Journal of Asia-Pacific Entomology 19 (2016) 1019–1025

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

Review Article

Seeking stability for research and applied uses of entomopathogenic fungi as biological control agents

Richard A. Humber

USDA-ARS Collection of Entomopathogenic Fungal Cultures Emerging Pests and Pathogens Research, Robert W. Holley Center for Agriculture and Health, 538 Tower Road, Ithaca, New York 14853-2901, USA

ARTICLE INFO

Article history:
Received 3 August 2016
Revised 15 September 2016
Accepted 20 September 2016
Available online 21 September 2016

Keywords:
Culture collections
Entomopathogenic fungi
Biological control
Biocontrol agent
Fungal taxonomy
Fungal nomenclature
Genome-based taxonomy

ABSTRACT

Future progress in research with entomopathogenic fungi depends on a number of diverse considerations that help to stabilize the state of knowledge while supporting research about the documentation of the biodiversity and systematics of these fungi as well as those studies about their actions as pathogens of major and minor pests, and even as biological curiosities rather than as serious agents for use in biological control. This review considers (1) the role of service culture collections in culturing, preserving, and providing essential germplasm resources of these fungi for any and all research purposes; (2) whether there is too much stability in the current spectrum of entomopathogenic fungi actually being used in a practical sense and of possible alternative strategies to exploit more fungal entomopathogens; and (3) the diverse and far-reaching impacts of new nomenclatural rules that are constricting the pool of names applicable to entomopathogenic fungi while also stripping away their underlying taxonomic concepts that have long guided our interpretation and understanding of these fungi at a time when so many more taxa are being recognized. Some urgent problems underlying the shift from traditional to genomically based taxonomic approaches and about issues about the rapidly growing mass of genomic data are also discussed.

Contents

Introduction

What is a life-long mycologist doing presenting information about fungal pathogens affecting insects and other invertebrates to an audience composed primarily of entomologists? There are some outstanding and even extremely important reasons behind this seemingly odd pairing of subject matter and audience to the extent that microbially based efforts to control insects and other invertebrate pests in many parts of the world very often focus strongly on the use of fungal pathogens such as *Beauveria bassiana* and *Metarhizium anisopliae*. Insect viruses show much narrower host ranges than do most fungi (Hunter-Fujita et al., 1998; Asgari and Johnson, 2010). Very few bacteria are used in applied biocontrol except for *Bacillus thuringiensis* var. *kurstaki* (which has comparatively broad activities against many insects), *B.t.* var. *israelensis* (with its specialized activity against mosquitoes and other dipteran pests), and *Bacillus sphaericus* (which is an infective pathogen of mosquitoes; Charles et al., 2000). Microsporidia

E-mail address: richard.humber@ars.usda.gov.

http://dx.doi.org/10.1016/j.aspen.2016.09.006
1226-8615/

have rarely proven to be effective pathogens able to reduce the populations of the insects that they affect (Bulla and Cheng, 1976; Weiss and Becnel, 2014). Nonetheless, the relatively few virulent microsporidia are discussed in a study of a Brazilian *Nosema* sp. that can be a major pathogen of highly damaging sugarcane borers, *Diatraea saccharalis* (Simões et al., 2015). Among all microbial entomopathogens, it seems that the fungi may be the most numerous, most diverse, and possibly the most potentially useful to control invertebrate pests.

In the very specialized world of insect biocontrol, entomopathogenic nematodes (Grewal et al., 2005) have widely been treated broadly as 'microbes' despite their placement among the Metazoa. Such nematodes have become very popular subjects for both research and applied biocontrol because such metazoan agents of insect mortality are also usually exempted from the strict governmental regulations usually applied world-wide to regulate the uses of viral, bacterial, microsporidian, protozoan, and fungal pathogens as biological control agents.

This review considers three diverse aspects of work with insect pathogenic fungi upon which most academic, commercial, and governmental laboratories dealing with these fungi throughout the world depend, and that the overall stability and health of current and future efforts with entomopathogenic fungi is strongly influenced by these areas of concern. The three points considered here include the following (without any implied order of importance): 1. The stable access to global culture collections both as sources of living fungi that might be needed for a vast number and range of purposes and a long-term, stable repositories for voucher samples of fungi being isolated or used in research programs is critically important. 2. While the choices of entomopathogenic fungi in commercial production and practical use may be appropriate under both governmental regulatory and corporate business models for the countries in which they are available, the constraints of the regulatory/commercial requirements for these fungi have needlessly, and perhaps unwisely, limited the choices of biocontrol fungi in actual use to a tiny list and rather restricted but stable (possibly *too* stable) list of species whose broad host ranges inevitably raise concerns for non-target hosts while also overlooking the potential utility of many highly effective, highly specific fungi that cannot or will not be registered and commercialized. 3. Fungal systematics and taxonomy–no matter how remote or even esoteric these subjects may seem to be–provide what should be expected to be stable means to assure fungal identifications that are accurate and unlikely to undergo arbitrary changes; these considerations are problematic because accurate identifications form the most fundamental basis for evaluating and comparing the results of research and applied use programs with these fungi.

Culture collections as sources and repositories for essential materials and research collaboration

Scientific progress builds and expands upon the foundations of past accomplishments and knowledge, and it is true that if foundations are well built, then large, impressive, durable and important edifices can be raised upon them. This is true whether those constructions are physical buildings or systems of intellectual concepts and knowledge that cross-link and expand established understandings. The advancement of knowledge in any broadly or even highly specialized field of endeavor depends on having access to a common set of established and accepted facts as well as materials to pursue their studies. In the large global community of scientists, students, regulators, and others dealing with fungal entomopathogens, the institutions and facilities providing cultures and assuring long-term preservation (Humber, 2012a) of germplasm resources are central to the strength of the scientific foundations on which progress is enabled. Many entomopathogenic fungi are deposited in such general service culture collections as the American Type Culture Collection (ATCC), CABI Genetic Resource Centre (IMI), Centraalbureau voor Schimmelcultures (CBS) and the German Collection of Microoganisms and Cell Cultures (DSMZ), as well as innumerable other collection in academic, governmental, and even corporate laboratories. However, the United States Department of Agriculture, Agricultural Research Service Collection of Entomopathogenic Fungal Cultures (ARSEF; Ithaca, New York) retains a unique status among these as the largest and most comprehensive repository for and source of fungi from insects and other invertebrates. As of the middle of 2016, ARSEF held more than 13,000 accessions representing at least 715 fungal taxa isolated from 1300 diverse hosts or other substrates, and collected from 2440 locations in more than 100 different countries and every continent, *including* Antarctica. All ARSEF cultures are stored in cryogenic dewars at −196 °C; additionally, the most-requested isolates of conidial and ascomycete fungi (that are able to survive freeze-drying) are also stored and shipped as lyophilized cultures. It is also worth noting that ARSEF now often ships live cultures by placing two or three blocks (ca 5–8 mm on a side) cut from the margins of colonies into dry, sterile Eppendorf tubes in order to minimize the volume, weight, and expense of shipments while providing excellent security against mechanical damage during shipment.

Sadly, many scientific journals are still publishing papers about fungal pathogens of invertebrates without insisting that voucher specimens or voucher cultures of the experimental microbes must be preserved in appropriate institutions. It is, of course, impossible to prove or to disprove any previous work unless that work can be replicated; it can also be argued that even if the materials and procedures used to do a study with living organisms are fastidiously documented, published studies still cannot be verified adequately unless the living organisms that were used are also available to check the reported results. Comparatively few studies about entomopathogenic fungi have an absolute need to restrict access to the living microbes reported in them, and this suggests that the fungal cultures discussed in the vast majority of publications could and probably should be deposited in culture collections that can more easily provide these cultures than can many authors of these studies. For the most valuable isolates–e.g., those with important and novel properties to be reported in manuscripts that are under preparation or for which patent applications might have been filed–culture collections can restrict access by third parties or may seek the depositor's permission to release a specific isolate for shipment.

The collection information about the microbes preserved in culture collections is one of the most valuable resources that any collection can provide. The online catalogs of most major culture collections usually publish very little information about their cultures. If one attempts to discover which fungi, for example, affect a particular host, or from what locations particular fungi collected, then such data may be available online. The value of such information resources is amplified dramatically both by increasing the size and diversity of the collection and, especially, when this information is presented in informatively indexed forms. The catalogs of the ARSEF culture collection are available online at *http://www.ars.usda.gov/Main/docs.htm?docid=12125* provide five separate and distinct indices to the collection that facilitate searches by fungal taxa, by the original hosts of isolates, or by their geographical origins, and also include alternative collection numbers (e.g., original collection designations), and alternative accession numbers for the cultures also available from other germplasm repositories. Sadly, no collection's online catalogs are able to compile and to publish information about all of the experimental work done with the collection's isolates. There is, for example, no good way for any culture collection to list, for example, which hosts have been shown experimentally to be susceptible a particular isolate, what biologically active compounds are produced, or even a listing of all of the gene sequences from a given culture that have been deposited in genomic databases.

The breadth of information available about the huge spectrum of pathogens held by ARSEF does provide a fundamental, invaluable information resource to the global scientific community. Beyond the isolate-specific information that is provided by the collection catalogs, this large and diverse culture collection is also a natural source for information about improved methods for isolating, maintaining, and preserving these fungi. For logistic reasons, most collections use very few methods