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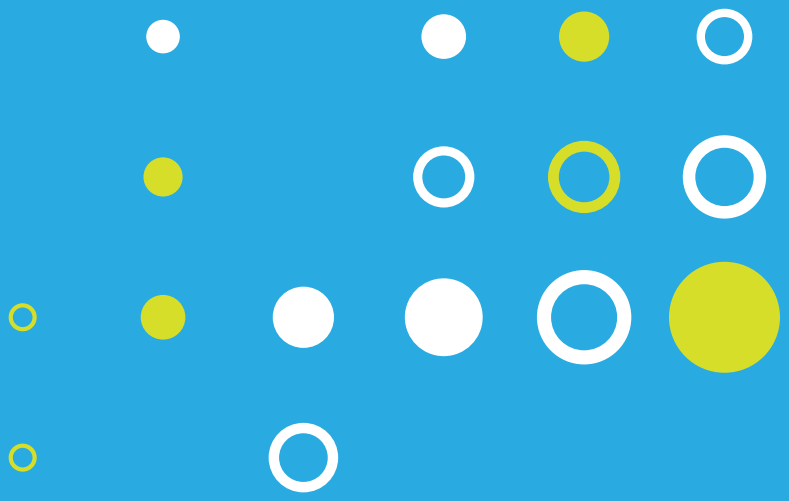

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BT-P09.
OPTIMIZED LOVASTATIN PRODUCTION BY SOLID-STATE FERMENTATION USING AGRO-INDUSTRIAL DERIVED SUBSTRATES

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Lovastatin is a drug that can inhibit the *de novo* synthesis of cholesterol by competitive inhibition of HMG-CoA reductase, which catalyzes the rate limiting step of cholesterol biosynthesis. It can be then effectively used for the hypercholesterolemia treatment. Nowadays, interest has focused in searching new statins and/or developing low-cost production strategies. This work aimed at optimizing lovastatin production by solid state fermentation (SSF) with *Aspergillus terreus* MEC, looking for low-cost and readily available substrates. Cultures consisted in textured deffated soy bean flour (HSDT) embedded with A. terreus-inoculated LP (whey protein concentrate) or SQ (whey) liquid media. Different initial pHs (4.3, 5.3, 6.3, 7.3), incubation temperatures (23, 25, 28, 30°C), moisture contents (CHI: 36, 46, 56, 66, 76%) and bed heights (1, 1.5, 2, 2.5, 3, 4 cm) were evaluated. Extracted lovastatin was analyzed by RP-HPLC with a Diode Array Detector and fungal growth was indirectly determined by ergosterol quantification. A lovastatin production of 1017.55 µg per g of dry weight was reached by using optimized SSF consisting in SQ liquid medium adsorbed on HSDT at an incubation temperature of 25°C, pH = 6.3, CHI = 46-56% and a bed height of 1.5-2.5 cm. These results highlight the relevance of SSF strategies for the production of high-added-value secondary metabolites.

BT-P10.
SUCCESSFUL FIBRINOLYTIC ENZYMES PRODUCTION BY SOLID STATE FERMENTATION

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Fibrin accumulation in blood vessels increases thrombosis risks promoting myocardial infarction and cardiovascular diseases. *Bionectria* sp. LY 4.1, a wild fungi isolated from Las Yungas Pedemontana rainforest, was recently reported by our group as a novel fibrinolytic enzyme source. In the present study we attempted the production of fibrinolytic enzymes by solid state fermentation (SSF) with *Bionectria* sp. LY 4.1 looking for low-cost substrates and simplified operational conditions. The different solid substrates (SS) evaluated (polyurethane foam - PUF, bagasse, textured soy flour, soy flour pellets and wheat bran), in adequate amounts were inoculated with fresh production medium containing 72 h-old *Bionectria* sp. culture. The effect of different conditions of moisture (9, 11, 13, 15, 17%), bed height (1, 2, 4, 6, 8 cm) and particle size (0.25; 0.5; 1 and 2 cm^2) was studied. Fibrinolytic activity was determined by the fibrin plate technique and protein concentration by the BCA method. The obtained results confirmed the possibility to produce fibrinolytic enzymes by SSF with *Bionectria* sp. LY 4.1. Furthermore, assays demonstrated the convenience of using PUF as SS under specific operating conditions over the other substrates tested. The findings herein presented offer an interesting alternative for the production of a high added-value pharmacological product.

BT-P11.
MALTOOLIGOSACCHARIDES PRODUCTION USING CYCLODEXTRIN GLUCANOTRANSFERASE FROM *Bacillus circulans* DF 9R

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Cyclodextrin glucanotransferase (CGTase, EC 2.4.1.19) catalyzes the starch conversion into cyclodextrins (CD), linear maltooligosaccharides (MOS) and limit dextrins. Many investigations are aimed at increasing the performance of CD production processes but there is little information regarding the use of CGTases on MOS production. The aim of this work was to study the optimal conditions to convert soluble potato starch into MOS by the action of CGTase from *Bacillus circulans* DF 9R. To study the reaction products obtained by CGTase activity on substrate in presence of carbohydrate acceptors, flasks containing 5 % soluble potato starch were incubated with 15 U of purified enzyme per gram of starch in a phosphate buffer 25 mM pH 6.4 at 56 ºC and 100 rpm. The effect of the addition of glucose or maltose as acceptors to reaction mixtures was evaluated in concentrations from 1.25 to 5.0% and the incubation time from 4 to 24 h. Products were analyzed by colorimetric reactions, paper chromatography and HPLC. When glucose or maltose concentrations increased, MOS production also increased. The best results were obtained using 5% soluble potato starch and 5% glucose. After 10 h of incubation, a highest yield of MOS and negligible CD concentration were obtained. This process might apply to MOS industrial production that nowadays is carried out using at least three different enzymes.

BT-P12.
ENGINEERING *Streptomyces coelicolor* FOR THE PRODUCTION OF FREE FATTY ACIDS

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Due to their high energy density and low water solubility, fatty acids are arguably the most appropriate biofuel precursors in a cell. Here we describe the design and construction of a *S. coelicolor* host strain suitable for the production of free fatty acids. To achieve this end, we engineered the cell by deleting genes of competitive pathways and inserted novel enzymatic activities, thus redirecting the malonyl-CoA flux to the desired product. We employed the CH999 strain, a widely used host for *in vivo* production of engineered natural products, and a mutant strain deficient in triglyceride biosynthesis. In this genetic background, we overexpressed the *S. coelicolor* Acetyl-CoA Carboxylase complex and the thioesterase TesA from *Escherichia coli*. The expressions of these genes were achieved using *ad hoc* customized vectors that consist in modular-combinable DNA features with BioBricks format. Using this approach, we constructed several plasmids carrying the genes coding for target enzymes under different endogenous *Streptomyces* promoters, active in late exponential or in stationary phase of growth. Under these conditions we were able to exert a temporal control of gene expression in order to minimize the interference with cell chassis and optimize the timing of gene induction accordingly to the metabolic state of the cell; finally improving the levels of production of free fatty acids.