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A microbial identification framework for risk assessment

Stéphane Bernatchez[a], Valar Anoop[a], Zeina Saikali[b], Marie Breton[a,*]

[a] Biotechnology Section, New Substances Control and Assessment Bureau, Health Canada, Ottawa, Ontario, K1A 0K9, Canada
[b] Regulatory Science and Policy, Biotechnology Section, Emerging Priorities Division, Environment Canada, Gatineau, Québec K1A 0H3, Canada

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ABSTRACT

Micro-organisms are increasingly used in a variety of products for commercial uses, including cleaning products. Such microbial-based cleaning products (MBCP) are represented as a more environmentally-friendly alternative to chemically based cleaning products.

The identity of the micro-organisms formulated into these products is often considered confidential business information and is not revealed or it is only partly revealed (i.e., identification to the genus, not to the species). That paucity of information complicates the evaluation of the risk associated with their use. The accurate taxonomic identification of those micro-organisms is important so that a suitable risk assessment of the products can be conducted.

To alleviate difficulties associated with adequate identification of micro-organisms in MBCP and other products containing micro-organisms, a microbial identification framework for risk assessment (MIFRA) has been elaborated. It serves to provide guidance on a polyphasic tiered approach, combining the data obtained from the use of various methods (i.e., polyphasic approach) combined with the sequential selection of the methods (i.e., tiered) to achieve a satisfactory identity of the micro-organism to an acceptable taxonomic level.

The MIFRA is suitable in various risk assessment contexts for micro-organisms used in any commercial product.

1. Context

The major use of microorganisms in commercial applications has been for the production of microbial fermentation products such as enzymes, which are used as processing aids and catalysts for a variety of commercial and industrial applications such as laundry detergents, textiles, food, beverages, feed and biofuels (Louter et al., 2012). Microorganisms are also used in various industrial and commercial applications such as waste-water treatments, bioremediation, floor cleaners, odour control, etc., as well as for the production of bioethanol, enzymes, etc. (Louter et al., 2012).

Recently, cleaning products in which active ingredients consist of various strains of micro-organisms, i.e., microbial-based cleaning products (MBCP), have become increasingly prevalent in many countries as an alternative to chemically based cleaning products (Arvanitakis, 2015). Those products appear to be increasingly sold for use in many domestic, commercial, or institutional settings as well as for a variety of cleaning activities (hard surface cleaning, odour control, degreasing, septic tank treatments, etc.) where chemically based cleaning products have traditionally been used (Arvanitakis, 2015). Many of those products are commonly advertised and described as "environmentally friendly", "biodegradable" and "non-toxic". Information on the microbial composition of MBCPs is often limited (i.e., limited to the genus) or considered as confidential business information (Arvanitakis, 2015; Spök and Klade, 2015).

The importance of accurate identification of micro-organisms, used for MBCPs and a variety of other purposes, was a recurring theme during the International Workshop to Address Risk Assessment and Risk Management Challenges and Opportunities Relating to Microbial-Based Cleaning Products held in Ottawa (Canada) in 2013 (Thomas and Versteeg, 2013). Throughout that workshop, microbial identification was highlighted as a key challenge by several presenters (Thomas and Versteeg, 2013). Furthermore, finding and/or developing the most appropriate test methods to ensure accurate taxonomic identification/designation of the microbial constituents of MBCPs was recognized as a major challenge by the participants. Those challenges had previously been recognized during the "Environmental uses of micro-organisms: An overview of the state-of-the-art and implications for biotechnology risk/safety assessment" OECD conference (Spök and Klade, 2015).

* Corresponding author.
E-mail address: marie.breton@canada.ca (M. Breton).

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2. The importance of microbial identification for the purpose of risk assessment

The safety evaluation of a microorganism begins with its identification. The taxonomic identification of a micro-organism constitutes the cornerstone in any risk assessment for a biotechnology product (OECD, 2003), regardless of its intended use. The identity of the micro-organism must be established as precisely as possible and it must be supported by reliable data. The name assigned to a micro-organism is essential for its basic characterization; it also forms the basis for any searches on subsequent hazard and exposure assessments (OECD, 2003).

The use of recognized methods to determine phenotypic and genotypic characteristics of a taxonomic group are crucial for effective identification of the notified micro-organism. The analysis of data obtained with such methods can also help to identify "closely-related" organisms (at the species or even subspecies-level), that can be used in providing complementary information to determine the hazard of the micro-organism under assessment and, if necessary, discriminate it from potential pathogens of clinical or environmental significance. Information from such "closely-related" organisms could be used as surrogate data in instances when there is a paucity of information on the micro-organism of interest. However, the validity of any extrapolation from surrogate data is underpinned by the taxonomic identification; furthermore, that extrapolation must also be validated with respect to the biological or functional similarities between the surrogate and the organism under assessment (Thomas and Versteeg, 2013).

3. Acceptable taxonomic level of identification

The objective of microbial identification is to select the relevant phenotypic and genotypic characteristics of the micro-organism of interest that will allow it to be placed within a recognized taxonomic group. From an overall risk assessment perspective, a reliable taxonomic designation for a given micro-organism is the most important determinant of its potential hazard to human health and environment (Environment Canada and Health Canada, 2011). The level of taxonomic designation will vary depending on the micro-organism, but in general, a designation to the species-level (Arvanitakis, 2015; OECD, 2003) or the subspecies/serotype/pathovar-level for a well-known species is a prerequisite for risk assessment.

The designation to the subspecies/serotype/pathovar-level is more appropriate where the micro-organism under assessment is closely-related to a clinical or environmental pathogen. For example, the bacterial species *Escherichia coli* comprises a number of pathogenic (for example, strains of the O157:H7 serotype; Riley et al., 1983) and non-pathogenic strains (for example, strains of the K-12 lineage which lack the O antigen; Kuhnert et al., 1995; Liu and Reeves, 1994)). Therefore, if an *E. coli* strain under assessment is claimed to be non-pathogenic, then the level of identification required to differentiate that particular *E. coli* strain from pathogenic relatives will be to a subspecies/serotype/pathovar-level. Species-level identification in the example of *E. coli* will result in uncertainties which must be taken into consideration in the risk assessment by assuming that the *E. coli* strain under assessment may be pathogenic since it was not distinguished from its pathogenic relatives. Such a conservative, but precautionary, assumption is likely to affect the conclusion of the risk assessment.

In instances in which a micro-organism can only be identified to a genus or sub-generic level (for example, a clade with multiple species) due to the lack of available information including lack of certainty in the original classification of the taxa, or lack of suitable methods to identify it as a particular species, then all species and sub-species within that genus will be considered as potential candidates for the identity of the micro-organism under assessment. The obvious uncertainty regarding the identity of the micro-organism will need to be addressed, either by conducting additional experiments to identify the micro-organism to the species- or sub-species level, by a review of the literature on all known species within the genus or sub-generic level where the micro-organism under assessment was placed, or by comprehensive characterization of the micro-organism and its effects. Those efforts will build the body of information that will be used for the risk assessment and if no closely-related pathogens are found within the clade or genus of interest, then for the purpose of risk assessment the clade designation may be adequate.

4. Identification challenges

Microbial species are part of a continuum of diversity and species delineation can be ambiguous (Konstantinidis et al., 2006; Liti et al., 2006). Despite this ambiguity, micro-organisms have been classified for practical purposes into different species based on the coherence of their genetic and phenotypic characteristics. Some of the factors that influence microbial identification and taxonomic classification include the concept of speciation, the pluralistic nature of microbial taxa, topological incongruence, horizontal gene transfers, and presence of multiple copies of conserved gene regions, especially in methods relying on genetic data. While the issues with the concept of species are acknowledged, it is nevertheless important to consider the species as a defined taxonomic entity and a practical component of a risk assessment.

Further complicating the challenge of achieving a proper identification of the micro-organism for risk assessment purposes, microbial taxonomy is in constant flux and new techniques are constantly providing new input in reclassifying or improving the specificity of the taxonomic grouping of a variety of micro-organisms (Beiko, 2015). The taxonomic designation of a micro-organism under assessment should follow the most current international codes of nomenclature and standard taxonomic sources, i.e., those that are officially recognized and accepted by international committees such as the International Committee on Systematics of Prokaryotes (http://www.the-icsp.org/) for bacteria; the International Commission on the Taxonomy of Fungi (http://www.fungaltaxonomy.org/) and the International Association for Plant Taxonomy (IAPT; http://www.iapt-taxon.org/nomen/main.php?page=pf) for algae and fungi; or the International Committee on the Taxonomy of viruses (http://www.ictvonline.org/codeOfVirusClassification.asp) for viruses.

The historical perspective of the taxonomy, nomenclature and phylogenetics of the micro-organism under assessment should also be taken into account when assigning it to specific taxonomic groups since the name of the organism may have changed over time. As an example, the bacterium initially named *Rhodopseudomonas sulfidophila* has undergone name changes over time and is currently designated as *Rhodovulum sulfidophilum* (Hiraishi and Ueda, 1994; Imhoff et al., 1984; Hansen and Veldkamp, 1973). The genera *Arcobacter*, *Clostridium* and *Pseudomonas* (respectively reviewed by Ferreira et al. (2016), Beiko (2015) and Peix et al. (2009)) constitute additional examples of taxonomic reorganizations that have occurred over time. When conducting a literature search for information about the micro-organism of interest, it is necessary to take the basis of such reorganization of taxa into consideration when determining the relevance of data and information related to superseded names. It is also important to consider the quality of the source of information, including the methods used to identify the original source material (eg., reference strains used in the analysis), as found in sources such as Bergey's Manual of Systematic Bacteriology, the List of prokaryotic names with standing in nomenclature (LPSN; www.bacterio.net) or StrainInfo (www.straininfo.net).

To be reliable, the identification of the micro-organism cannot be based on a single method (whether it is phenotypic, biochemical or genotypic), as it provides more opportunity for misidentifying bacterial species (Janda and Abbott, 2002). Identification of a taxon at any level should be based upon a polyphasic approach (Thompson et al., 2015; Vandamme et al., 1996), wherein the experiments include appropriate controls (i.e., type strains), and are performed and analyzed by trained