



Title	Versatile enzymatic system for the production of guanosine polyphosphates
Author(s)	Choi, MMY; Wang, Y; Watt, RM
Citation	The Apring 2010 Meeting of the Society for General Microbiology (SGM), Edinburgh, U.K., 29 March-1 April 2010. In Abstract Book of the SGM Spring 2010 Meeting, 2010, p. 99
Issued Date	2010
URL	http://hdl.handle.net/10722/129618
Rights	Abstract Book of the SGM Spring 2010 Meeting. Copyright © Society for General Microbiology.

for the prevention or control of infectious diseases. In this study, we screened soil samples for the isolation and identification of AHL-degrading bacteria. 13 samples were cultured in a minimal medium containing 1 mM N-(3-oxo-dodecanoyl) homoserine lactone and 2 mM N-(3-oxo-hexanoyl) homoserine lactone as the sole sources of carbon and nitrogen. Subsequently, the remaining AHL levels in the supernatant were determined in the *P. aeruginosa* QSIS2 biosensor assay. Strains growing on this minimal medium were isolated and identified by means of partial 16S rRNA gene sequencing. The soil samples tested yielded a total of 51 isolates that are, either alone or as part of a consortium, able to use AHL signal molecules as sole sources of carbon and nitrogen. The AHL levels in the supernatant of all the samples were significantly lower than in non-inoculated controls. Although characterization of the isolates is still ongoing, the first results indicate the presence of *Pseudomonas* spp., *Achromobacter* spp., *Streptomyces* spp., *Arthrobacter* spp. and *Stenotrophomonas maltophilia*.

ED02/20 Versatile enzymatic system for the production of guanosine polyphosphates

Mei Y. Choi, Ying Wang & RORY M. WATT

Oral Biosciences, Faculty of Dentistry, University of Hong Kong, Prince Philip Dental Hospital, 34 Hospital Road, Hong Kong;
 Email rmwatt@hku.hk

During periods of environmental stress, bacteria synthesize guanosine tetraphosphate (ppGpp, magic spot I) and guanosine pentaphosphate (pppGpp, magic spot II) in a process known as the stringent response. These intracellular allomone molecules 'reprogramme' the transcriptional and translational machinery to help the cell conserve scarce resources. Existing methods for the production of guanosine polyphosphates are either technically difficult or inefficient, hindering investigations into their biological roles. We have developed a simple and efficient one-step enzymatic method for the production of guanosine polyphosphates using a recombinant protein cloned from *Staphylococcus aureus*. The purified enzyme efficiently catalyses the formation of pppGpp (and AMP) from GTP + ATP; and ppGpp (and AMP) from GDP + ATP. Notably, it also catalyses the synthesis of pGpp (guanosine 5'-monophosphate 3'-diphosphate, and AMP) from GMP + ATP; albeit with reduced efficiency. The reverse reactions are not catalysed, leading to high conversion rates. Guanosine polyphosphate products can be obtained in a homogeneous form using a combination of anion exchange chromatography followed by desalting. Our approach can be used to produce guanosine polyphosphates on a multi-milligram scale. Furthermore, our results also suggest that a third 'magic spot' allomone may be formed within certain bacterial species.

ED02/21 Exon-intron structure of *Dictiostelium discoideum* and *Babesia bovis* genes

A.A. Kadbullina

Al-Farabi Kazakh National University, Kazakhstan

Background Exon and intron length varies considerably in genomes of *D. discoideum* and *B. bovis*. This work is devoted to study the distribution of exon and intron lengths from different intron number.

Methods Intron-containing genes of chromosomes I of both *D. discoideum* and *B. bovis* were arranged in groups of 1; 2; 3; 4; 5; 6–9 introns. For each group average length of exons and introns, total exon length (L_{ex}), gene length (N_g) and intron number (N_{in}) were evaluated.

Results The average exon length in groups of genes with one intron is 759 and 685 n, where as the exon length in groups of genes with 6–9 introns is 434 and 216 n of *D. discoideum* and *B. bovis*, respectively. Intron length reduced less significantly in genes with intron number augmentation for two organisms. Dependence between intron number and both total exon length and gene length was described by regression: $N_{in} = 0.0022L_{ex} - 2.679$ and $N_{in} = 0.0014L_{ex} - 1.520$ (*D. discoideum*) and $N_{in} = 0.0041L_{ex} - 2.217$ and $N_{in} = 0.0033L_{ex} - 1.666$ (*B. bovis*).

Conclusion Correlation between intron number in gene and both exon and intron lengths, also correlation between intron number in gene and total exon length and gene length was established.

ED02/22 Redox-sensitive cysteines in the mitogen-activated protein kinase (MAPK) StyI participate in a stress-specific mechanism to increase oxidative stress resistance

ALISON M. DAY & Elizabeth A. Veal

Institute for Cell & Molecular Biosciences, Newcastle University, Newcastle upon Tyne NE2 4HH

Mitogen-activated protein kinases (MAPK) are activated by and orchestrate responses to multiple, diverse stimuli. Although these responses involve the increased phosphorylation of substrate effector proteins eg. transcription factors, the mechanisms by which responses are tailored to particular stimuli are unclear. In the fission yeast *Schizosaccharomyces pombe* the StyI MAPK is crucial for changes in gene expression that allow adaptation to many forms of environmental stress. Here, we have identified two cysteine residues in StyI, Cys153 and Cys158, that are important for hydrogen peroxide-induced gene expression and oxidative stress resistance but