Ethical issues raised by genetically modified microorganisms

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(N.B.: all terms marked with an asterisk feature in the glossary at the end)

Unlike experiments carried out on humans and animals, applications involving microorganisms and their genetic modification do not cause pain and suffering as generally understood. The ethical problems raised by genetically modified microorganisms (GMMs) therefore mainly related to the impact they have on the Earth's biosphere. As microorganisms can be found everywhere, including in the ecological niches that are the most unsuited to life forms, the repercussions can be substantial and irreversible.

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In order to clearly grasp to what extent GMMs can be dangerous for humans and their environment, it is necessary, on the one hand, to define them and explain the context in which they are used or can be potentially used (this will be the subject of the first part of this text) and on the other hand, to specify the possible risks of these uses (this will be the subject of the second part). Next, having put these risks in perspective in the third part, we shall question the actual usefulness of these GMMs (can we do without them or not?) as, in the end, given the lack of knowledge currently available, the most effective way of solving this ethical problem once and for all is perhaps to reduce their use. We will end with the role of researchers in the emergence of these new organisms that they cannot yet completely control.

1. What is a genetically modified organism?

1.1. Which microorganisms are concerned*?*

In a phylogenetic* tree featuring all living organisms, the majority of these (in terms of diversity) are microorganisms: one branch of this tree includes true bacteria (also known as eubacteria) whereas the other two branches consist of archaebacteria and eukaryotic*

microorganisms. These notably include protozoa, unicellular algae, and yeasts and moulds (Bapteste *et al.*, 2004).

Within these three main categories of microorganisms, it is bacteria that have undergone the most genetic modification. However, within the eukaryotes, we do find moulds and yeasts that have also undergone genetic modification, although this is rarer.

This text will not cover the problem of viruses which, as they are not autonomous and require another organism to multiply, need to be studied separately.

1.2. What is a genetically modified organism?

A microorganism is considered to be genetically modified if:

- genetic material has been inserted into it by an unnatural method, i.e. other than natural conjugation, transformation or transduction.
- genetic material that has been modified *in vitro* has been inserted into it. In this case, even if a natural transfer method has been used, the microorganism is still considered to be genetically modified.

The gene that has been inserted into the bacteria can be homologous, i.e. it comes from the same species, or heterologous, in which case it has come from another species or bacterial genus (e.g. cloning of a *Bacillus thuringiensis* gene encoding an insecticide in *Pseudomonas*) (Wilson and Lindow, 1993). A heterologous gene can also come from an animal or plant cell (e.g. cloning the gene encoding human insulin in *Escherichia coli*).

DNA modified *in vitro* (foreign DNA) can be:

- either inserted into a cloning vector, i.e. a set of genes capable of enabling the genes that have been transferred to the bacteria to replicate in it; in this case the foreign DNA includes the cloned gene and the often heterologous genes making up the cloning vector.
- or inserted into the chromosome; in this case the foreign DNA is generally exclusively made up of the cloned gene.

1.3. In what cases can microorganisms and (*a fortiori*) genetically modified microorganisms be used? Are they useful?

There are five categories in which microorganisms — including or potentially including GMOs — can be used.

a) for producing molecules or biomass in a fermenter,
b) for producing fermented foods,
c) for various uses in the environment (in agriculture, for pollution control, etc.),
d) for producing strains for therapeutic purposes (e.g. live vaccines),
e) for gaining fundamental knowledge.

With certain technologies, living microorganisms can be released into the environment where they may multiply. To guard against this risk, many countries have legislated against these GM technologies.

1.3.1. Using microorganisms in a fermenter

Since the mid-20th century, microorganisms have been widely used to produce numerous molecules required by the pharmaceutical, agri-food and chemical industries. Moreover, they are produced in fermenters to develop the starter cultures required by the fermented food industry.

In the pharmaceutical industry, many molecules (such as antibiotics or vitamin B12, for instance) are produced by microorganisms which synthesise them naturally. There are also the even more numerous molecules whose gene has been cloned in a microorganism (e.g. human insulin, growth hormone, Hepatitis B vaccine). All these molecules have been marketed for many years and are part of developed countries' daily therapeutic arsenal (recombinant insulin has been produced since 1983) (Swartz, 2001).

In the agri-food industry, intermediate products such as enzymes* (e.g. amylases, rennin), amino acids*, organic acids and even nucleic acids* are produced by microorganisms. In the same way as above, these molecules can be naturally synthesised by microorganisms or come from GMMs.

Lastly, the chemical industry features many molecules (such as enzymes, organic acids and biofuels) produced by microorganisms.

Producing molecules in a fermenter involves culturing the microorganism responsible for producing the required molecule in a (generally) confined space containing a suitable nutritive medium. This operation is generally performed in a confined atmosphere and, in theory, does not cause microorganisms to be released into the environment. It must meet Community requirements (European directives 90/219 and 98/81). In order to master the technology, many GMM culture approvals have been granted over the past few decades (European directive 2001/18/EC). In theory, they are only granted once the risks to public health and the environment have been fully assessed.

There is little diversity in microbial species that are genetically modified to produce useful molecules, as they are often model microorganisms that have been studied over a long period and for which the recombinant DNA technologies are very sophisticated and well-known. They include mainly bacteria (e.g. *E. coli* (Baneyx, 1999), *Bacillus subtilis*) and yeasts (notably *Saccharomyces cerevisiae*) (Ostergaard *et al.*, 2000).

1.3.2. Producing fermented foods

Microorganisms are the main agents in the production of vast numbers of fermented foodstuffs. In Europe, many GMM strains used in this process have been available in research laboratories for a long time but are not yet used for legal reasons, unlike in the U.S.A.
Bread, wine, cheese, butter, creme fraiche, yoghurts, kefir, fermented meats (dry-cured sausage, salami) and fermented vegetables (sauerkraut, olives) are produced by the action of an extremely varied microbial flora. Some fermented foods are produced from a complex and little known microbial flora (that may be categorised as wild flora) found in raw materials and the environment (some unpasteurised cheeses, beers and sourdough bread); others are made from industrial starter cultures of simpler composition and identified flora (many cheeses made from pasteurised milk, for example) and lastly other fermented foodstuffs contain both complex wild flora and industrial flora, which has been added deliberately and in controlled

conditions (this is notably the case of fermented foodstuffs whose raw material cannot be sterilised, such as dry-cured sausage, etc.).

Whatever the case, apart from the majority of fermented drinks and bread, at the end of fermentation fermented foods generally contain roughly 10^9 microorganisms/gram. Depending on the type of food, this flora may or may not be living when the food is ingested by the consumer. Microbial flora then travels through the digestive tract and does not generally stay there, consequently ending up in the environment through faeces.

Why use GMOs for fermented foods?

Foods with complex flora are useful due to their elaborate organoleptic properties, but they can be the seat of pathogenic microorganism development. In addition, their production is difficult to control as microbial flora fluctuates according to the environmental conditions. On the other hand, foods with controlled flora are less useful from an organoleptic viewpoint but safer.

For several years, in order to produce fermented products with controlled flora that are as advantageous from an organoleptic* viewpoint as wild flora-derived foods, industrialists have added new bacterial species isolated from wild flora-derived foods to their starter cultures. But until very recently, they were unable to guarantee that these new species were totally harmless, which thus raised doubts about the safety of these foods. The use of GMMs to produce fermented foods would, on the one hand, solve the problem related to the use of little-known flora and, on the other hand, overcome the various technological problems thanks to the development of improved strains.

The use of GMMs to produce fermented foods is highly regulated, at least in the European Union. The presence of living microorganisms in foods ingested by humans is hampering the use of GMMs. Be that as it may, many bacterial strains and improved yeasts (in terms of industrial properties) are available in the research laboratories of numerous international teams.

1.3.3. Using microorganisms for various applications in the environment (in agriculture, pollution control, etc.)

Microorganisms come into play in many pollution control processes, the most common of which is sewage treatment, a process that involves highly complex wild flora. Methods for

controlling pollution of more specific compounds (hydrocarbons, slurry, various pesticides, etc.) have also been developed and involve selected flora, which is less complex (in terms of diversity). However, the action of this flora is far from optimal and therefore requires genetic improvement. Numerous microbial GMOs with properties that are compatible with the process (resistance to the substrate to be biodegraded, good establishment in the environment, etc.) have been developed in laboratories but they cannot be used for legal reasons, as there is a risk of uncontrolled dispersal into the environment.

Lastly, in agriculture, microbial strains are used to enhance the growth of plants and crop protection. In the same way as above, it has been necessary to develop genetically recombinant strains to optimise the processes. The use of these strains in the environment is currently banned in Europe but has been authorised in the United States for several years. Strains of *Sinorhizobium meliloti* that have been genetically improved to enable nitrogen fixation by the plant have been used since 1997 to seed legume crops.

Similarly, pesticides (Nogall and Mattch) using other genetically improved species (*Agrobacterium radiobacter*) are used in soils.

1.3.4. Production of strains for therapeutic purposes

Owing to their ability to survive or pass through human and animal mucosa, microorganisms can be used to treat or prevent certain diseases. For example, a strain of *Lactobacillus jensenii* has been modified to secrete the CD4 protein used by the HIV virus in the vaginal mucosa to penetrate lymphocytes. This secreted protein also traps viruses (Chang *et al.*, 2003). Even if these GMMs can solve major therapeutic problems, they are not always used because of their potential dispersal into the environment.

1.3.5. Use of microorganisms in laboratories to gain fundamental knowledge

Another no less important use of genetically modified microorganisms is in research laboratories, as they enable us to better understand how microorganisms function. Numerous genes belonging to a wide variety of microbial species have therefore been cloned and have given rise to thousands of GMM strains used as research material by researchers.

In short, in the Europe of today, genetically modified microorganisms are mainly used to produce molecules in fermenters. In this case, the microorganisms are in fact maintained in a confined atmosphere which theoretically prevents their release into the natural environment. They are used to produce the molecules used in the pharmaceutical, agri-food and chemical industries.
In the United States, the situation is somewhat different as some GMM strains are used to produce fermented foods and, more generally, in environmental processes.

2. What are the risks posed by genetically modified microorganisms?

The unicellular nature and relative simplicity of microorganisms means that they are able to multiply very rapidly: *Escherichia coli* is therefore able to give birth to two new cells in 20 minutes when it is cultured in an optimum culture medium. In the same way, *Lactococcus lactis* divides in 30 minutes when it is cultured in milk for the production of soft cheeses.
This property, together with their extremely small size which means that they are able to be dispersed efficiently into the environment by wind and water, allows microorganisms to reach a great variety of potential ecological niches.
The ease with which they are able to colonise these ecological niches allows microorganisms to adapt to a wide variety of environments which may pose difficulties for any other living organism. These bacteria demonstrate an extreme genetic adaptability due, on the one hand, to the fact that they are haploid (they only contain one chromosome, meaning that any mutation* is clearly expressed) and, on the other hand, to the fact that they may acquire genes from other microorganisms (horizontal transfers).
These mutations and acquisitions of new genes give rise to the appearance of a large number of microbial variants which are selected through pressure from the natural environment if the characteristics that they have acquired allow them to adapt more successfully to this environment.

The danger posed by these genetically modified organisms is therefore related both to their dispersal into the environment and to their potential for adaptation to a new environment. In so doing their development may, by altering the animal and plant microbial ecological balance, disrupt this environment to a greater or lesser extent. If, however, they are unable to develop in this environment, they may potentially transfer their modified genetic material to

other microorganisms, thus leading to the appearance of new variants which, themselves, may have the capability of greatly disrupting this environment.

2.1. What might happen when a genetically modified microorganism is released into the environment?

2.1.1. Possibilities of gene transfers to other organisms

Microorganisms are capable of acquiring new genes from other living microorganisms or microbial corpses in the natural environment. In the latter case, the free DNA in the environment is often damaged, although some habitats, such as marine sediments, limit this damage.
There are three main types of mechanism for gene transfer between microorganisms: conjugation, transformation and transduction.

Conjugation is a form of gene transfer between two bacterial cells. Certain genes, known as “transfer genes”, bring cells closer together and, through the formation of a cytoplasmic bridge*, allow DNA to travel between the two bacteria (one of the bacteria therefore becomes known as the “donor” and the other the “recipient”). In general, transfer genes are carried by plasmids (small loops of DNA which are capable of replication independently of the chromosome) which replicate both in the donor and the recipient bacteria. These plasmids do not only contain transfer genes but also other genes which often allow the bacteria to adapt to an ecological niche (genes encoding numerous resistance mechanisms or allowing the assimilation of nutritional elements, for example). This means that bacteria having acquired a plasmid by conjugation may, in turn, transfer it, thereby acquiring new properties. However, these plasmids (known as conjugative plasmids) are not able to be transferred to all bacterial species. There is a host specificity, meaning that some plasmids are able to replicate and be expressed in species which are very varied from a phylogenetic point of view, while others have a narrow host spectrum which only allows them to replicate and be expressed in a small number of species, or even just within a single bacterial species. In a way, this host spectrum controls and limits conjugative transfer in the bacterial world. In the case of GMMs, some genetic improvements are relatively frequently encoded and carried by a plasmid (known in

this case as a cloning vector). In order to limit gene transfers, these vectors do not carry transfer genes. Nevertheless, it is possible that they may be transferred to other bacteria if they are accompanied by a conjugative plasmid. During conjugation, plasmids which do not possess transfer genes may benefit from the enzymatic machinery established by the conjugative plasmid in order to cross into the recipient bacteria at the same time as the conjugative plasmid. However, vectors which are transferred in this way must then be capable of replicating and being expressed in the recipient bacteria.

Transformation is a mechanism which allows some bacteria to acquire exogenous DNA (DNA from dead and lysated cells which circulates freely in the natural environment) and to integrate it into its genome. This mechanism, which has been very well described for some bacterial species, allows bacteria to repair their genome when it is damaged (by exchanging damaged genes with others from dead bacteria) and also to acquire new genes from other bacteria. In the same way as above, this mechanism does not allow bacteria to acquire just any type of gene. The DNA which penetrates the bacteria must be able to be incorporated into the genome and must therefore have a certain sequence homology in order to allow recombination* with the chromosome. Moreover, this mechanism may be more or less effective depending on the species and it is not yet known whether all species possess this mechanism or one of a similar nature.

Transduction is the transfer of bacterial DNA to other bacteria by a bacteriophage, a virus specific to bacteria. When bacteria is infected by a bacteriophage, an event which occurs frequently in nature, this virus, when it multiplies in the bacterial cell, carries with it bacterial DNA instead of phagic DNA. When it reinfects new bacteria, it therefore injects it with this bacterial DNA. If it is plasmid DNA, it may potentially be able to survive in the bacteria. If it is chromosomal DNA, it may potentially become incorporated into the chromosome insofar as it is sufficiently homologous for a recombination to occur. For bacterial GMOs, the transfer of modified DNA may well take place by transduction; the capacity of the cloning vector to replicate in the recipient bacteria depends on its host spectrum and, in the case of chromosomal DNA, the ability to incorporate into the chromosome depends on the degree of homology with the DNA of the recipient bacteria.

These gene transfer mechanisms may allow a diverse flora to acquire new properties. This means that a genetically modified microorganism has the potential to transfer, whether dead or alive, genes which have been modified in laboratories.

The unregulated acquisition of new genes by microorganisms may potentially lead to the appearance of new pathogenic germs (for example through the acquisition of genes involved in virulence or in the colonisation of a new organism). These gene transfers may also lead to unwanted biochemical reactions which may have an impact on the environment (damaging important plant compounds, for example), or to the production of toxic compounds.

It is also conceivable that these gene transfers may lead to the emergence of new microbial variants which may acquire properties allowing them to colonise a new ecological niche and to replace the endogenous population, thus disrupting the microbial balance.

Although gene transfers between microorganisms are frequent and these mechanisms are partially identified, they are nevertheless a cause for concern in the case of GMMs. For example, researchers have shown that gene transfers between *Agrobacterium tumefaciens* (Demaneche *et al.*, 2001), the bacteria used to modify the genes of plant species, and *Pseudomonas fluorescens* most likely occur naturally in soils. Gene transfers between microorganisms and so-called "superior" organisms are less well-known, but nonetheless probably take place. Researchers have observed such transfers, for example, between a bacterium (*Wolbachia sp*) and an insect (*Callosobruchus chinensis*) (Kondo *et al.*, 2002).

2.1.2. The development of new microorganisms in the environment

Genetically modified microorganisms are often designed for a specific function. Nevertheless, we do not have sufficient knowledge of the microbial metabolic pathways in order to have complete control over the appearance of new properties resulting from the modification of genes in laboratories. If unforeseen metabolic modifications can occur in a microorganism whose genome has been modified, it is also highly likely that they can occur in other species in the environment which have acquired these modified genes through horizontal transfer.

It should be remembered at this stage that microorganisms exist in huge numbers in the environment (for example, there are at least 10^9 microorganisms per gramme in fertile humus, 10^5 per millilitre in the water of a river running through a town and 10^9 per millilitre at the outlet of a sewage treatment plant) and they are represented by a large number of diverse species (there are several hundred in the digestive tract of mammals, for example, or in the anaerobic reactor of a sewage treatment plant). The possibilities for gene transfers between

these microorganisms in a given ecosystem are therefore numerous and may lead to the emergence of more adapted variants.

If these new properties give a selective advantage to a species, it is possible that this species, being better adapted to a new environment, may colonise it, thus greatly disrupting the ecological balance, whether microbial, plant or animal. Such a problem is genuinely conceivable and was apparent even before the arrival of GMMs: some cases are already known in which microorganisms have found themselves in a new ecological niche as a result of (generally accidental) human intervention. They have subsequently colonised this niche, disrupting it to a great extent. A well-known example of this involves the toxigenic* unicellular alga *Chrysochromulina polylepis* (Belsher *et al.*, 2003) which, because of human activity (the release of nitrogenous substances into the sea), invaded part of the North Sea and the English Channel, leading to significant health problems as it produces toxins which are pathogenic for humans. Even though not a microorganism, this is also the case of the alga *Caulerpa taxifolia* (Belsher *et al.*, 2003) which, probably as a result of the accidental emptying of the aquariums of the Oceanographic Museum in Monaco, is currently colonising the Mediterranean sea bed and causing the death of the endogenous fauna.

2.1.3. Multiplication of pathogenic microorganisms

Research into pathogenic microorganisms requires, among other things, the cloning of genes encoding the pathogenicity factors in other microbial species, thereby giving rise to genetic events which would probably not naturally have taken place, and therefore the appearance of strains representing a new pathogenic power. Even though these laboratory experiments, concerning plant, animal and human sectors, do not directly aim to create strains which are more pathogenic but to aid the understanding of pathogenicity mechanisms, our current level of knowledge means that we are not able to ensure the complete harmlessness of these transgenic microorganisms. Thus US researchers, by inhibiting the activity of a virulence gene in *Mycobacterium tuberculosis*, created a variant which was much more virulent than the native strain (Shimono, 2003). The issue is even more crucial when it comes to the development of biological weapons: in this case, the primary objective is the creation of new pathogens against which an army or an enemy country is not able to defend itself. A US team thus recently modified the smallpox virus so that it would bypass the immune defences in humans due to vaccination or be resistant to available drugs. Another area of research involved the modification of the cowpox virus so that it might cross species barriers and infect

other species, such as humans. One of the viruses developed demonstrated an increased pathogenicity (*New Scientist*, 2003). Such GMOs threaten to escape the control of scientists and to have unpredictable consequences on animal and human species.

3. Things must be put into perspective

3.1 Genetic modifications of microorganisms also occur naturally

Nature does not need humans in order to modify the genetic inheritance of microorganisms. Their extremely short generation time (compared with that of superior beings such as animals), which allows them to reach a very high population size in a short space of time, means that microbial populations continuously generate a large number of mutants. A mutant may be defined as an individual which has a different genetic inheritance from that of its parents without having acquired new genes by horizontal transfer. A mutation may be a one-off variation in a nucleotide base within a genome or a genetic rearrangement (gene inversion, loss or duplication).

The phenomena which lead to mutations may be natural or caused by external agents of the natural environment. Some bases which make up genes may thus naturally change and be transformed into other bases. Elements from the external environment, such as radiation (ultraviolet rays, for example) or chemical molecules resulting from human activity or the activity of other organisms — including microorganisms — may be responsible for these mutations.

Such mutations are actually due to lesions in the genetic material which, if poorly repaired, give rise to a change in genetic information. The environment will then carry out a process of selection, allowing the emergence of the best adapted mutants. Consequently, when someone suffering from a bacterial infection is treated with an antibiotic, the use of the antibiotic may inadvertently lead to the selection of mutants which are resistant to it. These few mutants which are resistant to the antibiotic, and which constitute just a small proportion of the infecting bacterial population, will thus survive and replace all the original population.

This appearance of mutants with new properties is a phenomenon which has been well known for some time. Spontaneous yeast mutants have thus been used in brewing techniques for several decades.
As it is a natural and commonplace occurrence, mutagenesis is frequently used to improve the properties of microorganisms in all sectors where they are used (the pharmaceutical and food-processing industries, farming, pollution control, etc.). Strains modified in this way may be used and then released (or used) in the environment without posing any problems, ethical or otherwise.

The extreme diversity of microbial species and the large populations mean that genetic transfers between individuals are likely to be considerable in nature, thus contributing greatly to microorganisms' exceptional capacity for adaptation to environmental variations. If humans are able to carry out genetic modifications of microorganisms by the introduction of new genes, it should be remembered that this process also occurs naturally in the environment.

3.2. Recombinant microorganisms are not well adapted to the environment

During their time *in vitro*, "laboratory creatures" have a tendency to lose their capacity to colonise an environment or even to survive in their natural habitat. Several experiments have demonstrated that once the model strains which are used in laboratories (*Escherichia coli, Saccharomices cerivisae, Bacillus subtilis,* etc.) are removed from their test tubes, they have very little chance of surviving in their natural habitat. Successive culture on rich or selective media of these model strains used in laboratories, or genetic modifications carried out by researchers, mean that they have long since lost some of the faculties which are necessary for their adaptation to a complex environment colonised by a diverse flora. This observation does not just concern model species as, even when a strain taken from the natural environment and thus adapted to a complex flora is isolated and is cultivated for just a short time in a laboratory, it rapidly loses the faculties which allow it to settle back into the environment from which it comes. Nevertheless, these microorganisms, even if unable to survive in their natural habitat, are able, on their death and subsequent cellular lysis, to release DNA which may be captured by other microorganisms.

3.3. Gene transfers are limited

The process of gene transfer between microorganisms as described above does not allow gene flow between all species: some transfers may be limited to certain strains within a species, others take place between closely-related species or, more rarely, between phylogenetically distant species, and a small number occur between different bacterial genera. If a total mixing of genes seems impossible, this is because there are several genetic mechanisms which limit gene transfers. In the event of a GMM being released into the environment, these mechanisms therefore restrict the spread of modified genes to wild flora.

For example, transduction does not generally allow DNA transfers between very different species. The infection mechanisms of bacteriophages are specific to each strain. This means that, in general, bacteriophages are only able to infect strains of the same species, even if certain, quite rare, bacteriophages can cross species barriers. In the same way, for some species, the natural transformation of bacteria (foreign DNA from the external environment crosses the barrier into the bacterial cytoplasm) requires the presence of specific sequences on the exogenous genome. Some bacteria are therefore only able to acquire DNA comprising these particular sequences, which are often species-specific.

Finally, there are various bacterial mechanisms which are capable of damaging foreign DNA which enters into the bacteria (restriction-modification systems and the SMR system), making the bacteria unable to incorporate the foreign DNA.

In the same way, some genes are only able to express themselves in a certain type of bacteria. For example, it is known that the genes of Gram-positive* bacteria express themselves relatively easily in Gram-negative* bacteria, but the opposite can not always be observed; in particular, certain gene promoters allowing transcription are not recognised by the bacteria which have captured the DNA fragments containing them.

4. However…

4.1. Little is known about the adaptation potential of "lab creatures"

Although, through observations, it seems that model strains and strains modified in laboratories have lost their capacity for adaptation and colonisation, this does not rule out the possibility of a variant departing from this rule. For example, researchers have recently noted the survival of genetically improved strains of *Sinorhizobium meliloti* in soils for six years, even in the absence of the legumes with which they form a symbiotic relationship (Donegan *et al.*, 1999).

4.2. Gene transfers in nature are difficult to assess

The complex nature of the relationships between microbial populations within an ecosystem makes it very difficult to assess the importance of gene flow between microorganisms, as well as the nature and diversity of the genes involved. In-depth studies are necessary in order to assess the importance of these transfers for the evolution of microorganisms as well as the possibility that a recombinant microorganism might transfer its DNA to a microorganism that occurs naturally in the environment. Recent studies have shown that genetically improved strains of *Sinorhizobium meliloti* (Donegan *et al.*, 1999) transferred DNA to endogenous flora in the soil and that these transfers could be facilitated in the digestive tracts of arthropods present in the soil.

4.3. Genetic engineering causes modifications which are not naturally possible

Even if mutations and therefore modifications of genes occur spontaneously in nature and give new properties to microorganisms, some of the modifications carried out by humans are not at all spontaneously possible in nature. This is the case, for example, in the production of human hormones such as insulin using *Escherichia coli*, through the cloning of the human gene in the bacteria. The probability of this spontaneously occurring by genetic mutation or transfer in nature is practically zero.

In the same way, due to the mechanisms which limit the transfers of genes of different sizes, many genes cannot be exchanged between different species. Researchers, however, have the possibility of lifting these barriers between species and allowing gene transfers which would not naturally have been able to take place. Some researchers believe that, in so doing, the combination of transgenic harvests and genetically modified biopesticides (such as *Agrobacterium radiobacter* and *Pseudomonas fluorescens*) may create genetic recombinations which are likely to have devastating effects on the microflora and microfauna in soils.

5. Is it possible to avoid the use of genetically modified microorganisms?

5.1 Is the use of GMMs essential?

Microbial GMOs may potentially be used in the manufacture of recombinant molecules* (drugs, intermediate products in the agri-food industry), the production of fermented foodstuffs (alcoholic drinks, dairy products, etc.), pollution control (sewage, hydrocarbons), the improvement of crop production by facilitating symbiosis between plants and microorganisms (nitrogen fixation) and the substitution of various technologies (biolixiviation, etc.).
The reasons which are leading to the use of GMMs, or which may do so in the future, may be of an economic nature and/or may relate to the health of humans, animals or plant life. Every time a GMM is used, it is imperative that the risks involved in its use be compared to those which would exist if the GMM were not used.

In theory, in the case of molecules produced in fermenters, the GMM is not released into the natural environment. In order to ensure that there are no leaks, fermentation plants must be made more secure, therefore adding to the costs involved in the process. Nevertheless, this process is controllable and therefore considerably reduces the risks involved in the use of GMMs.
Some of the molecules produced in this way have enabled the treatment of conditions for which the pharmaceutical alternatives were far from comparable and even represented a

certain risk. For example, before it was produced using a recombinant *E. coli* strain, human growth hormone was produced using hypophyses taken from human corpses. Some batches, which were made from hypophyses contaminated with the agent responsible for Creutzfeldt-Jakob disease, were responsible for the transmission of this disease to children being treated for dwarfism with this hormone. Before recombinant insulin became commercially available, diabetics were treated with modified pig insulin against which, after a certain length of time, the human body produced antibodies.

In addition to these important advantages concerning the purity and quality of the molecule, such cloning of microorganisms offers, due to the ease of production, the twofold benefit of a considerable reduction in production costs, allowing more people to be treated, and the possibility of carrying out research into new uses. For example, growth hormone now has other applications (which are well-known in the field of competitive sport) over and above the restoration of growth in children suffering from dwarfism.

As far as the manufacture of drugs is concerned, there seems to be no doubt that the use of GMMs, insofar as they are not released into nature, represents progress for our society. On the other hand, several other molecules are produced to essentially economic ends in order to improve or stabilise a technological process. This is the case for many enzymes which are used in food processing, such as amylases which are used in brewing, proteases used in the meat industry and chymosin used during cheese production. There are often alternatives to the use of these molecules: they may be naturally synthesised by a microorganism which does not necessarily need to be a GMM (for example, *Bacillus subtilis* produces proteases) or they may be extracted from another organism (chymosin may also be extracted from calf rennet, which has traditionally been used for centuries in cheese-making technology). In the eyes of their critics, the use of GMOs in the production of molecules may be seen to be questionable if these can be produced by an alternative process. It must be borne in mind, however, that the process in question — cultivation in fermenters — does not lead, in theory, to the release of modified microorganisms into the environment. Moreover, the reduction in costs of a process through the use of molecules derived from GMMs may allow more people to consume the resulting food products.

If the risks involved in the use of fermenters seem to be controllable, the same can probably be said for the majority of molecules derived from microbial GMOs: where they are identical to naturally occurring molecules, the risks involved in their ingestion seem low. The consumption of cheeses made with recombinant chymosin is thus common (something which

proved to be very useful during the BSE crisis in order to replace calf rennet, which was likely to have been contaminated by the BSE agent).

As regards GMMs which are released or used in the environment, the risks involved in their use are greater than in the above example as it is conceivable that they may disrupt ecosystems, either because they may transfer their modified genes to another microorganism, providing the latter with "colonising" properties, or because their development may be to the detriment of endogenous species. This is why the use of modified strains in the manufacture of fermented food products for commercial purposes is currently banned in Europe, following the precautionary principle. The same is true for strains intended for use in pollution control or to improve the growth or resistance of plants. Developments in cloning technologies, such as the insertion of genes into the chromosome, may lead to a relaxation of legislation as has been the case in other countries (the United States has just authorised the use of a modified strain of *Rhizobium meliloti* in the environment) and to the authorisation of the use of genetically modified strains in food products and in the environment.

As far as GMMs which are available for use in the production of fermented foodstuffs are concerned, these essentially aim to improve production processes (the development of strains of *L. lactis* which are resistant to bacteriophages for the production of cheeses is a good example) or to improve the organoleptic, nutritional and hygienic properties of food. These improvements may, however, be achieved using other means such as the selection of appropriate non-modified strains or adherence to hygiene standards.

With regard to GMMs used in pollution control, the problem is more complex as the risks involved in their dispersal into the environment — which is also a form of pollution — must be compared with the risks of environmental pollution. In any case, it seems that even if GMMs provide ad hoc solutions to such problems, the long-term aim must be sustainable development and the prevention of pollution.

The issues surrounding GMMs which are used in conjunction or in symbiosis with plants are more delicate. In this context, GMMs are used to improve plant productivity or to develop the resistance of crops to harmful insects or pesticides. A solution which allows production costs to be lowered or productivity to be improved, especially in areas where crop production is difficult, cannot be immediately rejected out of hand. Even if alternative methods of cultivation — such as those which avoid the use of polluting fertilizers or pesticides — or sustainable development appear to be less effective than the development of techniques which use GMMs, it becomes difficult to justify rejection of these techniques.

However, as noted above, the risks involved in the release of GMMs into the environment must be assessed. As long as these risks are undefined, the precautionary principle, which advises against the use of GMMs, remains valid.

The most complex issue relates to the use of live GMMs to treat certain human and animal diseases. For example, species used in food production, such as *Lactococcus lactis*, have been genetically modified in such a way as to secrete molecules through the mucosa (the digestive membrane, for example) in order to treat medical conditions (Chang *et al.*, 2003). This form of treatment seems promising and should, in some cases, prove to be more effective than traditional allopathic medicine. These strains are ingested and passed through the human digestive tract and are therefore released, in living form, into the environment. This is why a current line of research involves the development of strains which will not be able to survive outside the digestive tract. In the meantime, however, if we compare the benefits they provide with the potential risks posed to the environment, it seems difficult at the current time to decide on whether these GMMs are absolutely necessary.

The final category, which concerns GMMs produced in laboratories, is by far the most extensive in terms of the diversity of the experiments carried out and the strains obtained. The cloning of microbial genes in model microorganisms such as *E. coli*, *B. subtilis* or *L. lactis*, which is often necessary in order to obtain data on their regulation and expression, is a procedure which is often very difficult to avoid. This is why the freezers of molecular biology laboratories are often overflowing with genetically modified strains. An understanding of the prokaryotic genome has often been a prerequisite for understanding more complex genomes such as the human genome, which is why it seems impossible to consider the abandonment of research using GMMs, insofar as the provisions set in place to avoid their dispersal into the environment are carefully followed.

5.2. Are researchers able to evaluate the legitimacy of their work?

As we have seen, fundamental research on genomes and on the way in which organisms work requires the use of genetic engineering, meaning that the development of GMOs in laboratories does not always directly comply with sometimes arguable economic imperatives. However, experience has taught us that these scientific advances also serve interests which go against the well-being of humans and their environment. Nonetheless, it seems impossible to

determine beforehand the consequences linked to the acquisition of new scientific knowledge which will be harmful to humankind.

It is indisputable that the acquisition of this fundamental knowledge is essential as it subsequently leads to applied research which aims, in particular, to improve the conditions in which humans live and to preserve their environment.

Nevertheless, researchers are entitled to call into question some lines of research which may increase risks to the health of humans, animals or plant life or to the ecological balance of the planet. The development of microbial strains with improved pathogenic capabilities in order to expand the arsenal of biological weapons provides a clear illustration of this. Even though it may appear difficult at first sight, every researcher has a responsibility to be concerned about the potential consequences of his or her work.

The freedom of researchers as to the purpose of their research is, however, becoming hindered to an ever greater extent by economic imperatives: in order to work, they need financial assistance which is dependent on the scientific guidelines dictated by the State or on the needs of businesses. If it can be said that the business community is directly motivated by economic imperatives and pays relatively little attention to humanitarian considerations, this is not necessarily true for the State. However, over the past few years in France, the State has tended to withdraw from the field of research, thus strengthening the ties between businesses and researchers.

6. Conclusion

Since the era of Pasteur, for practical reasons, the way in which microorganisms work has been studied *in vitro* on pure strains under the conditions and in the environment of the laboratory, thus revealing a mere fragment of the complexity of the microbial world. The scientific community has been aware for some years of the need to study microorganisms in their environment; however, the knowledge which is necessary to understand how microbial ecosystems work is insufficient. It is therefore very difficult at the current time to predict the influence that the dispersal of genetically modified microorganisms into our environment may have. Several studies, in particular relating to gene transfer, will be necessary over the coming years in order to give an accurate assessment of the risks and, consequently, to develop GMMs which do not pose a risk to humans and their environment. Several GMM strains are currently being used in conditions which limit this risk, such as production in fermenters. In

theory, the precautionary principle is applied in other cases, in particular in Europe, where such uses must be approved by the relevant authorities. However, in view of the real technical difficulties relating to the detection of these microorganisms, their unlawful use, in particular by industrialists, is a cause for concern. As regards fundamental research on microorganisms which gives rise to new modified strains or new technologies, the question of the real need for such research must be considered in the same way as with all research in all disciplines.

GLOSSARY

Amino acids: Molecules that make up proteins

Cytoplasmic bridge: Link between the two bacterial cells

Enzyme: Protein substance that catalyses biochemical reactions. It is effective in minute amounts and is not altered by the reaction

Eukaryotic: Organism whose chromosomes are confined to the nucleus

Gram-positive, Gram-negative: Two important bacterial groups that differ in cell wall composition

Mutation: Genome alteration

Nucleic acid: DNA, RNA

Organoleptic: Affecting the sense organs

Phylogenetics: Branch of genetics that studies genetic changes in species through evolution

Recombinant molecules: Molecules whose gene was cloned into another organism (in this case into a GMM)

Recombination: Gene rearrangement

Toxigenic: Produces one or more toxins

Bibliography

Baneyx F., 1999. Recombinant protein expression in Escherichia coli. *Current Opinion in Biotechnol.*, 10(5): 411-21.

Bapteste E., Boucher Y., Leigh J. and Doolittle W.F., 2004. Phylogenetic reconstruction and lateral gene transfer. *Trends in Microbiology*, 12(9): 406-11.

Belsher T., Lunven M., Le Gall E., Caisey X., Dugornay O. and Mingant C., 2003. Acquisition de données sur l'expansion de *Caulerpa taxifolia* et *Caulerpa racemosa* en rade d'Hyères et en rade de Toulon (France). *Oceanologica Acta*, 26(2): 161-166.

Bermudez-Humaran L.H., Langella P., Cortez-Perez N., Gruss A., Tamez-Guerra R.S., Oliveira S.C., Sauceda-Cardenas O., Montes de Oca-Luna R. and Le Loir Y., 2003. Intranasal Administration of recombinant *Lactococcus lactis* secreting Murine Interleukine-12 Enhances Antigen-specific Th1 Cytokine Production. *Infection and Immunity*, 71(4): 1887-96.

Chang T.L., Chang C.H., Simpson D.A., Xu Q., Martin P.K., Lagenaur L.A., Schoolnik G.K., Ho D.D., Hillier S.L., Holodniy M. and Lewicki J., 2003. Inhibition of HIV infectivity by a natural human isolate of *Lactobacillus jensenii* engineered to express functional two-domain CD4. *Proceedings of the national academy of sciences U S A.* 30, 100(20): 11672-7.

Demaneche S., Kay E., Gourbiere F. and Simonet P., 2001. Natural transformation of Pseudomonas fluorescens and Agrobacterium tumefaciens in soil. *Applied and Environmental Microbiology*, 67(6): 2617-21.

Donegan K.K., Seidler R.J., Doyle J.D., Porteous A., Digiovanni G., Widmer F. and Watrud L.S., 1996. A field study with genetically engineered alfalfa inoculated with recombinant Sinorhizobium meliloti: effects on the soil ecosystem. *Journal of Applied Ecology*, 36(6), p.920.

European Directive 2001/18/EC

European Directive 90/219 amended by Directive 98/81

Kondo N., Nikoh, Ijichi N., Shimada M. and Fukatsu T., 2002. Genome fragment of *Wolbachia endosymbiont* transferred to X chromosome of host insect. *Proceedings of the national academy of sciences U S A,* 29; 99(22): 14280-5. Epub 2002 Oct 17.4.

New Scientist issue, 1 November 2003.
http://www.eurekalert.org/pub_releases/2003-10/ns-udl102903.php

Ostergaard S., Olsson L. and Nielsen J., 2000. Metabolic engineering of *Saccharomyces cerevisiae*. *Microbiology and molecular biology reviews,* 64(1): 34-50.

Ruitton S., Javel F., Culioli J.M., Meinesz A., Pergent G. and Verlaque M., 2005. First assessment of the *Caulerpa racemosa* (*Caulerpales*, *Chlorophyta*) invasion along the French Mediterranean coast. *Marine pollution bulletin,* 2005 May 10.

Shimono N., 2003. Hypervirulent mutant of *Mycobacterium tuberculosis* resulting from disruption of the mce1 operon. *Proceedings of the national academy of sciences U S A,* 100(26): 15918-23.

Swartz J.R., 2001. Advances in Escherichia coli production of therapeutic proteins. *Current Opinion in Biotechnology,* 12(2,1): 195-201.

Wilson M. and Lindow S.E., 1993. Release of recombinant microorganisms. *Annual Review of Microbiology,* 47: 913-44.