2012

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Published (as Chapter 5) in Chris A. Wozniak and Alan McHughen, eds., *Regulation of Agricultural Biotechnology: The United States and Canada* (2012); doi: 10.1007/978-94-007-2156-2_5

The Status, Promise and Potential Perils of Commercially Available Genetically Modified Microorganisms in Agriculture and the Environment

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Abstract
Modern genetics has shown the power of modifying microbes, from viruses to bacteria to algae, to produce desirable agricultural products. Nevertheless, gene additions or modification have led to relatively few products in the marketplace due partly to costs of regulation, but also to the challenges of production, delivery and application. Some products with gene loss have been marketed, notably *Agrobacterium radiobacter* with a deletion for plasmid transfer, some veterinary vaccines and plants with one or a few genes from microbes for plant protection. Concerns over using live microbes are centered on recombination with wild type strains, potential for environmental risks, market acceptance, market scope, monitoring costs, and costs of production. The challenges in microbial agricultural plant biotechnology far outweigh those in medical and veterinary biotechnology because of pricing potential, larger markets and controlled environments in which modified microbes can function. Nevertheless, the promise and need for control of plant pathogens for which little or no plant resistance is available warrant continued efforts in this area. Veterinary uses of modified microbes will continue and be more widely accepted. Plants "vaccinated" with genes for plant protection are increasingly used but their safety is still questioned and debated. Products such as enzymes from GMOs will continue to enter the marketplace and be accepted with few questions.

Keywords: Genetic engineering, Commercial products, PIPs, Biocontrol, Vaccines

1 Introduction

Genetically modified microorganisms (GMOs) based on recombinant DNA techniques have been constructed since the 1970s. The potential for beneficial use was recognized very early, as was their potential risk through misuse, intentional or accidental. This dilemma remains with us in the twenty-first century. GMOs have also been known as genetically engineered microorganisms (GEOs) or genetically modified microbes (GMMs). Many of the issues related to the use of engineered microbes in agriculture have been presented earlier (Ryder 1994; Wilson and Lindow 1993; Wrubel et al. 1997), in a symposium on "The Scientist's Role in the Controversy Over Genetic Engineering, Regulation and Utilization of Microorganisms" (Vidaver 1989), and more recently in a study by the National Academy of Sciences (NRC 2004). However, the bulk of these presentations and discussions appeared two decades or more ago in the early days of modern genetics and genetic engineering. Today, the ease and lower cost of nucleic acid sequencing for genome analysis, improved methods of detection of microorganisms and specific sequences, new discoveries in genetic manipulation, and synthetic biology raise new issues to ponder and new approaches to assessing microbial ecology and the risks and benefits of GMOs in agriculture. In this chapter, we deal principally with commercialization of products used for plant and animal production and protection in agriculture.

2 Current Status of GMOs

2.1 Regulation

GMOs are regulated in the U.S. based on the intended use, whereas in Canada regulation is a part of novel product oversight. Microbial GMOs in plant agriculture agents, ranging from viroids to nematodes (Table 1), are under the jurisdiction of the Environmental Protection Agency (EPA) (see elsewhere in this book) if the objective of their use is for pest control. In Canada jurisdiction is by Health Canada. Protective veterinary products for animals

Table 1. Relative potential of microorganisms as GMOs for use in agriculture

Agent	Ease of genetic manipulation	Ease of production	Ease of application
Viroids	Variable	Challenging	Difficult
Viruses	Variable	Challenging	Challenging
Bacteria	Variable	Easy	Easy
Fungi	Challenging	Challenging	Challenging
Oomycetes	Difficult	Difficult	Difficult
Protozoa	Difficult	Difficult	Difficult
Algae	Difficult	Challenging	Difficult
Nematodes	Difficult	Difficult	Variable

and fish are regulated and licensed by the Center for Veterinary Biologics of the United States Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) in the U.S. and by the Canadian Food Inspection Agency (CFIA) in Canada. APHIS also has authority under the Plant Protection and Quarantine program over introduction and release into the environment of organisms that are or may be plant pests.

2.2 Commercial Product Overview

GMOs have a minor use in agriculture. Products are limited because of costs of regulation and marketplace determination of availability and applications. Since 1976, the U. S. EPA has compiled an inventory of about 82,000 chemicals produced in or imported into the U.S., many of which are used in agriculture (Schierow 2009). That period of over-three decades coincides with the rise of modern genetics and tools for modification of microorganisms. To date, there are 63 commercial products that we have identified, comprising living microbes, plants modified with microbial genes for pest protection, and veterinary vaccines. There are also a couple dozen enzymes derived from GMOs used in the food industry. This is hardly a robust number and cause for concern or alarm.

Bacteria, fungi and viruses are the major candidates of choice for genetic manipulation. Commercial products for protection could be in any form, but the most common is with the use of live or non-viable agents applied for competitive exclusion or direct competition for receptor sites on plant parts (Wilson and Lindow 1994), or as vaccines in animals (Jackwood et al. 2008; Meeusen et al. 2007). All of the agents approved thus far are primarily effective when used prior to exposure of the infectious agent. About 22 enzymes derived from GMOs are used in the food industry worldwide (Olempska-Beer et al. 2006).

2.3 Vaccines in Animal Agriculture

There are a number of genetically engineered veterinary viral and bacterial vaccines, including gene-deleted vaccines and live recombinant chimera viruses that combine parts of two infective viral genomes (Jackwood et al. 2008; Meeusen et al. 2007). These vaccines are in three categories: live genetically modified microbes (viruses or bacteria with one or more genes deleted or inactivated or carrying a foreign gene), recombinant inactivated vaccines (subunit vaccines containing only part of the whole organism) and genetic vaccines (nucleic acids or DNA with foreign genes). There are 21 of these commercially available for treatment of a wide variety of animals: ruminants, swine, poultry and companion animals. However, these are still a minor part of the commercial vaccine market. As of 2010, the USDA Center for Veterinary Biologics has listed 28 vaccines categorized as seven non-replicating recombinant antigen vaccines, two nucleic acid-mediated vaccines, four live gene-deleted vaccines and 15 live vectored vaccines.

Table 2. Registered genetically modified microbes and primary use in plant agriculture

Microorganism	Use
Agrobacterium radiobacter K1026	Protection of roots from crown gall
Pseudomonas fluorescens (killed) with *Bacillus thuringiensis* delta endotoxins (endotoxins from *B.t.* strains aizawai, Kurstaki, or San Diego)	Insecticidal spray (no reproduction of host bacterium)

2.4 Microorganisms Associated with Plants and Plant Pests

A small number of free-living GMOs have been approved for use since the last century (Amarger 2002); only those in current use are listed in Table 2. These have a Biopesticide Regulatory Action Document (BRAD) that indicates current status in the U.S. Note that strains are specifically mentioned. For example *Agrobacterium radiobacter* K1026 (Jones and Kerr 1989) is a Tra– (transfer negative) derivative of *A. radiobacter* K84, a naturally occurring bacterium effective against crown gall, caused by a tumorigenic relative, *A. tumefaciens*. A transferable plasmid in K84 also carries a gene for a specialized antibiotic or bacteriocin effective against *A. tumefaciens*. Deletion of the Tra+ gene prevents the rare transfer to *A. tumefaciens*, which could make it resistant to biocontrol. We were unable to find records of GMOs used outside the U.S., except for Australia where *A. radiobacter* K1026 originated; it has been used commercially since 1988 for reducing crown gall infection of stone fruits, such as peach and cherry and ornamentals, notably roses (Ryder 1994).

In the case of the *Bacillus thuringiensis* (*Bt*) derivatives, each has differing insecticidal properties. The corresponding wild-type (natural) strains have been used for about 70 years, including in organic farming. The modified strains or host bacteria attach to plant receptors more easily and are more resistant to UV light degradation than the parent strains. The host bacterium, dead or alive, has no known deleterious effects on animals, plants or humans. Extensive information on the analysis conducted by EPA of *Bt* in several formulations has been summarized by Mendelsohn et al. (2003).

There are a miniscule number of potential products that could provide protection for plants from infectious agents. The historic experiments with a *Pseudomonas syringae* ice-minus deletion summarized by Lindow (1989) and Wilson and Lindow (1993) did not lead to a viable product, although an unmodified strain is used now to protect plants from frost under a narrow temperature range. There was a transient commercialization of *Sinorhizobium* (*Rhizobium*) *meliloti* RMSPC-2 (EPA 1997, 1998) as seed inoculants for alfalfa. The strain had genes to enhance nitrogen fixation and nutrient utilization, as well as an antibiotic resistant marker gene (http://epa.gov/biotech_rule/pubs/factdft6.htm). The commercial transfer of the *Bt* delta endotoxin gene to the endophyte *Clavibacter xyli* for control of the corn ear worm (Tomasino et al. 1995) lost to competition with the development of *Bt* genes transformed as integral parts of the plant cell.

2.5 Microbial Genes as Plant Protectants

Microbial genes inserted into plants have been widely adopted since the mid-1990s. In the U.S., these genes for plant protection are classified as "PIPs" or plant-incorporated protectants, of which there are now about 40 registered with the EPA (http://www.epa.gov/oppbppd1/biopesticides/index.htm#pips). The majority use genes from *Bt* strains for insect control (Mendelsohn et al. 2003). Virus-protected papaya and cucurbit plants have been commercialized for several years but are not listed on the PIP website. The latest candidate is the coat protein gene of Plum pox virus used to protect stone fruit trees which has recently been approved for commercialization (http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/brad_006354.pdf).

3 Constraints

Not surprisingly, the use of dead or inactivated microbes (e.g. vaccines for animals) has been more widely accepted and commercialized than the use of live microbes or chemicals for plant protection (Table 3). Microbes are likely to be viewed more negatively in agriculture if they are able to replicate. Questions continue to be raised about their survival, persistence, contamination, spread, efficacy of expression of the beneficial trait(s), and gene transfer. This is the case even though no substantive differences have been found between GMOs and the corresponding parent strains (Amarger 2002; Wilson and Lindow 1994). Although substantial equivalence is becoming accepted for food safety/risk assessments (LeBlanc et al. 2010), we believe it is less likely to be used for agricultural and environmental applications.

4 Promises and Perils

Many opportunities and challenges remain. Containment remains an issue, but bioconfinement of microorganisms is possible genetically and physically (NRC 2004). Microbiologists and ecologists with little experience with plant

Table 3. Strategies and attributes of introduced microorganisms and chemicals used in plant health and protection

Strategy/attribute	Microorganisms	Chemicals
Replication	Yes (limited)	No
Shelf-life	Variable	Long
Ecological contamination	Rare	Variable
Cost (research, production, regulatory)	Variable	High
Persistence	Rare	Variable
Specificity	Common	Rare
Safety	Absolute (?); no reported adverse effects	Variable
Market prospects	Relatively limited	Wide

associated microbes remain concerned about reproduction, survival, and gene transfer to and from other microbes. When these questions have been dealt with experimentally and data provided for risk assessment, many candidate microbes may or may not be considered suitable for use. It is seldom recognized that thousands to millions of microbes colonize plants, including imported inspected plants and bulbs, and most do so in a beneficial or neutral manner. Even so, the public is reluctant to use microorganisms, compared with chemicals, because of the greater familiarity with germ and human disease causality rather than with the beneficial role of microbes in the environment (Table 3).

For animals, bio-engineered vaccines show great promise through using reverse genetics, non-replicating viral vectors, cytoplasmic replicating viruses (alpha viruses; positive stranded RNA viruses) and genetic vaccines, as well as benefiting from improved adjuvants and delivery systems (Patel and Heldens 2009).

New challenges and opportunities also lie with synthetic biology. For example, viruses can be readily constructed de novo from commercially available nucleotides, and a partially synthetic bacterium has been constructed. Due to the high monetary costs of research and regulation, such constructs are not likely to be available in the agricultural sector in the near future. However, there is promise through plant genomics and limiting pathogen invasion through novel resistance genes and RNAi approaches.

4.1 Challenges

Taxonomy is also a challenge to microbial production and use, and in risk assessment. The scope of GMO regulation targets "intergenerics," even though not all members of the same genus have similar habitats and traits. The use of taxa that include human and/or animal pathogens (e.g. *Burkholderia cepacia*) has met with opposition by several groups, even when there is no evidence of the strain's ability to cause harm. And, whether *Rhizobium* (*Bradyrhizobium*) inoculants that receive transgenes from other members of the species should be regulated under the Toxic Substances Control Act remains an open question, as it defines GMOs as intergeneric. This example is particularly pertinent because of its close taxonomic relationship to *Agrobacterium*, a genus composed largely of plant pathogens.

A number of critical needs must be met before more products are available and used in agriculture, including potential production of biofuels using GMOs (Glass 2008). It would be helpful to categorize microbes according to risk groups and show that there are many that are generally regarded as compatible with the environment. A GRACE classification (Generally Regarded as Compatible with the Environment) would demonstrate to the public that the commercial strains are in such a group, such as *Rhizobium* and *Bradyrhizobium*, among others. There should be clear differentiation between fears and risks. Risk assessments should be based on available science and, naturally, regulations and guidelines should be commensurate with the risk.

For small markets or specialty products, the equivalent of ORPHAN status might be considered. Delivery methodology needs to be improved in the plant sector, as is being done with human and animal medical vaccines. New technologies such as synthetic biology and nanotechnology need to be evaluated for safe introduction into the environment. And public and media education is essential.

Clearly, the marketplace for the private sector has been uneven. Few prosper with live GMOs. This appears to be largely due to insufficient sales commensurate with perceived usefulness by the applicator and regulatory costs and constraints. The likelihood of increased numbers of free-living products in plant agriculture, based on 35 years of product analysis, is not promising. Public acceptance of transgenes in the products themselves has been widespread, but continues to be challenged by certain sectors including organic foods. More research and education in multiple forums may alleviate such fears and enable more product development.

References

Amarger N (2002) Genetically modified bacteria in agriculture. *Biochimie* 84:1061–1072

EPA (1997) Fact sheet: commercialization of *Sinorhizobium* (*Rhizobium*) *meliloti*, RMBPC-2. http://www.epa.gov/biotech_rule/pubs/factdft6.htm . Accessed June 3, 2011

EPA (1998) Final rule: *Sinorhizobium meliloti* strain RMBPC-2: Significant new use rule. *Fed Regist* 63:29646–29648

Glass DJ (2008) Impact of government biotechnology regulations on biofuel development. *CleanTech* 2008, CSI Events, Boston

Jackwood MW, Hickle L, Kapil S, Silva R (2008) Vaccine development using recombinant DNA technology. Animal Agriculture's Future through Biotechnology, Part 7. CAST Issue paper 38, 12 pp. (http://www.cast-science.org)

Jones DA, Kerr A (1989) *Agrobacterium radiobacter* strain K1026, a genetically engineered derivative strain of strain K84, for biological control of crown gall. *Plant Disease* 73:15–18

LeBlanc JG, van Sinderen D, Hugenholtz J, Piard J-C, Sesma F, de Giori GS (2010) Risk assessment of genetically modified lactic acid bacteria using the concept of substantial equivalence. *Curr Microbiol* 61:590–595

Lindow SE (1989) Release and behavior of recombinant bacteria in field studies. *J Iowa Acad Sci* 96:71–73

Meeusen ENT, Walker J, Peters A, Pastoret P, Jungersen G (2007) Current status of veterinary vaccines. *Clin Microbiol Rev* 20:489–510

Mendelsohn M, Kough J, Vaituzis Z, Matthews K (2003) Are Bt crops safe? *Nat Biotechnol* 21:1003–1009

National Research Council (U.S.). Committee on Biological Confinement of Genetically Engineered Organisms (2004) *Biological confinement of genetically engi-*

neered organisms. National Academies Press, Washington, DC, 255 pp. (http://www.nap.edu)

Olempska-Beer ZS, Merker RI, Ditto MD, DiNovi MJ (2006) Food-processing enzymes from recombinant microorganisms—A review. *Regul Toxicol Pharmacol* 45:144–158

Patel JR, Heldens JGM (2009) Immunoprophylaxis against important virus diseases of horses, farm animals and birds. *Vaccine* 27:1797–1810

Ryder M (1994) Key issues in the deliberate release of genetically-manipulated bacteria. *FEMS Microbiol Ecol* 15:139–145

Schierow L-J (2009) The Toxic Substances Control Act (TSCA): Implementation and new challenges. Congressional Research Service, 7–5700, 35 pp

Tomasino SF, Liester RT, Dimock MB, Breach RM, Kelly JL (1995) Field performance of *Clavibacter xyli* subsp. *cynodontis* expressing the insecticidal protein gene crylA (c) of *Bacillus thuringiensis* against European corn borer in field corn. *Biological Control* 5:442–448

Vidaver AK (1989) Public policy on the introduction of genetically engineered microorganisms. *J Iowa Acad Sci* 96:74–77

Wilson M, Lindow SE (1993) Release of recombinant microorganisms. *Annu Rev Microbiol* 47:913–944

Wilson M, Lindow SE (1994) Ecological similarity and coexistence of epiphytic ice-nucleating (Ice^+) *Pseudomonas syringae* strains and a non-ice-nucleating (Ice^-) biological control agent. *Appl Environ Microbiol* 60:3128–3137

Wrubel RP, Krimsky S, Anderson MD (1997) Regulatory oversight of genetically engineered microorganisms: Has regulation inhibited innovation? *Environ Manage* 21:571–586