represent an improvement in hydrolysate treatments by reducing waste, volume and sugar loss. This work evaluated the microaerobic biodegradability of toxic compounds in the sugarcane hemicellulosic hydrolysate by the yeasts *Issatchenkia occidentalis* CCTCC M 2006097 and *Issatchenkia orientalis* CCTCC M 2006098. The yeast inocula were obtained by previous cell growth in semi-defined medium (containing glucose as carbon source). Biodegradability experiments were conducted in Erlenmeyer flasks containing 50 mL of hydrolysate (51.50 g/L xylose, 1.81 g/L glucose, 0016 g/L furfural, 0016 g/L HMF, 0.30 g/L ferulic acid, and 0.60 g/L syringaldehyde) or in semi-defined medium containing the same amount of individual hydrolysate inhibitors. All media were supplemented with 5 g/L $(NH_4)_2SO_4$,1 g/L KH_2PO_4,0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.1 g/L $CaCl_2$,0.1 g/L NaCl, 0.2 g/L yeast extract, and 2 g/L urea and incubated at 200 rpm and 30°C for 60 hours. Sugars and other compounds were quantified by HPLC. In 24 hours the results showed total furfural removal from hydrolysate for both yeasts. Also, total and partial (84%) removal of HMF by *I. orientalis* and *I. occidentalis*, respectively. The biodegradability of syringaldehyde and ferulic acid were 75% and 17% by *I. occidentalis* and *I. orientalis*, respectively. However, *I. orientalis* used xylose as a carbon source (10%), while *I. occidentalis* grew expressively in the hydrolysate even in microaerobic condition. In a semi-defined media, the total removal of individual inhibitor occurred in 48 hours. Therefore, the biodegradability of the furan and phenolic compounds by the genus yeast *Issatchenkia* in microaerobic conditions was possible in a single stage and by selectively removing the inhibitors from sugars in the sugarcane hemicellulosic hydrolysate.

The results showed the potential of these yeast strains in the biodegradability of the inhibitors present in sugarcane bagasse hydrolysate, which can be used in fermentative processes.

doi:10.1016/j.nbt.2009.06.300

2.1.054

Molecular identification of filamentous fungi from olive phyllosphere and investigation of their biotechnological properties

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A molecular approach was used for the identification of filamentous fungi isolated from olives. Fungi samples were isolated from olive fruits randomly taken from different olive plantations from Castilla La Mancha region (Spain). Samples from olive paste and olive pomace (*orujo*), a by-product from the processing of this raw material, were also collected. Molecular identification included comparison of their polymerase chain reaction amplicons of their 5.8S rRNA gene and internal transcribed spacers ITS1 and ITS2 (ITS1—5.8S—ITS2 region), followed by nucleotide sequence analysis. The results were compared to sequences held in public databases. From the 54 isolates analyzed, it was possible identify 14 different species, belonging to 7 different genus (*Aspergillus*, *Penicillium*, *Rhizomucor*, *Mucor*, *Rhizopus*, *Absidia* and *Galactomyces*), showing a heterogeneous diversity of species. *Aspergillus fumigatus*, followed by *Galactomyces geotrichum* and *Penicillium comune* were the most frequent species. These isolates were also approached regarding their biotechnological properties, where particular enzymes were evaluated by means of zymograms. From olive fruits, five isolates of *A. fumigatus* were selected for beta-glucosidase and CMCellulase and one isolate for lipase; one isolate of *Aspergillus* sp. for beta-glucosidase; one isolate of *Penicillium* sp. for beta-glucosidase and polygalacturonase and two isolates for *Mucor fragilis* for beta-glucosidase, CMCellulase and lipase. From olive paste, one isolate of *A. niger* was selected for beta-glucosidase, CMCellulase and polygalacturonase; one isolate of *Absidia corymbifera* for CMCellulase; one isolate of *Rhizopus oryzae* for beta-glucosidase and lipase; one isolate of *Penicillium commune* for lipase and one isolate of *Penicillium crustosum* for lipase activity. With respect of olive pomace, five isolates of *R. variabilitis* were selected by their strong activity for beta-glucosidase and also for CMCellulase and one isolate of *Galactomyces geotrichum* for lipase. Our study is the first one that describes filamentous fungi isolated from olive ecosystem from Castilla La Mancha and their ability in producing enzymes of industrial interest. Our work also shows the potential of some fungi isolated from olive pomace, suggesting that this olive by-product can also be used to take advantage for industrial biotechnology. Such enzymatic properties described are meaningful for further researches for olive oil industry and also other industrial applications.

doi:10.1016/j.nbt.2009.06.301

2.1.055

Recombinant protein expression of *Tetrahymena thermophila* glutathione-*S*-transferase zeta (6xHis-GSTz) in *E. coli* suggest a possible use as a GSTz or 6xHis-GSTz dual tag in *Tetrahymena* protein expression vectors

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Biologically, glutathione-*S*-transferase enzymes (GST) help to detoxificate effects of endogen and exogenous toxic compounds in living organisms. Biotechnologically, genetic engineers have used glutathione-*S*-transferase to create the GST gene fusion system. Results of the *Tetrahymena* macronuclear genome project showed that *T. thermophila* has about 19 different GST genes that represent only four subgroups as theta, omega, mu and zeta. The goal of this study is to develop a protein expression tag based on *Tetrahymena* GST family to be used in *Tetrahymena* expression vectors. A candidate mRNA sequence among *Tetrahymena* GST gene was selected as GST zeta based on the structural and sequence differentiation from the rest of the GST family. The full-length mRNA sequence of GST zeta gene was determined as 847 bp by using genomic sequence (77.m00138) from genome project, EST sequences of cDNAs from EST projects and clone sequences of 3′RACE. The region of GSTz cDNA gene between start and stop codon was cloned and sequenced to confirm the existence of the gene in *T. thermophila* SB210. Comparison of genomic and cDNA sequence of TtGSTz gene by PCR and agarose gel analysis showed that there

are totally 122 bp long intron in the genome copy. Multiple alignment of GSTz amino acid sequence with other GSTzeta showed the existence of conserved characteristic motif (SSCX[WH]RVIAL) of zeta subgroup in *Tetrahymena* GSTz. Change in expression of GSTz mRNA level under the hydrogen peroxide and cold (4°C) stresses with time intervals was analyzed with semi-quantitative RT-PCR method. While there was no change at the level of GSTz mRNA under the hydrogen peroxide stress, the amount of mRNA was increased with time under the cold stress. After incorporation of seven necessary multiple point mutations (TAA > CAA or TAG > CAG) on mRNA protein coding region of GSTz gene for the protein expression in *E. coli*, the recombinant fragment was ligated to pET16b expression plasmid and transformed into *E. coli BL21-DE3*. SDS-PAGE analysis of recombinant 6xHis-TtGSTz protein (24 kDa) showed that it is successfully purified not only by nickel agarose but also by glutathion-sepharose 4B. The Western blot analysis showed also that both anti-His and anti-GST antibodies recognized the purified recombinant 6xHis-TtGSTz. In conclusion, the preliminary results presented here suggest that 6xHis-TtGSTz could be used as a dual tag in the *Tetrahymena* expression vectors to improve the purification of proteins from *Tetrahymena*.

doi:10.1016/j.nbt.2009.06.302

2.1.056

Antimicrobial properties of TiO_2—SiO_2 thin films

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Titanium dioxide (TiO_2) is one of the most important and most widely used semiconductor photocatalyst due to its application in solar energy conversion and environmental purifications. In this study, SiO_2 used as support material and deposition of TiO_2 films on glass surfaces by using a SiO_2—TiO_2 binary system has been performed. The SiO_2 supported TiO_2 samples were synthesized by sol—gel technique by using SiO_2 colloidal solution and hydrolysis of TTIP. The thin films over soda glass plates were obtained by dip coating followed by calcination at 500°C. The photocatalytic activities of the samples were measured with methylene blue stain degradation and antimicrobial effect against *Escherichia coli* under artificial sunlight. It was found that the antimicrobial activity is enhanced by the increasing ratio of SiO_2/TiO_2. By contrast, the methylene blue degradation is suppressed on the surfaces with higher SiO_2/TiO_2 ratio in spite of their higher surface area. These results reveal that photocatalytic degradation and antimicrobial effect have different mechanisms and surface activity depends strongly on surface structure.

To observe effect of TiO_2 and light on the morphological changes of *E. coli* SEM analysis of *E. coli* cells on the coated and uncoated glasses was performed by Prof. Dr Zekiye Suludere and Dr Demet Suludere at Gazi University after one hour irradiation. After irradiation for one hour, no obvious morphological changes of *E. coli* cells were recognized on the uncoated glass. By contrast, the shape of the cells, which looks like sausage, disappeared on the coated glass after one hour irradiation.

The photocatalytic inactivation experiments with *E. coli* revealed that the addition of SiO_2 enhanced the photocatalytic activity of thin films. However, addition of SiO_2 reduced photocatalytic self-cleaning activity of thin films against MB stain. In addition, it was found that the specific surface area and superhydrophilic property of thin films increased with addition of SiO_2.

doi:10.1016/j.nbt.2009.06.303

2.1.057

Biosorptive removal of Cibacron Blue Cr by actively growing and inactivated *Aspergillus oryzae*

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Synthetic dyestuffs are used extensively in textile, paper, printing industries and dye houses. The effluents of these industries are highly colored and the disposal of these wastes into receiving waters causes damage to the environment. Toxic and genotoxic effects of the textile dyes on organisms suggest the need for remediation of dyes before discharging them into the environment. Biosorption has been continuously studied for the removal of heavy metals and other pollutants from wastewater, so it could be a promising alternative to replace or supplement present dye bearing wastewater treatment processes.

In this work, it was aimed to investigate uptake of Cibacron Blue CR (CB) that is used widespread in textile industries, by both actively growing and inactivated *Aspergillus oryzae* cells under different incubation conditions; to compare dye uptake data of actively growing and inactive *A. oryzae* by using adsorption isotherm models and to investigate the function of adsorption in decolorization mechanism. The Langmuir and Freundlich isotherm models were applied to the experimental data found at different temperatures; equilibrium data fitted well to Langmuir model. The maximum monolayer CB adsorption capacity of inactivated *A. oryzae* was found to be 63.69 mg/g. Under optimum conditions, maximum monolayer CB adsorption capacity of actively growing *A. oryzae* was found to be 58.82 mg/g. To determine the adsorption type and for the regeneration of adsorbent, desorption studies were carried out. 83.09% and 96.48% desorption efficiencies were obtained from homogenized actively growing *A. oryzae* and inactivated *A. oryzae* respectively by using NaOH. Findings of this study, showed that the basic mechanism of color removal by actively growing and inactivated *A. oryzae* is 'physical adsorption' and CB is removed by binding to the active sites of *A. oryzae*.

doi:10.1016/j.nbt.2009.06.304