Trends in Biotechnology July 2015, Vol. 33, No. 7

PERN: an EU–Russia initiative for rhizosphere microbial resources

Stéphane Declerck[1], Anne Willems[2], Marcel G.A. van der Heijden[3,4,5], Giovanna Cristina Varese[6], Olga Turkovskaya[7], Lyudmila Evtushenko[8], Irena Ivshina[9,10], and Philippe Desmeth[11]

[1] Université catholique de Louvain, Earth and Life Institute, Applied Microbiology, Mycology, Croix du Sud, 2 box L7.05.06, B-1348 Louvain-la-Neuve, Belgium
[2] Ghent University, Faculty of Sciences, Laboratory of Microbiology, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium
[3] Plant–Soil Interactions, Institute for Sustainability Sciences, Agroscope, 8046 Zürich, Switzerland
[4] Institute of Evolutionary Biology and Environmental Studies, University of Zurich, 8057 Zurich, Switzerland
[5] Plant–Microbe Interactions, Institute of Environmental Biology, Faculty of Science, Utrecht University, 3584 CH Utrecht, the Netherlands
[6] Mycotheca Universitatis Taurinensis (MUT), Department of Life Sciences and Systems Biology, University of Turin, viale Mattioli, 25, 10125 Turin, Italy
[7] Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, 13 Prospekt Entuziastov, 410049 Saratov, Russia
[8] All-Russian Collection of Microorganisms (VKM), G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russia
[9] Institute of Ecology and Genetics of Microorganisms, Ural Branch of the Russian Academy of Sciences, 13 Golev Street, 614081 Perm, Russia
[10] Microbiology and Immunology Department, Perm State University, 15 Bukirev Street, 614990 Perm, Russia
[11] Belgian Science Policy, Belgian Co-ordinated Collections of Micro-organisms (BCCM), Avenue Louise 231, 1050 Brussels, Belgium

Millions of microbial taxa inhabit the rhizosphere and could be used as biofertilizers, biopesticides, and/or for bioremediation. Only a fraction of these microbes have been described and/or are being utilized. Most are dispersed in collections, but coordination of their accessibility and availability is challenging. Here, we present the Pan-European Rhizosphere Resource Network (PERN), which is a transnational repository of microorganisms whose objectives are to facilitate access to rhizosphere resources and information and help users with technical and legal issues.

The soil microbial reservoir and the rhizosphere microbiome

Soil contains a tremendous diversity and abundance of microbes, such as phages and viruses (up to 10^9 g^{-1} soil), bacteria (4–20 $\times$ 10^9 cells cm^{-3}), protists (10^4–10^7 cells m^{-2}), and fungi (6.9 $\times$ 10^6–2.1 x 10^9 cells g^{-1}) with millions of species or ecotypes from each group [1–3]. The abundance and interactions of microbes largely depend on the environment, and microbes have a big impact on ecosystems, principally determining agroecological functioning and stability [4]. Many of these soil microbes inhabit the rhizosphere, that is, 'the interface between roots and soil where interactions among microbes and invertebrates affect biogeochemical cycling, plant growth and tolerance to biotic and abiotic stress' [5].

Corresponding author: Declerck, S. (Stephan.declerck@uclouvain.be)
Keywords: access and benefit sharing; biopesticide; biofertilizer; bioremediation; culture collection; rhizosphere; Nagoya protocol; plant microbiome.

0167-7799/
 http://dx.doi.org/10.1016/j.tibtech.2015.03.005

The need to preserve rhizosphere microbial diversity in culture collections

There are various reasons to preserve rhizosphere microbial diversity in a culture collection. A range of root-associated microbes has been reported to be beneficial for plant growth and nutrition [6]. For instance, estimates suggest that mycorrhizal fungi are responsible for up to 75% of the phosphorus that is acquired by plants annually, and many legumes rely on associations with nitrogen-fixing bacteria for nitrogen acquisition [6]. Moreover, root-associated microbes can improve plant resistance to pests and diseases [7]. This emphasizes the need to collect and preserve root-associated microbes because they can be used as biofertilizers, biopesticides, and/or for bioremediation.

The recent application of large-scale environmental DNA sequencing has revealed that only a fraction of microbes in soil or in the rhizosphere has been cultured by traditional techniques and a significant number of abundant microbial groups, such as the *Acidobacteria*, *Planctomycetes*, and *Verrucomicrobia*, are underrepresented among cultivated strains [8]. However, as more varied cultivation conditions are tested, representatives of these previously uncultured taxa are gradually being cultured [9]. These efforts should be continued, scaled up [10], and coordinated. A promising development in this respect is the recent success of culturomics (a method using high-throughput culture to enable the description of the microbial composition [11]) in human gut microbiome studies, which demonstrated a greater culturability when diverse culturing conditions were applied [12].

It is important that the microorganisms, which have been isolated at considerable expense, be preserved in optimal conditions so that they are available for future research and for the development of applications. This is not a trivial issue because many environmental strains may be sensitive to current preservation techniques [13]. The expected increase in the number of species and strains available in culture will require the simultaneous development and improvement of preservation methods to guarantee viability and optimal preservation of sensitive microorganisms without a change in their morphology, physiology, and genetics until use [14].

No single laboratory is currently set up to isolate, identify, preserve, characterize, or have an extensive knowledge of, all rhizosphere microorganisms. Microbial isolates may disappear from individual laboratories once research funding has ceased, emphasizing the need to preserve isolates in a coordinated effort and to promote exchange and cooperation between laboratories.

The Pan-European Rhizosphere Resource Network

In 2010, the European FP7 project BRIO was initiated to set up a virtual network of rhizosphere microbiological resource centers from Western Europe and Russia. The objective was to organize cooperation between specialized collections containing beneficial microorganisms isolated from the rhizosphere and to constitute a virtual, common, wide-range pool of microbial rhizosphere diversity that was exploitable for research and industry: PERN (http://www.PERN-BRIO.eu). PERN will ease the access to rhizosphere resources and information located in different collections via a single portal. It will also help users to comply with legal issues (Box 1) arising from: (i) the Convention on Biological Diversity (CBD; http://www.cbd.int); (ii) the Nagoya Protocol (NP) on Access and Benefit Sharing (ABS; http://www.cbd.int/abs/); and (iii) national and regional laws implementing this Protocol, such as the European Union (EU) Regulation 511/2014.

PERN is organized as a virtual collection, centrally managed in Brussels by the Belgian Coordinated Collections of Microorganisms (BCCM; http://bccm.belspo.be/) and enriched with microbes provided by culture collections in Belgium [BCCM/MUCL (http://bccm.belspo.be/about-us/bccm-mucl) and BCCM/LMG (http://bccm.belspo.be/about-us/bccm-lmg)], Italy (Mycotheca Universitatis Taurinensis (MUT; http://www.mut.unito.it/en), Switzerland (The Swiss Collection of Arbuscular Mycorrhizal Fungi; http://www.agroscope.admin.ch/bodenoekologie/08050/08067/08068/index.html? lang=en), and a consortium of Russian collections [All-Russian Collection of Microorganisms (VKM; http://vkm.ru/); Institute of Ecology and Genetics of Microorganisms (IEGM; http://www.iegm.ru/iegmcol/); and the Institute of Biochemistry and Physiology of Plants and Microorganisms (IBPPM; http://ibppm.ru/)].

Each culture collection within PERN is responsible for its own microbial resources [identification; preservation; viability, identity, and purity (VIP) check; distribution; and legal issues; i.e., compliance with CBD and the NP) and the implementation of a data set that comprises a taxon and strain property browser (Figure 1). The central management in Brussels hosts and updates the PERN website, facilitates access to microbial resources via the unique PERN portal, and coordinates and guarantees common standards.

Box 1. Access and use of microbial resources in PERN: the legal framework

Access and benefit sharing
ABS is a concept coined in the CBD and ruled by the NP under the CBD. Access to biological resources (e.g., deposited in culture collections) is granted provided that a fair and equitable share of generated benefit returns to the party providing these resources. Regulation EU 511/2014 translates the NP into European law.

Nagoya Protocol
The NP is a protocol that was adopted on October 29, 2010 and entered into law on October 12, 2014. It provides a legal framework for the fair and equitable sharing of benefits arising from the utilization of genetic resources, thus contributing to the conservation and sustainable use of biodiversity.

Material accession agreement
An MAA is a contract between the depositor of a microbial sample and the collection where the sample is preserved. This document records minimal data required by law and necessary for scientific labeling, such as place and date of sampling, etc. It specifies the respective rights and duties of the depositor and collection.

Material transfer agreement
An MTA is a contract between the providing collection and the purchaser of biological samples. 'MTA' is a generic term that includes shipment document, standard delivery notice, standard invoice containing standard requirements, or more specific contracts, including tailor-made mutually agreed terms.

Definitions available from http://bccm.belspo.be/projects/mosaicc and http://bccm.belspo.be/projects/trust

The total diversity within these collections currently exceeds 63 000 strains (many collected from food, feed, and agroenvironments), of which 1100 are rhizosphere microorganisms with documented properties available within PERN in compliance with international rules. These strains have been selected according to strict criteria for their biofertilizer potential (770 strains), biopesticide potential (81 strains), and bioremediation potential (291 strains). Their number is increasing constantly.

At strain deposit and distribution, a VIP check is performed (Figure 1). After the deposit of biological material, the organism is preserved following standard preservations techniques that differ from organism to organism, (i.e., by cryopreservation, lyophilization, or both). In some cases, storage under oil and water is also applied. Searches within this virtual collection are facilitated by a 'taxon browser' (e.g., accession number, organism name, etc.) and a 'strain property browser' (e.g., biofertilizer, biopesticide, and bioremediation). In both cases, an identity card presenting an exhaustive data set of information (Figure 1) is provided.

The website also presents a panel of international experts in applied microbial biotechnologies, such as biofertilizers, biopesticides, or bioremediation. An extensive list of protocols on how to isolate, culture, and preserve microbes from the rhizosphere as well as a list of documents (e.g., CBD, NP, EU as well as Russian legislation on intellectual Property Rights, and biosafety) is also provided to assist scientists and industries in the management and application of microorganisms.