Qualified Presumption of Safety
of Micro-organisms in Food and Feed
About EFSA

The European Food Safety Authority (EFSA) was established and funded by the European Community as an independent agency in 2002 following a series of food scares that caused the European public to voice concerns about food safety and the ability of regulatory authorities to fully protect consumers.

In close collaboration with national authorities and in open consultation with its stakeholders, EFSA provides objective scientific advice on all matters with a direct or indirect impact on food and feed safety, including animal health and welfare and plant protection. EFSA is also consulted on nutrition in relation to Community legislation.

EFSA’s work falls into two areas: risk assessment and risk communication. In particular, EFSA’s risk assessments provide risk managers (EU institutions with political accountability, i.e. the European Commission, European Parliament and Council) with a sound scientific basis for defining policy-driven legislative or regulatory measures required to ensure a high level of consumer protection with regards to food and feed safety.

EFSA communicates to the public in an open and transparent way on all matters within its remit.

Collection and analysis of scientific data, identification of emerging risks and scientific support to the Commission, particularly in case of a food crisis, are also part of EFSA’s mandate, as laid down in the founding Regulation (EC) No 178/2002 of 28 January 2002.

For more information about EFSA, please contact:

**Official seat:**
Palazzo Ducale
Parco Ducale 3
I-43100 Parma
Italy

**Operational and postal address:**
Largo N. Palli 5/A
I-43100 Parma
Italy

Tel: +39 0521 036 111
Fax: +39 0521 036 110
E-mail: info@efsa.eu.int
www.efsa.eu.int
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PREFACE

EFSA Scientific Colloquia aim to achieve a better understanding of the fundamental scientific issues in all areas of EFSA's mission and are organised in a way to provide opportunity for an interactive exchange of expert views. To that end the Scientific Colloquia are sufficiently informal to allow for substantial debates if needed. However, at the same time, they are adequately structured and managed to enable participants to reach conclusions and make recommendations as appropriate. The meeting on micro-organisms in food and feed qualified presumption of safety (QPS) was the second in the series of Scientific Colloquia.

The QPS approach is a system similar in concept and purpose to the GRAS (Generally Recognised As Safe) definition used in the USA, but modified to take account of the different regulatory practices in Europe. It represents a possible route to harmonisation of approaches for the safety assessment of micro-organisms used in feed/food production across the EFSA Panels and would ensure a better use of assessment resources by focussing on those organisms which represent the greatest risks or uncertainties. If introduced into Europe, QPS will permit the identification of what is required to make an adequate safety assessment. QPS is suggested as an operating tool within EFSA for safety assessment and priority setting. The objective of this Colloquium was to have an open scientific debate on the scientific principles behind the QPS approach as formulated in a working paper of a joint working group of 3 former DG SANCO (Directorate General for Health and Consumer Protection, European Commission) Scientific Committees including comments received on this working document during a public consultation period in 2003. In addition, the participants were asked to explore options on how the QPS concept may be further developed for possible implementation by EFSA in safety assessments within the framework of current and proposed legislation.

This present Summary Report is capturing the main discussion points raised by the participants of the Colloquium and does not necessarily express the view of EFSA or its Scientific Committee or Panels.
In the meantime, a working group of the EFSA Scientific Committee prepared an opinion on a generic approach to the safety assessment by EFSA of micro-organisms used in food/feed and the production of food/feed additives taking into account the suggestions made by the participants of the Colloquium and the former comments received during the consultation period of the QPS working paper in 2003. The opinion including a proposal on how the QPS approach may be implemented by EFSA has been adopted by the Scientific Committee in April 2005.

We are very pleased with the lively discussions and very constructive contributions by all participants of the meeting. Special appreciation is expressed to the overall chairs and rapporteurs of the Colloquium, the chairs and rapporteurs of the four Discussion Groups and in particular to Tine Rask Licht and John Heritage for drafting this Summary Report.

I INTRODUCTION

A variety of bacterial and fungal species are used in food and feed production. Some of these have a long history of safe use, while others may represent a putative risk for consumers. In order to capture important risk aspects without wasting resources on thorough investigations of microorganisms that are known to be safe, there is a need for development of a tool for setting priorities within the risk assessment of micro-organisms in the production of food and feed.

To explore the possibility of a system, similar in concept and purpose to the GRAS (Generally Recognized As Safe) definition used in the USA, a working paper for public consultation was prepared jointly by the former DG SANCO Scientific Committees on Food, Animal Nutrition and Plants. The proposed approach, referred to as QPS or Qualified Presumption of Safety (Annex 1) was published on the internet and was open for comments during 2003. Comments received this way are summarised in Annex 2.

With the exception of those encompassed by the Novel Food Regulation (1997), micro-organisms used for fermentation of food are presently not subject to community regulation. In contrast, micro-organisms used as feed additives or plant protection products are comprehensively regulated. This has led to illogical situations where the same strains used freely in human foods have been the subject of stringent safety assessments when seeking community approval as a feed additive. The QPS approach represents a possible route to harmonisation of approaches for the safety assessment of micro-organisms used in feed/food production without introducing unnecessary measures in areas where there has been no great concern about safety, while allowing more important safety concerns to be addressed. Importantly, QPS is suggested as an operating procedure within EFSA for risk assessment.

To have an open scientific debate on the QPS approach, EFSA organized its second Scientific Colloquium on 13-14 December 2004 in Brussels, Belgium. (The programme is given in Annex 3). About 100 participants (Listed in Annex 4) representing the scientific community, risk managers, risk
assessors and food/feed industry participated in an active debate. After a number of introductory presentations (Annex 5), participants were split up in smaller Discussion Groups each addressing different specific issues related to the QPS approach. Subsequently, the outcome of the debate from each group was presented and discussed in plenum (Annex 6). A summary of the discussion results is given in the following.

II SUMMARY OF THE DISCUSSION RESULTS

1 Traditional use of micro-organisms

The first topic for consideration was the traditional use of micro-organisms in food and feed to determine if a QPS safety assessment would be applicable. The questions to be debated were:

► is the safety evaluation of traditional uses necessary or desirable?
► if yes, could the QPS approach be adapted to include natural fermentations?
► if not, how could parameters like the presence of virulence factors and antibiotic resistances etc. be considered?

Although these questions were the starting point for discussions, the Colloquium recognised that there was merit in a QPS approach to risk assessment.

1.1 Is the safety evaluation of traditional uses necessary or desirable?

The Colloquium agreed that a long history of apparent safe use of a given micro-organism or micro-organisms for the making of a given food or feed product suggests a very high safety level for the consumption of such products.

The traditional use of micro-organisms may be placed in three categories:

A spontaneous fermentation processes *i.e.* without any micro-organisms added intentionally, and so-called back-slopping processes where an undefined mixture of micro-organisms, naturally present in a product, is recycled. Products of such processes include *e.g.* olives, cream and sourdough-based bread;

B processes based on deliberately added, but undefined microbial mixtures, which were not originally part of the natural flora in the raw material. An example of the use of an undefined microbial mixture is “Kefir”;

C processes using defined micro-organisms, which are identifiable at the strain level.
QPS is not applicable to traditional, undefined microbial mixtures belonging to Categories A and B. If such mixtures become defined, then they would be reclassified as belonging to Category C above. If an undefined microbial mixture has a long history of apparent safe use, the Colloquium agreed that no safety assessment is needed for this particular use. However, issues including the presence of virulence factors, toxic metabolites and antibiotic resistance may need to be addressed on a case-by-case basis (see 1.3).

If an undefined mixture belonging to Categories A or B can be defined at a later date, QPS will then become applicable. It was suggested that for microbial mixtures with a long history of safe use, identification at species level rather than at strain level would be required in order to obtain QPS status.

1.2 Defined strains
Based on this subdivision of the “traditional use” of micro-organisms, the Colloquium reached agreement as follows: for microbes belonging to Category C, QPS is applicable and represents a useful approach to the assessment of safety. It should be noted that this category includes complex microbial mixtures containing a large number of different strains, provided that each strain has been identified.

1.3 Undefined microbial mixtures
QPS is not applicable to traditional, undefined microbial mixtures belonging to Categories A and B, until the mixture becomes defined, and thus reclassified in Category C. If, however, the traditional undefined microbial mixture has a long history of apparent safe use, it was agreed that no safety assessment is needed for this particular use. However, certain issues could be addressed on a case-by-case basis, including the presence of virulence factors, toxic metabolites and antibiotic resistance determinants. The Colloquium agreed that further research in this area is needed.

It was envisaged that in the long term this pragmatic approach might raise the need to justify why fewer safety assessments are required for undefined microbial mixtures than for defined. However, this was not an immediate concern.

1.4 Novel uses
In the case of a novel use of a microorganism that also has a traditional use in food or feed production, the Novel Food Regulation covers the safety assessment of the products (Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel food and novel food ingredients1. It was the opinion of the Colloquium that for novel use of undefined microbial mixtures, where QPS is not possible, a full case-by-case assessment is needed. However, if the mixture can be defined, no matter how complex that mixture may be, QPS might be applicable, and necessary to optimise the safety assessment.

2 Taxonomy/familiarity
The questions relating to taxonomy and familiarity included:

- what evidence of taxonomic status is needed?
- what if a micro-organism that has been granted QPS would need to be reclassified? Will the QPS status be retained?
- what taxonomic level is appropriate for QPS?
- is a history of apparent safe use sufficient evidence of safety (and for all purposes)?
- is lack of clinical data evidence of a lack of pathogenicity?
- should taxonomic units which include pathogenic strains be excluded from QPS?

1 Official Journal of the European Communities L43,1-7
### 2.1 The taxonomic status of candidate organisms for QPS assessment

The experts were concerned that use of the term “familiarity” could cause confusion, particularly for people whose first language is not English. Rather, it was agreed that the term “body of knowledge” should be used. The latter term is more precise and reflects more accurately what is required to underpin any safety evaluation. At the Plenary session there was debate on who should define the “body of knowledge” required for a QPS safety assessment and who should decide what defines the boundaries to that body of knowledge. If QPS is to be introduced and applied successfully in the European Union, clarity is required in deciding who determines what needs to be assessed. There was general agreement that decisions on the body of knowledge and its limits would best be made by an Expert Panel.

Several elements will comprise the body of knowledge. In addition to the peer-reviewed scientific literature, these include understanding of history of use of a micro-organism, its industrial applications, its ecology, any clinical reports concerning the micro-organism and entries in public databases.

When considering “familiarity”, another term was considered likely to cause confusion. The term “traditional uses” should be excluded in favour of “established uses”, which implies a significant level of use, whether in terms of the period for which a micro-organism has been used or the number of individuals exposed to the micro-organism, or a combination of both.

It was agreed that undefined microbial mixtures are beyond the scope of a QPS safety evaluation because they are not a single taxonomic unit. It was proposed, however, that, in time, a better understanding of microbial mixtures could lead to their inclusion in a QPS assessment. There was a proposal that molecular tools could help to resolve the problems associated with microbial mixtures (see Section 3.1).

The Colloquium considered if it was appropriate to discuss “taxonomy”. Classification and identification are separate but related processes and it was agreed that it is essential for a QPS risk assessment to determine the identity of the micro-organism, rather than its position in a taxonomic scheme.

### 2.2 The reclassification of micro-organisms that have been granted QPS approval?

By shifting the focus onto the identity of an isolate, the issue of reclassification of a micro-organism is largely overcome. Identifying an isolate depends upon determining its biological activity and this will not change when an isolate becomes reclassified. Reclassification involves assigning different weightings to features, rather than altering the fundamental features themselves. Furthermore, the process of reclassification of a micro-organism will add to the body of knowledge relating to that organism, strengthening the QPS safety assessment. Where reclassification would group a species previously classified as QPS with other species that have additional hazards, e.g. virulence factor(s): this may necessitate the modification of the qualifications required to validate the safety of this species, or if the mechanisms responsible for these dangers are unknown...
When considering a history of apparent safe use, a number of factors require consideration. Vulnerable individuals within the population are increasing. These include the very young, the elderly and those whose host defences are compromised, particularly those individuals who suffer immuno-suppression. The rest of the product with which a micro-organism is associated may also influence the safety assessment. For instance, *Penicillium roquefortii* does not produce significant quantities of toxin when growing in blue cheeses but does produce toxin when present as a contaminant of bread. Bearing these factors in mind, a history of apparent safe use of a micro-organism will allow resources to be focussed where they are needed most.

2.3 Which taxonomic level is appropriate for QPS assessment?

With regard to the level of identity required for a QPS risk assessment, the Colloquium proposed that QPS should use the identity of an isolate set at the highest taxonomic unit that is appropriate for the purpose for which the evaluation is intended. This will depend upon the body of knowledge available for the micro-organism to be assessed and upon the nature of the micro-organism being assessed. It was considered that for lactobacilli, for example, assessment at the genus level could be appropriate, whereas for applications using fungi of the genus *Aspergillus*, the assessment might have to be at the level of individual strains. This is because of the occurrence of some strains in that genus that produce toxins. Yeasts pose a similar problem. For instance, for the genus *Saccharomyces* risk assessment at the level of genus would be appropriate, whereas it would be inappropriate to assess members of the genus *Candida*, since this genus is much less well defined than is the genus *Saccharomyces*. There is, as yet, no universally agreed classification system for filamentous fungi; to implement a QPS safety assessment of the fungi imperfecti successfully, particular care must be taken.

2.4 The suitability of a history of apparent safe use in safety assessment?

A history of apparent safe use does not simply mean use of a micro-organism over a long period; it would also include factors such as the level of exposure to a micro-organism. It was considered that a history of apparent safe use does not, in itself, constitute a risk assessment. Furthermore, no assurances can be made that something is absolutely safe. An appropriate history of safe use does, however, provide evidence in support of a “reasonable certainty of no harm”.

When considering a history of apparent safe use, a number of factors require consideration. Vulnerable individuals within the population are increasing. These include the very young, the elderly and those whose host defences are compromised, particularly those individuals who suffer immuno-suppression. The rest of the product with which a micro-organism is associated may also influence the safety assessment. For instance, *Penicillium roquefortii* does not produce significant quantities of toxin when growing in blue cheeses but does produce toxin when present as a contaminant of bread. Bearing these factors in mind, a history of apparent safe use of a micro-organism will allow resources to be focussed where they are needed most.

2.5 Is lack of clinical data evidence of a lack of pathogenicity?

Lack of clinical evidence is considered to be helpful in establishing the safety of a micro-organism, but this lack must be interpreted with caution. Lack of clinical evidence may be indicative of absence of pathogenic potential, but this is only the case where the population in question has in fact been exposed at a level at which any adverse events could be detected.

Another factor requires careful consideration when interpreting the absence of clinical evidence, which relates to methodology appropriate to identify an adverse effect. Lactic acid bacteria cannot all be considered to be entirely safe because occasionally they have been associated with clinical specimens. They may, however, be considered to be opportunist pathogens. In the case of the genus *Lactobacillus* isolated from clinical specimens, no virulence factors have been identified. This may be because such bacteria genuinely lack virulence factors and their presence in clinical samples is always adventitious. Alternatively, it may be that bacteria of the genus *Lactobacillus* do, at least in some conditions, express virulence factors that have yet to be recognised. The larger the body of knowledge on a strain, the less likely this alternative will prove to be the case.
2.6 Taxonomic units including pathogenic strains in QPS safety assessment?

The genus *Enterococcus* illustrates another issue relating to clinical evidence. These bacteria are frequent opportunistic pathogens of humans and clinical isolates of these bacteria often express adhesion factors. While the expression of an adhesion factor is considered to assist the isolate in producing disease, the same attribute may be desirable in strains to be used as probiotics. This is an important issue to resolve since bacteria of the genus *Enterococcus* are regarded as opportunistic pathogens, yet some strains are used as starter cultures in dairy products. Thus, providing that the level of identification is set appropriately, there is no compelling reason why species that include pathogenic strains should be excluded from a QPS risk analysis.

3 The role of molecular tools in QPS

The following topics were considered under the heading of the role of molecular tools in QPS:

- what is the role of molecular techniques in taxonomy and strain identification?
- to what extent do the molecular tools define the risk of transmissible antibiotic resistance?
- to what extent do the molecular tools define the risk of virulence?
- what are the issues for the validation of results obtained by molecular techniques?
- what is the potential of post-genomics tools?

3.1 The role of molecular techniques in taxonomy and strain identification

A range of techniques have been developed to underpin taxonomic studies and to aid the identification of micro-organisms. These DNA sequence-based tools overcome many of the problems encountered when applying classical character-based methods to the identification of micro-organisms. Molecular techniques applied to the identification of micro-organisms include, for example, 16S rRNA analysis, Pulsed-Field Gel Electrophoresis (PFGE) and Multi-Locus Sequence Typing (MSLT). With certain of these techniques, international databases are currently being developed and these may be of value in any QPS-like safety evaluation.

The most appropriate tool to use for a given application will depend on the nature of that application; the choice of which tool to use in a given application is best made by specialists with expertise in the field. Whichever method is chosen, however, it must be robust, reliable and reproducible. The need for validated tools was discussed.

The application of molecular tools could make a significant contribution to the characterisation of mixed microbial populations. This benefit may be seen at different levels. Molecular probes could determine whether undesirable organisms were present in a mixed population but may also be used to establish if genes encoding toxins or mobile resistance determinants are present in the mixture. Recognising that mixed microbial populations may be amenable to analysis using molecular tools indicates that there is a need to establish a vigilance plan to monitor for the emergence of micro-organisms with undesirable attributes in the mixture.

In addition to being able to establish that undesirable micro-organisms or their products are absent from mixed populations, molecular tools may be able to trace changes in the structure of such populations over time, permitting study of the dynamics of mixed populations. In this context, it is important to consider that in certain products, the micro-organism(s) used to initiate the production process are not present in the final product.
It should be stated, however, that taxonomy is fundamental to the identification of an organism and consequently is of great importance in safety assessment. Classical taxonomy based on morphological characteristics and physiological parameters has proven to be a significant tool and its value should not be underestimated.

### 3.2 The extent to which molecular tools define the risk of transmissible antibiotic resistance?

The nature of any antibiotic resistance determinant present in a candidate micro-organism for QPS evaluation needs to be determined. Antibiotic resistance per se is not a safety issue. It only becomes a safety issue when horizontal transfer is concerned. Intrinsic resistance may arise because the candidate micro-organism may lack the target for the antibiotic. This will raise less concern in a safety evaluation than a micro-organism that has acquired mobile DNA encoding an enzyme that modifies or destroys an antibiotic. It is also important to recognise that antibiotics differ in their clinical and veterinary importance. Risk assessment of antibiotic resistance determinants in micro-organisms must take this into account.

The application of gene probes in assessing antibiotic resistance should be used with caution. A number of resistance determinants are inducible and the presence of a DNA sequence that encodes antibiotic resistance does not necessarily imply that the host micro-organism will express that resistance. Another limitation of molecular tools to the study of antibiotic resistance is that they may only be used to detect resistance determinants where the genetic basis of the resistance determinant is known. There is no mechanism currently available that permits the detection of novel DNA sequences that encode antibiotic resistance where the mechanism of resistance is as yet uncharacterised.

Arising from these discussions was the recognition that transmissibility of DNA has implications for risk assessment. It was also highlighted that transmission of DNA is a two-way process. Strains that harbour the genes for antibiotic resistance or virulence determinants may act as a reservoir for the DNA encoding those traits. Strains that are initially devoid of such characteristics may, however, acquire and express DNA that encodes undesirable characteristics.

### 3.3 The extent to which molecular tools define the risk of virulence

There are close parallels between the issue of virulence determinants and the consideration of antibiotic resistance. Molecular tools are not the only methods by which virulence determinants may be characterised and, as with antibiotic resistance, they may only be applied to establish whether virulence determinants that have been characterised previously are present. As yet uncharacterised virulence determinants are not amenable to detection using molecular tools.

Molecular tools may, nevertheless, have a significant role in the detection of specific virulence determinants, even at the species level. If molecular tools are to be included in a QPS safety evaluation of virulence determinants, it is important that the determinants are ranked according clinical risk, assessed with respect to transfer and defined with respect to biological relevance.

### 3.4 Limitations of the results obtained by molecular techniques

While molecular tools may have a significant role to play in QPS risk assessment, it should be remembered that their application to certain issues may be inappropriate. With respect to the study of biogenic amines, for example, searching for the appropriate DNA sequence responsible for their production and then determining the level of expression of those sequences will, of necessity, be inferior to the direct detection of the undesirable substance.
3.5 The potential of post-genomics tools

“Omic” analysis provides the capacity to screen large numbers of genes fast but with limited specificity. New developments such as transcriptomics may become important for specific applications. An important question arises, however; “Should this high capacity be systematically used?” In the application of molecular tools it is important to differentiate that which we “need to know” from that which would be “nice to know”.

There was a consideration of the status of genetically modified micro-organisms in a QPS risk assessment. In particular, the case of self-cloning to introduce targeted changes in a strain was debated. Specific mutations may be introduced into DNA cloned into a suicide vector. This may then be introduced into the target strain and, on completion of the transformation process, the only trace of the genetic modification would be the presence of the desired mutation. This was compared with the selection of mutant using conventional selection procedures, where the recovered mutant may carry several cryptic mutations in addition to that which confers the desired trait. There appears to be no scientific basis for the exclusion of such self-cloned genetically modified micro-organisms from a QPS risk assessment.

4 Advantages and disadvantages of the QPS when used for safety assessment

Questions relating to the advantages and disadvantages of QPS included:

► what are the strengths and weaknesses of the QPS approach?

► are there better alternatives to the QPS approach? If so, what are the advantages and disadvantages of these alternatives when compared to QPS?

► should it be a requirement for QPS to deposit the given strain in a culture collection?

► could the QPS approach be extended to enzymes and other products of micro-organisms?

► could putative consequences be identified of implementing the QPS or any suggested alternatives for e.g. consumers, industry, risk assessors and risk managers?

These discussions were wide-ranging and at the Plenary meeting the report focussed on a general evaluation of the QPS approach, the benefits that may accrue if the European Union were to adopt QPS safety evaluation, weaknesses inherent in the proposals and issues to consider before QPS could be introduced, suggestions for modifications of the proposed QPS approach to safety evaluation, alternatives to QPS, the need for deposition of QPS strains in culture collections and the possible extension of the approach to include enzymes and other microbial products.

4.1 General evaluation

The participants agreed that there is a need to clarify the need, scope and objectives of the QPS approach. Even though the debate focussed on the scientific issues connected to the system, there was much discussion centred on the modality of functioning of the QPS system and its role in risk assessment versus its eventual use as a risk management tool.

The Colloquium agreed that the decision tree in the DG SANCO working document on QPS (Annex 1) is intended as a tool for risk assessors and it is not intended for use by notifiers. The QPS procedure may be used to create a list of taxonomic units that can be granted QPS status, with or without requested qualifications. Thus, the notifier would only be faced with the issue of whether his particular strain is granted “QPS”, “QPS with qualifications”, or “Not suitable for QPS”. In the last case, the strain in question and its proposed use would have to go through a full risk assessment.
In this form, the participants of the Colloquium were of the opinion that the QPS approach is a workable system. The scientific experts should decide what taxonomic grouping is considered, and how to define the demarcation of these groups. In addition, they should identify taxonomic groups where specific qualifications and additional information is required in order to obtain QPS status. It is envisaged that relatively few species need to be considered in order to cover the majority of the micro-organisms used in food and feed production.

4.2 Benefits of adopting the QPS approach

An important advantage of implementing the QPS approach is that it will make the evaluation more proportionate to the risk and allow risk assessors to focus on the more significant aspects of the assessment. As a result, the “any other concerns” issue in the proposed decision tree should be addressed effectively if the QPS approach is to be successful. Focussing on the most important issues leads to a better use of EFSA’s scientific resources. Furthermore, the human resources necessary for creation of dossiers etc. will be economised, and the need for experimental animals will be reduced.

Ideally, the QPS process will help to identify areas where additional knowledge is necessary, and thereby constitute a learning process for the scientific community inside as well as outside EFSA. This process should, however, distinguish clearly between what is an essential aid to the risk assessment process, and what is merely “nice to know”.

The implementation of an efficient assessment system such as QPS may facilitate the innovation of new food products. In this context, a putative benefit of the QPS approach may be increased consumer confidence. Standardisation of procedures within EFSA will increase transparency. It is, however, the responsibility of EFSA to provide and elaborate an effective system, and thereby to demonstrate to consumers that the approach is working. When addressing the food safety of products containing micro-organisms, it is crucial to educate consumers that the inclusion of “beneficial and safe bacteria” is at least as important to food and feed production as is the evasion of “bad bugs”, with which we are all too familiar. Currently, there is considerable attention on the occurrence of pathogenic bacteria in food, and it is therefore important not to create unreasoned anxiety about fermented products containing live bacteria. It was recognised that there is a need to communicate with consumers. Such a process is intended to reinforce the trust and confidence of the public in the safety of the products made with QPS micro-organisms found in food products that are normally considered to be among the safest to eat. Agreement was reached that the most important point to communicate is the focussing of resources on the greatest risks.

4.3 Issues to consider if the QPS approach is to be adopted

Initially, the Colloquium expressed anxiety that the QPS approach, and the idea of initiating any kind of risk assessment of microbes used for food production, would be an unreasonable burden for SMEs (small and medium sized enterprises), and thereby lead eventually to increased sectorisation. However, because QPS is envisioned only as a operational tool within EFSA to enable priorities to be set within the safety evaluation, there would be no link with risk management.

The concept of “familiarity” was discussed extensively, and it was agreed that the intention when establishing “familiarity” of a given strain is to link this strain to an existing body of knowledge. In this process, it is important to define not only what is known – but also what is not known about a given taxonomic unit for which “familiarity” is established.

There was agreement that the QPS approach is difficult to apply to undefined and/or complex natural microbial mixtures. This issue was discussed at length but remains a weakness or limitation of the approach.
Another difficulty identified was the determination of the requirements for molecular methods used in the risk assessment (e.g., to meet qualifications necessary to obtain QPS). It is necessary to base safety evaluation on validated methods. On the other hand, development of molecular methods is a rapidly evolving field, and it should not be the intention to prevent applicants/notifiers from using the best and newest methods available at a given time point.

It must be determined who is responsible for setting up the initial structure of the system. There was consensus that this should be done by EFSA. Also, a need to propose specific mechanisms for implementation was identified. This should be discussed within the EFSA Working Group on QPS.

4.4 Suggestions for modifications to QPS

In Paragraph 22 of the DG SANCO working paper on QPS (Annex 1), three possible end-uses of microbes in food and feed are envisaged, in short:

- a live organism is a component of the final product and is consumed directly;
- a live organism is not intended to enter the food chain but may enter it adventitiously;
- the organism is used only in the production and the final product is intended to be free of micro-organisms.

The Colloquium recommended a modification of the last bullet point to include also the presence of dead micro-organisms in the final product.

Additionally, it was suggested that there should be a modification of the decision tree outlined in the DG SANCO working paper on QPS, so that lack of familiarity for a given strain will directly lead to the status "Not suitable for QPS". This will in fact abolish the right side of the proposed decision tree.

4.5 Alternatives to QPS

A number of alternative approaches to the proposed QPS system exist at present. The US GRAS approach, which is connected to a specific use of a given micro-organism, was presented at the introductory session of the Colloquium. So was the French approach, which addresses only new organisms without a history of use. The Danish approach, which is a fully regulated process, was described. Another alternative is to refer to the industry to implement the safety assessment of their products. The main alternatives to consider are (i) a voluntary versus a regulatory system, and (ii) whether to include the approach in the risk assessment or in the risk management.
4.6 The requirement to deposit QPS strains in culture collections

The Colloquium did not reach agreement on the issue of whether or not it should be a requirement for QPS to deposit a given strain in a culture collection. A number of experts considered that the QPS concept will be difficult to implement without deposition of cultures in an approved culture collection. Other experts thought that acquiring the QPS status is more an issue of identification and characterisation of a strain, than of its deposition. The application of molecular tools to strain recognition and characterisation will be assisted considerably by the deposition of strains in recognised culture collections. If QPS safety evaluation is adopted, deposition of strains in culture collections should be encouraged to provide reference material; deposition could be in closed collections to protect intellectual property rights. The question was raised of what the deposited material will be used for. It was agreed that there should be a mechanism within the QPS approval procedure that takes into consideration the potential for genetic drift. It was suggested that the deposit of strains could be an additional element in the decision tree and there was a recommendation that it should be obligatory for new strains that achieve QPS status to be deposited in recognised culture collections, whether open or closed.

4.7 Extension of the QPS approach to include enzymes and other microbial products

In favour of such an extension is the argument that it will harmonise and simplify procedures within EFSA, and that the safety evaluation of mixed enzymes will be more easily dealt with than it is presently the case. An opposing argument is that it will lead to very cumbersome risk assessments for specific uses of enzymes. It was suggested that a micro-organism and its metabolites could be considered in a holistic assessment. This would imply a need for considering the level, processes and purpose for such an evaluation.

5 General Discussion

There were general points that arose during the deliberations of more than one Discussion Group at the Colloquium. Important among these was a discussion of the term “familiarity” and its need to be refined. Other common issues included the requirement for strains granted QPS status to be deposited in recognised culture collections and the importance when applying a QPS procedure of differentiating what would be “nice to know” from what it is necessary to know to complete the safety evaluation. Currently in Europe, decisions on risks are made on the basis of available knowledge. QPS, if introduced into Europe, will permit the identification of that which is required to make an adequate risk assessment.

There was a discussion around the question of the necessity and desirability of safety evaluation of traditional foods or feeds, the use of defined strains in foods and feeds, whether singly or in combination, issues concerning the use of undefined mixtures of microbes and whether QPS has a role in the safety assessment of novel products.

Some delegates did not see the need for deposition of strains that have achieved QPS status in recognised culture collections; others felt that deposition would be of benefit, particularly as the number of microorganisms qualifying for QPS status increased. This will be an important point for the Working Group on QPS to consider when making its recommendations to EFSA.

On the balance between what is “nice to know” and what needs to be known, any recommendation from the Working Group on QPS must recognise that resources are finite but that it is important to develop a transparent and effective safety assessment.

Related to the debate on defining the body of knowledge for a safety assessment, there was a discussion on the potential regulatory burden arising from the application of molecular tools to QPS evaluations, and the possibility that results obtained using these tools could be misinterpreted.
EFSA is currently required to assess micro-organisms for a variety of purposes; food, feed, plant protection, genetically modified organisms, etc. In addition, EFSA may be requested to conduct a risk assessment of a micro-organism, for example in response to concerns raised by a Member State. Because of this, a harmonised approach to the risk assessment of micro-organisms is desirable. It was agreed, however, that harmonisation is not the primary purpose of QPS, which is a tool to help evaluate effectively and efficiency the safety of a food or feed containing micro-organisms.

In the European Union, risk assessment of food and feed is separated from risk management. It was considered critical that this separation is maintained in the application of QPS. There is a need for consistent approach to the treatment of micro-organisms for feed use and for food use. EFSA has finite resources and these must be used in the most efficient manner possible without compromising safety assessment. It was generally agreed that QPS is a possible mechanism for achieving this.

Legislation defines clearly the need for risk assessment in many areas; how would QPS fit in those areas? There is no mechanism within European legislation for recognising what in the USA is “GRAS (Generally Recognised As Safe)”\(^\text{1}\); QPS is an attempt to provide a mechanism within the European Union for approving micro-organisms that are safe while focussing fuller assessments on those micro-organisms that are more hazardous, recognising that EFSA works in a different legislative framework than that of the USA.

Other advantages may accrue from the introduction of a QPS approach to food and feed safety assessment, for example harmonisation of assessment processes. It must be clearly understood, however, that QPS is primarily a tool for the efficient evaluation of safety of a food or feed containing a micro-organism. The QPS approach is an attempt to keep safety evaluations equal and fair in all areas. Regulations impose obligations, however, and there are intellectual property rights that may make the application of QPS difficult. This will be a matter that the Working Group on QPS will need to consider carefully in future.

The Colloquium addressed the need to proceed with caution if QPS is to be introduced successfully into the safety evaluation process. Care must be taken that its introduction does not raise public concerns about categories of foods that are among the safest that are consumed currently. There is an obligation on scientists to explain this process with great care and to communicate to the public that EFSA is not responding to some newly discovered risk.

In conclusion, it was agreed that QPS appears to be applicable to food, to feed and to microbial products from the point of view of safety assessment. There are, however, a number of issues that need careful consideration before QPS could be introduced into the European safety evaluation process. It is a system that may be developed to include not only micro-organisms in food and feed but also their metabolic products. Such an expansion of the system should, however, be a step-by-step process.

Issues on which the Colloquium did not reach agreement included:

- The need to deposit strains which have been granted QPS in a culture collection;
- The need for validation of molecular methods used to meet qualifications needed to obtain QPS.

On the other substantive issues that were discussed, however, consensus was reached as described.
III ANNEXES

Annex 1: On a generic approach to the safety assessment of microorganisms used in feed/food and feed/food production – working paper for public consultation (June 2003) prepared by a working group of the European Commission DG Health and Consumer Protection

Annex 2: Summary of issues raised before the Colloquium through the public consultation exercise

Annex 3: Programme of the EFSA Colloquium

Annex 4: Participants at the Colloquium

Annex 5: Presentations made at the Colloquium

Annex 6: Slides of Discussion Groups
Annex 1  On a generic approach to the safety assessment of micro-organisms used in feed/food and feed/food production

A working paper open for comment

Comments are invited from interested parties. Please send your comments before 30th June 2003 to the following e-mail address:

sanco-sc2-secretariat@cec.eu.int

This document for public consultation has been produced by a Working Group consisting of members of the Scientific Committee on Animal Nutrition, Scientific Committee on Food and the Scientific Committee on Plants of the European Commission.

Background

1. A wide variety of bacteria and microfungi are used to produce fermented foods in Europe and in other parts of the world. The bacterial genera involved include Lactobacillus, Lactococcus, Pediococcus, Leuconostoc, Carnobacterium, Enterococcus, Micrococcus, Streptococcus, Staphylococcus, Propionibacterium and Acetobacter. In addition to the well recognised use of Saccharomyces in the production of food and beverages, other fungal genera such as Kluyveromyces, Pichia, Kloeckera, Candida, Penicillium, Aspergillus and Mucor are used in the production of a variety of foods. Consequently, products of microbial action (alcoholic drinks, fermented milks, butter, cheeses, leavened and sourdough bread, pickled vegetables and fruit, cured meats, chocolate, tea and coffee) and/or the organisms themselves are part of an everyday
Scope and purpose

5. The purpose of this document is to explore the possibility of introducing a system, similar in concept and purpose to the GRAS (Generally Recognised As Safe) definition used in the USA, which could be applied to micro-organisms and eventually their products and to invite comments on its practicality. It is evident that such a scheme should not compromise on safety but should ideally improve, extend, clarify and make more consistent the approval procedures for micro-organisms and, where possible, allow a more generic approach to be taken in place of a full case-by-case assessment.

6. Such an approach should not seek to reproduce the GRAS system but should take account of the different social and regulatory climate present in Europe. This is necessary since issues of importance to Europe would not necessarily influence a GRAS listing. An example of this in the context of micro-organisms would be the presence of acquired antibiotic resistance factors, considered highly undesirable in Europe but currently of lesser issue in the USA.

7. Consequently it is proposed that any “generic listing” of a micro-organism should be qualified, allowing the general safety of the organism/group of organisms to be concluded provided that certain specific criteria are met. For example, for many of the live organisms currently used in the manufacture of, or added to, dairy products, this may simply be a requirement to demonstrate the absence of acquired antibiotic resistance factors.

diet. Some of these foods are manufactured using defined starter cultures, but many, even in industrialised processes, are produced either by spontaneous fermentation or by back-slopping.

2. With the exception of those micro-organisms not previously used to a significant degree in the preparation of a human food within the Community (captured by the Novel Foods Regulation\(^2\)), micro-organisms for food use are not subject to Community regulation. Implicit in this absence of any formal requirement for a safety assessment is the recognition that there has been a long history of presumed safe use.

3. This is in marked contrast to micro-organisms entering the food chain in association with animal feeds or as plant protection products, both of which are comprehensively regulated in Europe\(^3,4\). Although many of the organisms used in animal feed or as a source of processing aids are the same or closely similar to those used in human food production, there is currently no mechanism for extrapolating from the experience of the food industry. This is partly because there is no recognised means for a micro-organism formally to be considered as safe for human food applications.

4. This has already led to situations where the same or closely related strains used freely in human foods have been the subject of stringent safety assessments when seeking Community approval as a feed additive. Conversely, legitimate concerns, such as the presence of antibiotic resistance factors, which determined the need for a safety assessment for microbial feed additives, are not addressed when the same organism has only a traditional food use.

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Qualified presumption of safety

8. It is suggested that these requirements could be met by a system which would allow a:

Qualified presumption of safety (QPS), presumption being defined as “an assumption based on reasonable evidence” and qualified to allow certain restrictions to apply.

9. QPS would provide a qualified generic approval system that would harmonise the safety assessment of micro-organisms throughout the food chain. This could be done without either compromising the standards set for micro-organisms used in animal feedingstuffs or requiring all organisms used in food production with a long history of use to be subjected to a full and unnecessary safety review. Thereafter it would aid the consistency of assessment and make better use of assessment resources without compromising safety.

10. A case-by-case safety assessment then could be limited to only those aspects that are relevant for the organism in question (e.g. the presence of acquired antibiotic resistance determinants in a lactic acid bacterium or known virulence factors in a species known to contain pathogenic strains).

11. However, to have any value a QPS scheme must be seen as assuring safety both by food/feed manufacturers and consumers. Similarly, QPS must show clear advantages over a full case-by-case approach allowing notification to substitute for a repeat assessment when another use or production method is found for an organism already granted QPS status.

General considerations in a QPS scheme

12. Whatever the use and identity of the organism(s) there are a series of general conditions that would have to be met before QPS status could be established (see Figure 1). The starting point must be identity at whatever taxonomic level for which QPS status is sought. This could be at the genus level, but more likely would be for a named species, a recognised subgroup of a species or for a single well recognised/characterised strain (e.g. E. coli K12, a strain selected for its lack of pathogenic potential).

13. Thus a pre-requisite for QPS would be identity, unambiguously established at the taxonomic level claimed. The appropriate biochemical and molecular biological methods must exist to enable this to be done. The importance of taxonomy in the risk assessment of micro-organisms is recognised internationally and is the subject of a guidance document currently being produced by the OECD Working Group on Harmonization of Regulatory Oversight in Biotechnology.

14. Many industrial strains of micro-organisms will be a product of a selection/mutagenesis programme designed to improve their phenotype for a particular purpose. In the majority of cases cryptic mutation or selection for the same use (e.g. increased phage resistance or over-production of an enzyme) will not affect taxonomic status. Use of recombinant technology for strain improvement is the subject of separate existing legislation.

15. If the taxonomic unit cannot be related via the existing and any historic nomenclature to a body of knowledge, then QPS status is not applicable. This is most likely to occur when an isolate identified to the genus level cannot be assigned to an existing species/sub-species or when the species is a newly recognised taxonomic unit.

16. The second test that would have to be applied is the question of familiarity and, in particular, the degree of familiarity.

17. Familiarity in this context is taken to include practical experience of use of the organism(s) including its history of use for particular purposes and any body of literature on the biology of the taxonomic unit. Judgement as
to whether the organism(s) can be considered familiar should be based on a weight of evidence approach. This must be sufficient to provide adequate assurance that any potential to produce adverse effects in humans, livestock or the wider environment is understood and predictable.

18. For organisms not commonly used in food production or without a long history of use, this implies a need for experimental data on the genetics of the taxonomic unit and the growth and biochemical characteristics of the component strain(s) under a variety of relevant environmental conditions. This should provide sufficient material for the third test applied – that of pathogenic potential (human or animal).

19. Many micro-organisms can, under extreme conditions (e.g. in the severely immuno-compromised), be found associated with diseased tissue. The occasional clinical report of a micro-organism or group of micro-organisms being isolated from clinical specimens should not necessarily result in the taxonomic unit being treated as potential pathogens.

20. If a taxonomic group is commonly responsible for pathological conditions, then QPS does not apply. However, if pathogenicity is limited to selected strains and if the mechanism underlying the pathology is understood and testable, then the taxonomic unit might still be eligible for QPS status; the qualifications attached being used to exclude the pathogenic strains. This is important in relation to, for example, *Bacillus* species and their toxigenic potential or to the virulent/avirulent forms of enterococci.

21. For some groups of organisms, such as those used as plant protection products, a consideration of impact on the wider environment may be appropriate. However, this would exclude organisms considered either to be of healthy gut origin and regularly introduced into the wider environment or to be of soil/water origin. In both cases the organisms are naturally occurring and therefore free of any need for an environmental impact assessment.

22. The final general question relates to end use; and has, in essence, three possible outcomes:
   - A live organism is a component of a final product intended to enter the food chain directly (it is consumed);
   - A live organism is a component of a final product but is not intended to enter the food chain although it may enter it adventitiously (e.g. a plant protection product);
   - The organism(s) is used only as a production strain with the final preparation containing fermentation product(s) intended to be free of live organisms.

23. The end use will influence the nature and degree of familiarity needed to determine whether the taxonomic unit is suitable for QPS status. It will also influence the qualifications imposed. It is envisaged that for products of fermentation there would be separate considerations for QPS status for the production strain and the product itself. Thus QPS status for a production strain would allow a presumption of safety to be applied to the production system but not to the product.

Qualifications

24. It is envisaged that virtually all organisms considered suitable for QPS status would have qualifications attached. Possible exceptions to this generalisation are some fungal genera/species, such as *Saccharomyces* spp. and some *Kluyveromyces* strains.

25. Although each consideration for QPS status would have to be on a case-by-case basis and so some qualifications may be unique to a particular organism and its application, there are a number of qualifications likely to be more widely applied, particularly to bacteria. For example:
   - Live bacteria entering the food chain via animal feed, or live and dead bacteria directly consumed by humans should be free of any acquired resistance to antibiotics of importance in clinical and veterinary medi-
may be the ability to change production conditions (media etc.) with only a requirement for notification rather than generating a need for an additional safety assessment.

Requirements of QPS

30. For a Notifier with a production strain falling within a taxonomic unit already granted QPS status the only requirement would be:
   - Registration of a production strain with accompanying evidence of its taxonomic status and that the strain meets all of the qualifications imposed for the particular taxonomic unit;
   - Notification of any changes in use or to production conditions.
   Otherwise paragraph 28 might apply.

Figure 1. A general scheme for the assessment of suitability for QPS status of micro-organisms.
QPS as a practical exercise

31. The following are examples of how QPS might apply to micro-organisms widely used in the dairy food industry or to bacteria used primarily for bulk production by fermentation. These assessments are preliminary and designed only to illustrate how QPS might be applied. Any conclusions should not be considered definitive or binding.

Dairy lactobacilli

32. The genus *Lactobacillus* is a taxonomically heterogeneous group of organisms producing lactic acid, either as a sole fermentation product (homofermentative lactic acid bacteria -LAB) or together with acetic acid/ethanol and CO$_2$ (heterofermentative LAB). While certain species are obligatory homofermentative (*Lactobacillus acidophilus, L. helveticus, L. delbrückii*) or heterofermentative (*L. brevis, L. fermentum, L. buchneri, L. reuteri*), the majority metabolise hexose sugars homofermentatively and pentose sugars heterofermentatively. Although modern molecular biological classification methods have revealed clusters of species within the genus, these clusters do not correspond to the traditional grouping based on physiology, morphology and fermentation patterns. Nonetheless, species identification can in most cases be reliably done using few simple physiological and biochemical tests allowing connection to a long history of use and an extensive bibliography.

33. The lactobacilli have been traditionally used to produce a wide variety of fermented foods, including cheese, fermented milks, sourdough, cure meats and sausages etc. In dairy applications, particularly the obligate homofermentative species *L. delbrueckii ssp. bulgaricus* and *L. helveticus*, are well known as starters for yoghurt and Swiss cheese, respectively.

34. Besides the main fermentation end products, certain lactobacilli produce variable amounts of other metabolites such as, acetaldehyde (“yoghurt aroma”), formic acid and H$_2$O$_2$. Although lactobacilli are not known to produce actual antibiotics, production of protein or peptide bacteriocins active against other Gram positive bacteria is relatively common. Some species and strains also produce some low molecular weight antimicrobial substances, such as “reuterin” or 3-hydroxypropanal (produced by *L. reuteri*). Most of these are, however, chemically poorly defined. Production of biogenic amines by lactobacilli has been occasionally reported.

35. Lactobacilli are fastidious organisms requiring a milieu rich in nutrients, especially fermentable sugars, for growth. Consequently, their natural habitats include the mouth, intestinal and urogenital tract, decaying plant material and milk. No actually pathogenic lactobacilli are known, although they can be occasionally indicated in opportunistic infections, usually in cases where there has been a severe underlying disease.

36. Because common lactobacilli used in dairy applications can be readily identified to the species level, and because the dairy species or strains have only extremely rarely, if ever, been indicated even in the rare opportunistic infections caused by lactobacilli, species such as *L. delbrueckii* and *L. helveticus* could be reasonably considered for QPS-status. The only qualification that might be attached is evidence of the absence of acquired antibiotic resistance.

*Bacillus subtilis* and related bacteria

37. The guidelines for the delineation of a bacterial species require strains within a species to share more than 70% chromosomal DNA hybridisation and between species less than 70% hybridisation. The *B. subtilis* group traditionally comprises four species: *B. amyloliquefaciens, B. licheniformis, B. pumilus* and *B. subtilis* itself. These taxa all conform to the DNA hybridisation guidelines for bacterial species. More recent ecological studies have identified some very close relatives of *B. subtilis*, notably *B. atrophaeus, B. mojavensis* and *B. vallismortis*. 
38. The 16S rRNA gene sequences differ between representative species of the *B. subtilis* group, but such data are not available for the “ecological” group. Species of the traditional group can be distinguished phenotypically, but *B. mojavensis*, *B. subtilis* and *B. vallismortis* are indistinguishable and can only be identified by molecular means while *B. atrophaeus* is distinguished from *B. subtilis* only by pigmentation. One of the main implications of the inability to distinguish the members of the ecological group is that strains of “*B. subtilis*” being used by industry may actually belong to *B. mojavensis*, *B. vallismortis* or to other species.

39. The taxonomic status of the traditional members of the group is well established and allows connection to a sizeable body of information on their biology. *B. subtilis* was one of the first organisms to be fully sequenced and its genome is now extensively annotated. Although the species traditionally included in the *B. subtilis* group could be considered as a group for QPS purposes, in the first instance it would seem prudent to deal with them on an individual species basis.

40. Members of the “ecological group” may have considerable genetic similarity to the more generally recognised members, but little is known about their biology. It is unlikely that these species would be proposed for QPS unless a reassessment of taxonomic status led to the inclusion of an existing production strain.

41. Strains of *B. licheniformis*, *B. pumilus* and *B. subtilis* have occasionally been reported as causative agents in food poisoning. Both diarrhoeal and emetic types of outbreaks have been recorded, but the nature of the toxins associated with these species is not fully understood. In particular, it is not clear if the enterotoxins are the same as those of *B. cereus*, the common cause of *Bacillus* food poisoning, or if other enterotoxins are involved. The indirect evidence of the presence for genes similar to the *B. cereus* haemolytic toxin (Hbl) provided by PCR, and for Hbl and the non-haemolytic toxin (Nhe) by the commercial ELISA kits is not conclusive. Without purification of toxins and sequencing of the PCR products it is impossible to be sure about the presence of similar or identical virulence factors. However, *B. licheniformis* has been shown to produce a toxin that shows similar physico-chemical properties to cereulide although with a different pattern of biological activity.

42. Other indications of pathological conditions associated with the *B. subtilis* group are rare. However, in many clinical reports on opportunistic infections, the causative *Bacillus* has not been identified to species level. *B. pumilus* strains have been implicated in infections mimicking listeriosis. *B. licheniformis* is also associated with bovine toxemia and abortions, although it is evident that this species is only weakly virulent and usually will multiply freely only in animals which, for various reasons, are immune compromised.

43. *Bacillus* species are commonly isolated from gut contents but their presence appears to be due to constant re-inoculation rather than outgrowth and clonal expansion. Consequently, both animals and humans are constantly exposed to those *Bacillus* species encountered in the environment with no apparent ill effects. The lack of evidence for retention in the gut also reduces the likelihood of any genetic transfer occurring and any adverse consequences in the very unlikely event that such a transfer occurs.

44. The possible pathology of these organisms essentially is limited to a few strains able to produce symptoms of mild food poisoning and there is sufficient knowledge to allow this risk to be substantially reduced. Consequently, with the possible exception of *B. pumilus*, potential pathogenically is not a barrier for QPS at the species level.

45. Approximately half of the present commercial production of bulk enzymes derives from strains of bacilli, most from the *B. subtilis* taxonomic group. These include proteases and amylases (*from B. amyloliquefaciens, B. licheniformis*). Strains of *B. subtilis* are used for the preparation of nucleic acid bases such as inosine which are precursors of flavour enhancing nucleotides for use in the food industry. These bacteria also produce lipopeptide surfactants and a diversity of polypeptide “antibiotics” with activity against bacteria and fungi. Some of these...
Annex 2  Summary of issues raised before the Colloquium through the public consultation exercise

The draft QPS document (q.v.) formulated by the joint working group of a number of Scientific Advisory Committees was placed on the SANCO website with an invitation to interested parties to comment by the end of June 2003. Some 25 replies were received from individuals and trade organisations. These varied in extent from a detailed consideration of each part of the document, to comments addressed to the overall concept.

Among the replies four issues predominated:

- **The scope of the proposed QPS system.** While many were in favour of a harmonised approach to a safety assessment of micro-organisms used in food production, there was some uncertainty where the boundaries would be drawn. These concerns largely related to “traditional fermentations” in which the inoculum used is ill-defined or spontaneously derived. It was pointed out that some quite large production faculties used only partially defined strains of micro-organisms. Also it was thought unclear whether the proposal extended (or could be extended) to microbial products.

- **Antibiotic resistance determinants.** Particularly in relation to those organisms commonly used in the preparation of human foods. This was seen as one of the more difficult areas, partly because of the limited available data of patterns of antibiotic resistance amongst lactic acid bacteria and lack of standardisation, and partly because antibiotic resistance is known or suspected to be common amongst those LAB strains in common use. It was argued that antibiotic resistance has not been recognised as a problem in foods and there is no evidence to suggest that this form of human exposure has led to any measurable increase in resistance to antibiotics of clinical importance. Consequently, the continuing use of existing strain should not be placed in jeopardy.

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*Bacillus* species (*B. subtilis, B. licheniformis*) have also found use in the animal feed industry as live feed additives and have been used as probiotic preparation for humans.

46. Tools exist that allow the identity of strains to be established, the species comprising the *B. subtilis* group are familiar and their biology well understood and sufficient is known about their pathogenicity to exclude problem strains. Consequently, *B. subtilis, B. amyloliquefaciens* and *B. licheniformis* might reasonably be considered individually for QPS status. Strains falling within these taxonomic units could then be presumed safe providing the following qualifications were met:

- Provision of PCR-based evidence of the absence of a toxigenic potential and, because of doubts about the homology existing between genes encoding enterotoxins, evidence of an absence of effects in cytotoxicity assays.

- For production strains only in which the live organism is excluded from the final product, evidence of a capacity for toxin production would not necessarily exclude a strain from QPS. However, it would have to demonstrate that the strain failed to produce detectable levels of toxin under the production conditions employed. There would also be a requirement for the same evidence to be produced each time there was a change to the production system. This would not be necessary in the absence of a toxigenic potential.

- The strain should be shown to be free of any acquired resistance to antibiotics of importance in human and veterinary medicine. Again, the presence of antibiotic resistance would not exclude its safe use for production purposes but would exclude it from use as a live organism likely to enter the food chain.

- The absence of a capacity to produce antibiotics with structural similarities to those of importance in human and veterinary medicine likely to encourage development of resistance.
**Taxonomy and the need for an unequivocal identification.** There was uncertainty whether identity should always reflect the state of the art or whether identity based on phenotypic criteria would be sufficient. There were some concerns that smaller companies may not have the facilities to undertake extensive taxonomic studies. In addition, it was pointed out that several microbial groups which might be eligible for QPS status are relatively poorly understood and their taxonomy is undergoing almost continuous revision.

**Community Regulations.** Several groups had concerns about how QPS could be incorporated into Community legislation and whether this was desirable or necessary. Others recognised that this might be less of an issue once EFSA was functional.

Other issues were addressed including the need to make specific reference to virulence genes, the need to recognise vulnerable groups when determining suitability for QPS, and questions about the consequence and status of organisms not considered suitable for QPS. All of the replies received from the SANCO consultation, together with the conclusions and recommendation of this Colloquium, will be considered by the new EFSA QPS Working Group.
11.50-12.10 Use of micro-organisms in food and feed distribution Walter Hammes
12.10-12.20 Discussion
12.20-12.40 The GRAS Concept Laura Tarantino
12.40-12.50 Discussion
12.50-13.00 Instructions for Discussion Groups Juliane Kleiner
13.00-13.30 LUNCH
13.30-16.00 SESSION 2: DISCUSSION GROUPS (DG)

Four parallel Discussion Groups to address questions related to:

DG 1 Traditional use of micro-organisms Chair: Charles Daly
Rapporteur: Lorenzo Morelli
DG 2 Taxonomy/familiarity Chair: Wilhelm Holzapfel
Rapporteur: Stephen Forsythe
DG 3 The role of molecular tools in QPS Chair: Mike Gasson
Rapporteur: Pierre Renault
DG 4 Advantages and disadvantages of QPS? Chair: Pier Sandro Cocconcelli
Rapporteur: Jean-Louis Jouve
16.00-16.30 COFFEE/TEA BREAK

16.30-18.30 SESSION 3: REPORT BACK FROM DISCUSSION GROUPS TO PLENARY

16.30-16.45 Report back from Discussion Group 1 Lorenzo Morelli
16.45-17.00 Discussion
17.00-17.15 Report back from Discussion Group 2 Stephen Forsythe
17.15-17.30 Discussion
17.30-17.45 Report back from Discussion Group 3 Pierre Renault
17.45-18.00 Discussion
18.00-18.15 Report back from Discussion Group 4 Jean-Louis Jouve
18.15-18.30 Discussion
20.00 DINNER

Tuesday 14 December 2004

9.00-12.00 SESSION 4: CONTINUATION OF DISCUSSION GROUPS TO DISCUSS FEED BACK FROM PLENARY AND TO PREPARE CONCLUSIONS AND RECOMMENDATIONS

10.30-11.00 COFFEE/TEA BREAK
12.00-13.00 LUNCH

13.00-16.00 SESSION 5: FINAL PLENARY SESSION

13.00-14.00 Report back to Plenary Lorenzo Morelli
Stephen Forsythe
Pierre Renault
Jean-Louis Jouve
14.00-16.00 General discussion and conclusions and recommendations
16.00 COLLOQUIUM ADJOURNS
### Annex 4: Participants at the Colloquium

<table>
<thead>
<tr>
<th>NAME</th>
<th>Affiliation</th>
<th>Country</th>
<th>Discussion Group (DG)</th>
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<tbody>
<tr>
<td>Dr. Jens K. Andersen</td>
<td>Danish Institute for Food and Veterinary Research</td>
<td>DK</td>
<td>DG 2</td>
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<tr>
<td>Dr. Fabrizio Arigoni</td>
<td>Nestlé</td>
<td>CH</td>
<td>DG 3</td>
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<tr>
<td>Mrs. Isabelle Auger</td>
<td>Danisco France</td>
<td>FR</td>
<td>DG 1</td>
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<tr>
<td>Dr. Albert Bär</td>
<td>Bioresco Ltd</td>
<td>CH</td>
<td>DG 1</td>
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<tr>
<td>Dr. Jane Beal</td>
<td>University of Plymouth</td>
<td>GB</td>
<td>DG 1</td>
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<tr>
<td>Dr. Gerard Bertin</td>
<td>European Federation of the Animal Feed Additive Manufacturers (FEFANA)</td>
<td>BE</td>
<td>DG 3</td>
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<tr>
<td>Prof. Peter Brooks</td>
<td>University of Plymouth</td>
<td>GB</td>
<td>DG 4</td>
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<tr>
<td>Mrs. Coralie Bultel</td>
<td>French Food Safety Agency (AFSSA)</td>
<td>FR</td>
<td>DG 2</td>
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Annex 5: Presentations made at the Colloquium

INTRODUCTION TO EFSA

Herman B.W.M. Koëter
EFSA Deputy Executive Director and Director of Science
EFSA’s Mission and Tasks [Reg 178/2002]

... provide scientific advice and scientific and technical support... [Art.22.2]
... shall provide scientific opinions ... [Art.22.6]
... provide the best possible scientific opinions in all cases provided for by Community legislation and on any question within its mission ... [Art.23(a)]

EFSA’s Mission and Tasks [Reg 178/2002]

... collect and analyse data to allow the characterisation and monitoring of risks...[Art.22.4]
... promote and coordinate the development of uniform risk assessment methodologies ... [Art.23(b)]
... commission scientific studies ... [Art.23(d)]
... undertake action to identify emerging risks ... [Art.23(f)]
Provide scientific and technical assistance with a view to improving cooperation ... [Art.23(i)]

EFSA stands for

➤ Independency
➤ Scientific excellence
➤ Openness and transparency
➤ Co-operation

Scientific activities (work themes) (1)

Providing scientific opinions, guidance and advice in response to questions:
➤ Most questions from the Commission, but also from European Parliament and Member States
➤ Questions can be broad (risks and benefits of eating fish) or focused (e.g. risk assessment of SEM in baby food in glass)
➤ Questions have “terms of reference” and deadlines for response

Scientific activities (work themes) (2)

Assessing the risk of regulated substances and development of proposals for risk-related factors:
➤ Chemical categories include:
  ► Food additives, smoke flavourings, enzymes
  ► Additives for use in animal nutrition
  ► Pesticides
  ► Genetically modified food and feed
➤ Risk related factors include MRL’s
➤ Deadlines for opinions are strict and often very short (e.g. for GMO’s, feed additives)

Scientific activities (work themes) (3)

Monitoring of specific risk factors and diseases:
➤ Geographical BSE risk assessment
➤ BSE / TSE testing and validation of tests
➤ Monitoring of zoonoses and zoonotic agents
➤ Containment and eradication of animal diseases (e.g. AI, foot and mouth disease, rabies)
**Scientific activities (work themes) (4)**

Development, promotion and application of new and harmonized scientific approaches and methodologies for hazard and risk assessment of food and feed:

- Harmonization of detection methodology for chemical and microbiological contaminants in food/ feed
- Harmonized approach for environmental hazard and risk assessment
- Harmonized approach for human health risk assessment
- Harmonization of methodology for the monitoring and reporting of animal diseases
- Exposure assessment modeling (chemical and microbiological)
- Improving transparency of the risk assessment process

**Invest in fore-front science through**

Organisation of open scientific EFSA meetings, to discuss in-depth topical and sensitive issues related to EFSA’s mission: EFSA Scientific Colloquia. Adequate follow-up on EFSA Scientific Colloquia (*e.g.* development of Guidance Documents).

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**EFSA Colloquium 2**

“Micro-organisms in Food and Feed Qualified Presumption of Safety – QPS”

13-14 December 2004, Brussels, Belgium

**The Colloquium is:**

- An interactive event rather than a passive listening to lectures
- A platform for scientists to have in-depth discussions on methodology and principles related to QPS
- A way to build common views and understanding

**The Colloquium is not:**

- An attempt to agree on a harmonised QPS approach in risk assessment
- An attempt to finalise a Guidance Document
- A “who is right and who is wrong” discussion
QUALIFIED PRESUMPTION OF SAFETY (QPS)

Prof. Andrew Chesson
University of Aberdeen
Origin of QPS

Scientific Committees responsible for safety assessments within DG SANCO noted that, for micro-organisms deliberately associated with the food chain, there was a:
- Lack of harmonisation
- Lack of proportionality
- No mechanism for the formal recognition of familiarity

Basis of QPS

A group of organisms could be considered as safe for use provided that:
- Their identity could be established
- There is sufficient familiarity to establish safety
- There are no known pathogenic strains (or sufficient is known to exclude pathogenic strains)

Basis of QPS

Strains given QPS status would, in most cases, still be subject to specific qualifications
Safe provided that …
For example – free of known virulence or antibiotic resistance determinants, use restricted to a particular purpose.

Organisms not fulfilling the requirements for QPS

Still may be considered safe but would be assessed on a case-by-case basis.

Why the delay?

- Draft QPS document placed on SANCO website with comments invited before June 2003
- Mandate for SANCO Scientific Committees ended in March 2003
- Responsibilities transferred to EFSA and its Scientific Panels from April 2003
Advantages of QPS for EFSA

- Could be made operational without legislation – as an EFSA procedure
- Experience gained with micro-organisms could be extended to other categories
- Focuses assessments according to risk
- Provides option for a fast-track assessment

Remit given to the Scientific Committee

- Review and revise the SANCO document taking account of comments received
- Consider how QPS could be implemented within EFSA
- Explore with the Commission whether a more formal recognition of QPS is possible and/or desirable
Categories of uses of micro-organisms

- Food and/or feed containing living micro-organisms intentionally added: cheese, fermented drinks, bread, probiotics, etc.
- Food and/or feed ingredients resulting from micro-organisms metabolism: enzyme, amino acids, polysaccharides, vitamins, etc.
- Products containing added non-living micro-organisms

**Heterogeneity of standards**

“Domestic or European regulations do not cover all the fields where micro-organisms are used. In addition, only some regulations in force provide recommendations which allow the safety assessment of micro-organisms …”

<table>
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<th>Technological aids (enzymes)</th>
<th>Novel food</th>
<th>Plant protection</th>
<th>Food</th>
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The French background

“… As a result, both studies conducted by petitioners and requests of the experts who assessed these files are heterogeneous.”

- 1997: Harmonisation requirement expressed by experts (CSHPF)
- 1998: Draft of guidelines proposed by industrialists (AFNOR)
- 2001-2002: Reflexion by the French food safety agency (AFSSA)

2001-2002

**Expertise process of AFSSA**

- Pluridisciplinary working group
  - «Microbiology» panel
  - «Biotechnology» panel
  - «Feed» panel
  - «Food» panel

- Food operators consultation

- «Recommendations for presentation of data which will allow the safety assessment of micro-organisms used in food and feed sector” (November 2002)

**Objective: Harmonisation**

To establish guidelines for the presentation of data:

- presented by food operators for authorization of use of micro-organisms
- examined by authorities prior assessment (administrative admissibility of the file)
- analysed by experts who perform safety assessment
Applications
The proposed approach is a prerequisite:

- For the use of strains (new or modified) or
- For a different application of strains which are already used

= NOT “FAMILIAR”

Decision tree

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<th>Strain to be evaluated</th>
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<td>Is the strain sufficiently known?</td>
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<td>Yes: Is the species a known or documented hazard?</td>
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<tr>
<td>No: Generating data regarding the health risks related to the strain</td>
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<tr>
<td>Yes: Are the risks confirmed for the considered use?</td>
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<tr>
<td>No: Acceptable strain for the considered use</td>
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<tr>
<td>Yes: Unacceptable strain for the considered use</td>
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Divergent points with QPS approach

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<th>QPS guidelines</th>
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<td>«New micro-organisms»</td>
<td>«All micro-organisms»</td>
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<td>Precise guidelines for the presentation of data in a file</td>
<td>Global approach</td>
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<td>Case-by-case assessment</td>
<td>Case-by-case not systematic</td>
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<tr>
<td>Assessment is based on a file established by the petitioner</td>
<td>Key notion: «familiarity»</td>
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<tr>
<td></td>
<td>Assessment is based on a positive list initially established by risk assessors</td>
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Common points with QPS approach

- Pluridisciplinary approach
- Decision tree

- Harmonisation of data for safety assessment of micro-organisms used in the agro-food sector
THE USE OF MICRO-ORGANISMS
IN FOOD AND FEED

Prof. Dr. W. P. Hammes
Fields of use of micro-organisms in production of food and feed

- Production of fermented food and drinks
- Production of silage
- Micro-organisms are food and feed, e.g. algae, yeast and fungal mycelia
- Fermentative production of enzymes and metabolites used in food and feed, as ingredients or additives

Milestones in Food (and Feed) Fermentation

From Neolithic times until Louis Pasteur fermented food and silage were produced without any knowledge of micro-organisms:

- 1858 “levure lactique” described by L. P.
- 1878 Lister isolates “Bacterium Lactis”
- 1883 Hansen isolates Saccharomyces from beer
- 1890 Storch (DK), Weigmann (D) and Conn (USA) create the fundamentals of dairy starter cultures
- 1910 ”Reinzuchtsauer” (D) introduced for dough fermentation
- 1955 Niven (USA) introduces starter for fermented sausages

In the following starter cultures for fermentation of vegetables (and juices), fish, wine, as well as probiotic and protective cultures were introduced, but traditional fermentation processes are still widely in use.

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<td>- preservation</td>
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<td><strong>Probiotic cultures</strong></td>
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<tr>
<td>a) sensu stricto</td>
<td>Indication of hazard</td>
</tr>
<tr>
<td>b) indicative cultures</td>
<td>(“Emergency breaks” by acidification)</td>
</tr>
</tbody>
</table>
Fermented food and feed of plant origin
(selected number of products known in Europe)

<table>
<thead>
<tr>
<th>raw material</th>
<th>product</th>
<th>micro-organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olives, cabbage, cucumber, tomato and others</td>
<td>fermented olives, sauerkraut, pickled cucumber, etc.</td>
<td>LAB</td>
</tr>
<tr>
<td>dough and batters made from cereals</td>
<td>sourdough, yeast dough, kisra, etc.</td>
<td>LAB and yeasts vide infra</td>
</tr>
<tr>
<td>malt, koji, made from cereals and grains</td>
<td>beer, sake, spirits, etc.</td>
<td>LAB, fungi</td>
</tr>
<tr>
<td>beer, wine, spirits</td>
<td>vinegar</td>
<td>acetic acid bacteria</td>
</tr>
<tr>
<td>grapes and other fruits</td>
<td>wine</td>
<td>yeasts, LAB, (fungi)</td>
</tr>
<tr>
<td>soy, locust bean (oriental and African)*</td>
<td>soy sauce, tempe, natto, dawadawa, etc.</td>
<td>LAB, bacilli, fungi yeasts</td>
</tr>
<tr>
<td>grass, maize, potato, etc.</td>
<td>silage</td>
<td>LAB</td>
</tr>
</tbody>
</table>

* see Nout and Rambouts, 2000

Fermented food and feed of animal origin
(selected number of products known in Europe)

<table>
<thead>
<tr>
<th>raw material</th>
<th>product</th>
<th>micro-organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>milk*</td>
<td>sour milk products: curdled milk, sour cream, yoghurt, kefir, koumiss sour cream butter cheese</td>
<td>LAB, yeasts, acetic acid bacteria</td>
</tr>
<tr>
<td></td>
<td>fermented sausages</td>
<td>fungi, yeasts, LAB, propionibacteria, others: vide infra</td>
</tr>
<tr>
<td>meat**</td>
<td>fish silage (also from meat)</td>
<td>staphylococci, micrococci, Streptomyces</td>
</tr>
<tr>
<td></td>
<td>fish sauce, fermented fish silage</td>
<td>fungi, yeasts, LAB, staphylococci, Vibrio costicola, LAB</td>
</tr>
</tbody>
</table>

* Teuber, (2000)  
Ways to start a fermentation process

- indigenous fermentations, a spontaneous process
- back shuffling (of fermenting substrate)
- use of starter cultures
  - **undefined cultures**: similar to back shuffling but higher degree of microbial selection by continuous propagation, e.g. “Flora Danica” (>100 *Leuconostoc* and *Lactococcus* strains), “Reinzuchtsauer” (3 strains of two Lactobacillus species dominate)
  - **defined cultures**: multiple strain and single strain cultures, in use as dairy, dough, wine, meat, and vegetable cultures as well as in biotechnology for production of defined substances

Which micro-organisms have a Safe Tradition In Food Fermentation (STIFF)?

- Presence in the fermenting association. Examples are sourdough and red smear cheese

**In sourdough** the fermenting organisms are killed in the baking process. **Dead cells and metabolites are consumed.**

**In red smear cheese** living organisms are consumed together with their metabolites.

### Global production of food crops (2002, FAO)

<table>
<thead>
<tr>
<th>Crop</th>
<th>Production (million t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>400</td>
</tr>
<tr>
<td>Rice, Paddy</td>
<td>350</td>
</tr>
<tr>
<td>Wheat</td>
<td>250</td>
</tr>
<tr>
<td>Potatoes</td>
<td>300</td>
</tr>
<tr>
<td>Sugar Beets</td>
<td>200</td>
</tr>
<tr>
<td>Cassava</td>
<td>150</td>
</tr>
<tr>
<td>Soybeans</td>
<td>150</td>
</tr>
<tr>
<td>Sweet Potatoes</td>
<td>100</td>
</tr>
<tr>
<td>Oil Palm Fruit</td>
<td>100</td>
</tr>
<tr>
<td>Barley</td>
<td>100</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>50</td>
</tr>
<tr>
<td>Oranges</td>
<td>50</td>
</tr>
<tr>
<td>Cabbages</td>
<td>50</td>
</tr>
<tr>
<td>Sorghum</td>
<td>50</td>
</tr>
<tr>
<td>Coconuts</td>
<td>50</td>
</tr>
<tr>
<td>Oats</td>
<td>50</td>
</tr>
<tr>
<td>Millet</td>
<td>50</td>
</tr>
<tr>
<td>Rye</td>
<td>50</td>
</tr>
<tr>
<td>Carrots</td>
<td>50</td>
</tr>
<tr>
<td>Lettuce</td>
<td>50</td>
</tr>
<tr>
<td>Beans, Dry</td>
<td>50</td>
</tr>
<tr>
<td>Chick-peas</td>
<td>50</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>50</td>
</tr>
<tr>
<td>Quinoa</td>
<td>50</td>
</tr>
</tbody>
</table>

**Total global harvest of food crops: 3.6 billion t, 60% are cereals**

Industrialized countries: 70% are animal food. 30%, plus nearly the total harvest in developing countries serves human nutrition. The greater part is fermented. **Thus, cereals are the most important substrates for food fermentations.**
Why fermenting cereals?
To obtain products:
- Leavened baked goods (e.g. sourdough, yeast leavened dough)
- Acid fermented gruels (ogi, from manihot: fufu)
- Alcoholic drinks (e.g. beer, sake, spirits)
- Acid fermented drinks (e.g. boza, Berliner Weiße, kwass, mahewu (commonly in combination with alcoholic fermentation))
- Vinegar [secondary aerobic fermentation of e.g. alcoholic fermented rice (China), or beer (Europe)]
- Colorant (Angkak, Monascus purpureus, koji technology)
- Animal feed (silage)

Why fermenting cereals?
To affect the following:
- Conditioning for wet milling (steeping of maize and wild rice)
- Achieving sensory effects (aroma, taste, texture, colour)
- Saccharification (use of koji) prior to alcoholic fermentation or producing sweetened rice
- Preservation (e.g. by acidification and/or alcohol production)
- Enhancing safety (by inhibition of pathogens, e.g. Burkholderia gladioli (maize caused >200 death in China; Bonkrek poisoning, Staphylococcus aureus, Bacillus cereus). In silage L. monocytogenes and C. botulinum are of concern
- Improving the nutritive value
  - Removal of antinutritive compounds (e.g. phytate, enzyme inhibitors, polyphenols, tannins)
  - Enhancing bioavailability (e.g. affecting physico-chemical properties of starch and associations of fiber constituents with vitamins, minerals or proteins)
- Removal of undesired compounds (e.g. mycotoxins, endogenous toxins, lectins, cyanogenic compounds, flatulence producing carbohydrates)
- Reducing energy required for cooking
- Achieving the condition of bakeability, as it is required for producing leavened rye bread
**LAB isolated from sourdoughs (wheat and rye)**

<table>
<thead>
<tr>
<th>Species</th>
<th>Source</th>
<th>Sourdough</th>
<th>Sourdough</th>
<th>Sourdough</th>
<th>Sourdough</th>
<th>Sourdough</th>
<th>Sourdough</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. acidophilus</td>
<td>human, swine, poultry, cattle, horse,</td>
<td>012</td>
<td>12</td>
<td>L. mucosae</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. alimentarius</td>
<td>swine</td>
<td>S1</td>
<td>S1</td>
<td>L. murinus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. amylovorus</td>
<td>human, poultry, cattle</td>
<td>2</td>
<td>L. panis</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. brevis (L. pastorianus)</td>
<td>human, swine, poultry</td>
<td>S01</td>
<td>S0123</td>
<td>L. paraalimentarius</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. buchneri</td>
<td>mouse, rat</td>
<td>1</td>
<td>S1</td>
<td>L. paracasei (L. casei)</td>
<td>S01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. cellobiosus</td>
<td>human, swine, poultry, cattle, horse</td>
<td>S</td>
<td>S</td>
<td>L. plantarum</td>
<td>S01 S13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. coryniformis</td>
<td>human</td>
<td>1</td>
<td></td>
<td>L. reuteri</td>
<td>1 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. crispatus</td>
<td>human, swine, cattle</td>
<td>1</td>
<td></td>
<td>L. rhamnosus</td>
<td>01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. curvatus</td>
<td>human, swine, poultry, cattle, horse</td>
<td>01</td>
<td>1</td>
<td>L. sakei</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. delbrueckii ssp. lactis</td>
<td>human, swine, poultry, cattle, mouse, rat</td>
<td>0</td>
<td>2</td>
<td>L. sanfranciscensis</td>
<td>01 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. delbrueckii ssp. bulgaricus</td>
<td>human, swine, poultry, cattle, horse</td>
<td></td>
<td></td>
<td>L. xylosus</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. delbrueckii ssp. delbrueckii</td>
<td>human, swine, poultry, cattle, horse</td>
<td>01</td>
<td>1</td>
<td>Lc. lactis ssp. lactis</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. farcininis</td>
<td>human, swine, poultry, cattle, horse</td>
<td>S01</td>
<td>1</td>
<td>Leuc. citreum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. fermentshensis1</td>
<td>human, swine, poultry, cattle, horse</td>
<td>2</td>
<td></td>
<td>Leuc. mesenteroides</td>
<td>S0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. fermentum</td>
<td>human, swine, poultry, cattle, horse</td>
<td>01</td>
<td>12</td>
<td>Leuc. mesenteroides ssp. dextranicum</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. fructivorans</td>
<td>human, swine, poultry, cattle, horse</td>
<td>01</td>
<td>1</td>
<td>Pediococcus damnosus</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. frumenti</td>
<td>human, swine, poultry, cattle, horse</td>
<td>2</td>
<td></td>
<td>P. parvulus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. hilgardii</td>
<td>human, swine, poultry, cattle, horse</td>
<td>S1</td>
<td></td>
<td>P. pentosaceus</td>
<td>S01 S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. homohiocchi</td>
<td>human, swine, poultry, cattle, horse</td>
<td>S1</td>
<td></td>
<td>Enterococcus faecium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. johnsonii</td>
<td>human, swine, poultry, cattle, horse</td>
<td>2</td>
<td></td>
<td>Weissella confusa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. mali</td>
<td>human, swine, poultry, cattle, horse</td>
<td>W. viridescens</td>
<td>S0</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. maltaromaticus</td>
<td>human, swine, poultry, cattle, horse</td>
<td>Carnobacterium divergens</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Lactic acid bacteria found in sourdough as well as in the intestines of humans and animals**

(Hammes and Hertel, 2003; Dal Bello et al. 2003)

<table>
<thead>
<tr>
<th>Species</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. acidophilus</td>
<td>human, swine, poultry, cattle, horse,</td>
</tr>
<tr>
<td>L. amylovorus</td>
<td>swine</td>
</tr>
<tr>
<td>L. brevis</td>
<td>human, poultry, cattle</td>
</tr>
<tr>
<td>L. crispatus</td>
<td>human, swine, poultry</td>
</tr>
<tr>
<td>L. delbrueckii ssp. lactis</td>
<td>human, mouse, rat</td>
</tr>
<tr>
<td>L. fermentum</td>
<td>human, swine, poultry, cattle, mouse, rat</td>
</tr>
<tr>
<td>L. johnsonii</td>
<td>human, swine, poultry, cattle, horse</td>
</tr>
<tr>
<td>L. mucosae</td>
<td>swine</td>
</tr>
<tr>
<td>L. murinus</td>
<td>mouse, rat, dog</td>
</tr>
<tr>
<td>L. paracasei (L. casei)</td>
<td>human, cattle</td>
</tr>
<tr>
<td>L. plantarum</td>
<td>human, swine, cattle, horse</td>
</tr>
<tr>
<td>L. pontis</td>
<td>duck</td>
</tr>
<tr>
<td>L. reuteri</td>
<td>human, swine, cattle, poultry, mouse, hamster, horse</td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>human</td>
</tr>
<tr>
<td>L. sakei</td>
<td>human</td>
</tr>
<tr>
<td>Leuc. mesenteroides</td>
<td>human</td>
</tr>
<tr>
<td>P. pentosaceus</td>
<td>human</td>
</tr>
<tr>
<td>E. faecium</td>
<td>human, poultry, swine, cattle</td>
</tr>
</tbody>
</table>
Ecology of *Lactobacillus* species involved in human infections

<table>
<thead>
<tr>
<th>Species</th>
<th>Main habitat</th>
<th>probiotic food</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. acidophilus</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>L. brevis</em></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>L. casei</em></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>L. delbrueckii</em></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>L. fermentum</em></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>L. gasseri</em></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>L. jensenii</em></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>L. johnsonii</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>L. paracasei</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>L. rhamnosus</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>L. salivarius</em></td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* The early species identification may be doubtful (see also selection "Intestinal Tract – Lactobacilli in humans")

Micro-organisms in probiotic products

<table>
<thead>
<tr>
<th>Lactobacilli</th>
<th>Bifidobacteria</th>
<th>other LAB</th>
<th>non-LAB</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. acidophilus</em> (L. casei)</td>
<td><em>B. adolescentis</em></td>
<td><em>Ent. faecalis</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td><em>Bacillus cereus</em> (&lt;&quot;toyois&quot;&gt;)&lt;sup&gt;a,d&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>L. crispatus</em></td>
<td><em>B. animalis</em></td>
<td><em>Ent. faecium</em></td>
<td></td>
</tr>
<tr>
<td><em>L. gallinarum</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td><em>B. bifidum</em></td>
<td><em>Lactoc. lactis</em>&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><em>L. gasseri</em></td>
<td><em>B. breve</em></td>
<td><em>Leuconostoc mesenteroides</em>&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><em>L. johnsonii</em></td>
<td><em>B. infantis</em></td>
<td><em>Ped. acidilactici</em>&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><em>L. paracasei</em></td>
<td><em>B. lactis</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td><em>Sporolactobacillus inulinus</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td><em>B. longum</em></td>
<td><em>Strep. thermophilus</em></td>
<td></td>
</tr>
<tr>
<td><em>L. reuteri</em></td>
<td><em>P. acerocolon</em></td>
<td><em>Saccharomyces cerevisiae</em> (&lt;&quot;boulardii&quot;&gt;)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><em>L. rhamnosus</em></td>
<td><em>S. thermophilus</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* in animal nutrition, <sup>b</sup> Synonymous with *B. animalis*, <sup>c</sup> unknown probiotic effects, <sup>d</sup> in pharmaceutical preparations

Yeasts isolated from and adapted to doughs

<table>
<thead>
<tr>
<th>Species</th>
<th>Synonym</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida boidinii</em></td>
<td><em>Torulopsis glabrata</em></td>
</tr>
<tr>
<td><em>Candida glabrata</em></td>
<td><em>Torulopsis candida</em></td>
</tr>
<tr>
<td><em>Candida humilis</em></td>
<td><em>Candida milleri</em></td>
</tr>
<tr>
<td><em>Candida parapsilosis</em></td>
<td><em>Torulopsis candida</em></td>
</tr>
<tr>
<td><em>Candida stellata</em></td>
<td><em>Torulopsis candida</em></td>
</tr>
<tr>
<td><em>Debaryomyces Hansenii</em></td>
<td><em>Candida famata</em></td>
</tr>
<tr>
<td><em>Dekkeria bruxellensis</em></td>
<td><em>Brettanomyces</em></td>
</tr>
<tr>
<td><em>Galactomyces geotrichum</em></td>
<td><em>Geotrichum</em></td>
</tr>
<tr>
<td><em>Issatchenka orientalis</em></td>
<td><em>Candida kruzei</em></td>
</tr>
<tr>
<td><em>Kluuyveromycetes marxianus</em></td>
<td><em>Candida kruzei</em></td>
</tr>
<tr>
<td><em>Pichia anomala</em></td>
<td><em>Candida pelliculosa</em></td>
</tr>
<tr>
<td><em>Pichia fermentans</em></td>
<td><em>Hansenula anomala</em></td>
</tr>
<tr>
<td><em>Pichia ohmeri</em></td>
<td><em>Hansenula subpelliculosa</em></td>
</tr>
<tr>
<td><em>Pichia subpelliculosa</em></td>
<td><em>Hansenula subpelliculosa</em></td>
</tr>
<tr>
<td><em>Pichia minuta var. minuta</em></td>
<td><em>Hansenula minuta</em></td>
</tr>
<tr>
<td><em>Saccharomyces bayanus</em></td>
<td><em>Saccharomyces</em></td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td><em>Saccharomyces</em></td>
</tr>
<tr>
<td><em>Saccharomyces exiguus</em></td>
<td><em>Torulopsis</em></td>
</tr>
<tr>
<td><em>Saccharomyces kluyveri</em></td>
<td><em>Candida</em></td>
</tr>
<tr>
<td><em>Saccharomyces servazzi</em></td>
<td><em>Saccharomyces</em></td>
</tr>
<tr>
<td><em>Saccharomyces fibuligera</em></td>
<td><em>Endomyces</em></td>
</tr>
<tr>
<td><em>Saccharomyces fibuligera</em></td>
<td><em>Endomyces</em></td>
</tr>
<tr>
<td><em>Saturnispora saitoi</em></td>
<td><em>Pichia saitoi</em></td>
</tr>
<tr>
<td><em>Torulaspora delbrueckii</em></td>
<td><em>Torulopsis</em></td>
</tr>
<tr>
<td><em>Torulaspora delbrueckii</em></td>
<td><em>Candida colliculosa</em></td>
</tr>
<tr>
<td><em>Torulaspora delbrueckii</em></td>
<td><em>Saccharomyces</em></td>
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<td><em>Torulaspora delbrueckii</em></td>
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<td><em>Saccharomyces</em></td>
</tr>
<tr>
<td><em>Torulaspora delbrueckii</em></td>
<td><em>Saccharomyces</em></td>
</tr>
</tbody>
</table>

* in animal nutrition, <sup>b</sup> Synonymous with *B. animalis*, <sup>c</sup> unknown probiotic effects, <sup>d</sup> in pharmaceutical preparations
Microbial association on red smear cheese

- **Cheese types:** Münster, Romadour, Limburger, Harzer, Vacherin Mont d’Or, Tilsiter, Livarot, and many others
- **Bacteria:** *Arthrobacter nicotianum, Brevibacterium linens, Corynebacterium ammoniagenes, C. casei, C. variabile, Microbacterium gubbeenense, Rhodococcus fascians, Staphylococcus equorum, S. saprophyticus*, and many others
- **Yeast:** *Debaryomyces hansenii, Kluyveromyces marxianus, Pichia membranaefaciens*
- **Fungi:** *Galactomyces geotrichum*

Which micro-organisms have a **Safe Tradition In Food Fermentation** (STIFF)?

- Presence in the fermenting association. Examples are sourdough and red smear cheese
- **Taxonomic Position**
  - Examples are Lactobacilli and Staphylococci

### Phylogenetic relationship of lactobacilli to closely related genera (Hammes and Hertel, The Prokarytes)
**Consensus tree reflecting the relationships of Carnobacterium, Leuconostoc, Pediococcus, Weissella and major groups of the genus Lactobacillus**

By far the majority of lactobacilli are food associated, causing spoilage and/or fermentation.

At present 85 species validly described, more will certainly follow. Are these less safe?

---

**Table 1. List of the species of the genus Lactobacillus.**

The superscripts of the columns are: 
I, alphabetical numbering; II, species; III, type of glucose fermentation: 
A, obligately homofermentative; B, facultatively heterofermentative; 
C, obligately heterofermentative; IV, main habitat: D, food associated, 
usually involved in spoilage; F, involved in fermentation of food and feed; 
I, associated with humans and/or animals, e.g. oral cavity, intestines, vagina; 
S, sewage; (P), opportunistic pathogen; V, phylogenetic group**: bu, L. buchneri group; ca, L. casei group; de, L. delbrueckii group; pl, L. plantarum group; re, L. reuteri group; sa, L. sakei group; sl, L. salivarius group; u, unique.

<table>
<thead>
<tr>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td><em>L. acetotolerans</em> Entani et al. 1986</td>
<td>B</td>
<td>D</td>
<td>de</td>
</tr>
<tr>
<td>2</td>
<td><em>L. acidipiscis</em> Tanasupawat et al. 2000</td>
<td>B</td>
<td>F</td>
<td>sl</td>
</tr>
<tr>
<td>3*</td>
<td><em>L. acidophilus</em> (Moro 1900) Hansen and Mocquot 1970</td>
<td>A</td>
<td>I</td>
<td>de</td>
</tr>
<tr>
<td>4*</td>
<td><em>L. agilis</em> Weiss et al. 1981</td>
<td>B</td>
<td>S</td>
<td>sl</td>
</tr>
<tr>
<td>5*</td>
<td><em>L. algidus</em> Kato et al. 2000</td>
<td>B</td>
<td>D</td>
<td>sl</td>
</tr>
<tr>
<td>6*</td>
<td><em>L. alimentarius</em> (Reuter 1970) Reuter 1983</td>
<td>B</td>
<td>F</td>
<td>D</td>
</tr>
<tr>
<td>7*</td>
<td><em>L. amylolyticus</em> Bohak et al. 1998</td>
<td>A</td>
<td>F</td>
<td>de</td>
</tr>
<tr>
<td>8*</td>
<td><em>L. amylophilus</em> Nakamura and Crowell 1979</td>
<td>A</td>
<td>F</td>
<td>de</td>
</tr>
<tr>
<td>9*</td>
<td><em>L. amylivorans</em> Nakamura 1981</td>
<td>A</td>
<td>F</td>
<td>de</td>
</tr>
<tr>
<td>10*</td>
<td><em>L. animalis</em> Dent and Williams 1982</td>
<td>A</td>
<td>I</td>
<td>sl</td>
</tr>
<tr>
<td>11*</td>
<td><em>L. arizonensis</em> Swezey et al. 2000</td>
<td>B</td>
<td>F</td>
<td>pl</td>
</tr>
<tr>
<td>12a*</td>
<td><em>L. aviarius subsp. aviarius</em> Fujisawa et al. 1984</td>
<td>A</td>
<td>I</td>
<td>sl</td>
</tr>
<tr>
<td>12b</td>
<td><em>L. aviarius subsp. araffinosus</em> Fujisawa et al. 1984</td>
<td>A</td>
<td>I</td>
<td>sl</td>
</tr>
<tr>
<td>13*</td>
<td><em>L. bifemtans</em> (Pette and van Beynum 1943) Kandler et al. 1983a</td>
<td>B</td>
<td>D</td>
<td>u</td>
</tr>
<tr>
<td>14*</td>
<td><em>L. brevis</em> (Orla-Jensen 1919) Bergey et al. 1934</td>
<td>C</td>
<td>F</td>
<td>D</td>
</tr>
<tr>
<td>15*</td>
<td><em>L. buchneri</em> (Henneberg 1903) Bergey et al. 1923</td>
<td>C</td>
<td>F</td>
<td>D</td>
</tr>
<tr>
<td>16*</td>
<td><em>L. casei</em> (Orla-Jensen 1916) Hansen and Lessel 1971</td>
<td>B</td>
<td>I</td>
<td>F</td>
</tr>
<tr>
<td>17*</td>
<td><em>L. coleohominis</em> Nikolaitchouk et al. 2001</td>
<td>C</td>
<td>I</td>
<td>re</td>
</tr>
<tr>
<td>18*</td>
<td><em>L. collinoides</em> Carr and Davies 1972</td>
<td>C</td>
<td>D</td>
<td>pl</td>
</tr>
<tr>
<td>19a*</td>
<td><em>L. corynformis subsp. corynformis</em> Abo-Elnaga and Kandler 1965</td>
<td>B</td>
<td>F</td>
<td>u</td>
</tr>
<tr>
<td>19b</td>
<td><em>L. corynformis subsp. torquens</em> Abo-Elnaga and Kandler 1965</td>
<td>B</td>
<td>F</td>
<td>u</td>
</tr>
</tbody>
</table>
20* L. crispatus (Brygoo and Aladame 1953) Cato et al. 1983
21* L. curvatus subsp. curvatus (Troili-Petersson 1903) Abo-Elnaga and Kandler 1965
21b L. curvatus subsp. melibiosus Torriani et al. 1996
22* L. cypricasei Lawson et al. 2001a
23a* L. delbrueckii subsp. delbrueckii (Leichmann 1896) Beijerinck 1901
23b* L. delbrueckii subsp. bulgaricus (Orla-Jensen 1919) Weiss et al. 1983
24* L. diolivorans Krooneman et al. 2002
25* L. durianis Leisner et al. 2002
26* L. equi Morotomi et al. 2002
27* L. farcininis (Reuter 1970) Reuter 1983
28* L. ferriintoshensis Simpson et al. 2001
29* L. fermentum Beijerinck 1901
30* L. fornicialis Dicks et al. 2000
31* L. fructivorans Charlton et al. 1934
32* L. frumenti Müller et al. 2000a
33 L. fuchuensis Sakala et al. 2002
34* L. gallinarum Fujisawa et al. 1992
35* L. gasseri Lauer and Kandler 1980
36* L. graminis Beck et al. 1988
37* L. hamsteri Mitsuoka and Fujisawa 1987
38 L. helveticus (Orla-Jensen 1919) Bergey et al. 1925
39* L. hilgardii Douglas and Cruess 1936
40 L. homohiiochi Kitahara et al. 1957
41* L. iners Falsen et al. 1999
42* L. intestinalis (Hemme 1974) Fujisawa et al. 1990
43* L. Jensenii Gasser et al. 1970
44* L. johnsonii Fujisawa et al. 1992
45* L. kefiranofaciens Fujisawa et al. 1988
46* L. kefirgranum Takizawa et al. 1994
47* L. kefiri corrig. Kandler and Kunath 1983
48* L. kimchii Yoon et al. 2000
49* L. kunkeei Edwards et al. 1998
50* L. Lindneri (Henneberg 1901) Back et al. 1996
51* L. malefermentans (Russell and Walker 1953) Farrow et al. 1988
53* L. manihotivorans Morlon-Guyot et al. 1998
54* L. mucosae Roos et al. 2000
55* L. murinus Hemme et al. 1980
56* L. nagelii Edwards et al. 2000
57* L. oris Farrow and Collins 1988
58* L. panis Wiese et al. 1996
59* L. pantheris Liu and Dong 2002
60* L. parabuchneri Farrow et al. 1988
61a* L. paracasei subsp. paracasei Collins et al. 1989
62* L. parakefiri corrig. Takizawa et al. 1994
63* L. paralimentarius Cai et al. 1999
64* L. paraplantarum Curk et al. 1996
65* L. pentosus (Fred et al. 1921) Zanoni et al. 1987
66* L. perolens Back et al. 1999
67* L. plantarum (Orla-Jensen 1919) Bergey et al. 1923
68* L. pontis Vogel et al. 1994
69* L. psittaci Lawson et al. 2001b
70* L. reuteri Kandler et al. 1980
71* L. rhhamnosus (Hansen 1968) Collins et al. 1989
72* L. ruminis Sharpe et al. 1973a
73a* L. sakei subsp. sakei corrig. Katagiri et al. 1934
73b* L. sakei subsp. carnosus Torriani et al. 1996
74a* L. salivarius subsp. salivarius Rogosa et al. 1953
74b* L. salivarius subsp. salicinius Rogosa et al. 1953
75* L. sanfranciscensis corrig. (Kline and Sugihara 1971)
76* L. sharpeae Weiss et al. 1981
77* L. suebicus Kleynmans et al. 1989
78* L. vaccinostercus Okada et al. 1979
79* L. vaginalis Embey et al. 1989
80* L. zae (Kuznetssov 1959) Dicks et al. 1996

* Species for which at least 90% of the complete 16S rDNA sequences have been published. These species were considered for construction of the phylogenetic tree (Fig. 2 and 3). Partial sequences have been considered to attribute species to phylogenetic groups; ** Hertel, unpublished results.
Staphylococci involved in food fermentation

| Organism         | Xhl | Ahl | Bhl | Lip | Dhl | FAME | Ghl | TSS | SEA | SEF | FgBP | SEB | SEC | SED | SEE | ET | Coa | SAK | Cap | Nuc | Las | Spa | FnBP |
|------------------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|-----|----|-----|-----|-----|-----|-----|-----|
| S. aureus        |    |     |     |     |     |      |     |     |     |     |      |     |     |     |     |    |     |     |     |     |     |     |
| S. intermedius   |     |     |     |     |     | +    |     |     |     |     | +    |     |     |     |     |     |    |     |     |     |     |     |     |
| S. hyicus        |     |     |     |     |     | +    |     |     |     |     | +    |     |     |     |     |     |    |     |     |     |     |     |     |
| S. epidermidis   |     |     |     |     |     | +    |     |     |     |     |      |     |     |     |     |     |  + |     |     |     |     |     |     |
| S. xylosus       |     |     |     |     |     | +    |     |     |     |     |     |     |     |     |     |     |    |     |     |     |     |     |     |
| S. sciuri        |     |     |     |     |     | +    |     |     |     |     |      |     |     |     |     |     |    |     |     |     |     |     |     |
| S. lugdunensis   |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |    |     |     |     |     |     |     |
| S. warneri       |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |    |     |     |     |     |     |     |
| S. schleiferi    |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |    |     |     |     |     |     |     |
| S. haemolyticus  |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |    |     |     |     |     |     |     |
| S. saprophyticus |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |    |     |     |     |     |     |     |
| S. hominis       |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |    |     |     |     |     |     |     |
| S. simulans      |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |    |     |     |     |     |     |     |
| S. lentus        |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |    |     |     |     |     |     |     |
| S. capitis       |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |    |     |     |     |     |     |     |
| S. chromogenes   |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |    |     |     |     |     |     |     |
| S. equorum       |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |    |     |     |     |     |     |     |

Ahl, α-hemolysin; Bhl, β-hemolysin; Cap, type 5 or 8 capsule; Coa, coagulase; Dhl, δ-hemolysin; ET, esfoliative toxin; FAME, Fatty acid monoesterifying enzyme; FgBP, fibrinogen-binding protein; FnBP, fibrinogen-binding protein; Ghl, γ-hemolysin; Las, elastase; Lip, lipase; Nuc, staphylococcal nuclease; SAK, staphylokinase; SEA, enterotoxin A; SEB, enterotoxin B; SEC, enterotoxin D; SEE, enterotoxin E; Spa, protein A; TSS, toxic shock syndrome toxin-1; Xhl, uncharacterized hemolysin.
The safe tradition in food fermentation of strains of certain *Staphylococcus* species confirms that within a taxonomically related group of organisms safe strains or even species exist. The same applies *e.g.* to enterococci, streptococci (*S. thermophilus*), *Aspergillus oryzae* (used for koji production and closely related to *A. flavus*).

**The safety of micro-organisms in food**

<table>
<thead>
<tr>
<th>Long tradition and experience</th>
<th>Recent developments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food in general</td>
<td>Fermented food (uncontrolled)</td>
</tr>
<tr>
<td>Must not harm health. Germs are meaningful when pathogen or toxinogen</td>
<td>An effective fermenting association becomes dominating</td>
</tr>
<tr>
<td>Up to the limit of spoilage all micro-organisms accepted</td>
<td>Safety assessment needed; for N.F. legally required</td>
</tr>
</tbody>
</table>
Today I’ll Discuss …

THE GRAS CONCEPT:

- BACKGROUND AND ORIGINS
- FEATURES
- EVOLUTION
- ADVANTAGES/DISADVANTAGES

Food Drug & Cosmetic Act (1958)

- Defined “food additive”
- Required premarket approval of new uses of food additives
- Established the standard of safety

Statutory Definition of “Food Additive”
FD&C Act Section 201(s)

“The term ‘food additive’ means any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food …”

Scope of FDA’s Premarket Approval Authority

Certain classes of substances are explicitly excluded from requirement for FDA premarket approval under Section 409:

- Use of substances authorized by other laws: e.g., pesticides; animal drugs; dietary ingredients in dietary supplements; color additives
- Prior-sanctioned substances
- Substances “generally recognized as safe” (GRAS)

The “GRAS EXEMPTION”
FD&C Act Section 201(s)

“The term ‘food additive’ means any substance …

… if such substance is not generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or, in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use in food) to be safe under the conditions of its intended use …”

GRAS Status

- May be based only on the views of experts qualified by scientific training and experience to evaluate the safety of substances added to food
- Determination of GRAS status is not limited to FDA scientists
- Basis:
  - Scientific procedures
  - Experience based on common use in food (only if substance used in food prior to January 1, 1958)
Food Additive vs GRAS

- **Food Additive**
  - Information is privately held, sent to FDA
  - FDA reviews the data and makes a safety decision

- **GRAS Substance**
  - Data and Information are generally available
  - Reviewed by experts qualified by training and experience to evaluate the safety of the substance
  - Determination should reflect the consensus of experts
  - Not FDA’s decision

The Essence of GRAS

- **Information**: This is what distinguishes a GRAS substance from a food additive
  - The information relevant to the safe use of a GRAS substance is widely known
  - There is consensus among qualified experts that the available information establishes that the intended use of a GRAS substance is safe

GRAS: The First 40 Years

- 1959 ff ... The “GRAS List”: Impractical to list all GRAS substances – 21CFR Part 182
- 1969 – Cyclamate
  - Comprehensive Review – Presidential directive in response to removal of cyclamates from GRAS list
    - Codified procedures in CFR Part 170
    - GRAS Affirmation – 21 CFR Part 184

Common Use in Food

- 21 CFR 170.3(f), “[c]ommon use in food means a substantial history of consumption of a substance for food use by a significant number of consumers”
- 21 CFR 170.30(c)(1), “[g]eneral recognition of safety through experience based on common use in food prior to January 1, 1958, shall be based solely on food use of the substance prior to January 1, 1958, and shall ordinarily be based upon generally available data and information”
- The fact that a substance was added to food before 1958 does not, in itself, demonstrate that such use is safe

Scientific Procedures

- 21 CFR 170.3(h), “[s]cientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance”
- 21 CFR 170.30(b), “[g]eneral recognition of safety based upon scientific procedures ... shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient”
GRAS: The First 40 Years

- 1959 ff ... The “GRAS List”: Impractical to list all GRAS substances – 21CFR Part 182
- 1969 – Cyclamate
- Comprehensive Review – Presidential directive in response to removal of cyclamates from GRAS list
  - Codified procedures in CFR Part 170
  - GRAS Affirmation – 21 CFR Part 184

The GRAS (Notification) Proposal

- Proposed rule, published April 17, 1997
  - Clarify criteria for GRAS status
  - Eliminate GRAS affirmation petition process
  - Establish GRAS notification procedure

GRAS Criteria: Comparing a GRAS Substance to a Food Additive

- Food Additive
  - FDA
  - Technical Element
    - Common Knowledge Element
      - Generally available
      - Generally accepted

- GRAS Substance
  - Technical Element

Clarifications in GRAS Proposal

- Clarify criteria for GRAS status
  - Technical element
    - Reasonable certainty of no harm
  - Common knowledge element
    - General availability
    - General acceptance

GRAS Notification

- What it is
  - The notifier’s determination
  - A time-dependent determination, based on the information available at the time
- What it isn’t
  - Not an FDA approval
  - Not a license or company-based determination: i.e., companies other than the notifier with identical substances, identical uses, specifications may refer to a GRAS notice
- Other uses of a GRAS substance may also be GRAS, but are not necessarily so

GRAS Notification Procedure

- For further information on the GRAS Notification Procedure, including proposed regulation and a list of GRAS Notices
Summary

- **GRAS**
  - Determination of safety of the intended use by “qualified experts”, not FDA
  - History of common use in food before 1958 or Scientific Procedures
  - Current process by which a person making a GRAS determination can interact with the Agency: Notification – voluntary
  - It is information about the safety of the use of a substance that distinguishes GRAS vs food additive
Organisational Details

- 4 parallel Discussion Groups
- 13:30-16:00 DG 1st round – specific aspects
- 16:30-18:30 Touch base with Plenary
- 09:00-12:00 DG 2nd round – consideration of feedback & draft of conclusions and recommendations of DG
- 13:00-16:00 Final Plenary session – overall conclusions and recommendations

Discussion Group 1 – Traditional use of MO

Safety evaluation necessary or desirable?

Yes
QPS to be adapted to include natural fermentation?

No
Presence of virulence factors Antibiotic resistance

Discussion Group 2 – Taxonomy/familiarity (body of knowledge)

- Evidence of taxonomic status
- Level of taxonomy
- Duration of QPS status (new scientific evidence)
- History of apparent safe use → evidence of safety
- Lack of clinical data → lack of pathogenicity?
- Taxonomic units with pathogenic strains → exclusion from QPS

Discussion Group 3 – Role of molecular tools

- Role of MT in taxonomy and strain identification
- Risk of transmissible ABR: extent to be defined
- Risk of virulence: extent to be defined
- Validation of results
- Potential of post-genomics tools

Discussion group 4 – Advantages and Disadvantages

- Strengths and weaknesses of QPS approach
- Better alternatives?
- Deposit of strain a requirement for QPS?
- QPS approach to be extended?
- Consequences of implementing QPS approach for stakeholders
After the Colloquium

- Draft Summary Report of Colloquium to be prepared by rapporteurs (01/05)
- 1st Review by DG chairs and rapporteurs (02/05)
- Review of revised draft by all participants (03/05)
- Publication of Summary Report and power point presentation on EFSA website and in EFSA Scientific Colloquium Report Series
- EFSA Working Group on QPS to revise DG SANCO document taking into account conclusions and recommendations of the Colloquium and comments during the previous consultation period
- Consider options for implementation of QPS in EFSA as a practical tool for safety assessment and priority setting

Annex 6: Slides of Discussion Groups

DISCUSSION GROUP 1

TRADITIONAL USE OF MICRO-ORGANISMS

C. Daly
L. Morelli
Tuesday Dec 14th DG1

- 1st question: Is the safety evaluation of traditional uses of micro-organism necessary or desirable?

- IF THE USE OF MICRO-ORGANISMS IS TRADITIONAL NO SAFETY CONCERNS ARE ENVISAGED

CATEGORIES

- 1. Spontaneous (without any intentionally added micro-organism); back slopping (i.e. sourdough, olives, cream, etc.)
- 2. Undefined cultures (kefir, flora Danica)
- 3. Defined cultures (Known at the strain level)

QPS application to these 3 categories

- 3. Defined cultures (Known at the strain level)
- QPS applicable and represents a useful approach to the safety assessment of these cultures

But for Undefined cultures (Cat. 1 and 2)

TRADITIONAL MICRO-ORGANISMS

<table>
<thead>
<tr>
<th>VIABLE</th>
<th>UNVIABLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNDEFINED</td>
<td>QPS not possible not needed*</td>
</tr>
<tr>
<td></td>
<td>*Because of the long history of safe use</td>
</tr>
<tr>
<td>DEFINED</td>
<td>QPS possible but it needs a different decision tree</td>
</tr>
</tbody>
</table>

* Not suitable for QPS; issues with regard to AR, virulence factors, metabolites, etc. addressed on a case-by-case basis. The Discussion Group suggests that further research is needed. It should be noted that in the case of some products the microbes are not viable.

But for Undefined cultures (Cat. 1 and 2)

NOVEL USE OF MICRO-ORGANISMS

<table>
<thead>
<tr>
<th>VIABLE</th>
<th>UNVIABLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNDEFINED*</td>
<td>QPS not possible. Full, case-by-case assessment needed</td>
</tr>
<tr>
<td>DEFINED</td>
<td>QPS applicable and needed</td>
</tr>
</tbody>
</table>
Need to revise decision tree

DISCUSSION GROUP 2

Taxonomy / Familiarity

- Taxonomy adequately defined
  - Yes
  - No

- Familiarity
  - Yes
  - No

- Is the proposed use similar to traditional use?
  - Yes
  - No

- Are there known pathogenic variants?
  - Yes
  - No

- Can the pathogenicity be excluded?
  - Yes
  - No

- Any other concerns? (e.g. environment, resistance)
  - Yes
  - No

- Can the toxicity be excluded or eliminated or are the levels of exposure non-significant?
  - Yes
  - No

- Definable?
  - Yes
  - No

- Not suitable for QPS
  - Yes
  - No

- QPS (within the application)
  - Yes
  - No

- Not suitable for QPS
  - Yes
  - No
What taxonomic level is appropriate for QPS?

- QPS is not fixed *a priori*
- New information must be taken into consideration
  - The “Body of Knowledge” will increase with time
- Single species may be redefined as new species. But this is not as important as the “Body of Knowledge” of the strain
  - Redefinition of a species should have no consequences for the established features / technical properties / QPS status of a strain
- QPS should use the highest “taxonomic unit” that is definable and appropriate
  - Hence reduce “case-by-case” evaluation
- Level of “taxonomic unit” depends upon familiarity, supported (or defined) by the existing body of knowledge
- QPS should use the most up to date knowledge of taxonomy; *Future proof*. Molecular fingerprinting as important aid
- For example
  - *Lactobacillus* genus – good knowledge. Still, species level is important as some species (e.g. *Lb. rhamnosus*) are either classified as risk group 2 organisms, or have been isolated (rarely) from clinical specimens
  - *Aspergillus* – needs to define the strains due to potential toxin production

What evidence of taxonomic status is needed?

- Objective is more one of identification than taxonomy *per se*
- The “normal” taxonomic level is the species unit; however, the strain level identity has importance, both for technical reasons and for QPS

Mixed undefined cultures

- Historically “known” to be safe and “Body of Knowledge” *(experience)* does exist
- As “undefined” they are outside a definition of “taxonomic unit”
- It is plausible that in time they will be better understood *(e.g. using molecular markers; applying DGGE, etc.)*
- Refer to first question of Figure 1 (p7) in the QPS document.
  - “Identity” *(instead of taxonomy)* may be defined here in relation to “Body of Knowledge or Experience”
QPS status retained after reclassification?

- Yes in principle, retain QPS since its biological activity has not changed
  - Provided does not affect “familiarity”
  - However, reclassification from Risk Group 1 to 2 may require additional assessment of safety features; e.g. absence of virulence factors known for a particular species

Is history of apparent safe use sufficient?

- No, but it should remain a major criterion in safety evaluation
- No, but history of safe use is possibly the strongest evidence
- There is no such thing as “absolute safety”
- “Reasonable certainty of no harm” should be the objective
- If new information becomes available for a particular species, it might be necessary to determine for such a strain the presence/absence of undesired factors associated with the species (e.g. possible virulence factors; production of biogenic amines, etc.)

Issues to take into account

- Consideration of population susceptibility
- Young, immuno-compromised, and elderly persons increasing portion in general population
- Presence of potential opportunists in clinical samples does not necessarily indicate potential pathogenicity of the taxonomic entity

- Trying to focus resources to where they are most needed
- “Single”-step PCR techniques and/or molecular fingerprinting may be supportive in strain identification, and improve specificity of the existing “Body of Knowledge”

Is lack of clinical data evidence of safety?

- Evidence does not equal proof
- Lack of data – does this equate with “proof”?
- Lack of clinical data is indicative evidence for lack of pathogenicity provided the population has been exposed

Is lack of clinical data evidence of lack of pathogenicity?

- Can we consider all lactic acid bacteria as “safe”? No
- “BUT” particular strains have been associated with clinical specimens, probably in an opportunistic situation
- With exception of the genus Streptococcus and perhaps some representatives of the genus Enterococcus, none of the typical LAB (Lactobacillus, Lactococcus, Leuconostoc, Weissella) and either Bifidobacterium, fulfill the criteria for a pathogen
- No virulence factors known e.g. for the genus Lactobacillus. Hence lack of methodology? Definition of criteria for an opportunistic situation?
- Whereas with enterococci one can look at (adhesion) particular virulence factors, etc., necessary for translocation and invasion, and also transferable antibiotic resistance what is required to translocate. But this is typical virulence factors are not known for Lactobacillus species
Should taxonomic units which include pathogenic strains be excluded from QPS?
► No, but more careful investigation on the strain level may be necessary
► QPS: Condition non-pathogenic, non-toxigenic “taxonomic unit”
► Staphylococcus example
► Enterococcus faecalis risk group 2 used in some cheese starters – then it will require further consideration on the strain level
► Depends upon gene transfer potential / probability. Reference to antibiotic resistance particularly associated with transferable genes. Low level of transfer? Does it exclude from QPS? Level of significance may be defined under specified conditions
► Importance of typical environment / habitat

Other issues
► Mould starter cultures
  ▶ Species as “taxonomic unit”
  ▶ Identification
  ▶ Genes presence, expression and regulation
  ▶ Substrate specific production
  ▶ Aspergillus niger most strains either do not possess or do not express genes for ochratoxin production
  ▶ Penicillium roqueforte
    ▶ Presence of toxins on bread, not in cheese

Other issues
Open questions also related to the presence of “silent” genes (“pseudogenes”). e.g. known for Aspergillus oryzae (> 95 similarity with Asp. flavus) or Penicillium nalgiovense, from which strains with a long history of safe use in food have not been found to express mycotoxin synthesis. Reverse mutations not conceivable under typical conditions. These strains are therefore considered to be safe.

Modification of Figure 1 (p7) of the QPS discussion paper:
► Changed “familiarity” in the scheme. “Body of Knowledge”
► Qu. “Carryover” – inc. substrate specificity
► Metabolites – biogenic amines
  ▶ Could be part of “qualification” of QPS
  ▶ Refer to question in Fig.1 (p7) to be reconsidered
► Q1. “Taxonomy” “Identification”
► Q2. “Familiarity” “Body of Knowledge”
► Q3. “Traditional uses” “Established usage”
► Variations in environmental (intrinsic, extrinsic, implicit) conditions may result in modification of metabolic activities, e.g. resulting in the production of toxic or undesired metabolites such as biogenic amines
DISCUSSION GROUP 3

Role of molecular tools
Role of MT in taxonomy and strain identification
Risk of transmissible ABR: extent to be defined
Risk of virulence: extent to be defined
Validation of results
Potential of post-genomics tools
Application to mixed cultures
Status of GMOs self-cloning
What is possible and what is reasonable for safety?
Recommendation
Role of MT in taxonomy and strain identification

- Many strain typing methods available – must be robust / reliable / reproducible
- Let the specialist decide. Polyphasic approaches
  - Which technique: 16S PFGE, MLST, ...
  - Fingerprint databases, other current knowledge
  - Importance of the context
  - Problem of definition: strain, species! Bacteria/fungi
- Genome instability? Evolution of population/heterogeneity
- Limitations of the molecular approaches
  - Positive prove of identity / exclusion
- Availability of reference strains/ deposit in collections, to what extent?
- Need of MT for quantification? Available tools

Risk of transmissible ABR: extent to be defined

- What is the risk from ABR for QPS strains – rank ABR genes clinical risk – proportionality
  - Transmissible vs acquired ABR
  - Intrinsic / extrinsic
- Molecular tools only detect known genes for ABR
  - Should a list be made – current knowledge
  - Genotype vs phenotype – in practise do not always match
    - e.g. vancomycin resistance as intrinsic resistance plus potential presence of a transmissible gene – limited to known genes
- ABR is relevant with respect to transfer
  - Laboratory vs nature re ABR transfer risk

Risk of virulence, toxin and other: extent to be defined

- Not only MT are available to address this issue
- Molecular tools detect only known genes and products
- Virulence genes should be
  - ranked according clinical risk
  - assessed with respect to transfer
  - defined with respect to biological relevance
- virulence gene detection by molecular tools value and available techniques are species specific

Potential of "omics"

- Capability to screen large numbers of genes fast but with limited specificity
- New developments such as transcriptomics for specific application
- Should this high capacity be systematically used? Need to know vs nice to know

Validation of results from molecular tools

- Validation should be made, it is not an issue
  - sensitivity
  - threshold
  - normalisation needed
  - reliability
- Interpretation of the result extrapolation of genotype to phenotype, transmissibility
Application to mixed microbial cultures

- Apply MT to describe culture to the extent needed
- MT are available to detect the presence of undesirable strains, genes (ABR, virulence, toxin genes)
- MT can be used to follow dynamics of cultures

Status of GMOs self-cloning

- QPS could be used to assess certain strains made by recombinant techniques such as self-cloning

What is possible and what is reasonable for safety?

- Availability of MT does not imply the need for their use
- Strains present in the final products are not always those added by the producer
- Other tools than MT might be more relevant for certain assessment (biogenic amine?)
- Need to establish vigilance plan to monitor emergence of microbial strains with adverse effect
- Overuse of MT could lead to negative economic consequences
- Continuous development of MT should be taken into account
- MT more important in risk assessment than in law enforcement

Recommendation

- MT are valuable for QPS, but they are not the only ones
- Immediate application for taxonomy
- Overuse could lead to unnecessary regulatory burden
- Need to define an effective minimum for safety
What are the strengths and weaknesses of the QPS approach?

- Needs, scope and objectives of QPS
- Modality of functioning
- Role in risk assessment vs use as managerial tool

Is the risk assessment of microbial cultures needed?

Hazard identification

- Virulence factor – toxins
  - Fungi
- Acquired Antibiotic resistances
- New micro-organism without history of use

Hazard characterization

Exposure assessment

- Non traditional use – Higher exposure?

- QPS system is a fast track approach to safety assessment
- The system should be kept as simple as possible
- Other aspects might need to be considered (e.g. changing exposures, new use, consumer category …)
- Additional aspects would progressively lead to a more complete risk assessment

Three possible outcomes

- A live organism is a component of a final product intended to enter the food chain directly (it is consumed)
- A live organism is a component of a final product but is not intended to enter the food chain although it may enter it adventitiously (e.g. a plant protection product)
- The organism(s) is used only as a production strain with the final preparation containing fermentation product(s) intended to be free of live organisms

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- This sentence should be modified to include dead micro-organisms in food and feed
### Decision Tree Approach

- Decision tree is intended to be used by expert groups (not for notification)
- It is a workable system
- The scientists would decide on that basis
  - What grouping is considered
  - Where the group is to be split
  - What – where additional information is needed

### Benefits

- Focus on essential aspects – make the evaluation more proportional to the risk
- Increase consumer confidence (standardized procedures)
- **Harmonization and simplification of procedures for safety evaluation (suggest delete)**
- Learning process (focus on where additional knowledge is necessary)
- Better use of scientific resource
  - Economy of human resources
  - Reduced animal uses
- May contribute to the production of more innovative food
Focus on essential aspects – make the evaluation more proportional to the risk
- Any “other concerns” (see decision tree) should be effectively evaluated

Increase consumer confidence (standardized procedures)
- Consumers want a system that can be trusted
- It belongs to EFSA to elaborate a good system and show that the approach is working
- Balance the communication on “bad bugs” with “good and safe micro”

Learning process (focus on where additional knowledge is necessary)
- Identify were additional knowledge is needed
- What is essential to aid the risk assessment process and not merely what is nice to know

Better use of scientific resource
- Economy of human resources
- Reduced animal testing

(Weaknesses) Issues to consider
- If too heavily regulated might lead to increased sectorization
- Difficulty of characterizing what is intended by familiarity – condition of use
- Difficulties to address (mix) undefined cultures
- Methodological difficulties – validation vs innovation
- Onus for starting the process
- (Additional burden for SME)

(If too heavily regulated might lead to increased sectorization)
- Because QPS is envisioned as a tool for priority setting, there is no link with regulation

Difficulty of characterizing what is intended by familiarity – condition of use
- Familiarity: existing body of knowledge
- Important to define what is known and what is not known (uncertainties)

Difficulties to address (mix) – undefined cultures
- Same food produced using a natural or a selected starter cultures: different risk assessment
- Difficult to have QPS approach for undefined or natural complex or traditional cultures
Methodological difficulties validation vs innovation

- Molecular tool available
- Rapidly evolving field – problem of accessibility to information

Onus for starting the process (Additional burden for SME)

- Need to consider who is responsible for setting up the initial structure – consensus that it should be EFSA
- Need to propose specific mechanisms for implementation – reflection of the Discussion Group on QPS

Are there better alternatives to the QPS approach? If so, what are the advantages and disadvantages of these alternatives when compared to QPS?

- A number of approaches do exist
  - GRAS – for specified use
  - French Approach – for new micro
  - Danish Approach – fully regulated process
  - Industry traditionally implementing safety assessment
- Main alternatives are between:
  - Voluntary vs regulatory system
  - Part of risk assessment vs management

Should it be a requirement for QPS to deposit the given strain in a culture collection?

- Balanced approach from the group
  a. Yes – difficult to implement QPS concept without deposition in an approved Culture Collection
  b. More an issue of identification/characterization than deposition
- Specific difficulties
  a. Scope of QPS (Strain or species)?
  b. Use of deposited material?

- There should be a mechanism to take into consideration potential for genetic drift
- The deposit of strains could be an additional element in the decision tree

Could the QPS approach be extended to enzymes and other products of micro-organisms?

- Aspects to be considered
  1. Extension from micro. to other substances – metabolites as in new regulation for feed additives
     a. Pro: harmonization, simplification, deal more easily with mixed enzymes
     b. Con: cumbersome – specific uses
  2. For given micro. consider the micro and its metabolites in an holistic assessment
  3. Need for considering level-processes – purpose for such an assessment
Identify putative consequences of implementing the QPS or any suggested alternatives for *e.g.* consumers, industry, risk assessors, risk managers.

- Need to be clear about the implementation of QPS
  - Part of risk assessment vs managerial tool
- If direct impact on management decision need to involve stakeholders (*e.g.* acceptability of processes)

- QPS is a fast track procedure to safety assessment to be utilized within EFSA