in dry chamber for 2 days 0.7 mL of the cell suspension was inoculated into a vessel of model soil and kept at 30 °C. N-hexadecane was quantified with FID-GC after extraction with chloroform.

As the results, it was found that the degradation rate reached plateau over the hexadecane concentration of 1.1 mg $(g\text{-soil})^{-1}$. This suggests that the rate-limiting step in the degradation of hexadecane in soil is not the transportation of the hydrophobic substance through aqueous phase but the reactions that degrade the substance. Thus, we tried to clarify the rate-limiting reaction in the metabolic degradation pathway of hexadecane and on the other hand to prolong the degradation reaction of *Y. lipolytica*.

## EN-P42

### Characterization of thermostable polyester-degrading enzyme from *Thermobifida alba* AHK119

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Polyesters are categorized into three, that is, aliphatic, aromatic and aliphatic-aromatic ones. Aliphatic polyesters have high biodegradability, but low physical properties. Aromatic polyesters have excellent physical properties, but low biodegradability. Aliphatic–aromatic polyesters are designed for biodegradable plastics with physical properties enough for practical uses. *Thermobifida alba* AHK119 was isolated from compost, which are able to degrade one of commercially available aliphatic-aromatic polyesters with high melting temperature over 200 °C. The gene (*est*) encoding for a mature polyester depolymerase of 301 amino acids was cloned from *Thermobifida alba* AHK119 and showed the high identity with the genes encoding for lipolytic enzymes from several origins. The polyester depolymerase contains a catalytic triad formed by Ser, Asp and His, where Ser is conserved in an active site motif of lipase/esterase (GXSXGG). The presence of signal peptide (34 amino acids of N-terminus), and 3D modeling suggested that the gene codes for the extracellular esterase. The polyester depolymerase gene was expressed in different systems of pQE-80L and pET23a in *E. coli* BL21 (DE3) and Rosetta-gami (DE3). Most part of activity was expressed as pellets in BL21 (DE3), but as soluble form in Rosetagami (DE3). The 6xhistidine-tagged recombinant enzyme was purified from cell-free extracts of Rosetta-gami (DE3) harboring pET23a-est, using a Ni-NTA agarose column. The molecular mass of polyester depolymerase was determined to be approximately 30 kDa by SDS-PAGE, and gel filtration, suggesting monomer enzyme. The highest activity was observed when *p*-nitrophenyl hexanoate ($C_6$) was used as a synthetic substrate. The purified enzyme was characterized with regard to thermostability, substrate specificity and substrate binding ability. In addition, the inductivity of the enzyme by polyesters was compared with known polyester-degrading enzymes.

## EN-P43

### A novel endophytic bacterium, *Achromobacter xylosoxidans*, helps plants against pollutant stress and improves phytoremediation

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Phytoremediation has been considered as an in situ bioprocess to clean up environmental contaminated soil and water. Plants can act as solar-powered-pump treatment systems relying on their roots to take up contaminants and transport them through various plant tissues where the contaminants could be metabolized. However, using plants alone for remediation confronts many limitations. Recently, the application of endophytic bacteria (endophytes) has been extended to remediate contaminated soils in association with plants. In this study, 188 strains of the endophytes were isolated from reed (*Phragmites australis*), *Ipomoea aquatica*, and vetiver (*Vetiveria zizanioides* (L.) Nash). Some of the isolates demonstrated tolerance to heavy metals and aromatic compounds. Twenty-nine strains of endophytes grew well in the presence of naphthalene, catechol and phenol. While those strains performed not only tolerance but also can utilize aromatic compounds as sole carbon sources. One of the endophytic isolate *Achromobacter xylosoxidans* F3B was further studied to show its assistance for phytoremediation. Inoculation of *A. xylosoxidans* F3B to *Arabidopsis thaliana* resulted in a significant increase in root length, fresh weight in catechol and phenol-added agar. The presence of *A. xylosoxidans* F3B provides the protection against the phytotoxic effects of catechol and phenol. The results show the potential of endophytic bacteria helping plants to tolerate pollutant stress and improving the efficiency of phytoremediation.

Keywords: Phytoremediation, Endophytic bacteria, *Achromobacter xylosoxidans*, Biodegrading.

## EN-P44

### Prediction and classification of the modes of genotoxic actions using bacterial biosensors and cell array chip

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We report on a novel approach to predict the mode of genotoxic action of chemicals using a series of DNA damage specific bioluminescent bacteria. One such test popularly known is Ames test based on bacterial bioassay, which has limitation for determining the potential mode of DNA damage. In this study, we aim to classify genotoxic mode of toxic action and apply this for cell array chip using seven recombinant bioluminescence bacteria. For this, a group of seven different DNA-damage sensing recombinant bioluminescent strains were employed. Each of these strains were tested against model DNA damaging agents, such as mitomycin C (MMC), 1-methyl-1-nitroso-N-

methylguanidine (MNNG), nalidixic acid (Nal) and 4-nitroquinoline N-oxide (4-NQO). In a classification, we employed one of the sensing parameter, minimum detectable concentration (MDC), which can be used as an indicator of the assay's capability. The differential response patterns and its strength of these strains to various model genotoxicants allowed classifying the chemical's potential genotoxic mode. As a result, these biosensors were grouped based on their responses to a specific mode of genotoxic action, such as (a) DNA damage cascade response (biosensor with nrdA-, dinI- and sbmC-lux), (b) SOS response or DNA repair (strains carrying recA-, recN- and sulA-lux), and (c) DNA damage potentially by alkylation (biosensor with alkA-lux). The application of classified three groups was conducted and identified via 17 different chemical tests and construction of genotoxicity cell array chip. Finally, genotoxicity classification was configured different DNA damage mechanism and cell array chip approach showed the possible novel genotoxic assay for the pre-screening steps of new drugs or to characterize unknown or newly synthesized chemicals, food and environment samples.

## Analysis of protein expression profiles of As(III), As(V), Cd and binary heavy metal mixture in *Daphnia magna*

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Proteomics related toxicology has provided innovative approaches to novel biomarkers screening, toxic materials screening, and risk assessments. The application of proteomic analysis in the field of aquatic toxicology has recently started to increase, but until now, little attention has been given to the integrated analysis of molecular responses and higher-level effects within the same study. In this study, protein expression profiles provided significant insights into the toxicity mechanisms for the *Daphnia magna,* post exposure of heavy metals. We performed proteomic analysis of *D. magna* in response to As(III), As(V), Cd, As(III)+As(V), As(III)+Cd and As(V)+Cd as a heavy metal, by using two-dimensional gel electrophoresis (2-DE) and found that protein profiles from *D. magna* were shown different expression patterns for the each heavy metals. *D. magna* protein expression patterns of binary mixtures are not the simple sum of their individual compounds' fingerprint. For example, the changed spots are 42 and 46 in daphnia exposed to As(III) and Cd, respectively. In addition, the changed spots are 31 in daphnia exposed to binary mixture, As(III) and Cd. In comparison with results of single and binary mixture, the number of spots only found in As(III), Cd and binary mixture of As(III) and Cd are 23, 24 and 12, respectively. It means that the protein expression patterns of binary mixtures would be the results of additive molecular combinations of both individual compounds. Therefore, *D. magna* protein expression patterns of binary mixtures are not the simple sum of their individual compounds' fingerprint. The 23, 24 changed spots by As(III) and Cd can be used as novel biomarker candidates to detect As(III) and Cd.