BRIO - BANKING RHIZOSPHERE MICRO-ORGANISMS

European - Russian initiative to set up a network of rhizosphere microbiological resources centres network

Final report - 1 May 2011 to 30 April 2014

I. EXECUTIVE SUMMARY

Context

Soil microbes play key roles in ecosystems and exert multiple beneficial as well as detrimental functions impacting yield and quality of food.

Rhizosphere and endophytic microbes play a central role in promoting plant growth and health via acquisition and recycling of nutrients, modulation of plant hormonal balance, direct or indirect protection of the plant from detrimental organisms (e.g. pathogens), protection against abiotic stress (e.g. drought, heavy metals), and improvement of soil structure.

A holistic view of the rhizosphere microbiome is necessary to increase the knowledge about all micro-life interacting with the roots and with each other. Such understanding improves the effectiveness of solutions against stress induced by biological (pests and diseases) as well as non-biological (water shortage, pollutants, etc.) factors, offering opportunities for agro-environment sustainable management.

To support the study of the rhizosphere microbiome there is a need for optimised pool of microbial material, data, information and know how easily accessible, and technically as well as legally fit for use in biotechnology. BRIO aims at providing scientists with access to such a large array of rhizosphere micro-organisms, related data and expertise.

BRIO Objectives & Realization of PERN

The objectives of BRIO are:

I. to organize cooperation between specialised collections holding beneficial micro-organisms isolated from the rhizosphere,
II. to constitute a common wide-range pool of microbial diversity exploitable for research and industry: the **Pan-European Rhizosphere resources Network (PERN)**.

Two types of activities were performed to achieve the objectives:

- Activities aiming at optimising the structure and organisation of the network, i.e. the framework including a common policy regarding regulatory and ownership issues and the IT system. The preliminary database structure developed during the first year has been extended to other data related to possible uses of the material. Outcomes of work on common protocols, common procedures and joint data management are already visible on the website www.pern-brio.eu . In addition, network structuring and accession and distribution policies development are going on. Specific activities have been organised in common during the third year, including a field survey in Russia and specific training focused on culture collections needs. Contacts have been made with other collections in the Russian Federation, in connection with the World Data Center for Microorganisms (WDCM) and the World Federation for Culture Collection. A MoU has been signed between WDCM and the Skryabin Institute of Biochemistry and Physiology of Microorganisms, host institute of the VKM collection. The maintenance of the BRIO website is ensured by the SPP-PS BCCM webmaster. The maintenance of the database is ensured by the VKM computer scientist. Contents is updated by the PERN members. (Work Packages 5 to 8)

- Activities focused on the contents of the collection, i.e. the quality of the microbiological material and related data. All partners have biological material and related data, expertise, equipment and facilities to share and to organise in common. The work consists in organizing these assets to propose an optimum range of beneficial rhizosphere microbial resources organised around three main applications: biofertilisers, biopesticides, and bioremediation. The first assessment of the existing stocks made during the 1st year provided a set of 595 strains. Partners have designed a strategy to optimize every partner's holding and to build together a set of complementary stocks merged as one virtual collection. At the end of April 2014, the online catalogue posted on the project website www.pern-brio.eu listed more than 1100 strains. These BRIO collections constitute the Pan-European Rhizosphere resources Network (PERN) core, a significant modular repository of rhizosphere microbial resources publicly accessible for research. (Work Packages 1 to 4)

II. PROJECT CONTEXT

Soil microbes play key roles in ecosystems and exert multiple functions, from detrimental (as pathogens) to beneficial (e.g. plant growth promoters and pathogen antagonists), impacting yield and quality of food. They influence a large number of important ecosystem processes, including nutrient acquisition (Smith & Read 1997; Sprent 2001), nitrogen cycling (Tiedje 1988; Kowalchuk & Stephen 2001), carbon cycling (Hogberg et al. 2001) and soil formation (Rillig & Mummey 2006). Moreover, soil microbes represent the unseen majority in soil and comprise a large portion of the genetic diversity on Earth (Whitman et al. 1998).
Nowadays, increasing attention is devoted to rhizosphere and endophytic microbes which play a central role in promoting plant growth and health via:

- acquisition and recycling of nutrients important to plant growth,
- modulation of plant hormonal balance,
- direct or indirect protection of the plant from detrimental organisms (e.g. pathogens),
- protection against abiotic stress (e.g. drought, heavy metals), and
- improvement of soil structure.

It has been stated that "*the ultimate agricultural goal in studies of the biology of the soil-root interface, must be the manipulation of micro-organisms in this zone to increase plant health and growth*" (Rovira, 1979). Research should aim at improving our knowledge of the interactions between plants and microbes and of sustained management of these microbes to benefit the plant-food-consumer chain. This knowledge could help to reduce excessive use of agrochemicals alleviating hazardous effects of agricultural production on the environment.

Sound environmental and agricultural practices should favour balanced composition of micro-organisms population in the rhizosphere. A well balanced microbial population favours colonization of soil and nutrients uptake, limits pressure of pathogens (biotic stress), supports the development of commensal or symbiotic relationship between plant and micro-organisms, etc, with a good tempering effect regarding abiotic stress (good water retention capacity, available macro- and micronutrients).

A holistic view of the rhizosphere microbiome is necessary to increase the knowledge about all micro-life interacting with the roots and with each other. Such understanding improves the effectiveness of solutions against stress induced by biological (pests and diseases) as well as non-biological (water shortage, pollutants, etc.) factors, offering opportunities for agro-environment sustainable management.

To support the study of the rhizosphere microbiome there is a need for optimised pool of microbial material, data, information and know how easily accessible, and technically as well as legally fit for use in biotechnology. BRIO aims at providing scientists with access to such a large array of rhizosphere micro-organisms, related data and expertise.

III. OBJECTIVES

The objectives of BRIO are:

1. To establish **long term links and synergies between Russian and other European Biological Resources Centres** preserving microbial material isolated from plants' rhizosphere. It is expected that BRIO contributes to facilitate collaborations, to increase synergy between teams studying rhizosphere via the **Pan-European Rhizosphere resources Network (PERN).** The original core of PERN is made of the BRIO participants.

2. To constitute a **common pool of rhizosphere micro-biodiversity exploitable by research and industry.** BRIO participants have built an initial pool of rhizosphere microbial resources, related data and human expertise available via the BRIO website www.pern-brio.eu. The BRIO website is a **HUB** making these resources available for scientists. Facilitating the preservation and use of well documented, quality micro-organisms of the rhizosphere fosters the sustainable exploitation of renewable biological resources, a major objective of the European Knowledge Based Bio-Economy.

Collaboration between culture collections having material from West-European ecosystems and Russian Biological Resources Centres having micro-organisms from East-European biotopes forms a network of collections offering a wide range of micro-organisms coming from a broad spectrum of ecological zones spread over an extensive geographical area.

Two types of activities are performed to achieve the objectives:

- Activities aiming at optimising the structure and organisation of the network, i.e. the framework including a common policy regarding regulatory and ownership issues and the IT system. The preliminary database structure developed during the first year has been extended to other data related to possible uses of the material. Outcomes of work on common protocols, common procedures and joint data management are already visible on the website www.pern-brio.eu . In addition, network structuring and accession and distribution policies development are going on. Specific activities have been organised in common during the third year, including a field survey in Russia and specific training focused on culture collections needs. Contacts have been made with other collections in the Russian Federation, in connection with the World Data Center for Microorganisms (WDCM) and the World Federation for Culture Collection. A MoU has been signed between WDCM and the Skryabin Institute of Biochemistry and Physiology of Microorganisms, host institute of the VKM collection. The maintenance of the BRIO website will be ensured by the SPP-PS BCCM webmaster. The maintenance of the database will be ensured by the VKM computer scientist. Contents will continue to be provided by the PERN members. (Work Packages 5 to 8)

- Activities focused on the contents of the collection, i.e. the quality of the microbiological material and related data. All partners have biological material and related data, expertise, equipment and facilities to share and to organise in common. The work consists in organizing these assets to propose an optimum range of beneficial rhizosphere microbial resources organised around three main applications: biofertilisers, biopesticides, and bioremediation. The first assessment of the existing stocks made during the 1st year provided a set of 595 strains. Partners have designed a strategy to optimize every partner's

holding and to build together a set of complementary stocks merged as one virtual collection. At the end of April 2014, the online catalogue posted on the project website www.brionet.eu listed more than 1100 strains! Ultimately, these BRIO collections constitute the Pan-European Rhizosphere resources Network (PERN) core, a significant modular repository of rhizosphere microbial resources publicly accessible for research. (Work Packages 1 to 4)

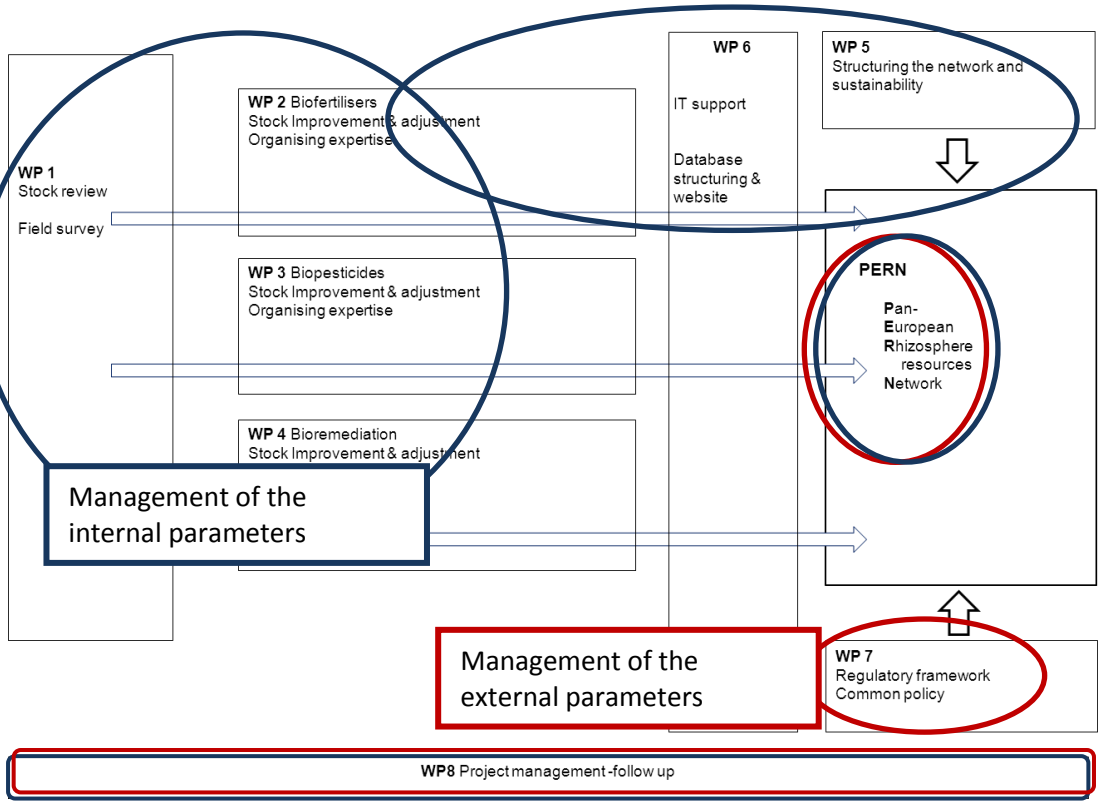
The project is organised in complementary modules.

These modules may be adjusted, added or deleted throughout the lifespan of the Pan-European Rhizosphere Network (PERN).

As described above, two kinds of activities are performed to set up PERN as a network focused on rhizosphere microbial resources::
- Building the microbial resources stock, consolidating the network and optimizing the coordination between the partners
- Opening the resources of the network to the users and linking with other initiatives in microbial resources' bio-banking

Work was performed along two axes:
- Consolidation of the network and optimizing the coordination between the partners
- Connection with other initiatives in microbial resources' bio-banking

Figure 1: "Graphical overview of the Work programme logic".



Connections and collaborations with global activities (World Data Centre for Microorganisms (WDCM), World Federation for Culture Collections (WFCC), or the TRUST initiative related to the implementation of the Nagoya Protocol in microbiology) are approved by the PERN participants.

Connection with the Microbial Resource Research Infrastructure-MIRRI infrastructure activities is ensured via the participation of BRIO partners VKM, UGent and SPP-PS to the MIRRI preparatory phase.

The various modules built during the 3 years of the EC subsidized part of project may be improved or deleted, other may be added depending on the needs of the users of the **Pan-European Rhizosphere resources Network.**

IV. RESULTS & ACHIEVEMENTS

The results are presented according to the activities that tend towards the two major objectives of the project.

Activities **under Objective 1** (WP 5 to 7 + WP8 for project management & dissemination)
To establish **long term links and synergies between Russian and other European Biological Resources Centres** preserving microbial material isolated from plants' rhizosphere. It is expected that BRIO contributes to facilitate collaborations, to increase synergy between teams studying rhizosphere via the **Pan-European Rhizosphere resources Network (PERN).** The original core of PERN is made of the BRIO participants.

WP5. NETWORK STRUCTURING AND LONG TERM SUSTAINABILITY

During the project 6 major meetings were held. All aspects of the future development of the network were discussed during three of these meetings. All Partners have taken part in discussions at the different BRIO project meetings where issues of network structuring and long term sustainability were discussed.

Partners 7 (IEGM) and 5 (UNITO) had exchanged their collection holdings. In particular, during Prof. Maria Kuyukina's visit to UNITO on 22 October 2013 to give the seminar "Importance of Hydrocarbon-Oxidizing *Rhodococcus* for Petroleum Microbiology and Bioremediation" and Practicum on morphological identification of *Rhodococcus* actinobacteria, six actinobacterial strains *Rhodococcus ruber* IEGM 70^{T}, *Rhodococcus ruber* IEGM 333, *Micrococcus luteus* IEGM 391^{T}, *Rhodococcus erythropolis* IEGM7^{T}, *Dietzia maris* IEGM 55^{T}, and *Rhodococcus fascians* IEGM 414^{T} were transferred to UNITO for educational purposes. Also, 26 fungal strains isolated from oil-contaminated soil and rhizosphere samples during the common field survey in Perm, as well as 2 fungal strains isolated from codeine-containing fermentation medium were transferred to UNITO on 28 April 2014, during the final BRIO meeting in Brussels, for research and educational purposes. Material Transfer Agreements (MTAs) for Non-Commercial Purposes were signed between IEGM and UNITO.

Partner 8 (IBPPM) hosted the 1st Annual meeting , Partner 7 (IEGM) hosted the 2nd annual BRIO meeting in Perm on 9-13 September 2013, Partner 6 (VKM) hosted the 3rd annual meeting. Coordinating partner 1 (SPP-PS) hosted the first and the final meeting in Brussels. Video-conferences using SPP PS bridging service facility for multipoint video conferencing were held in between.

Strain exchange between IBPPM collection and MUT collection and between IBPPM collection and LMG collection was carried out. In the first case the main objective of the exchange is a collaborative research concerning revealing of ligninolytic enzymes and their xenobiotic-degrading activities in *Schizophyllum commune* IBPPM 541, *Pleurotus ostreatus f. florida* IBPPM 540, *Fusarium oxysporum* IBPPM 543, *Geotrichum candidum* MUT 4803 and *Cladosporium herbarum* MUT 3238. In the second case the taxonomic belonging of *Azospirillum* sp. strains and newly rhizosphere strains isolated during the field surveys was clarified. Within the framework of collaboration between IBPPM Collection of Rhizosphere Microorganisms and LMG collection Dr. Ekaterina Dubrovskaya visited UGent in April 6-

13, 2014. During this visit Dr. Dubrovskaya received training on application of MALDI-TOF mass-spectrometry for bacterial identification and classification.

Collection of strains from common field survey

A collection of strains from the common field survey in Perm was created that included 12 actinobacterial strains and 26 fungal strains isolated from oil-contaminated soil and rhizosphere. The actinobacterial strains are maintained in IEGM, while the fungal strains are maintained in MUT (UNITO) according to collection specializations. The information on these strains is included into collection's databases and will be available in the BRIO catalogue.

Results and outcomes of the common field survey in Perm were presented by Partner 7 (IEGM) at the 2nd BRIO meeting (see partner 7 (IEGM) individual report) and discussed by all partners during the following BRIO meetings.

Set up of PERN

The participation of all BRIO Participants to the Pan-European Rhizosphere resources Network (PERN) is effective since the 3rd annual meeting organised in February 2014 (Month 34) in Moscow and Pushchino.

Various instruments and activities are already in place or in development such as
- General accession and distribution policies
- Common online catalogue and website
- Development of a network of experts related to PERN
- Programme of collaboration with global networking activities such as the Global Catalogue of Microorganisms
- Extension of the network in the Russian Federation
- Extension of the network

WP6. UPGRADING DATABASE STRUCTURE

All Partners have taken part in discussions at the different BRIO project meetings and in e-mail discussions where issues of **database structure** were discussed.

Partner 7 (IEGM) in collaboration with Partner 6 (VKM) has proposed the idea of presenting the strain property field in the BRIO catalogue in a format, which consists of two terms: a verb indicating the activity and a noun (or a word-combination) indicating the subject(s) of action (e.g. degrades polyaromatics or accumulates heavy metals). So, the user can search the particular strain of interest by both terms using the strain property search form at http://www.pern-brio.eu/catalogue/.

Partner 8 (IBPPM)'s list of appropriate collection strains for the BRIO database has been upgraded. This includes strains possessing high biodegrading abilities towards different pollutants, and plant-growth-promoting abilities towards different plants.

The dataset on 51 strains from the IBPPM collection potentially useful for biofertilization (39 strains) and bioremediation applications (39 strains) was created and sent to the Partner 6 (VKM) to be included into the BRIO catalogue. This dataset contains the taxonomic information, isolation and identification data, storage and growth conditions, and strain properties with literature references when available.

All Partners have taken part in discussions on the **project website structure** and all partners have delivered content for the website as agreed. It was decided to make the website largely bilingual (E/RU) and the Russian partners have taken on the task of translating much of the content to ensure a wider dissemination and easier use by Russian researchers.

Partner 1 SPP PS has written the source code to publish a searchable catalogue online, next to setting up the website itself.

Website

The domain name www.brionet.eu was the only one used during the project. There are now two domain names for PERN: www.brionet.eu and www.pern-brio.eu.

The BRIO website was designed and managed in house by SPP PS ICT expert François Guissart. It is developed on open source software.

The Alpha version of the website (D6.2) was made accessible to the participants for testing since spring 2013. The Beta version of the website (D6.3) was presented at month 30, six months before schedule. The website GO LIVE was made at the final BRIO meeting on 25 April 2014, on schedule.

The contents of the website is available **in two languages** (English and Russian) and comprises information on BRIO, its partners and funders, protocols for scientific experiments, a list of scientific experts and their fields of expertise, legal documentation, news, contact information, related links...

The website also contains the **BRIO Catalogue of biological material**. More than 1.100 well documented microbial strain records are available online. The catalogue data can be searched via a specialised web database application developed for BRIO by François Guissart.
The catalogue can also be browsed. Therefore 'taxonomic' and 'strain properties' catalogue browsers were implemented.

The BRIO website has be converted in PERN and its content **will be continuously maintained** after the end of the current BRIO project.

WP7. REGULATORY FRAMEWORK AND IPR MANAGEMENT

All Partners have taken part in discussions at the different BRIO project meetings and e-mail discussions where regulatory issues and issues of IPR management were discussed.

Partner 6 (VKM) collected information regarding laws and regulations impacting on exchanges and uses of microbial material in Russia and comparison between European and Russian legal framework related to access and transfer of microbiological material was done. Briefly, most of regulations and legal documents of EU and Russian Federation are similar especially with regard to international Agreements (Convention on Biological Diversity, Biological and Toxin Weapons Convention, Plants quarantine, Budapest Treaty, etc.**).**

The most significant differences between European and Russian regulations were observed in the area of biosafety. In Europe all microorganisms are divided into 4 risk classes. The risk class 1 includes non-pathogenic microorganisms (for humans, animal and plants) as well as opportunistic ones. As a result, according to European rules, all the microorganisms that are not included in risk classes 2-4 belongs to risk class 1. While handling the microorganisms of risk group 1 one should be aware that these microorganisms may be hazardous. In Russia only microorganisms known to induce diseases are qualified for their epidemiological risk. Group 1 encompasses the most hazarous, while the group 4 include the least hazarous microorganisms. The microorganisms that are not assigned to the pathogenicity groups 1-4 are assumed to be safe. Considering the items in lists of microorganisms belonging to analogous groups of risk/pathogenicity one can also see significant differences between them in European and Russian documents.

Based on the analysis of the documents for protection of intellectual property in area of biotechnology in EU and Russian Federation it may be concluded that the basic rules are quite similar. There are however, some differences in details. Thus, the Directive 98/44/EC Article 13 paragraphs 2 and 3 describe the rules for access to the deposited strains of microorganisms. In Russian Federation these rules are not yet provided. In practices, VKM collection requests official permission from depositors for this issue.

The laws for transfer of microorganisms are equal for Europe and Russia but there are differences in the departmental regulations. To illustrate this, Partners 3 (UGent), 6 (VKM) and 8 (IBPPM) have collaborated to test the Russian administrative requirements of sending strains from a Russian culture collection to a European research lab. A first shipment of two strains was requested by Partner 3 (UGent) from VKM collection on 6 Feb. 2014 and arrived on 31 March 2014 accompanied by 16 documents, nearly all in Russian; a second shipment of 11 strains was requested on 19 March 2014 and arrived on 10 April 2014 (accompanied by 35 documents for 11 strains).

Partner 6 VKM has also continued activities related to the development of the common access and distribution policy in the PERN network. It has organized a WFCC seminar at Pushchino (June, 2013) under the coordination of Partner 1 SPP-PS about the Global Catalogue of Microorganisms, the latest development of the Nagoya Protocol and the TRUST system, TRansparent Users-friendly System of Transfer designed to implement the Nagoya Protocol in microbiology. The second seminar, in Moscow (June, 2013), was timed to coincide the signing of a Memorandum of Understanding (MoU) between the World Data Center for Microorganisms (WDCM) from the World Federation for Culture Collection (WFCC) and the Skryabin Institute of Biochemistry and Physiology of Microorganisms,

Russian Academy of Sciences. The MoU is aiming at facilitating further cooperation directly between the VKM and WDCM, and structuring of the Russian network of collections. Relevant issues have been discussed. Partner 6 (Lev Kalakoutskii, Lyudmila Evtushenko and Svetlana Ozerskaya as members of relevant expert groups) have participated in several local meetings at the RF Ministry of Education and Science in Moscow and in subsequent e-mail discussions focused on legal access to biodiversity (concerned with the latest development of the Nagoya Protocol), establishing biological resource centers in Russia and the Russian network of microbial collections, as well as activities related to improvement Russian regulations in the area of biosafety. In addition, Lev Kalakoutskii provided presentation concerned with legal aspects of access to genetic resources and the required infrastructure at the round table conducted by the Committee on Science and High Technology of Russian State Duma (March, 2014).

Partner 7 (IEGM) has collaborated with Partner 5 (UNITO) to exchange strains from their culture collections. Two MTAs (of 22 October 2013 and 28 April 2014) produced using a model MTA designed by Partner 1 (SPP PS) for non-commercial purposes were signed between IEGM and UNITO, according to which 12 bacterial and 28 fungal strains were transferred from IEGM collection to MUT (UNITO) for educational and research purposes.

Partner 7 (IEGM) has continued working on establishing biological resource centres (BRCs) in Russia. Prof. Irina Ivshina as a member of the expert group on developing BRCs in Russia has participated in the group meeting at the RF Ministry of Education and Science in Moscow on 28 May 2014, where results of annual inventory of current biological collections and further activities related to the formation of national BRCs were discussed.

WP8. PROJECT MANAGEMENT AND DISSEMINATION

Reporting

Changes occurred in the Research Participants Portal website layout and functionalities. The Participant Portal User's Guide is exhaustive but for some particular tasks it was more efficient to have a digest of the instructions dealing with one single action. Therefore, like during the second period, coordination has issued digests of instructions. A set of Microsoft Word® files with step by step instructions concerning the use of the Participant Portal system. The latest digests concern:

Instructions for adding form C 3rd period.docx
Adjustment to previous form C.docx

Communication

Several videoconferences have been held by using the Gotomeeting™ software of Citrix® to solve specific issues and prepare the meetings.

Communication between the partners was excellent, making use of several tools:
- Email communication for written exchanges on particular topics,
- Videoconferencing for consultation on bottlenecks, focusing on sticking points
- Project Intranet providing for E-storage and sharing of files and documents

- Meetings
- The BRIO website

Website

The BRIO website (www.brionet.eu) was designed and managed in house by SPP PS ICT expert François Guissart. Ir. Guissart is a developer and a webmaster of the BCCM (Belgian Coordinated Collection of Microorganisms) website. His experience in managing a culture collections website with online catalogue facilitates the set-up of the different features of the webpages. He uses open source software.

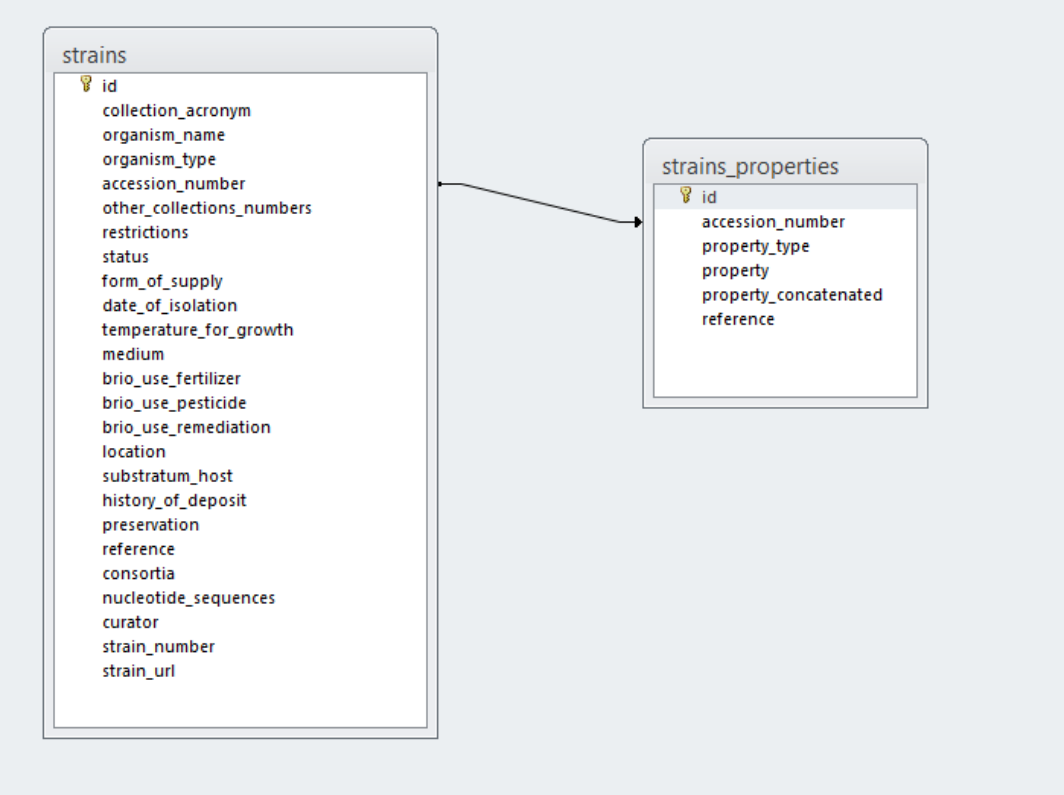
The Alpha version of the website (D6.2) was made accessible to the participants for testing since spring 2013. The Beta version of the website (D6.3) was presented at month 30, six months before schedule. The website GO LIVE was made at the final BRIO meeting on 25 April 2014, on schedule.

www.brionet.eu and www.pern-brio.eu home page

The contents of the website is available **in two languages** (English and Russian) and comprises information on BRIO, its partners and funders, protocols for scientific experiments, a list of scientific experts and their fields of expertise, legal documentation, news, contact information, related links...

Since the PERN network is set, the website has an additional domain name: www.pern-brio.eu .

The website also contains the **BRIO Catalogue of biological material**. More than 1.100 well documented microbial strain records are available online. The catalogue data can be searched via a specialised web database application developed for BRIO by François Guissart.
The catalogue can also be browsed. Therefore 'taxonomic' and 'strain properties' catalogue browsers were implemented.



Database structure of the BRIO online catalogue

The BRIO website and its content **will be continuously maintained** after the end of the current BRIO project.

Meetings

As foreseen during period 1 and 2, the original meeting planning has been reshuffled.

TABLE : Adjusted meeting agenda

	2011								2012								2013								2014			
	May	June	July	August	September	October	November	December	January	February	March	April	May	June	July	August	September	October	November	December	January	February	March	April	May	June	July	August	September	October	November	December	January	February	March	April
	Period 1								Period 2								Period 3																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Kick-off																																				
Stock review																																				
Collections & databases																																				
Accession & Distribution Policy																										3			4					5		6
Website and Catalogue																																				
Final																																				

Meeting 1 - BRIO Kick-off meeting

6 and 7 June 2011, Brussels, Belgium

It included sessions corresponding to the
Content task force (CTF) Meeting on extensive stock review preparation and
Framework task force (FTF) Meeting. It aimed at organising the consortium, in complement to talks related to specific work packages. Special sessions were devoted to:

- designing a dissemination and exploitation action plan,
- setting up contingency plan for the various stumbling block identified at administrative and technical levels,
- setting up the Extranet workspace as soon as possible,

Meeting 2 - BRIO first Annual meeting

From 8 to 11 October 2012, Saratov, Russian Federation

It was organised by participant IBPPM (Institute of Biochemistry and Physiology of Plants and Microorganisms). The meeting was originally planned at month 12 but postponed.

This meeting included:

- a closed workshop for the project participants only; during this day, in complement to the Work Package work summaries, the following theme were discussed :
  - the extensive stock review progress originally planned at month 12, but postponed to month 18. it is the Content task force (CTF) meeting
  - the plan for enhancement of individual collections
  - the field survey to be organised in August/September 2013
- a closed workshop with participation of representatives of Russian private companies as scheduled as:
  - Biofertilisers expert group meeting,
  - Biopesticides experts group meeting,
  - Bioremediation experts group,

All contributions and presentations are stored in the Extranet, except for confidential data presented by company's officials.
Most meetings were held with the participation of all participants and also third parties, private companies and other institutions.

Meeting 3 - BRIO intermediary *ad hoc* meeting.

17-21 June 2013, Moscow and Pushchino, Moscow Region, Russian Federation

This mission included three main activities:

- The signing of a Memorandum of Understanding (MoU) between the World Data Center for Microorganisms (WDCM) from the World Federation for Culture Collection (WFCC) and the Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences. The purpose of the MoU is to facilitate further cooperation directly

between the VKM All Russian Culture Collection and WDCM as well as indirectly with the PERN network. It is a step towards structuring of the Russian network of collections. It is also important because it facilitates the linkages between the BRIO database and the Global Catalogue of Microorganisms

- A work session dedicated more specifically to Regulatory framework. Comparison between Russian laws and EU laws as well as understanding the technical differences between the Russian and EU system of biosafety/biosecurity system is important.
- A WFCC Seminar at Pushchino, about the Global Catalogue of Microorganisms, the latest development of the Nagoya Protocol and the TRUST system, TRansparent Users-friendly System of Transfer designed to implement the Nagoya Protocol in microbiology. These activities are relevant for the development of the common access and distribution policy in the PERN network.

Participants to the meeting were from the coordination SPP-PS and participant VKM.

Meeting 4 - BRIO 2nd annual meeting

9 to 13 September 2013, Perm, Russian Federation

The programme included

A. the common field survey to collect strains in situ (Deliverable 5.2)
B. the follow up of the work packages' progresses
C. a seminar at the Perm State National Research University (PSNRU)
D. a training course in mycology

Representatives of all participants participated to the meeting

A. Common field survey to collect strains in situ (Deliverable 5.2)

This field trip and sampling for microbial strains was organised on 9 September 2013, with a visit to the Kokuy oilfield, with company "Priroda-Perm", a visit to the waste treatment plant and of a natural park. Samples were taken from these three sites. Deliverable 5.2 is dedicated to this activity.

B. The follow up of the BRIO work package progress

The programme of the 2 days closed meeting encompassed all work packages.

> The sketch of the website structure was proposed by François Guissart and approved. It was decided to wait for the beta version to make more precise adjustments. Exchange of protocols for rhizosphere microorganism, isolation-identification-preservation was done and a united protocol form to include into the website was prepared.

C. The public seminar

The public seminar was held on the 3rd day 12 September at the Perm State National Research University (PSNRU).

The rector of PSNRU introduced the seminar. Philippe Desmeth, BRIO coordinator, made a lecture on the Relationship between the microbial culture collections and their users, as a means to improve quality and reproducibility in scientific testing. These academic talks were followed by a discussion chaired by Prof. Irina Ivshina.

After a visit to the PSNRU laboratories and botanical garden, participant 7, IEGM, organised a round-table discussion concerning

- BRIO and the development of the network in Russia.
- Russian rhizosphere network connected to the WFCC Global Catalogue initiative.

D. Training course in mycology

The training course in mycology was held on September 13, at the IEGM collection laboratory and chaired by Prof. Cristina Varese, partner 5 (UNITO) and Dr Fritz Oehl, partner 4 (ART).

Meeting 5 - BRIO 3rd Annual meeting

10 to 14 February 2014, Moscow, Russian Federation

Representatives of all participants participated to the meeting.

The agenda of the meeting included:

A. Reviewing of the work packages progresses during closed meeting and the endorsement by the participants of the several deliverables, these steps constituted the milestones:
B. Scientific conference

A. Review of work packages and endorsement of deliverables, reaching milestones

- Regulatory framework and IPR management
  Information on Russian and European laws impacting on access to and transfer of microbiological resources.
  Discussion and endorsement of the Common accession and distribution policy proposal
- Network structuring and long term sustainability.
  Meeting on survey outcomes. The field trip organised in September 2013 during the previous annual meeting was considered fully successful because the collected samples yielded several strains of relevant microorganisms.
  The administrative workflow to get these strains exported from Russia and imported in another European country was tested. All strains have not been characterized. It has been agreed to work on it after the end of the EC subsidized project phase.
  Future developments on long term sustainability were discussed.
- Exchange of protocols for rhizosphere microorganism isolation/identification/ preservation. Discussion on a unique format of the protocols for the website
- Parallel programming of the activities.
- Set of common fields between database of rhizosphere microbes and databases of relevant parameters (geographical distribution of diseases, soil information, target plants for microbial biofertilizers, etc.)

- Database subset of microbiological resources (for use as biofertilizers, biopesticides and bioremediation agents).
- Extensive stock review: Presentation of Catalogue.
- Round-table discussion: Proposals for the future development of the project.

B. Scientific conference

- Conference at the G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow Region. *Culture Collections at the crossroad of biodiversity and biotechnology activities*

- Visit of VKM and other laboratories of the G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Pushchino, Moscow Region

- Visit to Radio Astronomy Observatory, Russian Academy of Sciences, Pushchino

Meeting 6 - Final meeting

From 24 to 28 April 2014, Brussels, Belgium

Programme

Visit of BCCM/ MUCL collection and Laboratory of Microbiology.

Project outcomes overview
WP 1 Extensive stock review, **WP 2** Biofertilisers, **WP 3** Biopesticides and disease prevention, **WP 4** Bioremediation
WP 5 Network structuring and sustainability and WP7 Regulatory framework and IPR management
WP 6 Databases
WP8 Project management and dissemination

The central communication instrument is the BRIO website that hosts the online catalogue. The updated catalogue was put online in March 2014. Firstly access was limited to the BRIO participants, then after testing, it was made publicly accessible. Milestone 15 was reached on time.

BRIO website check and design of maintenance, updating and optimization programme.

The meetings were highly productive and constructive. They were absolutely necessary to get the final version of deliverables approved.
Visa for travel to Russia were not always easy to get. One should ask for a multiple entries visa open for the length of the project.
Thanks to the possibility of SPP-PS to hold the meetings in its own facilities, costs of the meetings in Brussels were limited.
Some partners have benefited from their joint participation to other international conferences to improvise work sessions. This helped to move faster, especially on the structure of the database and on the subsets of strains for biofertilization, biopesticides and bioremediation.

All participants consider that having meetings in Russia is very important for the Russian participants:

- It helps the teams based in relatively remote city of Russia to feel fully part of the project;
- It raises the profile of the Russian teams;
- It allows a better understanding by the European Union's participant of the working environment of their Russian counterparts
- Through the organization of public information sessions such as the one organized in Saratov, it better disseminate the project outcomes as well as other information related to the Framework programme of the European Commission, among others, the MIRRI ESFRI initiative.
- Finally, it has permitted the participation of representatives of local Russian SME to the meeting. This participation improved dramatically the understanding of the situation of these companies in this difficult economic time, focusing on the necessity to shorten the delay between upstream research outputs and downstream developments supporting innovative commercial activities.
- It has also highlighted unexpected impact of minor scientific progresses on the operation of SME. For example, change in taxonomical name of specific micro-organisms can result in costly additional administrative burden such as the obligation to renew authorization for commercialization of products because a new taxonomical name induces a new formulation of biofertilizers or biopesticides.

Activities **under Objective 2** (Work Packages 1 to 4)
To constitute a **common pool of rhizosphere micro-biodiversity exploitable by research and industry.** BRIO participants have built an initial pool of rhizosphere microbial resources, related data and human expertise available via the BRIO website www.brionet.eu. The BRIO website is a **HUB** making these resources available for scientists. Facilitating the preservation and use of well documented, quality micro-organisms of the rhizosphere fosters the sustainable exploitation of renewable biological resources, a major objective of the European Knowledge Based Bio-Economy.

WP1. EXTENSIVE STOCK REVIEW

1.1. Inventory of the partner's holdings

The inventory of microbiological material already available in the collections of every participant was made. The information was reported according to an assessment grid ultimately structured as a Minimum Data Set (MDS).

The list of strains selected during the stock review has been consolidated into one database under coordination of partner 6 (VKM) and forms the core of the PERN database, publicly available at www.pern-brio.eu. At month 35, when made publicly accessible for the first time, the catalogue comprised 770 strains with biofertilizer potential, 81 strains with biopesticide potential, and 291 strains with bioremediation potential.

The collection of partner 7 (IEGM) has been enhanced by microbial cultures isolated from soil and rhizosphere samples taken during the common field survey on 9 September 2013 in Kungur region, Perm Krai, Russia. In total, 12 strains of hydrocarbon-oxidizing actinobacteria and 26 strains of hydrocarbon-oxidizing filamentous fungi were isolated from oily waste, oil-contaminated soil and plant rhizosphere using mineral media supplemented with hydrocarbons as the sole carbon source. Taxonomic positions of plants were determined from which rhizosphere microorganisms were isolated. Actinobacterial strains were identified using partial 16S rRNA gene sequencing, PCR tests with specific primers for ecologically significant *Rhodococcus* species, as well as by phenotypic, chemotaxonomic and immunochemical analyses. Fungal cultures were partly purified and maintained on malt extract agar, and transferred for identification to Prof Cristina Varese from Partner 5 (UNITO) during the 3rd annual BRIO meeting in Moscow on 9-14 February 2014. All cultures isolated and identified were deposited in the IEGM collection under accession numbers and stored in lyophilized state and cryoconserved at -84°C. The information on these new strains is included into the collection database.

The IBPPM collection currently maintains over than 300 strains of bacteria. A part of IBPPM-collection is not presented in the on-line catalogue.
During the third period the IBPPM collection has been enlarged with newly isolated rhizosphere strains having agriculture and/or ecological significance. Thirteen strains were isolated in the course of field survey in Saratov region, 5 strains were isolated in the course of field survey in Perm, and 4 strains were isolated from root zone of *Cucurbita pepo* and *Cucumis sativus*. The taxonomic position of these isolates is recognized. *Cucurbita pepo*- and *Cucumis sativus*-associated isolates were identified on basis of their morphological, cultural, physiological, biochemical characteristics and 16S rRNA gene sequences. They were assigned to *Bacillus pumilus* (3 strains) and *Bacillus licheniformis* (1 strain). "Strain

passports" for these bacteria were generated and formalized according to the provisional standards worked out in the framework of the BRIO project. All strains will be verified for their ability to be used as biofertilizers, or bioremediation agents.

1.2. Sharing and improving of protocols

Sharing of methods relevant for isolation, preservation and study of rhizosphere microorganisms. All partners have provided a number of protocols that are now publicly available at http://www.brionet.eu/protocols/ . The protocols were divided under three main sections which are:

- Sampling and isolation
- Preservation and maintenance
- Characterization and identification

Among others, IBPPM has prepared protocols for detection and isolation of plant-growth-promoting rhizobacteria, for azospirilla, for PAH-degrading and surfactant degrading microorganisms using selective media, for preservation and characterization have been set up. IEGM has prepared a protocol of identification of ecologically significant *Rhodococcus* species based on free fatty acid composition was developed, its English and Russian versions were sent to partner 6 (VKM). Partner 1 (SPP-PS) has published the protocols on the website.

1.3. Pilot Field survey

The field survey was held at the occasion of the second project meeting in Perm, Russia, 8-13 Sept. 2013. Partner 7 (IEGM) organized a common field survey on 9 September 2013 in Kungur region, Perm Krai, Russia. This field survey was aimed to visit the Priroda-Perm Company waste treatment & bioremediation site at Kokuy and to collect samples of oil-contaminated soil/waste, oil-resistant plant rhizosphere, endemic plant rhizosphere and nodule-carrying plants. Totally, 11 samples in triplicate were collected with the appropriate sampling information recorded (e.g. geographical origin, including GPS coordinates, isolation substrate, taxonomically identified plants). The samples collected were preserved at the IEGM collection in the air-dried state and intended to be transferred to all partners for isolation of specific groups of agro-environmentally relevant microorganisms (according to WP2, WP3, and WP4) to increase partners' collection holdings. Additionally, small portions of the freshly taken samples were cryo-preserved at -84^{o}C for future metagenomic studies. Because of the non-harmonized European-Russian postal regulations on environmental samples (in particular soil samples), timely sample transfer to European partners was not possible. Therefore, the samples were transferred within Russia to partners 6 (VKM) and 8 (IBPPM); and partner 6 (VKM) was requested to provide documents necessary for the sample release from Russia to abroad.

The partner 8 (IBPPM) collection has been enlarged with newly isolated rhizosphere strains having agriculture and/or ecological significance. Thirteen strains were isolated in the course of field survey in Saratov region, 5 strains were isolated in the course of field survey in Perm, and 4 strains were isolated from seedlings roots of *Cucurbita pepo* and *Cucumis sativus* grown under lab conditions.

1.4 The online catalogue of strains is available at http://www.brionet.eu/catalogue/ . It is effectively modulated into subsets pertinent to the main biotechnological fields of application: biofertilizers and bioremediation. It is searchable for several phenotypic and technical features.

After the lifespan of the EC subsidized part of the project, an objective quantitative indicator of the health of the network will be the number of strains incorporated regularly in the public catalogue.

WP2. BIOFERTILISERS

2.1. Organising the collections.

All partners have surveyed their holdings for strains with biofertilizer potential and contributed these to the BRIO catalogue.

Partner 7 (IEGM) has provided plant rhizosphere and nodule-carrying plant samples to partners 6 (VKM) and 8 (IBPPM) for isolation of microorganisms with potential biofertilizer properties.

The list of potential experts, including Russian biotechnology companies operating in the field of biofertilisers, was prepared by partner 3 (UGent). The expert group on biofertilizers has been organized virtually: experts are listed in an online expert list at http://www.brionet.eu/experts/

2.2. Enhancement of specific collections.

The following groups of biofertilizers are represented in the partners' collections:

Frankia: N_2-fixing actinomycete symbionts of diverse non-leguminous (actinorhizal) plants. Partner 6 (VKM) maintains about 30 *Frankia* strains (along with many other actinobacteria isolated from root nodules of actinorhyzal plants of different geographical origin).

Rhizobia: N_2-fixing symbionts of legumes. Partner 3 (UGent) has expertise in isolation and identification and holds a rich representation of rhizobial reference strains in the BCCM/LMG bacteria collection that is hosted by UGent.

Azospirilla: N_2-fixing plant-growth-promoting rhizobacteria that dwell under various soil-climatic conditions. Partner 8 (IBPPM), maintains the largest Russian collection of *Azospirillum*, including isolates that degrade various classes of hydrocarbons.

Arbuscular mycorrhizal fungi:
Partner 2 (UCL), hosts the Glomeromycota IN vitro COllection (GINCO) embedded in the BCCM/MUCL collection. GINCO is the world largest collection of AMF (the Glomeromycota Phylum) grown *in vitro* on various hosts (carrot, Medicago, crotalaria, chicory). The collection is about 30 strains. It also has a large collection (± 400 strains) of these organisms maintained on the host plant *Plantago lanceolata* in the greenhouse. The strains originate from temperate to tropical and Andean ecosystems (via several EU-funded

projects such as VALORAM and COMMINANDES), and a number have been evaluated for improved nutrition and growth of agricultural important crops (e.g. banana, potato) under greenhouse and in the field and for *in vitro* biotization of plants.

Partner 4 (ART), has another remarkable, growing collection of 214 strains of AMF. Strains originate from arable land and grassland in Middle Europe and recently also from new habitats or niches (e.g. deeper soil layers, or AMF strains with a very long life cycle). The ART AMF collection complements the collection at MUCL in that the isolates originate from Middle Europe and its strong focus on the functional diversity of AMF. Several AMF isolates have been found as promising biofertilisers (see below).

Saprotrophic fungi:
Partner 5 (UNITO), has a large collection (about 1000 strains) of fungi isolated from the rhizosphere, the rhizoplane and inside the roots of many herbaceous and woody plants. These will be further characterized for important features to be exploited as biofertilisers.

The BRIO catalogue holds 775 strains of biofertilizer strains, composed of subsets from the different partners:
Partner 2 UCL: 213 MUCL strains
Partner 3 UGent: 325 LMG strains
Partner 4 ART: 116 ART strains
Partner 5 UNITO: 41 MUT strains
Partner 6 VKM: 41 VKM strains
Partner 8 IBPPM 39 strains

The collection of Partner 3 (UGent) holds many rhizobial strains in this category. Most of these are type strains or strains from other continents. None of them originates from Belgium. Therefore as part of this task Partner 3 (UGent) has enhanced its holdings by depositing in the public collection a number of rhizobial isolates from Belgium isolated over the past five years. A representative selection of 52 strains was chosen for inclusion in the BCCM/LMG collection and the BRIO catalogue in consultation with the Curator, Claudine Vereecke. These strains were revived from frozen state, the purity was checked by plating out and the identity was checked by partial 16S rRNA gene sequence analysis. Finally 43 viable strains with confirmed identification were passed on to BCCM/LMG for inclusion in the collection. Viability of the lyophilized cultures was performed as final control. Strains will be made available in the BRIO catalogue.

Further experiments were performed to result in further enhancements to the collection of biofertilizers with novel strains that were received from other scientists:

- Three novel *Rhizobium* strains from Bangladesh
- Three novel *Bosea* and *Tardiphaga* strains from Russia (St. Peterburg)
- Three novel *Azospirillum* strains from India
- Two novel *Azospirillum* strains from Russia (Saratov)

These organisms have been checked for purity, genus identification and determination of closest relatives by partial 16S rRNA gene analysis and DNA-DNA hybridization to establish species identity or novel species status. This work is going on (see below, Task 2.3) and results indicate that several new species are indeed among these strains. They will be described and deposited in the BCCM/LMG bacteria collection and be made available in the BRIO database once their characterization is completed.

IBPPM collection has been enhanced by seven type strains of *Azospirillum* genus (*A. halopreference, A. doberanire, A. canadense, A. melinis, A. oryzae, A. palatum, and A. zeae*). These strains have been received from LMG collection within the framework of strain exchange. In its turn LMG collection has been received seven rhizosphere strains including newly isolated during the field survey in Saratov region.

2.3. Taxonomic characterisation of strains of particular interest.

Partner 3 (UGent) has characterized and identified several other sets of strains with the objective to further enrich its collection (Task 2.2) or at the request of other partners.

1. Three novel *Rhizobium* strains from Bangladesh were received from Dr. Harun-o-Rashid, Soil Microbiology Laboratory, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh. These strains were checked for purity and identity was verified by partial 16S rDNA sequencing. DNA-DNA hybridizations were performed to establish the species affiliation with closest relatives *Rhizobium etli* and *Rhizobium phaseoli*. Genomic %GC was also determined in the process of verifying DNA quality.
The results indicate that the three strains represent three novel species distinct from their closest relatives. These results will contribute to the description and naming of these species in a separate publication, provided sufficient other discriminating information is available. This is being done in collaboration with Dr. Harun-o-Rashid and a manuscript is in preparation. M. Harun-or Rashida, J. Peter W. Young, Isobel Everall, Pia Clercx, Anne Willems, M. Santosh Brown and Michael Wink. *Rhizobium lense* sp. nov. *Rhizobium bangladeshi* sp. nov. and *Rhizobium binae* sp. nov. from lentil (*Lens culinaris*) nodules. Manuscript in preparation.

2. Three novel *Bosea* and *Tardiphaga* strains from were received from Dr. Vera Safronova, All Russian Research Institute for Agricultural Microbiology, St. Peterburg, Russia. These strains were checked for purity and identity was verified by partial 16S rDNA sequencing. DNA-DNA hybridizations were performed to establish the species affiliation with closest relatives *Tardiphaga robiniae* and *Bosea lathyri*. Genomic %GC was also determined in the process of verifying DNA quality.
The results indicate that one of the strains represents a novel *Bosea* species and here as well, once sufficient descriptive data are available, we will describe this new species in collaboration with Dr. Safronova. For the other two strains the results are not conclusive and further experiments will be performed to resolve their species identification. One manuscript is already in preparation. Vera I. Safronova, Irina G. Kuznetsova, Anna L. Sazanova, Anastasiia K. Kimeklis, Andrey A. Belimov, Evgeny E. Andronov, Alexander G. Pinaev, Elena P. Chizhevskaya, Andrey R. Pukhaev, Konstantin P. Popov, Anne Willems, Igor A. Tikhonovich. *Bosea vaviloviae* sp. nov. a new species of slow-growing rhizobia isolated from nodules of the relict species *Vavilovia formosa* (Stev.) Fed. Manuscript in preparation.

3. Three novel *Azospirillum* strains were received from India from Dr. Mahesh S. Dharne
NCIM Resource Centre, CSIR-National Chemical Laboratory, Maharashtra, India. These strains were checked for purity and identity was verified by partial 16S rDNA sequencing. DNA-DNA hybridizations were performed to establish the species affiliation with closest relatives *Azospirillum oryzae* and *Azospirillum zeae*. Genomic %GC was also determined in the process of verifying DNA quality.

For one of the strains the identity check showed it was not *Azospirillum* but *Micrococcus*. It was therefore not studied further. The other two strains were found to be distinct from *Azospirillum zeae*, but the experiments with *Azospirillum oryzae* will be repeated as they were inconclusive. This work will continue in collaboration with Dr. Dharne.
4. During the final month of the project we received two novel *Azospirillum* strains from Dr. Anna Muratova, IBPPM, Saratov, Russia (Partner 8). These strains had been sent via the VKM collection in Pushchino and had gone through the lengthy administrative process to receive all the required permits and approvals. Since they arrived at the end, the work done so far is limited to a purity check and verification of identity by partial 16S rRNA gene sequencing. Results show that both are related to, but distinct from *Azospirillum oryzae*. The characterization of these strains will continue in collaboration with Dr. Muratova.

Among the newly isolated microorganisms from the field survey, 13 strains manifested biofertilizer properties. All of them were capable of growing on nitrogen-free medium, and may be considered as potential nitrogen-fixers. Nine strains were capable of solubilisation of $Ca_3(PO_4)_2$ demonstrating the appearance of clearing zones around the colonies grown on selective agar medium, and may be considered as Pi-solubilizers.
In addition, taxonomic and functional characterizations have been supplemented for some strains with distinct biofertilizer properties. In particular, the taxonomic position of *Ensifer meliloti* P221 that is IAA-producer has been confirmed by 16S rRNA gene sequence analysis. Direct evidence for ability to promote growth of *Medicago sativa* and *Sorghum bicolor* has been obtained for this strain in the pollutant-free and phenanthrene-contaminated environment, respectively. It was shown that the *Sorghum bicolor*–phenanthrene–*Ensifer meliloti* P221 interactions are mediated by bacterial IAA under heavy pollution. *Ensifer meliloti* P221 has indole-3-pyruvate pathway of IAA biosynthesis because both indole-3-pyruvate and indole-3-acetaldehyde are intermediates in the strain's auxin anabolism.
The taxonomic position of other plant-growth-promoting bacteria *Enterobacter cloacae* K7 has been confirmed by 16S-23S rRNA intergenic spacer sequence analysis. This strain possessing such PGP-traits as fixation of atmospheric nitrogen, solubilisation of inorganic phosphates and synthesis of the phytohormone IAA can improve the growth of *Helianthus annuus* L.

2.4. Capacity building.

Partner 2 (UCL) has prepared a protocol for the long-term preservation at ultra-low temperature of AMF grown both in vivo and *in vitro*. This protocol is of upmost importance in culture collections as these organisms needs to be cultured in association with a suitable host plant (they are obligate symbionts). Sub-cultivation (the most common way to preserve these organisms) is time and space-consuming and offers no guarantee of purity and physiological stability. Therefore cryopreservation at ultra-low temperature (-130°C) is a must. The protocol has been published in a review paper in 2014 (Lalaymia I, Cranenbrouck S., Declerck S. (2014). Maintenance and preservation of ectomycorrhizal and arbuscular mycorrhizal fungi. Mycorrhiza 24: 323-337).

In the same paper, a protocol for the long-term preservation at ultra-low temperature (-130 °0C) of ectomycorrhizal fungi was proposed. This protocol was assayed on 100 strains and worked for more than 95% of these.
The protocols for AMF and ECM are nowadays used in MUCL as the unique collection where these organisms can be maintained at -130 °C.

Partner 3 (UGent) has prepared a protocol for the isolation of rhizobia from legume root nodules. This protocol is now available from the project website at http://www.brionet.eu/protocols/

Partner 4 (ART) investigated how mycorrhizal fungi and different mycorrhizal strains influence nutrient cycling. In particular it was tested whether mycorrhizal fungi can I. enhance plant nutrient uptake and II. contribute to the reduction of nutrient leaching losses (e.g. induced by rain). This later aspect is important because mycorrhizal fungi could help to enhance the nutrient use efficiency in (agricultural) ecosystems, a fact that will become increasingly important as phosphorus fertilisers are expected to become depleted in the next 50-200 years and nitrogen fertilisers will become more expensive. First results indicate that mycorrhizal fungi can enhance nutrient use efficiency and thus contribute to the sustainability of farming systems. However, results are soil type dependent. Three publications are in preparation:
1) Bender SF, Conen F and van der Heijden MGA Mycorrhizal effects on nutrient cycling, nutrient leaching and 1 N2O production in experimental grassland (to be submitted)
2) Olbrecht L, van der Heijden MGA. Differential effects of mycorrhizal fungal isolates on plant growth and nutrient leaching (working title)
3) Säle V, Palenzuela J, Azcón-Aguilar C, Sánchez-Castro I, Aguilera P, Seitz B, Silva GA, van der Heijden MGA, Oehl F (2015) Efficacy of arbuscular mycorrhizal fungi for promoting leek (*Allium porrum*) growth (working title).

Furthermore, partner 4 (ART) has isolated about 100 strains of bacteria and fungi from the rhizosphere of clover and from the soil. Currently, experiments are initiated to investigate the ecological function of these isolates (activities as biofertiliser or biopesticides and effects on plant growth). Isolates with activities as biofertilisers or biopesticides will be included in the BRIO database.

One of the bioremediation experts invited by Partner 7 (IEGM) and listed in the online expert list at http://www.brionet.eu/experts/, Prof. Irina A. Arkhipchenko from the All-Russia Research Institute for Agricultural Microbiology, has also biofertilizer specialization.

The taxonomic identification of two rhizosphere strains newly isolated during the field survey in Saratov region is being performed by Partner 3 (UGent) in BCCM/LMG. Within the framework of the collaboration with BCCM/LMG collection Dr. E.V. Dubrovskaya has got training on application of MALDI-TOF mass spectrometry for bacterial identification and classification. Dr. Dubrovskaya has visited UGent from 6 till 13 April 2014. It is expected that cooperation between IBPPM and LMG collections will be continued.

Standard protocols for plant-growth-promoting rhizobacteria and for azospirilla detection and isolation using selective media, preservation and characterization have been set up in view of the common field survey. These protocols will be available in the website.

Metagenomic data.

No metagenomic data from soils are currently available to the partners, or have been analysed for biofertilizer presence or activity.

Database subset of collections used as biofertilisers. Biofertilisers can be selected through the search options developed for the online catalogue accessible at the BRIO webportal http://www.brionet.eu/catalogue/. Updates are made over time, including, when available, links to metagenomic data.

WP3. BIOPESTICIDES AND DISEASES PREVENTION

All partners have surveyed their holdings for strains with biopesticide potential and contributed these to the BRIO catalogue.

The expert group on biopesticides has been organized virtually: experts are listed in an online expert list at http://www.brionet.eu/experts/

Partner 2 (UCL) confirms that the AMF strains documented for biofertilization (see above) also present potential as biopesticide. A limited number of strains documented in the data base have been specifically and extensively documented and tested for their bioprotection effects against *Rhizoctonia solani* and *Phytophthora infestans*, two major pathogens in potato and against nematodes (*Radopholus similis*, *Meloidogyne* spp. and *pratylenchus goodeyi*) and phytopathogenous fungi (C*ylindrocladium spathiphylli*) in banana. About 10 papers have been published on these organisms by the laboratory of mycology in the past few years (see selected papers below). Interestingly the AMF strain used is also the only strain of AMF which is completed sequenced.

The laboratory of mycology is leading a national programme on the utilization of filamentous fungi, yeasts and arbuscular mycorrhizal fungi on the control of major plant pathogens. This project is first focused on potato and *Phytophthora infestans*. 150 fungi were tested for their biocontrol properties against fungi plant pathogens. 92 presented strong inhibition towards *P. infestans.* Interestingly 8 strains were particularly efficient (*Chaetomium globosum, Emericella nidulans, Gliocladium catenulatum, Hypomyces rosellus, Lecanicillium dimorphum, Penicillium aurantiogriseum, Talaromyces flavus,Trichoderma Virens*). 4 strains inhibit *P. infestans* with the fungal extract (*Clavispora reshetovae*, *Phytophthora parasitica*, *Saccharomyces cerevisiae*, *Ustilago maydis*). These strains will be included in the BRIO catalogue.

Partner 3 (UGent) has documented its strain holdings for strains used in applications in the areas of biopesticides or disease prevention. So far there are 34 strains with documented biocontrol properties that are available at the LMG collection and that have now been included in the BRIO catalogue. Of these 34 strains, two strains have anti-nematode properties, while 30 strains have antifungal properties, and two strains have antibacterial properties.

For partner 4 (ART), the Swiss Collection of Arbuscular mycorrhizal fungi (SAF), entered the WFCC data base (WDCM, World Data Centre for Microorganisms, Culture Collections World Wide) under the registered number 1032: http://www.wfcc.info/ccinfo/collection/by_id/1032. Until April 2014, 213 AM fungal isolates, comprising 21 AM fungal species, have been registered and maintained in SAF, respectively. Another major progress was achieved in February 2014: SAF went online at Agroscope homepage (http://www.agroscope.admin.ch/grandes-cultures-systemes-pastoraux/05911/07581/index.html) with 132 available isolates comprising the 21 AM fungal

species. These 132 isolates accordingly entered the BRIO catalogue. One formerly undescribed AM fungal species, *Glomus compressum*, was published online on 3.6.2014, and could be added to the SAF collection (SAF203):

Forty-eight of the SAF isolates had been tested with six replicates per treatment for their potential to suppress three agriculturally important weeds (*Echinochloa grus-galli*, *Solanum nigrum* and *Papaver rhoeas*). Up to three isolates per AM fungal species were screened including 11 AMF genera and about 20 AM fungal species, e.g. *Gigaspora margarita*, *Cetraspora helvetiva*, *Scutellospora calospora*, *Funneliformis mosseae*, *Fu. caledonius*, *Fu. geosporus*, *Glomus compressum*, *Gl. diaphanum*, *Gl. intrradices*, *Gl. invermaium*, *Setoglomus constrictum*, *Claroideoglomus claroideum*, *Entrophospora infrequens*, *Diversispora celata*, *Di. epigaea*, *Archaeospora trappei* and *Paraglomus laccatum*. In earlier work with the same plant species, but in a different soil, we observed that the AM fungus *Rhizophagus irregularis* suppressed growth of these three weeds (Veiga et al. 2012). However, none of the tested AM fungal isolates have shown a potential to suppress any of these three weed species. On the other hand, about half of the isolates significantly improved plant growth of *Allium porrum;* these isolates were of the family Glomeraceae. Thus, these isolates have potential as biofertiliser but their importance as biopesticide is unclear and probably not strong enough for application. The first 15 isolates, generated at Agroscope have been sequenced on the ribosomal gene by Oehl and co-workers in the frame of a Swiss project in April 2014. The data will be continuously added to the BRIO catalogue within the next months after publication of this AMF-*Allium porrum* screening study (tasks 3.4 and 3.6). The publication about the growth response of *A. porrum* is in preparation (see above).

Two additional isolates from another fungal collection at Agroscope were added to the BRIO catalogue that are registered in Switzerland as biopesticides, INH Myc 8 (*Beauveria brongniartii*) and INH Myc 36 (*Metarhizium anisopliae*). These isolates are on the Swiss markets under the product names Beauveria-Schweizer and Metarhizium Schweizer (see BRIO catalogue). However, no correlation can so far be made between the biogeography of the pests (the harmful insects *Melolontha melolontha* and *Agriotes* spp.) and the two biopesticides controlling these pests (task 3.5).

Partner 5 (UNITO) selected 4 fungal strains with documented biocontrol properties that are available at the MUT collection and that have now been included in the BRIO catalogue. One of these strains has anti-nematode properties, while three strains have antifungal properties.

Partner 6 (VKM) selected more then 50 strains with documented or potential biocontrol properties that have been included in the BRIO catalogue. These encompass *Streptomyces* strains with high insecticide activities against spider mites, mosquito larvae and flies. The effective biocontrol activity against peach aphid and greenhouse whitefly was shown for *Streptomyces* sp. VKM Ac-2626 (water extract of mycelium). Some actinobacteria have antifungal activity against phytopathogenic fungi (*Fusarium moniliforme, Fusarium oxysporum, Drechslera sorokiniana, Puccinia albescens).* Antifungal activities was also shown for some yeast strains (*Cryptococcus* spp.) that produce 'micocins'. Several bacterial strains presumably assigned to a new non-pathogenic subspecies of *Clavibacter michiganensis* and two strains from plant pathogenic species/subspecies (*Rathaibacter tritici* and *Clavibacter mihiganensis* subsp. *insidiosus*) showed antagonism towards many plant pathogenic actinobacteria (genera *Clavibacter, Curtobacterium, Rathayibacter*) due to production of antibacterial agents ('bacteriocins'). Some above actinobacterial strains from rhizosphere and plant tissues (e.g., representatives of *Clavibacter, Curtobacterium,*

Microbacterium) may also be biofertilizers. Members of these taxa from plant microbiomes are well known to produce phytohormone auxins (controlling plant growth and tissue differentiation), 1-aminocyclopropane-1-carboxylate deaminase (modulating ethylene levels in plants), facilitate the uptake of certain nutrients from the soil, etc.

Partner 7 (IEGM) has reviewed its collection holdings for actinobacterial strains with potential applications in the areas of biopesticides or disease prevention. This work is on-going, and *Rhodococcus erythropolis* strains with documented *N*-acyl homoserine lactone degrading activity will be included in the catalogue.

Partner 8 (IBPPM) has reviewed its strain holdings for strains which can be used in applications in the areas of biopesticides or disease prevention. There are 4 strains with documented biocontrol properties that are available at the IBPPM collection and they are included in the BRIO catalogue.

WP4. BIOREMEDIATION

4.1 Taxonomic and functional characterization of rhizospheric microorganisms from polluted environments

All partners have surveyed their holdings for strains with bioremediation potential and contributed to the BRIO catalogue. In detail, 291 strains of microorganisms belonging to different genera of bacteria, yeasts, and filamentous fungi have been included in the catalogue.

The expert group on bioremediation has been organized virtually: experts are listed in an online expert list at http://www.brionet.eu/experts/

Partner 7 (IEGM) has studied taxonomic and functional characteristics of isolated strains of actinobacteria (mentioned above in WP1), such as oxygenase activity, resistance to heavy metal salts, biodegrading activities towards individual hydrocarbons, crude oil and oil products, and other pollutants (nitrogen-containing heterocyclic compounds, phenol derivatives, etc.). Young researcher Dr. Anastasiia Krivoruchko visited Partner 3 (UGent) for MALDI-TOF characterisation of actinobacteria in November 2013. These results were used to confirm the taxonomic status of actinobacteria isolated from contaminated soils. Particular gene systems for degradation of aromatic and polycyclic hydrocarbons were revealed and corresponding metabolic pathways were described in newly isolated representatives of *Rhodococcus erythropolis* and *Rhodococcus ruber*. Relevant information on strain properties useful for bioremediation was included into the BRIO catalogue.

The work on to assessment which strains from the collection holdings have applications in the area of bioremediation is on-going. The strains with documented applications will be included in the BRIO catalogue.
Bioremediation properties were found among 9 newly isolated strains. Degradative activity toward crude oil (21 to 51% for 7 days) has been shown for 5 of them by determining the residual oil concentration in the culture medium. One strain was able to utilize phenanthrene according to Kiyohara plate test.
In addition, taxonomic and functional characterizations have been supplemented for some strains with distinct pollutant-degrading properties. In particular, the taxonomic position of

Ensifer meliloti P221 that is PAH-degrader has been confirmed by 16S rRNA gene sequence analysis. This microorganism is capable of bioconversion of phenanthrene via two parallel pathways. The first, major pathway is through terminal aromatic ring cleavage (presumably at the C3-C4 bond) producing benzocoumarin and 1-hydroxy-2-naphthoic acid, whose further destruction with the formation of salicylic acid is difficult or is very slow. The second pathway is through the oxidation of the central aromatic ring at the C9-C10 bond, producing 9,10-dihydro-9,10-dihydroxyphenanthrene, 9,10-phenanthrenequinone, and 2,2'-diphenic acid. This is the first time that the dioxygenation of phenanthrene at the C9 and C10 atoms, proven by identification of characteristic metabolites has been reported for a bacterium of the *Ensifer* genus.
In addition, it was established that *Ensifer meliloti* P221 has the phnJ gene, encoding the key catalytic component of the C–P lyase multi-enzyme system, which is used by bacteria to utilize phosphonates.
The taxonomic position of *Enterobacter cloacae* K7 degrading the organophosphorus herbicide glyphosate has been confirmed by 16S-23S rRNA intergenic spacer sequence analysis. It was established that the catabolism of glyphosate by *Enterobacter cloacae* K7 is carried out via sarcosine, which is then oxidized to glycine.
In the course of screening studies four new strains manifesting tolerance to Cd^{2+} have been isolated from rhizosphere of heavy metals accumulating plants *Solanum nigrum* and *Bidens tripartite*. These strains are tolerance to 0.2-1.0 mM Cd^{2+}. Preliminary identification of them showed that two of them are Gram-positive spore-forming rods and the third one is Gram-positive non spore making rods.
Standard protocols for isolation of PAH-degrading rhizospheric microorganisms using selective media, for preservation and characterization have been set up. These protocols will be available in the website.

4.2 Evaluation of the ecologically significant factors limiting the bioremediation processes

Partner 5 (UNITO) has characterized and identified several strains of fungi and bacteria with the objective to further enrich its collection or at the request of other partners. Il detail, during the stage carried out by Dr. Maria Kuyukina in December 2013, several strains of *Rhodococcus* belonging to the IEGM collection have been characterized for the capability to produce oxidative enzymes i.e. laccases and peroxidases and 29 fungal strains isolated from polluted soil and able to degrade organopollutants have been identified by means of morphological and molecular tools at species level. Moreover, 4 fungal strains isolated from Russian polluted soil and preserved at the IBPPM, Saratov where identified at species level by the Russian team that took advantages of different protocols kindly provided by MUT.

During the whole period of the project Participant 8 together with Scientific Production Association "Ecosphere" Ltd. conducted field surveys of oil-contaminated sites in the Saratov region. As a result of monitoring the evaluation of the ecologically significant factors limiting the bioremediation process have been done. It was found that the most significant factor limiting the bioremediation process in the Saratov region is wet, because this region is in the arid zone. Its soils are mainly chernozems (on the right banks of the Volga River – ordinary, southern, and typical chernozems) and chestnut soils (on the left banks of the Volga River) and are characterized by a large buffer capacity and a high number of microorganisms.

Field observations and isolation of strains degrading petroleum hydrocarbons from soils of Saratov region allow us to conclude that these soils have a high potential for self-healing: the number of hydrocarbon-oxidizing microorganisms reaches 10^5 (in bulk soil) and 10^6 (in rhizosphere soil) cells per gram. Phytoremediation or biostimulation are often preferable bioremediation approaches, excluding introduction of active biopreparations, and aimed at stimulating indigenous pollutant degrading populations. Monitoring of plant species most frequently encountered in oil-polluted areas allowed us to identify promising for phytoremediation plants such as *Medicago sativa*, *Medicago falcate*, *Lolium perenne*, *Calamagrostis epigeios*, *Sorghum bicolor*. Analysis of prevalence of rhizospheric hydrocarbon-degrading microorganisms allowed us to identify the plant species that support the highest population of these microorganisms in their root zone: *Calamagrostis epigeios, Medicago sativa*, *Medicago falcate*, *Phragmites australis*, *Atriplex tatarica*.

Capacity building.

Partner 5 (UNITO) has prepared 2 protocols for the isolation and detection of filamentous fungi potentially involved in soli bioremediation. Partner 7 (IEGM) has prepared 2 protocols about the isolation of PHA-degrading and surfactant degrading microorganisms. These protocols are now available from the project website at http://www.brionet.eu/protocols/

Partner 3 (UGent) has hosted a researcher from partner 7 (IEGM), Dr. Anastasiia Krivoruchko, twice for training in MALDI-TOF characterisation of bacteria (13 November – 5 December 2012 and Nov. 2013). This work contributed to the characterization and identification of rhodococci from contaminated soils.

4.3 Development of rhizosphere-enhanced bioremediation associations for multi-functional remediation.

Partner 2 (UCL) has a number of AMF listed in the catalogue which offer special interest for bioremediation of pollutants such as hydrocarbons.

Partner 2 (UCL) is also leading a national initiative on the isolation, identification and application of arbuscular mycorrhizal fungi and other filamentous fungi from the Yasuni national park in Ecuador. This park is a hotspot of diversity but is also exploited for hydrocarbons. In this project, partner 2 (UCL) is sampling polluted soils for fungi. Several species has been isolated and are in the process of identification. In a further step, these strains will be tested for bioremediation and included in the BRIO catalogue.

During the final month of the project the MUT team received one fungal strain from the IEGM team, Perm, Russia. These strains had been isolated as “contaminants” from a trials devoted to identify bacteria able to degrade pharmaceutical organopollutants. The Russian isolated revealed to be a “fungal consortium” of two species endowed of bioremediation capabilities: *Trametes versicolor* and *Aureobasidium pullulans*. The two strains are currently under evaluation at IEGM to assess their potential in bioremediation and their possible cooperation with bacterial strains.
Partner 7 (IEGM) has developed actinobacterial associations based on IEGM collection strains suitable for oil-contaminated soil and water bioremediation. One association consisted of *Rhodococcus erythropolis* IEGM 275 and *Rhodococcus ruber* IEGM 231 was tested in laboratory soil microcosms and provided an effective reduction in oil contamination under

cold conditions. The association developed was recommended for multifunctional remediation (restoring all natural functions of soil) of oil-contaminated soils in cold climate regions (Urals, Russia) and is currently field tested by the Priroda-Perm company under the joint demonstration project on field-scale bioremediation. This demonstration project is aimed to evaluate the efficacy of bioremediation using a selected bacterial association, and to demonstrate its advantages to SMEs and large industrial companies dealing with the oil-contaminated soil clean-up. Another actinobacterial association of *Rhodococcus opacus* IEGM 275 and *Rhodococcus ruber* IEGM 231 was developed for oil-contaminated wastewater purification and is currently under laboratory bioreactor testing.

Partner 7 (IEGM) has also studied actinobacterial associations made of the IEGM strains able to degrade pharmaceutical pollutants, in particular codeine-containing waste, to be used in the wastewater treatment process. During laboratory trials, an air-born fungus culture was isolated from the fermentation medium, which was able to efficiently degrade codeine. This culture was sent to Partner 5 (UNITO) for taxonomic characterization and deposition in the MUT collection. Partners 5 and 7 intend to curry out further collaboration research using selected actinobacterial and fungal cultures to develop associations useful for pharmaceutical wastewater purification and contaminated site bioremediation.

Some strains from Partner 8 (IBPPM) collection were studied on their capacity to improved phytoremediation effectiveness. Rhizosphere-enhanced plant-microbial associations for phytoremediation of contaminated soil were developed.
For PAH-contaminated soil: *Medicago sativa – Sinorhizobium meliloti* P221 (IBPPM 383) and *Sorghum bicolor – Sinorhizobium meliloti* P221 (IBPPM 383).
For petroleum-contaminated soil: *Secale cereale – Azospirillum brasilense* SR80 (IBPPM 24) and *Lolium perenne – Azospirillum brasilense* SR80 (IBPPM 24).
For glyphosate-contaminated soil: *Heianthus annuus – Enterobacter cloacae* K7 (IBPPM 476).
For soil contaminated with Pb, Cd, Ni and As *Sorghum sudanense – Aeromonas* sp. MG3 (IBPPM 462).

To detect *Ensifer meliloti* P221 in hydrocarbon-contaminated soils immunochemical approaches on the basis polyclonal rabbit antibodies specific to the strain's surface carbohydrate antigens were developed. Dynamics of in situ detection of introduced strain's antigens was estimated with using optimized ELISA. In oil-sludge-contaminated soil the number of strain P221 was highest in ryegrass rhizosphere. In diesel fuel-contaminated soil strain P221 survived successfully both in ryegrass and sorghum rhizospheres. With above mentioned approach it was found that *Sorghum bicolor* supported selectively the development of the *Ensifer meliloti* P221 population in PAH-contaminated root zone.
This immunochemical method was also applied for detection of oil-degrading *Diezia maris* AM3 population in hydrocarbon-contaminated soil.
The taxonomic position of other plant-growth-promoting bacteria *Enterobacter cloacae* K7 has been confirmed by 16S-23S rRNA intergenic spacer sequence analysis. This strain possessing such PGP-traits as fixation of atmospheric nitrogen, solubilisation of inorganic phosphates and synthesis of the phytohormone IAA can improve the growth of *Helianthus annuus* L. It was established that the catabolism of glyphosate by *Enterobacter cloacae* K7 is carried out via sarcosine, which is then oxidized to glycine. Mini-antibodies specific to strain K7 were obtained using affine selection from combinatorial phage library of sheep's antibodies. Possibility for application of these mini-antibodies was demonstrated to detect these bacteria in the root zone.

IBPPM collection has been enhanced by five fungal strains with ligninolytic enzymes xenobiotic-degrading activities. These strains have been isolated in Saratov region during the field survey. These strains were identified as *Schizophyllum commune* IBPPM 541, *Pleurotus ostreatus* f. *florida* IBPPM 540, *Fusarium oxysporum* IBPPM 543, *Geotrichum candidum* MUT 4803 and *Cladosporium herbarum* MUT 3238 in MUT Partner 5(UNITO) within the framework of collaboration with Dr. Cristina Varese.

4.4. Metagenomic data.

No metagenomic data from soils are currently available to the partners, or have been analysed for biofertilizer presence or activity.

Oil-contaminated soil and rhizosphere samples taken during the common field survey in Perm were cryopreserved by Partner 7 (IEGM) for future metagenomic studies. Also, total bacterial DNA isolated from 2 fresh soil and 2 rhizosphere samples was cryopreserved and tested by direct PCR using species-specific primers designed for ecologically significant hydrocarbon-oxidizing *Rhodococcus* species.

Database subset of IBPPM collection used for bioremediation includes: 34 oil-degrading strains; 12 PAH-degrading strains; 8 synthetic surfactant-degrading strains; 10 heavy metals resistant strains and 10 phosphonates-resistant or degrading strains.

The BRIO catalogue available at http://www.brionet.eu/catalogue/ contains several hundred strains isolated from polluted sites and potentially useful for bioremediation. For each strain, the information on contaminated substrate and geographic origin is provided. These strains can be searched through the BRIO catalogue for bioremediation properties or found using the ***taxon browser*** or the ***strain properties browser***.

V. IMPACT

V.1 CONNECTIONS AND COLLABORATIONS

V.1.1 BRIO objective 1

To organize cooperation between specialised collections. BRIO aims at establishing **long term links and synergies between major Russian and EU Biological Resources Centres.**

BRIO not only contributes to increase the synergy and to foster collaborative efforts within the group of BRIO, but also acts as an initial core around which complementary efforts crystallize and develop into further collaborative efforts. It opens new perspectives of collaboration between Russian and EU teams who will access the resources of and collaborate with the Pan-European Rhizosphere resources Network (PERN).

V.1.2 Outcomes related to objective 1, direct and future impact

PERN - Core group of the network

BRIO partners have networked to establish the PERN - **Pan-European Rhizosphere resources Network.**

Connections and collaborations with global activities

- Via the coordinator (SPP-PS) and Russian partner 6 (VKM) connections have been established with the World Data Centre for Microorganisms (WDCM) in the field of bioinformatics.

- All partners are registered in the World Federation for Culture Collections (WFCC). All partners also join the Global Catalogue of Microorganisms for connecting catalogue into one global portal.

- Via the coordination (SPP-PS), partners have incorporated the developments of the TRUST (1) initiative related to the implementation of the Nagoya Protocol in the acquisition and distribution policy (Accession & Transfer). It included the organization of an intermediary *ad hoc* meeting of June 2013 in Moscow were approved by the participants.

- Connection with the European Infrastructure MIRRI - Microbial Resource Research Infrastructure is ensured via the participation of BRIO partners VKM, UGent and SPP-PS to the MIRRI preparatory phase.

V.2 WINDOW TO THE RHIZOSPHERE RESEARCH & INNOVATION COMMUNITY

V.2.1 BRIO objective 2

To constitute a common pool of micro-biodiversity exploitable for research and industry: the **Pan-European Rhizosphere resources Network (PERN)**.

BRIO participants are associated to create a pool of common rhizosphere microbial resources, related data and human expertise. They manage a one-stop shop HUB making

these resources available for scientists. Putting together these material, data and expertise facilitates multidisciplinary research.

The focus is laid on biofertilisers, biopesticides and bioremediation to help scientists to cope with challenges in agriculture, forestry and environment under changing climate conditions. Facilitating the access to and the use of well documented, quality micro-organisms of the rhizosphere fosters the exploitation of renewable biological resources, a major objective of the European Knowledge Based Bio-Economy (2).

V.2.2 Outcomes related to objective 2, direct and future impact

The project impacts on cumulative cutting-edge research via:

1. Collaboration in scientific research:
- development of long-term links between Russian and other partners beyond the project life;
- development of collaborative synergies with industries to help develop eco-efficient products using rhizosphere micro-organisms and communities in promoting plant growth, suppressing soil borne disease and priming plant biotic defences.

2. Conservation of the natural resources:
- underpinning cumulative research by ensuring long term preservation of the microbial components of the rhizosphere in reputed West European and Russian collections;
- use the expertise of PERN partners for management of fungal and bacterial germplasm collections;
- providing soil microbial micro-flora for R&D;
- linking the consortium with industry for transfer of technology.

PERN & website

Conceived as a resources hub, according the OECD recommendations (3)

(a) PERN ensures conservation of micro-organisms associated in the plant-microbes-soil systems, facilitating further use by scientists from academies and enterprises (including SME) in EU and Russia.
=>The website host a searchable catalogue

(b) PERN contributes to research on better understanding of rhizosphere diversity and its effect on soil fertility and suppression of pathogens. That will generate research outcomes useful for the development of eco-efficient technologies and management practices, reducing the adverse effects of human activities on the environment.
=> The website posts article relevant to the rhizosphere study.

(c) PERN shares knowledge and strengthen capacities in the area of upstream and downstream research in soil microbiology. It organises and generates resources and knowledge applicable globally. It promote exploitation of the beneficial properties of rhizosphere micro-organisms For instance, oil-polluted areas are a problem encountered in all continents, BRIO partners and thus PERN members have experience in different part of the globe, in different eco-systems, under different climatic and edaphic conditions. Combining expertise and comparing solutions applied for particular situation brings innovative approach. PERN members are scanning the possibilities for funding and partnership to further extent the impact of the newly developed tools..
=> The website proposes lists of experts in the three core field of application: bio-remediation, bio-fertilization, and bio-control.

In summary the project outcomes have been incorporated into the website. They constitute the structure and the contents of the website.
As explained in the project activity report, website update and maintenance, including catalogue database optimization is ensured by Russian partner VKM and coordinator SPP-Ps with the voluntary contribution of the PERN members.

V.3 CONTRIBUTION TO THE EU POLICY AND OBJECTIVES IN R& I.

PERN participates in the exploration and subsequent exploitation of renewable biological resources (i.e. micro-organisms, plants and animals), contributing to the European knowledge based bio-economy.

BRIO will further contribute to the commitments of the EU by conforming to the Convention of Biological Diversity (CBD) and the **Nagoya Protocol**, more particularly in the implementation of the **EU Regulation 511/2014** (4).
PERN hosts EU and Russian world-classes international culture collections which follow these rules and more particularly the recommendations of the EU funded code of conduct MOSAICC (5) and the guidelines of the TRUST system.

VI. DISSEMINATION AND EXPLOITATION OF PROJECT RESULTS

Website and online catalogue. Since one major objective of BRIO is to provide for publicly available rhizosphere resources, the project website is the central instrument to provide access to the projects results, mainly via the **online catalogue of strains**, and also through complementary sources of information and links.

The online catalogue is the basis instrument for exploitation of the project results since it will allow users to access well-documented technically and legally fit-for-use rhizosphere microbial resources and related information.

A part of the website, the project Intranet serves as internal communication only accessible to partners and the European Commission.

Contacts with private companies have been made, more specifically concerning existing applications in the fields of biofertilisers, biopesticides and bioremediation. Contacts are maintained via the PERN members who usually collaborate with them.

Contacts with other Russian resources centres are ongoing. the Russian members of PERN are actively promoting the necessary structuration of the Russian network of microbial collections. Depending on available funding and opportunities PERN partners will invite other research teams to joint activities as in a capacity building effort.

Other activities include participation to workshop, training sessions. It is worth noting that although not scheduled in the project life, training sessions have been already organised by taking advantages of the travel of the PERN members to BRIO meeting or to other conference.

Fair and equitable sharing of benefits

Since this project is by essence cross-border and focused on microbial diversity resources it has to abide to the provisions of the Convention of Biological Diversity (CBD) and the Nagoya Protocol. Like already explained in the previous section of the report, the acquisition and distribution policy designed to be common to the PERN members design a non-discriminatory framework for the access to genetic resources, and for the fair and equitable sharing of the benefits arising out of their utilization.

This sharing of benefits can be monetary and/or preferably non-monetary, directed in such way as to promote conservation and sustainable use of microbial resources of the rhizosphere. Therefore it will follow the recommendations of TRUST and EU Regulation 511/2014.

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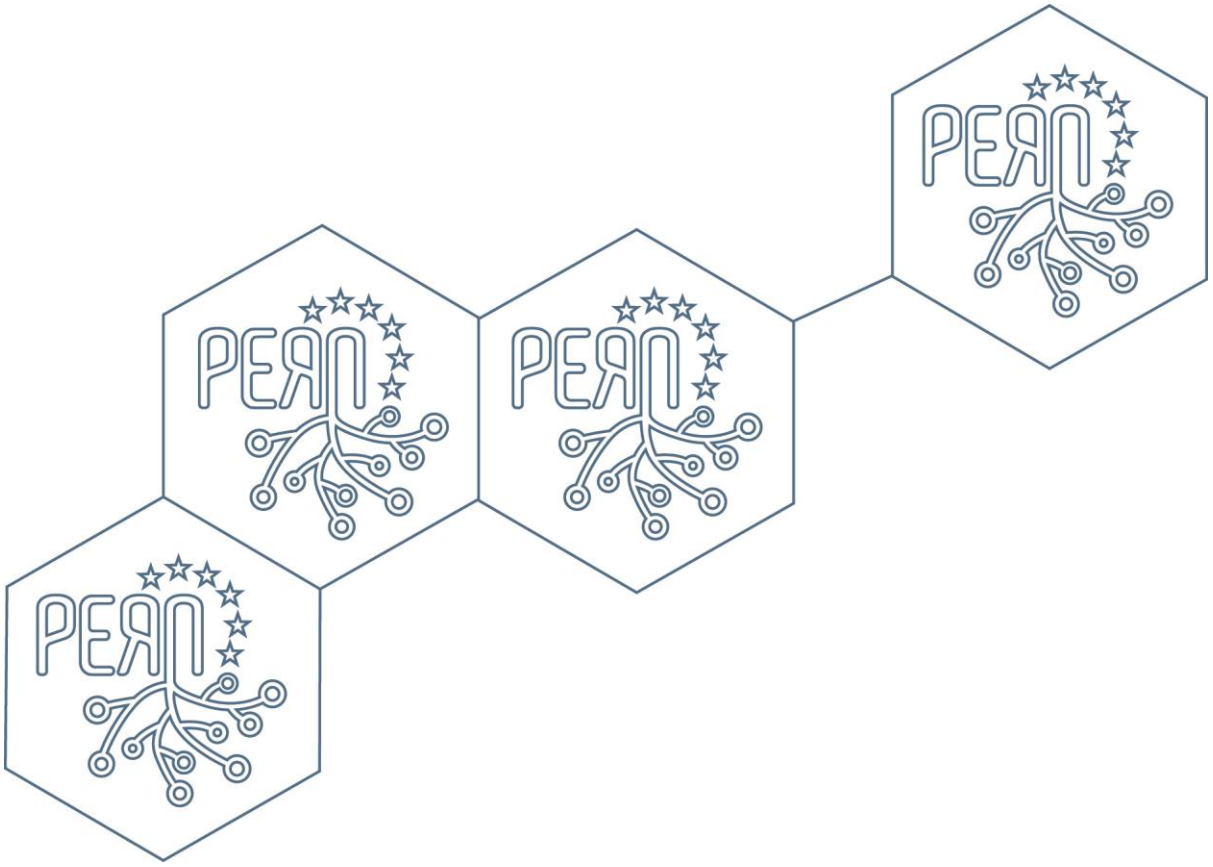

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(1) TRUST stand for Transparent User-friendly System of Transfer
see http://bccm.belspo.be/projects/trust

(2) Bio-economy is defined as that part of economic activities "which captures the latent value in biological processes and renewable bio-resources to produce improved health and sustainable growth and development".

(3) See recommendations in document: OECD Best Practice Guidelines for Biological resource Centre, 2007, OECD, Paris. http://www.oecd.org/dataoecd/6/27/38778261.pdf

(4) Regulation (EU) No 511/2014 of the European Parliament and of the Council of 16 April 2014 on compliance measures for users from the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization in the Union

(5) MOSAICC stands for "Micro-organisms Sustainable use and Access regulation International Code of Conduct". It is a voluntary Code of Conduct to support the implementation of the Convention on Biological Diversity (CBD, Rio de Janeiro, 5 June 1992) at the microbial level, in accordance with other relevant rules of international and national laws
See http://www.cbd.int/abs/instruments/ or http://bccm.belspo.be/projects/mosaicc.