Biotechnology
Paper alert

A selection of interesting papers that were published in the two months before our press date in major journals most likely to report significant results in biotechnology.

Current Opinion in Biotechnology 2000, **11**:109–114

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• of special interest
•• of outstanding interest

Analytical biotechnology

Selected by Tony Cass
Imperial College of Science, Technology and Medicine, London, UK

Screening unlabeled DNA targets with randomly ordered fiber-optic gene arrays. Steemers FJ, Ferguson JA, Walt DR: *Nat Biotechnol* 2000, **18**:91-94.

•• **Significance**: DNA microarrays have been developed in several formats and to further improve their performance advances need to be made with respect to sample volume, 'upstream' sample handling and probe density. In this paper, an optical fibre array is combined with molecular beacons to address these issues.

Findings: Molecular beacons with probe sequences corresponding to variants of the cystic fibrosis transmembrane conductance regulator (CFTR) were bound to beads via a biotin–streptavidin link and the beads were encoded with different combinations of fluorophores such that a particular probe sequence corresponded to a particular encoding scheme. Beads were then randomly dispersed into 3μm wells at the end of an optical fibre bundle such that each bead was individually addressable. Using this array, the different CFTR targets could be identified with detection limits in the nM region and hybridisation times of around five hours.

Anchored multiplex amplification on a microelectronic chip array. Westin L, Xu X, Miller C, Wang L, Edman CF, Nerenberg M: *Nat Biotechnol* 2000, **18**:199-204.

• **Significance**: One of the problems with multiplex PCR of several genes in a mixture is primer–primer interactions as well as different optimal conditions for the amplification of different sequences. This paper describes a way to circumvent this problem, that is, to spatially localise probes and target DNA in different regions on an electronic chip. Electrophoretic migration also accelerates the subsequent hybridisation reaction.

Findings: Isothermal amplification of target DNA using the strand displacement assay (SDA) was found to be compatible with the electronic chip structures. To demonstrate this approach, a 10-plex (i.e. 10 different genes) amplification of a mixture of human and bacterial targets was performed. Typical detection limits were around 10^4 molecules of target with low cross reactivity between the different targets.

Intracellular measurements in mammary carcinoma cells using fiber-optic nanosensors. Cullum BM, Griffin GD, Miller GH, Vo-Dinh T: *Anal Biochem* 2000, **277**:25-32.

• **Significance**: Biochemical analysis of single cells is attracting considerable interest and a variety of approaches have been used, including mass spectrometry and microelectrode methods. Optical sensing techniques have been extended into this domain. This paper describes the use of optical fibre nanosensors that can be inserted into single cells for fluorescence measurements.

Findings: Optical fibres with a diameter of 40nm were coated on their tips with an antibody to benzo[a]pyrene tetrol (BPT). The fibres were then inserted into individual rat liver epithelial or mammary carcinoma cells. After bathing the cells in a solution of BPT, the latter's uptake into the cells could be measured by fluorescence using the optical fibres and a detection limit of around 6 pM was determined.

Rapid nanopore discrimination between single polynucleotide molecules. Meller A, Nivon L, Brandin E, Golovchenko J, Branton D: *Proc Natl Acad Sci USA* 2000, **97**:1079-1084.

• **Significance**: High-throughput analysis of DNA at low copy number has important applications in human genotyping. Ideally, methods to do this would not require labelling of the DNA and would be rapid.

Findings: Diffusion of single DNA molecules through an α-hemolysin pore was found to produce a transient ion blockade which could be measured by patch-clamp methods. In a mixture of polynucleotides of different size and base composition, the components showed distinct behaviour in terms of the transit time through the pore and the magnitude of the ion blockade current. Even where the size and composition were the same, different sequences could be distinguished. An analysis of the distributions of transit time and current could be used to identify the different nucleotides present.

Activity-based protein profiling: the serine hydrolases. Liu Y, Patricelli MP, Cravatt BF: *Proc Natl Acad Sci USA* 1999, **96**:14694-14699.

•• **Significance**: The parallel determination of multiple enzyme activities in cell extracts is a valuable complement to the measurement of gene expression and protein levels. Variations in the profiles of enzymatic activity between tissues or between diseased and healthy cells could guide the discovery of new drugs. The authors demonstrate a method for tissue-specific profiling of serine proteases.

Findings: A fluorophosphate (FP)–biotin derivative was synthesised and used to irreversibly modify serine proteases in tissue extracts. As the compound only modifies catalytically active proteases its degree of reaction is a measure of the amount of active enzyme present. Modified proteins are detected by avidin blotting following electrophoretic separation.

Tissue-specific profiling of serine proteases was demonstrated with this method.

Plant biotechnology

Selected by Jim Dunwell
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Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Ye X, Al-Babili S, Kloti A, Zhang J, Lucca P, Beyer P, Potrykus I: *Science* 2000, **287**:303-305

•• **Significance**: Vitamin A deficiency affects millions worldwide, is frequent where rice is the staple diet, and can lead to juvenile blindness. This report provides a method to increase the amount of vitamin A in rice and may therefore help to overcome an important public health problem.

Findings: The authors used *Agrobacterium*-mediated transformation to introduce the entire β-carotene biosynthetic pathway into rice endosperm. The genes comprised phytoene synthase and lycopene β-cyclase from daffodil, and phytoene desaturase from *Erwinia uredovora*. Fertile plants were produced and their endosperm contained up to 1.6 μg/g carotene.

Cadmium tolerance and accumulation in Indian mustard is enhanced by overexpressing gamma-glutamylcysteine synthetase. Zhu YL, Pilon-Smits EA, Tarun AS, Weber SU, Jouanin L, Terry N: *Plant Physiol* 1999, **121**:1169-1178.

• **Significance**: These results extend the evidence that plants can be used for bioremediation. This study describes the use of genetic engineering to facilitate the growth of plants on metal contaminated sites.

Findings: The *Escherichia coli gsh1* gene encoding γ-glutamylcysteine synthetase (ECS) was introduced into Indian mustard (*Brassica juncea*). Compared with controls, the transgenic seedlings showed fivefold higher levels of ECS, had increased tolerance to Cd and accumulated 40–90% more Cd in their leaves.

***Arabidopsis* ecotypes and mutants that are recalcitrant to *Agrobacterium* root transformation are susceptible to germ-line transformation.** Mysore KS, Kumar CT, Gelvin SB: *Plant J* 2000, **21**:9-16.

• **Significance**: These results may be of value in understanding the factors that control the transformation capacity of recalcitrant crops species, such as soybean and sugar beet.

Findings: Using a germ line (vacuum infiltration) method, the authors transformed many *Arabidopsis* strains in which the vegetative material is deficient in its ability to bind bacteria and integrate T-DNA. Results suggest that germ line tissue may have higher amounts of 'factors' that allow transformation.

Biochemical engineering

Selected by Andrew J Daugulis and the Biochemical Engineering Laboratory
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Use of sublimation to prepare solid microbial media with water-insoluble substrates. Alley J, Brown L: *Appl Environ Microbiol* 2000, **66**:439-442.

• **Significance**: The low solubility of many chemicals presents a considerable challenge to assessing their bioavailability to microorganisms growing on solidified aqueous-based media. The authors present a novel method for preparing solid media with water-insoluble substrates that overcomes many of the disadvantages of traditional methods, particularly the need for solvents, which often have toxic effects or act as alternative carbon sources.

Findings: The proposed method uses sublimation to deposit a thin, even and visible layer of the water-insoluble compound onto the agar surface. The system utilizes a heated sand bath to sublime the chosen compound onto an inverted plastic petri-plate containing inoculated mineral salts agar. During the process, the agar is kept cool by placing an aluminum dish containing ice on the bottom of the petri-plate. With this method, both the amount of compound deposited and the time required to deposit a visible layer (based on vapour pressure) can be quantitatively determined.

Hydroxybenzotriazole increases the range of textile dyes decolorized by immobilized laccase. Reyes P, Pickard MA, Vazquez-Duhalt R: *Biotechnol Lett* 1999, **21**:875-880.

• **Significance**: In the development of a biological process to decolorize textile dyes, it is important to have a biocatalyst that can decolorize a wide range of dye classes under a wide variety of conditions. The authors showed that hydroxybenzotriazole will increase the range and rate of decoloration of textile dyes by immobilized laccase and identified some of the conditions under which decoloration will occur.

Findings: Laccase from *Coriolopsis gallica* was immobilized in agarose and evaluated for repeated decoloration of 50 mL of dye solutions circulated through a packed column. Reactive Blue 198 was decolorized for ten cycles with the laccase retaining 85% of its initial activity. When an industrial effluent (containing three dyes, sodium sulfate, sodium carbonate, soap and a dispersant) was evaluated under the same conditions, the immobilized enzyme retained only 14% of its initial activity after 10 cycles of decoloration. Although the reason for inactivation was not identified, it is was not found to be an individual component of the effluent. Immobilized laccase decolorized only 13 of 38 industrial dyes tested but in the presence of hydroxybenzotriazole, a free radical mediator, 26 dyes were decolorized and the rates of decoloration increased.

Environmental biotechnology

Selected by Lawrence P Wackett
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Generation of novel bacterial regulatory proteins that detect priority pollutant phenols. Wise AA, Kuske CR: *Appl Environ Microbiol* 2000, **66**:163-169.

•• **Significance**: Biological methods for detecting environmental pollutants have merit for convenience, cost and the ability to monitor the bioavailable, as opposed to the total, chemical concentration in a given environment. Biological methods also offer a high degree of selectivity for monitoring complex chemical mixtures. This study combines the use of a natural protein capable of binding phenols and PCR mutagenesis methods to change the recognition specifity of the protein. This indicates that a range of selective biological environmental monitoring systems can be generated by combining protocols widely available to many laboratories.

Findings: This paper describes the use of the regulatory gene *dmpR* fused to the luminescent gene cassette *lux* for detecting phenols available to the cell and quantitation of the response via measuring light output. Further, it describes the alteration of the DmpR protein's specificity for different phenols via mutagenic amplification of its gene using PCR.

Genetic analysis of a bacterial genetic exchange element: the gene transfer agent of *Rhodobacter capsulatus*. Lang AS, Beatty JT: *Proc Natl Acad Sci USA* 2000, **97**:859-864.

•• **Significance**: Gene transfer among bacteria is important in the environment and for biotechnology. The three known mechanisms for gene transfer – conjugation, transformation and transduction – have all been used for basic research and biotechnological applications. The present paper describes gene transfer agent (GTA) from *Rhodobacter capsulatus,* which differs from the previously known mechanisms and could prove to be a useful addition to the methods for transferring genes between bacteria in the laboratory.
Findings: This paper describes a mobile genetic element, GTA, that transfers random locations of the total genome of *Rhodobacter* strains to other *Rhodobacter*. GTA carries 4.5 kilobase double-stranded DNA fragments in an assemblage resembling a small, tailed phage, but it does not induce plaque formation or transfer viral genes as a phage would. GTA appears to transfer only short DNA fragments. The genes *cckA* and *ctrA* are required for expression of GTA genes and may function in a sensor kinase/response regulator signaling pathway.

Engineering *Deinococcus radiodurans* for metal remediation in radioactive mixed waste environments. Brim H, McFarlan SC, Fredrickson JK, Minton KW, Zhai M, Wackett LP, Daly MJ: *Nat Biotechnol* 2000, **18**:85-90.
•• **Significance**: Hazardous waste sites containing mixed organic and radioactive wastes are the most difficult to contain and clean up. The cost of such clean-ups using physicochemical methods is estimated to be $265 billion. This necessitates the development of new and cheaper biotechnological approaches; however, the high level radiation at such waste sites would prove lethal to virtually all bacteria that are commonly used in bioremediation. The bacterium discussed in this paper, *Deinococcus radiodurans*, is known to survive high radiation fluxes and could potentially prove useful for transforming metals to less toxic forms.
Findings: The *mer* operon genes, encoding proteins for transport and reduction of toxic mercury (II), were cloned and expressed in *D. radiodurans*. The recombinant strain was shown to be resistant to mercury and to transform ionic mercury to less toxic mercury (0). Moreover, a recombinant strain with both *mer* and *tod* genes was shown to oxidize the organic solvent toluene in the presence of mercury.

Pantocin B, an antibiotic from *Erwinia herbicola* discovered by heterologous expression for cloned genes. Brady FC, Wright SA, Lee JC, Sutton AE, Zumoff CH, Wodzinski RS, Beer SV, Clardy J: *J Am Chem Soc* 1999, **121**:11912-11913.
•• **Significance**: Microbial pathogens cause extensive damage to crop plants. A particularly devastating disease of apples, pears and other rosaceous plants is caused by the bacterium *Erwinia amylovora*. Another bacterium, *Erwinia herbicola*, appears to control the pathogen. This might be exploited directly or the chemical that mediates pathogen control might be used as an important low-toxicity agricultural chemical. This study was conducted to identify the specific control agent.
Findings: A cosmid library of *Erwinia herbicola* DNA was prepared in *Escherichia coli* and screened for pathogen-containing antibiotics. The structures of two antibiotics, designated pantocin A and B, were elucidated. Pantocin B inhibited *E. amylovora* at picomolar concentrations.

Enzymatic conversion of carbon dioxide to methanol: enhanced methanol production *in silica* sol-gel matrices. Obert R, Dave BC: *J Am Chem Soc* 1999, **121**:11912-11913.
•• **Significance**: An ideal industry would use energy from sunlight and obtain carbon from carbon dioxide, either by using plant biomass or using synthetic steps derived from plants and microbes. This study investigates the use of enzymes to reduce carbon dioxide to methanol.
Findings: Formate and formaldehyde dehydrogenases were used in solution with reduced pyridine dinucleotide to produce methanol from carbon dioxide. Sol–gel encapsulated samples were prepared and showed significantly higher yields of methanol.

Expression vectors and delivery systems

Selected by Thomas A Kost and Patrick Condreay
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Cationic microparticles: a potent delivery system for DNA vaccines. Singh M, Briones M, Ott G, O'Hagan D: *Proc Natl Acad Sci USA* 2000, **97**:811-816.
•• **Significance**: A method is reported that improves the efficiency of direct injection of DNA as a method of vaccination to induce immunity to an encoded antigen.
Findings: Biodegradable particles were formulated with cationic surfactants to give them a net negative surface charge. Plasmid DNA formulated on the surface of these particles was released in intact form slowly over the course of 14 days, and gave rise to higher levels of reporter gene expression than naked DNA after intramuscular injection. DNA on microparticles demonstrated an enhanced ability to induce antibody and cytotoxic T lymphocyte responses.

Molecular mechanism for silencing virally transduced genes involves histone deacetylation and chromatin condensation. Chen WY, Townes TM: *Proc Natl Acad Sci USA* 2000, **97**:377-382.
• **Significance**: This report provides evidence for a molecular model for the effect of histone acetylation on transgene expression, an understanding of which might lead to methods to circumvent this problem to retain long term expression.
Findings: Expression of silenced transgenes that had been stably introduced into cells with an AAV vector can be reactivated by the addition of an inhibitor of histone deacetylation, trichostatin A (TSA). It is demonstrated by sensitivity to DNase and endonucleases that the chromatin around the transgene becomes condensed when it is silenced, and that this condensation is reversed upon treatment with TSA. Silencing is also correlated with hypoacetylation of histones. Reactivation of the transgene by TSA results in acetylation of specific lysine residues of histone H4, and maintenance of the open chromatin appears to depend upon acetylation of lysine residue K8 of histone H4.

Highly efficient electro-gene therapy of solid tumor by using an expression plasmid for the herpes simplex virus thymidine kinase gene. Goto T, Nishi T, Tamura T, Dev SB, Takeshima H, Kochi M, Yoshizato K, Kuratsu J-I, Sakata T, Hofmann GA, Ushio Y: *Proc Natl Acad Sci USA* 2000, **97**:354-359.
• **Significance**: A promising method that avoids some of the problems of virally mediated *in vivo* gene delivery is demonstrated in an animal tumor model.
Findings: Widespread expression of a reporter gene in subcutaneous tumors in mice was demonstrated by injection of plasmid DNA followed by electroporation. The same procedure was used to deliver therapeutic genes, either the diptheria toxin A subunit, or the herpes simplex virus thymidine kinase gene followed by ganciclovir administration. These treatments resulted

in significant reductions in tumor volumes as compared to untreated controls. Furthermore, the treatment could be repeated on residual tumors to maintain the antitumor effect.

Use of the EF-1α promoter for expression can significantly increase success in establishing stable cell lines with consistent expression: a study using the tetracycline-inducible system in human cancer cells. Gopalkrishnan RV, Christiansen KA, Goldstein NI, DePinho RA, Fisher PB: *Nucleic Acids Res* 1999, **27**:4775-4782.
• **Significance**: The use of promoters from cellular housekeeping genes for heterologous gene expression, as demonstrated in this report, can help to overcome silencing of expression from viral promoters.
Findings: In attempting to construct cell lines with inducible expression of particular gene products, these investigators failed to obtain stable derivatives that expressed the chimeric transactivator protein of the tetracycline-inducible system under the transcriptional control of the CMV promoter/enhancer. It was reasoned that this might be due to silencing of the viral promoter in these cell lines; therefore, they replaced that promoter with one from the elongation factor 1α (EF-1α) gene. Cell lines were derived from several human tumor lines that stably expressed the transactivator and demonstrated tetracycline-inducible gene expression.

A genetically encoded, fluorescent indicator for cyclic AMP in living cells. Zaccolo M, De Giorgi F, Cho CY, Feng L, Knapp T, Negulescu PA, Taylor SS, Tsien RY, Pozzan T: *Nat Cell Biol* 2000, **2**:25-29.
• **Significance**: This technique provides a novel means for monitoring fluctuations of cAMP in living cells.
Findings: The tagging of the cAMP effector protein kinase A (PKA) with two green fluorescent protein mutants generated a probe in which the fluorescence resonance energy transfer between the two fluorescent moieties is dependent on the levels of intracellular cAMP. The GFP-tagged PKA was used to detect cAMP changes induced by treatment with forskolin and norepinephrine.

The optimal use of IRES (internal ribosome entry site) in expression vectors. Attal J, Théron M-C, Houdebine LM: *Gen Anal Biomol Eng* 1999, **15**:161-165.
• **Significance**: This report describes a new IRES found in the HTLV-1 genome and demonstrates the importance of IRES location in optimizing gene expression in dicistronic constructs.
Findings: IRES sequences found in human T-cell leukemia virus-1, poliovirus and encephalomyocarditis virus work optimally when added about 100 nucleotides after the termination codon of the first cistron. These IRES elements became totally inefficient when added after 300–500 nucleotides spacers.

Genomic integration and gene expression by a modified adenoviral vector. Zheng C, Baum BJ, Iadarola MJ, O'Connell CO: *Nat Biotechnol* 2000, **18**:176-180.
•• **Significance**: The gene transfer vector described in this report combines the high titer and broad host range of adenoviral vectors with the long-term gene expression and integration of retroviral vectors. It can integrate into genomic DNA at a much higher frequency, and express much longer, than conventional adenoviral vectors.
Findings: A replication-deficient recombinant adenovirus encoding luciferase was constructed that contained the 5′ and 3′ long terminal repeat (LTR) sequences of Moloney murine leukemia virus. Luciferase expression was observed in cultured cells and for as long as three months in infected rats. The vector integrated randomly into the genome of both dividing and non-dividing cells, in the absence of trans-complementing reverse transcriptase or integrase activity.

Intracellular trehalose improves the survival of cryopreserved mammalian cells. Eroglu A, Russo MJ, Bieganski R, Fowler A, Cheley S, Bayley H, Toner M: *Nat Biotechnol* 2000, **18**:163-167.

AND

Trehalose expression confers desiccation tolerance on human cells. Guo N, Puhlev I, Brown DR, Mansbridge J, Levine F: *Nat Biotechnol* 2000, **18**:168-171.
• **Significance**: These two reports present results that demonstrate the utility of the sugar trehalose for preserving mammalian cells in the frozen and dry states. These findings can be translated rapidly into improved methodologies for preserving and storing mammalian cells.
Findings: The authors used a loading approach and a cellular engineering approach to load trehalose into cultured cells. Low concentrations of trehalose loaded into cells permitted long-term post-thaw survival of more than 80% of 3T3 fibroblasts and 70% of human keratinocytes. Human primary fibroblasts infected with a recombinant adenovirus vector expressing the *E. coli* genes encoding the trehalose biosynthetic enzymes could be maintained in the dry state for up to five days.

Food biotechnology

Selected by Jeroen Hugenholtz and Michiel Kleerebezem
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Detection and identification of gastrointestinal *Lactobacillus* species by using denaturing gradient gel electrophoresis and species-specific PCR primers. Walter J, Tannock GW, Tilsala-Timisjarvi A, Rodtong S, Loach DM, Munro K, Alatossava T: *Appl Environ Microbiol* 2000, **66**:297-303.
• **Significance**: In view of the increasing use of *Lactobacilli* as probiotic microorganisms in food products, the methodology described here adds a powerful tool in the studies that aim to elucidate the fate and behavior of these bacteria in this complex environment.
Findings: The methodology described in this paper is based on denaturing gradient gel electrophoresis separation of PCR-amplified V2–V3 regions of 16S ribosomal DNA (rDNA). A number of different *Lactobacillus* species could be identified directly on basis of the migration of their V2–V3 sequences, which could be substantiated by application of species-specific primer pairs. This means that a complex isolate (e.g. GI tract samples) needs to be tested only with two or three primer pairs to obtain a specific identification. This method will contribute to future studies of the composition of the intestinal microflora and to a better understanding of *Lactobacillus* taxonomy.

An explosive antisense RNA strategy for inhibition of a lactococcal bacteriophage. Walker SA, Klaenhammer TR: *Appl Environ Microbiol* 2000, **66**:310-319.
•• **Significance**: This paper describes a successful and effective phage defense strategy based on antisense RNA technology, which is in clear contrast to the mainly negative results of similar strategies described in the past.

Findings: Previous attempts to use antisense technology to protect *L. lactis* from phage attack were largely unsuccessful. The effectiveness of the strategy described here is to a large extent dependent on the use of the 'explosive expression system' that was previously developed by the same group. This explosive expression system (O'Sullivan, Walker SA, West SG, Klaenhammer TR 1996, Bio/Technology 1996,14:82-87) couples phage infection to massive plasmid amplification and gene, or in this case antisense RNA, expression. Thereby an extremely enhanced ratio of antisense RNA to sense RNA at the appropriate time in the phage lytic cycle is generated and this is shown to effectively block phage proliferation.

Selection and application of peptide-binding peptides. Zhang Z, Zhu W, Kodadek T: *Nat Biotechnol* 2000, **18**:71-74.
•• **Significance**: Description of a simple genetic selection protocol by which peptide libraries can be screened for molecules that bind a particular target peptide sequence and can be used as antibody replacement in Western blot and affinity purification procedures.
Findings: By using a lambda repressor reconstitution assay, a random peptide library is very effectively screened for molecules that bind selectively to a chosen target peptide sequence. The selected peptides form complexes with the target peptides with dissociation constants that are in the micromolar range, which is much higher than those observed for antibody–peptide epitope binding. Nevertheless, the peptide–peptide interactions are shown to support 'immunoblot' analysis and affinity purification. These small sized peptides are easy to select and can be synthesized in large amounts, and could in some applications replace specific antibodies, which are tedious and expensive to generate.

Pulsed-electric field treatment enhances the bactericidal action of nisin against *Bacillus cereus*. Pol IE, Mastwijk HC, Bartels PV, Smid EJ: *Appl Environ Microbiol* 2000, **66**:428-430.
• **Significance**: This paper describes an elegant example of the so-called hurdle concept, where the exposure of undesirable bacteria to two or more different stress conditions results in a larger (inhibitory) effect than the sum of individual stress effects.
Findings: Vegetative cells of *Bacillus cereus* were subjected to low doses of nisin (0.06 µg/ml) and mild pulsed-electric field (PEF) treatment (16.7 V/cm, 50 pulses each of 2 µs duration). The effect of nisin treatment alone and of the PEF-treatment alone was both a 10-fold decrease of the viable count of *Bacillus cereus*. Combination of both mild treatments resulted in a 10,000-fold decrease of the viable count. This shows a clear synergy between the effects of PEF and nisin on *Bacillus cereus* and opens interesting possibilities for mild preservation techniques.

Pharmaceutical biotechnology

Selected by Steve Projan
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Drug target validation: lethal infection blocked by inducible peptide. Tao J, Endler P, Connelly G, Lim A, Zhang J, King M, Li T, Silverman JA, Schimmel PR, Tally FP: *Proc Natl Acad Sci USA* 2000, **97**:783-786.
• **Significance**: Many potential antibacterial targets are being revealed by a myriad of genomic and molecular genetic discovery programs. Although gene 'knockout' experiments can address the essential nature of a given target, they do not demonstrate that the inhibition of a given target can have a therapeutic effect in a bacterial infection. The authors of this paper have combined two techniques (phage display and inducible expression) to address both the ability to target a given gene product and to eventually develop an assay to find inhibitors of that putative target. This work describes a general method for antibacterial target validation.
Findings: Using phage display, the authors first identified specific binders to prolyl-tRNA synthetase, one of these inhibited the *in vitro* aminoacylation of tRNA by the enzyme with a K_i of 250 nM. The authors then fused a coding sequence for the most active peptide inhibitor (called 'Pro-3') to a sequence coding for glutathione-*S*-transferase (GST) and put it under the transcriptional control of a tetracycline-inducible promoter in *Escherichia coli*. Induction was accomplished using anhyrdotetracycline (which functions as an inducer but has no antibacterial activity). Expression of the Pro-3–GST fusion in culture demonstrated a marked inhibition of bacterial growth. The authors then successfully protected mice from an intraperitoneal challenge with a virulent strain of *E. coli* carrying the Pro-3–GST expression system, but only when expression of the Pro-3–GST fusion was induced. It should be noted that when a specific peptide binder has been validated *in vivo* (as with Pro-3 and prolyl-tRNA synthetase) then a 'function blind' ligand displacement assay can be formatted to identify small molecular inhibitors of the target.

Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. Hazuda DJ, Felock P, Witmer M, Wolfe A, Stillmock K, Grobler JA, Espeth A, Gabryelski L, Schleif W, Blau C, Miller MD: *Science* 2000, **287**:646-650.

AND

Structure and absolute stereochemistry of HIV-1 integrase inhibitor integric acid. A novel eremophilane sesquiterpenoid produced by a *Xylaris* sp. Singh SB, Zink D, Polishook J, Valentino D, Shafiee A, Silverman K, Felock P, Teran A, Vilella D, Hazuda D, Lingham RB: *Tetrahedron Lett* 1999, **40**:8775-8779.
•• **Significance**: To date, therapeutic agents used to treat HIV-1 infections have targeted either reverse transcriptase or protease. Despite the efficacy of highly active anti-retroviral therapy (HAART) in driving down viral load in infected individuals, resistance to the current anti-HIV agents eventually arises. Althought HIV integrase is essential for viral replication and several inhibitors of integrase have been described previously, none of these inhibitors block viral replication in infected cells in culture. These two papers describe specific HIV-1 intergrase inhibitors.
Findings: The first of these two articles describes a series of diketo acids that are HIV integrase inhibitors that do prevent HIV replication in infected cells. These compounds appear to specifically inhibit the catalysis of strand transfer (a reaction in which the 3′ end of the viral DNA is joined covalently to cellular DNA). The best of these compounds was shown to have an IC_{50} of 50 nM in an *in vitro* strand transfer reaction and a 1.0 µM IC_{50} in a viral infectivity assay. These IC_{50} values for replication inhibition are probably too high for these compounds to be considered as potential therapeutic agents but the values do provide a proof of principle that integrase is a 'drugable' target. The second paper describes a natural product derived from *Xylaria* that also inhibited integrase activity: it blocks at least two of the steps of integration, including strand transfer, but has an IC_{50} of 3–10 µM.

Technical comment: activation and inhibition of the staphylococcal *agr* system. Novick RP, Ross HF, Figuerefo AMS, Abramochkin G, Muir T, and response by Balaban N, Sngh B, Goldkorn T, Rasooly A, Torresand JV, Uziel O: *Science* 2000, **287**:391a.

• **Significance**: The accessory gene regulator (*agr*) system of *Staphylococcus aureus* encodes an autocrine, quorum-sensing regulatory system that controls the expression of a large number of virulence factors. Previously, Balaban *et al.* (*Science* 1998, 280:438.) reported that a 38 kD a protein (RAP) produced by an *agr*-null strain (RN6911), functioned as the agr activator and that this protein had immunoprotective activity in a murine abscess model. In addition, Balaban *et al*. reported that a linear heptapeptide (RIP), presumably derived from RAP, inhibited *agr* activation and inhibited infectivity in the murine abscess model. These findings are at odds with previous work by Novick and co-workers (*Proc Nat Acad Sci USA* 1999, 96:1218) demonstrating that the activator/inhibitor peptides are seven or eight amino acids in length, contain a five-membered thiolactone ring and are uniformly encoded by *agrD*. Resolving this controversy will be important in the design and development of novel anti-staphylococcal agents and vaccines.

Findings: Novick *et al*. reported an inability to replicate the work of Balaban *et al*., failing to demonstrate *agr*-inducing activity with preparations of RN6911 supernatant or inhibition of *agr* by the heptapeptide RIP. In response Balaban *et al*. take issue with the preparation and/or handling of the material by Novick *et al*. and suggest that the β-lactamase reporter system used by Novick *et al*. is lacking in sensitivity compared with the northern blots used by Balaban *et al*. Although the handling of the respective samples may be a legitimate difference in the work described by both groups, it should be pointed out that the use of reporter gene enzyme assays tend to be both more sensitive and more reproducible than northern blots of bacterial mRNA. The failure of RIP to inhibit in the experiments Novick *et al.* is in direct contrast to the continued success of RIP to inhibit *agr* expression *in vitro* and virulence *in vivo* in the experiments of Balaban *et al.* Resolution of these disparate findings will now require studies in other, independent laboratories.

Chemical biotechnology

Selected by Andrew J Daugulis and the Biochemical Engineering Laboratory
Queens University, Kingston, Ontario, Canada

Generation of novel bacterial regulatory proteins that detect priority pollutant phenols. Wise A, Kuske C: *Appl Environ Microbiol* 2000, **66**:163-169.

•• **Significance**: Effective detection and monitoring of contamination release during manufacturing or from chemical spills and leaks is important both from environmental and legal standpoints. This paper presents developments in the application of whole-cell bacterial biosensors as pollutant detectors through adaptation of the genetic systems, enabling microbial utilization of organic contaminants as carbon and energy sources.

Findings: The detection of a variety of phenols, which in the test biosensor (*Pseudomonas putida*) is mediated by the transcriptional activator DmpR, is evaluated. Modifications in the sensor domain of the transcriptional activator are shown to both help increase the sensitivity to chemical effectors, as well as expand the substrate range beyond that of the natural system. Depending on the mutation and specific effector molecule, induction levels as great as 170-times background were detected, even for some normally poorly detected disubstituted and *para*-substituted phenols. This type of protein engineering within biosensors presents a novel, inexpensive and easily applied alternative for improved pollutant detection.

Ferric iron reduction by bacteria associated with the roots of freshwater and marine macrophytes. King GM, Garey MA: *Appl Environ Microbiol* 1999, **65**:4393-4398.

• **Significance**: Intrinsic biodegradation of organic compounds frequently depends on the availability of a terminal electron acceptor other than oxygen. Our knowledge of biogeochemical cycles is important in understanding the mechanisms underlying intrinsic biodegradation. This paper presents data that indicate that the activity of iron reducers in the rhizosphere of aquatic macrophytes limit organic carbon availability to other heterotrophs such as methanogens.

Findings: This paper shows that the roots of freshwater and marine macrophytes harbour iron-reducing microorganisms. The amount of iron reduction in freshwater roots (>100 μmol/g[dry weight]/day) far exceeded methanogenesis (1–4 μmol of CH_4 produced/g[dry weight]/day) previously measured under similar conditions. This indicated that iron reduction might be an important activity in freshwater rhizospheres. Sodium molybdate did not inhibit iron reduction by roots of marine macrophytes, whereas roots of a freshwater macrophyte reduced iron oxyhydroxide when incubated with anthroquinone disulfate (AQDS), a humic acid precursor. Bacterial enrichments and isolates used acetate, ethanol, succinate and toluene as a source of carbon and energy, and iron oxyhydroxide, ferric citrate, uranate and AQDS as terminal electron acceptors.