Microbes and disturbance

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The microbiology of disturbed environments is a vigorous area of research [1] that has benefited from cultivation-independent molecular techniques. Our challenge now is to embrace the concept of 'microbial landscapes' in all its complexity and recognise the multidisciplinary nature of molecular and microbial ecology.

In conventional studies microbes in disturbed environments are overlooked if they cannot be cultured. Similarly, molecular approaches that involve gene selection and amplification using the polymerase chain reaction (PCR) have limited capacity to detect novel organisms if they have divergent gene sequences or occur at very low levels [2]. The literature concerned with extreme and disturbed environments abounds with reports of new microbes with novel gene arrangements and so it is highly likely that there will indeed be organisms overlooked by cultivation and PCR dependent techniques [3-7]. However, researchers are increasingly attempting to address this bottleneck [8] using techniques that bypass [9] or refine the PCR step [10-11]. The latter is particularly important because PCR is such a useful tool in microbial ecology. A set of protocols for analysing microbial diversity that brings together the most efficient diversity assessment tools [7] would address some concerns.

There is a strong link between naturally extreme and disturbed environments, and although this article focuses on human induced extremes there are many other examples of disturbance that have not been covered here [1]. Acid mine drainage (AMD) is a serious environmental issue (Figures 1 and 2) that can be a natural process leading to the production of sulfuric acid [12]. However, in mining the process can be accelerated and microbes contribute to acid formation [13]. For a long time it was believed that two bacterial species – *Acidithiobacillus ferrooxidans* and *A. thiooxidans* – were the main species associated with AMD [6], but molecular techniques have shown that many microbes are involved [3,6]. Sulfate reducing bacteria (SRB) can reduce the acidity resulting from AMD and can react with metals to form insoluble metal sulfides that precipitate out of solution. As a result of this buffering and metal precipitation there is a growing interest in the use of engineered anaerobic SRB to remove heavy metals. These bioreactors have been successful in the laboratory [14-15] and in the field [16].

In environments contaminated with hydrocarbons, recovered molecular sequences are related to organisms that can degrade these compounds [4] (Streten-Joyce *et al,* unpublished data). Similarly, a striking feature of heavy metal introduction to the environment is the ensuing modification of microbial communities and their activities [17]. Bacteria show resistance to almost all toxic metal ions of environmental concern but there appears to be no general mechanism of metal resistance [18-20]. Although it is known that microbes can immobilise metals, evidence now suggests that they may themselves be subject to mobilisation [21]. This has implications for subsurface transport models and mechanisms of metal remobilisation, and provides a case for factoring microbes into these models.

There is mounting evidence that gene insertions under the control of integrons are a fundamental aspect of microbial

Figure 1. East Branch of the Finniss River, Northern Territory, Australia, looking towards Rum Jungle, a legacy uranium/copper mine site leaching acid. The white material on the left-hand bank are evaporites from the AMD, the cobbles in the creek are algal mats and the pH is around 3.8. Photo courtesy Jenny Brazier, Charles Darwin University and Environmental Research Institute of the Supervising Scientist (eriss) – Darwin Office.

Figure 2. Evaporites (salts containing a range of metals) resulting from AMD from the Rum Jungle mine site. Photo courtesy of Jenny Brazier, Charles Darwin University and eriss.

adaptation and evolution [2,7]. This also raises questions about the current reliance on organism identification using 16S rRNA genes, which may be evolving at different rates from genes involved in metabolism [7,22]. An analysis of a microbial community using only organism identification may be less informative than an analysis of community using genes and proteins directly associated with the disturbance. This leads to the somewhat vexed issue of ecological relevance and *in situ* monitoring using microbes – i.e. which genes and proteins should be targeted to satisfy the requirement for ecological relevance?

Microbes are widely used to assess ecological health because they provide a trophic link to higher organisms [23,24] and they respond quickly to the bio-available fraction of contaminants [25]. Bacteria have the highest surface area to volume ratio of any living organism, and only a thin membrane forms the boundary between them and their environment. Thus, bacteria are very sensitive to even slight environmental fluctuations and as such should be excellent early indicators of environmental degradation [26]. Microbial assays are commonly used as indicators of hydrocarbon [27] and heavy metal [28] contamination. Despite their obvious sensitivity to change, one of the biggest impediments to the routine use of bacteria is their significant temporal and spatial variation [29]. This variation may be more manageable if microbes are viewed at a community level and the focus shifts to functionally significant genes rather than tracking particular species.

Recent advances have provided us with the tools to catalogue genomes, proteins and metabolic pathways of the total microbiota in environmental samples. There is a growing awareness that most microbes are actually part of a complex and highly coordinated microbial 'city' or biofilm [30]. This concept of 'microbial landscapes' opens the door to a more rigorous approach to microbial community analysis through the application of classical ecological principles [30]. Added to this is our increasing capacity to study genes, proteins and metabolic pathways at the community level, and this provides an opportunity to learn about functional significance and mechanisms of tolerance or resistance. By understanding how microbes survive and function in disturbed environments, we should gain an appreciation of their ecological relevance and their capacity to act as early indicators of change.

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