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Highlight

# Sugar (ribose), spice (peroxidase) and all things nice (laccase hair-dyes)

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The removal of pollutants from the environment has been declared a priority by a number of Environmental Protection Agencies (Roze *et al*., 2009). A great number of aerobic pathways have been deciphered and their relevance in microbiology and biotechnology has been reviewed several times (Garmendia *et al*., 2008; Siezen and Galardini, 2008; Govantes *et al*., 2008; Atlas and Bragg, 2009). In the area of biodegradation the role anaerobes and fungi play in removal of pollutants is of mounting interest. *Microbial Biotechnology* is publishing a number of new titles in this area, and here we have extracted some of the main conclusions.

Taş and colleagues (2009) have dealt with mineralization of polychlorinated chemicals, which are harmful contaminants due to their persistence and their chronic toxicity to living organisms. *Dehalococcoides* spp. can anaerobically transform chlorinated xenobiotics to less- or even non-noxious derivatives via reductive dechlorination. Taş and colleagues (2009) have reviewed the biology of this genus, focusing on its genetic peculiarities, its variability and, of course, its biodegradative properties. *Dehalococcoides* can replace chlorine by hydrogen atoms in recalcitrant halogenated compounds, using them as electron acceptors during anaerobic respiration. More than 100 16S rRNAs from environmental *Dehalococcoides* spp., are available, most of them corresponding to uncultured strains. In addition to the standard problems of cultivating anaerobic microbes, these coccoids usually grow in microbial communities where they can find a $H_2$ supplier needed for thriving. The full genome sequences of several *Dehalococcoides* strains show that they have very small genomes which are highly similar. Moreover, they exhibit a large number of putative dehalogenase-encoding genes (*rdh*), reaching up to 1.7% of the coding sequences in *Dehalococcoides* sp. Further work, combining transcriptional and proteomic techniques, will identify which proteins are really essential for the degradation of polychorinated xenobiotics.

Jeon and colleagues (2009) also report in *Microbial Biotechnology* issues related with the attack of halogenated chemicals. They detail the discovery of four HAD (Halodehalogenases) defluorinases from different microbial genomes. Some of these dehalogenases have enhanced activities and this appears to arise from their sequence diversity (less than 30% sequence identity for HADs) (Prudnikova *et al*., 2009; Rye *et al*., 2009). The set of new dehalogenase were elucidated via biochemical characterization of 163 potential dehalogenases from the sequenced genomes of five common soil bacteria. Their discovery and characterization will be imperative to the future use of these enzymes in the biodegradation of halogenated chemicals.

Another area of interest is the anaerobic degradation of monoaromatic compounds such as benzene, toluene, ethylbenzene and the xylene isomers (BTEX; Dou *et al*., 2008a; Wolicka *et al*., 2009). Anaerobic BTEX degradation has been shown to occur under denitrifying, sulfate-reducing, iron-reducing, manganese-reducing and methanogenic conditions (Dou *et al*., 2008a,b; Barton and Fauque, 2009). These activities are of the relevance in removal of pollutants from contaminated aquifers and soils, and they are considered an important remediation strategy for hydrocarbon-contaminated sites. New approaches based on isotopes are being taken, in fact, recently, compound-specific isotope analysis was successfully used to distinguish between the effects of non-degradative processes of mass loss such as sorption, volatilization, and dilution and those of biodegradation for aromatic hydrocarbons in the field (Fischer *et al*., 2008; Vogt *et al*., 2008). Compound-specific isotope analysis is based on the fact that, in most chemical reactions, lighter

isotopomers react faster than heavier ones, leading to a kinetic isotope effect. Herrmann and colleagues (2008) in *Environmental Microbiology Reports*, suggest that two-dimensional isotope fractionation analyses are a valuable tool for identifying and monitoring anaerobic biodegradation of xylene isomers. They explored the carbon and hydrogen isotope fractionation of benzylsuccinate synthase (Bss)-initiated degradation pathways for xylene isomers in order to obtain further information on the variability of isotope fractionation processes associated with Bss that might be important for the assessment of anaerobic degradation of xylene and toluene in the environment. The use of combined carbon and hydrogen isotope fractionation analyses may therefore be useful to monitor anaerobic xylene degradation at contaminated sites; this sort of technology will allow invaluable *in situ* monitoring of bioremediation processes.

**Eco hair-dyes**

In the Early View articles online at the *Microbial Biotechnology* website a fantastic example of 'green' chemistry is demonstrated in the publication by Jeon and colleagues. Using a laccase enzyme from *Trametes versicolor* and natural plant-derived phenolic compounds they were able to produce a colourful array of eco-friendly dyes (Salame *et al.*, 2010). This novel approach allowed them to overcome one of the major stumbling blocks in this technology as previous uses for laccase and flavonoid-based pigment formation had been limited to the staining of wood and textiles in various shades of brown (Kim *et al.*, 2007). They found that the colour range could be broadened significantly by using mixtures of natural phenols that had been derived from edible plant fibres such as lignin and tannin; the result being colourful polymer synthesis and the discovery of desirable colours that may be useful in the cosmetic industry as eco-friendly organic pigments. The overall process mimics the synthesis of plant fibres and is very attractive because such reactions fulfil the basic requirements of 'green' chemistry, in that toxic waste is reduced as the monomers are eco-friendly and an enzyme is a 'green' catalyst while the polymer synthesis mimics a natural synthesis utilized for flavonoid polymers such as tannins and proanthocyanidins. Once the authors had developed the novel products they were tested by *in situ* dying of grey hair and the colour permanence to conventional shampooing was assayed. The newly produced polymeric dyes were shown to colour hair the expected shade and the dyeing showed remarkable resistance to conventional shampooing. The possible uses of laccase-based polymer synthesis are enormous and future use of this newly developed system could reduce the use of hydrogen peroxide-based dying methods involving potentially carcinogenic phenylenediamines.

**Protein expression systems**

In the same Early View section there appears a Minireview by Schlegel and colleagues (2009), regarding the revolutionizing of membrane protein overexpression in bacteria. This in-depth review emphasizes the cutting edge techniques that are being utilized to overcome the problem of low level expression of membrane proteins in standard *E. coli* systems. One of the major topics covered relates to the engineering or selection of *E. coli* strains with improved membrane protein overexpression characteristics and the new analytical methods for monitoring of membrane protein overexpression which have been central to these new developments. The use of bacterial expression hosts other than *E. coli* is also covered and the positive results obtained from strains such as *Lactococcus lactis* in which the expression of the human KDEL receptor and Na+/tyrosine transporter (Tyt1) of *Fusobacterium nucleatum* could be achieved – neither of which could be expressed in *E. coli*. In addition, the topic of *in vitro* protein expression is discussed in relation to the use of *E. coli*-based cell-free systems. Although these methodologies have been around for a number of years it is only relatively recently that systems based on both *E. coli* extracts and purified *E. coli* components have become readily available. Previous screens comparing the expression of $>100$ *E. coli* membrane proteins in a cell-free expression system with expression *in vivo*, indicated that more proteins could be expressed by the cell-free system (Savage *et al.*, 2007); making these systems a serious alternative for the production of membrane proteins. One expects that with these advances and others, such us the development of bacterial strains that can specifically glycosylate heterologous membrane proteins we will see a boom in structural and functional studies of this vital set of proteins.

**Bifidobacteria**

D-Ribose is a common sugar present in the human gut and is mainly derived from ribonucleotide degradation. Pokusaeva and colleagues (2009) shed light on the genetic regulation of the ribose catabolic pathway in *Bifidobacterium breve* UCC2003. This article constitutes a great example of a complete study on the subject, because the analysis includes: (i) transcriptional studies comparing cultures grown on ribose to others grown on glucose, which showed upregulation of the *rbs* operon, encoding putative ribose degradation enzymes, and downregulation of the putative repressor gene *rbsR*; (ii) complementation studies proving that these *rbs* genes are able to complement a ribokinase-negative *E. coli* strain; (iii) mutational studies showing that their disruption impedes growth on ribose; (iv) protein–DNA interaction studies confirming that purified $RbsR_{His}$ binds to the pro-

 

moter of the *rbs* operon and that this binding is specifically inhibit by D-ribose; and (v) biochemical characterization of the RbsK ribokinase demonstrating that the enzyme specifically phosphorylates D-ribose. Furthermore, by comparative genome hybridization, the authors show that the *rbs* gene organization, although similar to *E. coli* and *Bacillus subtilis*, was not present in other *Bifidobacteria* suggesting differences in their ribose degradation capabilities. This in-depth characterization of pentose metabolism in bifidobacteria extends our understanding of carbohydrate utilization by this important human commensal and shows its adaptation to the available carbon sources present in the mammalian intestine.

## References


Atlas, R., and Bragg, J. (2009) Bioremediation of marine oil spills: when and when not – the *Exxon Valdez* experience. *Microbial Biotechnol* **2:** 213–221.

Barton, L.L., and Fauque, G.D. (2009) Biochemistry, physiology and biotechnology of sulfate-reducing bacteria. *Adv Appl Microbiol* **68:** 41–98.

Dou, J., Liu, X., and Hu, Z. (2008a) Anaerobic BTEX degradation in soil bioaugmented with mixed consortia under nitrate reducing conditions. *J Environ Sci* **20:** 585–592.

Dou, J., Liu, X., Hu, Z., and Deng, D. (2008b) Anaerobic BTEX biodegradation linked to nitrate and sulfate reduction. *J Hazard Mater* **151:** 720–729.

Fischer, A., Herklotz, I., Herrmann, S., Thullner, M., Weelink, S.A.B., Stams, A.J.M., *et al.* (2008) Combined carbon and hydrogen isotope fractionation investigations for elucidating benzene biodegradation pathways. *Environ Sci Technol* **42:** 4356–4363.

Garmendia, J., Heras, A., Calcagno Galvao, T., and de Lorenzo, V. (2008) Tracing explosives in soil with transcriptional regulators of *Pseudomonas putida* evolved for responding to nitrotoluenes. *Microbial Biotechnol* **1:** 236–246.

Govantes, F., Porrúa, O., García-González, V., and Santero, E. (2008) Atrazine biodegradation in the lab and in the field: enzymatic activities and gene regulation. *Microbial Biotechnol* **2:** 178–185.

Herrmann, S., Kleinsteuber, S., Neu, T.R., Richnow, H.H., and Vogt, C. (2008) Enrichment of anaerobic benzene degrading microorganisms by *in situ* microcosms. *FEMS Microbiol Ecol* **63:** 94–106.

Jeon, J.-R., Kim, E.-J., Murugesan, K., Park, H.-K., Kim, Y.-M., Kwon, J.-H., *et al.* (2009) Laccase-catalysed polymeric dye synthesis from plant-derived phenols for potential application in hair dyeing: enzymatic colourations driven by homo- or hetero-polymer synthesis. *Microbial Biotechnol* (in press): doi: 10.1111/j.1751-7915.2009.00153.x.

Kim, S., Moldes, D., and Cavaco-Paulo, A. (2007) Laccases for enzymatic colouration of unbleached cotton. *Enzyme Microb Technol* **40:** 1788–1793.

Pokusaeva, K., Rute Neves, A., Zomer, A., O'Connell-Motherway, M., MacSharry, J., Curley, P., *et al.* (2009) Ribose utilization by the human commensal *Bifidobacterium breve* UCC2003. *Microbial Biotechnol* (in press): doi: 10.1111/j.1751-7915.2009.00152.x.

Prudnikova, T., Mozga, T., Rezacova, P., Chaloupkova, R., Sato, Y., Nagata, Y., *et al.* (2009) Crystallization and preliminary X-ray analysis of a novel haloalkane dehalogenase DbeA from *Bradyrhizobium elkani* USDA94. *Acta Crystallogr Sect F Struct Biol Cryst Commun* **1:** 353–635.

Roze, E., Meijer, L., Bakker, A., Van Braeckel, K.N., Sauer, P.J., and Bos, A.F. (2009) Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age. *Environ Health Perspect* **117:** 1953–1958.

Rye, C.A., Isupov, M.N., Lebedev, A.A., and Littlechild, J.A. (2009) Biochemical and structural studies of a L-haloacid dehalogenase from the thermophilic archaeon *Sulfolobus tokodaii*. *Extremophiles* **13:** 179–190.

Salame, T.M., Yarden, O., and Hadar, Y. (2010) *Pleorotus ostratus* manganese-dependent peroxisdase silencing impairs decolourization of Orange II. *Microb Biotechnol* **3:** 93–106.

Savage, D.F., Anderson, C.L., Robles-Colmenares, Y., Newby, Z.E., and Stroud, R.M. (2007) Cell-free complements *in vivo* expression of the *E. coli* membrane proteome. *Protein Sci* **16:** 966–976.

Schlegel, S., Klepsch, M., Gialama, D., Wickström, D., Slotboom, D.J., and de Gier, J.-W. (2009) Revolutionizing membrane protein overexpression in bacteria. *Microbial Biotechnol* (in press): doi: 10.1111/j.1751-7915.2009.00148.x.

Siezen, R.J., and Galardini, M. (2008) Genomics of biological wastewater treatment. *Microbial Biotechnol* **1:** 333–340.

Taş, N., van Eekert, M.H.A., de Vos, W.M., and Smidt, H. (2009) The little bacteria that can – diversity, genomics and ecophysiology of *Dehalococcoides* spp. in contaminated environments. *Microbial Biotechnol* (in press): doi: 10.1111/j.1751.7915.2009.00147.x.

Vogt, C., Cyrus, E., Herklotz, I., Schlosser, D., Bahr, A., Herrmann, S., *et al.* (2008) Evaluation of toluene degradation pathways by two-dimensional stable isotope fractionation. *Environ Sci Technol* **42:** 7793–7800.

Wolicka, D., Suszek, A., Borkowski, A., and Bielecka, A. (2009) Application of aerobic microorganisms in bioremediation in situ of soil contaminated by petroleum products. *Bioresour Technol* **100:** 3221–3227.


 