Project no. NMP-3-CT-2003-505790

Project acronym **HIPERMAX**

Project title **High Performance Industrial Protein Matrices through Bioprocessing**

Instrument **STReP**

Thematic Priority **Priority 3 - NMP**
NANOTECHNOLOGIES AND NANO-SCIENCES, KNOWLEDGE-BASED MULTIFUNCTIONAL MATERIALS, AND NEW PRODUCTION PROCESSES AND DEVICES

39 Months Activity Report

Publishable executive summary

DWI, DE	PECCI, I	SETA, I
VTT, FIN	TDS, I	BLC, UK
DPPT, UK	TNTU, UK	PDM, UK
UNIMAN, UK	ABEG, DE	ASTON, UK
TTX, I	ROAL, FIN	POLITO, I
UNIPI, I	BUTE, H	

Period covered: from 01.08.2006 to 31.05.2007 Date of preparation:

Start date of project: 01.03.2004 Duration: 39 months

Elisabeth Heine
DWI at RWTH Aachen e.V. Revision [FINAL VERSION]

Publishable executive summary

The project aimed to develop novel enzymatic technologies for the production of high performance, economically viable protein matrices with tailored properties leading to enhanced surface/bulk characteristics. These novel materials are to be exploited in textile, leather, materials & medical industry. The choice of matrices wass focused on materials Nature has already engineered. Scientifically the objective was to discover novel enzymes capable of modifying and grafting functional groups onto protein matrices, to generate knowledge on enzymatic reaction mechanisms in molecular level using model substrates and to apply this knowledge to real substrates i.e.materials like wool, silk, leather, feather in order to produce novel tailored materials. Specifically the objectives were: -to define accessibility and reactivity of target groups in heterogeneous protein matrices, -to develop screening methods and screen for novel enzymes catalysing modification of protein matrices, -to produce novel enzymes in pilot scale, -to exploit novel enzymes in order to develop tailored materials e.g. grafting antimicrobials, hydrophobic agents; crosslinking, restructuring, reinforcing to design materials with new properties and to produce added-value products from waste material. The project integrated complementary know-how from 6 EU countries. The combination of different expertises of microbiology, biochemistry, enzymology, fiber research, material science and industry into an interdisciplinary project created efficient synergy. The STRP project corresponds to NMP-3.4.2.2-2 generating fundamental knowledge, developing generic technologies, fostering strong position of EU industry in area of smart coatings and thin films functionality by implementing enzyme-catalysed processes & to NMP-3.4.2.3-1 defining the accessibility of specific target groups for enzyme-catalysed functionalisation in protein matrices through reaction modelling & to NMP-3.4.3.2-1 developing sustainable solutions through bioprocesses aiming at creating knowledge-based industries.

Partners VTT, ABEG, ASTON, BUTE and ROAL worked in close co-operation on the development of screening methods, the screening for novel enzymes catalysing modification of protein matrices, and on the cloning of novel genes for the production of novel enzymes in pilot scale.

Within their co-operation to screen and to produce novel enzymes partners had different main foci of R&D. At VTT screening and characterisation of novel sulfhydryl oxidases (SOXs) with the aim of enzymatic grafting i.e. incorporating certain molecules with desired functionalities into proteinaceous fibres were performed. Furthermore the potential of a novel fungal Tyrosinase to catalyse grafting of functionalities to wool and silk was assessed in comparison to a mushroom tyrosinase.

At ABEG work was performed on the identification of novel Transglutaminase (*tg)* genes and on the development of suitable expression systems (*Bacillus subtilis*-based) for industrial scale production of TGases.

ASTON worked on the development of sensitive assays for screening of microorganisms for the production of novel TGases, on the elaboration of optimal growth conditions and enzyme purification and characterisation.

Screening of large culture collections and isolates for production of TGases was performed at BUTE.

ROAL worked on the cloning of *tg* genes from microorganisms and on the expression of the genes in production hosts (*Trichoderma reesei* based).

Partners performed cooperative R&D work to define accessibility and reactivity of target groups in heterogeneous protein matrices. In order to develop tailored materials e.g. grafting antimicrobials, hydrophobic agents; crosslinking, restructuring, reinforcing to

design materials with new properties and to produce added-value products from waste material at first commercially available enzymes were used. Different partners concentrated on specific materials and the introduction of discrete functionalities. In partners cooperation synergies were achieved by combining different protein matrices and enzymes as well as by combining chemical / physical and biological (enzymatic) treatments of material and moreover, by exchanging samples and analyses.

Modelling of TGase-catalysed grafting and crosslinking of amines to protein substrates was performed by DWI. Furthermore, TGase catalysed grafting of antimicrobial substances to protein materials was worked out.

At SETA Tyrosinase-catalysed production of protein-polysaccharide bioconjugates and surface functionalization of protein materials was elaborated.

TNTU studied the TGase- and Tyrosinase-catalysed grafting of small molecules to model and real protein substrates. Furthermore, TGase and Tyrosinase were applied to improve tensile strength and shrink resistance of untreated and and air plasma treated wool.

The potential of mTGase and tyrosinase to effect the grafting of amino-functional surface treatments (amino-polyethers and silk sericin) on to PMS-pretreated wool has been studied at DPPT, and the evaluation of these systems for the improvement of the mechanical properties.

At UNIMAN the chemical reactivity and accessibility of target residues in wool were analysed and a database was set up. Transglutaminase- and tyrosinase-catalysed grafting of small molecules to protein substrates was monitored and confirmed via mass spectrometry. Commercially feasible products from poultry feather were prepared in collaboration with PDM.

Plasma-pretreatment to enhance efficiency of TGase-catalysed modification of protein material was operated at TTX and industrially realised at PECCI and TdS.

UNIPI and POLITO developed and utilized enzyme technology to realize functionalized protein-based devices for tissue engineering / medical devices. Furthermore, they realized enzyme-catalysed functionalisation of hide powder and leather.

BLC carried out studies for enzyme-catalysed modification of leather properties.

Several papers have been published in the framework of the project and will be made available by contacting the coordinator.

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