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Highlight

Evolution of antibiotic resistance, catabolic pathways and niche colonization

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Evolution and adaptation are an important and basic part of biology and with the advancement of high-throughput technologies new approaches are available to exploit these properties for biotechnological applications. In this highlight we underscore a number of recent articles that we believe are highly relevant to biotechnology, including the discovery of new antibiotics, the evolution of catabolic pathways and biodiversity in extreme niches.

Antibiotics are a group of compounds that due to their importance in fighting infections have significantly influenced human and animal life expectancy. Chemical modifications of natural antibiotics have improved their effectiveness, leading to an excellent repertoire of new clinical drugs (Singh and Barrett, 2006). However, it is also true that the misuse of antibiotics has led to the acquisition by bacteria of new mechanisms of resistance to these drugs. This increasing phenomenon represents a serious threat to human lives, and has therefore led to an urgent search for new antibacterial agents.

Phillips and colleagues (2011) present in *Chemistry & Biology* the discovery of a novel antibiotic from the family of novomyocin that has been found using high-throughput screening technologies combined with the search for new sources of natural products from different locations in the world. The basis of the screening is the so-called *Staphylococcus aureus* fitness test assay that consists of a series of 245 inducible antisense RNA strains engineered for reduced expression of essential genes for cell growth; a differential sensitivity response of cells to compounds that inhibit the targeted gene product is the readout of the system. The analysis involves the combination of these variants grouped into pools that are grown together in the presence of the chemicals under test; differences in growth should correspond to specific antisense strains either being depleted or being enriched by the pool. Subsequently, the abundance of the strains at the end of the experiment is compared with controls, and the profile that is obtained is used as an indication of the potential mechanism of action of the tested compound(s). Phillips and colleagues (2011) use the above approach to find and characterize a new chemical, subsequently named kibdelomycin, which is produced by a *Kibdelosporangium* strain from a soil sample collected from a forest in the Central African Republic. The molecular formula of the new drug was determined by NMR. As the spectrum of activity of kibdelomycin was similar to the aminocoumarin antibiotic novobiocin, their mechanism of action was supposed to be analogous; inhibition of bacterial DNA gyrase and topoIV leading to suppression of DNA synthesis and cell death. This assumption was confirmed by *in vitro* assays. Kibdelomycin did not present cross-resistance with other major classes of DNA gyrase inhibitors and bacteria displayed a significantly lower frequency of resistance to kibdelomycin than to other antibiotics. This antibiotic is said to be the first compound discovered from a new class of natural-product bacterial gyrase inhibitors since the cyclothialidines were described in the early 1990s. In addition it is the first antibiotic with potent whole-cell antibacterial activity since the discovery of novobiocin and other coumarin antibiotics in the 1950s. Clearly this important discovery emphasizes and proves the efficiency of the fitness test assay in the hunt for novel antibacterial compounds.

Xenobiotic pollutants have been present in the environment for only a relatively short time, and, subsequently, only a few microorganisms have been able to evolve catabolic pathways to degrade these compounds. These

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pathways can emerge *via* vertical evolution, as such, a new pathway is assembled based on existing metabolic modules, or through horizontal acquisition of genes (Ramos *et al.*, 2011). The development of a new metabolic pathway can also occur from mutations affecting a pre-existing route, and in this case in addition to the 'new' enzymes, the regulatory systems often evolve, allowing the expression of the desired catabolic enzymes in the right place and at the right time. Nitroaromatic compounds are essentially new structures in the natural environment; however, microbes that attack these chemicals have already been described and the catabolic pathways implicated have been identified (Garmendia *et al.*, 2008; Ju and Parales, 2010); findings which provide an excellent opportunity to investigate the evolutionary processes leading to novel metabolic routes. de las Heras and colleagues (2011) suggest in *Molecular Microbiology* that the 2,4-dinitrotoluene (DNT) metabolic system from *Burkholderia* sp. has evolved from the naphthalene degradation pathway of *Ralstonia* sp. U2 by gene duplication. The authors show that the expected regulator, DntR, has not yet acquired the capacity to impart fine control of gene expression in the new pathway. In fact, they found that the DNT metabolic pathway is not transcriptionally induced in the presence of DNT but the regulator still responded to salicylate, a non-substrate of the pathway as an inducer (Devesse *et al.*, 2011; de las Heras *et al.*, 2011). The authors argue that the evolution of a degradation pathway requires first the evolution of novel substrate specificities by the catalytic enzymes and subsequently changes in the regulatory system. As a final point, the paper also includes an interesting argument regarding the selective advantages of maintaining regulators activated by effectors that are metabolically useless.

Silva-Rocha and colleagues (2011) have used 'electric engineering' concepts based on the adoption of Boolean models, to develop 'logic gates' that lead to the LOGICOME of a regulatory circuit that controls a catabolic pathway. The key is to use binary values (1 or 0) to reflect the activity of the nodes while the regulatory interactions are represented in the network as logical gates that execute Boolean functions such as AND, OR, NOR, NOT, etc. This modelling allows the description of expression of any gene as a result of the presence or absence of other genes and small molecules that act as regulators. The LOGICOME of the TOL plasmid catabolic pathways for biodegradation of *m*-xylene by *Pseudomonas putida* has been used as a proof of concept and the authors show that the TOL logicome reflects well-established experimental data and can collect information for: one exogenous signal (*m*-xylene), six endogenous inputs (IHF, HU, σ^{70}, σ^{54}, σ^{38} and σ^{32}), one inborn signal carrier molecule (3MB) and one single output (TCA). How LOGICOME models could be used to 'integrate information' at a global level will however require further studies.

Metagenomic comparison of microbial communities in the Red Sea

The Red Sea is an aquatic environment with unique biological characteristics that are mostly unknown. Qian and colleagues (2011) have examined the biodiversity in the water column overlaying two of its Deeps: Discovery and Atlantis II. The ecosystems in the Red Sea are characterized by both high temperature and salinity, due to the high rate of evaporation, low level of precipitation and lack of major river inflows. In addition, there is a clear vertical stratification of environmental parameters in the water column, with different gradients of temperature, conductivity, salinity and fluorescence intensity, with the deepest areas being relatively stable. Qian and colleagues (2011) selected four sampling points at depths of 20, 50, 200 and 1500 m. Their metagenomic studies show that there is a vertical stratification of the microbial communities, with significant differences in both bacterial and archaeal diversity observed between the upper (2 and 50 m) and the deeper layers (200 and 1500 m) that were attributed to environmental adaptation. The upper zone, extending from the surface to 200 m deep, was characterized by drastic environmental changes, whereas the lower zone, situated below 200 m to above the two deep-sea brine pools, had relatively stable surroundings. As expected, microbial (archaeal + bacterial) composition was more similar between the two locations at the same profundity, than in a location at different depths. The remarkable characteristics of the Red Sea and its endemic habitants may well prove to be a major source of new and important enzymes.

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