. Microbiol. 67, 10, 4520-4530.

Gunther NWT, Nunez A, Fett W and Solaiman DK, 2005. Production of rhamnolipids by *Pseudomonas chlororaphis*, a nonpathogenic bacterium. Appl. Environ. Microbiol. 71, 5, 2288-93.

Hagens S and Offerhaus ML, 2008. Bacteriophages - New Weapons for Food Safety. Food Technol. 62, 46-54.

Han SH, Lee SJ, Moon JH, Park KH, Yang KY, Cho BH, Kim KY, Kim YW, Lee MC, Anderson AJ and Kim YC, 2006. GacS-dependent production of 2R, 3R-butanediol by *Pseudomonas chlororaphis* O6 is a major determinant for eliciting systemic resistance against *Erwinia carotovora* but not against *Pseudomonas syringae* pv. *tabaci* in tobacco. Mol. Plant Microbe Interact. 19, 8, 924-30.

Hanlon GW, 2007. Bacteriophages: an appraisal of their role in the treatment of bacterial infections. Int. J. Antimicrob. Agents. 30, 118-128.

Heikens E, Leendertse M, Wijnands LM, van Luit-Asbroek M, Bonten MJ, van der Poll T and Willems RJL, 2009. Enterococcal surface protein Esp is not essential for cell adhesion and intestinal colonization of *Enterococcus faecium* in mice. BMC Microbiol. 9, 19.

Heimpel AM, Thomas ED, Adams JR and Smith LJ, 1973. The presence of nuclear polyhedrosis viruses of *Trichoplusia ni* on cabbage from the market shelf. Environ. Entomol. 2, 72-75.

Hendrickx AP, Bonten MJ, van Luit-Asbroek M, Schapendonk CM, Kragten AH and Willems RJ, 2008. Expression of two distinct types of pili by a hospital-acquired *Enterococcus faecium* isolate. Microbiol. 154, 10, 3212-23.

Henker J, Laass M, Blokhin BM, Bolbot YK, Maydannik VG, Elze M, Wolff C and Schulze J, 2007. The probiotic *Escherichia coli* strain Nissle 1917 (EcN) stops acute diarrhoea in infants and toddlers. European J. Pediatrics 166. 4, 311-318.

Herniou EA and Jehle JA, 2007. *Baculovirus* phylogeny and evolution. Curr. Drug Targets 8, 1043-50.

Hof H, 2008. Will resistance in fungi emerge on a scale similar to that seen in bacteria? Eur J Clin Microbiol Infect Dis. 2008. 27, 5, 327-34.

Hu Y, 2006. *Baculovirus* vectors for gene therapy. Adv. Virus Res. 68, 287-320.

Huang DB, Mohanty A, DuPont HL, Okhuysen PC and Chiang T, 2006. A review of an emerging enteric pathogen: enteroaggregative *Escherichia coli*. J. Medical Microbiol. 55, 10, 1303-1311.

Huber J, 1986. Use of baculoviruses in pest management programs. In: The biology of baculoviruses. Volume II: Practical applications for insect control (Granados RR and Federici BA, Eds.), CRC Press, Boca Raton, Florida. pp. 181-202.

Hudson JA, Billington C, Carey-Smith G and Greening G, 2005. Bacteriophages as biocontrol agents in food. J. Food Prot. 68, 426-437.

Hughes LE and Marks J, 1985. *Serratia rubidaea* isolated from a silastic foam dressing. Infection 13, 2, 90.

Hunter-Fujita FR, Entwistle PF, Evans HF and Crook NE, 1998. Insect viruses and pest management. John Wiley and Sons, Inc., New York.

Hwang YH, Kim MS, Song IB, Park BK, Lim JH, Park SC and Yun HI, 2009. Subacute (28 day) Toxicity of Surfactin C, a Lipopeptide Produced by *Bacillus subtilis*, in Rats. J. Health Sci. 55, 3, 351-355.

ICTV, 2005. 8th Report, *Potyviridae*. In: Virus Taxonomy (Fauquet CM, Mayo MA, Maniloff J, Desselberger U and Ball LA (Eds.).

ICTV, 2008. International Committee on Taxonomy of Viruses (ICTV). 2008 update. www.ictvonline.org

Ignoffo CM, 1973. Effects of entomopathogens on vertebrates. Annals of the New York Academy of Sciences 217, 141-172.

Isu NR and Njoku HO, 1997. An evaluation of the microflora associated with fermented African oil bean (*Pentaclethra macrophylla* Bentham) seeds during ugba production. Plant Foods for Human Nutrition 51, 2, 145-157.

Jaureguy F, Landraud L, Passet V, Diancourt L, Frapy E, Guigon G, Carbonnelle E, Lortholary O, Clermont O, Denamur E, Picard B, Nassif X and Brisse S, 2008. Phylogenetic and genomic diversity of human bacteremic *Escherichia coli* strains. Bmc Genomics 9, 560.

Jehle JA, Blissard GW, Bonning BC, Cory JS, Herniou EA, Rohrmann GF, Theilmann DA, Thiem SM and Vlak JM, 2006. On the classification and nomenclature of baculoviruses: a proposal for revision. Arch. Virology 151, 1257-1266.

Jofre J, 2009. Is the replication of somatic coliphages in water environments significant? J. Appl. Microbiol. 106, 1059-1069.

Johnson JR and Russo TA, 2005. Molecular epidemiology of extraintestinal pathogenic (uropathogenic) *Escherichia coli*. Int. J. Medical Microbiol. 295, 6-7, 383-404.

Joondeph HC and Nothnagel AF, 1983. *Serratia rubidae* endophthalmitis following penetrating ocular injury. Ann. Ophthalmol. 15, 12, 1138-40.

Kalbe C, Marten P and Berg G, 1996. Strains of the genus *Serratia* as beneficial rhizobacteria of oilseed rape with antifungal properties. Microbiol. Res. 151, 4, 433-9.

Kaper JB, Nataro JP and Mobley HLT, 2004. Pathogenic *Escherichia coli*. Nature Rev. Microbiol. 2, 2, 123-140.

Konstantinidou-Doltsinis S, Markellou E, Kasselaki AM, Siranidou E, Kalamarakis A, Tzembelikou K, Schmitt A, Koumakis C and Malathrakis M, 2007. Control of powdery mildew of grape in Greece using Sporodex (R) L and Milsana (R). J. Plant Dis. Prot. 114, 256-262.

Kruis W, Fric P, Pokrotnieks J, Lukas M, Fixa B, Kascak M, Kamm MA, Weismueller J, Beglinger C, Stolte M, Wolff C and Schulze J, 2004. Maintaining remission of ulcerative colitis with the probiotic *Escherichia coli* Nissle 1917 is as effective as with standard mesalazine. Gut 53, 11, 1617-1623.

Kurztman CP and Fell JW (eds.) 2000. The Yeasts: A taxonomic study. Fourth revised and enlarged edition. Elsevier, Amsterdam.

Lavaggi ML, Cabrera M, Gonzalez M and Cerecetto H, 2008. Differential enzymatic reductions governing the differential hypoxia-selective cytotoxicities of phenazine 5,10-dioxides. Chem. Res. Toxicol. 21, 9, 1900-1906.

Leathers TD, 2003. Biotechnological production and applications of pullulan. Appl. Microbiol. Biotechnol. 62, 5-6, 468-73.

Lecoq H, Lemaire JM and Wipf-Scheibel C, 1991. Control of zucchini yellow mosaic virus in squash by cross protection. Plant Dis. 75, 208-211.

Lecoq H, Wipf-Schneibel C, Chandeysson C, Lê Van A, Fabre F and Desbiez C, 2009. Molecular epidemiology of zucchini yellow mosaic virus in France: an historical overview. Virus Res. 141, 190-200.

Letarov A and Kulikov E, 2009. The bacteriophages in human- and animal body-associated microbial communities. J. Appl. Microbiol. 107, 1-13.

Li D and Calderone RA, 2004. Antifungal Drugs, Targets and Targets Discovery. In: Pathogenic Fungi. Hosts Interactions and Emerging Strategies for Control (Ed: G. San-Blas and R.A. Calderone). Caister Academic Press, England.

Li B, Ravnskov S, Xie G.L and Larsen J, 2007. Biocontrol of *Pythium* damping-off in cucumber by arbuscular mycorrhiza-associated bacteria from the genus *Paenibacillus*. Biocontrol 52, 6, 863-875.

Li B, Ravnskov S, Xie GL and Larsen J, 2008a. Differential effects of *Paenibacillus* spp. on cucumber mycorrhizas. Mycological Progress 7, 4, 277-284.

Li J, Halgamuge SK and Tang SL, 2008b. Genome classification by gene distribution: an overlapping subspace clustering approach. BMC Evol. Biol. 8, 116-131.

Lima-Mendez GJ, Van Helden A, Toussaint A and Leplae R, 2008. Reticulate representation of evolutionary and functional relationships between phage genomes. Mol. Biol. Evol. 25, 762-777.

Lisa V and Lecoq H, 1984. Zucchini yellow mosaic virus. In: Murant, A. F. and Harrison, B. D. (Eds) CMI/AAB Description of plant viruses, No. 282. AAB, UK.

Liu J, Glazko G and Mushegian A, 2006. Protein repertoire of double-stranded DNA bacteriophages. Virus Res. 117, 68-80.

Liu H, He Y, Jiang H, Peng H, Huang X, Zhang X, Thomashow LS and Xu Y, 2007. Characterization of a phenazine-producing strain *Pseudomonas chlororaphis* GP72 with broad-spectrum antifungal activity from green pepper rhizosphere. Curr. Microbiol. 54, 4, 302-6.

Livermore DM and Woodford N, 2006. The beta-lactamase threat in *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter*. Trends Microbiol. 14, 9, 413-420.

Lucas PM, Claisse O and Lonvaud-Funel A, 2008. High frequency of histamine-producing bacteria in the enological environment and instability of the histidine decarboxylase production phenotype. Appl. Environ. Microbiol. 74, 3, 811-7.

Mahajan-Miklos S, Tan MW, Rahme LG and Ausubel FM, 1999. Molecular mechanisms of bacterial virulence elucidated using a *Pseudomonas aeruginosa* Caenorhabditis elegans pathogenesis model. Cell 96, 1, 47-56.

Maiorano AE, Piccoli RM, da Silva ES and Rodrigues MFD, 2008. Microbial production of fructosyltransferases for synthesis of pre-biotics. Biotechnol. Lett. 30, 11, 1867-1877.

Martignoni ME and Iwai PJ, 1986. A catalogue of virus diseases in insects, mites and ticks (4th ed). General Technical report PNW-195 US Department of Agriculture, Forestry Service, Pacific Northwest Research Station, Portland, Oregon, USA.

Matthews REF, 1949. Studies on potato virus X. II. Criteria of relationship between strains. Ann. Appl. Biol. 36, 460-474.

Matthews AM and Novick RP, 2005. Staphylococcal phages. In: Waldor MK, Friedman DI and Adhya SL (Eds.) Bacteriophages. Their role in bacterial pathogenesis and Biotechnology. Washington D.C. USA, ASM Press.

Matsuyama T, Kaneda K, Ishizuka I, Toida T and Yano I, 1990. Surface-active novel glycolipid and linked 3-hydroxy fatty acids produced by *Serratia rubidaea*. J. Bacteriol. 172, 6, 3015-22.

Matsuzaki S, Rashel M, Uchiyama J, Sakurai S, Ujihara T, Kuroda M, Ikeuchi M, Tani T, Fujieda M, Wakiguchi H and Imai S. 2005. Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. J. Infect. Chemother. 11, 211-219.

Mazodier P, Petter R and Thompson C, 1989. Intergeneric conjugation between *Escherichia coli* and *Streptomyces* species. J. Bacteriol. 171, 3583-3585.

McCall KE and Duncan CJ, 1995. The action of phenazine methosulphate in causing cellular damage in the isolated mouse soleus muscle preparation. Pathobiol. 63, 5, 278-282.

McGrath S, Fitzgerald GF and van Sinderen D, 2007. Bacteriophages in dairy products: pros and cons. Biotechnol. J. 2, 450-455.

McGrath S and van Sinderen D, 2007. Bacteriophages: Genetics and molecular biology. Norfolk, UK, Horizon Scientific Press.

McKinney HH, 1929. Mosaic diseases in the Canary Islands, West Africa and Gibraltar. J. Agricultural Res. 39, 557-578.

McClure P, 2005. *Escherichia coli*: virulence, stress response and resistance. In: Understanding pathogen behaviour. M. Griffiths. Woodhead Publishing Ltd, Cambridge, 240-278.

Mehrabi S, Ekanemesanga UM, Aikhionbareb FO, Kimbroa KS and Bendera J, 2001. Identification and characterization of *Rhodopseudomonas* spp., a purple, non-sulfur bacterium from microbial mats. Biomolecular Engineering 18, 49-56

Miller RV, 2001. Environmental bacteriophage-host interactions: factors contribution to natural transduction. Antonie Van Leeuwenhoek 79, 141-147.

Miltenburger HG, 1978. No effect of NPV on mammalian cells in vivo and in vitro (cell proliferation; chromosome structure). In: Safety aspects of baculoviruses as biological insecticides (H.G. Miltenburger, Ed.), pp. 185-202. Bundesministerium für Forschung und Technologie, Bonn, Darmstadt, Deutschland.

Moscardi F, 1999. Assessment of the application of baculoviruses for control of *Lepidoptera*. Ann. Rev. Entomol. 44, 257-289.

Mugula JK, Narvhus JA and Sørhaug T, 2003. Use of starter cultures of lactic acid bacteria and yeasts in the preparation of togwa, a Tanzanian fermented food. Int J Food Microbiol. 83, 3, 307-18.

Nakkeeran S, Kavitha K, Chandrasekar G, Renukadevi P and Fernando WGD, 2006. Induction of plant defence compounds by *Pseudomonas chlororaphis* PA23 and Bacillus subtilis BSCBE4 in controlling damping-off of hot pepper caused by *Pythium aphanidermatum*. Biocontrol Sci. Technol. 16, 4, 403-416.

Nallapareddy SR, Singh KV and Murray BE, 2008a. Contribution of the collagen adhesin Acm to pathogenesis of *Enterococcus faecium* in experimental endocarditis. Infect. Immun. 76, 9, 4120-8.

Nallapareddy SR, Singh KV, Okhuysen PC and Murray BE, 2008b. A functional collagen adhesin gene, acm, in clinical isolates of *Enterococcus faecium* correlates with the recent success of this emerging nosocomial pathogen. Infect. Immun. 76, 9, 4110-9.

Nelson D, 2004. Phage taxonomy: we agree to disagree. J. Bacteriol. 186, 7029-7031.

Nielsen DS, Teniola OD, Ban-Koffi L, Owusu M, Andersson TS and Holzapfel WH, 2007. The microbiology of Ghanaian cocoa fermentations analysed using culture-dependent and culture-independent methods. Int. J. Food Microbiol. 114. 2, 168-86.

Novick RP, Edelman I and Lofdahl S, 1986. Small *Staphylococcus aureus* plasmids are transduced as linear multimers that are formed and resolved by replicative processes. J. Mol. Biol. 192, 209-220.

Oda Y, Larimer FW, Chain PS, Malfatti S, Shin MV, Vergez LM, Hauser L, Land ML, Braatsch S, Beatty JT, Pelletier DA, Schaefer AL and Harwood CS, 2008. Multiple genome sequences reveal adaptations of a phototrophic bacterium to sediment microenvironments. Proc. Natl. Acad. Sci. U.S.A. 105, 47, 18543-18548.

O'Connor SP, Dorsch M, Steigerwalt AG, Brenner DJ, Stackebrandt E, 1991. 16S rRNA sequences of *Bartonella bacilliformis* and cat scratch disease bacillus reveal phylogenetic relationships with the alpha-2 subgroup of the class Proteobacteria. J. Clin. Microbiol. 29, 10, 2144-50

Okada T, Yokota E and Matsumoto I, 2002. Community acquired sepsis by *Serratia rubidaea*. Kansenshogaku Zasshi 76, 2, 109-12.

Oliveira L, Alonso JC and Tavares P, 2005. A defined in vitro system for DNA packaging by the bacteriophage SPP1: insights into the headful packaging mechanism. J. Mol. Biol. 353, 529-539.

Ongena M and Jacques P, 2008. *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. Trends Microbiol. 16, 3, 115-125.

Parment PA, Ursing J and Palmer B, 1984. *Serratia rubidaea* isolated from a silastic foam dressing. Infection 12, 4, 268-9.

Parvathi A, Krishna K, Jose J, Joseph N and Nair S, 2009. Biochemical and molecular characterisation of *Bacillus pumilus* isolated from coastal environment in Chohin, India. Brazilian J. Microbiol. 40, 269-275.

Peix A, Valverde A, Rivas P, Igual JM, Ramírez-Bahena M-H, Mateos PF, Santa-Regina I, Rodríguez-Barrueco C, Martinez-Molina E and Velázquez E, 2007. Reclassification of *Pseudomonas aurantiaca* as a synonym of *Pseudomonas chlororaphis* and proposal of three subspecies, *P. chlororaphis* subsp. *chlororaphis* subsp. nov., *P. chlororaphis* subsp. *aureofaciens* subsp. nov., comb. nov. and *P. chlororaphis* subsp. *aurantiaca* subsp. nov., comb. nov. Int. J. Syst. Evol. Microbiol. 57, 1286-1290.

Perring TM, Farrar CA, Blua MJ, Wang HL and Gonsalves D, 1995. Cross protection of cantaloupe with a mild strain of zucchini yellow mosaic virus: effectiveness and application. Crop Protection 14, 601-606.

Pfosser MH and Baumann H, 2002. Phylogeny and geographical of Zucchini yellow mosaic virus isolates (*Potyviridae*) based on molecular analysis of the coat protein and part of the cytoplasmic inclusion protein genes. Archives Virol. 147, 1599-1609.

Plumed-Ferrer C and von Wright A, 2009. Fermented pig liquid feed: nutritional, safety and regulatory aspects. J. Appl. Microbiol. 106, 351-368.

Prasad R, Panwar SL and Krishnamurthy S, 2002. Drug Resistance Mechanisms of Human Pathogenic Fungi. In: Fungal Pathogenesis: Principles and Clinical Applications. Calderone RA and Cihlar RL (eds.) Marcel Dekker, Inc. USA.

Prasad R and López-Ribot JL, 2004. Fungal Biofilms and Drug Resistance. In: Pathogenic Fungi. Hosts Interactions and Emerging Strategies for Control. San-Blas G and Calderone RA (eds.) Caister Academic Press, England.

Quiberoni A, Guglielmotti D and Reinheimer J, 2008. New and classical spoilage bacteria causing widespread blowing in Argentinean soft and semihard cheeses. Int. J. Dairy Technol. 61, 4, 358-363.

Qi Zizhong, Zhang Xiao-Hua, Nico B and Peter B, 2009. Probiotics in aquaculture of China - Current state, problems and prospect. Aquaculture Nut. 290, 15-21.

Rast ATB. 1972. MII-16, an artificial symptomless mutant of tobacco mosaic virus for seedling inoculation of tomato crops. Netherlands J. Plant Pathol. 78, 110-112.

Ratcliff FG, MacFarlane SA and Baulcombe DC, 1999. Gene silencing without DNA RNA-mediated cross protection between viruses. Plant Cell 11, 1207-1215.

Raya R, Varey P, Oot RA, Dyen MR, Callaway TR, Edrington TS, Kutter EM and Brabban AD, 2006. Isolation and characterization of a new T-even bacteriophage, CEV1, and determination of its potential to reduce *Escherichia coli* O157:H7 levels in sheep. Appl. Environ. Microbiol. 72, 6405-6410.

Reimann R and Miltenburger HG, 1983. Cytogenetic studies in mammalian cells after treatment with insect pathogenic viruses [*Baculoviridae*]. II. In vitro studies with mammalian cell lines. Entomophaga 28, 33-44.

Rice LB, Carias L, Rudin S, Vael C, Goossens H, Konstabel C, Klare I, Nallapareddy SR, Huang W, and Murray BEA, 2003. A potential virulence gene, hylEfm, predominates in *Enterococcus faecium* of clinical origin. J. Infect. Dis. 187, 508-512.

Rodriguez-Jerez JJ, Giaccone V, Colavita G and Parisi E, 1994. *Bacillus macerans* - a new potent histamine producing microorganism isolated from Italian cheese. Food Microbiol. 11, 5, 409-415.

Rodríguez J, Saavedra J, Fernández-Jurado A and Prados D, 1999. *Leuconostoc pseudomesenteroides* bacteremia. Sangre (Barc) 44, 1, 82-3.

Rogoff MH, 1975. Exposure of humans to nuclear polyhedrosis virus during industrial production. In: Baculoviruses for Insect Pest Control: Safety Considerations (Summers MD, Engler R, Falcon LA, Vail PV, Eds.) American Society of Microbiology, Washington, D.C., pp. 102-103.

Rohwer F and Edwards R, 2002. The Phage Proteomic Tree: a genome-based taxonomy for phage. J. Bacteriol. 184, 4529-4535.

Roman-Blanco C, Sanz-Gomez JJ, Lopez-Diaz TM, Otero A and Garcia-Lopez ML, 1999. Numbers and species of *Bacillus* during the manufacture and ripening of Castellano cheese. Milchwissenschaft-Milk Sci. Int. 54, 7, 385-388.

Ron EZ, 2006. Host specificity of septicemic *Escherichia coli*: human and avian pathogens. Current Opin. Microbiol. 9,28–32.

Rossetti L, Carminati D, Zago M and Giraffa G, 2009. A qualified presumption of safety approach for the safety assessment of Grana Padano whey starters. Int. J. Food Microbiol. 130, 70-73.

Rossi F and Dellaglio F, 2007. Quality of silages from Italian farms as attested by number and identity of microbial indicators. J. Appl. Microbiol. 103, 5, 1707-1715.

Roy B, Ackermann HW, Pandian S, Picard G and Goulet J, 1993. Biological inactivation of adhering *Listeria monocytogenes* by listeriaphages and a quaternary ammonium compound. Appl. Environ. Microbiol. 59, 2914-2917.

Sanz-Penella JM, Tamayo-Ramos JA, Sanz Y and Haros M, 2009. Phytate reduction in bran-enriched bread by phytase-producing bifidobacteria. J. Agri. Food Chem. 57, 21,10239–10244.

Schroeder B, Duncker S, Barth S, Bauerfeind R, Gruber AD, Deppenmeier S and Breves G, 2006. Preventive effects of the probiotic *Escherichia coli* strain nissle 1917 on acute secretory diarrhea in a pig model of intestinal infection. Digestive Dis. Sci. 51, 4, 724-731.

Sekhsokh Y, Arsalane L, El Ouenass M, Doublali T, Bajjou T and Lahlou Amine I, 2007. *Serratia rubidaea* bacteremia. Med. Mal. Infect. 37, 5, 287-9.

Simmons HE, Holmes EC and Stephenson AG, 2008. Rapid evolutionary dynamics of zucchini yellow mosaic virus. J. Gen. Virol. 89: 1081-1085.

Skerman VBD, McGowan V and Sneath PHA, 1980. Approved list of names. Int. J. System. Bacteriol. 30, 225-420.

Stock I, Burak S, Sherwood K J, Gruger T and Wiedemann B, 2003. Natural antimicrobial susceptibilities of strains of 'unusual' *Serratia* species: *S. ficaria*, *S. fonticola*, *S. odorifera*, *S. plymuthica* and *S. rubidaea*. J. Antimicrob. Chemother. 51, 4, 865-85.

Sulakvelidze A and Kutter E, 2005. Bacteriophage therapy in humans. In: E. Kutter and A. Sulakvelidze (Eds.), Bacteriophages: Biology and Applications, Chapter 14, pp. 381-436. CRC Press.

Summers MD, Engler R, Falcon LA and Vail PV (Eds.) 1975. Baculoviruses for insect pest control: Safety considerations. American Society of Microbiology, Washington, D.C., USA.

Sun JB, Gunzer F, Westendorf AM, Buer J, Scharfe M, Jarek M, Gossling F, Blocker H and Zeng A P, 2005. Genomic peculiarity of coding sequences and metabolic potential of probiotic *Escherichia coli* strain Nissle 1917 inferred from raw genome data. J. Biotechnol. 117, 2, 147-161.

Suwannakham S, Huang Y and Yan S-T, 2005. Construction and characterization of ack knock-out mutants of *Propionibacterium acidipropionici* for enhanced propionic acid fermentation. Biotecnol. Bioeng. 94. 383-395,

Tien P and Wu G, 1991. Satellite RNA for the biocontrol of plant diseases. Advances in Virus Res. 39, 321-339.

Uraz G, Simsek H and Maras Y, 2001. The inhibitory effects of *Lactobacillus casei* and *Lactobacillus helveticus* on *Bacillus* species isolated from raw milk in various salt concentrations. Int. J. Dairy Technol. 54, 4, 146-150.

Ursua PR, Unzaga MJ, Melero P, Iturburu I, Ezpeleta C and Cisterna R, 1996. *Serratia rubidaea* as an invasive pathogen. J. Clin. Microbiol. 34, 1, 216-7.

Van Oers MM and Vlak JM, 2007. *Baculovirus* genomics. Current Drug Targets 8, 1051-1068.

Van Rij ET, Wesselink M, Chin-A-Woeng TFC, Bloemberg GV and Lugtenberg BJJ, 2004. Influence of environmental conditions on the production of phenazine-1-carboxamide by *Pseudomonas chlororaphis* PCL1391. Mol. Plant Microbe Interactions 17, 5, 557-566.

Vanberg C, Lutnaes BJ, Langsrud T, Langsrud T, Nes I and Holo H, 2007. *Propionibacterium jensenii* produces the polyene pigment granadaene and has haemolytic properties similar to those of Streptococcus agalactiae. Appl. Environ. Microbiol. 73. 5501-5506.

Vlak JM, de Gooijer CD, Tramper J and Miltenburger HG (Eds.) 1996. Insect cell cultures: Fundamental and applied aspects. Kluwer Academic Publishers, Dordrecht. pp. 309.

Waldor MK, Friedman DI and Adhya SL, 2005. Bacteriophages. Their role in bacterial pathogenesis and Biotechnology. ASM Press, Washington D.C. USA.

Walkey DGA, Lecoq H, Collier R and Dobson S, 1992. Studies on the control of zucchini yellow mosaic virus in courgettes by mild strain protection. Plant Pathol. 41, 762-771.

Wang Y and Jehle JA, 2009. Nudiviruses and other large, double-stranded circular DNA viruses of invertebrates: New insights on an old topic. J. Invertebrate Pathol. 101, 187-93

Wang J and Fung DYC, 1996. Alkaline-fermented foods: A review with emphasis on pidan fermentation. Crit. Rev. Microbiol. 22, 2, 101-138.

Wiles TJ, Kulesus RR and Mulvey MA, 2008. Origins and virulence mechanisms of uropathogenic *Escherichia coli*. Experiment. Mol. Pathol. 85, 1, 11-19.

Willems RJL and van Schaik W, 2009. Transition of *Enterococcus faecium* from commensal organism to nosocomial pathogen. Future Microbiol. 4, 9, 1125-1135

Wilson SJ, Everts RJ, Kirkland KB and Sexton DJ, 2000. A pseudo-outbreak of *Aureobasidium* species lower respiratory tract infections caused by reuse of single-use stopcocks during bronchoscopy. Infect. Control Hosp. Epidemiol. 21, 7, 470-2.

Yamazaki G, Nishimura S, Ishida A, Kanagasabhapathy M, Zhou X, Nagata S, Morohoshi T and Ikeda T, 2006. Effect of salt stress on pigment production of *Serratia rubidaea* N-1: a potential indicator strain for screening quorum sensing inhibitors from marine microbes. J. Gen. Appl. Microbiol. 52, 2, 113-7.

Yan F and Polk DB, 2004. Commensal bacteria in the gut: learning who our friends are. Curr. Opin. Gastroenterol. 20, 6, 565-571.

Yarden G, Hemo R, Livne H, Maoz E, Lev E, Lecoq H and Raccah B, 2000. Cross protection of Cucurbitaceae from zucchini yellow mosaic virus. I: Proceedings of the 7th EUARPIA meeting on cucurbits genetics and breeding. Acta Horticultura, Katzir N and Paris HS (Eds.), p. 349-356.

Zerza L, Hollis RJ and Pfaller MA, 1996. In vitro susceptibility testing and DNA typing of *Saccharomyces cerevisiae* clinical isolates. J. Clin. Microbiol. 34, 3031-3034.

Zhang T, Breitbart M, Lee WH, Run JQ, Wei CL, Soh SWL, Hibbert ML, Liu ET, Rohwer F and Ruan Y, 2006. RNA viral community in human feces: prevalence of plant pathogenic viruses. PloS Biol. 4, 1, e3.

Zhang Y and LeJeune JT, 2008. Transduction of *bla*$_{CMY-2}$, *tet*(A), and *tet*(B) from *Salmonella enterica* subspecies *enterica* serovar Heidelberg to *S.* Typhimurium. Vet. Microbiol. 129, 418-425.

Zhang A and Yang S-T, 2009. Engineering *Propionibacterium acidipropionici* for enhanced propionic acid tolerance and fermentation. Biotechnol. Bioeng. 104, 4, 766-73.

Zhao X and Calderone RA, 2002. Antifungals Currently Used in the Treatment of Invasive Fungal Diseases. In: Pathogenic Fungi. Hosts Interactions and Emerging Strategies for Control. San-Blas G and Calderone R (eds.) Caister Academic Press, England.

Xu-Xia Zhou, Yuan-Jiang Pan, Yan-Bo Wang and Wei-Fen Li, 2007. In vitro assessment of gastrointestinal viability of two photosynthetic bacteria, *Rhodopseudomonas palustris* and *Rhodobacter sphaeroides*. J. Zhejiang University - Science B 8, 9, 686-692.

APPENDICES

APPENDIX A: THE FORMER 2008 LIST OF QPS GRANTED MICROORGANISMS (EFSA, 2008a)

Gram-Positive Non-Sporulating Bacteria			
Species			**Qualifications *****
Bifidobacterium adolescentis *Bifidobacterium animalis*	*Bifidobacterium bifidum* *Bifidobacterium breve*	*Bifidobacterium longum*	
Corynebacterium glutamicum			QPS status applies only when the species is used for production purposes.
Lactobacillus acidophilus *Lactobacillus amylolyticus* *Lactobacillus amylovorus* *Lactobacillus alimentarius* *Lactobacillus aviaries* *Lactobacillus brevis* *Lactobacillus buchneri* *Lactobacillus casei* ** *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 paracasei* *Lactobacillus paraplantarum* *Lactobacillus pentosus* *Lactobacillus plantarum* *Lactobacillus pontis* *Lactobacillus reuteri* *Lactobacillus rhamnosus* *Lactobacillus sakei* *Lactobacillus salivarius* *Lactobacillus sanfranciscensis*	
Lactococcus lactis			
Leuconostoc citreum	*Leuconostoc lactis*	*Leuconostoc mesenteroides*	
Pediococcus acidilactici	*Pediococcus dextrinicus*	*Pediococcus pentosaceus*	
Propionibacterium. freudenreichii			
Streptococcus thermophilus			
Bacillus			
Species			**Qualifications**
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 stearothermophillus*	Absence of food poisoning toxins*. Absence of surfactant activity.* Absence of enterotoxic activity.*

The former QPS 2008 list (continued).

Yeasts [19]			
Species			**Qualifications**
Debaryomyces hansenii			
Hanseniaspora uvarum			
Kluyveromyces lactis	*Kluyveromyces marxianus*		
Pichia angusta	*Pichia anomala*	*Pichia jadinii*	QPS status applies only when the species is used for production purposes.
Pichia pastoris			
Saccharomyces bayanus	*Saccharomyces cerevisiae*	*Saccharomyces pastorianus (synonym of Saccharomyces carlsbergensis)*	†
Schizosaccharomyces pombe			
Xanthophyllomyces dendrorhous			

** When strains of these QPS units are to be used as seed coating agents, testing for toxic activity is not necessary, provided that the risk of transfer to the edible part of the crop is very low*

** The previously described species "*Lactobacillus zeae*" has been included in the species *Lactobacillus casei*

*** For all QPS bacterial Units, the strains should not carry any transferable antimicrobial resistance, unless cells are not present in the final product, as described in EFSA (2008b).

† *S. cerevisiae*, subtype *boulardii* is contraindicated for patients of fragile health, as well as for patients with a central venous catheter in place.

EFSA, 2008a. Opinion: The maintenance of the list of QPS microorganisms intentionally added to food or feed - Scientific Opinion of the Panel on Biological Hazards (Question number: EFSA-Q-2008-006) www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902221481.htm The EFSA Journal, 2008, 923, 1-48

EFSA, 2008b. Technical guidance. Update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance. Prepared by the Panel on Additives and Products or Substances used in Animal Feeds. The EFSA Journal 732. 1-15.

[19] Yeast Synonyms commonly used in the feed/food industry
Pichia anomola: synonym *Hansenula anomala*, *Saccharomyces anomalus*
Pichia jadinii: anamorph *Candida utilis*; synonyms *Hansenula jadinii*, *Torulopsis utilis*
Saccharomyces cerevisiae synonym *S. boulardii*

APPENDIX B: MICROBIAL SPECIES FROM PREVIOUS NOTIFICATIONS AND AS NOTIFIED TO EFSA

EFSA Panel/ Unit Or as notified previously	Genus and species of microorganism as notified (current taxonomy where different)	Intended use	EFSA question number and published opinion [additional information]	Comments
	Bacteria			
FEEDAP	*Actinoplanes utahensis*	Production of acarbose		No body of knowledge, therefore not considered for QPS
FEEDAP	*Alcaligenes acidovorans* = *Ralstonia* sp.	Biomass for animal feed	EFSA-Q-2004-171 www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620784006.htm	No body of knowledge, therefore not appropriate for QPS
FEEDAP	*Bacillus amyloliquefaciens*	Feed additive	EFSA-Q-2007-190 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902039267.htm	Qualification: Absence of toxin production etc. (see EFSA Opinion, 2008)
FEEDAP	*Bacillus brevis* = *Aneurini bacillus sp.*	Biomass for animal feed	EFSA-Q-2004-171 www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620784006.htm	No body of knowledge, therefore not appropriate for QPS

EFSA Panel/ Unit Or as notified previously	Genus and species of microorganism as notified (current taxonomy where different)	Intended use	EFSA question number and published opinion [additional information]	Comments
(70/524/EEC) FEEDAP	*Bacillus cereus var. toyoi* = *B. cereus*	Feed additive	EFSA-Q-2003-086 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620783486.htm EFSA-Q-2005-021 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620783657.htm EFSA-Q-2006-037 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620781828.htm EFSA-Q-2007-090 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178647331659.htm EFSA-Q-2008-287 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902299515.htm	QPS status inapplicable for the group of *B. cereus* strains (see EFSA Opinion 2007, Appendix B)
1831/2003	*Bacillus coagulans*	Feed additive		Qualification: Absence of toxin production etc. (see EFSA Opinion, 2008)
FEEDAP	*Bacillus firmus* = *Brevibacillus agri*	Biomass for animal feed	EFSA-Q-2004-171 www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620784006.htm	No body of knowledge, therefore not appropriate for QPS
Reg(EC)1831/2003	*Bacillus lentus*	Feed additive		Qualification: Absence of toxin production etc. (see EFSA Opinion, 2008)
SCF Opinion 22 June 2000	*Bacillus licheniformis*	Production of b-cyclodextrin (food additive carrier and stabiliser of food flavours, food colours and some vitamins)		Qualification: Absence of toxin production etc. (see EFSA Opinion, 2008)

EFSA Panel/ Unit Or as notified previously	Genus and species of microorganism as notified (current taxonomy where different)	Intended use	EFSA question number and published opinion [additional information]	Comments
Reg(EC)1831/2003	*Bacillus licheniformis* MBS-BL-01	Feed additive		Qualification: Absence of toxin production etc. (see EFSA Opinion, 2008)
Reg(EC)1831/2003	*Bacillus pumilus*	Feed additive		Qualification: Absence of toxin production etc. (see EFSA Opinion, 2008)
Reg(EC)1831/2003	*Bacillus subtilis*	Feed additive		Qualification: Absence of toxin production etc. (see EFSA Opinion, 2008)
PRAPeR	*Bacillus subtilis* Strain QST 713	Plant protection product	EFSA-Q-2008-492 (In progress)	Qualification: Absence of toxin production etc. (see EFSA Opinion, 2008)
PRAPeR	*Bacillus* subsp. *thuringiensis aizawai* (strains ABTS 1857 and GC-91) = *Bacillus thuringiensis* serovar aizawai	Plant protection product	EFSA-Q-2009-00121 (In progress) EFSA-Q-2009-00247 (In progress) [http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_006494.htm]	Already considered as not appropriate for QPS (see EFSA Opinion, 2007)
PRAPeR	*Bacillus* subsp. *thuringiensis israelensis* (serotype H-14), strain AM 6552 = *Bacillus thuringiensis* serovar israelensis	Plant protection product	EFSA-Q-2009-00122 (in progress) EFSA-Q-2009-00248 (In progress) [http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_006476.htm]	Already considered as not appropriate for QPS (see EFSA, 2007)

EFSA Panel/ Unit Or as notified previously	Genus and species of microorganism as notified (current taxonomy where different)	Intended use	EFSA question number and published opinion [additional information]	Comments
PRAPeR	*Bacillus* subsp. *thuringiensis kurstaki* (strains ABTS 351, PB 54, SA11, SA 12, EG 2348) = *Bacillus thuringiensis* serovar kurstaki	Plant protection product	EFSA-Q-2009-00123 (in progress) EFSA-Q-2009-00249 (In progress) [http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_006452.htm]	Already considered as not appropriate for QPS (see EFSA, 2007)
PRAPeR	*Bacillus* subsp. *thuringiensis tenebrionis* (strain NB176 (TM 141)) = *Bacillus thuringiensis* serovar tenebrionis	Plant protection product	EFSA-Q-2009-00124 (in progress) EFSA-Q-2009-00250 (In progress)	Already considered as not appropriate for QPS (see EFSA, 2007)
FEEDAP	*Bifidobacterium animalis subsp. Animalis*	Feed additive		Already QPS
Reg(EC)1831/2003	*Bifidobacterium animalis subsp. Lactis*	Feed additive		Already QPS
Reg(EC)1831/2003	*Bifidobacterium longum*	Feed additive		Already QPS
FEEDAP	*Clostridium butyricum*	Feed additive	EFSA-Q-2008-303 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902496474.htm	No history of use, therefore not appropriate for QPS
GMO	*Corynebacterium glutamicum* (formerly: *Brevibacterium lactofermentum*)	Dried killed biomass for feed	EFSA-Q-2007-157 (Additional data requested)	The recipient species is QPS, but not for this application, therefore not appropriate for QPS (EFSA 2008 Opinion)

EFSA Panel/ Unit Or as notified previously	Genus and species of microorganism as notified (current taxonomy where different)	Intended use	EFSA question number and published opinion [additional information]	Comments
FEEDAP	*Corynebacterium glutamicum*	Production of L-Arginin	EFSA-Q-2006-031 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620781637.htm	QPS status applies only when the species is used for production purposes (EFSA Opinion, 2007)
Reg(EC)1831/2003	*Enterococcus faecium*	Feed additive		No taxonomical unit within *Enterococcus* can be considered as free of infectious strains. Therefore no recommendation for QPS status (EFSA, 2007)
Reg(EC)1831/2003	*Enterococcus mundtii*	Feed additive		No taxonomical unit within *Enterococcus* can be considered as free of infectious strains. Therefore no recommendation for QPS status (EFSA Opinion, 2007)
GMO	*Escherichia coli*	Dried killed biomasses for feed	EFSA-Q-2008-412a and EFSA-Q-2008-669a (Additional data requested)	QPS 2009 update
FEEDAP	*Escherichia coli*	Dried killed biomasses for feed	EFSA-Q-2008-412b and EFSA-Q-2008-669b (Additional data requested)	QPS 2009 update
FEEDAP	*Escherichia coli*	Feed additive, L-cystein production		QPS 2009 update
FEEDAP	*Escherichia coli*	Feed additive (horses)	EFSA-Q-2005-167 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902391773.htm	QPS 2009 update
FEEDAP	*Eubacterium* sp. DSM 11798	Reduce toxicity of mycotoxins	EFSA-Q-2003-052 www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620782757.htm	No body of knowledge. Already given a negative assessment by FEEDAP. Not appropriate for QPS (EFSA Opinion 2008)
Reg(EC)1831/2003	*Lactobacillus acidophilus*	Feed additive		Already QPS
Reg(EC)1831/2003	*Lactobacillus amylolyticus*	Feed additive		Already QPS

EFSA Panel/ Unit Or as notified previously	Genus and species of microorganism as notified (current taxonomy where different)	Intended use	EFSA question number and published opinion [additional information]	Comments
Reg(EC)1831/2003	*Lactobacillus amylovorans*	Feed additive		Already QPS
Reg(EC)1831/2003	*Lactobacillus brevis*	Feed additive		Already QPS
Reg(EC)1831/2003	*Lactobacilllus buchneri*	Feed additive		Already QPS
Reg(EC)1831/2003	*Lactobacillus bulgaricus* = *L. delbrueckii* subsp. *bulgaricus*	Feed additive		Already QPS
Reg(EC)1831/2003	*Lactobacillus casei* (note: this species is very rare and its identity might need to be verified)	Feed additive		Already QPS
Reg(EC)1831/2003	*Lactobacillus casei rhamnosus* = *Lactobacillus rhamnosus*	Feed additive		Already QPS
Reg(EC)1831/2003	*Lactobacillus cellobiosus*	Feed additive		Not given QPS status (see EFSA Opinion 2008)
Reg(EC)1831/2003	*Lactobacillus collinoides*	Feed additive		Not given QPS status (see EFSA Opinion 2008)
FEEDAP	*Lactobacillus delbrueckii subsp. lactis*	Feed additive		Already QPS
Reg(EC)1831/2003	*Lactobacillus farciminis*	Feed additive		Already QPS
Reg(EC)1831/2003	*Lactobacillus fermentum*	Feed additive		Already QPS
Reg(EC)1831/2003	*Lactobacillus helveticus*	Feed additive		Already QPS
Reg(EC)1831/2003	*Lactobacillus mucosae*	Feed additive		Already QPS

EFSA Panel/ Unit Or as notified previously	Genus and species of microorganism as notified (current taxonomy where different)	Intended use	EFSA question number and published opinion [additional information]	Comments
Reg(EC)1831/2003	*Lactobacillus paracasei*	Feed additive		Already QPS
Reg(EC)1831/2003	*Lactobacillus pentosus*	Feed additive		Already QPS
Reg(EC)1831/2003	*Lactobacillus plantarum*	Feed additive		Already QPS
Reg(EC)1831/2003	*Lactobacillus reuteri*	Feed additive		Already QPS
Reg(EC)1831/2003	*Lactobacillus rhamnosus*	Feed additive		Already QPS
Reg(EC)1831/2003	*Lactobacillus sakei*	Feed additive		Already QPS
Reg(EC)1831/2003	*Lactobacillus salivarius*	Feed additive		Already QPS
Reg(EC)1831/2003	*Lactococcus lactis*	Feed additive		Already QPS
2001/122/EC	*Leuconostoc mesenteroides*	Production of dextran as NF ingredient for bakery industrial and food fermentations		Already QPS
Reg(EC)1831/2003	*Leuconostoc oeno* = *Oenococcus oeni*	Feed additive		QPS 2009 update
Reg(EC)1831/2003	*Leuconostoc pseudomesenteroides*	Feed additive		Not proposed for QPS status (see EFSA Opinion 2007, Appendix A)
FEEDAP	*Methylococcus capsulatus*	Biomass for animal feed	EFSA-Q-2004-171 www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620784006.htm	No body of knowledge, therefore not appropriate for QPS (EFSA, 2008)
Opinion SCF adopted on 22/06/2000	*Paenibacillus macerans*	b-cyclodextrin production (food additive)		QPS 2009 update

EFSA Panel/ Unit Or as notified previously	Genus and species of microorganism as notified (current taxonomy where different)	Intended use	EFSA question number and published opinion [additional information]	Comments
FEEDAP	Astaxanthin-rich *Paracoccus carotinifaciens*	Production of red carotenoids	EFSA-Q-2006-173 www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178650355146.htm	No body of knowledge, therefore not considered for QPS (EFSA, 2008)
Reg(EC)1831/2003	*Pediococcus acidilactici*	Feed additive		Already QPS
Reg(EC)1831/2003	*Pediococcus pentosaceus*	Feed additive		Already QPS
Reg(EC)1831/2003	*Propionibacterium acidipropionici*	Feed additive		Not proposed for QPS status (see EFSA Opinion 2007, Appendix A)
Reg(EC)1831/2003	*Propionibacterium freudenreichii shermanii*	Feed additive		Already QPS
Reg(EC)1831/2003	*Propionibacterium globosum*	Feed additive		Not proposed for QPS status (see EFSA Opinion 2007, Appendix A)
PRAPeR	*Pseudomonas sp.* DSMZ 13134	Plant Protection Product	no dossier received yet – new active substance	Species to be verified when MS report on dossier is received
PRAPeR	*Pseudomonas chlororaphis*	Plant Protection Product	EFSA-Q-2008-618 [http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_006478.htm]	QPS 2009 update
Reg(EC)1831/2003	*Rhodopseudomonas palustris*	Feed additive		QPS 2009 update
Reg(EC)1831/2003	*Serratia rubidaea*	Feed additive		QPS 2009 update
Reg(EC)1831/2003	*Streptococcus cremoris = L. lactis subsp. Cremoris*	Feed additive		Already QPS
Reg(EC)1831/2003	*Streptococcus faecium =Enterococcus faecium*	Feed additive		No taxonomical unit within *Enterococcus* can be considered as free of infectious strains. Therefore no recommendation for QPS status (EFSA Opinion, 2007)
Reg(EC)1831	*Streptococcus thermophilus*	Feed additive		Already QPS

EFSA Panel/ Unit Or as notified previously	Genus and species of microorganism as notified (current taxonomy where different)	Intended use	EFSA question number and published opinion [additional information]	Comments
PRAPeR	*Streptomyces* strain K 61 formerly *S. griseoviridis*	Plant protection product	EFSA-Q-2009-00134 (In progress) EFSA-Q-2009-00295 (in progress) [http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet_129069.htm]	*Streptomyces* spp. produce antibiotics, are therefore inappropriate for QPS (EFSA Opinion, 2008)
FEEDAP	*Streptomyces albus*	Production of salinomycin sodium	EFSA-Q-2003-009 www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620783414.htm	*Streptomyces* spp. produce antibiotics, are therefore inappropriate for QPS (EFSA Opinion 2008)
FEEDAP	*Streptomyces aureofaciens*	Production of polyether monocarboxylic acid	EFSA-Q-2003-046 www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620783396.htm	*Streptomyces* spp. produce antibiotics, are therefore inappropriate for QPS (EFSA Opinion 2008)
FEEDAP	*Streptomyces cinnamonensis*	Production of monensin sodium	EFSA-Q-2005-024 www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620783743.htm	*Streptomyces* spp. produce antibiotics, are therefore inappropriate for QPS (EFSA Opinion 2008)
FEEDAP	*Streptomyces lasaliensis*	Production of lasalocid sodium	EFSA-Q-2004-076 www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620783432.htm	*Streptomyces* spp. produce antibiotics, are therefore inappropriate for QPS (EFSA Opinion 2008)
	Yeasts			
PRAPeR	*Aureobasidium pullulans* strains DSM 14940 and DSM 14941	Plant Protection Product	no dossier received yet – new active substance	QPS 2009 update
Reg(EC)1831/2003	*Candida glabrata*	Feed additive		Unsuitable for QPS (see EFSA Opinion 7007, Appendix C)

EFSA Panel/ Unit Or as notified previously	Genus and species of microorganism as notified (current taxonomy where different)	Intended use	EFSA question number and published opinion [additional information]	Comments
PRAPeR	*Candida oleophila* strain O	Plant protection product	EFSA-Q-2009-00338 (in progress) [http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_021008.htm]	Body of knowledge insufficient, therefore not appropriate for QPS (EFSA Opinion 2008)
FEEDAP	*Hansenula polymorpha* = *Pichia angusta*	Production of enzymes	EFSA-Q-2005-030 www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620769671.htm	Already QPS status applies only when species is used for production purposes (EFSA Opinion 2008)
2148/2004/EC	*Kluyveromyces marxianus* var. *lactis* K1	Feed additive		Already QPS
Reg(EC)773/2006 Corrigendum CS	*Kluyveromyces marxianus-fragilis*	Feed additive		Already QPS
FEEDAP	Astaxanthin rich *Phaffia rhodozyma* = *Xanthophyllomyces dendrorhous*	Production of astaxanthin	EFSA-Q-2004-148 www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620783707.htm EFSA-Q-2003-112 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620783707.htm	No body of knowledge, therefore not appropriate for QPS (EFSA Opinion 2008)
FEEDAP	*Pichia pastoris*	Production of enzymes	EFSA-Q-2006-025 www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178685303625.htm	Already QPS
PRAPeR	*Pseudozyma flocculosa*	Plant protection product	EFSA-Q-2009-00315 (in progress) [http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_119196.htm]	Mycelial fungi: already considered as not appropriate for QPS (see EFSA, 2007)
FEEDAP	*Saccharomyces cerevisiae*	Feed additive		Already QPS

EFSA Panel/ Unit Or as notified previously	Genus and species of microorganism as notified (current taxonomy where different)	Intended use	EFSA question number and published opinion [additional information]	Comments
GMO	*Saccharomyces cerevisiae*	Dried killed biomass for feed	EFSA-Q-2007-156 (Waiting for full dossier)	Already QPS
FEEDAP	*Schizosaccharomyces pombe*	Production of enzymes	EFSA-Q-2008-272 www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620769568.htm EFSA-Q-2005-080 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620782208.htm EFSA-Q-2005-063 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620769568.htm http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620783151.htm	Already QPS
	Fungi			
Reg(EC)1831/2003	*Aspergillus niger*	Feed additive		Potential for mycotoxin production, therefore not suitable for QPS status (see EFSA Opinion 2007, Appendix D)
Reg(EC)1831/2003	*Aspergillus oryzae*	Feed additive		Not suitable for QPS status (see EFSA Opinion 2007, Appendix D)
PRAPeR	*Beauveria bassiana*	Plant protection product	EFSA-Q-2009-00125 (in progress) EFSA-Q-2009-00251 (in progress) [http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_128818.htm http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_128924.htm]	Mycelial fungi: already considered as not appropriate for QPS (see EFSA, 2007)
PRAPeR	*Beauveria brongniartii*	Plant protection product	EFSA-Q-2009-00017 (in progress)	Mycelial fungi: already considered as not appropriate for QPS (see EFSA, 2007)

EFSA Panel/ Unit Or as notified previously	Genus and species of microorganism as notified (current taxonomy where different)	Intended use	EFSA question number and published opinion [additional information]	Comments
ACF (as mentioned in the register of questions)	*Blakeslea trispora*	Production of lycopene (food colorant) Production of b-carotene (food colorant)	EFSA-Q-2004-102 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620764493.htm EFSA-Q-2007-001 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178700117557.htm	Can not be proposed for QPS status (see EFSA Opinion 2007, Appendix D)
NDA	*Blakeslea trispora*	Food ingredient	EFSA-Q-2004-169 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620765774.htm EFSA-Q-2008-697 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902228574.htm	QPS 2009 update
PRAPeR	*Coniothyrium minitans*	Plant protection product	EFSA-Q-2008-515 (in progress) [Review report for the active substance *Coniotyrium minitans*, SANCO/1400/2001-final, July 2003] [http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_028836.htm]	QPS 2009 update
FEEDAP	*Duddingtonia flagrans* Alternative name: *Trichothecium flagrans*	Feed additive	EFSA-Q-2004-115 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620783270.htm EFSA-Q-2005-051 under consideration	QPS 2009 update
PRAPeR	*Gliocladium catenulatum* = *Clonostachys rosea forma catenulata*	Plant protection Product	EFSA-Q-2008-559 (in progress) [http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet_021009.htm]	QPS 2009 update
PRAPeR	*Lecanicillium muscarium* formerly *Verticillium lecanii*	Plant protection product	EFSA-Q-2009-00130 (in progress) EFSA-Q-2009-00255 (in progress)	Mycelial fungi: already considered as not appropriate for QPS (see EFSA, 2007)

EFSA Panel/ Unit Or as notified previously	Genus and species of microorganism as notified (current taxonomy where different)	Intended use	EFSA question number and published opinion [additional information]	Comments
PRAPeR	*Metarhizium anisopliae* var.Anisopliae formerly *M. anisopliae*	Plant protection product	EFSA-Q-2009-00131 (in progress) EFSA-Q-2009-00253 (in progress)	Mycelial fungi: already considered as not appropriate for QPS (see EFSA, 2007)
PRAPeR	*Paecilomyces fumosoroseus*	Plant protection product	EFSA-Q-2008-599 (in progress) EFSA-Q-2009-00323 (in progress) [http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_115002.htm]	Mycelial fungi: already considered as not appropriate for QPS (see EFSA, 2007)
PRAPeR	*Paecilomyces lilacinus*	Plant protection product	EFSA-Q-2008-600 (in progress) Conclusion on the peer review (2007): http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178623095016.htm [http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_028826.htm]	Mycelial fungi: already considered as not appropriate for QPS (see EFSA, 2007)
PRAPeR	*Phlebiopsis gigantea*	Plant protection product	EFSA-Q-2009-00132 (in progress) EFSA-Q-2009-00285 (in progress)	Mycelial fungi: already considered as not appropriate for QPS (see EFSA, 2007)
PRAPeR	*Pythium oligandrum*	Plant protection product	EFSA-Q-2009-00133 (in progress) EFSA-Q-2009-00287 (in progress) [http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_028816.htm]	Mycelial fungi: already considered as not appropriate for QPS (see EFSA, 2007)
PRAPeR	*Trichoderma atroviride* formerly *T. harzianum*	Plant protection product	EFSA-Q-2009-00137 (in progress) EFSA-Q-2009-00297 (in progress)	Mycelial fungi: already considered as not appropriate for QPS (see EFSA, 2007)

EFSA Panel/ Unit Or as notified previously	Genus and species of microorganism as notified (current taxonomy where different)	Intended use	EFSA question number and published opinion [additional information]	Comments
PRAPeR	*Trichoderma asperellum* formerly *T. harzanum* and *T. viride*	Plant protection product	EFSA-Q-2009-00136 (in progress) EFSA-Q-2009-00300 (in progress)	Mycelial fungi: already considered as not appropriate for QPS (see EFSA, 2007)
PRAPeR	*Trichoderma gamsii* formerly: *Trichoderma viride*	Plant protection product	EFSA-Q-2009-00138 (in progress) EFSA-Q-2009-00300 (in progress)	Mycelial fungi: already considered as not appropriate for QPS (see EFSA, 2007)
PRAPeR	*Trichoderma harzianum Rifai*	Plant protection product	EFSA-Q-2009-00139 (in progress) EFSA-Q-2009-00298 (in progress) [http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_128902.htm]	Mycelial fungi: already considered as not appropriate for QPS (see EFSA, 2007)
Reg(EC)1831/2003	*Trichoderma longibrachiatum*	Feed additive		Ineligible for QPS status (see EFSA Opinion 2007, Appendix D)
PRAPeR	*Trichoderma polysporum*	Plant protection product	EFSA-Q-2009-00140 (in progress) EFSA-Q-2009-00299 (in progress) [http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_128902.htm]	Mycelial fungi: already considered as not appropriate for QPS (see EFSA, 2007)
Reg(EC)1831/2003	*Trichoderma reesei*	Feed additive		Ineligible for QPS status (see EFSA Opinion 2007, Appendix D)
PRAPeR	*Verticillium albo-atrum* formerly *Verticillium dahliae*	Plant protection product	EFSA-Q-2009-00141 (in progress) EFSA-Q-2009-00303 (in progress)	Mycelial fungi: already considered as not appropriate for QPS (see EFSA, 2007)
	Algae			
FEEDAP	*Haematococcus pluvialis*	Production of astaxanthin		No body of knowledge except for this strain. Therefore not considered for QPS (EFSA Opinion 2008)

EFSA Panel/ Unit Or as notified previously	Genus and species of microorganism as notified (current taxonomy where different)	Intended use	EFSA question number and published opinion [additional information]	Comments
	Bacteriophages			
1831/2003	*Clostridium sporogenes* phage	Feed additive		QPS 2009 update
1831/2003	*Clostridium tyrobutyricum* phage	Feed additive		QPS 2009 update
	Viruses			
PRAPeR	*Adoxophyes orana granulovirus* strain BV-0001	Plant protection product	EFSA-Q-2009-00324 (in progress)	QPS 2009 update
PRAPeR	*Cydia pomonella granulovirus* Mexican isolate	Plant protection product	EFSA-Q-2009-00126 (in progress) EFSA-Q-2009-00254 (in progress) [http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_107300.htm]	QPS 2009 update
PRAPeR	*Helicoverpa armigera nucleopolyhedrovirus*	Plant protection product	EFSA-Q-2009-00341 (in progress)	QPS 2009 update
PRAPeR	*Spodoptera littoralis nucleopolyhedrovirus*	Plant protection product	EFSA-Q-2008-630 (in progress)	QPS 2009 update
PRAPeR	Zucchini yellow mosaic virus, weak strain	Plant protection product	EFSA-Q-2009-00346 (in progress) [http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_244201.htm]	QPS 2009 update

Yeast Synonyms commonly used in the feed/food industry
Pichia anomola: synonym *Hansenula anomala*, *Saccharomyces anomalus*
Pichia jadinii: anamorph *Candida utilis*; synonyms *Hansenula jadinii*, *Torulopsis utilis*
Saccharomyces cerevisiae synonym *S. boulardii*

1. EFSA 2007 Opinion: Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA - Opinion of the Scientific Committee (Question number: EFSA-Q-2005-293) www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178667590178.htm The EFSA Journal, 2007, 587, 1 – 16
2. EFSA 2008 Opinion: The maintenance of the list of QPS microorganisms intentionally added to food or feed - Scientific Opinion of the Panel on Biological Hazards (Question number: EFSA-Q-2008-006) www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902221481.htm The EFSA Journal, 2008, 923, 1-48

APPENDIX C: SCIENTIFIC REPORT ON THE ASSESSMENT OF FILAMENTOUS FUNGI

1. INTRODUCTION

Filamentous fungi are flexible microorganisms that can show different properties depending on the external factors (substrate, growth conditions, biotic/abiotic conditions). A consequence is the ability of a microorganism to produce different types and quantity of secondary metabolites depending on the growth conditions. Mycotoxins are well-known secondary metabolites, and *Penicillium roqueforti,* for example, is safely used for cheese production but could start to produce a lot of mycotoxins if the substrate is changed, *e.g.* to rye bread. Regarding the QPS status, the ability of fungal species to produce toxic metabolites represents the greatest difficulties. Based on the assumption that each of the estimated 1.5 million fungal species (Hawksworth, 1991) can produce at least two unique secondary metabolites, there may be as many as 3 millions unique fungal metabolites. Approximately 10% of the secondary metabolites listed up till now have been classified as mycotoxins. Thus, there are potentially up to 300,000 unique mycotoxins (CAST, 2003). The number of fungal metabolites and mycotoxins still undiscovered is therefore quite large and the diversity of toxic mechanisms will be equally as great. There is, unfortunately, no standardised method to consider fungal metabolites and their toxicity such as effect-based bioassay methods. The regulation of metabolites and their possible interactions are therefore poorly understood. Just as for other types of microorganisms, the toxic effect of a fungus used for food production will only be detected in case of acute toxicity, but not if it shows long term (chronic) toxicity (*e.g.* carcinogenic properties). In addition, the number of validated analytical methods for mycotoxins and other fungal metabolites is low and even for those available, analytical quality assurance procedures are often lacking (van Egmond, 2004).

As reported for bacteria, the spread of antifungal resistance in filamentous fungi has become an issue of concern. For example, resistance among *Aspergillus* species to azole antifungals is increasingly being reported (Howard et al., 2009). There has been a sudden rise in the frequency of azole resistance in *Aspergillus* since 2004, and many isolates have shown cross-resistance between all the currently licensed azole options. In The Netherlands the emergence of resistance to clinically used triazoles of *Aspergillus fumigatus* isolates has been linked to the use of azole antifungal in agriculture (Snelders et al., 2009).

The use of fungi as biocontrol agents has the potential to replace many of the toxic chemicals currently in use and represents a very promising challenge. Fungal biological control agents have several mechanisms of action that allow them to control pathogens, including mycoparasitism, production of antibiotics or enzymes, competition for nutrients and the induction of plant host defences. Application could be directly into an agricultural field, in soil or *via* injection in the xylems of some trees. The risk identification procedure linked to the use of fungal biocontrol agents includes Environmental and Human Health Assessment. Focuses are on allergic properties, risks of toxic metabolites, genetic recombination and displacement of natural strains, effect on biodiversity (*i. e.* impact on non-target organisms). One essential point is the investigation of the stability and fate of the fungal biocontrol agent and its metabolites, in order to check the lack of contamination of ground water or of plant products that enter the food and feed chain. This investigation requires the availability of specific molecular and analytic methods that allow monitoring metabolites and fungal agents in the environment.

1.1. Mycological methods to be used for identification of moulds

The market-research study published by Sunesen and Stahnke (2003) illustrates the difficulty of getting precise information on the identity of organisms used in food, and therefore to evaluate their safety.

Filamentous fungi are traditionally identified to genus level by phenotypic characters, such as morphological and cultural characteristics. Unfortunately, there is not one universal mycological textbook or reference compendium which is used for identification of moulds, which makes identification to genus level a highly subjective task. This is further complicated by the necessity to identify fungal strains to the species level as each species within a genus may have very different functional characters, *e.g.* mycotoxin profiles and physiological properties. Again, traditional methods like morphological and cultural characteristics are widely used, profiles of secondary metabolites have also been used within some genera. Phenotypic characteristics do vary according to growth conditions, which make it difficult to construct robust identification keys. No identification key covers all species, so it is recommended seeking advice for identification procedures by contacting specialists in food, feed and industrial mycology – *e.g.* via the International Commission on Food Mycology (ICFM (Anonymous, 2007a)), which can direct inquires to recommended specialists.

For filamentous fungi the use of molecular biology based methods is less developed than for bacteria and yeasts. On the other hand, in combination with phenotypic studies, numerous phylogenetic studies using gene sequences have changed the systematics within mycology and will play an increasing role in the future by changing our understanding of species delimitations and relationships. As a spin-out from molecular biology, some sequenced-based identification schemes have been developed (*e.g.* for *Trichoderma* – see below) along with various PCR detection systems. However, the latter systems often are intended for a limited number of species, at times only a minor part of a genus. The molecular methods developed so far are not based on the same gene(s) for different genera and need further improvement (Paterson, 2006).

At the end of the 20th century, several reviews describing the state of knowledge on biosynthetic pathways of mycotoxins have been published (Desjardins et al., 1993; Keller and Hohn, 1997; Steyn, 1995). Since then, an enhanced effort at identifying biosynthetic steps and genes involved in mycotoxins production and regulation has been initiated, mainly as a result of the availability of complete fungal genomes (Xu et al., 2006) and gene expression sequence databases. The resulting significant insights have been recently covered (Desjardins and Proctor, 2007; Stadler and Keller, 2008, Georgianna and Payne, 2009). The genomic data have also illustrated that fungi may have many more secondary metabolite pathways than was previously thought. As an example, analysis of the *Emericella nidulans* (anamorph *Aspergillus nidulans*) genome indicated that *E. nidulans* has 50 genes clusters that could be involved in the synthesis of secondary metabolites (Georgianna and Payne, 2009). The increased understanding in mycotoxin's biosynthetic pathways has allowed identification of genes, which are required for toxin production. These genes are currently exploited in the development of improved molecular-based detection methods for mycotoxigenic fungi in feed and food.

Many recent phylogenetic studies and molecular detection systems are based on a Multi-Locus Sequence Typing (MLST) concept, where sequences from several genes are used simultaneously. Typical targets chosen for MLST typing are "housekeeping" genes, without which the host organism will be unable to function. Again, there will be differences among fungal genera regarding the loci used in MLST studies and advice should be obtained by consulting specialists – *e.g.* via the International Commission on Food Mycology. The majority of the published sequences are accumulated in freely accessible databases and as such easy to use as an identification system. The pitfall is that there is no quality control facility on the information labelled to each sequence, which in this case would be information on strain identity and culture collection number. This means that the validity is in the hand of the depositor or through the identity of the specific strains by the tagged culture collection number. This may require reading an extended list of scientific literature due to the rapid development in fungal classification these years. In many cases, a consultation of a specialist or reliable reference cultures would be needed, despite the easiness of using sequence data for identification.

2. *ASPERGILLUS*

This genus is among the best known filamentous fungi, as *Aspergillus* species are widely used for production of chemicals (*e.g.* citric acid), enzymes and for biotransformations. On the other hand, *Aspergillus* species are also known to be among the most toxic spoilers of food and feed; some species are pathogenic to man and food producing animals. A recently published comprehensive monograph addresses many aspects regarding taxonomy, identification, pathogenicity, clinical manifestations and treatment (Latge and Steinbach, 2008). Specific reviews will be cited for the relevant species in the paragraphs that follow. A recent update on *Aspergillus* systematics with full text articles was published (Anonymous, 2007b).

2.1. *Aspergillus* section *Nigri* (the black Aspergilli)

The taxonomy of the section *Nigri* (the black Aspergilli) is not fully resolved as the number of accepted species depends on the methodology used. So far there has not been complete agreement between morphological, chemical and molecular data, but some generally acceptance has been proposed (Schuster et al., 2002; Abarca et al., 1994; Accensi et al., 2004; Samson et al., 2004b); however species identification remains problematic. The section *Nigri* includes 16 species *A. niger, A. foetidus, A. tubingensis, A. aculeatus, A. brasiliensis, A. carbonarius, A. costaricaensis, A. ellipticus, A. heteromorphus, A. homomorphus, A. ibericus, A. japonicus, A. lacticoffeatus, A. piperis, A. sclerotiniger,* and *A. vadensis;* however only the first four species listed will be evaluated for a possible QPS status as they have been used for food or feed purposes, including enzyme production. *Aspergillus* section *Nigri* are used in biotechnology, for the production of enzymes (such as amylases), acids (in particular citric acid), and pectinases for fermentation. Products of *A. niger* are considered GRAS by the FDA for use in the food industry.

2.2. *Aspergillus niger*

In general *Aspergillus niger* sensu lato has a long history of apparent safe use in biotechnology, *e.g.* for the production of chymosin and other enzymes or citric acid (Schuster et al.,, 2002; van Dijck et al., 2003). *A. niger* is not known to be used as food or feed in Europe, even though this species has been evaluated for use as a source of single-cell protein (Christias et al., 1975; Hang 1976; Singh et al., 1991; Oboh and Akindahunsi, 2002). Were a strain of *A. niger* to be allowed in Europe, it would fall under the Novel Food Regulation (258/97/EC) and would thus require a risk assessment under that legislation (Anonymous, 1997).

The full nucleotide sequences of the genomes of three strains of *Aspergillus niger sensu stricto* have been determined and information are available at two web sites (Anonymous, 2009a; Anonymous, 2009b).

It is well documented that some strains of this species produce the mycotoxins ochratoxin A (Abarca et al., 1994; Samson et al., 2004a; Serra et al., 2006) and fumonisin B2 (Frisvad et al., 2007). Other metabolites with poorly documented biological activity from *A. niger* are: pyranonigrin, kotanins and naphtho-γ-pyrones (Samson et al., 2004b). Other than their industrial and agricultural significance, *A. niger* is also recognized as a human pathogen. It is often reported as the third most frequently occurring *Aspergillus* species associated with invasive pulmonary aspergillosis (Richardson and Hope, 2009). Many of these cases are associated with immunosuppression or severe illness (Richardson and Hope, 2009). Aspergillomas may subsequently produce oxalic acid whilst *in situ* which can result in renal complications. It is also a recognised opportunistic pathogen for animals and there have been reports of natural aspergillosis in various species of mammals and birds (Smith, 1989). Nevertheless, black aspergilli are a relatively rare cause of invasive aspergillosis. It is believed that the *A. niger* complex is less adept at causing disease than *A. fumigatus*, conceivable due to inferior virulence, large conidia and the propensity for the conidia to adhere to each other.

Despite the long history of apparent safe use in biotechnology, where strain improvement combined with cleaning and purification steps have been added to processes to eliminate metabolites other than

the product of interest (van Dijck et al., 2003; Blumenthal, 2004), industrial strains of *A. niger* have been proven to produce ochratoxin A (Schuster et al., 2002) which makes *A. niger* ineligible for an inclusion on the QPS list.

2.3. *Aspergillus aculeatus, Aspergillus foetidus* and *Aspergillus tubingensis*

These species are used for enzyme production. Even though *A. aculeatus* is from Section *Nigri,* it can be distinguished morphologically from *A. niger* and the other species in the *Nigri* section (Pitt and Hocking, 1997). Nevertheless, due to the confused taxonomy of the Section *Nigri* in the past, many reports on enzyme production by *A. niger* should probably be attributed to isolates of *A. foetidus, A. tubingensis* or *A. aculeatus.* These species are known to produce many metabolites with poorly described biological activity. For *A. foetidus*, these include pyranonigrin, naphtho-γ-pyrones, asperazine, and anatafumicin (Samson et al., 2004b). *A. tubingensis* has been reported to produce pyranonigrin, naphtho-γ-pyrones, and asperazine, (Samson et al., 2004b), and *A. foetidus* to produce ochratoxin A (Teren et al., 1996; Bragulat et al., 2001; Abarca et al., 2004). One metabolite from *A. aculeatus*, secalonic acid, is known to be a mycotoxin (Samson et al., 2004b).

Despite the long history of apparent safe use in biotechnology, the body of knowledge concerning the toxicological aspects of the metabolites is insufficient, which makes *Aspergillus foetidus, A. tubingensis* and *A. aculeatus* ineligible for an inclusion on the QPS list.

2.4. *Other Aspergilli*

2.4.1. *Aspergillus candidus*

A. candidus can be found in meat products (sausages) as a starter culture with a long history of traditional use with regard to the house mycobiota (Sunesen and Stahnke, 2003). This species is not produced commercially as starter culture (for application by spraying or dipping), hence it is not declared. *A. candidus* can also be found as a contaminant (food spoiler) in cereals and many other food products (Pitt and Hocking, 1997).

Despite the frequent occurrence of this species, the body of knowledge is considered as insufficient; it produces known metabolites, some of them showing cytotoxic activity: AcT1 (Chattopadhyay et al., 1987), xanthoascin (Ito et al., 1978), terphenyllin (Marchelli and Vining, 1975; Stead et al., 1999). However, there remains metabolites that are not yet identified and classified (Samson et al., 2004b; Andersen and Thrane, 2006). The toxicology of the metabolites of *A. candidus* is unknown, so the safety concerns cannot be excluded. Even if it is possible to get rid of most of the fungal biomass by washing the surface of the product, there is the possibility that fungal metabolites will remain on the product. Moreover, possible interactions between these metabolites have yet to be investigated. Some rare case of infections linked to *A. candidus* can be found in the literature (Kwon-Chung and Bennett, 1992; Ribeiro et al., 2005).

In conclusion, considering that *Aspergillus candidus* is known to produce secondary metabolites with poorly understood toxicity for which there is no data on possible interactions, and that *A. candidus* is mainly used for food production as a house starter culture and therefore mixed with other fungi, *A. candidus* is ineligible for an inclusion on the QPS list.

2.4.2. *Aspergillus oryzae*

In Asia a long tradition of using fungal cultures to produce fermented food such as sake (rice wine), shoyu (soy sauce) and miso (soybean paste) exists. These products are fermented by 'koji-moulds', which consist principally of *Aspergillus oryzae*, but may also contain *A. 'awamori'* (*=A. niger*), *A. sojae* and *A. tamarii*. The consumption of these fermented foods in Japan has been considered as

safe (Tanaka et al., 2006). Recent genomic approaches have demonstrated that *A. oryzae* and *A. tamarii* are taxonomically closely related to *A. flavus*, while *A. sojae* and *A. awamori* are genetically related to *A. parasiticus* and *A. niger*, respectively (Machida et al., 2005).

Aspergillus oryzae has a long history of apparent safe use, both in food outside Europe (it is one of the main species used in Asia for the production of soy sauce, which is exported worldwide), and for enzyme / protein production (cell factory), however, this is as GM organisms (Archer, 2000). *A. oryzae* is accepted as a domesticated form or atoxigenic variant of *A. flavus* (Pitt and Hocking, 1997; Heydayati et al., 2007), which is an aflatoxin producer. The phenotypic distinction between *A. oryzae* and *A. flavus* is difficult as only fine details in conidial ornamentation and colony characteristics (*i.e.* colour of conidial mass and colour of colony reverse on Aspergillus Flavus Parasiticus Agar) separate the two (Samson et al., 2004a). However, by several molecular methods it has not been possible to separate the two into distinct species (Cary and Ehrlich, 2006; Chang et al., 2006). *A. oryzae* has the gene cluster for aflatoxin but has a minute change in the sequence for a regulatory gene, *aflR*, which is believed to the reason for the absence of aflatoxin production by *A. oryzae* (Lee et al., 2006). A recent review of the occurrence of aflatoxins and their production by various koji-moulds (Tanaka, 2006) demonstrated that 212 strains used for fermentation of different foods were negative for aflatoxin production. Aflatoxins were not detected in any of the 289 food samples analysed (rice, soy sauce, soybean paste).

Since the fine distinction between *A. oryzae* and *A. flavus* is often difficult it is important to present an overview of *A. flavus* and its impact on animal and human health. After *A. fumigatus*, *A. flavus* is the second leading cause of invasive aspergillosis and it is the most common cause of superficial infection (Richardson and Hope, 2009). Experimental invasive infections in mice show *A. flavus* to be 100-fold more virulent than *A. fumigatus* in terms of inoculum required. Particularly common clinical syndromes associated with *A. flavus* include chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and osteomyelitis following trauma and inoculation. Outbreaks associated with *A. flavus* appear to be associated with single or closely related strains, in contrast to those associated with *A. fumigatus*. In addition, *A. flavus* produces aflatoxins, the most toxic and potent hepatocarcinogenic natural compounds ever characterized. Accurate species identification within the *A. flavus* complex remains difficult due to overlapping morphological and biochemical characteristics and much taxonomic and population genetics work is necessary to better understand the species complex.

Strains of *A. oryzae* do produce the mycotoxins cyclopiazonic acid, which is a neurotoxic and immunosuppressive compound, and β-nitropropionic acid and kojic acid (Samson et al., 2004a). Four of 36 *A. oryzae*-strains used commercially were found to be producers of cyclopiazonic acid (Goto et al., 1987), whereas kojic acid was found to be produced by 85 of 149 koji-mould strains used commercially (Shinshi et al., 1984). The strains producing toxin were removed from commercial use and Tanaka et al. (2006) concluded that the risk for mycotoxin contamination of typical Japanese fermented food can be classified as very low. *A. oryzae* is also used as feed for dairy cows and beef cattle in growth finishing stages; however, potential production of cyclopiazonic acid and β-nitropropionic acid were not taken into consideration (EFSA, 2006a).

Despite the long history of apparent safe use in food and biotechnology, where cleaning and purification steps have been added in the process to get rid of all metabolites but the product of interest (Blumenthal, 2004), the body of knowledge concerning the formation of well-known mycotoxins, cyclopiazonic acid and β-nitropropionic acid, under production conditions as well as any long-term toxicological aspects of these toxins is insufficient. In addition, no universally accepted method for an unambiguous identification of *A. oryzae* exists, which makes *Aspergillus oryzae* not suitable for the QPS list.

3. *BEAUVERIA BRONGNIARTII*

Within the anamorphic genus *Beauveria* (Ascomycota ; Hypocreales), several species have been reported as entomopathogenic soil fungi and tested as pest biocontrol agents. Among these, *B. brongniartii* is described as a virulent pathogen of *Melolontha melolontha* (Coleoptera: Scarabaeidae), the European cockchafer (Dolci et al., 2006). Conidia of a related species, *B. bassiania,* can survive in the soil and these fungi also occur as endophytes of plants (White et al., 2002). There are however no reports on the germination, invasion or growth on plant and foodstuff in preliminary field trials. One of the main metabolites produced by *B. brongniartii* in submerged cultures is oosporein (Michelitsch et al., 2004). Oosporein, a 2,5-dihydrobenzoquinone derivative, is a mycotoxin that may cause nephrotoxicity through either mitochondrial dysfunction or lipid peroxidation. Even though there is no evidence of oosporein transferred to plants nor of signicant levels produced in soil, risks of oosporein exposure for users and consumers can not be excluded and *Beauveria brongniartii* cannot be proposed for the QPS list.

4. *BLAKESLEA TRISPORA*

Blakeslea trispora is used to produce carotenoids in well established commercial products; these naturally produced food colorants are usually not purified. An extensive literature search did not reveal any information on toxic metabolites from this species. In addition, the AFC Panel of EFSA concluded that the toxicity data on lycopene from *B. trispora* is not of concern as long as the mean intake of lycopene from coloured food does not exceed the intake from natural sources (EFSA, 2005).

Despite the apparent safe use as a colorant producing organism, it has not been possible through extensive literature searches to find work verifying a general absence of biological active secondary metabolites, including allergenic compounds, from *Blakeslea trispora*. Thus, this species cannot be recommended for the QPS list.

5. *CLONOSTACHYS ROSEA FORMA CATENULATA (SYN. GLIOCLADIUM CATENULATUM)*

The current name in use for *Gliocladium catenulatum* is *Clonostachys rosea* f. *catenulata* and the taxonomic relationship as well as nomenclature is described in detail by Schroers (2001). *Clonostachys rosea* and its green form (= *G. catenulatum*) are used as biocontrol agents and are reported to produce peptaibols (Jaworski and Bruckner, 2000), which is a common term for linear peptide antibiotics with a molecular weight between 500-2200 Da, a high content of α-aminoisobutyric acid (Aib) and an amide-bonded 1,2-amino alcohol as a C-terminal residue. Peptaibols form pores in bilayer lipid membranes and can inhibit mitochondrial ATPase, suppress immune systems and show neuroleptic effects (Degenkolb et al., 2003). This species also produces polyketide antibiotics (Okuda et al., 2000) and compounds with unknown biological activity (Joshi et al., 1999).

Considering the capacity of this species to produce many biological active compounds, it makes *Clonostachys rosea* including the green form *C. rosea* f. *catenulata* ineligible for the QPS list.

6. *CONIOTHYRIUM MINITANS*

Coniothyrium minitans is a filamentous fungus that belongs to the class "Coelomycetes" within the "Deuteromycotina" subdivision. Up to now, no sexual stage of *C. minitans* has been identified. *C. minitans* is a soil autochthonous microorganism that occupies a specialized niche by parasitizing the sclerotia of several *Sclerotinia* and *Botrytis* species. The use of *C. minitans* as a biocontrol agent of diseases caused by sclerotium-forming pathogens has been extensively investigated and its efficiency to control *S. sclerotiorum* in numerous crops including lettuce, celery, sunflower and oilseed rape successfully demonstrated (Whipps

et al., 2008). The application of *C. minitans* follows generally one of two ways: either soil application to reduce the sclerotial inoculum-potential or spore-sprays on crop debris. Recently in May 2009, the Health Canada's Pest Management Regulatory Agency has granted full registration for the sale and use of *C. minitans* to control fungal diseases in a variety of field and greenhouse vegetables (RD 2009/07, Health Canada). Due to the high host-specificity of *C. minitans*, effects on other non-target organisms are not expected and there are no published reports of disease associated with *Coniothyrium minitans* in birds, wild mammals, fish, insects, soil micro organisms and plants except for the intended pest and its close relatives, i.e. *Sclerotinia* species. When *C. minitans* strain was administered orally to rats, no signs that it caused toxicity or disease were observed (Dewhurst, 2004). However, certain strains of *C. minitans* produce secondary metabolites such as macrosphelide A (McQuillen et al., 2003). This macrolide compound characterized by a 16-membered ring structure is an antifungal metabolite, the production of which explains a part of the biocontrol efficiciency of *C. minitans* against *S. sclerotiorum.* No acute toxicity has been ascribed to macrosphelide A. Moereover, macrosphelide A is an inhibitor of cell-cell adhesion molecule and its use as a antimetastatic agent is currently investigated (Ishara et al., 2004).

Despite their apparent safe use as biocontrol agents, it has not been possible through extensive literature searches to find work confirming a general absence of toxic biological active secondary metabolites or allergenic compounds from *Coniothyrium minitans.* Moreover the body of knowledge is limited to its usage as a biocontrol agent. Thus, this species cannot be recommended for the QPS list.

7. *CRYPHONECTRIA PARASITICA (SYN. ENDOTHIA PARASITICA)*

Cryphonetria parasitica is the valid name for *Endothia parasitica* (Barr 1978) and this fungus is used to produce protease with rennet-like activity (Pariza and Johnson, 2001). WHO has evaluated enzymatic preparations from *Cr. parasitica* and concluded that no adverse effects could be observed (WHO, 1975a). However, this species has been reported to produce rugulosin and skyrin (Frisvad and Thrane, 1993). These compounds with poorly described biological activity have also been found in the fermentation batches and the body of knowledge is limited.

Despite the long history of apparent safe use of enzyme production by *Cryphonetria parasitica,* the capacity of this microorganism to produce biological active compounds under production conditions makes it ineligible for the QPS list.

8. *DUDDINGTONIA FLAGRANS*

An Opinion of the Panel on additives and products or substances used in animal feed (FEEDAP) on the safety of the micro-organism preparation of *Duddingtonia flagrans*, for use as a feed additive for calves in accordance with Council Directive 70/524/EEC was adopted on 7th March 2006 (EFSA, 2006b). In this Opinion: *Duddingtonia flagrans* belongs to a group of nematophagous fungi that physically entrap nematodes by means of a specialised adhesive hyphal net. Chlamydospores from cultures of the fungus are fed to the target species. Ingested spores pass through the digestive tract without germinating and are deposited on pasture with the faeces where they germinate and produce mycelium with its hyphal traps. This in turn reduces the number of nematodes able to migrate to herbage and re-infect the grazing animals.

The detection and identification of *D. flagrans* is reliant on morphological methods, which can be laborious, time-consuming, and error prone. Kelly et al. (2008) have developed a PCR assay using species-specific primers located in the ITS regions for the rapid and accurate identification of *D. flagrans*. The PCR assay was specific to five different isolates of *D. flagrans* and was capable of

detecting a minimum concentration of 100 chlamydo-spores per gram of soil. In contrast to cultured-based detection and identification methods, this assay is amenable to high throughput screening of environmental samples. The assay detected *D. flagrans* in faecal, leaf litter, and soil samples collected from 80% of the Irish farms tested indicating that the fungus is abundant in Ireland. *D. flagrans* produces the following secondary metabolites with antibiotic activity: flagranones A (2), B (3) and C (4) (Anderson et al., 1999). These antibiotics are structurally related to the farnesylated cyclohexenoxides of the oligosporon group recently isolated from the nematode-trapping fungus *Arthrobotrys oligospora*, and show similar antimicrobial activity.

As there is no history of use of the product, just the experimental trials mentioned in the FEEDAP Opinion, there is insufficient body of knowledge on the generic safety of *Duddingtonia flagrans*, thus it cannot be recommended for QPS.

9. *FUSARIUM*

Currently, the genus *Fusarium* contains about 150 species; however the systematics is now changing rapidly due to the rapid developments in molecular biology. Many recently-described *Fusarium* species have been discovered by molecular tools used in phylogenetic studies, followed by a formal description of the species (Skovgaard et al., 2003; O'Donnell et al., 2004; Aoki et al., 2005). Introductions to *Fusarium* are available (Leslie et al., 2001; Summerell et al., 2003; Samson et al., 2004a; Leslie and Summerell, 2006) along with extended information on the mycotoxin production by *Fusarium* species (Marasas et al., 1984; Thrane, 2001; Sewram et al., 2005; Andersen and Thrane, 2006). In the last two years, significant progresses have been performed in the specific detection and quantification of *Fusarium spp.* occurring in cereals. Most of these developments use real-time polymerase chain reaction. Specific molecular tools are now available to quantify the most prevalent *Fusarium* species contaminating cereals products (Nicolaisen et al., 2009). The recent access to *F. graminearum* [www.broad.mit.edu/annotation/genome/Fusarium_graminearum/Home.htm] and *F. verticillioides* [www.broad.mit.edu/annotation/genome/Fusarium_verticillioides/Home.htm] genomes has led to an increased knowledge of the molecular organisation of fumonisins and trichothecenes biosynthetic pathways together with the identification of genes involved in regulation mechanisms (Desjardins and Proctor, 2007). The zearalenone biosynthetic pathway remains today less understood although significant insights have been recently published by Lysøe et al. (2009). One of the key challenges that research on *Fusarium* has to answer in the next years is the elucidation of the effects of environmental factors on initiation or repression of toxins biosynthesis.

Only one species *(F. venenatum)* is used in food production and one species extensively studied as a potential biocontrol agent (*F. oxysporum*).

9.1. *Fusarium venenatum*

The only commercial mycoprotein products for human food are based on *Fusarium venenatum* biomass. The biotechnological development of these products is well described (Wiebe 2002). The major concern is that *F. venenatum* is a potential producer of mycotoxins, such as trichothecenes (diacetoxyscirpenol [DAS], nivalenol and fusarenonX), butenolide and culmorin (Thrane and Hansen 1995; Miller and MacKenzie, 2000; Nielsen and Thrane, 2001), which are carefully controlled and monitored during mycoprotein production (Johnstone, 1998). Strains of *F. venenatum* which are used to produce enzymes are genetically modified (Royer et al., 1995; Royer et al., 1999; Pedersen and Broadmeadow, 2000; Ahmad et al., 2004).

Considering that *F. venenatum* is very toxic as wild-type, and that all strains used for enzyme production are genetically modified, this species is ineligible for the QPS list.

10. *ISARIA FUMOSOROSEA (SYN. PAECILOMYCES FUMOSOROSEUS)*

Isaria fumosorosea is the valid name for *Paecilomyces fumosoroseus* as the anamorphic genus *Paecilomyces* is separated in 2 sections of which the section *Isarioidea* contains mesophilic species which are mostly pathogens of invertebrates (Madsen et al., 2007). The other section, section *Paecilomyces*, comprises thermophilic species. *Paecilomyces* species are often difficult to identify and in some studies are placed in a group called "other fungi with hyaline spores". Madsen et al. (2007) present the arguments for assigning the species *P. fumosoroseus* and *P. farinosus* to the genus *Isaria* (as *I. fumosorosea* and *I. farinosa*) comprising the species found in the section *Isarioidea*. In contrast, it is proposed that the nematode pathogen *P. lilacinus* should not be included in *Isaria* based on the current data. This taxonomical revision thus leaves species in the section *Paecilomyces* as members of the genus *Paecilomyces*, while most insect pathogens should be included in *Isaria*.

I. fumosorose is a naturally occurring insect fungus found in infected and dead insects, and in some soils. The fungus infects the host by penetrating the outer layer (cuticle) of the insect, and proceeding to grow until the insect dies. This species has been reported to produce beauvericin, which is a known mycotoxin from many *Fusarium* species, as well as dipicolinic acid and beauverolides, however there is limited information on metabolites from this species as reviewed by Zimmermann (2008).

Despite the history of apparent safe use of *I. fumosorosea* as insecticide, the capacity of this microorganism to produce biological active compounds makes it ineligible for the QPS list.

11. *LECANICILLIUM MUSCARIUM*

Lecanicillium muscarium is widely known as an insect pathogen and has been developed into a biocontrol agent (Bardin et al., 2008, Cuthbertson et al., 2008). Despite the apparent safe use as a biocontrol product, it can be assumed based on scientific knowledge that a potential risk might be present even though an extensive literature searches failed to find work verifying a presence of biological active secondary metabolites, including allergenic compounds, from *L. muscarium.* Moreover the body of knowledge is limited to this usage as a biocontrol agent. Thus, this species cannot be proposed for the QPS list.

12. *METARHIZIUM ANISOPLIAE*

Metarhizium anisopliae is widely known as an insect pathogen and has been developed into biocontrol agents by several companies. The safety of this species as a biocontrol agent has been reviewed in details by Zimmermann (2007). This species is able to produce destruxins, cytochalasins (cytotoxic) and swainsonine (potential effect in cancer treatment). Considering the capacity of this species to produce many biological active compounds makes *Metarhizium anisopliae* ineligible for QPS.

13. *MONASCUS*

Monascus species (*M. purpureus, M. ruber, M.* spp*)* are known to produce yellow, orange and red pigments. Traditionally, *Monascus* has been cultured on rice and other cereals by solid sate fermentation. The red-coloured rice (Anka or Ang-kak) has been used for centuries in Asia as natural food colorant for bean curd, meat, wine and other foods. Nowadays, the purified pigments are widely used as colorants in processed seafood, sausages and sauce in Asia. In addition, extracts and other red-mould rice preparations are sold through the internet as nutritional additives with claims that they will lower blood cholesterol levels. No direct adverse health aspects have been reported. However, several studies have shown the presence of the mycotoxin citrinin, which is nephrotoxic and therefore an undesirable toxic secondary metabolite, among the pigments of *Monascus* and in commercial

Monascus-preparations (Blanc et al., 1995; Dietrich et al., 1999; Xu et al., 1999). The allergenic relevance of *M. purpureus* was the first time shown in 2002 (Hipler et al., 2002).

The European Community legislation on food additives is based on the principle that only those additives that are explicitly authorised may be used. Pigments from *Monascus* and *Monascus* preparations are not included in the list of permitted food colours of the European Parliament and Council Directive. As no toxicological safe and technologically efficient strains of *Monascus* are available for general use, no species within this genus is eligible for QPS.

14. *PAECILOMYCES LILACINUS*

P. lilacinus is mainly soil-borne and is known to be a nematode pathogen, but has also been found, in, for example, infested building materials, in the soil of potted plants in hospitals. In indoor air (hospital) and an outdoor environment in Europe, low exposures to *P. lilacinus* have been recorded (Madsen et al., 2007). It has been proven that this species can produce biological active compounds of the peptaibol family (Degenkolb and Brückner, 2008). Morphological identification of *Paecilomyces* at species level is difficult, and molecular characterization is achieved by sequencing of the ITS region (Castelli et al., 2008). There is one PRAPeR report (2007) on a specific strain of *Paecilomyces lilacinus* (EFSA, 2007).

In general, there have been numerous reports of human invasive infections by *Paecilomyces lilacinus* causing endophthalmitis, keratitis, chronic sinusitis, skin and soft tissue infections, and catheter-related infections. These infection shave been reviewed by Pastor and Guarro (2006). In summary, *P. lilacinus* is an emerging pathogen that causes severe human infections, including devastating oculomycosis. Usually, it shows low susceptibility to conventional antifungal drugs in vitro, and variable susceptibility to novel triazoles. A review of the published literature identified 119 reported cases of human infection by *P. lilacinus* between 1964 and 2004. Most were cases of oculomycosis (51.3%), followed by cutaneous and sub-cutaneous infections (35.3%), and a smaller group of miscellaneous infections (13.4%). Lens implantation is the most frequent predisposing factor for oculomycosis. Cutaneous and sub-cutaneous infections occur mainly in solid organ and bone marrow transplant recipients, although surgery and primary or acquired immunodeficiency are also relevant predisposing factors. Infections in apparently immunocompetent patients have also been reported. Surgical debridement combined with antifungal drug therapy, or the correction of predisposing factors, such as neutropenia, are usually required to obtain improvement. Treatment with traditional antifungal drugs often fails. Voriconazole has demonstrated good activity in both cutaneous and ocular infections in the few cases in which this drug has been used. The new triazoles ravuconazole and posaconazole show good in-vitro activity against *P. lilacinus* and could be promising therapeutic alternatives.

Despite the history of apparent safe use of *Paecilomyces lilacinus* as a nematode pathogen, the capacity of this microorganism to produce biological active compounds and the numerous reports of human invasive infections, makes it ineligible for the QPS list.

15. *PENICILLIUM*

Among the most frequently encountered fungi in food and feed systems are species of the genus *Penicillium,* which are very well-known as spoilers and mycotoxin producers but also as starter cultures for products like *e.g.* white- and blue-mould cheeses and mould-ripened meat products. The modern systematics of the genus *Penicillium* was initiated by a monograph more than 25 years ago (Pitt, 1979) and has developed dramatically since then. Today the genus is divided into four subgenera (Pitt and Hocking, 1997; Samson et al., 2004c) and may contain more than 500 species. Many species, however, are soil fungi and has never been related to food and feed systems, except as occasional spoilers. All *Penicillium* species are good producers of mycotoxins and other biological

active metabolites, however the available literature is overwhelming and difficult to interpret as the identification of *Penicillium* cultures is not trivial and has resulted in numerous misidentifications (Frisvad et al., 2006). Partly as a consequence of this, starter cultures are often vaguely labelled as "*Penicillium* spores" (Sunesen and Stahnke, 2003). Among the *Penicillium* strains used routinely in the food industry, toxigenic strains are frequent. In a study of 249 *Penicillium* strains originally isolated from food products and used as starter cultures only 13 isolates were found to meet the demands on technological suitability and toxicological safety, which includes the testing of the strains with regard to the production of antibiotic, cytotoxic and mutagenic metabolites (Gareis et al., 1999).

Based on literature reviews, only species within the subgenus *Penicillium* have been used as starter cultures for food and feed. Recently this subgenus has been the subject of a monograph (Frisvad and Samson, 2004; Samson et al., 2004c; Smedsgaard et al., 2004) including an extensive review on the related secondary metabolites (Frisvad et al., 2004). An interactive identification key based on phenotypic characters and β-tubulin gene sequences is available at: http://www.cbs.knaw.nl/penicillium/DefaultPage.aspx.

15.1. *Penicillium camemberti*

Penicillium camemberti has a long history of use in cheese production (camembert cheese and white mould cheeses in general) often declared by the use of invalid synonyms as *P. album, P. candidum*, *P. casei* or *P. caseicola*. It is also found as a spontaneous coloniser on fermented sausages originating from the local mycobiota of the production plant (Sunesen and Stahnke, 2003) and as a starter culture to give aroma to fermented meat products. This species is also used for enzyme production (Pariza and Johnson 2001). The taxonomy of *P. camemberti* is well known and this species is accepted as a domesticated form of *P. commune.*

There are no reports of an adverse health effect for cheese or meat produced with *P. camemberti*, *i.e.* no acute toxicity associated with food produced by *P. camemberti* has been reported. This species is however known as a producer of cyclopiazonic acid (CPA), this being a neurotoxic and immunosuppressive compound (Frisvad et al., 2004); unknown cytotoxic metabolites are also produced when this fungus is used as a starter culture for mould-ripened meat products (Gareis 1999). A few strains also produce metabolites with poorly described biological activity, such as cyclopaldic acid, rugulovasine A & B and palitantin (Frisvad et al., 2004). CPA has been detected in cheeses at 0.25-0.37 mg/kg cited by (Pitt and Hocking, 1997) and in meat products cited by (Sunesen and Stahnke, 2003). Naturally occurring mutants that do not produce this mycotoxin have been reported (Geisen et al., 1990). There are not enough toxicological data available to set a threshold under which the consumption of cyclopiazonic acid does not pose any risk. It is important to note that *P. camemberti* (mostly cited as *P. casei*) is known as the aetiological agent of the "cheese worker's lung" associated with hypersensitivity pneumonitis (Campbell et al., 1983; Marcer et al., 1996).

Despite the long history of apparent safe use of *P. camemberti,* the capacity of this microorganism to produce cyclopiazonic acid, even under known production conditions, makes *P. camemberti* ineligible for QPS.

15.2. *Penicillium chrysogenum*

Penicillium chrysogenum is used as a starter culture for the production of dry sausages (Sunesen and Stahnke, 2003) and is also used in the pharmaceutical industry to produce penicillin. It is also known to produce roquefortine C, PR-toxin and secalonic acids, which are mycotoxins, in addition to secondary metabolites with poorly described biological activity: chrysogine, xanthocillin, sorrentanone and sorbicillin (Frisvad et al., 2004).

Considering the capacity of this species to produce unwanted antibiotics in food, each strain should be investigated in detail, which makes *P. chrysogenum* ineligible for QPS.

15.3. *Penicillium funiculosum*

P. funiculosum is used as a producer of enzyme preparation intended for animal feed (Das and Singh, 2004) and also as a host for the production of heterologous proteins. The promoter of the histone H4.1 gene was successfully used to drive the expression of an intracellular bacterial enzyme, β-glucuronidase, and a secreted homologous enzyme, xylanase C (Belshaw et al., 2002). In general, no known mycotoxins are associated with this species; however a single strain has been shown to produce the mycotoxin secalonic acid (JC Frisvad, pers. comm.). Cultures of *P. funiculosum* do produce many secondary metabolites of unknown structure and unknown biological activity, hence *P. funiculosum* is ineligible for QPS.

15.4. *Penicillium nalgiovense*

Penicillium nalgiovense is widely used as starter culture for the production of dry sausages (Sunesen and Stahnke, 2003). Wild-type isolates from meats and cheeses have green conidia, whereas starter cultures have white conidia. This species produces penicillin and a broad range of secondary metabolites with poorly described biological activity: nalgiovensin, nalgiolaxin, diaporthins and dipodazin (Frisvad et al., 2004). Typically isolates from meats are good producers of penicillin, while cheese isolates produce penicillin in low amounts. In addition, some strains have been found to produce cytotoxic metabolites on nutrient agar and mould-ripened salamis (Gareis et al., 1999).

Despite the long history of apparent safe use of *P. nalgiovense,* the capacity of this species to produce unwanted antibiotics and cytotoxic metabolites in food makes *P. nalgiovense* ineligible for QPS.

15.5. *Penicillium roqueforti*

Penicillium roqueforti has a long history of apparent safe use in the production of blue-moulded cheeses, but is also often isolated from rye bread, silage and other acid preserved products. *P. roqueforti* has also been reported as a source for enzymes used in food processing (Pariza and Johnson, 2001).

Thirteen years ago, two closely related species, *P. paneum* and *P. carneum*, were discovered (Boysen et al., 1996). All three share many ecological and morphological features, which makes it difficult to interpret older literature, however their profiles of secondary metabolites are distinct (Frisvad et al., 2004; Nielsen et al., 2006).

P. roqueforti sensu stricto produces the mycotoxins roquefortine C & D, PR-toxin, mycophenolic acid, isofumigaclavine A & B and metabolites with poorly described biological activity: citreoisocoumarin and α-amino butyric acid peptides (peptaibols) (Frisvad et al., 2004). The related species *P. carneum* produces the mycotoxins mycophenolic acid, patulin, roquefortine C, penitrem A, isofumigaclavine A, as well as cyclopaldic acid with a poorly described activity. *P. paneum* produces the mycotoxins patulin, roquefortine C & D, botryodiploidin and metabolites with poorly described biological activity: marcfortines and citreoisocoumarin (Frisvad et al., 2004). For *P. roqueforti sensu stricto* roquefortine and PR-toxin productions are occurring in cheese but at amounts that are not considered as toxic for humans (Pitt, 1997). There is no data on possible long term toxic effects. In general toxicological data for *P. roqueforti* metabolites are insufficient to set a threshold for regulatory purposes.

Despite the long history of apparent safe use of *P. roqueforti*, this species is ineligible for QPS as no validated analytical methods for the mycotoxins exist to qualify for the absence of mycotoxins under production conditions.

16. *PHLEBIOPSIS GIGANTEA*

Within the genus *Phlebiopsis* (Basidiomycota; Polyporales; Phanerochaetacea), the *P. gigantea* is an aggressive saprophytic white rot fungus that invades cut tree surfaces preventing the subsequent invasion by the rot-causing organisms, particularly *Heterobasidion annosum*. The biofungicide efficiency of spore suspensions of *P. gigantea* has been investigated for different conifer species in many countries (Annesi et al., 2005). The mechanism of action is reported to be competition for nutrients and not rely on secretion of secondary metabolites. *P. gigantea* suspension was one of the world's first fungal biocontrol agent registered as pesticide in the UK (Pratt et al., 1999). Use of *P. gigantea* as a fungal pre-treatment for logs processing has also been investigated and could result in substantial benefits to the pulp and papermaking industry (Behrendt and Blanchette, 1997). *P. gigantea* is not pathogenic to plants or animals and up to now a toxin production was not reported which represents not a confirmation that the species does not have this potential. There is also no evidence that it is not able to produce toxins. As reported by Dewhurst (2004), no adverse reactions in human exposed to *P. gigantea* from natural occurrence in forests or in operators applying it has been described. Moreover, no indication of hypersensitivity was reported in individuals that had been formulating the product for 40 years. No body of knowledge for food and feed use is available.

Due to the insufficient knowledge concerning the capacity of *Phlebiopsis gigantea* to produce biological active secondary metabolites, this species cannot be proposed for the QPS list.

17. *PYTHIUM OLIGANDRUM*

Pythium oligandrum is a well characterized, naturally occurring fungus common in soil and in or on plants which acts as a hyperparasite by colonizing other pathogenic fungi in and around seeds and the rhizosphere of treated plants, thereby suppressing the growth of at least 20 soil-born pathogenic fungi, including *Alternaria, Botrytis, Fusarium, Gaeumannonyces, Ophiostoma, Phoma, Pseudocercosporella, Pythium, Sclerotinia* and *Sclerotium*.

P. oligandrum produces the namesake protein oligandrin and other compounds that stimulate plants cell walls to fend off pathogen invasion, and also stimulates natural plant defence mechanisms called pathogenesis-related (PR) proteins which help plants resist disease, without harming the plant. *P. oligandrum* can grow within the roots of certain plants, including tomato and sugar beet. Production of auxin-like substances stimulates plant growth. Defence responses can be induced in the plant, which primes the plant from further infection by pathogenic fungi, oomycetes or bacteria. An Opinion on *P. oligandrum*, based on US EPA FactSheet, was published in 2007 (EPA, 2007):

Some other *Pythium* species can cause disease in mammals however no toxicological or pathogenic effects of *P. oligandrum* in mammals have been reported in available public literature or in the submitted data. However, there are related *Pythium* species that can cause diseases in mammals. Pythiosis is a life-threatening infectious disease caused by the oomycete, fungus-like, aquatic organism *Pythium insidiosum*, which is the only *Pythium* species of the kingdom Stramenopila known to infect humans and some animals, such as horses, dogs, cats, and cattle, in tropical and subtropical countries (Kaufman, 1998; Mendoza et al., 1996; Franco et al., 2009).

It has been impossible through extensive literature searches to find work demonstrating the presence of biological active secondary metabolites, including allergenic compounds, from *Pythium oligandrum,* however the body of knowledge is limited to this usage as a biocontrol agent. Thus, this species cannot be proposed for the QPS list.

18. *RHIZOMUCOR*

Rhizomucor miehei and *Rh. pusillus* are the valid names for the thermophilic fungi *Mucor miehei* and *M. pusillus*, respectively (Schipper, 1978) and both are used to produce chymosin, dextranase and protease with rennet-like activity (Pariza and Johnson, 2001). Extensive literature searches have not retrieved any information on toxic compounds produced by these two species. In addition, the WHO has evaluated enzymatic preparations from *Rh. miehei* and *Rh. pusillus* and concluded that no adverse effects could be observed (WHO 1975 b;c).

Despite the apparent safe use as an enzyme producing organism, it has not been possible through extensive literature searches to find work demonstrating the presence of biological active secondary metabolites, including allergenic compounds, from *Rhizomucor* species. Moreover the body of knowledge is limited. Thus, species from this genus cannot be proposed for the QPS list.

19. *TRICHODERMA*

There have been many developments within the taxonomy and systematics of this genus lasting recent years (Druzhinina and Kubicek, 2005; Samuels, 2006) and interactive identification key to the more than 90 species of *Trichoderma* and its teleomorph, *Hypocrea,* has been developed based on molecular methods (Druzhinina et al., 2005; Kopchinskiy et al., 2005) located at http://www.isth.info/index.php.

Another interactive key based on morphological and cultural characters for identification of *Trichoderma* and some of its teleomorphs is also available and has many illustrations. This key is located at http://nt.ars-grin.gov/taxadescriptions/keys/TrichodermaIndex.cfm

The available literature on bioactive compounds from *Trichoderma* species is extensive and was reviewed some years ago (Sivasithamparam and Ghisalberti,1998). Since then, numerous reports have been published, however no production of compounds classified as mycotoxins have been reported. For many years, the production of trichothecene mycotoxins has been associated with several *Trichoderma* species, but it has now been clarified that the trichothecene producing species is a newly described species, *T. brevicompactum*, and not any of those species listed below (Nielsen et al., 2005). *Trichoderma* species are known to be aggressive and are used as biocontrol agents, however the difficult systematics is a challenge when it comes to identifying exactly which species is involved (Kullnig et al., 2001; Hermosa et al., 2004). An EU sponsored initiative to evaluate biological control agents, REBECA, has been launched – see http://www.rebeca-net.de for details.

A recently published and very well documented review describes the numerous practical and applied uses of *Trichoderma sp.* and reports the list of *Trichoderma* strains that have been commercially exploited up to 2007 (Verma et al., 2007). *Trichoderma sp.* are widely used as biocontrol agents due to their antagonistic properties against several pests (fungi, bacteria, invertebrates and weeds) as well as their ability to act as plant growth enhancers. Their mechanism of action includes mycoparasitism, spatial and nutrient competition, production of active metabolites and induction of plant defence reactions. *Trichoderma sp.* are also widely used for their production of cellulose-degrading enzymes. According to Verma et al. (2007), commercial fungal biocontrol agents manufactured with *Trichoderma* preparations share, in 2005, about 60% of all fungal biocontrol agents. Moreover an increasing number of *Trichoderma spp.* based biocontrol products are registered regularly.

19.1. *Trichoderma atroviride*

Due to the taxonomic confusion in the past around *T. atroviride* and the two species to which *T.atroviride* strains often have been assigned, *T. harzianum* and *T. viride* (Dodd et al., 2002), it is impossible to judge the capability of *T. atroviride* to produce biological active compounds previously

reported as produced by *T. harzianum* or *T. viride*. These species have been reported to produce peptaibols (Oh et al., 2000). Thus, this species cannot be proposed for the QPS list.

19.2. *Trichoderma asperellum*

Due to the taxonomic confusion in the past around, *T. asperellum* and the two species to which *T. asperellum* strains often have been assigned, *T. harzianum* and *T. viride* (Samuels et al., 1999), it is impossible to judge the capability of *T. asperellum* to produce biological active compounds previously reported as produced by *T. harzianum* or *T. viride.* It has not been possible through an extensive literature search to find work demonstrating a presence of biological active secondary metabolites, including allergenic compounds, from *T. asperellum.* However the body of knowledge is limited to this usage as a biocontrol agent. Thus, this species cannot be proposed for the QPS list

19.3. *Trichoderma gamsii*

Trichoderma gamsii is a newly discovered species as a result of a reassessment of the species complex around *T. viride* (Jaklitsch et al., 2006). Due to the taxonomic development, it is impossible to judge the capability of *T. gamsii* to produce biological active compounds previously reported as produced by *T. viride* in the old and broad species concept. It has not been possible through an extensive literature search to find work demonstrating a presence of biological active secondary metabolites, including allergenic compounds, from *T. gamsii.* However the body of knowledge is limited to this usage as a biocontrol agent. Thus, this species cannot be proposed for the QPS list.

19.4. *Trichoderma harzianum*

Trichoderma harzianum is mainly used as a biocontrol agent (Harman et al., 2004) and some strains are known to be very aggressive to (plant pathogenic) mushrooms (Samuels et al., 2002). A recent patent concerning a preparation of *Trichoderma harzianum* granules has been recently published (WO/2009/083819). This preparation is reported as a microbial fertilizer and microbial biocontrol agent to protect olive trees against the wilt (*Verticillium dahliae*). *T. harzianum* is known to produce a high number of secondary metabolites with partly characterised biological activity (Sivasithamparam and Ghisalberti, 1998; Hanson, 2005); however it is known that 6-n-pentyl-α-pyrone (coconut smell) is responsible for at least part of the biological aggressiveness of this species and that highly biologically active α-amino butyric acid cyclic peptides (peptaibols) are involved in the apoptosis mechanism, in addition to anthraquinones, azaphilones, harzianolide and harzianopyrione which have different activities towards plant pathogens (Vinale et al., 2006).

Considering the capacity of this species to produce unwanted biological active compounds, each strain should be investigated in detail, which makes *T. harzianum* ineligible for QPS.

19.5. *Trichoderma longibrachiatum*

Trichoderma longibrachiatum has been reported as a potential biocontrol agent (Kullnig et al., 2001; Vizcaino et al., 2005). This species is considered very aggressive and has been reported to produce several biologically active α-amino butyric acid cyclic peptides (peptaibols) (Mohamed-Benkada et al., 2006) in addition to many secondary metabolites with poorly described biological activity (Sivasithamparam and Ghisalberti, 1998; Sperry et al., 1998; Vicente et al. 2001). Possibly many production strains are misidentified according to an updated taxonomy; however this cannot be proven as many strains are no longer available. *T. longibrachiatum* has been associated with human fungal infections (Kuhls et al., 1999; De Miguel et al. 2005).

Considering the capacity of this species to produce many biological active compounds, each strain should be investigated in detail, which makes *T. longibrachiatum* ineligible for QPS.

19.6. *Trichoderma polysporum*

This species has been reported to produce peptaibols (Fujita et al., 1981). Thus, this species cannot be proposed for the QPS list.

19.7. *Trichoderma reesei*

Trichoderma reesei is widely used for enzyme production and the toxicological evaluations that need to be taken into consideration have been reported (Blumenthal, 2004). However, the potential production of trichothecenes can be neglected as this species cannot produce these mycotoxins (Nielsen et al. 2005). *T. reesei* is reported to produce peptaibol compounds which are known to disintegrate cell membranes, causing therefore apoptosis (Bruckner and Graf, 1983), as well as other biological active cyclopeptides (Sun et al., 2006). The *Trichoderma reesei* genome paper was recently published in Nature Biotechnology (Martinez et al., 2008). Genome analysis led to the identification of numerous genes encoding biosynthetic pathways for secondary metabolites, which will allow a better prediction of the panel of biogical active compounds this species is able to produce.

Considering the capacity of this species to produce unwanted biological active compounds, each strain should be investigated in detail, which makes *T. reesei* ineligible for QPS.

19.8. *Trichoderma viride*

Trichoderma viride has been evaluated for single cell production (Hang, 1976; Youssef and Aziz, 1999), but this has never been commercialised. This species is not used as a biocontrol agent but is considered very aggressive and has been reported to produce 6-n-pentyl-α-pyrone (coconut smell) and several biologically active α-amino butyric acid cyclic peptides (peptaibols) in addition to many secondary metabolites with poorly described biological activity (Sivasithamparam and Ghisalberti, 1998). Possibly many production strains are misidentified according to an updated taxonomy; however this cannot be proven as many strains are no longer available. *T. viride* has been associated with human fungal infections (De Miguel et al., 2005).

Considering the capacity of this species to produce many biological active compounds, each strain should be investigated in detail, which makes *T. viride* ineligible for QPS.

20. *VERTICILLIUM ALBOATRUM*

Verticillium species (Deuteromycota, Hyphomycetes) are soil-borne fungi with worldwide distribution, causing vascular disease that results in severe yield and quality losses in fruit and nut crops, legumes, vegetables, forest trees, and woody and herbaceous ornamentals. Most crop diseases are caused by the two species *Verticillium alboatrum* and *V. dahliae*. Spread of disease results either by growth of the pathogen from diseased to healthy susceptible plants by root contact or by the dissemination of infected plant material. The possibility, in some cases, of the disease being spread by air-blown spores could also occur. Few published data are available concerning the metabolites from *Verticillium alboatrum*: alboatrin is a phytotoxin (Ichihara et al., 1988) and VD toxins are also phytotoxins, but their exact structure remains to be elucidated (Buchner et al., 1989). In addition, a set of volatile compounds has been identified (Aissami et al., 1999). A white mutant of naturally occurring *V. alboatrum* has been reported as an efficient biocontrol agent of Dutch Elm disease (Elgersma et al., 1993). The specific *V. alboatrum* strain was originally isolated from a potato field in the Netherlands, and has lost most of its pathogenic properties and is not able to induce wilting symptoms on trees.

Despite the apparent safe use of a single strain of *Verticillium alboatrum* as a biocontrol agent, there is an insufficient body of knowledge on the generic level for this species, thus *V. alboatrum* cannot be proposed for the QPS list.

REFERENCES

Abarca M L, Accensi F, Cano J and Cabañas FJ, 2004. Taxonomy and significance of black aspergilli. Antonie Van Leeuwenhoek 86, 1, 33-49.

Abarca ML, Bragulat MR, Castella G and Cabañes FJ, 1994. Ochratoxin A production by strains of *Aspergillus niger* var. niger. Appl. Environ. Microbiol. 60, 7, 2650-2.

Accensi F, Abarca ML and Cabañes FJ, 2004. Occurence of *Aspergillus* species in mixed feeds and component raw materials and their ability to produce Ochratoxin A. Food Microbiol. 21, 623-627.

Ahmad SK, Brinch DS, Friis EP and Pedersen PB, 2004. Toxicological studies on Lactose Oxidase from Microdochium nivale expressed in *Fusarium venenatum*. Regul. Toxicol. Pharmacol. 39, 3, 256-70.

Andersen B and Thrane U, 2006. Food-borne fungi in fruit and cereals and their production of mycotoxins. Adv. Exp. Med. Biol. 571, 137-52.

Anderson MG, Rickards RW and Lacey E, 1999. Structures of Flagranones A, B and C, cyclohexenoxide antibiotics from the nematode-trapping fungus *Duddingtonia flagrans*. J. Antibiot. 52, 1023-1028.

Annesi T, Curcio G, D'Amico L and Motta E, 2005. Biological control of *heterobasidion annosum* on *Pinus pinea* by *Phlebiopsis gigantea*. Forest Pathology, 35,127-134.

Anonymous, 1997. Regulation (EC) 258/97/EC of the European Parliament and of the Council (OJ L43, p1, 14/02/1997) of 27 January 1997 concerning novel foods and novel food ingredients.

Anonymous, 2007a. International Commission on Food Mycology (ICFM). (http://www.foodmycology.org)

Anonymous, 2007b. Proceedings of the International Workshop '*Aspergillus* Systematics in the Genomic Era'. Studies in Mycology 59, www.aspergilluspenicillium.org/SIM59.html.

Anonymous, 2009a. http://genome.jgi-psf.org/Aspni1/Aspni1.home.html

Anonymous, 2009b. www.aspergillus.org.uk/indexhome.htm?secure/sequence_info/index.php~main

Aissami A, El Amri H, Mrabet N, Chekti R and Lahlou H, 1999. Contribution to the study of the chemical composition of *Verticillium alboatrum* secretions in liquid media. Mycopathologia, 144, 93-95.

Aoki T, O'Donnell K and Scandiani MM, 2005. Sudden death syndrome of soybean in South America is caused by four species of *Fusarium*: *Fusarium brasiliense* sp. nov., *F. cuneirostrum* sp. nov., *F. tucumaniae*, and *F. virguliforme*. Mycoscience 46, 162-183.

Archer DB, 2000. Filamentous fungi as microbial cell factories for food use. Curr. Opin. Biotechnol. 11, 5, 478-83.

Bardin M, Fargues J and Nicot PC. 2008. Compatibility between biopesticides used to control grey mould, powdery mildew and whitefly on tomato. Biological Control 46: 476-483.

Barr ME, 1978. The Diaporthales in North America, with emphasis on Gnomonia and its segregates. Mycologia Memoir 7, 1-232.

Behrendt CJ and Blanchette RA, 1997. Biological processing of pine logs for pulp and paper production with *Phlebiopsis gigantea*. Appl. Environ. Microbiol. 63, 5, 1995-2000.

Belshaw N J, Haigh NP, Fish NM, Archer DB and Alcocer MJ, 2002. Use of a histone H4 promoter to drive the expression of homologous and heterologous proteins by *Penicillium funiculosum*. Appl. Microbiol. Biotechnol. 60, 4, 455-60.

Blanc PJ, Laussac JP, Le Bars J, Le Bars P, Loret MO, Pareilleux A, Prome D, Prome JC, Santerre AL and Goma G, 1995. Characterization of monascidin A from Monascus as citrinin. Int. J. Food Microbiol. 27, 2-3, 201-13.

Blumenthal CZ, 2004. Production of toxic metabolites in *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*: justification of mycotoxin testing in food grade enzyme preparations derived from the three fungi. Regul. Toxicol. Pharmacol. 39, 2, 214-28.

Boysen M, Skouboe P, Frisvad J and Rossen L, 1996. Reclassification of the *Penicillium roqueforti* group into three species on the basis of molecular genetic and biochemical profiles. Microbiology 142, 3, 541-9.

Bragulat MR, Abarca ML and Cabañes FJ, 2001. An easy screening method for fungi producing ochratoxin A in pure culture. Int. J. Food Microbiol. 71, 2-3, 139-44.

Bruckner H and Graf H, 1983. Paracelsin, a peptide antibiotic containing alpha-aminoisobutyric acid, isolated from *Trichoderma reesei* Simmons. Part A. Experientia 39, 5, 528-30.

Buchner V, Burstein Y and Nachmias A, 1989. Comparison of *Verticillium dahliae* – produced phytotoxic purified from culture fluids and infected potato stems. Physiol. Mol. Plant Pathology, 35, 253-269.

Campbell JA, Kryda MJ, Treuhaft MW, Marx JJ Jr and Roberts RC, 1983. Cheese worker's hypersensitivity pneumonitis. Am. Rev. Respir. Dis. 127, 4, 495-6.

Cary JW and Ehrlich KC, 2006. Aflatoxigenicity in *Aspergillus*: molecular genetics, phylogenetic relationships and evolutionary implications. Mycopathologia 162, 3, 167-77.

Council for Agricultural Science and Technology (CAST) 2003. Mycotoxins: Risk in Plant, Animal and Human Systems. Task Force Report n°139. Ames (Iowa, USA).

Castelli MV, Alastruey-Izquierdo A, Cuesta I, Monzon A, Mellado E, Rodriguez-Tudela JL and Cuenca-Estrellaet M, 2008. Susceptibility Testing and Molecular Classification of *Paecilomyces* spp. Antimicrobial Agents and Chemotherapy 52, 8, 2926-2928.

Chang P K., Ehrlich KC and Hua SS, 2006. Cladal relatedness among *Aspergillus oryzae* isolates and *Aspergillus flavus* S and L morphotype isolates. Int. J. Food Microbiol. 108, 2, 172-7.

Chattopadhyay, SK, Nandi B, Ghosh P and Thakur S, 1987. A new mycotoxin from *Aspergillus candidus* Link isolated from rough rice. Mycopathologia 98, 1, 21-6.

Christias C, Couvaraki C, Georgopoulos SG, Macris B and Vomvoyanni V, 1975. Protein content and amino acid composition of certain fungi evaluated for microbial protein production. Appl. Microbiol. 29, 2, 250-4.

Cuthbertson AGS, Blackburn LF, Northing P, Luo WQ, Cannon RJC and Walters KFA, 2008. Further compatibility tests of the entomopathogenic fungus *Lecanicillium muscarium* with conventional insecticide products for control of sweetpotato whitefly, Bemisia tabaci on poinsettia plants. Insect Sci. 15, 355-360.

Das H and Singh SK, 2004. Useful byproducts from cellulosic wastes of agriculture and food industry--a critical appraisal. Crit. Rev. Food Sci. Nutr. 44, 2, 77-89.

Degenkolb T, Berg A, Gams W, Schlegel B and Grafe U, 2003. The occurrence of peptaibols and structurally related peptaibiotics in fungi and their mass spectrometric identification via diagnostic fragment ions. J. Peptide Sci. 9, 11-12, 666-678.

Degenkolb T and Brückner H, 2008. Peptaibiomics: towards a myriad of bioactive peptides containing C(alpha)-dialkylamino acids? Chem. Biodiversity 5, 9, 1817-1843, .

De Miguel D, Gómez P, González R, García-Suárez J, Cuadros JA, Bañas MH, Romanyk J and Burgaleta C, 2005. Nonfatal pulmonary *Trichoderma viride* infection in an adult patient with acute

myeloid leukemia: report of one case and review of the literature. Diagn. Microbiol. Infect. Dis. 53, 1, 33-7.

Desjardin A, Hohn T and Mac Cormick S, 1993. Trichothecene biosynthesis in *Fusarium* species: chemistry, genetics, and significance. Microbiol. Rev. 57, 3, 595-604.

Desjardins A and Proctor RH, 2007. Molecular Biology of *Fusarium* mycotoxins. Int. J. Food Microbiol. 119, 1-2, 47-50.

Dewhurst IC, 2004. Microbial pesticides. In: Mars T, Ballantyne B, Wiley (Eds) Pesticide Toxicology and International Regulation, 349-365.

Dietrich R, Usleber E, Martlbauer E and Gareis M, 1999. Detection of the nephrotoxic mycotoxin citrinin in foods and food colorants derived from *Monascus* spp. Archiv für Lebensmittelhygiene 50, 17-21.

Dodd SL, Lieckfeldt E, Chaverri P, Overton BE and Samuels GJ, 2002. Taxonomy and phylogenetic relationships of two species of *Hypocrea* with *Trichoderma* anamorphs. Mycological Progress 1, 409-428.

Dolci P, Guglielmo F, Secchi F and Ozino OI, 2006. Persistence and efficacy of *Beauveria Brogniartii* strains applied as biocontrol agents against *Melolontha meolontha* in the valley of Aosta (northwest Italy). J. Appl. Microbiol. 100, 1063-1072.

Druzhinina I and Kubicek CP, 2005. Species concepts and biodiversity in *Trichoderma* and *Hypocrea*: from aggregate species to species clusters? J. Zhejiang Univ. Sci. B 6, 2, 100-12.

Druzhinina IS, Kopchinskiy AG, Komoń M, Bissett J, Szakacs G and Kubicek CP, 2005. An oligonucleotide barcode for species identification in Trichoderma and Hypocrea. Fungal Genet. Biol. 42, 10, 813-28.

EFSA, 2005. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to an application on the use of a-tocopherol containing oil suspensions and cold water dispersible forms of lycopene from Blakeslea trispora as a food colour. The EFSA Journal 275, 1-17.

EFSA, 2006a. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on safety and efficacy of the product "Amaferm" as a feed additive for dairy cows and cattle for fattening in accordance with Regulation (EC) No 1831/2003. The EFSA Journal 337, 1-17.

EFSA, 2006b. An Opinion of the Panel on Additives and Products or Substances used in animal feed (FEEDAP) on the safety of the micro-organism preparation of *Duddingtonia flagrans*, for use as a feed additive for calves. The EFSA Journal, 334, 1-8.

EFSA, 2007, Conclusion on peer review of *Paecilomyces lilacinus* strain 251. EFSA Scientific Report, 103, 1-35.

Elgersma DM, Roosien T and Scheffer RJ, 1993. Biological control of Dutch elm disease by exploiting resistance in the host. In: Sticklen MB, Sherald JL, eds. Dutch Elm Disease Research, Cellular and Molecular Approaches. New York USA, Springer-Verlag, 188-192.

EPA, 2007. *Pythium oligandrum* DV 74 (028816) Fact sheet. U.S. Environmental Protection Agency. http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_028816.htm

Franco DM, Aronson JF, Hawkins HK, Gallagher JJ, Mendoza L, McGinnis MR and Williams-Bouyer N, 2009. Systemic *Pythium insidiosum* in a pediatric burn patient. Burns. 2009 Oct 27. Epub ahead of print.

Frisvad, J. C., Nielsen KF and Samson RA, 2006. Recommendations concerning the chronic problem of misidentification of mycotoxigenic fungi associated with foods and feeds. Adv. Exp. Med. Biol. 571, 33-46.

Frisvad JC and Samson RA, 2004. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. Studies in Mycology 49, 1-173.

Frisvad JC, Smedsgaard J, Larsen TO and Samson RA, 2004. Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium*. Studies in Mycology 49, 201-242.

Frisvad, J. Smedsgaard, R. A. Samson, T. O. Larsen and U. Thrane. Fumonisin B2 production by *Aspergillus niger*. 2007. J. Agric. Food Chem. 55, 23, 9727-9732, .

Frisvad JC and Thrane U, 1993. Liquid column chromatography of mycotoxins. Chromatography of mycotoxins: techniques and applications. V. Betina. Amsterdam, Elsevier Publishers B.V.**:** 253-372.

Fujita T, Takaishi Y, Okamura A, Fujita E, Fuji K, Hiratsuka N, Komatsu M and Arita I, 1981. New peptide antibiotics, Trichopolyns I and II, from *Trichoderma polysporum*. J. Chem. Soc., Chem. Commun., 1981, 585 - 587.

Gareis M, Rotheneder R and Roedel W, 1999. Mould-ripened meat products: New selection scheme for non-toxigenic *Penicillium* spp. Mycotoxin Res. 15, 61-66.

Geisen R, Glenn E and Leistner L, 1990. Two *Penicillium camembertii* mutants affected in the production of cyclopiazonic acid. Appl. Environ. Microbiol. 56, 11, 3587-90.

Georgianna D and Payne G, 2009. Genetic regulation of aflatoxin biosynthesis: from gene to genome. Fungal Genet. Biol. 46, 2, 113-125.

Goto T, Shinshi E, Tanaka K and Manabe M, 1987. Production of cyclopiazonic acid by koji molds and possibility of cyclopiazonic acid conatmination of Japanese fermented foods. Rep. Natl. Food Res. Institute. 51, 23-28.

Hang YD, 1976. Fungal treatment of beet waste. Progress in Water Technology 8, 325-327.

Hanson JR, 2005. The chemistry of the bio-control agent, *Trichoderma harzianum*. Sci. Prog. 88, 237-248.

Harman GE, Howell CR, Viterbo A, Chet J and Lorito M, 2004. *Trichoderma* species--opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol. 2, 1, 43-56.

Hawksworth DL, 1991. The fungal dimension of biodiversity: Magnitude, significance, and conservation. Mycological Research 95, 641-655.

Hermosa MR, Keck E, Chamorro I, Rubio B, Sanz L, Vizcaíno JA, Grondona I and Monte E, 2004. Genetic diversity shown in *Trichoderma* biocontrol isolates. Mycol Res 108(Pt 8): 897-906.

Heyayati MT, Pasqualotto AC, Warn PA, Bowyer Pand Denning DW, 2007. *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. Microbiology 153, 1677-1692.

Hipler UC, Wigger-Alberti W, Bauer A and Elsner P, 2002. Case report. *Monascus purpureus*--a new fungus of allergologic relevance. Mycoses 45, 1-2, 58-60.

Howard SJ, Cerar D, Anderson MJ, Albarrag A, Fisher MC, Pasqualotto AC, Laverdiere M, Arendrup MC, Perlin DS and Denning DW, 2009. Frequency and evolution of Azole resistance in *Aspergillus fumigatus* associated with treatment failure. Emerg Infect Dis. 15,1068-76.

Ichihara A, Nonaka M, Sakamura S, Sato R and Tajimi A, 1988. Structure and synthesis of alboatrin, a novel phytotoxic metabolite from *Verticillium albo-atrum*. Chemistry Letters 1, 27-30.

Ishara K, Kawaguchi T, Matsuya Y, Sakurai H, Saiki I and Nemoto H, 2004. Synthesis and Biological Evaluation of Macrosphelide Core. Eur. J. Org. Chem. 3973-3978.

Ito Y, Ohtsubo K, Yoshihira K, Sekita S, Natori S and Tsunoda H, 1978. Toxic effects of rice culture of *Aspergillus candidus* and its metabolite, xanthoascin, on Japanese quails. Jpn. J. Exp. Med. 48, 3, 187-91.

Jaklitsch WM, Samuels GJ, Dodd SL, Lu BS and Druzhinina IS, 2006. *Hypocrea rufa*/*Trichoderma viride*: a reassessment, and description of five closely related species with and without warted conidia. Studies in Mycology 56, 135-177.

Joshi BK, Gloer JB and Wicklow DT, 1999. New verticillin and glisoprenin analogues from Gliocladium catenulatum, a mycoparasite of *Aspergillus flavus* sclerotia. J. Natural Products 62, 730-733.

Johnstone L, 1998. Molecular phylogenetic, morphological, and mycotoxin data support reidentification of the Quorn mycoprotein fungus as *Fusarium venenatum*. Fungal. Genet. Biol. 25, 1, 75.

Kaufman, L. 1998. Penicilliosis marneffei and pythiosis: emerging tropical diseases. Mycopathologia 143, 3-7.

Keller N and Hohn T, 1997. Metabolic pathway gene clusters in filamentous fungi. Fungal Genet. Biol. 21, 1, 17-29.

Kelly P, Good B, Fitzpatrick R, Hanrahan JP and de Waal TD, 2008. Development and application of a PCR diagnostic assay for the accurate and rapid identification of the nematophagous fungus *Duddingtonia flagrans*. Mycol. Res. 112, 1026-30.

Kopchinskiy A, Komoń M, Kubicek CP and Druzhinina IS, 2005. TrichoBLAST: a multilocus database for *Trichoderma* and *Hypocrea identifications*. Mycol. Res. 109, 6, 658-60.

Kuhls K, Lieckfeldt E, Börner T and Guého E, 1999. Molecular reidentification of human pathogenic *Trichoderma* isolates as *Trichoderma longibrachiatum* and *Trichoderma citrinoviride*. Med. Mycol. 37, 1, 25-33.

Kullnig CM, Krupica T, Woo SL, Mach RL, Rey M, Benitez T, Lorito M and Kubicek CP, 2001. Confusion abounds over identities of *Trichoderma* biocontrol isolates. Mycological Research 105, 770-772.

Kwon-Chung KJ and Bennett JW, 1992. Medical Mycology. Philadelphia, Lea and Febiger.

Latge JP and Steinbach WJ (eds.) 2008. *Aspergillus fumigatus and Aspergillosis*. pp.568, ASM Press, American Society for Microbiology, Washington DC.

Lee C Z, Liou GY and Yuan GF, 2006. Comparison of the aflR gene sequences of strains in *Aspergillus* section Flavi. Microbiology 152, 1, 161-70.

Leslie JF and Summerell BA, 2006. The *Fusarium* Laboratory Manual. Ames (Iowa, USA), Blackwell Publishing.

Leslie JF, Zeller KA and Summerell BA, 2001. Icebergs and species in populations of *Fusarium*. Physiolog. Mol. Plant Pathol. 59, 107-117.

Lysøe E, Bone F and Klemsdal S, 2009. Real-time quantitative expresssion studies of the zearalenone biosynthetic gene cluster in *Fusarium graminearum*. Phytopathology 99, 2, 176-184.

Machida M, Asai K, Sano M, Tanaka T, Kumagai T, Terai G, Kusumoto K, Arima T, Akita O, Kashiwagi Y, Abe K, Gomi K, Horiuchi H, Kitamoto K, Kobayashi T, Takeuchi M, Denning DW, Galagan JE, Nierman WC, Yu J, Archer DB, Bennett JW, Bhatnagar D, Cleveland TE, Fedorova ND, Gotoh O, Horikawa H, Hosoyama A, Ichinomiya M, Igarashi R, Iwashita K, Juvvadi PR, Kato M, Kato Y, Kin T, Kokubun A, Maeda H, Maeyama N, Maruyama J, Nagasaki H, Nakajima T, Oda K, Okada K, Paulsen I, Sakamoto K, Sawano T, Takahashi M, Takase K, Terabayashi Y, Wortman JR, Yamada O, Yamagata Y, Anazawa H, Hata Y, Koide Y, Komori T, Koyama Y,

Minetoki T, Suharnan S, Tanaka A, Isono K, Kuhara S, Ogasawara N and Kikuchi H, 2005. Genome sequencing and analysis of *Aspergillus oryzae*. Nature 438, 7071, 1157-61.

Madsen AM, Hansen VM, Meyling NV and Eilenberg J, 2007. Human exposure to airborne fungi from genera used as biocontrol agents in plant protection. Ann. Agric. Environ. Med. 14, 5-24

Marasas WFO, Nelson PE and Toussoun TA, 1984. Toxigenic *Fusarium* Species. Identity and Mycotoxicology, The Pennsylvania State University Press, University Park and London.

Marcer G, Franchini M, Gemignani C, Zancanaro A, Semenzato G, Tassinari C, Cipriani A, Festi G and Saia B, 1996. Cheese workers' lung. Allergy 51, 12, 959-60.

Marchelli R and Vining LC, 1975. Terphenyllin, a novel p-terphenyl metabolite from *Aspergillus candidus*. J. Antibiot. (Tokyo) 28, 4, 328-31.

Martinez D, Henrissat B, Saloheimo M, Arvas M, Baker SE, Chapman J, Chertkov O, Coutinho PM, Cullen D, Danchin EGJ, Grigoriev IV, Harris P, Jackson M, Kubicek CP, Han CS, Ho I, Larrondo LF, de Leon AL, Magnuson JK, Merino S, Misra M, Nelson B, Putnam N, Robbertse B, Salamov AA, Schmoll M, Terry A, Thayer N, Westerholm-Parvinen A, Schoch CL, Yao J, Barabote R, Nelson MA, Detter C, Bruce D, Kuske CR, Xie G, Richardson P, Rokhsar DS, Lucas SM, Rubin EM, Dunn-Coleman N, Ward M and Brettin TS, 2008. Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*). Nature Biotechnology, 26, 553-560.

McQuillen MP, Gemmel J, Hill R, Whipps J, 2003. Production of Macrosphelide A by the mycoparasite *Coniothyrium minitans.* FEMS Microbiology Letters 219, 27-31.

Mendoza L, Ajello L and McGinnis MR, 1996. Infection caused by the oomycetous pathogen *Pythium insidiosum*. J. Mycol. Med. 6, 151-164.

Michelitsch A, Rückert U, Rittmannsberger A, Seger C, Strasser H and Likussar W, 2004. Accurate determination of oosporein in fungal culture broth by differential pulse polarography. J. Agric. and Food Chem. 52, 6, 1423-1426.

Miller JD and MacKenzie S, 2000. Secondary metabolites of *Fusarium venenatum* strains with deletions in the Tri5 gene encoding trichodiene synthetase. Mycologia 92, 764-771.

Mohamed-Benkada M, Montagu M, Biard JF, Mondeguer F, Verite P, Dalgalarrondo M, Bissett J and Pouchus YF, 2006. New short peptaibols from a marine *Trichoderma* strain. Rapid Commun. Mass Spectrom. 20, 8, 1176-80.

Nicolaisen N, Suproniene S, Nielsen L, Lazzaro I, Spliid N and Justesen A, 2009. Real-time PCR for quantification of eleven individual *Fusarium* species in cereals. J. Microbiol. Meth. 76, 3, 234-240.

Nielsen KF and Thrane U, 2001. Fast methods for screening of trichothecenes in fungal cultures using gas chromatography-tandem mass spectrometry. J. Chromatogr .A 929, 1-2, 75-87.

Nielsen KF, Gräfenhan T, Zafari D, Thrane U, 2005. Trichothecene production by *Trichoderma brevicompactum*. J Agric. Food Chem. 53, 21, 8190-6.

Nielsen KF, Sumarah MW, Frisvad JC and Miller JD, 2006. Production of metabolites from the *Penicillium roqueforti* complex. J. Agric. Food Chem. 54, 3756-3763.

O'Donnell K, Ward TJ, Geiser DM, Corby Kistler H and Aoki T, 2004. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal. Genet. Biol. 41, 6, 600-23.

Oboh G and Akindahunsi AA, 2002. Nutrient and anti-nutrient contents of *Aspergillus niger*-fermented cassava products (flour and gari). J. Food Compos. Analysis 15, 617-622.

Oh SU, Lee SJ, Kim JH and Yoo ID, 2000. Structural elucidation of new antibiotic peptides, atroviridins A, B and C from *Trichoderma atroviride*. Tetrahedron Letters 41, 61-64.

Okuda T, Kohno J, Kishi N, Asai Y, Nishio M and Komatsubara S, 2000. Production of TMC-151, TMC-154 and TMC-171, a new class of antibiotics, is specific to '*Gliocladium roseum*' group. Mycoscience 41, 239-253.

Pastor FJ and Guarro J, 2006. Clinical manifestations, treatment and outcome of *Paecilomyces lilacinus* infections. Clin. Microbiol. Infect. 12, 948-960.

Pratt JE, Gibbs JN and Webber JF, 1999. Registration of *Phlebiopsis gigantea* as a forest biocontrol agent in the UK recent experience. Biocontrol Sci. Technol. 9, 113-118.

Pariza MW and Johnson EA, 2001. Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. Regul. Toxicol. Pharmacol. 33, 2, 173-86.

Paterson RRM, 2006. Identification and quantification of mycotoxigenic fungi by PCR. Process Biochemistry 41, 1467-1474.

Pedersen PB and Broadmeadow A, 2000. Toxicological studies on Thermomyces lanuginosus xylanase expressed by *Fusarium venenatum*, intended for use in food. Food Addit. Contam. 17, 9, 739-47.

Pitt JI, 1979. The Genus *Penicillium* and Its Teleomorphic States *Eupenicillium* and *Talaromyces*. London, Academic Press.

Pitt JI and Hocking AD, 1997. Fungi and Food Spoilage. London, Blackie Academic & Professional.

Ribeiro SC, Santana AN, Arriagada GH, Martins JE and Takagaki TY, 2005. A novel cause of invasive pulmonary infection in an immunocompetent patient: *Aspergillus candidus*. J. Infect. 51, 4, 195-7.

Richardson MD and Hope WW, 2008. Aspergillosis. In: Clinical Mycology, 2nd Edition. (Eds. Anaissie E, McGinnis MR, Pfaller MA). Elsevier, New York, pp. 271-296.

Royer JC, Moyer DL, Reiwitch SG, Madden MS, Jensen EB, Brown SH, Yonker CC, Johnston JA, Golightly EJ, Yoder WT and Shuster J R, 1995. *Fusarium graminearum* A 3/5 as a novel host for heterologous protein production. Biotechnology (N Y) 13, 13, 1479-83.

Royer JC, Christianson LM, Yoder WT, Gambetta GA, Klotz AV, Morris CL, Brody H and Otani S, 1999. Deletion of the trichodiene synthase gene of *Fusarium venenatum*: two systems for repeated gene deletions. Fungal Genet. Biol. 28, 1, 68-78.

Samson RA, Hoekstra ES and Frisvad JC, 2004a. Introduction to Food- and Airborne Fungi. Utrecht, Centraalbureau voor Schimmelcultures.

Samson RA, Houbraken JAM.P, Kuijpers AFA, Frank JM and Frisvad JC, 2004b. New ochratoxin A or sclerotium producing species in *Aspergillus* section Nigri. Studies in Mycology 50, 45-61.

Samson RA, Seifert KA, Kuijpers AFA, Houbraken JAMP and Frisvad JC, 2004c. Phylogenetic analysis of *Penicillium* subgenus *Penicillium* using partial b-tubulin sequences. Studies in Mycology 49, 175-200.

Samson RA, Hong SB and Frisvad JC, 2006. Old and new concepts of species differentiation in *Aspergillus*. Medical Mycology 44, suppl, 133-148.

Samuels GJ, Lieckfeldt E, Nirenberg HI, 1999. *Trichoderma asperellum*, a new species with warted conidia, and redescription of *T. viride*. Sydowia 51, 71-88.

Samuels GJ, Dodd SL, Gams W, Castlebury LA and Petrini O, 2002. *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. Mycologia 94, 146-170.

Samuels GJ, 2006. Trichoderma: Systematics, the sexual state, and ecology. Phytopathology 96, 195-206.

Schipper MAA, 1978. On the genera *Rhizomucor* and *Parasitella*. Studies in Mycology 17, 53-71.

Schuster E, Dunn-Coleman N, Frisvad JC and van Dijck PWM, 2002. On the safety of *Aspergillus niger*--a review. Appl. Microbiol. Biotechnol. 59, 4-5, 426-35.

Schroers HJ, 2001. A monograph of Bionectria (Ascomycota, Hypocreales, Bionectriaceae) and its *Clonostachys anamorphs*. Studies in Mycology 46, 1-214.

Serra, R., Cabanes F. J. , Perrone G., Castellá G., Venâncio A., Mulè G., Kozakiewicz Z. 2006. *Aspergillus ibericus*: a new species of section Nigri isolated from grapes. Mycologia 98(2): 295-306.

Sewram V, Mshicileli N, Shephard GS, Vismer HF, Rheeder JP, Lee YW, Leslie JF and Marasas WF, 2005. Production of fumonisin B and C analogues by several fusarium species. J. Agric. Food Chem. 53, 12, 4861-6.

Shinshi E, Manabe M, Goto T, Misawa Y, Tanaka K and Matsuura S, 1984. Studies on the fluorescent compounds in fermented foods Part VII. The degradation of added kojic acid during soy sauce fermentation. Nippon Shoyu Kenkyusho Zasshi 10, 151-155.

Singh A, Abidi AB, Agrawal AK and Darmwal NS, 1991. Single cell protein production by *Aspergillus niger* and its evaluation. Zentralbl. Mikrobiol. 146, 3, 181-4.

Sivasithamparam K and Ghisalberti EL, 1998. Secondary metabolism in *Trichoderma* and *Gliocladium*. Basic biology, taxonomy and genetics. 12. (Kubicek CP and Harman GE, Eds) pp. 139-191. Taylor & Francis Ltd, London, UK.

Skovgaard K, Rosendahl S, O'Donnell K and Nirenberg HI, 2003. *Fusarium commune* is a new species identified by morphological and molecular phylogenetic data. Mycologia 95, 630-636.

Smedsgaard J, Hansen ME, Frisvad JC, 2004. Classification of terverticillate Penicillia by electrospray mass spectrometric profiling. Studies in Mycology 49, 243-251.

Smith JMB, 1989. Opportunistic Mycoses of Man and Other Animals. p. 193. Wallingford, CAB International. Wallingford, United Kingdom.

Snelders E, Huis In 't Veld RA, Rijs AJ, Kema GH, Melchers WJ and Verweij PE, 2009. Possible environmental origin of resistance of *Aspergillus fumigatus* to medical triazoles. Appl. Environ. Microbiol. 75, 4053-7.

Solla A and Gil L, 2003. Evaluating *Verticillium dahliae* for biological control of *Ophiostoma novo-ulmi* in *Ulmus* minor. Plant Pathology, 52, 579-585.

Sperry S, Samuels GJ and Crews P, 1998. Vertinoid polyketides from the saltwater culture of the fungus *Trichoderma longibrachiatum* separated from a Haliclona marine sponge. J. Organic Chemistry 63, 10011-10014.

Stadler M and Keller N, 2008. Paradigm shifts in fungal secondary metabolite research. Mycological Research, 112, 47-50.

Steyn P, 1995. Mycotoxins, general view, chemistry and structure. Toxicology letters, 82-83, 843-851.

Stead P, Affleck K, Sidebottom PJ, Taylor NL, Drake CS, Todd M, Jowett A and Webb G, 1999. Isolation and characterisation of a prenylated p-terphenyl metabolite of *Aspergillus candidus* possessing potent and selective cytotoxic activity; studies on mechanism of action. J. Antibiot. (Tokyo) 52, 2, 89-95.

Summerell BA, Salleh B and Leslie JF, 2003. A utilitarian approach to *Fusarium* identification. Plant Diseases 87, 117-128.

Sun Y, Tian L, Huang YF, Sha Y, Pei YH, 2006. A new cyclotetrapeptide from marine fungus Trichoderma reesei. Pharmazie 61(9): 809-10.

Sunesen, L. O., Stahnke, L.H. (2003). Mould starter cultures for dry sausages - selection, application and effects. Meat Science 65: 935-948.

Tanaka K, Kushiro M and Manabe M, 2006. A review of studies and measures to improve the mycotoxicological safety of traditional Japanese mold-fermented foods. Mycotoxin Research 22, 153-158.

Teren J, Varga J, Hamari Z, Rinyu E, Kevei F, 1996. Immunochemical detection of ochratoxin A in black *Aspergillus* strains. Mycopathologia 134, 3, 171-6.

Thrane U, 2001. Developments in the taxonomy of *Fusarium* species based on secondary metabolites. *Fusarium*. B. A. Summerell, Leslie, J.F., Backhouse, D., Bryden, W.L., Burgess, L.W. St. Paul, Minnesota, APS Press**:** 29-49.

Thrane U and Hansen U, 1995. Chemical and physiological characterization of taxa in the *Fusarium sambucinum* complex. Mycopathologia 129, 3, 183-90.

van Dijck PW, Selten GC and Hempenius RA, 2003. On the safety of a new generation of DSM *Aspergillus niger* enzyme production strains. Regul Toxicol Pharmacol 38, 1, 27-35.

van Egmond HP, 2004. Natural toxins: risks, regulations and the analytical situation in Europe. Anal. Bioanal. Chem. 378, 5, 1152-60.

Verma M, Brar S, Tyagi R, Surampalli R, Valero J, 2007. Antagonistic fungi, *Trichoderma spp*: panoply of biological control. Biochemica Engineering Journal 37,1-20.

Vicente MF, Cabello A, Platas G, Basilio A, Díez MT, Dreikorn S, Giacobbe RA, Onishi JC, Meinz M, Kurtz MB, Rosenbach M, Thompson J, Abruzzo G, Flattery A, Kong L, Tsipouras A, Wilson KE and Peláez F, 2001. Antimicrobial activity of ergokonin A from Trichoderma longibrachiatum. J. Appl. Microbiol. 91. 5. 806-13.

Vinale F, Marra R, Scala F, Ghisalberti EL, Lorito M and Sivasithamparam K, 2006. Major secondary metabolites produced by two commercial *Trichoderma* strains active against different phytopathogens. Lett. Appl. Microbiol. 43, 2, 143-8.

Vizcaino JA, Sanz L, Basilio A, Vicente F, Gutiérrez S, Hermosa MR and Monte E, 2005. Screening of antimicrobial activities in *Trichoderma* isolates representing three trichoderma sections. Mycol. Res. 109, 12, 1397-406.

Whipps JM and Davies KG, 2000. Biocontrol of plant pathogens and nematodes by microorganisms. In: Gurr G and Wratten, SD (eds.). Measures of success in biological control. Kluwer, Dordrecht, 231-269.

Whipps J, Sreenivasaprasad S, Muthumeenakshi S, Rogers C.W and Challen MP, 2008. Use of *Coniothyrium minitans* as a biocontrol agent and some molecular aspects of sclerotial mycoparasitism. Eur. J. Plant Pathol. 121,323-330.

White JF, Belanger F and Meyer W, 2002. Clavicipitalean fungal epibionts and endophytes-development of symbiotic interactions with plants. Symbiosis, 33, 201-213.

WHO, 1975a. Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain food additives. Microbial rennet (Endothia parasitica). WHO Food Additives Series 6, 386.

WHO, 1975b. Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain food additives. Microbial rennet (Mucor miehei). WHO Food Additives Series 6, 387.

WHO ,1975c. Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain food additives. Microbial rennet (Mucor pusillus). WHO Food Additives Series 6, 388.

Wiebe MG, 2002. Myco-protein from *Fusarium venenatum*: a well-established product for human consumption. Appl. Microbiol. Biotechnol. 58, 4, 421-7.

Xu GR, Lu C, Mu XQ, Chen JL, Chen Y, Gu YM, Wu YP, Sheng F and Wu MY, 1999. A study on the production of citrinin by *Monascus* spp. Archiv für Lebensmittelhygiene 50, 88-91.

Xu J, Peng Y, Dickman M and Sharon A, 2006. The Dawn of Fungal Pathogen Genomics. Ann. Rev. Phytopathol. 44,337-366.

Youssef BM and Aziz NH, 1999. Influence of gamma-irradiation on the bioconversion of rice straw by *Trichoderma viride* into single cell protein. Cytobios 97, 171-183.

Zimmermann G, 2007. Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. Biocontrol Sci. Technol. 17, 879-920.

Zimmermann G, 2008. The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosus*) and the *Isaria fumosorosea* species complex (formerly *Paecilomyces fumosoroseus*): biology, ecology and use in biological control. Biocontrol Sci. Technol. 18, 865-901.