Chapter 14

Reflection on environmental risk assessment of micro-organisms

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Due to the inter-dependent network of organisms, a microbe's interaction with the physical environment and its continuous evolution through mutation and horizontal gene transfer, microbial diversity is never static, which makes an analytical approach almost impossible for assessing the risk of the environmental use of microbes. One possible alternative approach could build on concepts developed by the OECD in the early 1990s: familiarity and substantial equivalence.

According to the Cartagena Protocol on Biosafety, *"*t*he objective of* a *risk assessment is to identify and evaluate the potential adverse effects of living modified organisms on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account risks to human health"*. The "*p*otential adverse effects" are not always easy to identify *and i*nterpreting them in different circumstances has been a long-standing question. *Ambiguity surrounding this key word appears to have caused regulatory uncertainty.*

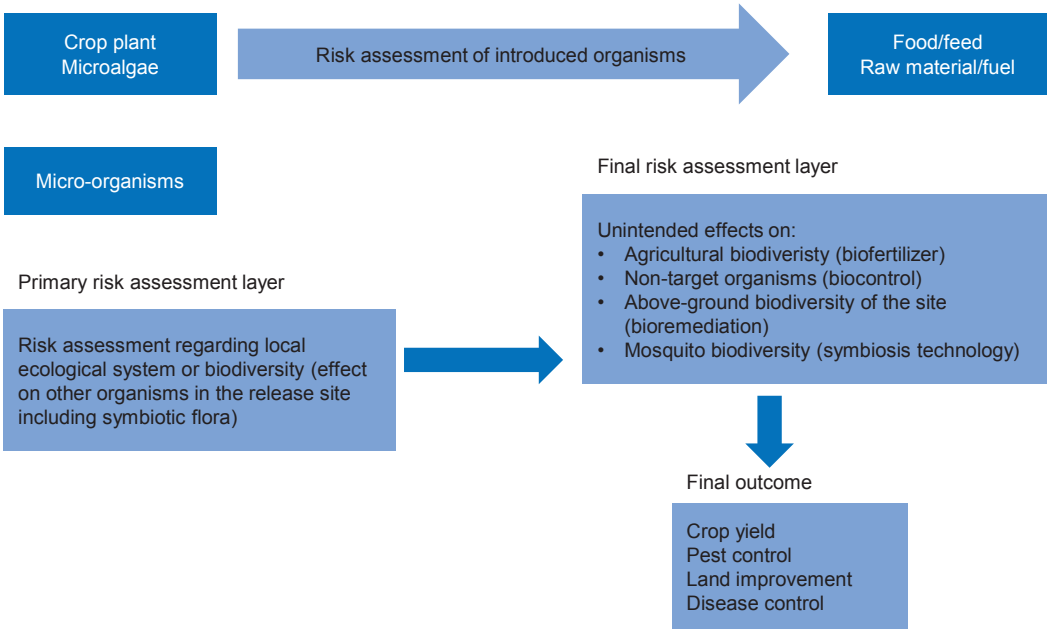
This chapter *addresses the t*arget of risk assessment of environmental application of microbes *and the difficulty of using an analytical approach in assessing the risk of micro-organisms used in the environment.*

Target of risk assessment

For crop plants or microalgae for food, feed, fuel or feedstock, the plants or microalgae introduced into the environment are clearly the target of risk assessment. However, for biofertilizer, biocontrol, bioremediation or mosquito control through paratransgenesis, this is not necessarily the case. For example, for paratransgenesis for mosquito control, while the biodiversity of living organisms impacted by the presence of mosquitoes is the main target, the biodiversity primarily affected is that of the symbiotic microbial flora of mosquitoes. For biocontrol or biofertilizer, while the non-target effect on the above-ground organisms could be the main target, the biodiversity primarily affected is the underground microbial flora (bacteria, fungi, nematodes, insects, plant roots, etc.). Thus, in these cases, the risk assessment consists of two layers. Such a bi-layered structure inherent in the risk assessment of microbes will be an important consideration in structuring the risk assessment of the environmental use of microbes (Figure 14.1). To which layer should we focus more in the risk assessment?

It should be noted that very often where risk assessment cannot identify the main target, such as in case of biofertilizer or bioremediation, risk assessment tends to focus excessively on the effect on microbial ecology.

Figure 14.1. **Bilayered structure inherent in risk assessment related to micro-organisms**



Difficulty of using an analytical approach in assessing risk of micro-organisms used in the environment

Problems encountered in assessing the "microbial diversity" is seen in Baas Becking and Beijerinck's statement, "Everything is everywhere, but the environment selects" (De Wit and Bouvier, 2006).

"Environment selects" refers to the fact that microbes are always under the influence of environmental factors, such as light, temperature, humidity, water, carbon, nitrogen, phosphate, sulphate, minerals, organic matters or organisms with which the microbes interact. Effective use of microbes in the environment requires the appropriate

environmental conditions. The introduced microbes alone do not determine the environmental consequences of releasing such microbes into the environment, but the combination of the microbes and the environment do. This consideration is particularly important in view of the changing climate and increasing human population which enhances the anthropogenic consequences (Smol, 2012).

"Everything [in terms of microbes] is everywhere" relates to the inherent difficulty of assessing microbial ecology. For example; "a pond 1 ha in area and 10 m deep will host 10^{18} bacteria, 10^{16} protists, 10^{11} small animals; species with 10^{7} individuals or less in the pond are unlikely ever to be detected" (Fenchel and Finlay, 2004). There will be ten-fold more viruses in addition to the ones that are inventoried. Use of metagenomics may reveal further biodiversity of microbes in the environment, but its capacity will be far short of what is required for its full understanding. Every microbe may be everywhere but it may not be noticed because its population remains small.

Microbes, i.e. bacteria (*Archaea* and *Eubacteria*), viruses (bacterial, animal and plant viruses), fungi, nematodes, arthropods and underground animals, make a complex interacting community. They constitute a metabolic and genetic consortium. Such a consortium is under the influence of the physical environment, such as temperature, water supply, nutritional content of the soil or water, etc, which are, in their turn, affected by microbial activities through cycling of carbon, nitrogen, phosphorus, sulphur and other molecules. Human activities such as agriculture, building cities and industries, strongly change the soil and water environments through pollution and land/water use.

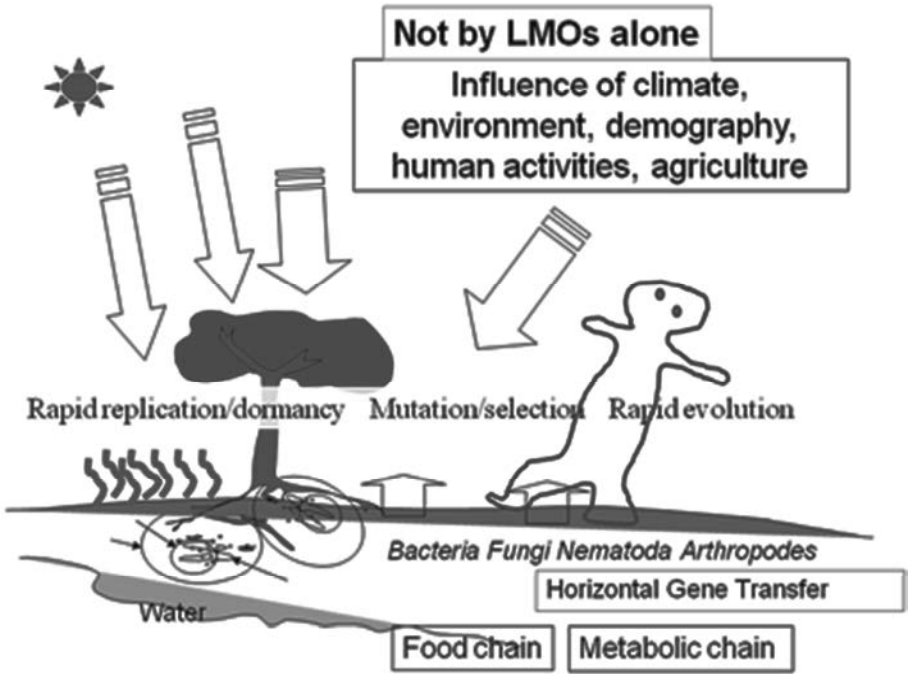
Microbes, owing to their rapid growth and capacity of clonal expansion and through mutation and selection, are undergoing a constant evolutionary process, which is enhanced by horizontal gene transfer, which occurs every moment even between different species (Figure 14.2). In fact, "horizontal gene transfer is essential for the evolution of prokaryotes and can be legitimately viewed as a necessary condition of the long-term survival of archaea and bacteria. Any asexual population is headed for eventual extinction because it does not possess effective means to eliminate the inevitably accumulating deleterious mutations" (Koonin, 2012).

In short, microbes do not exist independently; they are part of an inter-dependent network of primary production, degradation of organic material, prey-predator relation, etc. Microbes are under continuous evolution through mutation, horizontal gene transfer and selection in response to environmental change. Microbial diversity is never static.

All these situations make the environmental risk assessment of micro-organisms difficult, which is already addressed in the consensus documents (Table 14.1), i.e.:

- Incomplete information on the number of existing microbial species (OECD, 2003).
- Viable but non-culturable microbes (OECD, 2004).
- Frequent inter-species horizontal gene transfer including that of pathogenicity-related genes (OECD, 2010). Species name is an inexact marker of risk (OECD, 2011).
- The new knowledge has not always brought us closer to understanding of speciation in bacteria. There is no best method for taxonomy, not even if we restrict ourselves to aspects of taxonomy that are meaningful in risk assessment (OECD, 2003).

Figure 14.2. **Many factors other than LMOs influencing on biodiversity**



As a consequence, we may get into a situation where: "Because most GM micro-organisms cannot reveal their potential until release, and because some testing relevant to risk assessment cannot be done until release, one can't test without release, but one can't get permission to release without testing" (OECD, 2003). There is surely a limitation to the analytical approach in assessing the risk of environmental use of microbes.

Table 14.1. **Past OECD work on the environmental risk assessment of micro-organisms**

Regulatory oversight series
Guidance documents on:
– The Use of Taxonomy in the Risk Assessment of Micro-Organisms: Bacteria (No. 29)
– Horizontal Gene Transfer Between Bacteria (No. 50)
– Detection of Micro-Organisms Introduced into the Environment: Bacteria (No. 30)
– Pathogenicity Factors in Assessing the Potential Adverse Health Effects of Micro-Organisms (No. 52)
– Information used in the assessment of environmental applications involving:
– *Acinetobacter* (No. 46)
– *Acidithiobacillus* (No. 37)
– *Pseudomonas* (No. 6)
– *Baculovirus* (No. 20)
Important concepts:
Substantial Equivalence or Comparative Safety Assessment: Safety Evaluation of Foods Derived by Modern Biotechnology: Concepts and Principles (1993)
Familiarity and Stepwise Scale-Up: Safety Considerations for Biotechnology: Scale-Up of Crop Plants (1993)

An alternative approach is needed. One approach could be going back to the two complementary concepts developed by OECD in early 1990s: familiarity and substantial equivalence.

The concept of "familiarity" was proposed in combination with a scale-up process to gain "familiarity" (OECD, 1993a). It could be a process "from trial-and-error to earnest engineering" as expressed by Prof. Victor de Lorenzo.

"Substantial equivalence" or "comparative safety assessment" uses, as a reference, a "conventional counterpart with history of safe use" (OECD, 1993b). The principle is appropriate use of experience; it was first developed in relation to food safety assessment, but may be adjusted conceptually to an environmental risk assessment. Uncertainty is removed or reduced only through experience. The concept was successfully used in the Codex Alimentarius Commission that agreed on a series of texts on foods derived from modern biotechnology (Codex Alimentarius Commission, 2009).

The OECD's Working Group on Harmonization of Regulatory Oversight in Biotechnology has developed consensus documents since its first session in 1995 (Table 14.1). Examples in such documents shown in Table 14.1 will be an important information source on the history of the safe use of microbes (Table 14.2).

Table 14.2. **Environmental use of microbes in the past**

Acinetobacter spp.
– removal of phosphates
– bioremediation of sites contaminated with hydrocarbons, heavy metal, pesticides
– plant growth promoters and biocontrol agents against bacteria and fungi
– biosensors for pesticides metaphos, sumithion, etc.
Acidithiobacillus
– removal of sulphides from industrial waters, heavy metals from sludge and mine waters
– bioleaching of copper, uranium, etc.
– desulphurisation (remove sulphur from coal); bioleaching of pyrite from oil shale
– agricultural fertilisation (through involvement in sulphur cycle)
Pseudomonas
– *P. aeruginsa*: washing hydrocarbons from soil (biosurfactant)
– *P. fluorescens*: ice-minus; plant and fish disease control by inhibiting growth of fungi; degradation of chlorinatedaliphatic hydrocarbons, etc.
– *P. putida*: degradation of PCB, etc.
Baculoviruses
– biological control as insecticides (moth, cotton bollworm, etc.; no negative or unintended effects)
– registered insecticides: Adoxophyes orana (GV); Agrotis segmentum (GV); Anticarsia gemmatalis (MNPV)

If microbial biotechnology is to be used for the improvement or conservation of the environment, we should not miss timing. The underground microbial community is interacting with the above-ground community. Once the land becomes barren, the introduction of any microbes requiring above-ground plants will not work.

References

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Annex 14.A1

Text of paragraphs 8 and 9 of the Annex III to the Cartagena Protocol on Biosafety

8. To fulfill its objective, risk assessment entails, as appropriate, the following steps:

- (a) An identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health.
- (b) An evaluation of the likelihood of these adverse effects being reali[s]ed, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism.
- (c) An evaluation of the consequences should these adverse effects be reali[s]ed.
- (d) An estimation of the overall risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being reali[s]ed.
- (e) A recommendation as to whether or not the risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks.
- (f) Where there is uncertainty regarding the level of risk, it may be addressed by requesting further information on the specific issues of concern or by implementing appropriate risk management strategies and/or monitoring the living modified organism in the receiving environment.

Points to consider

9. Depending on the case, risk assessment takes into account the relevant technical and scientific details regarding the characteristics of the following subjects:

- (a) Recipient organism or parental organisms. The biological characteristics of the recipient organism or parental organisms, including information on taxonomic status, common name, origin, centres of origin and centres of genetic diversity, if known, and a description of the habitat where the organisms may persist or proliferate.
- (b) Donor organism or organisms. Taxonomic status and common name, source and the relevant biological characteristics of the donor organisms.
- (c) Vector. Characteristics of the vector, including its identity, if any, and its source or origin, and its host range.
- (d) Insert or inserts and/or characteristics of modification. Genetic characteristics of the inserted nucleic acid and the function it specifies, and/or characteristics of the modification introduced.

- (e) Living modified organism. Identity of the living modified organism, and the differences between the biological characteristics of the living modified organism and those of the recipient organism or parental organisms.
- (f) Detection and identification of the living modified organism. Suggested detection and identification methods and their specificity, sensitivity and reliability.
- (g) Information relating to the intended use. Information relating to the intended use of the living modified organism, including new or changed use compared to the recipient organism or parental organisms.
- (h) Receiving environment. Information on the location, geographical, climatic and ecological characteristics, including relevant information on biological diversity and centres of origin of the likely potential receiving environment.