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Highlight

Microbial Biotechnology: biofuels, genotoxicity reporters and robust agro-ecosystems

Craig Daniels,[1] Carmen Michán[2] and Juan-Luis Ramos[3]*

[1]*Structural Proteomics in Toronto, UHN and University of Toronto, Banting and Best Department of Medical Research; C.H. Best Institute 112 College Street, M5G 1L6, Toronto, Ontario, Canada.*

[2]*Department of Biochemistry and Molecular Biology, University of Cordoba, Campus de Rabanales, E-14071 Córdoba, Spain.*

[3]*Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, C/ Prof. Albareda, 1, E-18008 Granada, Spain.*

In Meschke and Schrempf (2010), the authors present a very colourful research article on the potential of *Streptomyces lividans* as a biocontrol agent. *Streptomyces* are well-characterized soil microorganisms that have attracted the attention of scientists because of their potential as producers of secondary metabolites that are of interest to the pharma industry (Chater *et al*., 2010). In the current article the authors explore the potential of *S. lividans* as a biocontrol agent and show that *S. lividans* inhibits the proliferation of the ascomycetes *Verticillium dahliae*. *Verticillium* are plant pathogens that threaten the yield of a number of herbaceous plants and trees, including olive trees (Prieto *et al*., 2009; Segura *et al*., 2009). It has previously been reported that *Verticillium* wilt was suppressed by certain bacteria from the genus *Pseudomonas* (Prieto *et al*., 2009); now the observation has been extended to *Streptomyces*. The studies reported by Meschke and Schrempf (2010) are based on very detailed and well-processed video-imaging of *Streptomyces* and *Verticillium* colonizing seeds of *Arabidopsis* that have been used as a model plant. *Streptomyces lividans* spores germinated earlier than the ascomycetes' conidia and bacterial hyphae progressed and developed very well within the *Arabidopsis* mucilage. The ascomycetes damage the seeds when germinated in the absence of *Streptomyces*, but in the presence of the bacteria fungal hyphae development was inhibited and fungal damage notably decreased. In this study, it is also shown that *S. lividans* is able to colonize and cover plant roots producing a protective layer along the outer surface. All of this is illustrated through a series of marvellous pictures that allow the reader to visualize the protective effects of *S. lividans*. The discussion of the article by Meschke and Schrempf (2010) conveys some interesting hypotheses related to the potential mechanisms involved in suppression of fungal development in the presence of *S. lividans*. The authors noted that all of the effects observed in regard to fungal inhibition in the presence of *S. lividans* correlated with the phenotypes observed in a *Verticillium* mutant devoid of VMK1; a mitogen-activated protein kinase that may be important in several processes such as tolerance to cold and desiccation (Rauyaree *et al*., 2005). Further studies are being performed in the author's laboratory to provide new insights into this relevant biocontrol characteristic.

Microbes are able to proliferate and form structured complexes called biofilms in association with both biotic and abiotic surfaces. Interest in the biology of bacteria associated in biofilms derives from what can be learnt from a basic point of view as well as because understanding biofilm formation can have important implications to combat microbes that corrode materials or cause disease (Karatan and Watnick, 2009; Ma and Wood, 2009). In the March issue of *Microbial Biotechnology*, Hong and colleagues (2010) showed that the global regulator H-NS is involved in the control of expression of a number of genes related to biofilm formation; Rac prophage excision and modulation of the expression of the killing protein HokD. Using an elegant genetic approach Hong and colleagues (2010) have identified a mutant variant of H-NS in which Lys57 was replaced by Asn, which had a profound effect on biofilm formation, Rac prophage excision and *hokD* gene expression. It is interesting that a single amino acid substitution causes such a dramatic physiological change. The authors quantified the effects and found that the H-NS K57N mutant exhibited a near 10-fold reduction in biofilm formation, which appears to be in part mediated through its interaction with Cnu and StpA proteins, which in turn modulates expression of a number of genes as demonstrated by the use of transcriptomic arrays. The

*For correspondence. E-mail juanluis.ramos@eez.csic.es; Tel. (+34) 958 181608; Fax (+34) 958 129600.

role of H-NS silencing of horizontally acquired AT rich genes was previously elegantly described by Madrid and colleagues (2007). In the study reported by Hong and colleagues here in *Microbial Biotechnology* it is clear that H-NS K57N induces prophage excision and expression of the killing protein HokD, which *per se* can have a direct effect on biofilm formation.

Natural, abundant and renewable resources such as lignocellulosic materials are an essential requirement for the continued functioning of our industrial societies; their full exploitation is likely critical to the development of a sustainable global economy. One of the stumbling blocks in the effective use of lignocellulose, which is composed mainly of cellulose, hemicellulose and lignin, is the build-up of toxic fermentation intermediates during the conversion to more useful fuels and compounds. Wierckx and colleagues (2010) have published an important article in *Microbial Biotechnology* reporting a finding that will facilitate the complete utilization of these materials. They describe the isolation of a novel bacterium *Cupriavidus basilensis* HMF14 capable of metabolizing many of the toxic constituents of lignocellulosic hydrolysate – including furfural, HMF, acetate, formate and a host of aromatic compounds. One of the amazing discoveries regarding *Cupriavidus basilensis* HMF14 is that it does not use the most abundant sugars in the biomass material as it has a preference for the utilization of the toxic constituents and the aromatic compounds present in the mix. This unique property allows the important sugars, glucose, xylose and arabinose to be utilized by ethanol producing yeasts *Saccharomyces cerevisiae*, and microorganisms such as *Zymomonas mobilis*.

The biological detoxification of lignocellulosic hydrolysates is an effective method of inhibitor removal and over the past few years numerous microorganisms have been isolated that either degrade inhibitors or convert them into less toxic compounds. Unfortunately, many of the previously isolated microorganisms also metabolized the important sugars required for the fermentative production of biofuels and biochemicals; making them less useful as implements for bio-abatement. Thus, the isolation of *C. basilensis* HMF14 is an important biotechnological discovery that should aid in combating the constraints currently impacting the effective use of biomass as an ethanol fuel. Additionally, because the genus *Cupriavidus* is well known for its ability to efficiently produce polyhydroxyalkanoates (PHAs) the researchers tested the newly isolated *C. basilensis* HMF14 for the ability to produce PHAs. Indeed, the strain could produce PHA granules; a capacity which may further contribute to its future cost-effectiveness because the biomass generated in the bio-abatement treatment could be employed for the production of bioplastics.

Genetically engineered microbial reporter strains were pioneered almost 20 years ago and during the preceding two decades there have been many reports describing the construction of new sensor cells capable of reporting on the presence of either specific compounds or global stress factors (King *et al*., 1990). The basic principle behind most of these reporter systems is the fusion of a readily quantifiable reporter element to a gene regulatory component (promoter), which is able to respond to the compounds or stress that is to be detected. Several reporter systems have been used; LacZ, eGFP and Lux and numerous publications have targeted the engineering of these components and/or the manipulation of the gene regulatory elements to improve the utility of the reporter strains. In *Microbial Biotechnology*, Yagur-Kroll and colleagues describe several general specific approaches, which may be utilized to improve reporter output and augment sensor performance (Yagur-Kroll *et al*., 2010). By modifying the length of the promoter-containing DNA fragment they were able to achieve significant improvements in detection sensitivity, while implementing random mutagenesis of the promoter enhanced the response kinetics. The random mutagenesis studies were complemented via site-directed mutagenesis of consensus elements in the promoter and by promoter duplication, which allowed superior signal intensity to be achieved. Using these four independent strategies of molecular manipulation they showed that whole-cell biosensor optimization can be accomplished with no previous knowledge of the specific promoter regulation, indicating that they may be applied to a broad range of promoter–reporter fusions. This is an important finding because it permits the use of powerful techniques such as random mutagenesis to select for enhanced performance in these systems.

Continuing on the topic of biosensors in *Microbial Biotechnology* is the mini-review of Biran and colleagues (2010) on bacterial genotoxicity bioreporters. Here the authors provide a thorough review on genotoxicity assays that employ genetically engineered microorganisms, which have been customized to generate a quantifiable signal that reflects the genotoxic efficacy of the sample being tested. These assays hold significant advantages over more traditional approaches; such as rapid response times, high reproducibility, and low operational cost. However, one of the main deficiencies of bacterial-based assays is their inability to perform the complex biochemical reactions collectively known as ‘metabolic activation’; reactions which often take place in mammalian liver cells during which xenobiotics are transformed into genotoxic forms. Topics taken into account by Biran and colleagues are the sensing elements and reporter systems used, the importance of appropriate and well tested cytotoxicity controls and improved detection performance, including

the enhancement of sensitivity via the expansion of the response spectra. The authors then go on to discuss devices that incorporate these bacterial genotoxicity reporters; several have been reported however, it appears that the long-term use of such devices will require further research and development of systems with increased life span and which incorporate either internal or external metabolic activation processes. Clearly, the rising global demand for safe drinking water will command additional progress in this important field of microbial biotechnology.

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