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Effective redesign of oxidative enzymes for green chemistry-FP7 OXYGREEN

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experiments, the biosurfactant stimulated IL-1 β , IL-12, IL-18 and TNF- α production of human monocytes and displayed no cytotoxicity against human lymphocytes. Recently developed optimization procedures for the biosurfactant synthesis and recovery would broaden its potential applications in new advanced technologies, such as environmental bioremediation, improved polymeric material construction and biomedicine.

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[I.38]

Kojic acid production from glycerol: Optimization using central composite rotatable design

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Keywords: Kojic acid; Glycerol; Experimental design; Fermentation

Glycerol is the main by-product from biodiesel production. With the fast growth of biodiesel industry, new uses for the excess glycerol generated must be developed. The biotransformation of glycerol into value added products by microorganisms is an alternative that provides both economic and environmental benefits. In this study we evaluated the use of glycerol as substrate for the production of kojic acid, a widely used tyrosinase inhibitor with applications in the cosmetics, pharmaceutical and food industries. A number of carbon and nitrogen sources were initially tested for relevant kojic acid production by *Aspergillus flavus* NRRL 626 and glycerol was identified as a promising option. In the preliminary tests up to 18.8 g l⁻¹ of kojic acid was produced after 22 days using a basal media formulation described in literature. A central composite rotatable design (CCRD) was then carried out in order to optimize a low cost media formulation containing glycerol as sole carbon source and corn steep liquor as nitrogen source. The variables studied were glycerol concentration, corn steep liquor concentration and initial pH. Up to 29 g l⁻¹ of kojic acid were produced after 20 days among the runs. The optimal formulation was determined as 220 g l⁻¹ of glycerol and 7 g l⁻¹ of corn steep liquor. The initial pH showed no significant influence over kojic acid production. The maximum kojic acid concentration after 20 days of fermentation was predicted to be 34.4 g l⁻¹, which was about 1.8-fold higher than the obtained before optimization. These results indicate that glycerol might be successfully used as a low cost feedstock for the production of kojic acid.

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[I.39]

Effective redesign of oxidative enzymes for green chemistry—FP7 OXYGREEN

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Keywords: Enzyme redesign; Monooxygenase; Baeyer-Villiger; Biocatalysis

Enzymes are extremely powerful natural catalysts able to perform almost any type of chemical reaction while being mild by nature and highly specific. The catalytic potential of enzymes is more and more appreciated by the industry as many industrial processes rely on these sophisticated biocatalysts. However, the number of reactions catalyzed by enzymes is restricted as enzymes only have evolved to catalyze reactions that are physiologically relevant. Furthermore, enzymes have adapted to the cellular environment in which they have to function. This excludes the existence of enzymes that do not fit within the boundaries set by nature. It is a great challenge to go beyond these natural boundaries and develop methodologies to design 'unnatural' tailor-made enzymes. Ideally it should become possible to (re)design enzymes to convert pre-defined substrates. Such designer enzymes could theoretically exhibit unsurpassed catalytic properties and, obviously, will be of significant interest for industrial biotechnology.

The OXYGREEN project (www.oxygreen.org) aims at the design and construction of novel oxidative enzymes for the production of bioactive compounds that can be used in medicine, food and agriculture and targets the development of novel powerful and generic enzyme redesign tools for this purpose. We specifically aim to redesign cytochrome P450 monooxygenases, Baeyer-Villiger monooxygenases, and non-heme iron dioxygenases. These enzymes can potentially catalyze a large range of reactions that are extremely useful for the chemical and pharmaceutical industry. The project will yield a broadly applicable platform of newly designed oxidative biocatalysts that are suited for novel and 'green' synthesis routes. It will also provide new and rapid strategies and technologies for enzyme redesign to tailor an enzyme for a specific biocatalytic process. Recent results in the area of monooxygenase redesign will be presented, with emphasis on engineering effective Baeyer-Villiger monooxygenases.

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[I.40]

Protein fusions for high level expression and purification of pharmaceutical and industrial proteins in plants

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Keywords: Molecular farming; Recombinant protein production; Recombinant protein purification; Protein bodies

The production of recombinant proteins in plants is an active area of research and many different high-value proteins have now been produced in plants. We have worked on several pharmaceutical and industrial proteins such as interleukin-10 for the treatment of inflammatory bowel disease, interleukin-24 for the treatment of cancer, erythropoietin for tissue protection, spider silk proteins for the production of high tensile strength fibers and enzymes for biofuels production including cellulases and amylases. However, two major challenges for economical production