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Biological control agents: from field to market, problems, and challenges

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Global food security is vulnerable due to massive growth of the human population, changes in global climate, the emergence of novel/more virulent pathogens, and demands from increasingly discerning consumers for chemical-free, sustainably produced food products. Bacterium-based biological control agents (BCAs), if used as part of an integrated management system, may satisfy the above demands. We focus on the advantages, limitations, problems, and challenges involved in such strategies.

Background

An ever-increasing human population (9 billion by 2050) and global climate change will place huge demands on natural resources, including water and land availability for food-crop production. Crop diseases have been a serious problem over many years and remain a major threat to food production [1]. Heightened consumer awareness coupled with EU legislation limiting the availability of some agrichemicals and the lack of consumer acceptance of genetically modified crops, particularly in Europe, alongside the strict regulatory procedures regarding their registration, drive the search for new, sustainable agricultural practices. The immediate task facing stakeholders is how to institute a sustainable crop-production system that ameliorates the above problems threatening global food security. An example of such an integrated sustainable crop-production system is one that incorporates beneficial microorganisms. These beneficial organisms include bacteria that can have an effect on plant growth; for example, directly through biofertilisation and phytostimulation activities or indirectly through pathogen suppression. These organisms therefore act as BCAs.

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BCAs

A biological approach that includes the use of bacterium-based agents can provide an opportunity to minimise the use of agrichemicals [2]. The company BCC Research reported a figure of $2.1 billion in 2011 for the global biocontrol market and this is expected to rise to $3–4 billion by 2017 (Table 1), in part influenced by the increasing demand for organic products. A list of commercial bacterial-based BCAs listed in EU Annex I (Table 1) is shown in Table 2.

BCAs from field to market: problems and challenges

Bringing BCAs to market begins in the field, with the identification of the target crop and the pathogen and gaining an understanding of the epidemiology of the disease and current disease-control strategies. Multiple populations of bacteria can be isolated from selected sites, purified, and identified to genus, species, subspecies, or strain level [3], although current taxonomic classification can change following scientific progress. The best available technologies for these processes include 16S rRNA gene sequencing and multilocus sequence analysis (MLSA), often referred to as multilocus sequence typing (MLST). The efficacy of bacterial isolates is determined by a rigorous *in vitro* screening regimen (e.g., antagonism tests) followed by greenhouse and controlled field trials. To ensure consistency of performance in the field, BCAs must be tested at different geographical locations, under different climatic conditions, and on different crops against a range of pathogens to evaluate their potential for broad-spectrum activity. The most widely recognised indirect multiple mechanisms for biological control include the production of antibiotics (e.g., phenazines and 2,4-diacetylphloroglucinol by *Pseudomonas* sp., lipopeptides such as iturin and fengycin by *Bacillus* sp.), competition for nutrients (e.g., iron) and space, lytic enzyme production (chitinase and glucanase), and induced systemic resistance (ISR) in the host plant [2–5].

The next step is concerned with the preservation of the BCAs in reputable culture collections; that is, those that are members of the World Federation of Culture Collection (Table 1) and work under the quality and safety rules recommended by the Organisation for Economic

Table 1. List of websites

http://www.bccresearch.com
http://ec.europa.eu/sanco_pesticides/public
http://www.wfcc.info/
http://www.oecd.org/health/biotech/oecdbestpracticeguidelinesforbiologicalresourcecentres.htm
http://www.cbd.int/convention/text/
https://www.cbd.int/abs/text/
http://bccm.belspo.be/about-us/bccm-lmg
http://bccm.belspo.be/db/lmg_search_form.php
http://www.dsmz.de
http://bccm.belspo.be/db/lmg_search_form.php
http://www.oecd.org
http://www.epa.gov
http://www.senasag.gob.bo
http://www.agrocalidad.gob.ec
http://www.senasa.gob.pe

Co-operation and Development (OECD) guidelines for biological resource centres (BRCs) (Table 1). Once the isolate is deposited in the culture collection, the quality and authenticity of the material is checked according to VIPS criteria (i.e., viability, purity, identity, and stability) and the sequence information (e.g., 16S rRNA or housekeeping gene sequences) is provided by the depositor. The bacterial collection (LMG) of the Belgian Coordinated Collections of Microorganisms (BCCM) is such a collection, following the internationally accepted rules of intellectual property (IP) rights and access and benefit sharing (ABS) according to the Convention of Biological Diversity (CBD) of Rio (1992) (Table 1) as well as the Nagoya protocol (2013) (Table 1). A so-called Material Transfer Agreement (MTA) typically accompanies the retrieval of biological material (subcultures of deposited isolates) from culture collections in countries that have implemented these international conventions. An example of such an MTA document can be found at the BCCM/LMG website (Table 1). The cultures are preserved for long periods under stable conditions and, depending on the formulation of the material, will be distributed as lyophilised (ampoules) or living (preserved in liquid nitrogen) material. With respect to the deposition of BCAs that are intended for commercialisation, the BCCM/LMG and nearly all culture collections offer 'safe-deposit facilities' to protect the material. This material will not be distributed to third parties without prior authorisation.

Once preliminary BCA efficacy is determined, pilot-scale production on an industrial small scale using liquid (bacteria) fermentation techniques can be advanced and further field trials commenced. Shelf life, compatibility with application practices, cost, and ease-of-application issues need consideration during the development of a commercial formulation [6,7]. Using BCAs in agriculture requires strict pathogenicity testing before product development. The biosafety of bacteria can be evaluated using various laboratory-based methods: (i) growth at 37 °C; (ii) categorisation of risk groups above one (Table 1); and (iii) fast, inexpensive bacterial pathogenicity tests such as the *Caenorhabditis elegans* assay. The potential of the BCA to be pathogenic in humans can be determined by assessing the mortality of *C. elegans* in the latter assay [8]. These tests should be used in combination with human toxicological tests and plant pathogenicity (hypersensitivity) tests, a necessity for registration [9].

Registration: a case for the EU and North and South America

The OECD (Table 1) has issued regulations relating to the import and use of BCAs for all countries. A harmonisation process, in terms of the regulation of biocontrol agents, is currently under review for South East Asian countries [10]. The USA and Europe are considered the most extensive regional markets for biocontrol products, followed by South America, and the procedures governing the registration of bacterium-based BCAs in these regions are outlined below.

The differences in regulations between these continents explain to some extent the discrepancies in the number of biocontrol products that have come to market. In Europe, commercialisation is regulated by revised Regulation (EC) No. 1107/2009 adopted on 21 October 2009. The directive employs a two-tier registration system that involves an assessment of the BCA (information provided in Annexes IIB and IIIB) followed by the addition of the BCA to the listing in Annex I (Table 1). Annex IIB lists the requirements for the active substance (e.g., identification, mode of activity, toxicity testing), whereas IIIB highlights the microbial product requirements (e.g., formulation). An additional EU-specific requirement is validation of the formulation efficacy. In every EU member state where a product is awaiting authorisation, data certifying product efficacy and safety must be provided over a 24-month period. An active substance is appended to Annex I based on peer review by all EU member states, the European Food Safety Authority (EFSA), and the European Commission. The BCAs listed in Annex I that can be used in the EU include approximately 14 strains. Institutions officially accredited by the legislative authorities of each country are in charge of overseeing field trials in accordance with Good Experimental Practice (GEP). This approach distinguishes European regulations from those of other nations.

Putative BCAs often fail to demonstrate consistency under different environmental conditions. Currently, some bacterial strains that have passed the toxicity tests are awaiting registration because the field results were variable. The registration file for *Pseudomonas chlororaphis* (Cedomon), targeting seed-borne diseases of barley and wheat, was submitted in December 1994 in accordance with EU Directive 91/414 and received authorisation 10 years later (October 2004) with an investment of more than €2.5 million. The expensive registration procedures for putative BCAs are discouraging for small and medium-sized companies. In general, only larger companies are in a position to afford the research required to prepare a registration file. By contrast, a single regulatory procedure for the marketing of BCAs has been in use in the USA whereby the registration, effected by the Environmental Protection Agency (EPA) (Table 1), relies on the *a priori* assumption that biocontrol products are safer than chemical products. This considerably simplifies the registration process, which is completed in a minimum of 12–24 months (compared with 84 months in Europe). Certain health and