



Best practices in the use and exchange of microorganism biological control genetic resources

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Abstract The Nagoya Protocol actions the third objective of the Convention on Biological Diversity and provides a framework to effectively implement the fair and equitable sharing of benefits arising out of the use of genetic resources. This includes microorganisms used as biological control agents. Thus biological control practitioners must comply with access and benefit-sharing regulations that are implemented

by countries providing microbial biological control agents. A review of best practices and guidance for the use and exchange of microorganisms used for biological control has been prepared by the IOBC Global Commission on Biological Control and Access and Benefit-Sharing to demonstrate commitment to comply with access and benefit-sharing requirements, and to reassure the international community that biological control is a very successful and environmentally safe pest management strategy that uses biological resources responsibly and sustainably. We propose that best practices include the following elements: collaboration to facilitate information exchange about

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the availability of microbial biological control agents and where they may be sourced; freely sharing available knowledge in databases about successes and failures; collaborative research with provider countries to develop capacity; and production technology transfer to provide economic opportunities. We recommend the use of model concept agreements for accessing microorganisms for scientific research and non-commercial release into nature where access and benefit-sharing regulations exist and where regulations are not restrictive or do not exist. We also recommend a model agreement for deposition of microbial biological control agents into culture collections.

Keywords Biological control · Access and benefit-sharing · Model concept agreements · Information exchange · Microbial biological control agent

Introduction

The Nagoya Protocol (NP), the instrument that further develops the third objective of the Convention on Biological Diversity (CBD), provides guidelines to enable the fair and equitable sharing of the benefits arising out of the utilization of genetic resources including microorganisms (CBD 2011). The NP entered into force on 12 October 2014, and now has 138 Parties (plus the European Union), one ratified non-Party and 60 non-Parties (CBD 2023a). The NP provides guidance for its contracting Parties to implement measures for access to genetic resources, benefit-sharing and the requirements for compliance. Provided they decide to regulate access to their genetic resources (some Parties may choose not to regulate access), the Parties are required to introduce domestic measures to give legal certainty, clarity and transparency to implement the protocol nationally. As of 7 March 2023, 98 of the Parties have reported on implementation of national laws whilst 133 countries have established access and benefit-sharing (ABS) National Focal Points (CBD 2023b). The objective of the NP is to provide clear rules for prior informed consent (PIC), laying down mutually agreed terms (MAT) and promoting and encouraging research contributing to biodiversity conservation and its sustainable use. Although often overlooked, it is essential for Parties to consider the importance of genetic resources for food and agriculture for food security.

However, no guiding principles were provided by the NP to assist in the development of legislation or procedures to access and utilise genetic resources. As Silvestri et al. (2020) determined, implementation of ABS measures has led to repercussions on access to, exchange and utilization of biological control agents. Thus, the biological control community needs to develop appropriate best practices that will guide this community to meet the challenges of ABS and to demonstrate leadership to those developing measures for accessing and utilizing their biodiversity.

The International Organization for Biological Control (IOBC) Global Commission on biological control and ABS endorses and recommends using best practices for biological control genetic resources. Mason et al. (2018) provided guidance on best practices for invertebrate biological control genetic resources. A similar approach applies to microbial biological control agents, but with some differences (e.g., importance of *ex situ* culture collections to identify potential agents, high probability to develop commercial products, type of benefit-sharing). Here we review best practices in the use and exchange of microorganisms and provide guidance to comply with ABS measures for those microorganisms used in biological control.

The main challenges

Not all access to genetic resources triggers domestic measures implementing the Nagoya Protocol. In most cases the genetic resource has to be ‘utilised’, meaning to conduct research and development on the genetic and/or biochemical composition of genetic resources, including through the application of biotechnology as defined in Article 2 of the CBD. Taxonomy, including identification, is normally out of scope and some regulations allow deposit of specimens into culture collections and culture maintenance. Many countries chose not to control access to their genetic resources, for example European Union member states where EU ABS Regulation (EU 2022) leaves access control to the member states. The majority have not implemented access controls but have made compliance commitments when genetic resources of other countries are used. Compliance obligations include taking measures to ensure that genetic resources from another contracting Party have been

accessed in compliance with their measures, cooperation in cases of alleged violation of another contracting party's requirements (Article 15) and measures to monitor the use of genetic resources after they leave a country including by designating effective checkpoints at any stage of the value-chain: research, development, innovation, pre-commercialization or commercialization (Article 17). Sharing is subject to mutually agreed terms and benefits may be monetary or non-monetary such as royalties and the sharing of research results (Article 5.4). There are examples of monetary benefit-sharing but in the majority of cases benefits shared are non-monetary, for example joint research and publications, capacity building, sharing knowledge, and technologies (Smith et al. 2021; Mason et al. 2023). The regulations accompanying NP adoption have unfortunately resulted in delayed access to genetic resources through time taken to negotiate access and diversion of funding for innovative research to access costs, with little indication of monetary benefits getting back to the conservation and sustainable use of biodiversity.

Thirty years after enactment of the CBD and almost 12 years since the Nagoya Protocol the debate on how to implement access and benefit-sharing continues. Negotiations on a new set of global goals and targets for biodiversity were reported in the summary report of the 4th Meeting of the Open-ended Working Group on the post-2020 global biodiversity framework, 21–26 June 2022 (CBD 2022a). Parties and stakeholders have submitted position statements and comments to support the review of the proposed goals and targets of the post-2020 global biodiversity framework and digital sequence information (DSI) (CBD 2022b). The Kunming-Montreal Global Biodiversity Framework was adopted in December 2022 which includes a new target to reduce the impact of invasive alien species (Target 6) as well as to provide for the fair and equitable benefit-sharing derived from the use of biodiversity (Target 9) (CBD 2022c). There is continuing debate on whether DSI should be included in benefit-sharing requirements of the CBD and/or the Nagoya Protocol (CBD 2022d; Silvestri and Mason 2023). Given that sequencing of DNA and RNA and indeed the proteins and metabolites of microorganisms is now how we identify and characterise them, the outcomes of these discussions are important. They could have huge impact on how the biological control community carries out their science

and, in particular, how they identify and assess biological control agents. It is important that the biological control community have a voice in these discussions to ensure that access to biological control agents are not impeded and that they are recognised as being for the public good and help to deliver the Sustainable Development Goals (SDGs) (UN 2022). Consensus has not been reached and there seems to be irreconcilable extremes of the argument (Silvestri and Mason 2023). Some believe DSI is strictly information and should be excluded from the CBD and the Nagoya Protocol. Others believe that using DSI enables utilization and avoiding access to the genetic resource, therefore it should be included. Currently, a multilateral benefit-sharing mechanism drawing 1% of the retail price of all commercial income resulting from the use of genetic resources and DSI is being considered (CBD 2022e). Because food security is an important objective of the United Nations the question that arises is: should biological control be excluded from monetary benefits and the beneficial output of reduced losses to pest and diseases and the shared knowledge and use of biological control agents considered to be sufficient?

Further challenges for access and use of microbial biological control agents are presented when there are multiple source countries with different approaches to ABS. For example, biological control of the 24 invasive weeds in the UK involved accessing microorganisms from more than 20 countries of origin with a range of ABS requirements. In this case, Australia, Canada, Chile, Iran, New Zealand, Paraguay, Russia and the USA are non-parties to the NP; Argentina and China are parties; Brazil, India, Japan, Malaysia, Mexico, South Africa, Ukraine, and Uruguay are parties with law; and Poland is a non-party with law. This demonstrates the complexity that ABS measures introduce to biological control projects.

To summarize, the main challenges to access and use microorganisms for biological control include a clear understanding of: (1) whether or not a source country has ABS measures in place, (2) what is considered a genetic resource (the physical microorganism, information associated with the microorganism), (3) what conditions for use are in place, (4) how to ensure compliance with measures required by the source country when conducting research and development, and (5) the types of benefits to be shared (monetary, non-monetary). Navigating multiple

requirements is time consuming and slows progress of biological control projects (Mason et al. 2023).

Microorganism use in biological control

Microorganisms contribute as agents in natural, conservation, classical (importation), augmentative and commercial biological control. Their role in natural and conservation biological control is not as well understood as it is in classical and augmentative biological control. Furthermore, microorganisms play a significant role in commercial biological control through development of biopesticides.

Natural biological control

Natural biological control is an ecosystem service (Buitenhuis et al. 2023) and microorganisms play a key role by providing an existing level of mortality. For example, seven species of pathogenic fungi were found to be associated with soybean aphid, *Aphis glycines* L. (Hemiptera: Aphididae), of which *Pandora neoaphidis* (Remaudière and Hennebert) Humber (Entomophthoraceae) was the primary species infecting the host in the epizootic that caused the crash of a field population in northeastern USA (Neilson and Hajek 2005).

Conservation biological control

Manipulation of the soil habitat can encourage microbial biological control agents in the soil that reduce attack by plant pathogens, or perhaps conservation of adjacent vegetation can encourage the right individual microorganisms or microbiomes (Collinge et al. 2022). These authors cite two examples: lowering the local soil pH by applying elemental sulphur discourages *Streptomyces scabies* Lambert and Loria (Streptomycetaceae), which causes scab on potato (Vlitos and Hooker 1951), and irrigating potato plants during tuber formation, stimulates colonization of new lenticels by antagonistic bacteria (Cook and Papendick 1972). Steinkraus (2007) developed a surveillance strategy to conserve the fungus *Neozygites fresenii* (Nowakowski) Renaudière and S. Keller (Neozygita-ceae) so growers could take advantage of the annual widespread epizootics that occur every year that kill up to 100% of cotton aphids, *Aphis gossypii* Glover

(Hemiptera: Aphididae), in the southern USA. This conservation biological control program delayed spraying for cotton aphids and conserved not only the fungus but also natural enemies of other pests such as the cotton bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae).

Classical biological control

Microorganisms are important agents used in classical (importation) biological control. Some have successfully controlled insect and mite pests where approximately 49 species have been intentionally released (Hajek et al. 2021) and invasive alien plant species where 36 fungal pathogens have been authorised for introduction across 18 countries (Morin 2020). The goal of classical (importation) biological control, a non-commercial activity, is to establish populations of a natural enemy that are self-propagating to suppress pest populations in the environment to which they are introduced (Stenberg et al. 2021). Fungi, viruses and nematodes have been the most commonly introduced microorganisms, although microsporidia, bacteria and oomycetes have also been introduced as classical biological control agents (Hajek et al. 2007). Target organisms include insects, mites, nematodes, weeds, plant disease causing fungi or bacteria, and vertebrates (Sundh and Goettel 2013).

The rhinoceros beetle, *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae), is a highly destructive insect pest of coconut and oil palms (Bedford 2013). Native to South and Southeast Asia, *O. rhinoceros* was accidentally introduced in 1909 into the Pacific, spreading rapidly throughout Pacific Island nations and territories to become a major economic problem (Paudel et al. 2021). The highly virulent *Oryctes rhinoceros* nudivirus (*OrNV*) (Nudiviridae: Alphanudivirus) was discovered in 1963 during surveys in Malaysia, the area of origin of *O. rhinoceros*, and initially introduced as a biological control agent into Western Samoa (Huger 2005). *OrNV* has subsequently been released in nine additional countries where rhinoceros beetle has invaded, significantly reducing damage by this pest in some (Hajek et al. 2016, 2021).

Since the 1970s, plant pathogens have played an important role in weed biological control and 36 fungal pathogen species have been released globally

for the classical biological control of weeds (Morin 2020). For example, blackberry, *Rubus constrictus* Lefevre and P. J. Mull. (Rosaceae) is a serious problem weed in agricultural and forest areas of Argentina, Australia, New Zealand, USA, Chile, and some islands of the Azores archipelago (Vargas-Gaete et al. 2019). The rust fungus *Phragmidium violaceum* (Schultz) G. Winter (Phragmidiaceae) from Germany was released in 1973 in Chile (Winston et al. 2014), where it has established and causes infections that hasten defoliation by several months, with severe attacks reducing seed production by 45% (Morin and Evans 2012). Plants infected over successive years become reduced in height and become considerably less competitive allowing colonisation by other species (Morin and Evans 2012).

Augmentative biological control

Bacteria, fungi and nematodes have been used for augmentative biological control. Augmentative biological control aims to periodically introduce into a specific environment mass-produced natural enemies that are not expected to establish to suppress pest—and pathogen—populations (Stenberg et al. 2021). Entomopathogens such as *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Cordycipitaceae) can be seeded into crops using pollinators such as honeybees, *Apis mellifera* L. (Hymenoptera: Apidae), or bumble bees *Bombus* spp. (Hymenoptera: Bombidae) (Lacey et al. 2015). The predatory mites, *Typhlodromips* (= *Amblyseius*) *swirskii* (Athias-Henriot) and *Neoseiulus cucumeris* (Oudemans) (Mesostigmata: Phytoseiidae) have also been shown to disseminate *B. bassiana* conidia onto leaves infested with *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Lin et al. 2017). Fungal inoculants can be incorporated into seed coatings to introduce fungi, such as *Trichoderma* spp., into the rhizosphere where they establish and prevent losses to root diseases (Lacey et al. 2015). Furthermore, entomopathogens such as the rhizo-competent *M. anisopliae* may establish on the developing roots of seedlings, reducing insect damage, and the endophytic *B. bassiana* may colonise the plant providing resistance to plant pathogens (Lacey et al. 2015).

Microorganism isolates that are highly effective against plant pathogens can be mass-produced on

artificial media and applied during a growing season as augmentative biological control agents (Köhl et al. 2019). These pathogens may act in several ways: by inducing or priming resistance in plant tissues to infections by a pathogen without direct antagonistic interaction with the pathogen, through competition for nutrients and space, by interacting with the pathogen directly via hyperparasitism or by antibiosis (Köhl et al. 2019).

Commercial biological control

Biopesticides are formulations of microorganisms used in augmentative biological control that are registered, similar to chemical pesticides, and sold commercially. Inundative application of entomopathogens is most commonly used for control of pest arthropods with more than 50 viruses, bacteria, fungi, and nematodes produced as commercial biopesticides (Lacey et al. 2015). Bioherbicide use on invasive alien weeds has relied completely on plant pathogens already existing in the region of introduction of the target weeds and thus constitute new associations. Seventeen bioherbicides have been produced for the control of invasive alien weeds, of which seven bioherbicides are still registered, and only two are commercially available (Bailey 2014; Morin 2020). Stumbling blocks to commercialisation is the size of the prospective market. There are approximately 31 biopesticide products derived from at least 37 microorganism species in use for augmentative biological control of plant pathogens (Collinge et al. 2022). Overall, there are more than 200 registered products derived from 94 microorganism species (van Lenteren et al. 2018).

The best known of the biopesticides are products that have been derived from *Bacillus thuringiensis* (*Bt*) Berliner sub-species (Bacillaceae). Approximately 90% of the microbial biopesticides on the market are represented by *Bt* products (Kumar et al. 2021). The success of *Bt* products can be attributed to the facts that they are fast acting, easily produced at low cost, readily formulated, the shelf life is long and can be applied with conventional equipment (Lacey et al. 2015).

Exploration for microbial biological control agents

Traditionally, discovery of new microorganisms for use in classical/introduction biological control was

made through exploration in the area of origin of the invasive alien plant, pathogen or pest, to develop a list of potentially specific and effective species (Sheppard et al. 2020). As described by Huger (2005) for discovery of *Oryctes* virus, surveys are carried out in the native range of the target pest species with the aim to find natural populations that show signs of disease. Once discovered, *in situ* observations are made to understand the disease process and samples are analysed to identify the microorganism involved. In order to conduct such surveys, countries where exploration is to be conducted must be contacted regarding the requirements to obtain collecting permits and this may be challenging because some countries include natural enemies as part of their genetic resources (Hajek and Eilenberg 2018).

Novel microbial biological control agents may also be isolated from the habitat invaded by the non-native species (i.e., where the agent would be used) and then screened directly for activity on the target species (Collinge et al. 2022). They may also be discovered during surveys for dead or dying insects or plants in heavily infested habitats. *Enterobacter cloacae* (Jordan) Hormaeche and Edwards (= *Coccobacillus acridiorum* d'Herrelle) (Enterobacteriaceae) was discovered from diseased *Schistocerca pallens* (Thunberg) (Orthoptera: Acrididae) in Yucatan Mexico (Sweetman 1936). *Bacillus thuringiensis* serotype *israelensis* Barjac (Bacillaceae) was discovered during a survey for biological control agents of mosquitoes in the Negev desert in Israel (Margalit and Dean 1985). The rust *Maravalia cryptostegiae* (Cummins) Ono (Raveneliaceae) was discovered during surveys in Madagascar for biological control agents of rubber vine, *Cryptostegia grandiflora* Roxb. ex R. Br. (Asclepiadaceae) (Evans 1993; Palmer and Vogler 2012). For plant pathogens, discovery can involve sampling healthy plants in areas with high disease pressure and identifying beneficial microorganisms in their associated microbiome (Collinge et al. 2019). Pathogen agents targeted for the classical biological control of weeds undergo comprehensive host-specificity testing, usually in the region of introduction, to assess pathogenicity and any risks they pose. Promising pathogens are usually deposited in collections where they can be accessed for further research.

Microbial culture collections are a major resource for discovering biological control agents. They were established to preserve and study *ex situ* the

biodiversity in ecosystems, and to distribute promising microbial strains for production of goods and services (Díaz-Rodríguez et al. 2021), including new biological control agents. They serve as repositories for strains that are used for patent requirements, provide safe and confidential storage for key microorganisms for research and industry, and are sources of organisms cited in scientific papers for verification of research results (Smith 2003). There are numerous microbial culture collections around the globe with holdings of 1000's of species and strains (see below).

Historically as best practice, microorganisms, like invertebrates, have been freely shared among parties. Even though the target country might be in competition with the provider country the latter may have already benefited, or anticipates to benefit in turn, when access to a biological control agent is needed (Cock et al. 2009, 2010). An important difference is that microorganisms are usually deposited as live cultures in collections and upon request samples are freely provided for research, unless there are biosecurity concerns (WFCC 1999; Stackebrandt et al. 2014). More recently the use of Material Transfer Agreements (MTAs) has become a mean to set out terms and conditions whereby the recipient of the culture collection sample has a responsibility to ensure the microbial resources are properly used. Free sharing of live specimens and effective networking of biological control practitioners globally are the principles that deserve special consideration with regards to ABS (Mason et al. 2018). A standard MTA could be used for microorganisms with potential for use as biological control agents deposited into culture collections to provide the necessary documentation to enable free use and exchange. The 'deposition' MTA would acknowledge the source of the microorganism and indicate any associated conditions (i.e., development of a commercial product requires negotiation with the source or that release into the environment is freely allowed).

In response to concerns about the possible impacts of the NP on biological control practice, the IOBC formed a Global Commission on Biological Control and ABS in October 2008. The first task of the Commission was to write a contribution requested by the Food and Agriculture Organization Commission on Genetic Resources for Food and Agriculture (FAO-CGRFA) summarising the practices used for exchange of biological control genetic resources. The

result was a background study paper focusing on how biological control agents are accessed and utilised (Cock et al. 2009). Among the recommendations provided was to develop best practices for ABS in relation to biological control. A first contribution on this topic was Mason et al. (2018). Since then, there have been requests to develop a similar document for microbial biological control agents.

Article 20 in the Nagoya Protocol encourages the development, update and use of voluntary codes of conduct, guidelines and best practices and/or standards. The Access and Benefit-Sharing Clearing House (ABSCH) holds 408 records of best practices with 36 concerning microorganisms although these are general and do not address biological control agents specifically. The IOBC Global Commission on biological control and ABS recognizes the importance of the MTA approach for sharing microorganisms and recommends the following best practices for microorganisms used in biological control.

Best practices for exchange of microorganism biological control genetic resources

Best practices ensure that access to microorganisms used as biological control agents comply with the ABS requirements implemented by the country providing the genetic resource. These practices consider the best approaches and tools that provide for the fair and equitable sharing of the benefits yet allow for efficient access to microorganisms used for biological control.

Collaboration to facilitate information exchange on microbial biological control agents

Networks

Informal cooperative networks involve scientists associated with government agencies, international agricultural research centres, universities, inter-governmental organisations, industries, and others from around the world (Cock et al. 2009). These networks have enabled exchange of invertebrate biological control agents (Mason et al. 2018), particularly when redistributing known agents from where they have been introduced to another country that has been newly invaded by the target (Cock et al. 2009). These

networks are best able to assist practitioners to freely exchange microbial biological control agents. However, since microorganisms are normally housed in living culture collections, institutional networks continue to play a major role in the exchange of microorganisms with potential as biological control agents.

The IOBC is an international network of biological practitioners that provides the opportunity to participate in biological control activities and to contribute to the promotion of biological control worldwide (IOBC 2022). The IOBC is well-positioned to play a role in facilitating best practices, including the use and exchange of biological control agents.

Institutional/organisation practices

Of the best practices and codes of conduct concerning microorganisms on the ABSCH website (<https://absch.cbd.int/en/about/infoTypes>) several were produced by or for culture collection organisations, with several of their members holding and distributing biological control organisms. Some example are is the Microbial Resource Research Infrastructure (MIRRI) Best Practice Manual on Access and Benefit-Sharing produced as guidance for the microbial domain Biological Resource Centers (mBRCs). It was primarily designed for the management of collections of living microorganisms (MIRRI 2016). MOSAICC (Micro-Organisms Sustainable use and Access regulation International Code of Conduct) was produced for the World Federation for Culture Collections at the initiative of the Belgian Coordinated Collection of Microorganisms (BCCM) through the EU project ‘Elaboration and diffusion of a ‘code of conduct’ for the access to and sustainable use of microbial resources within the framework of the convention on biological diversity’ number BIO4972206 (<https://cordis.europa.eu/project/id/BIO4972206/de>). The code of conduct is available at: (<https://bccm.belspo.be/projects/mosaicc/>). TRUST (TRAnsparent User-friendly System of Transfer), is a modular system having the Global Catalogue of Microorganisms (<https://gcm.wdcm.org/>) as its backbone. It uses the expertise gained by MOSAICC, Micro-Organisms Sustainable use and Access management Integrated Conveyance System (MOSAICS) and other initiatives to incorporate the legal obligations and the ethical standards of the CBD and NP into the activities of microbiologists.

The document is available at: <https://bccm.belspo.be/documents/files/projects/trust/trust-march-2016.pdf>.

Another best practice is provided by the European Culture Collections' Organisation (ECCO). ECCO produced model documents that comply with the Nagoya Protocol for Material Deposit and Transfer Agreements (Verkley et al. 2020).

Each organization has provided these guidance documents for their members to adopt best practices in ABS. Some practices have been officially recognised as best practice by the European Commission to comply with the EU ABS Regulation or by other National Authorities. One such recognised best practice is that of the Consortium of European Taxonomic Facilities (CETAF). These were developed to fully support the operations of taxonomic collections and non-commercial biological research institutions to comply with the Nagoya Protocol. Other research communities (e.g., Global Genome Biodiversity Network and the IOBC) have published best practices to encourage exchange and use of genetic resources legitimately. CAB International (CABI) (<https://www.cabi.org/about-cabi/>) houses one of the UK National Culture Collections and is both a user and a provider of genetic resources. CABI has published its ABS policy (<https://www.cabi.org/wp-content/uploads/PDFs/AboutCABI/Cabi-Abs-Policy-Draft-For-Website-May2018.pdf>) where it states that it will put in place best practices to comply with national legislation and will perform due diligence regarding ABS in all its activities involving those resources. CABI's goal is to engender trust that will facilitate science and ensure that benefits are shared. CABI has had to generate a separate set of best practices built on the same principles for each of its Research Centres which are based in 11 countries each with different requirements (Smith et al. 2018). CABI has aligned its best practices as a user of genetic resources with host country requirements. It is also negotiating access agreements with all provider countries to ensure compliance with the NP not only locally but globally. CABI Best Practice for the Centre in Switzerland is recognised by the Swiss national authority Bundesamt für Umwelt (BAFU) and CABI country-specific best practices have been drafted for Brazil, China (where an interim agreement is in place until national regulation is enacted), Ghana (where an MoU is in place with the competent national

authority), India, Kenya, Malaysia, Pakistan, Trinidad and Tobago, the UK and Zambia.

Sharing knowledge on availability of microbial biological control agents

There are not many publicly accessible catalogues or databases specifically providing information about agents, targets and outcomes for biological control agents. However, several studies in the scientific literature provide this type of information (e.g., van Lenteren et al. 2018; Hajek et al. 2021; Buitenhuis et al. 2023). Culture collections, particularly those from the agricultural sector, have been supplying microbial biological control agents for many years and present their holdings online as individual resources or have contributed their catalogues to the Global Catalogue of Microorganisms (Wu et al. 2013). A search of the latter for the application 'biocontrol' gave 188 species from a total of 56,258 species held by 146 collections in 51 countries. These species are held by 12 collections (Supplementary table S1). However, these figures miss many of the agriculture sector collections. For example CABI, Agriculture and Agri-Food Canada (AAFC), Agricultural Research Council, Plant Health Protection (ARC-PHP) in South Africa and the United States Department of Agriculture, Agriculture Research Service Culture Collection (NRRL) are missing from the list and all hold biological control agents (Supplementary table S2).

Contacting individual curators for access to microorganisms of interest is the current practice and should be encouraged as a best practice. In future, the IOBC could play a role as a central clearing house providing information on microbial biological control agents.

Gaining access to microbial biological control agents through collaboration

Discovery of new microorganisms requires that field collections be made, the species studied, cultured (usually) and transported to the receiving country/countries following protocols set out by the World Fungal Collections Consortium (WFCC) (e.g., Smith and Ryan 2019). National regulations outline what permits are required for field surveys and export of microorganisms and partnering with local collaborators has been key to achieving these activities. Where

ABS requirements are in place PIC and MAT may also need to be negotiated to gain access. As Mason et al. (2018) noted, governments tend to focus on protecting and enhancing the value of their biodiversity and put in place legislation based on that interest, although emphasising economic aspects in their ABS regulations may impede the use of their biodiversity. These authors proposed a concept benefit-sharing agreement for accessing invertebrate biological control agents that would safeguard a provider country's biodiversity protection and enhancement of its value but also maximise research and development (Mason et al. 2018). This concept benefit-sharing agreement would certainly be useful for accessing microbial biological control agents.

Where there are no ABS requirements, permits to collect and export may still be required but restrictions on use do not exist. However, to keep track of activities some sort of documentation to ensure that the microorganism was obtained legally should be obtained/kept/stored. Since microorganisms are deposited in culture collections such documentation is of high value to ensure that when a request is made to a collection manager there is certainty about where the culture originated and sets out the conditions under which a microbial biological control agent can be provided or should not be provided. The use of a Material Deposit Agreement (MDA) would then protect the culture collection that provides the microorganism from later actions by parties to claim ownership. A model MDA is provided by Verkley et al. (2020). It includes core elements usually included in a 'deposit form' that provide information necessary for assessment of the status of the material under ABS legislation as well as a set of example clauses for inclusion in 'terms and conditions of use' for managing the culture collection and for third parties. When using this form, we recommend that in Section C under "Other relevant details of strain history" it should be stated that the material is a biological control microorganism.

Collaborative research and opportunities for benefit-sharing

Microorganisms are being increasingly used as biological control agents with a focus on five commercialised species in 1970 that rose to 94 in 2018 (van Lenteren et al. 2018, 2020, 2021). A review carried

out for the Commission on Genetic Resources for Food and Agriculture (CGRFA 2021) assessed the extent to which microorganisms were used in biological control. The background study paper not only discussed those microorganisms that were used commercially but also the thousands of potential biological control agents in collections around the world and considered that the majority were yet to be discovered. In general, microorganisms are poorly known: approximately 99% still remain to be discovered (Locey and Lennon 2016; Smith et al. 2018). The more than 800 collections listed by the World Data Centre for Microorganisms (WDCM) hold over 3.3 million strains (as of March 2023) (<https://ccinfo.wdcm.org/statistics>). The CABI collection which numbers 28,000 fungi and 2000 bacteria has in excess of 3000 potential biological control agents (Smith et al. 2022) and there are several other collections registered with the WDCM that have an agricultural focus or, for example, purport to be collections of insect pathogens (25 collections). There are 905 species represented by 3647 strains from the 25 collections that have been isolated from insects (CGRFA 2021).

The majority of discoveries of new species of microorganisms are now made through genomic analysis of environmental and host samples. However, this results in names being applied to sequences rather than isolated organisms, for bacteria these are given *Candidatus* status, a category used since the 1990s to accommodate uncultured taxa defined by DNA sequences (Pallen 2021). Although culturomics (Lagier et al. 2012) is offering improved ways of culturing fungi the majority of the microorganisms being discovered are yet to be grown. Molecular methods are not only used to identify microorganisms, but they are increasingly used to get a better understanding of their capacity and properties and it is now possible to target those having traits that are best suited for biological control (Dang et al. 2019; Leung et al. 2020; Bridge et al. 2021; Tang et al. 2022).

The molecular technologies open up access to the 99% of microbial diversity yet to be discovered and microbiome studies allow the observation of microorganisms working in communities. It is already known that the microbiome of a plant, animal or human can improve the health and immunity of its host and in effect offer some level of biological control. For example, elucidating the fruit microbiome is

important to develop effective strategies for biological control of post-harvest diseases (Zhang et al. 2021). The integration of microbiome studies provides an opportunity to develop biological control strategies and approaches for product optimization. They can be implemented during product development at different stages, from finding for new candidates in their natural environment to risk assessments that are required for registration (Rändler-Kleine 2020). They are not only useful in identifying strong and weak attributes of biological control agents, but can also be used for improving their field performance (Cernava 2021).

There are numerous proposals on how countries can address ABS concerning their genetic resources. Indeed, countries are implementing processes to meet their specific interpretation of the Nagoya Protocol and to deliver their own specific requirements. Microorganisms are part of the biodiversity a country protects and extends its sovereign rights over but are often the element of biodiversity that the country knows least about (Mannazzu et al. 2020; Morses 2021; Thaler 2021). Countries are implementing ABS regimes with a hope to generate funds to prevent biodiversity loss but to date there is little evidence that sufficient funds can be generated. Countries have overly optimistic expectations (Correa 2005). The administrative and transaction costs have been beyond levels of benefits that are being shared currently from ABS regimes to date. For example, the effort in Costa Rica reported extensive non-monetary benefits but little monetary benefit (Richerzhagen and Holm-Mueller 2005). The World Intellectual Property Organization (WIPO) reported that the National Institute of Biodiversity of Costa Rica (INBio) agreed to provide Merck with 10,000 samples of plants, animals and soil including exclusive rights to conduct research on these samples for two years and to retain the rights for any resulting patents. In exchange, Merck made an upfront one million US\$ payment to INBio and provided the institute with personnel training and laboratory equipment. Merck also agreed to pay royalties for any drugs developed but no products had seemingly reached the market at the time of the report (WIPO 2006). Additionally, a verbal communication within the informal advisory group for DSI of the open-ended working group reported that in Brazil 11,000 products had reached the market netting around seven million US\$. Monetary benefits have generally, come from a share of revenue from

products on the market. If you remove funding from the discovery process, you only reduce the innovation and public good that can come from it. Access and use of genetic resources for uses such as biological control of pest and diseases that improves yields and addresses Sustainable Development Goals such as SDG 2 Zero Hunger should not be subject to monetary benefit-sharing. Benefits can be shared in other ways such as capacity building, exchanging information on how to develop biological control agents and their application to reduce chemical use thus improving the environment as well as reducing losses. These benefits have been identified in the Commission on Genetic Resources for Food and Agriculture, FAO, Background Study Paper No. 47 (Cock et al. 2009). The main beneficiaries of classical biological control being the farmers indirectly or directly where biological control agents are established as well as the public interest. The reduced crop losses from pests also serves the public good through improved food security and livelihoods. Additionally, there is the added benefit of reducing pesticide use, and thus lower residues in food. The use of augmentative and classical biological control in place of pesticides enables producers to meet the standards of profitable export markets, creating jobs for growers and a very significant influx of foreign revenue in developing countries (Cock et al. 2009).

Although few examples of monetary benefit-sharing have been documented, numerous examples of non-monetary benefit-sharing exist. For example, CABI has summarised Nagoya Protocol triggered benefit-sharing from projects running in the UK Centre (Smith et al. 2022). The benefits shared are summarised as: (1) sharing of R&D results relevant to country needs; (2) collaboration in education, training, research, development programmes; (3) joint authorship of publications and joint ownership of intellectual property rights; (4) access to *ex situ* facilities and to databases; (5) transfer of scientific information, knowledge and technology; and (6) institutional capacity-development to help build or maintain local collections.

Production technology and technology transfer considerations for use and exchange of microbial biological control agents

Patenting and production

Microorganisms in their native form cannot be patented unless they have been genetically modified or the processes in which they are used are novel (often dependent on the country). However, as with all patents there must be novelty, an “invention” step and in many countries, non-patentable categories may include scientific theories, aesthetic creations, mathematical methods, plant or animal varieties, discoveries of natural substances, commercial methods, methods for medical treatment (as opposed to medical products) or computer programs (https://www.wipo.int/patents/en/faq_patents.html). There is also a requirement for the microorganism concerned to be made available because a simple written description of the application will not suffice if a specific strain is needed for the process patented. The WIPO Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure 1977 (<https://www.wipo.int/treaties/en/registration/budapest/>) was implemented for this purpose. This avoids the need to deposit in each country in which protection is sought. International Depositary Authorities (IDA) are established in contracting states to receive these deposits, and these are recognised by all Parties to the Treaty. The European Patent Office (EPO), the Eurasian Patent Organization (EAPO) and the African Regional Intellectual Property Organization (ARIPO) have made such declarations. An IDA is a scientific institution—typically a “culture collection”—which is capable of storing microorganisms, appointed by the contracting host state. On 7 July 2022, 48 such authorities were known: seven in the UK, four in the Republic of Korea, three each in China, India, Italy and the USA, two each in Australia, Japan, Poland, the Russian Federation and Spain, and one each in Belgium, Bulgaria, Canada, Chile, the Czech Republic, Finland, France, Germany, Hungary, Latvia, Mexico, Morocco, the Netherlands, Slovakia and Switzerland.

WIPO (2017, 2018, 2019) provide answers to key questions on patent disclosure requirements for genetic resources and traditional knowledge (<https://tind.wipo.int/search?ln=en&p=patent%20disclosure%20requirements%20for%20genetic%20resources&f=&sf=&so=d&rg=10&fti=0>).

WIPO explains that policymakers and other stakeholders often raise operational questions and seek practical and empirical information about patent disclosure requirements in relation to genetic resources and traditional knowledge.

Particular strains of microorganisms have been patented. For example, a patent survey of *Trichoderma* species (Hypocreaceae) demonstrated the wide range of applications that this fungus can be utilised in, including as a biopesticide and biological control agent (Al-Ani 2019). The patents include many new *Trichoderma* strains, for example, several that showed high activity in reducing levels of plant diseases, enabling the development of biopesticides involving mixtures of organisms. The microbial-based solutions that can be used for the biological control of the primary microbial spoilers, phytopathogens, and human food-borne pathogens that affect fruits and vegetables during the production and storage phases have been reviewed (De Simone et al. 2021). It covered the most recent patents in this area and innovations, particularly those approaches that integrate biological control agents to minimise spoilage phenomena and microbiological risks. They conclude that there is a growing interest in biological control strategies that will counteract the growth of spoilage and/or pathogenic microorganisms suggesting that there will be a considerable increase in new commercial products and patents worldwide based on innovative biotechnological solutions in the sector. Ortega et al. (2020) review 185 patents associated with endophytic fungi (from January 1988 to December 2019) and consider their applicability for abiotic stress tolerance and growth promotion of plants, as agents for biological control of herbivores and plant pathogens and bio- and phyto-remediation applications.

Another example of biological control agent use in agriculture is the WO1994019950A1 (<https://patents.google.com/patent/WO1994019950A1/en>) patent which recognises ‘prior art’ and describes how this is overcome through the application of: (1) a mixed culture of a yeast component and a bacterial component, and (2) a substrate for the mixed culture. The patent also references other patents which describe uses of yeasts or fungi and bacteria obtained from a natural source. For example: (1) EP 485,440 a new yeast strain obtained from the surface of citrus fruits

and which may be used to control fruit rot pathogens; (2) US Patents 5,047,239 and 4,764, a strain of *Bacillus subtilis* (Ehrenberg) Cohn (Bacillaceae) used for biological control of fruit rot; (3) US Specification 4,377,571 *Pseudomonas syringae* van Hall (Pseudomonadaceae) used for treatment of Dutch elm disease; (4) US Specification 4,950,472 a new strain of *Acremonium breve* (Sukapure & Thirum.) W.Gams (Hypocreaceae) used to control grey mould infection of pome fruit; and (5) US Specification 4,975,277 an isolate of *Burkholderia* (= *Pseudomonas*) *epacian* (Palleroni and Holmes) Yabuuchi et al. (Burkholderiaceae) used for biological control of post-harvest disease in fruit. In Japanese Patent JP 3,077,803 reference is made to *Pseudomonas* bacteria selected from *B. epacian*, *B. gladioli* (Zopf 1885) Yabuuchi et al., *Ralstonii picketti* (Ralston et al.) Yabuuchi et al., *P. vorans* (Burkholderiaceae), *Brevundimonas diminuta* (Leifson and Hugh) Segers et al. (Caulobacteraceae) and *Bacillus* bacteria selected from *B. cereus* Frankland & Frankland (Bacillaceae), *B. mycoides* Flügge, *B. anthracis* Cohn (Bacillaceae) and *B. thuringiensis* for biological control of soil borne diseases.

Modified microorganisms used as biological control agents have also been patented (<https://patents.justia.com/patent/10508280>). For example, biological agents and populations of such agents that are modified to be herbicide-tolerant or resistant are selected or engineered. The patent lists 46 ways the organism is selected (modified) and lists extensive numbers of species (e.g., pathogens, biological control agents). One of these ways is to introduce antagonistic factor genes from a known biological control agent by combining them with promoters, derived from the recipient microorganism to create a new agent. For example, the naturally occurring epiphytic bacterium *Erwinia ananas* Serrano (Enterobacteriaceae) strain NR1 was genetically modified by introducing the chitinolytic enzyme gene from the bacterium *Serratia marcescens* Biszio (Yercinicaceae) strain B2 to control the phytopathogenic fungus *Pyricularia oryzae* (T.T. Hebert) M.E. Barr (Magnaporthaceae), the cause of rice blast disease (Soymea and Akutsu 2006).

Licensing production

Microorganisms that are developed as biopesticides are subject to registration and must follow

requirements set out by individual countries where they are intended to be used. Once a product is registered production may be licensed to a third party. Green Muscle™ is based on a specific isolate of *Metarhizium acridum* (Driver & Milner) J.F. Bisch., Rehner and Humber (Clavicipitaceae) which infects locusts and grasshoppers. The discovery and development of this biopesticide was funded by a collaboration among the governments of Canada, the Netherlands, Switzerland, Britain and the USA with CABI being involved in the 1990s. The product was licenced to Eléphant Vert and CABI provided the starter cultures. Eléphant Vert is mass producing and marketing Green Muscle™ in Africa and Asia for the control of devastating locust swarms (CABI 2022). Companies like Eléphant Vert have production operations around the globe (one of which is in Mali) which provide employment opportunities in communities where they are located. *Metarhizium acridum* was first isolated in the Sahel region of Africa Niger (Niassy et al. 2011) and a spore production facility was constructed at the International Institute of Tropical Agriculture (IITA) in Cotonou, Benin (Cherry et al. 1999; Lomer et al. 2001) and would have employed local people.

Conclusion

ABS regulations have changed the practice of biological control using microorganisms. Best practices are key to ensuring that access to new and existing microorganisms for biological control continues. The use of a benefit-sharing agreement for accessing microbial biological control agents (see Mason et al. 2018) would safeguard the biodiversity of a provider country and enhance its value by maximising the research and development of that biodiversity. To protect culture collections from subsequent actions by parties claiming ownership, the use of a MDA that clearly indicates the status of the material under ABS legislation is recommended (see Verkely et al. 2020). The best practices outlined here will contribute to ensuring that high standards are in place for use and exchange of microbial biological control agents that are essential to protect biodiversity while providing solutions for important pest problems.

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