

PROTECTOR 514082 – FINAL RESULTS

Summary description of project objectives, contractors involved, work performed and end results

W P	Project Objectives	Work performed Contractors involved	End results and Main achievements	Degree (%)
DEVELOPMENT OF RECYCLING STRATEGY FOR ORGANIC WASTE STREAMS ANIMAL BONE CHARCOAL (ABC) CARRIER DEVELOPMENT				
1	Development of recycling/upgrading strategy for organic waste streams from the food industry.	Market evaluated and analysed in the EU and USA Ongoing and future trends established, where PROTECTOR fits in. Legislative factors identified P1, P11, P12	Strategy identified for organic waste streams from the food industry by full chain follow up (slaughterhouse, bone meal processing, bone char processing). Development of strategy for thermal recycling of the phosphorous rich bone meal into safe and sterile animal bone charcoal, which is suitable for specific microbiological carrier.	100
1	Food industrial organic waste material and processing characterization.	Food industrial organic waste materials and processing characterized by P1, P11	Food industrial organic waste materials and processing characterized.	100
1	Animal bone charcoal carrier characterization	Detailed chemical analysis of the ABC by P1, P6	Detailed chemical analysis of the ABC	100
MICROBIOLOGICAL STRAIN SELECTION				
2	Selection of bacteria compatible with Animal Bone Charcoal for phosphate mobilisation and biocontrol	Ninety-seven bacterial isolates belonging to different species were tested for their potential to mobilize phosphate from animal bone char (ABC) by P2 PRI. Of the bacterial isolates 60 % showed positive scores. Further selection was based on growth ability and survival in ABC, as well as <i>in vitro</i> inhibition of plant pathogens <i>Pseudomonas chlororaphis</i> , and <i>Bacillus pumilus</i> were the best bacterial phosphate mobilizers in tests with ABC, followed by <i>Paenibacillus polymyxa</i> , <i>Burkholderia pyrrocinia</i> and two <i>Streptomyces</i> isolates. <i>In planta</i> tests to evaluate the biocontrol properties of twelve selected bacteria grown in ABC were performed with the plant pathogens Pythium and Rhizoctonia in the greenhouse and climate	Two most promising bacteria were selected: a strain of <i>Pseudomonas chlororaphis</i> and <i>Bacillus pumilus</i> . These strains were able to grow and survive in ABC, having biocontrol potential and P-mobilisation capacity.	100

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		room. The most potential bacterial strains concerning P-mobilisation as well as biocontrol potential were selected: <ol style="list-style-type: none"> 1. <i>Pseudomonas chlororaphis</i> 4.4.1 2. <i>Bacillus pumilus</i> 4.4.2 3. <i>Paenibacillus polymyxa</i> 12.4.11 4. <i>Streptomyces pseudovenezuelae</i> 13.4.2 		
2	Selection of a capable <i>Trichoderma</i> strain compatible with ABC for mobilization of phosphorus and positive biocontrol effects.	Twenty <i>Trichoderma</i> strains of P4 UoR (<i>T. harzianum</i> , <i>T. saturnisporum</i> , <i>T. viride</i> strains) with origin from soil and rhizosphere were used for the selection of suitable strains for Protector. P4 (UoR) tested 20 <i>Trichoderma</i> strains and selected three <i>Trichoderma harzianum</i> strains (DAR5, DAR7 and DAR14), which were successful in the colonisation of ABC. The other selection criteria were: high spore production on the surface of the ABC particles, <i>in vitro</i> P mobilisation and <i>in vitro</i> antagonism.	All <i>Trichoderma</i> strains were able to grow with ABC as sole P source in solid and liquid culture. The abilities of the <i>Trichoderma</i> strains to stop the growth of <i>Fusarium oxysporum</i> strains were higher than to stop the growth of <i>Pythium ultimum</i> possibly caused in part by the fast growth of this species.	100
DEVELOPMENT OF INNOVATIVE SOLID SUBSTRATE FERMENTATION AND FORMULATION TECHNOLOGY				
3	Manufacturing of bone meal and animal bone charcoal (ABC) carrier samples for validation test.	Manufacturing of bone meal P11 Manufacturing of ABC carrier samples with alternative qualities (cattle bone, pig bone from low to high qty) up to no 15 types of samples P1 Design and preparation of meal meal air selector, to avoid abundant PAH generation under thermal treatment P1	Manufacturing of 5000 kg bone meal.(P11) Design and preparation of innovative meal meal air selector, to avoid abundant PAH generation under thermal treatment P1. All total 1500kg ABC carrier has been manufactured during the project life time. 22 different types of ABC has been manufactured to optimized bone char surface characteristics best for microbiological requirements. ABC SM15 seria has been the most successful and used for tests. (P1)	100
3	Laboratory scale optimization of solid substrate fermentation and	Growth of the two selected bacteria were optimised in liquid as well as solid stage fermentation by P2-PRI. Scanning electron microscopy (SEM) pictures	Growth of the two selected bacteria was optimised in small scale solid substrate fermentation. Survival of <i>Bacillus</i> is perfect. Survival of <i>Pseudomonas</i> still has to be improved.	100

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	formulation 'SSFF' process for the selected bacteria (P2-PRI) and fungi (P4-UOR)	were prepared to visualize the growth of the bacteria inside and outside of ABC particles. Survival of the bacteria was evaluated in the dried PROTECTOR product.	All selected micro-organisms, 2 fungi and 4 bacteria grow very well in a <u>two stage fermentation process</u> , based on the addition of a cheap liquid medium with proper C and N contents to the solid ABC.	
3	Development of sustainable nutrient strategies for the 'SSFF' fermentation process.	<p>Recycling and application of food industrial waste stream (molasses, corn steep liquor, tomato byproducts) to provide an organic nutrient source for the microbiological growth in the 'SSFF'.</p> <p>Technical and economical evaluation of the nutrient(s) were made before application and optimization of fermentation conditions.</p> <p>For determination of the optimal media composition for liquid fermentation of <i>Trichoderma</i> strains several combinations have been tested.</p>	<p>Nutrient selection criteria and strategy has been set up.</p> <p>The most effective nutrient – for economical and applicability point of view was the tomato pulp. Tomato pulp was selected for liquid and solid fermentation test program and for production of PROTECTOR prototypes.</p>	100
3	Industrial like optimization and scale up of the 'SSFF' process	<p>Two microbiological strains were used in the optimization and scale up program in Polgardi, Hungary</p> <ul style="list-style-type: none"> ● DAR5 <i>Trichoderma harzianum</i> – selected in WP2 by P 4. ● ST4B <i>Trichoderma harzianum</i> – TERRA (P1) own strain, used as reserve strain <p>Comprehensive scientific, technical and economical modelling of the SSFF process. Optimization of liquid and solid phase fermentation.</p>	<p>Economically and technological optimized SSFF fermentation process for the prime selected strains up to 400 kg/batch capacity.</p> <p>Cheap industrial medium has been selected</p>	100
3	Development, design,	Development, design, manufacturing and ready	Installed pilot SSFF model equipment with the following main	100

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	manufacturing and ready installation of an 400 kg/batch innovative pilot SSFF model, which is in product like performance for the prime selected strain.	installation of an 400 kg/batch innovative pilot SSFF model, which is in product like performance. The equipment is fully stainless steel made, including driver for agitation by 1-5 rpm. Comprehensive engineering design made, which is highly innovative. (P1)	components: <ul style="list-style-type: none"> ● Deep tank fermentor with 160 l capacity (stainless) ● Deep tank fermentor filter unit with 120 l capacity (stainless) ● Deep tank fermentor temporary storage unit with 100 l capacity (stainless) ● ABC mixer (stainless) ● SSFF formulation unit with 400 kg capacity, including discharge units ● Sterile air supply system and drier ● Pneumatic supply and transport system 	
3	Research test production of PROTECTOR fermented with bacteria for the larger scale experiments.	PROTECTOR with bacteria was produced for large scale field and greenhouse experiments in 2007 and 2008; up to 20 kg (P2-PRI)	Developed solid substrate fermentation process for two selected bacteria up to 20 kg scale.	100
3	Research test production of PROTECTOR formulated substances by SSFF method <ul style="list-style-type: none"> ● ABC-DAR5 <i>Trichoderma harzianum</i> ● ABC-ST4B <i>Trichoderma harzianum</i>, for control and comparison 	TEST production of two PROTECTOR prime strain prototypes by SSFF methods in Polgardi, Hungary by P1 (TERRA). The developed „SSFF” method is consisting of STAGE 1: Liquid phase deep tank fermentation and STAGE 2 Solid fermentation.	The results of the SSFF tests clearly confirmed our assumption that the ABC is advantageous support material for microbial colonization. The results have been demonstrated, that the developed two stage SSFF fermentation system works very well for several <i>Trichoderma</i> strains. Altogether 1500 kg ABC/ABC formulated substance has been produced during the research test production.	100
ASSESSMENT AND MATHEMATICAL DESCRIPTION OF PROTECTOR PHOSPHOROUS DISSOLUTION KINETICS				
4	Assessment and mathematical description of the PROTECTOR phosphorous dissolution kinetics and the impacts of PROTECTOR on P and	Mathematical description of dissolution kinetics of PROTECTOR. Predictions and process level explanations of agronomic effectiveness of PROTECTOR Data sets on reactions and turnover of SOM fractions following PROTECTOR application	Predictions and process-level explanations of agronomic effectiveness of PROTECTOR. In a direct comparison with GPR, which is well-characterised and widely accepted as a relatively soluble phosphate rock, ABC was better in providing available P, assessed by the Olsen method. It could even supply some available P immediately after application to soils of high	100

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	organic matter cycles in soil.	Data sets on the ability of AMF to transfer P from soil/PROTECTOR to plants. Characterization of microbial biomass and functionality. Contractors involved: P3, P4 and P5	pH, in which GPR was ineffective. Thus ABC has a wider range of application than PR.	
4	Elucidation of the 'P-bridge' function of arbuscular mycorrhizal fungi (AMF) between the PROTECTOR and host plant.	The general approach to investigate the P-bridge function of AMF between ABC particles and plant roots was to grow different plants in greenhouse experiments offering no other P-source than the ABC material. Contractor involved: P5.	The results clearly document the ability of arbuscular mycorrhizal fungi to extract P from ABC material and deliver it to their host plants. AMF can supply plants with P from ABC particles via their extraradical hyphae acting as a P-bridge. MF isolate could be selected that can be used together with biocontrol micro-organisms in combined applications without any disadvantages.	100
DETAILED RISK ASSESSMENT OF ALL COMPONENTS OF THE RECYCLING PROCESS AND THE FINAL PRODUCT				
5	Development of a rational approach for a risk assessment strategy for the agricultural use of material based on animal bone char;	Development of integrated risk assessment scheme that covers both ecotoxicological and human health aspects associated with using ABC as fertilizer and plant protection product. (P6-UFZ)	Targeted ecotoxicological test strategy has been developed for the risk assessment of ABC material.	
5	Risk assessment of the meat industrial Technical By-product concerning TSE	The BSE Risk assessment falls into three parts: 1- Risk assessment of input material (i.e estimate infectious load in applied MBM). 2- Risk reduction due to processing into product and cross-contamination risks. 3- Risk at use as fertilizer on various crops. Contractors involved: (P2 - PRI)	The final <u>conclusion</u> of the assessment was that the risk of transmitting BSE to cattle or humans via the use of PROTECTOR on crops is negligible, both for human food and for cattle feed.	100
5	Safety evaluation of selected micro-organisms	Safety of the selected bacteria and fungi were evaluated on the basis of literature and registration of similar strains. Material Safety Data Sheets were prepared for the selected micro-organisms. Basic safety and registration information was gathered. (P2-PRI, P4-UOR)	All selected isolates are non-GMO, and isolated from natural agricultural fields within Europe. The selected 4 bacteria and fungi belong to safety class 1 following 2000/54/EC Regulation. No harm is expected for humans to work with these strains. These bacteria and fungi can be used under standard safety regulations.	100

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			A Material Safety Data Sheet (MSDS) has been developed for the four bacteria and fungi.	
5	Chemical analysis of ABC material	More than 10 different batches of the ABC material and sub samples were chemically and ecotoxicologically analysed in the laboratories of the UFZ to identify the most critical contaminants and to estimate the quality of the different ABC batches. (P6-UFZ)	Quality assessment of the different ABC batches. The ABC carrier is safe for soil application.	100
5	Ecotoxicological Characterization of the ABC material.	The ecotoxicological assays used in the risk assessment were selected on the basis of their scientific meaningfulness and their current or future acceptance by regulatory institutions in the different European countries. (P6-UFZ) Using the bioassays ranging from soil bacteria, luminescent bacteria to microalgae, from Daphnia, zebrafish embryos, earth worms to higher plants, a wide range of ecologically relevant organisms was analyzed. (P6-UFZ)	Overall the quality of the ABC material was so good, that acute effects in the different analyzed test organisms and test conditions could not be observed. Effects on soil microflora could not be seen also. There was no toxicity to earthworms observable. No mutagenicity and toxicity of the PROTECTOR substance found.	100
5	Evaluation of the impact of the PROTECTOR on the soil microflora	The impact of PROTECTOR (animal bone charcoal with the microorganisms) on the soil microflora will be evaluated. The PROTECTOR product should survive long enough to be effective; however, this should not harmfully influence the soil microorganisms. The aim of this task is to analyze the potential shifts in the bacterial and fungal populations in soil due to the application of PROTECTOR. Community structures of fungi and bacteria can be compared by the use of PCR-DGGE techniques. (P4)	The influence of the PROTECTOR product (i.e. ABC fermented with microorganisms) on general soil communities is little. As a consequence, the environmental risk of this product will be low.	100
VALIDATION OF PROTECTOR EFFECTS				

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6	Validation of PROTECTOR effects	<p>(1) Evaluation of effects of PROTECTOR on soilborne pathogens in greenhouse; (2) Identification of mode of action of PROTECTOR; (3) Qualitative and quantitative evaluation of effects of PROTECTOR crop development and yield; Two formulations of ABC (ABC SM 7, ABC SM 15) and seven formulation of Protector (ABC SM 15 + microorganisms: DAR 7, DAR5, T22, <i>Paenibacillus polymixa</i>, <i>Pseudomonas chlororaphis</i>, <i>Bacillus pumilus</i>, <i>Streptomyces pseudovenezuelae</i>) were tested small, semi field and field scale. ABC and Protector where tested for their efficacy in different simulated conditions, both at nursery stage and growing stage directly in the soil, against <i>Fusarium oxysporum</i> f.sp. <i>radicis lycopersici</i> and <i>Pythium</i> sp. on tomato.</p> <p>Totally were carried out:</p> <ul style="list-style-type: none"> ● 7 laboratory tests; ● 12 trials in greenhouse; ● 5 field trials. <p>Contractors involved: P2, P7, P9</p>	<p>(1) PROTECTOR stimulates and increase active microbial biomass, competing consequently with the pathogen for space, carbon and other nutrients; (2) PROTECTOR is a carrier of beneficial microorganisms, such as <i>Trichoderma</i> spp., that are able to directly parasitize pathogens, produce antibiotics substances inhibiting pathogens, colonize harmful propagules and reduce the disease potential; (3) PROTECTOR enhance phosphorus availability in the soil, providing slow releasing of the nutrient and inducing tillering, root development, early flowering, ripening and fruit quality; (4) PROTECTOR enhance nutrient availability in the soil and, consequently, is responsible to induce disease resistance in the plant.</p>	100
6	Detection of the introduced microorganisms	Development of selective detection technique for detection of introduced organisms within a complex microbial population. (P2)	A detection system was successfully developed for <i>Pseudomonas</i> , which showed extremely good root colonizing capacities of this strain in greenhouse experiments. Detection of <i>Bacillus</i> in the vicinity of complex microbial populations is not possible yet.	100
6	Biological efficiency investigation of Protector substance	Additional Authority efficiency trial of the PROTECTOR substance have been executed.	On the basis of the complex Hungarian Plant Protection Authority test program Protector treatments (600 kg/ha, 1000 kg/ha) influenced favourably the soil structure. No any phytotoxic changes observed. It can be stated that Protector in all rates favourably influenced the yield	100

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			results. Recommended Protector dose: 1000 kg/ha.	
JUSTIFICATION OF PROTECTOR SOCIO-ECONOMIC AND TECHNICAL VIABILITY				
7	Justification of Protector socio-economical and technical viability by execution of CBA, SWOT and socio-economic assessment of Protector impacts, including the evaluation of its acceptance by consumer and retailers.	SWOT (strengths, weakness, opportunities, threats), CBA and consumer retailer acceptance evaluation. Socio-economic analysis and feasibility of PROTECTOR. Contractors involved: P7, P4, P1, P11	Detailed SWOT, CBA and consumer retailer acceptance evaluation of PROTECTOR end product and its application.	100
DEVELOPMENT OF CLEAR PRODUCT- AND TECHNOLOGY-ORIENTED DISSEMINATION AND MARKETING STRATEGY				
8	Development of dissemination technology transfer plan and marketing strategy and management of dissemination program.	Development of dissemination, technology transfer plan and marketing strategy. Efficient management of dissemination program.	Participation at international conferences, an oral presentations. Four newsletters have been issued and circulated in English and Hungarian language. Continuous contact with the HUNGARIAN NATIONAL AUTHORITY for SOIL and PLANT PROTECTION. 4 languages and abstract information on PROTECTOR project have been linked to the PROTECTOR web site. WEB PUBLICATION: http://www.terrenum.net/protector . WEB and electronic communications	100