

Presentation of the funded projects in 2010 for the « Blanc
 International SVSE 7» Programme

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Blanc Inter SVSE 7 Programme

YEAR 2010

Project title	BIOFILTREE – Filtration biologique pour la réduction des éléments traces dans la biomasse des arbres
Abstract	<p>Due to wastes, agricultural and industrial activities, large sites highly contaminated by trace elements (TE) are found in France and Canada imposing hazard(s) to environment and human health. Phytoremediation has become an attractive alternative to other clean up technologies due to their relatively low cost, potential effectiveness and the inherently aesthetic nature of using plants to clean up contaminated sites. This project aims at defining and demonstrating integrated bioremediation technologies for the control of TE transfer into harvestable woody biomass. We propose an original implementation of an integrated bioremediation strategy that combines different tree species in an intercropping strategy together with rhizospheric microorganisms, exploiting the complex interactions evolved for the mutual benefit of both organisms, in which plant roots provide habitat, nutrients and exudates to microbial populations, whereas microbes facilitate mineral nutrition of plants. It is now widely accepted that rhizospheric microorganisms may actively participate to phytoremediation processes, and may be useful in extending the application of phytoremediation to additional TE contaminated sites. Hosting different mycorrhizal types might be of functional importance for plant nutrition and has been shown to contribute to metal tolerance of host plants as fungi can reduce the metal uptake by the plant by sequestration, extracellular precipitation and biosorption to the cell walls. Indeed mycorrhizal fungi play a filtering/sequestering role on plant roots, enhancing root to shoot metal ratio and increasing survival rate in harsh conditions. Moreover, nitrogen fixing species (<i>Frankia</i>) are also good candidates for remediation by phytostabilization due to an improvement in soil properties and the retention of TE in nodulated roots. The tripartite associations among <i>Frankia</i> (N-fixing bacteria), ECM fungi and <i>Alnus</i> could improve the growth, nitrogen fixation and mineral acquisition (rock solubilization) of <i>Alnus</i> species, but also</p>

that of the neighbouring poplar species. Thus, the problem of nutrient limitation of yield in short rotation coppice can be alleviated by microbial retrieval of nitrogen and phosphorus from soil organic material and from atmospheric N. The main objectives of the proposal are: i) The original implementation of large-scale phytostabilization field trials based on intercropping poplar and alder short rotation coppices. ii) The use of microbial symbionts that will reduce transfer of TE in harvestable aboveground parts of trees. Biofiltration analyses of TE transfer to aboveground parts of trees will be performed and mechanisms involved in TE sequestration by fungal cells will be investigated at the physiological and molecular level. iii) The retrieval of woody biomass in an energetic sector, by combustion or gasification. Moreover another innovative aspect of the project will deal with a detailed study of the technological and economical feasibility of the different tasks. This project is therefore characterized by the cooperation of foresters and microbiologists, researchers in the field of forest and mycorrhizal genomics and phytoremediation industries to support the development of an improved scheme of phytostabilisation, with the use of woody biomass in an energy path. All steps of the phytostabilization process – from analysis of site pollution, via tree implementation, TE transfer analysis, to the economic use of woody biomass- will be covered and will therefore allow an economical/regulatory assessment to be proposed to future actors of the phytoremediation area.

Partners**CNRS - UMR 249 UFC**

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ANR funding

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and duration**

- 36 months

Reference

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Project title

BIOPUNTIA – Opuntia spp comme source de nouveaux composés à visée pharmaceutique. Caractérisation de composés bioactifs et de leur évolution lors de la domestication.

Abstract

Medicinal herbs have been used for many thousands of years as alternative therapies to complementary medicines. Hence, there is an increasing demand for Traditional Herbal Medicine (THM). The multi-component and synergistic nature of THMs means that it is beneficial to analyze complex extracts. Among the several Mexican plants that have been claimed as medicinal, Opuntia spp. is one with higher biotechnological potential : they can grow in arid areas, are feed and food resources, and the most important, it has been used as a folk medicine for the treatments of diabetes, gastric ulcer, inflammatory, and several other illnesses. But still the compound(s) that are responsible of the beneficial effect are not well known. In addition, there are not reports about the possible biopeptides participation on the medicinal effects of Opuntia plants. The lacks of information about type of proteins presents in cladodes are mainly due to technical manipulation of protein extraction. Nowadays, modern technologies, as higher resolution HPLC columns, powerful software's, and the ability to determine the amino-acid sequences by Mass Spectrometry, gives the tools to analyse hundreds or even thousands of individual chemical entities present in a wide range of concentration levels with the result of a better characterization of proteins (peptides) and phytochemical compounds. One of the challenges for use plants as source of active compounds is, that the active compounds are not enough for a treatment, or the technical processing during products elaboration has a detrimental effect on the active molecules. For this reason, plant cell culture has great potential as an alternative system for the production of phytochemicals normally extracted from whole plants. This system can overcome many of the problems associated with industrial production of these phytochemicals extracted from field grown plants. In cultures, the production of natural compounds can be carried out throughout the year, unaffected by the season. The risk of crop failure due to natural hazards and the danger of extinction of some species due to their mass extraction from natural populations are eliminated. Cell cultures not only provide means for de novo synthesis of natural products but also serve for bioconversion of low

value compounds into high value products. Taking in account that wild Opuntia species are widely distributed along the semiarid regions of Mexico where hard environmental conditions prevail, they must express a higher level of metabolites than domesticated species *O. ficus indica*. For that reason, we have the main goal to characterize the metabolites and proteins profile from wild Opuntia species where at least five levels of domestication are found. To avoid variations due to different environmental conditions and/or culture conditions, the plants will be collected from the CHAPINGO University/ Agriculture, localized at Orito municipality, Zacatecas state, México. With the used of novel technologies for the study of plant metabolomics and proteomics, at least ten compounds will be tested on several biological/pathological end-points (colon cancer, atherosclerosis, obesity). With the results of the present proposal we expect to generate novel molecular information that could give to Opuntia species an increased biotechnological value. This information will help to protect the wild species and to developed strategies to produce metabolites with high potential, as new bioactive compounds against several illnesses.

Partners

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227 394 €

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and duration**

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Reference

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Cluster label

Cancer-Bio-Santé Industries et agro-ressources

Project title

IMMORTEEL – Impacts des contaminations métalliques et organiques des systèmes de la Gironde et du St Laurent sur deux espèces en déclin, l'anguille européenne et américaine.

Abstract

The European and American eels are two economically, ecologically and culturally important fish species currently considered in decline and, in the case of the European eel, threatened of extinction. These fish exhibit a complex life cycle. They are born and breed in the Sargasso Sea. Larvae then drift towards the European and American continents, where they metamorphose and invade freshwater systems to grow (yellow eel phase), before departing for a long migration back to where they were born to breed and die (silver eel phase). Among the causes mentioned to explain their decline, global climate change, overfishing, physical obstacles to freshwater migration such as hydroelectric dams and turbines, parasitism and exploitation of the Sargasso Sea have been mentioned. The role of pollution in the decline of these fish is still largely unknown. This research project is a joint initiative of researchers from Québec and France. Its main objective is to examine the relationships between pollution, both inorganic and organic, and the health of Atlantic eels. Indeed, contaminants released in water by urban, industrial, mining and agricultural activities accumulate in yellow eels during their long phase of somatic growth and could affect their growth rate as well as cause tumors and lesions. Furthermore, during their reproductive migration, silver eels mobilize their energy reserves to fuel migration and for gonad maturation. Accumulated contaminants could then be released massively and cause toxicity in the adult or be transferred to embryos. For eels from both continents, this project will examine the relationships between accumulation of contaminants and genetic diversity, health and reproductive potential..

Partners

CNRS - UMR 5805 EPOC

Cemagref

CNRS - UMR 7208 BOREA

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and duration** 01/01/2011 - 36 months

Reference ANR-10-INTB-1704

Cluster label Aerospace Valley

Project title**MACBI – Megastigmus et conifères : Biologie de l'invasion****Abstract**

Forests are an enormous economic resource in Canada and France. Not only do they represent an immediately exploitable resource, they are renewable and sustainable. The health of forests is highly susceptible to environmental effects. These range from abiotic factors, such as changes in weather patterns, and biotic factors, such as pests and diseases. Insect invasions due to the global seed trade are now becoming extensive. Seed parasites that destroy conifer seed crops are essentially invisible as the parasites move undetected within seed lots shipped from continent to continent. An added risk is that invasions by one species are often occasions for invasion complexes to develop. Diseases such as fungi travel with new insects, doubling the threat to forest health. Our ability to predict the influence of insects and diseases on future landscapes depends on how well we understand the relationship between immobile trees and highly mobile insects. Tree seed insects damage forests and plantations in both countries, affecting native species of plants as well as the animals that depend on them. The success of seed insects is due to their ability to bypass barriers in the developing plant ovule. If we wish to manage seed insects, we require basic information about how the insect takes over a seed's physiology, then moves through a species into other ones, and finally, how it then moves across a landscape. We will look at the highly invasive species of chalcid wasps in the genus *Megastigmus* (Hymenoptera; Torymidae), which cause extensive damage. *M. spermotrophus* is found on Douglas-fir. We will deep sequence the seeds and the insects to determine putative mechanisms of physiological control of the plant by the insect. In addition, we will investigate both the insect's and tree's phenologies to decipher how *Megastigmus* species are able to infest trees in the Pinaceae and Cupressaceae. This information, coupled with phylogeny of the chalcids, will be used to develop models of future invasion. To tackle these international problems caused by global seed exchange we have assembled a team of Franco-Canadian experts in *Megastigmus*-conifer seed interactions including those specialized in plant and animal reproduction, forest pest surveys, entomology and modeling.

Partners	INRA Zoologie Forestière Department of Biology, University of Victoria (Canada) Canadian Forest Service (Canada) INRA - UPR 629 Ecologie des Forêts Méditerranéennes
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Référence	ANR-10-INTB-1704

Project title**MiOxyFun – Biodégradabilité des composés oxygénés des essences (ETBE et MTBE): Micro-organismes - Mono-oxygénases - Fonctionnalité****Abstract**

Ethers, methyl tert-butyl ether (MTBE) and ethyl tert-butyl ether (ETBE), are added to gasoline to enhance the octane index. MTBE and ETBE are highly soluble in water (40 and 10 g.L⁻¹, respectively). Their use at a huge scale required to get information about their fate in the environment and in impacted aquifers. MTBE was found in several aquifers as a contaminant after releases of MTBE-supplemented gasoline due to its poor biodegradability. To our knowledge, the groundwater contamination by ETBE was not documented in the countries that use it, like France and Hungary. Studies are required to understand the environmental impact of MTBE and ETBE and to characterize the microorganisms, enzymes and genes involved in their biodegradation. The project is based on a unique common collection of MTBE- or ETBE- biodegrading microcosms from different geographic areas that was obtained in France and Hungary. This collection will allow to study: -The oxygenases responsible for the first enzymatic attack on MTBE or ETBE. We will characterize the biodegradation capacities of the oxygenases active towards ETBE and MTBE by determining (i) the degradation kinetics, (ii) the production of tert-butyl alcohol or TBA, an intermediate of MTBE and ETBE biodegradation, (iii) the interactions with other components of gasoline (mono-aromatics compounds or BTEXs and n-alkanes). We will also characterize the biodegradation capacities towards a wide range of chemicals with different structures to understand the specificity of the oxygenase and its capacity to attack compounds with a high steric hindrance (tertio-butyl group). The activity of these oxygenases will also been studied by expressing the genes encoding these oxygenases through heterologous expression in engineered microbial systems which is essential for proving the role and function of enzymes. -The phylogenic composition of the microcosms (i) by using "Denaturing Gradient Gel Electrophoresis" (DGGE) and (ii) by isolating microorganisms and determining their MTBE or ETBE biodegradation capacities. Then, (iii) the structure of the functional microbial communities will be studied by coupling a SIP (Stable Isotope Probing) approach to NanoSIMS observation, which principle relies on the labelling of the microbial cells with ¹³C-MTBE or ¹³C-ETBE; this study will determine which microorganisms metabolize the ¹³C-labelled

substrate in situ and if the microorganisms isolated correspond to those active in situ in the consortia. Two significant MTBE- or ETBE-degrading microorganisms will be chosen for further genomic DNA sequencing and annotation. -The genes induced on MTBE or ETBE. Different genes involved in the MTBE or ETBE biodegradation are known: (i) ethB encoding a cytochrome P450 oxidizing ETBE, (ii) mdpA, encoding a hydroxylase related to the Alkane Hydroxylases (AHs), (iii) mpdB and mpdC encoding dehydrogenases responsible for the production of 2-hydroxyisobutyric acid (HIBA) in the TBA pathway and (iv) icmA encoding a mutase responsible for HIBA assimilation. We will search for the presence of this panel of genes in the microcosms or strains isolated. Then, we will study their differential expression during growth on MTBE or ETBE by comparison to a classical substrate (RTqPCR). A transcriptomic study will be carried out using high throughput on the two strains sequenced.. It will allow to get an important databank on the diversity of MTBE-and ETBE-biodegraders, the mode of action of the oxygenases and the genes involved in the MTBE- and ETBE-biodegradation and will bring new insights on the environmental impact of the fuel oxygenates.

Partners

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- 36 months

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Cluster label