Chapter 15

Risk assessment considerations of genetically modified micro-organisms for releases

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The environmental risk assessment of a genetically modified micro-organism (GMM) needs to consider its potential interactions with indigenous microbial communities in a given habitat. Interactions can relate to the survival of the GMM and/or the transfer of recombinant genes to indigenous community members. While there is already considerable knowledge about the biology and ecology of some species used as hosts for genetic modifications to inform their environmental risk assessments, in-depth studies on the biology, genetics and eco-physiology of other GM species may still be required before considering their use in not-strictly contained systems, for example for biofuel production or as biocontrol agents. Containment can be achieved when using GMM symbionts which are non-viable outside of their hosts, as demonstrated with Wolbachia sp. and insects. Given the potential of non-symbiotic micro-organisms to spread in the environment, it appears desirable that a GM should not persist after its intended purpose of application has been achieved, even if it’s presence does not necessarily translate to a risk, as it may have no adverse properties. In summary, in addition to a detailed characterisation of the genetic and biological properties of a GMM, in-depth knowledge about its interactions with its target and non-target environments is not only crucial to improve its efficiency, but also important to assess their environmental risks.
Microbial community networks and resilience

The metabolic activity of micro-organisms is crucial for life on Earth. The cycling of atoms and molecules, which provide the basis for life, is only possible due to the metabolic versatility, niche colonisation, environmental persistence and overall abundance of microbial cells. As much as micro-organisms modify their immediate environment by transforming nutrients and excreting metabolites, the surrounding environmental conditions select for specifically structured microbial communities. While a huge part of the diversity of micro-organisms on Earth is still unknown in terms of species identity and particular physiological properties and potentials, the increasing speed of new nucleic-acid sequencing technologies and their high-throughput bioinformatic analyses opens access to many of them. These technologies increasingly allow viewing and appreciation of the complexity of microbial communities as they occur in ecosystems, e.g. soils, gastrointestinal tracts or intercellular niches provided by plants (Barriuso et al., 2011; Shokralla, 2012). Due to their long evolutionary history of millions or billions of years, the complexity of microbial communities is not random, but extremely stabilised in networks of interactions among their individual members. This microbial networking not only provides ecosystem services such as the biogeochemical cycling of elements, but is also required to directly protect humans, animals or plants against pathogenic micro-organisms (for an example for plants, see Van Bruggen et al., 2006).

While microbiologists do not doubt that microbial communities and their networking lay the foundation for life on Earth, it is still controversial whether or not they require protection and how this would be done, or whether they are self-regulated and highly stable in the first place. In this context, the characterisation and evaluation of resilience and robustness of such communities is a crucial factor (Allison and Martiny, 2008; Silva-Roche and de Lorenzo, 2010). This resilience of microbial community structure and function to disturbance can, in fact, be highly variable depending on the type of community and its environment, e.g. the buffering capacity of a highly diverse microbial community in a clay soil with organic matter is much higher than a community in a low pH sandy soil (Griffiths et al., 2008). Resilience will be different in systems with natural perturbation, e.g. variable amounts and qualities of inflowing nutrients, e.g. the human gut (De La Cochetiere et al., 2005; Dethlefsen et al., 2008), than in systems which are stratified and mainly undisturbed, i.e. biological soil crusts in desert ecosystems (Berard et al., 2011; Kuske et al., 2012).

The issue of resilience and buffering capacity of indigenous microbial communities translates into the problem of assessing the risks which would be associated with a release of genetically modified micro-organisms (GMM). There are several peculiarities about GMM as compared to genetically modified plants – for instance, their release is irreversible. While plants may be removed from a site with appropriate soil management strategies, the elimination of GMM from larger areas, e.g. agricultural fields or contaminated industrial soils, is hard to achieve. A practical example of the environmental persistence of a GMM was given by the first deliberate field release of two strains of the GM soil bacterium *Sinorhizobium meliloti* conducted in Germany in the early 1990s. These strains were tagged with a chromosomally inserted luciferase marker gene derived from a firefly. *S. meliloti* is capable of colonising the roots of certain legumes causing nodulation, i.e. lucerne (alfalfa) in which they mediate biological fixation of atmospheric nitrogen. But the species can also survive and grow in soil independent of such symbiotic partners. The strains, released in conjunction with seeding
of lucerne, were detectable for several years after their soil inoculation. Populations could be stabilised in the presence of lucerne in soil, but they were also maintained at a small, but rather stable, size independent of lucerne (Selbitschka et al., 2006) over several years. There were no indications that the inoculation affected the overall abundance and diversity of the dominant indigenous soil microbial community or the microbiological soil functions (Schwieger and Tebbe, 2000; Tebbe and Miethling-Graff, 2006). In environments with low resilience microbial communities, the presence of a GMM may already cause a structural or functional shift, even at lower population sizes, but in highly robust communities, huge amounts of GMMs would have no effect if they fail to occupy a niche within such systems, as demonstrated with the above-mentioned field release.

**Risk assessments based on information on recipients**

Chapters in the present volume give a good impression of how diverse the properties of GMM and their targeted environments can be. It is evident that the resilience of natural microbial communities may, in fact, limit the efficiency of a GMM to survive and perform its task, e.g. to promote plant growth in the rhizosphere or to degrade a pollutant in soil. A major challenge for the safe environmental application of a GMM is to construct on one side a competitive GMM but on the other side to limit its capacity to interfere with ecosystem services provided by the natural microbial communities. An environmental risk assessment should therefore consider how resilient or vulnerable an existing microbial community would be. Depending on the expected resilience of the natural microbial community, the level of scrutiny required in the risk assessment procedure could be different.

The severity of challenging the resilience of natural microbial communities also depends on the particular physiological and genetic properties and potentials of the GMMs themselves. Consideration of the environmental impact of the release of GMMs in general began with the advent of the possibility to generate them, as already described in the OECD “Blue Book” published more than 25 years ago (OECD, 1986). To date, there are a number of well-elaborated national and international consensus documents and guidelines for the environmental risk assessments of GMMs, including several documents issued by the OECD. For risk assessment of GMMs associated with the food/feed sector, a new guideline by the European Food Safety Authorities (EFSA) has been published (EFSA Panel on Genetically Modified Organisms, 2011b). All types of risk assessment consider the following aspects: molecular characterisation, hazard identification, exposure, direct and indirect effects of a GMM, intended and unintended effects on target and non-target organisms, comparisons with closely related non-GM organisms, and the availability of monitoring tools. As a basic requirement, the risk assessment and the derived risk management should be clear about protection goals on which they are based. Finally, risk assessments are not finished in advance of a field application or commercialisation, but continue to monitor effects, anticipated or not, once the GM organism or product is used. For GM plants, guidelines for such a post-market environmental monitoring have been developed (EFSA Panel on Genetically Modified Organisms, 2011a) and the principles would equally apply to GMMs for environmental use.

The molecular characterisation of the GMM and comparison to its non-modified counterpart, e.g. the non-modified parental strain, is an important starting point in risk assessment. Knowledge about the biology of a GMM can be gained from familiarity with the counterpart. Due to the fact that genetic modifications are preferentially done on
genetically very well-studied micro-organisms with a history of safe use, information on their biology and ecology is often available, e.g. in OECD consensus documents on certain micro-organisms, i.e. *Pseudomonas*, *Acinetobacter* or *Acidithiobacillus* (OECD, 1997; 2006; 2008). More recently, however, insights from cultivation-independent community analyses combined with novel techniques of cultivation of micro-organisms (Janssen et al., 2002; Stevenson et al., 2004), result in an increasing access to a novel diversity of environmental micro-organisms with the potential for biotechnological use. In such cases, additional in-depth studies on the biology, genetics and eco-physiological properties of a novel microbial species appear to be fundamental in the context of predicting their performance and assessing their environmental risks. GM cyanobacteria, as well as eukaryotic algae for the production of food, feed, chemicals or biofuels, were discussed at the OECD conference and it was indicated that due to a lack of familiarity with specific species, additional knowledge would be desirable in regard to their potential to colonise niches outside of their immediate application (contained use) in order to gain information for environmental risk assessments. This could also apply to GMMs used as biocontrol agents, exhibiting a capacity to colonise a target environment, e.g. a plant or the gut of an insect.

**Environmental performance and containment**

While knowledge of the biology and environmental behaviour, including pathogenicity, of a GMM is of crucial importance in a risk assessment, the recombinant genes also need to be assessed, since they may dramatically change the potential of an organism to survive outside of the laboratory. However, increasing the capacity of a GMM to survive in the environment is not a risk per se, as it may be an intended effect of the modification, e.g. to persist in a contaminated soil and degrade organic pollutants. Results with current bacterial inoculants, in fact, indicate that the risk of failure of a GMM to perform its desired activity in such soils is much higher than the risk it would impose on natural microbial communities (de Lorenzo, 2009). Similar constraints are likely to limit the success of bacterial inoculants in agriculture, e.g. to replace chemical fertilisation by biological nitrogen-fixation or phosphate mobilisation. A potential approach to enhance the viability and desired biological activities of bacterial inoculants could be to alter the expression of their natural genes by engineering their own promoters (Ryan et al., 2009). The huge gain of knowledge due to high throughput DNA-sequencing and bioinformatics delivers the tools which will probably allow progress from “spray and prey” to the successful design of GMM for more effective and reliable environmental applications (de Lorenzo, 2008). Should their survival and environmental exposure be enhanced through these practices, then the environmental risk assessment could differ in its level of required scrutiny from those applied before.

Ideally, GMM, once they have finished the job (for which they were designed), should disappear from the environment. A number of such concepts for containment, including bacteria with decreased fitness to repair mutations or substrate-inducible suicide-systems, have been developed and tested in the field and this principle of biological containment may become important for future applications (Molin et al., 1993; Schwieger et al., 2000; Torres et al., 2000). Due to the potential for mutational changes or other factors, such containment systems may not be 100% secure. On the other hand, bacterial symbionts, i.e. *Wolbachia* appear to be highly efficient containment systems, suggesting that for the control of insect-borne diseases the environmental spread of a GMM would be negligible (Alphey et al., 2002; Moreira et al., 2009).
An unintended environmental persistence of a GMM does not immediately and necessarily present a risk, since micro-organisms may be in resting cell stages, thus, metabolically inactive outside of their natural niche, or their metabolic activity may not interfere with the ecosystem functions provided by the existing microbial communities (see above the example of *S. meliloti*). The environmental persistence of a GMM may, however, correlate with its potential to travel beyond the immediate areas of application and thus enter non-target environments and ecosystems, which consequently would require an extended risk assessment of non-target effects. In this respect, GMMs with a tight symbiotic relationship, i.e. *Wolbachia* with insects or *S. meliloti* with certain legumes, could be preferable species for environmental applications.

**Horizontal gene transfer**

Horizontal gene transfer (HGT) is the stable transfer of genetic material between organisms without reproduction (OECD, 2010). The risk assessment of recombinant genes of a GMM must therefore not exclusively be linked to the GM host but require consideration of what those genes could do in another biological and ecological context (Davison, 1999; Snow et al., 2005). The environmental persistence of a recombinant gene may totally change once it has been transferred from one organism, e.g. a bacterium optimised to grow and survive in the gut, to another bacterium, capable of growing under nutrient limitation in soils or surface waters or a surface-colonizer with resistance to sunlight. Thus, because of the spread of microbial cells and because of HGT, an environmental risk assessment of a GMM should not only look at target environments but also at relevant non-target environments.

The likelihood of HGT depends on temporal and spatial aspects, densities of donor and recipient cells and their *in situ* physiological status, but also on molecular characteristics of the recombinant genes and their genetic context, e.g. the presence of homologous DNA stretches which may serve as sites for recombination, and whether the genes are located on mobile genetic elements and what the host range of such elements would be (Brigulla and Wackernagel, 2010; Thomas and Nielsen, 2005). Even though these factors have a dramatic influence on the likelihood of HGT in the range of ten or more orders of magnitude, the crucial question to be answered in risk assessing a GMM is what a hazard of the recombinant gene could be in any imaginable host, including potential pathogens, thus following a worst-case scenario approach. It should be noted that the HGT event itself has, in principle, no immediate consequences as it normally would occur between single cells within a background of billions. To become detectable and environmentally significant, growth in competition with indigenous micro-organisms would be required. Thus, the consideration of whether a selective advantage would be provided by the genetic modification is a crucial component in considering the environmental risks of horizontal gene transfer.

**Conclusion**

In addition to direct biological effects of a GMM, including their potential for HGT, hazards may also be caused by indirect effects. Indirect effects may include consequences of the replacement of an existing technology by utilising one linked to the use of a GMM. The assessment of indirect effects can be complex and may require interdisciplinary approaches, including modelling to making predictions from small-scale experiences and contained uses to broader non-contained applications. This effort incorporated into a risk assessment of a GMM, as described above, is clearly rewarding if GMM technologies, as
presented in this volume, are developed to improve the efficiency and environmental friendliness compared to current applications.

References


