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REVIEW ARTICLE

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Development, registration and commercialization of microbial pesticides for plant protection

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Abstract Plant protection against pathogens, pests and weeds has been progressively reoriented from a therapeutic approach to a rational use of pesticide chemicals in which consumer health and environmental preservation prevail over any other productive or economic considerations. Microbial pesticides are being introduced in this new scenario of crop protection and currently several beneficial microorganisms are the active ingredients of a new generation of microbial pesticides or the basis for many natural products of microbial origin. The development of a microbial pesticide requires several steps addressed to its isolation in pure culture and screening by means of efficacy bioassays performed in vitro, ex vivo, in vivo, or in pilot trials under real conditions of application (field, greenhouse, post-harvest). For the commercial delivery of amicrobial pesticide, the biocontrol agent must be produced at an industrial scale (fermentation), preserved for storage and formulated by means of biocompatible additives to increase survival and to improve the application and stability of the final product. Despite the relative high number of patents for biopesticides, only a few of them have materialized in a register for agricultural use. The excessive specificity in most cases and biosafety or environmental concerns in others are major limiting factors. Non-target effects may be possible in particular cases, such as displacement of beneficial microorganisms, allergenicity, toxinogenicity (production of secondary metabolites toxic to plants, animals, or humans), pathogenicity (to plants or animals) by the agent itself or due to contaminants, or horizontal gene transfer of these characteristics to non-target microorganisms. However, these non-target effects should not be evaluated in an absolute manner, but relative to chemical control or the absence of any control of the target disease (for example, toxins derived from the pathogen). Consumer concerns about live microbes due to emerging food-borne diseases and bioterrorism do not help to create a socially receptive environment to microbial pesticides. The future of microbial pesticides is not only in developing new active ingredients based on microorganisms beneficial to plants, but in producing self-protected plants (so-called plant-incorporated pesticides) by transforming agronomically high-value crop plants with genes from biological control agents

Keywords Biological control · Plant disease · Pest · Weed · Fermentation · Formulation patents

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Introduction

According to the Food and Agriculture Organization (FAO), the estimate of the world population for 2001 was 6.134×10^9 inhabitants (http://apps.fao.org) and the projection towards 2025 is nearly 8.5×10^9 inhabitants. Such an increase, which will occur mainly in developing countries, will inevitably require an additional agricultural production of 2.4×10^9 t/year. However, this additional production should not be based on an increase in the arable surface taken from temperate or rain forest, but on the improvement of crop productivity. This can be achieved in part by suitable control of losses due to biotic agents (pests, diseases, weeds), which on average are estimated to be 38–42% of the potential production [1, 35]. Currently, the control of plant pests, diseases and weeds is achieved mainly by spraying crops with a vast amount of synthetic chemical pesticides [1, 7]. However, an increase in the use of chemical pesticides to support the derived increase in agricultural activity needed to sustain the expected population growth can severely deteriorate the planet's health because of non-target effects.

Microbial pesticides in the new scenario of crop protection

Social and political concerns have influenced the practice of crop protection that has been progressively reoriented to a rational use of pesticides and to a reduction in the number of registered active ingredients to those certainly unavoidable, more selective, less toxic and with lower negative environmental impact [15, 38]. Under this objective, the European Union and several countries, including the United States, have undertaken regulatory changes in pesticide registration requirements. The European Union has established directive 91/414/CEE for harmonizing the register of pesticides. This projects a reduction for the year 2008 from nearly 900 active substances existing in 1991 to less than 400 (http://europa.eu.int/comm/food) and require the presentation of a very large dossier for each substance, providing scientific data on toxicological studies in mammals, ecotoxicology, traceability and environmental impact. In the United States, the Environmental Protection Agency (EPA) registers pesticides under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) of 1988, which includes regulatory requirements to prevent unreasonable, adverse effects on consumer health or the environment, and the Federal Food, Drug and Cosmetic Act (FFDCA), which establishes tolerances for residues in food. The Food Quality Protection Act, approved in 1996, amended the FIFRA and FFDCA with new safety standards. From 610 recognized active ingredients registered in 1995, only 382 will be retained in 2004 (http://www.epa.gov/oppfead1/trac/factshee.htm). However, implementation of the new regulations has been difficult, due to limitations in scientific knowledge and for other reasons, and they progress slowly, especially in the European Union [12]. In a 5-year study (1996–2000) performed in the European Union, monitoring pesticide residues in fresh products of plant origin, although pesticides were detected in 60–61% of samples, in 32–37% their levels were below or at the maximum residue limit (MRL) and in 3–4% of the cases were the levels above MRL [11].

Under this context, consumer health and environmental preservation prevail over any other productive or economic considerations. Consequently, the therapeutic approach used in the past in plant protection is shifting to a total system or sustainable pest management approach [22, 27]. However, this change in crop protection methods generates additional problems caused by the lack of chemical pesticides for the control of some plant diseases of economic importance (e.g. elimination of methyl bromide as a pesticide for soil disinfection).

New methods of crop protection are based on historical observations in agriculture and forestry of the benefits obtained from naturally occurring microbial communities, which exert a biological control of pests and diseases [7, 8, 9, 20, 25, 39]. Biological control is sustained by beneficial interactions resulting from competition, antagonism and hyperparasitism of certain microorganisms against plant pathogens, insects and weeds [3, 31, 32]. Currently, several microorganisms involved in such processes are the active ingredients of a new generation of microbial pesticides [6, 18, 33, 41], or are the basis for many natural products of microbial origin (e.g. elicitors) [17] or after chemical modification (e.g. phenylpyrrole fungicides) [26]. This paper discusses only biopesticides based on living microorganisms.

Screening and development of microbial pesticides

The development of a microbial pesticide requires several steps. The procedure consists first of isolation in pure culture or enrichment of the microorganism, then identification, characterization and performance of efficacy bioassays, which can be in vitro, ex vivo, or in vivo depending on the target pathogen or pest organism, and finally pilot trials under real conditions of application (field, greenhouse, post-harvest; Fig. 1).

The first stage consists of the isolation of bacteria, fungi, virus, or nematodes able to interfere with the biological cycle of plant pathogens or pests. A proper sampling can increase the probability of obtaining useful microorganisms. For this reason, samples are taken at places or in certain materials where there is evidence of the presence of beneficial microorganisms, such as dead arthropods, disease-suppressive soils, or healthy plants in epidemic areas.

However, isolation in pure culture needs suitable methods for either cultivation in synthetic media or enrichment in a given biological system (cellular

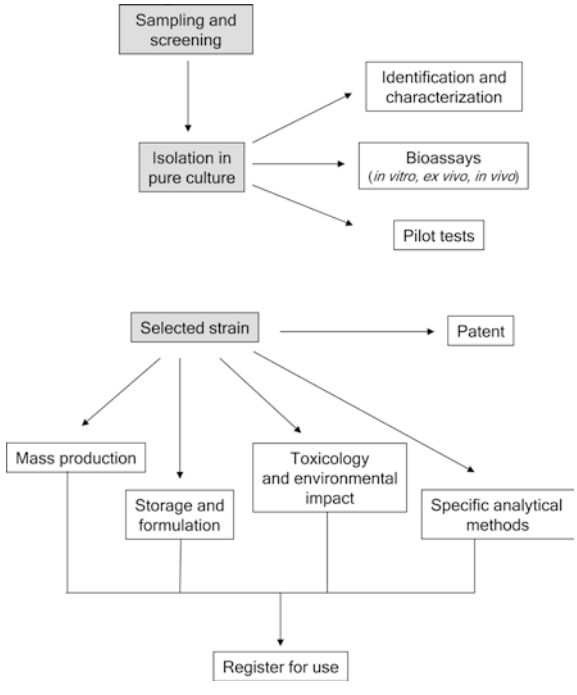

Fig. 1 A procedure for the screening and development of microbial pesticides

cultures, trap organisms). Among the microbial pesticides that are easily cultivable are the entomopathogenic fungus *Paecilomyces* and the biofungicidal bacterium *Pseudomonas chlororaphis*, which are produced by liquid fermentation in synthetic media. Other beneficial microorganisms are non-cultivable, such as the bacterium *Pasteuria penetrans*, which is strictly entomopathogenic [1], and the insecticidal viruses that are strict cellular parasites and cause granulosis or nuclear polyhedrosis in some arthropod larvae.

Screening for antagonistic activity against the target pest or pathogen is the critical step, because the type of microorganism selected depends on the method used. It usually addresses a given mechanism of action, such as antibiosis or toxinogenesis towards the target, parasitism, competition, or induction of plant defenses. During this stage, a collection of hundreds or thousands of isolates is performed. The isolates are submitted to efficacy bioassays in small-scale controlled-environment laboratory trials towards the target pathogen, pest, or weed, using in vitro, ex vivo, or in planta tests. However, this process to find suitable biological control agents (BCAs) is time-consuming and rather random. Certainly, there is a need for easy and inexpensive screening methods whose test conditions approach as much as possible the real system where the biocontrol has to be applied. A three-partner model system (putative BCA, target organism, host plant) is recommended. A knowledge of the mechanism of action of a BCA in combination with an analysis of the sequence of the corresponding genes can provide gene targets to develop high-throughput screening procedures. Modern techniques of assisted selection using phenotypic or genotypic markers can improve the productivity of the screening stage. Recent advances with DNA arrays can also contribute to develop potent screening methods. Unfortunately, an association between some markers and the capacity for biocontrol has been proved only in a few cases [32]. During the selection process, additional criteria apart from efficacy and consistency of results between bioassays are used to retain isolates, such as the presentation of low effective doses [34], the production of specific anti-metabolites or toxins against the target organism and a tolerance to some pesticides commonly used in agriculture. In general, fewer than 1% satisfy the expectations, due to the fact that the capacity of biological control is a strain-dependent subspecific characteristic. Therefore, only a few strains within a given species naturally develop such a capacity.

Pilot trials are performed under conditions as close as possible to the real conditions under which biological control will be developed in practice. It is convenient to perform this stage with several pathogens or pest systems to verify the action spectrum and under environmental conditions diverse enough to guarantee a wide range of applicability, which is the most attractive property for the pesticide industry. These limiting conditions permit working only with a few isolates. Unfortunately, most of the BCAs discovered fail at this stage, due to their too-narrow spectrum of action, some inconsistency in results from trial to trial, or a low efficacy under real conditions [19, 36, 42].

Apart from the research steps mentioned above, additional studies are needed. Biosafety of the microbial pesticide is extremely important to avoid non-target effects, both on plants and animals and on the environment. In the case of bacteria, the absence of a reaction of hypersensitivity in solanaceous plants, especially in tobacco, is an indication of a lack of plant pathogenicity. In the case of insects and other pest pathogens, it is recommended also to test for the absence of negative effects on auxiliary organisms. Toxicological studies in mammals are necessary to guarantee health of consumers and handlers of the microbial pesticide, especially if the production of secondary metabolites by the microorganism is suspected [24]. When the identification of the microorganism has been sufficiently documented and if there is no clinical or veterinary history, the toxicological studies do not need to be exhaustive. The most frequent toxicological tests are oral acute toxicity in rat, with the objective of determining the median lethal dose (LD_{50}) and the limit lethal dose. In the case of biocontrol agents based on bacteria, an acceptable LD_{50} should be higher than 10^{11} colony-forming units/kg of animal weight. Other tests are oriented to prove the safety of manipulation of the concentrated product; and it is advisable to verify the absence of dermal, eye and inhalation irritation in guinea pig or rabbit. In certain cases, depending on the results of acute toxicity, the contact-delayed hypersensitivity test of Magnusson and Klingman has to be checked. Additional tests are aimed at detecting toxic secondary metabolites that could be synthesized by the biocontrol agent, depending on the fermentation conditions. In certain cases, genotoxicity tests are also performed, usually by the Ames mutagenicity test.

For the commercial development of a microbial pesticide, the biocontrol agent should be produced at the industrial scale (fermentation), preserved, stored and formulated [37]. In general and depending of the agent's nature (bacteria, fungi or yeast, nematodes, or viruses), the methods used for industrial scale-up are solid- or liquid-phase fermentation, which can profit from the advanced technology in the pharmaceutical and food industries. Bacteria and yeast are usually produced by liquid fermentation using continuously stirred tank bioreactors, but many fungi are fermented in a solid state [13, 14, 23]. Independent of the method used for fermentation, the aim is to achieve the highest yield possible with the lowest cost of culture medium, which is achieved by using molasses, peptones, or industrial-grade protein hydrolysates. After liquid fermentation, the microbial cells are concentrated by filtration or centrifugation. In cases in which the microorganisms cannot be cultured directly on synthetic media (nematodes, strict parasitic bacteria, viruses), the scale-up process consists of using an alternative host or even cellular cultures or tissue culture. An interesting example

is the production of entomopathogenic nematodes that feed on bacteria. The nematode *Steirnema* transports bacteria of the genus *Xenorhabdus* and the nematode *Heterorhabditis* transports bacteria of the genus *Photorhabdus* [41]. Once the nematode has invaded the insect larva, the symbiotic bacteria produce its septicemia and feeds the nematode within the insect. The industrial production of this type of nematode is based on a first step of associated bacterial fermentation and a second step of nematode scale-up, feeding on the previously produced bacteria [10].

The need for storage and preservation of the microbial pesticide for commercialization requires an increase in the shelf-life, which is one of the main limiting factors [23, 37]. The treatment consists of stabilizing the viability of the microorganism. This can be achieved in liquid state and maintained by refrigeration, by freezing in the presence of cryoprotectant substances, or by keeping it as a dehydrated product. A general recipe does not exist and each method is established empirically, but there are some starting-point conditions. The methods based on dehydration are the best from a practical point of view, because they allow optimum conditions for storage, handling, distribution and formulation of the microorganism. Lyophilization is the method that best maintains viability, but its cost is very high. Encapsulation of microbial cells is an alternative method: it consists of mixing cells with a matrix-forming material, such as gelatinized polysaccharide or an emulsion in lipid material, which is finally diluted and spray-dried to obtain particles [21]. At the industrial level and in order to obtain a low-cost product, the methods preferred are spray- or fluidized bed- drying. Many products are obtained by spray-drying, but this method produces a high loss of viability in some microorganisms (e.g. Gram-negative bacteria), due to the thermal treatment.

Independent of the method used for the preservation of microbial cells, the final product should be formulated before use, by means of biocompatible additives that increase survival, improve application and stabilization of the final product, or attract or stimulate feeding in the target pest [5]. The additives consist of wetting and dispersal agents, nutrients and ultraviolet light- or osmotic-protection agents. Some of these products help the microorganism cells to survive under field conditions where there is damage by ultraviolet light, low water potential, nutrient limitation or other stress conditions [2, 28]. In many cases, this technology is similar to that used for the formulation of chemical pesticides and pharmaceutical products.

Knowledge of the mechanism of action of the BCA can help to develop and improve formulation and application systems [4, 16]. In many microbial pesticides, the application system is similar to chemical pesticides and is based on inundative treatment, i.e. either spraying or drenching at high rates. However, the ideal biological control system is based on innoculative (applied at very low concentration and with an autonomous population increase) or augmentative performance (low concentration but the environment is modified to favor its development) [7, 24]. Application methods vary across spraying or drenching plants, local application (sticks, tablets), seed-coating and insect dispersion (bees).

Specific analysis methods are necessary not only to control the quality of the microbial pesticide active ingredient (the BCA) but also to study its traceability, residue analysis and environmental impact [30, 40]. Classic microbiological methods are generally not suitable, because they do not distinguish the biocontrol agent strain from its wild type present in the natural microbiota. In certain cases, the selection of spontaneous mutants resistant to rifampicin (or other suitable antibiotics for which it is rare to find resistance in nature) may suffice. However, genotypic markers are preferable because they are more stable and their expression does not depend on the type of culture media used for analysis. Getting specific genotypic markers is a difficult, time-consuming process that can be accomplished by DNA fingerprinting of the biocontrol agent strain. These methods are based on the amplification of gene sequences by means of the polymerase chain reaction (PCR; e.g. RAPD, REP, ERIC, AFLP) or by means of DNA digestion with restriction enzymes (RFLP) [30]. By comparing the fingerprint patterns of the biocontrol strain with isolates of the same species, strain-specific fragments can be identified, sequenced and characterized (sequence-characterized amplified regions; SCARs). Knowledge of the nucleotide sequence of these SCAR fragments may form the basis for designing primers for PCR analysis. Currently, real-time PCR (quantitative PCR) is a strong tool for quantifying in a highly specific way the strain of the biocontrol agent.

Patenting, registration and commercialization of microbial pesticides

Before the commercial exploitation of a microbial pesticide, its legal protection as a biotechnological invention can be assured by means of a patent. A patent is a temporary privilege for industrial or commercial exploitation given by the administration to the owner for 20 years after the application date, according to a series of claims. Biopesticide patents are considered biotechnological inventions because they include microbial products and processes. However, a patent is not an authorization for commercial use (phytosanitary registration).

As with other inventions, the filing of patents for microbial pesticides is regulated by a legal series of national and international treaties. International treaties include the Treaty of the Union of Paris of 1883, which established a 12-month priority after the application has been subscribed by 140 countries, the European Patent Agreement of 1973, by which an application is valid in 18 European countries, and the international agreement or Patent Cooperation Treaty

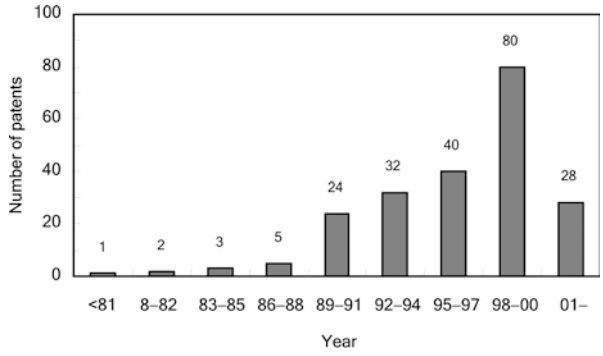

Fig. 2 Number of patents of microbial pesticides approved in the United States, Europe or world-wide (Patent Cooperation Treaty) from 1979 to 2001

(PCT) of 1970, by which the validity of a patent filed in one of the signatory countries is valid in the other 102 countries, and an 18-month priority is established, in addition to the 12 months in each country (a total of 30 months). The patents of pesticides based on microorganisms are regulated by the Budapest Treaty, signed by all countries pertaining to the World Intellectual Property Organization, which specifies the need for a pure culture of the strain of the microorganism to be deposited in an officially recognized microbial collection. In the European Union, the legal framework is regulated by Directive 98/44CEE on the patentability of biotechnological inventions. Because the patents reflect the applied interest of a given technology, I made a study of 215 patents involving

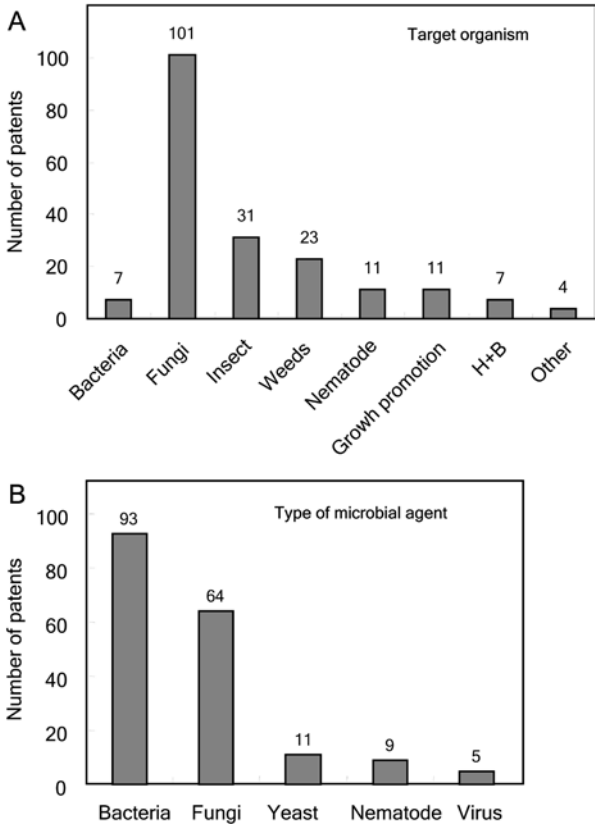

Fig. 3 Distribution of microbial pesticide patents according to the nature of the target organism (**A**) and the type of microbial agent (**B**)

Table 1 Biopesticides based on bacteria that are registered in some countries. *I* Insecticide, *F* fungicide, *B* bactericide, *N* nematicide, *H* herbicide

Species/strain	Type	Target
Bacillus popilliae	I	*Popilla japonica*
B. thuringiensis var. *aizawai*	I	*Galleria melonella*
B. thuringiensis var. *israeliensis*	I	Dipteran larvae
B. thuringiensis var. *kurstaki*	I	Lepidopteran larvae
B. thuringiensis var. *xentari*	I	Lepidopteran larvae
B. thuringiensis var. San Diego	I	Coleopteran larvae
B. thuringiensis var. *tenebrionis*	I	Coleopteran larvae
B. thuringiensis EG2348	I	*Lymantria dispar*
B. thuringiensis EG2371	I	Lepidopteran larvae
B. thuringiensis EG2424	I	Coleopteran larvae
Burkholderia cepacia	F	Soil-borne fungi, nematodes
Pseudomonas fluorescens	F	Soil-borne fungi
P. syringae ESC-10, ESC-11	F	Post-harvest fungi
P. chlororaphis	F	Soil-borne fungi
P. aureofaciens Tx-1	F	Antracnose, soil-borne
Bacillus subtilis	F	Soil-borne fungi
B. subtilis FZB24	F	Soil-borne
B. subtilis GB03	F	Soil-borne and wilt
B. subtilis GB07	F	Soil-borne fungi
Streptomyces griseoviridis K61	F	Various fungi
S lydicus	F	Soil-borne
Agrobacterium radiobacter K84, K1026	B	Crown gall *A. tumefaciens*
Ralstonia solanacearum non-pathogenic	B	Pathogenic *R. solanacearum*
Pseudomonas fluorescens A506	B	Frost damage, fire blight (*E. amylovora*)
Bacillus firmus	N	Nematodes
Pseudomonas syringae pv. *tagetis*	H	*Cirsium arvense*
Xanthomonas campestris pv. *poae*	H	*Poa annua*

Table 2 Biopesticides based on fungi that are registered in some countries. *I* Insecticide, *F* fungicide, *N* nematicide, *H* herbicide

Species/strain	Type	Target
Beauveria bassiana	I	White fly
Verticillium lecanii	I	White fly
Paecilomyces fumosoroseus	I	White fly
Metarrhizium anisopliae	I	Black beetle
Lagenidium giganteum	I	Mosquitoes
Trichoderma polysporum, *T. harzianum*	F	Soil-borne fungi
T. harzianum KRL-AG2	F	Soil-borne fungi
T. harzianum	F	Foliar fungi
T. harzianum, *T. viride*	F	Various
T. viride	F	Various
T. lignorum	F	Vascular wilt
Trichoderma spp	F	Soil-borne
Ampelomyces quisqualis M-10	F	Powdered mildew
Talaromyces flavus V117b	F	Soil-borne fungi
Gliocladium virens GL-21	F	Soil-borne fungi
G. catenulatum	F	Soil-borne fungi
Fusarium oxysporum non-pathogenic	F	Pathogenic *Fusarium*
Pythium oligandrum	F	*Phytium ultimum*
Phlebiopsis gigantea	F	*Heterobasidium*
Coniothyrium minitans	F	*Sclerotinia sclerotiorum*
Candida oleophila I-182	F	Post-harvest rot
Myrothecium verrucaria	N	Nematodes
Paecilomyces ilacinus	N	Nematodes
Phytophthora palmivora MWV	H	*Morrenia odorata*
Colletotrichum gloeosporioides	H	*Cuscuta* and various
C. gloeosporioides f.sp. *malvae*	H	*Malva pulsilla*
C. g. f.sp. *aeschynomene*	H	*Curty indigo*
C. coccodes	H	*Abutilon theophrasti*
C. truncatum	H	*Sesbania exalta*
Alternaria cassia	H	*Senna obtusifolia*

Table 3 Biopesticides based on viruses that are registered in some countries. *I* Insecticide, *F* fungicide

Species/strain	Type	Target
Granulosis virus	I	*Bytiscus betulae*, codling moth
Pine sawfly NPV	I	*Diprion similis*
Heliothis NPV	I	*Helicoverpa zeae*
Gypsy moth NPV	I	*Lymantria dispar*
Tussok moth NPV	I	*Orgyia pseudotsugata*
Mamestria brassicae NPV	I	*Heliothis*
Spodoptera exigua virus	I	*S. exigua*
Bacteriophage of *P. tolaasii*	F	Bacterial rot of mushroom

strains of microorganisms with potential application as microbial pesticides (fungicides, insecticides, herbicides, or plant growth stimulants). The study includes PCT (world patents), USPTO (United States patents) and EPO (European patents; http://ep.espacenet.com/). From 1986 to 2000, the number of microbial pesticide patents increased at an average of five patents/year and a maximum of 80 patents were approved over the period 1998–2000, with an actual tendency to decrease (Fig. 2). As shown in Fig. 3, most of the biopesticides patented have fungicide activity and are made of bacteria. Most patents for biopesticides were initially deposited in the United States (141), United Kingdom (18) and Australia (14).

In spite of the relatively high number of patents for biopesticides, only a few have materialized in a register for agricultural use. The registration depends on specific rules within each country. In the United States, the register is authorized by the Office of Pesticide Programs in the EPA. The EPA has authorized for commercialization 34 bacterial strains (14 biofungicides, 2 bactericides, 16 insecticides), 17 fungi (8 fungicides, 4 herbicides, 5 insecticides) and 8 viruses (mainly insecticides; see http://www.epa.gov/pesticides/biopesticides/). In the European Union, the register is kept by the Directorate of the Consumer Health Protection and is regulated by Directive 91/414/CEE, which was been amended specifically for biopesticides by Directive 2001/36/EC. To date (2003), only nine applications for microbial pesticides have been presented, including two bacteria (biofungicides), six fungi (two insecticides, four fungicides) and one virus (insecticide; see http://europa.eu.int/comm/food/). However, several are expected to be registered in the future.

Tables 1 and 2 show biopesticides based on bacteria (Table 1) and fungi (Table 2) which are registered and available on the market in some countries, with their

target organisms. The biopesticides based on viruses (Table 3) are mainly insecticides. They include different types of granulosis and nuclear polyhedrosis virus and a bactericide based on the bacterium *Pseudomonas tolaasii*.

Future prospects

In spite of the relatively abundant number of patents for microbial pesticides, the number of commercial applications has not been as dramatic as expected. Analysis of the patents of microbial pesticides indicates that the research has usually originated from universities or public administrations. However, the number of private companies interested in registering new biopesticides is increasing, especially in the United States; and they correspond to a typical profile of small- to medium-sized companies. In Europe, the limiting factor for registration, apart from the cost, is undoubtedly the slow process of decision-taking. As an example, the first application for patenting a biopesticide, *Paecilomyces fumosoroseus*, was submitted to the European Union in 1994 and approved only in 2001. In most cases, excessive specificity is a problem difficult to solve because it is intrinsic to the biological control system. In fact, success depends on three living systems: the pathogen or pest, the BCA and the host plant.

Biosafety and environmental concerns are also major limiting factors for microbial pesticide prospects. Non-target effects have been object of a review by Mathre et al. [32], who pointed out possible problems, such as displacement of beneficials including micorhizae or symbiotic rizobacteria, allergenicity, toxinogenicity (production of secondary metabolites toxic to plants, animals, or humans), or pathogenicity (to plants or animals) by the agent itself or due to contaminants. Problems may also derive from horizontal gene transfer of these characteristics to non-target microorganisms. All these non-target effects, however, should not be evaluated in an absolute manner, but in a case-by-case manner, relative to chemical control or the absence of any control of the target disease (for example toxins derived from the pathogen).

Consumer concerns about live microbes related to limited information about safe and harmful microbes cannot be overlooked. Certainly, emerging food-borne diseases and the threat of bioterrorism do not help to create a socially receptive environment for microbial pesticides. The future of microbial pesticides is not only in developing new active ingredients based on microorganisms beneficial to plants, but in producing self-protected plants (so-called plant-incorporated pesticides) by transforming agronomically high-value crop plants with genes from BCAs [29].

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