[P-E.33]

Luminescent bacteria from a brackish environment

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Keywords: Bioluminescence; Vibrionaceae; 16S rDNA

Bioluminescent bacteria are ubiquitous in the marine environment and are also found in freshwater, brackish water, and soil environments. Luminous bacteria have been observed in marine environment living as planktonic or free-living, saprophytic, light organ- and gut-symbiotic, and pathogenic. Bioluminescent bacteria are used in biotechnological applications, in tests of water quality and toxicity, and as biosensors in environmental biomonitoring.

Although phenotypic characterization is considered a valuable method in taxonomic investigations, it is time-consuming and often can be compromised by bacterial variability in the responses to biochemical tests.

Bioluminescent bacteria were isolated from water samples of the meromictic Faro lake (Messina, Italy) and characterised phenotypically and genotypically by cultural and molecular assays. All strains were members of the family *Vibrionaceae*. Forty-four bioluminescent isolates were pre-screened by Amplified Ribosomal DNA Restriction Analysis (ARDRA) with five enzymes (*Eco*RI, *Dde*I, *Hha*I, *Hinf*I, and *Rsa*I) to clusterise them into homology groups before 16S rDNA gene sequencing.

Culturable luminous bacteria in the water of Faro Lake were dominated by *Vibrio harveyi* and *Photobacterium phosphoreum*. For analysis of 16S rDNA gene sequences, 29 strains were selected as representative strains of the each ARDRA group. Phylogenetic analysis showed that eight strains were closely (98-99% sequence similarity) related with *V. harveyi*, and other eight strains were similar (98-99%) to different luminescent *Vibrio* spp. Four strains were affiliated (98-99%) with *Photobacterium phosphoreum*. Nine strains, possessing sequence similarity levels below 97% with yet described species, could be considered new bacterial species.

Five strains remained luminescent after one year of subculturing. The applications in biotechnological assays of the present luminous bacteria remain to be evaluated.

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[P-E.34]

Isolation and characterization of lipid bodies from a marine bacterium *Alcanovirax borkumensis*

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Keywords: lipid bodies; *Alcanivorax borkumensis*; marine biotechnology

A cosmopolitan marine hydrocarbonoclastic bacterium *Alcanivorax borkumensis* strain SK2 posseses a specialised metabolism ensuring the survival in harsh conditions of a marine nutriet-limited environment where carbon source is a limiting factor and thus has to be stored when in abundance. *Alcanivorax borkumensis* is able to store carbon in the form of various lipid compounds such as wax esters and polyhydroxyalkanoates. These compounds are deposited inside the cell in specialised organelles called lipid bodies. Other important feature of *A. borkumensis* is its remarkable substrate spectrum which consists almost entire spectrum of aliphatic hydrocarbons. This specialised metabolism, mainly the ability to degrade oil and lipid production, determines the use of *A. borkumensis* in several biotechnological processes such as clean-up of marine oil spills or production of biodegradable plastics. We have focused our attention on lipid storing organelles, lipid bodies, namely on evolving the protocol for their purification and subsequently their characterization. Several floatation technics have been tested for isolation of lipid bodies, techniques being evaluated by the purity of purified lipid bodies in the means of lipid and protein content and their concentration ratio. Further purified lipid bodies were characterized by their lipid and protein profile, lipids being characterized by TLC and GC/MS techniques and protein profile being characterized using SDS-PAGE. In close future we also plan the protein characterization using mass spectrometry. All above mentioned informations allow to improve and expand the use of *A. borkumensis* in biotechnological processes.

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[P-E.35]

Metal extraction from wastes by biotechnological strategies

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Keywords: waste; metal; bioleaching

This work deals with biotechnologies aimed at metal extraction from spent hydroprocessing catalysts and from fluorescent powders coming from cathode ray tube recycling processes. The exhaust catalyst was rich in nickel (45 mg/g), vanadium (94 mg/g) and molybdenum (44 mg/g). The fluorescent powder was rich in zinc (5 mg/g) and yttrium (0.5 mg/g). Both these wastes had a sulphide component which bound the metals. Involved microorganisms were iron/sulphur oxidizing bacteria Acidithiobacillus spp. and Leptospirillum ferrooxidans, chemo-autotrophic bacteria able to oxidise both iron and sulphur. Investigated factors were the initial redox potential and solid concentration. The performed experiments evidenced the effectiveness of bioleaching with respect to chemical controls and the inhibition of bacterial growth in the presence of ferric iron at the beginning of the treatment. The achieved results offer new eco-sustainable technologies for the use of waste as resources.

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[P-E.36]

Development of a bacterial biosensor for arsenite detection

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Keywords: Arsenic drinking water pollution; gene reporters; microfluidics

Contamination with arsenic is a recurring problem in both industrialized and developing countries Diesel et al., 2009. Of par-

ticular concern is the contamination of potable water sources by arsenic in Southeast Asia (Bangladesh, Vietnam). In order to provide alternative measurement tools for detection arsenic contamination, our group has developed a number of bioassays with so-called reporter bacteria Stocker et al., 2003; Trang et al., 2005. These bacteria synthesize an easily measurable protein (such as green fluorescent protein) in response to arsenic. The response is proportional to the amount of arsenic applied to the cells.

The goal of the overall project is to miniaturize the arsenic bioassay and embed the reporter cells in microfluidics chips to have an easy portable unit by which samples could be measured for arsenic in the field. Small cavities on the chip (50 x 50 x 10 μm) hold several hundreds of cells and the fluorescence of the cells can be observed Diesel et al., 2009.

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[P-E.37]

Upgrading and detoxification of aqueous extracts from dry olive mill residues by white-rot fungi

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Keywords: Dry olive mill residues; White-rot fungi; Upgrading; Phenoloxidase enzymes

Dry olive mill residue (DOR) is the waste from the two-phase manufacturing process of olive oil. Despite its potential fertilizer value, its application onto soil results in a variety of negative effects mainly due to its toxicity. Most of toxicity is due to the presence of water-soluble phenols. At the same time, it can be considered a useful resource that could be up-graded *via* recovery of fine-chemicals or *via* fermentation. The low moisture content of DOR (13%) allows a long-term storage prior to its possible use for upgrading purposes. Among them, DOR might be resuspended in water and used as a growth medium for microbial bioconversions.

Thus, the objective of the present study was to screen for white-rot fungi able to grow on the aqueous extracts of DOR, to produce enzymes of commercial interest, such as phenoloxidases, and to reduce, at the same time, both its organic load and toxicity.

To this aim, both plate tests and shaken cultures were preliminarily performed. The selection criteria were based on capability of removing phenols, color and toxicity and to produce extracellular enzyme involved in the degradation of both monomeric and polymeric aromatic compounds of the waste. Among the six fungal strains screened, both *Phlebia* sp. DABAC 9 and *Lentinus (Panus) tigrinus* CBS 577.79 were most effective. To gain preliminary indications on process transfer, the two fungi were grown in a bubble-column reactor with 2-l working capacity. With regard to the production of aromatics-degrading enzymes, the two fungi did not differ each one another, since they mainly released Mn-dependent peroxidase activity (576 ± 44 and 631 ± 53 IU l^{-1}, respectively) on aqueous DOR and, to a lesser extent, laccase and mono-phenolase. Color and phenol content were also significantly removed at the end of fermentation processes (more than 90% in the case of phenols).

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[P-E.38]

Effect of inoculum formulation and contaminant bioavailability on PAH degradation performances of *lentinus tigrinus* on contaminated solid matrices

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Keywords: Mycoremediation; *Lentinus tigrinus*; PAH contaminated soil; Creosote-impregnated shavings

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants that have been included among priority pollutants for toxic, mutagenic and, in some cases, carcinogenic properties. Their recalcitrance in the environment is mainly due to the physical properties, such as, *e.g.*, low aqueous solubility and high solid/water distribution ratios, that limit PAH bioavailability. Also, aging phenomena can further decrease contaminant bioavailability thus reducing bioremediation effectiveness.

Objective of the work was to assess the impact of three lignocellulosic inoculum carriers (*i.e.*, wheat straw, WS; corn cobs, CC and commercial pellets, CP) on both growth and PAH degradation performances of *Lentinus tigrinus* CBS 577.79. To this aim, two highly PAH-contaminated matrices were selected, a historically polluted soil (HPS) and creosote-impregnated shavings (CIS) (Sobeslav, southern Bohemia). These matrices significantly differed for both the contents of each PAH contaminants and the amount of the relative PAH bioavailable fractions. To better elucidate the effect of the contaminated matrix and its interaction with both inoculum carrier and fungus, data obtained with *L. tigrinus* were compared with those of *Irpex lacteus* CCBAS 238/617, the PAH-degrading capacity of which is well known.

Although degradation performances of *L. tigrinus* were not significantly affected by the type of the support, they were invariably better than those of *I. lacteus* on both HPS and CTS. Although degradation efficiencies of all fungal microcosms were highly and significantly correlated with bioavailability, certain PAHs, such as chrysene and benzo[a]pyrene, were removed by *P. tigrinus* from HPS at amount that exceeded about 2.3-fold their respective bioavailabilities. Degradation of PAHs was negatively correlated with their organic carbon sorption coefficients (K_{oc}) and hydrophobicity (log P); the strength of linear association of former variable, however, was not affected by the type of contaminated matrix for *L. tigrinus* while it was significantly larger in HPS than CIS for *I. lacteus*-based microcosms.

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