HIGH-LEVEL EXPRESSION, HIGH-THROUGHPUT SCREENING AND DIRECT RECOVERY OF NITROREDUCTASE ENZYMES FROM METAGENOME LIBRARIES

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Key Words: New tools; biomedical applications; enzyme promiscuity; metagenome screening

We have developed generally applicable library generation methods to maximize expression of cloned environmental genes, enabling screening for weak phenotypes in metagenome libraries. Our method also permits direct recovery of the encoded enzymes, providing rapid access to an almost unlimited diversity of previously unexplored biocatalysts. We have exemplified this for nitroreductases, members of a diverse family of oxidoreductase enzymes that can catalyze the bioreductive activation of nitroaromatic prodrugs such as metronidazole. These capabilities have diverse applications in medicine and research, including anti-cancer gene therapy and targeted ablation of nitroreductase-expressing tissues in transgenic animal models. However, research in these fields has largely been focused on the canonical nitroreductase NfsB from *Escherichia coli*, which exhibits sub-optimal levels of metronidazole activity. In previous work we have investigated alternative nitroreductase enzymes, sourced from genome-sequenced bacteria. To complement this work we have now turned to the discovery of novel nitroreductases from metagenomic DNA fragments, derived from the uncultivable bacteria present in New Zealand soil and lichen species.