

enzymes. DAAO turned out to be a versatile biocatalyst and a robust scaffold to evolve novel variants active on natural and unnatural amino acids. For this reason, this enzyme was widely exploited in biotechnology. On the other hand, biotechnological exploitation of LAOs turned out to be an awkward task because of the difficulty in their recombinant overexpression. The recent characterization of an L-amino acid deaminase from *Proteus myxofaciens* (PmaLAAD) allowed to close the circle and to extend the application of chiral biocatalysis to the production of pure D-amino acids. PmaLAAD is specific for large hydrophobic natural L-amino acids (e.g., L-Phe, L-DOPA) and, to a lesser extent, on unnatural substituted alanines. Based on the 3D structure of the enzyme and following sequential rounds of site-saturation mutagenesis, enzyme variants with an improved activity on L-1-naphthylalanine were isolated. The evolved enzyme variants were used in biotransformation reactions on the L-enantiomer and on the racemic amino acid mixture, in the latter case in combination with the M213G DAAO variant. The combined use of evolved variants of DAAO and PmaLAAD represented a competitive and versatile biocatalytic system for  $\alpha$ -keto acid production, deracemization and stereoinversion of amino acids of biotechnological relevance.

### P.07-038-Tue

#### Optimization of fermentation condition for $\alpha$ -amylase production

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The study is concerned the growth conditions of *Bacillus amyloliquefaciens* MDC 1974 isolated from soil samples (Microbial Depository Center of SPC "Armibiotechnology" NAS RA) for maximum production of thermostable  $\alpha$ -amylase. The strain was identified as *Bacillus amyloliquefaciens* by 16S rDNA phylogenetic analysis. The sequence of 16S rDNA gene was submitted to GenBank under the accession number MF953984.1. With consumers growing increasingly aware of environmental issues, industries find enzymes as a good alternative over other chemical catalysts. Among all groups of hydrolytic enzymes, amylases have been used extensively because of their potential of application in the biotechnology-based industries.  $\alpha$ -Amylase can be produced by plant or microbial sources. Due to the advantages that microbiological production offers, to  $\alpha$ -amylases of microbial origin was paid great attention by researchers. Strains of *Bacillus* genus are the main sources of  $\alpha$ -amylases having the industrial application. Five incubation temperatures (30, 35, 40, 45 and 50 °C), two incubation times (24 and 48 h) have been studied. The growth media contains salts (KH<sub>2</sub>PO<sub>4</sub> 0.05 M, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.005 M and CaCl<sub>2</sub> 0.001 M), different carbon (glucose, starch and wheat flour (1% w/v)) and a nitrogen (yeast extract (0.5% w/v)) sources. The fermentation was carried out at 7.0 pH in 500 ml Erlenmeyer flasks containing 100 ml growth media by laboratory shaker (Innova 43 Shaker "New Brunswick", USA), 200 rpm. For optimization of each parameters, three sets of independent experiments were carried out and the average values were reported. According to the obtaining results the starch was chosen as a best carbon source. Maximum specific activity of  $\alpha$ -amylase (9.2 U/mg) was obtained at 40 °C after 48 h of incubation. The total activity yield was 3770 U. Thus, the  $\alpha$ -amylase of *Bacillus amyloliquefaciens* strain will have industrial application.

### P.07-039-Wed

#### Optimization of laccase production by fungi co-cultivation

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In this work production of fungal laccase was optimized using solid state fermentation and kiwifruits peels as support-substrate. Fungi *Irpex lacteus* (MUM 94.08) and *Trichoderma viride* (EXF-8977) were cultivated separately and with each other on kiwi fruits peels, which was pre-treated with base to neutralize organic acid and distilled water, with and without soil for period of 21 days at 25° C by taking samples on 7, 14 and 21 days. Extracellular laccase activity was measured spectrophotometrically using 0.5 mM 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) as substrate, concentration of protein was estimated with Bradford's assay as well as amylase and protease enzyme activities were detected under same conditions. Subsequently, purification and optimization of culture conditions for laccase production was studied with the effect of temperature, pH and moisture on production. Comparison of laccase activity among the designed fungi and combination shows increasing of enzyme production. *Trichoderma viride* (EXF-8977) produces on kiwi peels 518.56 UI<sup>-1</sup>, co-cultivation on same condition produce 947.45 UI<sup>-1</sup> of laccase. *Irpex lacteus* (MUM 94.08) produces on substrate 196.02 UI<sup>-1</sup>, while mixed with soil is able to produce 1366.2 UI<sup>-1</sup>. However, co-cultivation of fungi on peels and soil give us 2295.81 UI<sup>-1</sup>. According to the results co-cultivation of these two strains on kiwifruits peels and soil increase laccase production.

### P.07-040-Mon

#### A modified E3 ubiquitin ligase CHIP of *Arabidopsis thaliana* for the target degradation of GFP

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Over 5% of *A. thaliana* genes are involved in the ubiquitinylation process. More than a thousand genes are E3 ubiquitin ligases (UL) that perform specific degradation of target proteins. Modification of one of these genes for the recognition of a specific target makes it possible to regulate the level of selective protein degradation in cells using the ubiquitin-proteasome system (UPS). To this end, we have modified the gene of E3 UL Chip for the recognition of GFP and used it for the selective degradation of the GFP-expressing transgenic plants of *N. benthamiana*. We have analyzed two variants of the modified gene of E3 UL Chip, lacking 100 or 140 amino acids at the N-terminus, and fused in the reading frame with the anti-GFP monoclonal antibody gene. For transient expression in plant cells we have created vectors based on plasmid pCXSN in which the gene encoding Ch100mb or Ch140mb was placed under the control of the strong constitutive 35S promoter of the cauliflower mosaic virus. The resulting vectors were used to transform the *A. tumefaciens* strain C58. Fluorescence microscopy analysis of GFP degradation was carried out on the 6th day after the infiltration of the leaves of the transgenic *N. benthamiana* expressing GFP with agrobacterium carrying the Ch100mb or Ch140mb genes. No changes in the level of GFP fluorescence were identified in the control samples - in the zone without infiltration, in the infiltration zone with agrobacterium containing the empty vector pCXSN or the pCXSN-E3 vector of UL Chip. In both infiltration zones with