European Cooperation
in Science and Technology
- COST -

Secretariat

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COST 4126/11

MEMORANDUM OF UNDERSTANDING

Subject :	Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action FA1103**:** Endophytes in Biotechnology and Agriculture

Delegations will find attached the Memorandum of Understanding for COST Action FA1103 as approved by the COST Committee of Senior Officials (CSO) at its 182nd meeting on 17 May 2011.

MEMORANDUM OF UNDERSTANDING

For the implementation of a European Concerted Research Action designated as

COST Action FA1103

ENDOPHYTES IN BIOTECHNOLOGY AND AGRICULTURE.

The Parties to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above and described in the technical Annex to the Memorandum, have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 4154/11 Rules and Procedures for Implementing COST Actions, or in any new document amending or replacing it, the contents of which the Parties are fully aware of.

2. The main objective of the Action is to identify bottlenecks limiting the use of endophytes in biotechnology and agriculture and to provide solutions for the economically and ecologically compatible exploitation of endophytes.

3. The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 52 Million in 2011 prices.

4. The Memorandum of Understanding will take effect on being accepted by at least five Parties.

5. The Memorandum of Understanding will remain in force for a period of 4 years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of Chapter IV of the document referred to in Point 1 above.

A. ABSTRACT AND KEYWORDS

Plants are associated with micro- and nanoorganisms: Endophytic bacteria and fungi, which live inter- and intracellularly in plants without inducing pathogenic symptoms, interact with the host biochemically and genetically. Endophytic microorganisms (EMOs) may function as plant growth and defense promoters by synthesising phytohormones, producing biosurfactants, enzymes or precursors for secondary plant metabolites, fixing atmospheric nitrogen and CO_2 or control plant diseases as well as providing a source for new bioactive natural products with utility in pharmaceutical, agrochemical and other LifeScience applications. The use of these EMOs to control plant-pathogenic bacteria and fungi is receiving increasing attention as a sustainable alternative to synthetic pesticides and antibiotics. Furthermore, these EMOs are likely to be adapted to the presence and metabolism of complex organic molecules and therefore show useful biodegradation activities. In order to reduce the input of pesticides and fertilizers and to bring European added value to eco-friendly agriculture, it will be important to develop inocula of biofertilizers, stress protection and biocontrol agents. The aim of the Action is to identify bottlenecks limiting the use of endophytes in biotechnology and agriculture and to provide solutions for the economically and ecologically compatible exploitation of endophytes.

Keywords: Endophytes, plant growth promoters, secondary metabolites, agriculture, plant-microbe-interaction

B. BACKGROUND

B.1 General background

Plants should be considered as super- rather than single organisms, because they are associated with micro- and nanoorganisms within their cells and tissues. Endophytic bacteria and fungi, which live inter- and intracellularly in plants without inducing pathogenic symptoms, while being investigated, interact with the host biochemically and genetically. They show a range of properties playing a mostly unknown, but important role in plant production, including promoting plant growth, improving of nutrient uptake, increasing stress tolerance, antagonizing plant pathogens and inducing of systemic resistance. Furthermore they exhibit a huge potential for biotechnical application as a source for bioactive enzymes and secondary metabolites (SM). Concerning plant production, many experiments in the past were carried out with focus on the existence and negative influence of endophytic microorganisms (EMOs) in tissue culture and forage plants. However, within the last few years it became obvious that some endophytic bacteria and fungi can be beneficial for growth and development of plants, so-called competent endophytes. Based on this knowledge, first attempts were made to isolate, cultivate and characterize some of these strains.

Currently, EMOs are thought to play an important role in the adaptation of plants to different environments. They may act as plant growth promoters by synthesising phytohormones, producing biosurfactants or enzymes, fixing atmospheric nitrogen or controlling plant diseases. The use of EMOs to control plant-pathogenic bacteria and fungi is receiving increasing attention as sustainable alternative to synthetic pesticides. In addition, endophytes have been shown to improve phytoremediation of xenobiotics. Endophytes contribute to the fixation of atmospheric CO_2 as they may increase its fixation by plants. It has been even postulated that axenic plants suffer from low stress tolerance due to the absence of endophytes. Furthermore, these EMOs are likely to be adapted to the presence and metabolism of complex organic molecules and therefore show useful biodegradation activities.

Development of proper inocula of EMOs as biofertilizers and biocontrol agents of plant pathogens is crucial for reduction of pesticides and hazardous fertilizer use. This will undoubtedly entail progress of eco-friendly agriculture in Europe. The market for these bioinoculants is not yet developed in Europe compared to North America. Their role in agriculture, especially crop production and germplasm preservation, remains underestimated. New cultivation protocols are necessary, because the majority of all recognized endophytes is currently non-cultivable. However, although several studies have highlighted the evident effects provided by individual microbial partners, still little is known about the function of endophytic assemblages in overall plant performance.

The COST Action will combine activities of different European laboratories to compare protocols and technologies for the analysis and utilization of bacterial and fungal endophytes, mainly for plant production, but also as sources for other applications. For this multi- and interdisciplinary approach the COST framework is ideal.

B.2 Current state of knowledge

Studies on species diversity of endophytic bacteria have been mainly approached by cultivation-based methods. Investigations which applied cultivation-independent approaches showed that individual plants are colonized by phylogenetically very different bacteria and that only a subset of endophytes is accessible by cultivation. Endophytic fungi are ubiquitous in all plants studied to date and considered one of the planet’s most hyperdiverse groups of organisms. Cultivation independent studies of fungal endophytes also show a similar divergence between culturable and non culturable forms; however, the relative contribution of technical versus truly biologically cause remains ambiguous.

EMOs form an intimate relationship with the host plant which provides a protected ecological niche. However, an inoculant strain has to survive in the plant’s interior, and furthermore it must effectively compete with existing endophytic microbiota. The composition of the bacterial and fungal community usually differs among different host species, among geographically distinct growing individuals of the same host species, and among different tissues or organs within a host plant individual.

As the majority of endophytic bacteria probably originally derive from the rhizosphere, their diversity is determined by the soil in which the plant grows, but also depends on the plant genotype. This is much different from endophytic fungi, which can be disposed by wind or insect vectors as well. Obviously, the ecology of these organisms and their interaction with plants has to be understood in order to exploit their potential to be used in agriculture and environmental protection.

Numerous EMOs have been obtained up to now, by surface sterilisation of healthy plant tissues and plating on different media. After separation from their plant host, some isolates exhibit vigorous growth *in vitro* under conditions amenable for successful scale up. Characteristics of endophyte competence have first been tested in mechanistic *in vitro* models. Examples of such assays included N_2 fixation by bacterial endophytes or organic phosphate solubilisation. For more than 20 years, pharmaceutical and agrochemical screening programs have identified many potent antifungal, antibacterial, and insecticidal metabolites from endophytic fungi. For example, *Neotyphodium* and *Epichloë* endophytes produce antiherbivore alkaloids which protect plants, and these fungi are increasingly being used by the seed industry for the improvement of forage and turf grass cultivars.

The application of microorganisms (MO) in sustainable plant production systems and their capacity for biosynthesis of new compounds have been mentioned in a number of calls of EU frameworks and is also a topic in different EU fora. However, the explicit identification and use of MOs living inside plant tissues has not been emphasized.

Most research aimed at identifying endophytes has so far been directed towards fungi. Simple methods can assign unknown isolates that have either been isolated or detected *in planta* by PCR-based methods, to a suprageneric taxon (family, order). In those cases where reliable reference data have become available due to polyphasic taxonomic studies, the fungal isolates can even be identified to genus or species level by means of comparing their 5.8S/ITS nrDNA sequences. According to estimates, approx. 10 % of the fungal endophytes which DNA sequences are recorded in GenBank may even belong to yet undiscovered genera or families.

Different primer pairs (from ITS region and small and large subunit of ribosomal DNA) are used to amplify marker genes of fungi and bacteria, but unfortunately they do not work efficiently for amplification of endophytic DNA in plant material, resulting in false positive signals in PCR analysis. Therefore, more specific primer pairs for DNA of EMOs in plants are needed.

Although some endophytes can be characterised solely by molecular biology, *in vitro* cultivation is still essential to understand their physiology and discover new applications. Some are easily cultured on synthetic or complex media, including complex media with host plant components. Nevertheless, more effective culturing media and techniques are needed to fully explore the potential of microbial endophytes.

Plant's response to bacterial quorum signals (Acyl-Homoserine-Lactone, AHL) includes induction of glutathione S-transferases and peroxidases, thus moderate stress reaction. The nature of the signalling, and the extent of plant reactions are more or less unknown, but endophytic colonisation may positively mediate agrochemical tolerance in crops and phytoremediation.

Different stress conditions have been used to screen for endophytes able to increase the tolerance or the resistance against stressful environmental parameters. Under nutrient deficient conditions, biofertilizing fungi have been identified that were able to bridge the roots' depletion zone for low-mobility mineral nutrients such as phosphate. Others could help the plant to overcome drought or high salinity. Most of the investigations, however, have targeted endophyte-induced systemic resistance against different pathogens. However, field trials, in most cases, have failed until now to show significant efficiency and reproducibility to merit transfer into cultural practice.

Several reports also indicate that endophytic fungi can functionally replace or support mycorrhizal fungi under conditions where they are unable to grow. It has been shown that some endophytes can confer tolerance to stress to their host plants, and are responsible for their survival in environments under conditions of high selective pressure. The ability of the endophytic fungi to confer stress tolerance may provide a new strategy to mitigate the impacts of global climate change on agriculture and natural plant communities. This can be achieved by developing genomic and metagenomic approaches to establish the molecular basis for these complex relationships coupled with evaluation of novel plant-endophyte partnerships under greenhouse and field conditions.

The pharmaceutical industry has intensively examined fungal endophyte secondary metabolites (SM) resulting in numerous novel compounds. In the past decade, the discovery of interesting and unique compounds from endophytic fungi has expanded, especially in Asia and Latin America. To date, the only marketed fungal endophyte metabolite is a semisynthetic derivative of PF-1022A that is being commercialised as antiparasitic agent.

B.3 Reasons for the Action

The COST Action will impact a number of socio-economic matters as well as EC directives that affect the quality of life of EU citizens and EU contributions to global issues.

A co-ordinated effort involving scientists from different disciplines and several countries is imperative to identify innovative scientific solutions for multidisciplinary questions of endophyte biology and use. To solve problems of such dimension, a COST Action is the most appropriate tool to build a fruitful synergy at the European and partly also at global level (as non-European scientists will be involved). Through its multi-disciplinary networking approach, the COST Action will deal with all aspects of the topic, identifying current bottlenecks and providing solutions to overcome the problems stated above. Furthermore, the multidisciplinary research is needed to solve questions of fundamental character and the transformation into applied research approaches may not be within the reach of the institutions and companies or even entire countries. Therefore, the Action aims to create added value at all levels and to ensure research excellence in the European Research Area (ERA) and development on production and exploitation of endophyte use.

The EC Directorate-General for Research adopted an Action Plan on Environmental Technologies (ETAP) with the aim to stimulate their development and uptake. This plan recognised the "potential of [environmental] technology to create synergies between environmental protection and economic growth" and that "investing in [environmental] research, from both public and private sources, is vital for the EU economy". The goal of the COST Action is to generate new and innovative plant/microbial technologies, helping to raise new interest and possibilities (i.e. to stimulate investment and innovations). This will increase the competitiveness of European SMEs and generate new products for a highly promising market. The latter goal is particularly important, considering that mainly SME are focusing on the target markets for products that may arise from the research; e.g., the Biocontrol/Biofertilizer niche markets are considered too small for large Agro companies to invest substantial efforts.

The COST Action conforms to the vision paper and the Strategic Research Agenda (SRA) set by the European Technology Platform (Plants for the future) by advancing the knowledge in sustainable agriculture by reducing environmental impact of crop production and managing biodiversity. The COST Action also complies with the Water Supply and Sanitation Technology Platform by ensuring the quality of water (e.g by reducing N, P and pesticides leaching in ground water) and with the Nitrate Directive that support the utilization.

Finally, the COST Action complies with the European knowledge based bio-economy by exploring and exploiting renewable biological resources (e.g. microorganisms).

B.4 Complementarity with other research programmes

The COST Action will increase competitiveness of European SMEs, through the development of strategies favouring microbial-based products and improved application technologies. The number of SMEs involved in microbial inoculum production is increasing in the EU and worldwide. A significant percentage of them are involved in other COST Actions which do, however, cover only some aspects of endophyte biology and application.

In addition, participants were involved in COST Action 870 (‘From production to application of arbuscular mycorrhizal fungi in agricultural systems: a multidisciplinary approach’), COST Action 865 (‘Bioencapsulation Multiscale Interaction Analysis’) and COST Action CM804 (‘Chemical Biology with Natural Products’).

The COST Action will develop cost-effective and quality-enhancing, eco-efficient products based on the encapsulation of selected endophytes, which will improve the commercialization opportunities for new products. The outcomes of the COST Action will reduce dependency of growers on EU/national subsidies under reformed Common Agricultural Policy. This will be possible through the expansion of environmentally-friendly production methods.

C. OBJECTIVES AND BENEFITS

C.1 Main/primary objectives

The aim of the Action is to identify bottlenecks limiting the use of endophytes in biotechnology and agriculture and to provide solutions for the economically and ecologically compatible exploitation of endophytes. The main aims are: 1. Identification of bottlenecks limiting the use of endophytes in biotechnology and agriculture and to provide solutions for the economically and ecologically compatible exploitation of endophytes. 2. Gain further knowledge of ecology of endophytes in plant-soil ecosystems and in plant-plant and plant-animal interactions. 3. Identification of new competent endophytes from different plant hosts in Europe with importance for agriculture, environment and industry enabling a Central European web-based database of endophytic strains. 4. Development of new microbial inocula for increase of biomass together with improved plant quality, plant-microbe interaction in soil, protection against biotic and abiotic stress, and phytoremediation as well as elucidation of endophyte recognition, mode-of-action and function of effects on plant growth, development and health (in vitro, post vitro, conventional). 5. Increased cooperation and exchange of knowledge about endophytes (composition of networks) between research institutions and companies for development of new marketable products of endophytes for Europe and overseas.

The key deliverables are: 1. Central European strain database of isolated endophytes on StrainInfo bioportal. 2. Software tools for in silico knowledge-based screening of EMOs for their possible use. 3. Novel detection tools for endophytes to track them in the host plant spatio-temporally (16S rRNA gene-targeted PCR, functional microarrays). 4. Novel insights in the role of endophytes in plant ecology and their dynamic systems in soils, including the role of SMs. 5. Isolates that can be applied for plant growth promotion, for phytoremediation or that can be used as novel sources of therapeutic agents. 6. Elucidation of the balance of endophytes during biotechnical plant propagation. 7. Information about the usage, storage and quality control of inocula for the use in plant production and agriculture (seed coating, dry beads). 8. Information on regulatory domains, control, risk assessment and legal aspects. 9. Integrated safety guidelines for humans, animals and plants with a publicly accessible StrainInfo bioportal. 10. Awareness and improvement of rational accessibility of endophytes and their SMs for industrial applications enabling discovery of new product candidates for the LifeScience and Biotechnology industry that can be procured in a sustainable manner.

C.2 Secondary objectives

The secondary objectives are:

- Guidelines of management practices for protection of endophytic flora in Europe
- Economic utilisation of competent endophytes for agricultural plants and plant tissue cultures in Europe
- Elucidation of life cycles of competent endophytes in and outside of plants as base for an optimal production of new inocula

C.3 How will the objectives be achieved?

The interest in this COST Action was enormous: 51 experts from European institutions in 13 countries already have responded to join this Action. The scientists involved are familiar to endophyte research and represent the top expertise in biotechnology, botany, microbiology, molecular biology, mycology, plant physiology and plant pathology and hence having all the expertise and infrastructure (laboratory, modern equipment and other experimental facilities) needed to reach the goal laid out in this COST Action. The topic has numerous interconnections, both horizontal and vertical: e.g. bacterial and fungal endophytes, endophytes of plants, animals and fungi, application from lab to field scale.

Exchange between member laboratories with different expertise and working on different study (model) systems will efficiently facilitate progress and will also help to distribute or apply important techniques to answer key questions in endophyte research. The state of the art and strategies for further research will be presented and discussed in thematically focussed workshops. European and worldwide key scientists not directly involved in the COST Action, having important specific expertise, will be invited to contribute to the workshops.

C.4 Benefits of the Action

Farmers, public consumers, horticulture an others: Endophytes have been shown to induce systemic resistance, improve growth of the host plant and/or synthesize metabolites that are toxic to plant pathogens or herbivores. Their use as biocontrol agents is of special interest to antagonize human pathogenic microorganisms associated with crops, and to reduce the input of synthetic pesticides. Understanding the basic mechanisms of growth promoting effects of endophyte-plant interactions will offer great benefits for agricultural practises; e.g. increased yields and nutritional value of important cash crops as wheat or corn as well as higher biomass yields of energy crops.

Research and industry: The elimination of pathogens and control of covert organisms which have potential to be harmful under stress conditions during plant tissue culture will reduce the loss of individuals. The described benefits are of interest for both, research and industry. Selected endophytes, particularly those which are seed transmitted, can be used for the improvement of commercial cultivars of agricultural species. This aspect is interesting for the seed industry, which is already using some endophytes for turfgrass cultivar improvements. Biofertilizers could also lead to reduced input of mineral fertilizers, whose production is accompanied by large energy demands and CO_2-emissions. Especially with fungal endophytes, the elucidation of the life cycle by means of molecular ecology also appears to be an important issue in the selection process for new potential biocontrol and biofertilizer candidate organisms. It would be rather crucial to decide whether an endophytic fungus is a potentially harmful, latent parasite, or whether it exerts beneficial effects even when the host is subjected to stress. Likewise, the production of toxins and other secondary metabolites would need to be monitored in the pre-selection process, which can ideally be accomplished by synergistic research among the members of the four WG. Endophyte metabolites could also be important for red and white biotechnology: e.g. medicine in anti-cancer research and industrial enzymes.

The transfer of basic knowledge about the mechanisms allowing bacteria and fungi to enter a plant and the development of improved inoculation techniques will help exploiting endophytes for biotechnical and agricultural application.

Applied and basic research and practice: Researchers from universities and other research institutions in Europe could cooperate together via research projects about endophytes in order to elevate the economical importance of endophytes in biotechnology and agriculture in Europe. With respect to patenting new endophyte-mediated processes, the EU lags behind endophyte innovation in America and Asia. Therefore, this COST Action helps to initiate researcher groups for seeking new patents in this area.

C.5 Target groups/end users

The results derived from the COST Action will be targeted to farmers, plant breeders, food and seed industry, pharmacy, medicine, public consumers and local and national governments in developed and developing countries but the recommendations derived from the result will also be shared with international agencies such as the World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO) and organisations of environment.

The interdisciplinary approach unites biologists, analytical chemists and phamacologists, many of whom will be active in the other projects creating a high level of synergy. Several members have decades of experience in microbial natural product research, including experience with EMOs and maintain close collaborations with companies that are active in various sectors of the Life Science and biotechnology industries (Pharma, Agro, Food, Cosmetics) These contacts will attract industrial partners for exploitation of the results of this Action. Representatives of all relevant industries from the member countries, in particular SME, who can either contribute complementary knowledge and assets or may become responsible for development of the lead compounds resulting from the Action, will be invited to join.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

To achieve the objectives, four Working Groups will be created:

WG1. Ecology of endophytes

A key component of the Action aims at understanding the ecology of microbial endophytes hosted by the natural vegetation and important crop plants. Based on this knowledge, it will be possible to identify and isolate endophytes, which colonize a specific plant and exhibit desirable effects on plant growth/health and which can persist under various environmental stresses.
Execution of following tasks will achieve a better understanding of endophytes, which is of importance for further application:

1. Survey of endophyte populations in key plants for European agriculture and ecosystems taking into account different environmental conditions, stresses and management practices. The Action also aims at identifying management practices to protect endophyte populations and warranting their beneficial effects in European ecosystems.
2. Experiments to better understand endophytic colonization, life cycle and expression of plant beneficial characteristics *in planta*. Efficient colonization of various plant parts is of primary importance for the application of endophytes, however, colonization (particularly of above ground plant parts) is not well understood. Furthermore, plant growth promoting characteristics have been mostly investigated under laboratory conditions, and therefore a better understanding is needed to how the plant and the environment effect the expression of these beneficial traits under field conditions.

WG2. Identification of new competent endophytes

The aim of this WG is the isolation and characterization of bacterial and fungal endophytes that are beneficial to host plants following re-inoculation. Consequently, they will be analysed with regard to their antagonistic activities against other microorganisms, their impact on plant performance under various conditions, and their metabolomic profiles. Following tasks will be carried out in order to identify new competent endophytes:

1. Different methods for efficient isolation of EMOs from plant tissues will be compared among the participants of the COST Action. This should result in a high throughput technology for establishing an initial collection of potentially useful bacterial and fungal endophytes. They will be identified according to their structural and molecular characteristics.
2. Standard systems using selected model plants and pathogenic MO will be set up to test the bacterial and fungal isolates of this initial collection for their establishment inside plant tissues and their capacities to combat certain plant pathogens.
3. Proteomic and genomic analysis will clarify the mechanisms responsible for beneficial attributes of colonization.

The consensus will be a catalogue of standardised methods, with a list of endophytes that are competent for practical applications, and which can be used for the development of new inocula (WG3).

WG3. Development of new microbial inocula

The scientific focus will target the development of inoculants (both bacteria and fungi) and inoculation technology. The screening process will span from *in vitro* tissue culture assays through lab-scale, greenhouse and field trials, as it is the ultimate goal to apply the potential of endophyte inoculants. In addition the impacts of the endophyte inoculants on population dynamics of pathogens, autochthonous MOs and communication in plants will be investigated. Epigenetically inherited positive changes in plants after inoculation will be taken into account and studied, as far this information leads to novel technologies.

Different inoculation methods will be investigated as the development of a suitable procedure for the production of bio-preparations by cultivation and stable formulation is a necessary precondition for the practical application and shelf life of endophytes.

Under laboratory conditions, plant metabolism of xenobiotics and agrochemicals in the presence and absence of endophytes and endophytic signals will be investigated as well the durability of endophytic colonisation, in order to quantify the beneficial effects of inoculation / horizontal gene transfer of desired traits.

WG4. New industrial products in life sciences

The network will continue seeking pharmaceutical and agrochemical indications, but will expand endophyte exploitation into other industrial applications. Recently, interest for innovative products has arisen in other life sciences industries, including the food and cosmetics sectors as well as white biotechnology. Novel enzymes, natural colorants and preservatives, probiotics and other food additives from renewable sources are being sought to replace traditional synthetic additives. The beneficial properties of endophytes employed for biocontrol or “mycofumigants” are often related to SM production; however, new products must be scrutinized for mycotoxins and proven advantageous over former synthetic chemicals. Rapidly evolving antibiotic resistance, and now affecting Gram negative pathogens, has been recognised by the EU as a major public health threat.

Massive funding is being dedicated to the discovery of novel antibiotics. As most antibiotics have been developed from natural products, the vast reservoir of unknown endophytic microbes may be among the most exciting sources for new antibiotic scaffolds.

1. SMs can mediate interorganism communication and ecological interactions and can determine biocontrol efficacy. Therefore efforts on model organisms employed by other WGs will be prioritized and model endophytes based on production of potent bioactive molecules will be suggested.
2. In interaction with WG2, new organisms by focusing on phylogenetic groups that are known to be creative SM producers will be found. The search will be accomplished by best-practice isolation protocols including high throughput culturing and baiting techniques, or selection of known endophytes from culture collections.
3. Correlations between SM production and phylogeny based on model organisms, e.g. *Xylariaceae* will be exploited, that are prolific SM producers and have yielded important new chemistry, e.g. PF-1022 and nodulisporic acid mentioned in Part B.
4. Expression of SM pathways during establishment of fungus-plant or fungus-insect vector interactions will be followed and the ecological advantage of these compounds during the endophyte life cycle will be understood. The model organisms described above should also be included here.
5. Endophyte SMs have not been systematically screened outside the large pharmaceutical companies. Expose endophyte SMs to biochemical mechanism assays relevant for lead discovery in the food and cosmetics industry (e.g. food preservatives; antioxidant and anti-aging mechanisms).
6. Useful SMs will be optimized for scale up in bioreactors employing modern downstream processing equipment and preparative chromatography systems to in collaboration with industrial partners and assuring sustainable production.

D.2 Scientific work plan: methods and means

Working Group 1 'Ecology of endophytes'

1. Meta-analysis of bacterial and fungal endophytes encountered so far in the most important crops / native plant species in Europe.

2. Survey of endophyte populations in key plants for European agriculture and ecosystems taking into account different environmental conditions and stresses. Such a survey will particularly involve cultivation-independent analysis as all MO irrespective of their culturability can be addressed. Surveys will be established preferentially in zones more likely to present important patterns of variation of endophytic communities (e.g. environmental gradients). This will enable the assessment of possible correlations between endophytic assemblages' occurrence and symbiotic interactions with plants.
3. Experiments to better understand endophytic colonization and expression of plant beneficial characteristics *in planta*. Colonization characteristics under various conditions will be studied in detail with model MO defined by the scientists participating in the COST Action. The whole genome sequences of such strains will be ideally available allowing the analysis of the expression of various plant growth characteristics at different stages of colonization and environmental conditions.

Working Group 2 'Identification of new competent endophytes'

The scientific work plan will establish the European Endophyte Database (EEDB), as genebank European genebanks will be provided with the strains (f.e. DSMZ, MUCL). The following methods and means will be used and different variations and alternatives will be compared. The EEDB will catalogue these methods:

1. Isolation of endophytes (surface sterilisation, imprint techniques, high-throughput culturing, catalogue of media). Rely on the strongholds and established techniques previously established by the partners (e.g., antimicrobial, anticancer and biochemical assays are established in several labs of the WG) and will be used to achieve a broad characterisation of the SM resulting from the Action.
2. Characterisation of morphology and growth
3. Molecular phylogeny (link to sequence database)
4. *In vitro* characterisation
    1. media for the identification of particular enzymatic activities
    2. co-cultivation assays (set of pathogens as indicator organisms)
5. *In planta* characterisation
    1. inoculation techniques
    2. staining and microscopy techniques

    3. catalogue of pathosystems for screening for induced resistance
    4. platform of transgenic plants with promoter-reporter constructs (e.g. phytohormone-regulated promoters)
    5. chemical analytical methods for estimating plant product quality
6. In depth characterisation of model endophytes on high throughput platforms
    1. Genome analysis, Transcriptomics, Proteomics, Metabolomics

Working Group 3 'Development of new microbial inocula'

1. Inoculants will be characterised and inoculated to a variety of plant systems. Experiments will be lab-scale, greenhouse and field-scale and will assess the intended impact of the desired traits (biocontrol, biofertilisers, stress tolerance, biodegradation etc.) on the system.
2. Application of genomic and metagenomic approaches will identify genetic functions that might be important for successful establishment of endophytes within a plant and that might be involved in plant growth promotion and improvement of abiotic stress tolerance of plants. Based on these findings experiments such as; expression studies, knock-out mutations, etc. will determine the role of certain genetic characteristics.
3. Tools for monitoring and localization of inoculants within plants as well as molecular sensors for *in vitro* and *in planta* detection of bacterial activities relevant for plant growth promotion and phytoremediation will be developed.
4. Gene expression patterns associated with plant-fungal endophyte mutualism will be determined using selected endophytes and model plants, by means of transcriptome analysis (microarrays, RNAseq etc). The goal will be to identify both plant and endophyte genes important for the establishment of the symbiotic interaction.
5. Endophytic bacteria with potential to enhance phytoremediation will be inoculated to target plants and the phytoremediation efficiency will be evaluated by analysing phytotoxicity (biomass, activity of oxidative stress-related enzymes, stomatal resistance), the metal uptake, biodegradation of herbicides and the evapotranspiration of volatile organics.

6. Biochemical analysis of intra- and extraradical enzymes, hormones and important signal molecules and the analysis of possible transport processes of nutrients by endophytes are important to characterize these organisms for usage as bioinoculum, here is a connection to WG2. For commercial application it is important to formulate the inoculum in liquid or powder/gel (alginate) substances and to look for successful inoculation techniques. Another aspect is the shelf life of inoculum.

Working Group 4 'New industrial products in life sciences'

1. DNA barcodes and HPLC profiling to concisely pre-select non-redundant endophyte strains prior to construction of screening libraries will be used.
2. An array of environmental simulations, fermentation and epigenetic methods to induce cryptic SM pathways in endophytes to produce metabolite-enriched sample libraries will be applied.
3. State-of-the-art HPLC-DAD/MS profiling (comprehensive dereplication libraries are available from some partners), HR-MS and high field 2D-NMR for physico-chemical characterisation of the SM profiles will be used.
4. Endophyte SMs will be exposed to a broad array of cellular and biochemical assays for biological characterisation of the samples; a first line evaluation will be whole-cell antimicrobial, antiparasitic, and anticancer targets. However, the newest targets in human, animal and plant health will be sought by promoting the network to large and small enterprises and to those readily available via consortium partners.
5. Integration with current molecular phylogeny, DNA barcoding (links to Barcode of Life)
6. *In vitro* manipulations
    1. media for secondary metabolite expression
    2. induction of cryptic secondary metabolite pathways
    3. identification of secondary metabolites by analytical chemistry
    4. extraction protocols and library construction
7. *In planta* characterisation:
    1. identifying the role of secondary metabolites in plants
    2. chemical analytical methods detecting secondary metabolites in plants

8. Opportunities for new chemical applications
    1. basic profiling for antibiosis and cytoxicity and invertebrate toxicity
    2. strategies for collaboration in new chemotherapeutic areas, food and cosmetic industries
9. Linking secondary metabolite pathways to their products
    1. genome mining
    2. transcriptomics
    3. metabolomics

E. ORGANISATION

E.1 Coordination and organisation

The Action will be managed by a Management Committee (MC) representing the participating countries, which will meet twice yearly. During the first MC meeting, a Chairperson, Vice Chairperson and WG leaders will be elected, they form the Core Group. The MC is responsible for planning and controlling the activities within the available budget. The MC will integrate the reports of the WG, will take care of the annual reporting and evaluate regularly the activities and results in relation to the Action's activities. The MC will monitor and evaluate the achievement of objectives and all on-going activities based on Quality Management System and will create and maintain contacts with appropriate on-going COST Actions for synergies.

The MC will appoint a person responsible for generating and maintaining a dedicated web site for the COST Action. Additionally an e-mail reflector and white–board facilities for timely and efficient exchange of ideas between the members and for dissemination of the results generated in the Action will be provided. Information generated during Action's activities will be documented and placed into the web, together with the minutes of the MC meetings, and other information such as announcements of seminars and workshops in areas relevant to the Action. The Action Web pages will be updated regularly (typically every two weeks). A member's section on the scientists' network platform ResearchGATE (www.researchgate.net) will be initiated. An email newsletter will be published for members twice a year.

E.2 Working Groups

Leading European experts elected by the MC will set up the Working Groups (WG) within this Action to deal with the five objectives. It is of special interest that anyone involved in different research aspects of endophytes and willing to take part in a European research network, is welcome to join this Action. The different EU partners are funded on a national basis, the reason for joining this Action are possible synergies within the research network.

The WG leaders will be responsible for organising the WG scientific meetings to meet their objectives and assess their progress. Moreover, the WGs will strive to achieve trans-WG cooperation and exchange of novel insights. One aim is to set up joint research activities. The results of the activities of the WGs will be reported to the Action Chairperson and MC. Two technical workshops will be organized during the Action’s period to be held in conjunction with one of the MC Meetings. In the final year of the Action, a symposium will be organised for disseminating the results of the Action to specific end users and to a wider audience, as well as for obtaining inputs from other COST Actions and international experts world-wide. A book will be published based on the Symposium presentations in order to reach even a wider audience and to expose the results of the Action to international review.

E.3 Liaison and interaction with other research programmes

The COST Action ‘Endophytes in Biotechnology and Agriculture’ will be a significant addition to a number of existing COST Actions and research projects, namely COST Action 870 ‘From Production to Application of Arbuscular Mycorrhizal Fungi in Agricultural Systems: A Multidisciplinary Approach’, which also includes information about MO and their effects on plant growth and health, but is not focussed on endophytes. Furthermore the Action complements COST Action CM0804 ‘Chemical Biology with Natural Products’, COST Action 871 ‘Cryopreservation of crop species in Europe’ (CRYOPLANET), COST Action 865 ‘Bioencapsulation Multiscale Interaction Analysis’, COST Action 837 (‘Plant biotechnology for the removal of organic pollutants and toxic metals from wastewaters and contaminated sites'), COST Action 859 ('Phytotechnologies to promote sustainable land use and improve food safety') and COST Action FA0905 ('Mineral-improved Crop Production for Healthy Food and Feed'). The already ended COST Action 859 'Phytotechnologies to promote sustainable land use and improve food safety' and COST Action 830 'Microbial inoculants in agriculture and the environment' delivered valuable results (e.g. formulation and quality aspects of inocula) which will be taken into account for this Action.

Several scientists are members and/or MC members in those Actions, which will encourage additional knowledge transfer. The possibility of organising Inter-COST Workshops with other appropriate on-going COST Actions will also be explored to address topics of common interest.

Members of the COST Action are (resp. have been) involved in several research projects, e.g. 'Commercial Production of Enhanced Bio-Control Agents for Combating Soil Borne Pathogens in Egypt' (EU-Egyptian Innovation Funds), 'Establishing Cryopreservation Methods For Conserving European Plant Germplasm Collections' (QLK5-CT-2002-01279, CRYMCEPT), 'Search for new targets and antibiotics against Gram negative pathogenic bacteria' (FP7 Health, AntiPathoGN), 'Traditional food in combating foodborne pathogens (LLP, TRADFOOD), FP7 KBBE 'Valorizing Andean microbial diversity through sustainable intensification of potato-based farming systems' (VALORAM), 'Gentle remediation of trace element contaminated land' (FP7 GREENLAND), 'Good practice in traditional Chinese medicine' (Coordinative Action EU Program 'GP-TCM'), 'Using MicroBes for the REgulation of heavy metaL mobiLity at ecosystem and landscape scAle: an integrative approach for soil remediation by geobiological processes' (FP7 226870 UMBRELLA), 'PlantPower – Living plants in microbial fuel cells for clean, renewable, sustainable, efficient, *in situ* bioenergy production' (PlantPower FP7 Project Nr. 226532), 'INTERREG 3: Alps-BioCluster project', ERA-Net Pathogenomics (BFU2008-04709-E/BMC 'Development of novel antifungal compounds against *Aspergillus* spp') and the FP7 project AntiPathoGN (http://www.antipathogn.eu/).

None of these projects will significantly overlap with this Action.

E.4 Gender balance and involvement of early-stage researchers

An important matter for this Action is to achieve a good gender balance among the participating researchers. This COST Action will respect an appropriate gender balance in all its activities and the Management Committee will place this as a standard item on all its MC agendas. In the list of experts interested in this COST Action, female scientists make up nearly 40 % of the total which is already a good gender balance.

The MC will be responsible for assessment of the STSM (Short Term Scientific Mission) applications and will particularly encourage younger scientists and scientists from less scientifically favoured areas to be involved in the STSM scheme. Members of the WG will have the opportunity to advance their knowledge in workshops and/or secondments. The Action will run training schools for one week, bringing together PhD students and early stage researchers (ESR) from the partner institutions of the Action. The Action is also committed to considerably involve early-stage researchers (STSM, training schools, Action think tank, and conference grants). This item will also be placed as a standard item on all MC agendas. Participation in all of these activities will be strictly monitored by the MC in order to ensure a fair and equal gender balance.

For all recruitments the ‘Code of Conduct for the Recruitment of Researchers’ will be respected, all member institutions will respect the ‘European Charter for Researchers’ in their scientific work.

F. TIMETABLE

The MC will be appointed after the approval of the Action during the first Management Committee meeting. The detailed Scientific Programme for the first two years of the Action will be established by the Scientific Committee and approved by the Management Committee within 2 months after the first Management Committee. The outline of the main milestones and deliverables of the Action is given in the table below. Scientific seminars, conference attendance, paper/poster presentations, journal and non-technical publications, training and exchange visits, networking, and outreach events will take place on a regular basis for the whole duration of the Action.

Task	Month due
Nomination of the MC	M1
Starting Plenary MC1 meeting	M2
Set-up of the internal web site	M2
Scientific Programme	M3
Set-up of the electronic communication network	M3
Set-up of the public web site	M3
First meeting of the Working Groups	M4-M5
1st Annual Workshop, General Meeting, and MC meeting	M12
Approval of first-year report	
Separate or combined meetings of WGs and STSM	M14-20
2nd Annual Workshop and MC Meeting	M24
Approval of second-year report	
Scientific meeting within or between WGs and STSM	M 26-M34
3rd Annual Workshop and MC Meeting	M36
Approval of third-year report	
Scientific meeting within or between WGs and STSM	M37-M46
Symposium	M42
4th Annual Workshop and Final Plenary Meeting, MC meeting	M48
Approval of the final results and reports of the Action.	

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: AT, BE, DE, DK, EL, ES, FI, FR, IE, IT, NL, PL, UK. On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at 52 Million € for the total duration of the Action. This estimate is valid under the assumption that all the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

H. DISSEMINATION PLAN

H.1 Who?

The objectives stated below will be realized by disseminating the results to the following target audiences:

A. Different scientific communities involved in plant breeding
B. National Health Institutes and Ministries of Health Care
C. National governments and regional authorities
D. Nutritionists and farmers all over Europe
E. Industry (Pharma, Agro, Food, Cosmetics)
F. Other research frameworks COST

H.2 What?

The main outreach objectives of the Action are as follows:

- to increase the awareness of the general public, the agronomical, and other scientific communities about the possibilities of endophyte use
- to initiate and catalyse the interest of the relevant authorities at national and regional levels, and thereby to contribute to raising national funding for development of new endophyte exploitation strategies in Europe
- to promote the collaboration between the different scientific communities (e.g. agronomists, analytical chemists, botanists, microbiologists, molecular biologists, mycologists, pharmacologists), which is necessary for successful development and improvements of endophyte use at national and European levels
- to foster links with European industrial partners (large companies as well as SMEs), potentially interested in manufacturing the devices and equipment needed

H.3 How?

At each member institution: Presentation of the results to supervisory team and to colleagues during laboratory/institute meetings. Preparation of flyers or posters of the Action and of major results for visitors or during events, such as the European Researcher Night.

Within the COST Action: During WG meetings speakers will be required to present their work progresses and future plans orally and in a written report. It will be also an opportunity to discuss their results with all the participants involved in the Action. Within the network, the members will also be asked to implement the intranet webpages to favour exchange of knowledge between the participants of the COST Action and local scientists and discuss the results of the projects, or to plan future collaborative work.

During events organized by the COST Action: These events will provide an ideal means to present the results of the project through oral presentations, posters and proceedings to leading international scientists in the field, journalists, policy-makers and stakeholders. External attendees will also be invited to present their research results.

At a national and international level:

Webpages: Will offer the opportunity to the members to present their research programmes and main results. This webpage will be advertised in the partner institutional websites.

Publication: Publications of research results in prestigious international peer-review journals will be at the forefront of the members' agenda. To achieve this, they will benefit from the experience of the MC in preparing high quality high impact articles based on highly novel and innovative research findings. Publications in wide circulation / non-expert journals will also be encouraged to present the public, stakeholders and policy makers the main results of the project.

The table below shows the methods, which will be used for carrying out the dissemination plan of the Action to which target audiences:

Dissemination Tools	**Target audiences (H.1)**
Posting of general information on the public website	B, C, D, E, F
Posting of working documents and interim reports on the internal website	A
Electronic communication network	A, B
Publications	A, B, C, D, E, F
Workshops and conferences	A, B, C, E, F
Non-technical publications	B, C, D