Indexing & Abstracting
Of
Microbiology

YEAR 2017

Published by

LIBRARY & INFORMATION CENTRE
INDIAN PHARMACOPOEIA COMMISSION
Ministry of Health & Family Welfare
Government of India
Ghaziabad (U.P.)

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Volume 163, Issue 3, March 2017

Volume 163, Issue 4, April 2017

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Volume 163, Issue 11, November 2017

Volume 163, Issue 12, December 2017

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Volume 163, Issue 1, January 2017

Microbe Profile: Escherichia coli O157:H7 – notorious relative of the microbiologist's workhorse

David L. Gally, Mark P. Stevens

ABSTRACT

Escherichia coli O157:H7 is a zoonotic diarrhoeal pathogen of worldwide importance. It belongs to a subset of Shiga toxin-producing E. coli that can form attaching and effacing lesions on intestinal epithelia via the action of a type 3 secretion system that injects bacterial effectors into enterocytes. Infections in humans often arise from contaminated food or direct environmental exposure and can involve life-threatening Shiga toxin-dependent sequelae. In the three decades since E. coli O157:H7 was first recognized intensive research has helped to unravel the basis of pathogenesis, but few effective options for prevention and treatment of infections exist.

Interaction and signalling networks: a report from the fourth 'Young Microbiologists Symposium on Microbe Signalling, Organisation and Pathogenesis'

Clare L. Kirkpatrick, Olivier Lesouhaitier, Jacob G. Malone, Shi-Qi an, Delphine L. Caly

ABSTRACT

At the end of June, over 120 microbiologists from 18 countries gathered in Dundee, Scotland for the fourth edition of the Young Microbiologists Symposium on 'Microbe Signalling, Organisation and Pathogenesis'. The aim of the symposium was to give early career microbiologists the opportunity to present their work in a convivial environment and to interact with senior world-renowned scientists in exciting fields of microbiology research. The meeting was supported by the Microbiology Society, the Society of Applied Microbiology and the American Society for Microbiology with further sponsorship from the European Molecular Biology Organisation and the Royal Society of Edinburgh. In this report, we highlight some themes that emerged from the many interesting talks and poster presentations, as well as some of the other activities that were on offer at this energetic meeting.

Overexpression of the fratricide immunity protein ComM leads to growth inhibition and morphological abnormalities in Streptococcus pneumoniae

Daniel Straume, Gro Anita Stamsås, Zhian Salehian, Leiv Sigve Håvarstein

ABSTRACT

The important human pathogen Streptococcus pneumoniae is a naturally transformable species. When developing the competent state, it expresses proteins involved in DNA uptake, DNA processing and homologous recombination. In addition to the proteins required for the transformation process, competent pneumococci express proteins involved in a predatory DNA acquisition mechanism termed fratricide. This is a mechanism by which the competent pneumococci secrete a muralytic fratricin termed CbpD, which lyses susceptible sister cells or closely related streptococcal species. The released DNA can then be taken up by the competent pneumococci and integrated into their genomes. To avoid committing suicide, competent pneumococci produce an integral membrane protein, ComM, which protects them against CbpD by an unknown mechanism. In the present study, we show that overexpression of ComM results in growth inhibition and development of severe morphological abnormalities, such as cell elongation, misplacement of the septum and inhibition of septal cross-wall synthesis. The toxic effect of ComM is tolerated during competence because it is not allowed to accumulate in the competent cells. We provide evidence that an intra-membrane protease called RseP is involved in the process of controlling the ComM levels, since ΔrseP mutants produce higher amounts of ComM compared to wild-type cells. The data presented here indicate that ComM mediates immunity against CbpD by a mechanism that is detrimental to the pneumococcus if exaggerated.

Natural Escherichia coli isolates rapidly acquire genetic changes upon laboratory domestication

Bin Liu, Gustavo Eydallin, Ram P. Maharjan, Lu Feng, Lei Wang, Thomas Ferenci

ABSTRACT

The adaptation of environmental bacteria to laboratory conditions was analysed through the exploration of genomic changes in four strains of Escherichia coli freshly isolated from their natural habitats and belonging to different taxonomic clusters. Up to 25 mutations were present in all cultures of natural isolates within 10 days of transfer in rich media or with a single growth cycle involving an extended stationary phase. Among numerous individual mutations, two genes were affected in parallel in distinct backgrounds. Mutations in rpoS (encoding sigma factor RpoS), altering a multiplication–survival trade-off in E. coli, were present in isolates derived from all four different ancestors. More surprisingly, two different natural isolates acquired mutations in mutL, affecting DNA mismatch repair, and a third also involved higher mutation rates. The elevated mutation rates in these isolates indicate the danger of increased genetic instability arising from laboratory domestication. Neither rpoS nor mutator mutations were detected in the already-acclimatized MG1655 laboratory strain; only one or no new mutations were present in the laboratory strain under the same culture conditions. Our results indicate rapid adaptation to the laboratory environment. Ancestor-specific responses also arise in the laboratory and mutational events are also sensitive to culture conditions such as extended stationary phase. To maintain natural isolates in a stable state, our data suggest that the transition of strains to the laboratory should minimize culture cycles and extended stationary phase.

Inversion of Correia repeat enclosed elements in Neisseria gonorrhoeae

Firat Elbeyioglu, Sabrina B. Roberts, Russell Spencer-Smith, Madhuri Pulijala, Marta A. Zelewska, Jean-Christophe Nebel, Lori A. S. Snyder

ABSTRACT

Neisseria gonorrhoeae is capable of causing gonorrhoea and more complex diseases in the human host. Within the gonococcal genome are over 100 copies of the insertion sequence-like Correia repeat enclosed element (CREE), which has been predicted to be mobile within the neisserial genomes. Although there is evidence of ancestral movement of these elements, no previous study has provided evidence for current mobilization. CREE has the ability to alter gene expression and regulation in many ways: by insertional mutagenesis, by introducing promoter elements, by generating mRNA processing sites and by association with non-coding RNAs. Previous studies have compared the genomic locations of CREEs in the Neisseria spp., demonstrating that otherwise identical regions have either the element or the target TA insertion site. In this study, we report for the first time, to our knowledge, movement of CREEs, through inversion of the element at its chromosomal location. Analysis of Ion Torrent generated genome sequence data from N. gonorrhoeae strain NCCP11945 passaged for 8 weeks in the laboratory under standard conditions and stress conditions revealed a total of 37 inversions: 24 were exclusively seen in the stressed sample, 7 were seen in the control sample and the remaining 3 were seen in both samples. These inversions have the capability to alter gene expression in N. gonorrhoeae through the previously determined activities of the sequence features of these elements, potentially resulting in reversible phase-variable gene expression.

Characterization of two new putative adhesins of Leptospira interrogans

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ABSTRACT

We here report the characterization of two novel proteins encoded by the genes LIC11122 and LIC12287, identified in the genome sequences of Leptospira interrogans, annotated, respectively, as a putative sigma factor and a hypothetical protein. The CDSs LIC11122 and LIC12287 have signal peptide SPII and SPI and are predicted to be located mainly at the cytoplasmic membrane of the bacteria. The genes were cloned and the proteins expressed using Escherichia coli. Proteinase K digestion showed that both proteins are surface exposed. Evaluation of interaction of recombinant proteins with extracellular matrix components revealed that they are laminin binding and they were called Lsa19 (LIC11122) and Lsa14 (LIC12287), for Leptospiral-surface adhesin of 19 and 14 kDa, respectively. The bindings were dose-dependent on protein concentration, reaching saturation, fulfilling the ligand-binding criteria. Reactivity of the recombinant proteins with leptospirosis human sera has shown that Lsa19 and, to a lesser extent, Lsa14, are recognized by antibodies, suggesting that, most probably, Lsa19 is expressed during infection. The proteins interact with plasminogen and generate plasmin in the presence of urokinase-type plasminogen activator. Plasmin generation in Leptospira has been associated with tissue penetration and immune evasion strategies. The presence of a sigma factor on the cell surface playing a secondary role, probably mediating host –pathogen interaction, suggests that LIC11122 is a moonlighting protein candidate. Although the biological significance of these putative adhesins will require the generation of mutants, our data suggest that Lsa19 is a potential candidate for future evaluation of its role in adhesion/colonization activities during L. interrogans infection.

Down-regulation of PE11, a cell wall associated esterase, enhances the biofilm growth of Mycobacterium tuberculosis and reduces cell wall virulence lipid levels

Shivangi Rastogi, Amit Kumar Singh, Garima Pant, Kalyan Mitra, Koneni V. Sashidhara, Manju Y. Krishnan

ABSTRACT

PE11 (Rv1169c or LipX) is a cell wall associated esterase/lipase of Mycobacterium tuberculosis (Mtb). Evidences suggest that PE11 is expressed by Mtb both in vitro and in vivo. Previous studies have shown that PE11 leads to modification in cell wall lipid content and enhanced virulence when expressed in the non-pathogenic surrogate Mycobacterium smegmatis. Since cell wall lipids often play different roles in pathogenic and non-pathogenic mycobacteria, we investigated the role of PE11 in its host, Mtb. Mtb with lowered expression of PE11 (PE11 knock-down) displayed significant changes in colony morphology and cell wall lipid profile, confirming the role of PE11 in cell wall architecture. In addition, the levels of phthiocerol dimycocerosates, a cell wall virulence factor, were decreased. Levels of trehalose esters and free mycolic acids were increased. In contrast to M. smegmatis expressing Mtb PE11, a role reversal was observed in Mtb with respect to pellicle/biofilm formation. The PE11 knock-down Mtb strain showed significantly enhanced aggregation and early biofilm growth in detergent-free medium, compared to the wild-type. Knock-down strain also showed nearly 27-fold up-regulation of a fibronectin attachment protein (Rv1759c), linking biofilm growth with over-expression of bacterial proteins that help in aggregation and/or binding to host extracellular matrix. The knock-down also resulted in poor virulence of Mtb in PMA (phorbol 12-myristate 13-acetate) treated and PMA+IFN-γ treated THP-1 macrophages. Therefore, the study not only links PE11 to cell wall virulence lipids but also reveals the involvement of this cell wall associated esterase in down-regulation of biofilm in Mtb.

Methanosarcina acetivorans utilizes a single NADPH-dependent thioredoxin system and contains additional thioredoxin homologues with distinct functions

Addison C. McCarver, Faith H. Lessner, Jose M. Soroeta, Daniel J. Lessner

ABSTRACT

The thioredoxin system plays a central role in the intracellular redox maintenance in the majority of cells. The canonical system consists of an NADPH-dependent thioredoxin reductase (TrxR) and thioredoxin (Trx), a disulfide reductase. Although Trx is encoded in almost all sequenced genomes of methanogens, its incorporation into their unique physiology is not well understood. Methanosarcina acetivorans contains a single TrxR (MaTrxR) and seven Trx (MaTrx1–MaTrx7) homologues. We previously showed that MaTrxR and at least MaTrx7 compose a functional NADPH-dependent thioredoxin system. Here, we report the characterization of all seven recombinant MaTrxs. MaTrx1, MaTrx3, MaTrx4 and MaTrx5 lack appreciable disulfide reductase activity, unlike previously characterized MaTrx2, MaTrx6 and MaTrx7. Enzyme assays demonstrated that, of the MaTrxs, only the reduction of disulfide-containing MaTrx7 is linked to the oxidation of reduced coenzymes. NADPH is shown to be supplied to the MaTrxR–MaTrx7 system through the oxidation of the primary methanogen electron carriers F420H2 and ferredoxin, indicating that it serves as a primary intracellular reducing system in M. acetivorans. Bioinformatic analyses also indicate that the majority of methanogens likely utilize an NADPH-dependent thioredoxin system. The remaining MaTrxs may have specialized functions. MaTrx1 and MaTrx3 exhibited thiol oxidase activity. MaTrx3 and MaTrx6 are targeted to the membrane of M. acetivorans and likely function in the formation and the reduction of disulfides in membrane and/or extracellular proteins, respectively. This work provides insight into the incorporation of Trx into the metabolism of methanogens, and this reveals that methanogens contain Trx homologues with alternative properties and activities.

Characterization of cis-elements in the promoter of trz2 encoding Schizosaccharomyces pombe mitochondrial tRNA 3′-end processing enzyme

Jinyu Liu, Linting Huang, Yirong Wang, Ying Huang

ABSTRACT

The endonuclease tRNase Z is responsible for the 3′-end processing of tRNA precursors, which is one of the essential steps in tRNA maturation. The fission yeast Schizosaccharomyces pombe contains two essential tRNase ZL genes (trz1 and trz2) involved in nuclear and mitochondrial tRNA 3′-end processing, respectively. Our previous studies suggest that trz2 is expressed at a very low level. Here we report characterization of the trz2 promoter. Using lacZ as a reporter, we show that the trz2 promoter contains a HomolD box and a very weak diverged TATA element. The HomolD box is usually found in the promoters of S. pombe ribosomal protein genes. lacZ reporter assays suggest that the HomolD box regulates the expression of both trz2 and the ribosomal protein gene rps2501, which are arranged head-to-head on opposite strands. Overexpression of Rrn7, a candidate HomolD box-binding protein, up-regulates expression of lacZ under the control of the trz2 promoter or the rps2501 promoter. Functional complementation studies suggest that the TATA-like element is essential for trz2 expression, whereas the HomolD box may play a nonessential regulatory role. We also demonstrate that a 57 nt negative regulatory element (NRE) located between the HomolD box and the TATA-like element represses the expression of lacZ under the control of the trz2 promoter. Our results suggest that the low-level trz2 expression may arise from a low level of transcription caused by lack of a strong TATA box and the NRE. Our analysis also suggests that trz2 and rps2501 may be coregulated by the HomolD box.

High concentrations of intracellular Ap4A and/or Ap5A in developing Myxococcus xanthus cells inhibit sporulation

Yoshio Kimura, Chihiro Tanaka, Katsuho Sasaki, Masashi Sasaki

ABSTRACT

Diadenosine polyphosphates (Ap n A) are thought to act as signalling molecules regulating stress responses and biofilm formation in prokaryotes. However, Ap n A function in Myxococcus xanthus remains unknown. Here, we investigated the role of Ap n A in M. xanthus, using the wild-type and Ap n A hydrolase (apaH) mutant strains exposed to various stress conditions. In both wild-type and apaH mutant cells cultured on starvation medium (CF agar), the levels of intracellular diadenosine tetraphosphate (Ap4A) and pentaphosphate (Ap5A) increased several fold during the first 16 h of development and decreased gradually thereafter. The levels of Ap4A and Ap5A in the apaH mutant were about 5- and 11-fold higher than those in the wild-type strain at 16 h, respectively. Ap n A hydrolase activity of the wild-type strain increased 1.5-fold during the first 8 h of development, and it then gradually decreased. The apaH mutant formed spores 1–2 days after the wild-type strain did, and the yield of viable spores was 5.5 % of that in the wild-type strain 5 days after inoculation onto CF agar. These results suggest the possibility that high intracellular levels of Ap4A and/or Ap5A may inhibit M. xanthus sporulation at the early stage of development and that the bacteria reduce intracellular Ap4A and Ap5A accumulation through Ap n A hydrolase activity.

Disruption of MiaA provides insights into the regulation of phenazine biosynthesis under suboptimal growth conditions in Pseudomonas chlororaphis 30-84

Jun Myoung Yu, Dongping Wang, Leland S. Pierson III, Elizabeth A. Pierson

ABSTRACT

Many products of secondary metabolism are activated by quorum sensing (QS), yet even at cell densities sufficient for QS, their production may be repressed under suboptimal growth conditions via mechanisms that still require elucidation. For many beneficial plant-associated bacteria, secondary metabolites such as phenazines are important for their competitive survival and plant-protective activities. Previous work established that phenazine biosynthesis in Pseudomonas chlororaphis 30-84 is regulated by the PhzR/PhzI QS system, which in turn is regulated by transcriptional regulator Pip, two-component system RpeA/RpeB and stationary phase/stress sigma factor RpoS. Disruption of MiaA, a tRNA modification enzyme, altered primary metabolism and growth leading to widespread effects on secondary metabolism, including reduced phenazine production and oxidative stress tolerance. Thus, the miaA mutant provided the opportunity to examine the regulation of phenazine production in response to altered metabolism and growth or stress tolerance. Despite the importance of MiaA for translation efficiency, the most significant effect of miaA disruption on phenazine production was the reduction in the transcription of phzR, phzI and pip, whereas neither the transcription nor translation of RpeB, a transcriptional regulator of pip, was affected. Constitutive expression of rpeB or pip in the miaA mutant completely restored phenazine production, but it resulted in further growth impairment. Constitutive expression of RpoS alleviated sensitivity to oxidative stress resulting from RpoS translation inefficiency in the miaA mutant, but it did not restore phenazine production. Our results support the model that cells curtail phenazine biosynthesis under suboptimal growth conditions via RpeB/Pip-mediated regulation of QS.

Lantibiotics produced by Actinobacteria and their potential applications (a review)

Karen Machado Gomes, Rafael Silva Duarte, Maria do Carmo de Freire Bastos

ABSTRACT

The phylum Actinobacteria, which comprises a great variety of Gram-positive bacteria with a high G+C content in their genomes, is known for its large production of bioactive compounds, including those with antimicrobial activity. Among the antimicrobials, bacteriocins, ribosomally synthesized peptides, represent an important arsenal of potential new drugs to face the increasing prevalence of resistance to antibiotics among microbial pathogens. The actinobacterial bacteriocins form a heterogeneous group of substances that is difficult to adapt to most proposed classification schemes. However, recent updates have accommodated efficiently the diversity of bacteriocins produced by this phylum. Among the bacteriocins, the lantibiotics represent a source of new antimicrobials to control infections caused mainly by Gram-positive bacteria and with a low propensity for resistance development. Moreover, some of these compounds have additional biological properties, exhibiting activity against viruses and tumour cells and having also potential to be used in blood pressure or inflammation control and in pain relief. Thus, lantibiotics already described in Actinobacteria exhibit potential practical applications in medical settings, food industry and agriculture, with examples at different stages of pre-clinical and clinical trials.

Exploring the parameters of post-segregational killing using heterologous expression of secreted toxin barnase and antitoxin barstar in an Escherichia coli case study

Dorien S Coray, Brigitta Kurenbach, Jack A Heinemann

ABSTRACT

Post-segregational killing (PSK) is a phenotype determined by plasmids using a toxin and an antitoxin gene pair. Loss of the genes depletes the cell's reserve of antitoxin and allows the toxin to act upon the cell. PSK benefits mobile elements when it increases reproductive success relative to other mobile competitors. A side effect of PSK is that plasmids become refractory to displacement from the cell during growth as a monoculture. Most PSK systems use a cytoplasmic toxin, but the external toxins of bacteriocins also have a PSK-like effect. It may be that any toxin and antitoxin gene pair can demonstrate PSK when it is on a plasmid. The secreted ribonuclease barnase and its protein inhibitor barstar have features in common with PSK modules, though their native context is chromosomal. We hypothesized that their recruitment to a plasmid could produce an emergent PSK phenotype. Others had shown that secreted barnase could exert a lethal effect on susceptible bacteria similarly to bacteriocins. However, barnase toxicity did not occur under the conditions tested, suggesting that barnase is toxic to neighbouring cells only under very specific conditions. Bacteriocins are only produced under some conditions, and some conditionality on toxin function or release may be advantageous in general to PSKs with external toxins because it would prevent killing of potential plasmid-naive hosts. Too much conditionality, however, would limit how advantageous the gene pair was to mobile elements, making the genes unlikely to be recruited as a PSK system.

The whcD gene of Corynebacterium glutamicum plays roles in cell division and envelope formation

Dong-Seok Lee, Younhee Kim, Heung-Shick Lee

ABSTRACT

In this study, we analysed the whcD gene from Corynebacteriumglutamicum, which encodes a homologue of whiB, a Streptomycescoelicolor gene required for the sporulation of aerial hyphae. Deletion of the gene (ΔwhcD) severely affected cell growth in C. glutamicum. The ΔwhcD strain exhibited a large filamentous, branched and bud-shaped morphology with multiple septa. The transcription levels of the cell division genes involved in Z-ring assembly and septal peptidoglycan synthesis, including ftsZ, sepF, ftsQ and ftsI, were markedly decreased in the ΔwhcD strain. The divIVA gene, which is responsible for apical growth, also showed decreased transcription in the ΔwhcD strain. However, genes involved in the later stages of cell division, such as cell separation and chromosome segregation, did not show notable changes in their transcription levels. Moreover, the mutant strain was susceptible to inhibitors of transpeptidation, including penicillin and vancomycin. In addition, the transcription of genes fas-IA, fas-IB and accD1, which participate in the synthesis of fatty acid and cell envelope component mycolic acid, was altered in the ΔwhcD strain. This increased the cell surface hydrophobicity in the mutant strain, apparently leading to cell aggregation in liquid media. These findings indicate that whcD is a whiB-like gene with roles in the early stages of cell division and fatty acid synthesis, and the pleiotropic phenotypes of the ΔwhcD strain suggest that whcD may be a global regulatory gene.

Deletion of a putative NlpC/P60 endopeptidase BAS1812 affects germination, long-term survival and endospore formation in Bacillus anthracis

Se Kye Kim, Yun Min Park, Kyoung Hwa Jung, Young Gyu Chai

ABSTRACT

Bacillus anthracis, an aetiologic agent of the zoonotic disease anthrax, encodes a putative NlpC/P60 endopeptidase BAS1812. It harbours a signal peptide, three bacterial SH3 domains and an NlpC/P60 family domain. Previous studies showed that BAS1812 is immunogenic in infected hosts and is a potential biomarker for anthrax treatment. To date, however, little information is known about its function and involvement in anthrax pathogenesis. Here we describe the phenotypic effect of BAS1812 deletion in B. anthracis Sterne strain. Transcriptional analysis showed that BAS1812 expression in a host-like environment was enhanced at the end of log phase, started to diminish after entry to stationary phase and increased again late in stationary phase. The constructed BAS1812 mutant showed impaired long-term survival in the stationary growth phase, less resilience to detergent, lesser endospore formation and delayed germination. The mutant also showed diminished ability to degrade peptidoglycan, but its ability to produce anthrax exotoxins was not affected. We hypothesize that BAS1812 is a cell wall hydrolase involved in biological activities related to maintaining cell wall integrity, sporulation and spore germination.

Viscosity-dependent variations in the cell shape and swimming manner of Leptospira

Kyosuke Takabe, Hajime Tahara, Md. Shafiqul Islam, Samia Affroze, Seishi Kudo, Shuichi Nakamura

ABSTRACT

Spirochaetes are spiral or flat-wave–shaped Gram-negative bacteria that have periplasmic flagella between the peptidoglycan layer and outer membrane. Rotation of the periplasmic flagella transforms the cell body shape periodically, allowing the cell to swim in aqueous environments. Because the virulence of motility-deficient mutants of pathogenic species is drastically attenuated, motility is thought to be an essential virulence factor in spirochaetes. However, it remains unknown how motility practically contributes to the infection process. We show here that the cell body configuration and motility of the zoonotic spirochaete Leptospira changes depending on the viscosity of the medium. Leptospira swim and reverse the swimming direction by transforming the cell body. Motility analysis showed that the frequency of cell shape transformation was increased by increasing the viscosity of the medium. The increased cell body transformation induced highly frequent reversal of the swimming direction. A simple kinetic model based on the experimental results shows that the viscosity-induced increase in reversal limits cell migration, resulting in the accumulation of cells in high-viscosity regions. This behaviour could facilitate the colonization of the spirochaete on host tissues covered with mucosa.

The intestinal proteome of diabetic and control children is enriched with different microbial and host proteins

Elsa Pinto, Marisol Anselmo, Manuela Calha, Andrew Bottrill, Isabel Duarte, Peter W Andrew, Maria L Faleiro

ABSTRACT

In this study, the intestinal microbial proteome of children with established type 1 diabetes (T1D) was compared with the proteome of healthy children (Control) with the aim to identify differences in the activity of the intestinal microbiota that not only will contribute to a deeper knowledge of the functionality of the gut in these children but also may provide new approaches to improve the control of the disease. Faecal protein extracts collected from three T1D children (aged 9.3±0.6 years) and three Control children (aged 9.3±1.5 years) were analysed using a combination of 2D gel electrophoresis and spectral counting. The results evidenced markedly differences between the intestinal proteome of T1D children and the Control. The T1D microbial intestinal proteome was enriched with proteins of clostridial cluster XVa and cluster IV and Bacteroides. In contrast, the Control proteome was enriched with bifidobacterial proteins. In both groups, proteins with moonlight function were observed. Human proteins also distinguished the two groups with T1D children depleted in exocrine pancreatic enzymes.

Identification of lysophospholipase protein from Spiroplasma eriocheiris and verification of its function

Huanxi Zhu, Peng Liu, Jie Du, Jian Wang, Yunting Jing, Jia Zhang, Wei Gu, Wen Wang, Qingguo Meng

ABSTRACT

Spiroplasma eriocheiris is known to cause tremor disease in the Chinese mitten crab Eriocheir sinensis; however, the molecular characterization of this pathogen is still unclear. S. eriocheiris has the ability to invade and survive within mouse 3T6 cells. The invasion process may require causing damage to the host cell membrane by chemical, physical or enzymatic means. In this study, we systematically characterized a novel lysophospholipase (lysoPL) of S. eriocheiris TDA-040725-5T. The gene that encodes lysoPL in S. eriocheiris (SE-LysoPL) was cloned, sequenced and expressed in Escherichia coli BL21 (DE3). Enzymatic assays revealed that the purified recombinant SE-LysoPL hydrolysed long-chain acyl esterases at pH 7 and 30 °C. SE-LysoPL was detected in the membrane and cytoplasmic protein fractions using the SE-LysoPL antibody in Western blot. The virulence ability of S. eriocheiris was effectively reduced at the early stage of infection (m.o.i.=100) by the SE-LysoPL antibody neutralization test. To the best of our knowledge, this is the first study to identify and characterize a gene from S. eriocheiris encoding a protein exhibiting lysoPL and esterase activities. Our findings indicate that SE-LysoPL plays important roles in the pathogenicity of S. eriocheiris.

Lack of formylated methionyl-tRNA has pleiotropic effects on Bacillus subtilis

Yanfei Cai, Pete Chandrangsu, Ahmed Gaballa, John D Helmann

ABSTRACT

Bacteria initiate translation using a modified amino acid, N-formylmethionine (fMet), adapted specifically for this function. Most proteins are processed co-translationally by peptide deformylase (PDF) to remove this modification. Although PDF activity is essential in WT cells and is the target of the antibiotic actinonin, bypass mutations in the fmt gene that eliminate the formylation of Met-tRNAMet render PDF dispensable. The extent to which the emergence of fmt bypass mutations might compromise the therapeutic utility of actinonin is determined, in part, by the effects of these bypass mutations on fitness. Here, we characterize the phenotypic consequences of an fmt null mutation in the model organism Bacillus subtilis. An fmt null mutant is defective for several post-exponential phase adaptive programmes including antibiotic resistance, biofilm formation, swarming and swimming motility and sporulation. In addition, a survey of well-characterized stress responses reveals an increased sensitivity to metal ion excess and oxidative stress. These diverse phenotypes presumably reflect altered synthesis or stability of key proteins involved in these processes.

CRISPR-Cas system presents multiple transcriptional units including antisense RNAs that are expressed in minimal medium and upregulated by pH in Salmonella enterica serovar Typhi

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ABSTRACT

The CRISPR-Cas system is involved in bacterial immunity, virulence, gene regulation, biofilm formation and sporulation. In Salmonella enterica serovar Typhi, this system consists of five transcriptional units including antisense RNAs. It was determined that these genetic elements are expressed in minimal medium and are up-regulated by pH. In addition, a transcriptional characterization of cas3 and ascse2-1 is included herein.

Poly (A) polymerase I participates in the indole regulatory pathway of Pantoea agglomerans YS19

Zihua Li, Jing Jiang, Xuemei Yu, Cunxiang Wu, Delong Shen, Yongjun Feng

ABSTRACT

Pantoea agglomerans YS19 is a preponderant endophytic bacterium isolated from rice. It is characterized by the formation of symplasmata, a type of multicellular aggregate structure, contributing to a strong stress resistance and specific adaptation of YS19 in endophyte–host associations. Indole is an important signal molecule in intra- or interspecies relationships, regulating a variety of bacterial behaviours such as cell aggregation and stress resistance; however, the regulatory mechanism remains an ongoing area of investigation. This study selected YS19 as a model strain to construct a mutant library, utilizing the mTn5 transposon mutagenesis method, thus obtaining a positive mutant with an indole-inhibited mutation gene. Via thermal asymmetric interlaced PCR, the mutational site was identified as the gene of pcnB, which encodes the poly(A) polymerase I to catalyse the polyadenylation of RNAs. The full length of the pcnB sequence was 1332 bp, and phylogenetic analysis revealed that pcnB is extremely conserved among strains of P. agglomerans. The expression of the gene was significantly inhibited (by 36.6% as detected via quantitative PCR) by indole (0.5 mM). Many physiological behaviours of YS19 were affected by this mutation: the cell decay rate in the post-stationary growth phase was promoted, symplasmata formation and motility were inhibited in the late stationary growth phase and the colonization ability and growth-promoting effect of YS19 on the host plant were also inhibited. This study discusses the indole regulatory pathways from the point of RNA post-transcriptional modification, thus enriching our knowledge of polyadenylation and expanding current research ideas of indole regulation.

Functional analysis of a biosynthetic cluster essential for production of 4-formylaminooxyvinylglycine, a germination-arrest factor from Pseudomonas fluorescens WH6

Rachel A Okrent, Kristin M Trippe, Maciej Maselko, Viola Manning

ABSTRACT

Rhizosphere-associated Pseudomonas fluorescens WH6 produces the germination-arrest factor 4-formylaminooxyvinylglycine (FVG). FVG has previously been shown to both arrest the germination of weedy grasses and inhibit the growth of the bacterial plant pathogen Erwinia amylovora. Very little is known about the mechanism by which FVG is produced. Although a previous study identified a region of the genome that may be involved in FVG biosynthesis, it has not yet been determined which genes within that region are sufficient and necessary for FVG production. In the current study, we explored the role of each of the putative genes encoded in that region by constructing deletion mutations. Mutant strains were assayed for their ability to produce FVG with a combination of biological assays and TLC analyses. This work defined the core FVG biosynthetic gene cluster and revealed several interesting characteristics of FVG production. We determined that FVG biosynthesis requires two small ORFs of less than 150 nucleotides and that multiple transporters have overlapping but distinct functionality. In addition, two genes in the centre of the biosynthetic gene cluster are not required for FVG production, suggesting that additional products may be produced from the cluster. Transcriptional analysis indicated that at least three active promoters play a role in the expression of genes within this cluster. The results of this study enrich our knowledge regarding the diversity of mechanisms by which bacteria produce non-proteinogenic amino acids like vinylglycines.

The function of the three phosphoribosyl pyrophosphate synthetase (Prs) genes in hyphal growth and conidiation in Aspergillus nidulans

Ping Jiang, Wen-fan Wei, Guo-wei Zhong, Xiao-gang Zhou, Wei-ran Qiao, Reinhard Fisher, Ling Lu

ABSTRACT

Phosphoribosyl pyrophosphate synthetase, which is encoded by the Prs gene, catalyses the reaction of ribose-5-phosphate and adenine ribonucleotide triphosphate (ATP) and has central importance in cellular metabolism. However, knowledge about how Prs family members function and contribute to total 5-phosphoribosyl-α-1-pyrophosphate (PRPP) synthetase activity is limited. In this study, we identified that the filamentous fungus Aspergillus nidulans genome contains three PRPP synthase-homologous genes (AnprsA, AnprsB and AnprsC), among which AnprsB and AnprsC but not AnprsA are auxotrophic genes. Transcriptional expression profiles revealed that the mRNA levels of AnprsA, AnprsB and Anprs C are dynamic during germination, hyphal growth and sporulation and that they all showed abundant expression during the vigorous hyphal growth time point. Inhibiting the expression of AnprsB or AnprsC in conditional strains produced more effects on the total PRPP synthetase activity than did inhibiting AnprsA, thus indicating that different AnPrs proteins are unequal in their contributions to Prs enzyme activity. In addition, the constitutive overexpression of AnprsA or AnprsC could significantly rescue the defective phenotype of the AnprsB-absent strain, suggesting that the function of AnprsB is not a specific consequence of this auxotrophic gene but instead comes from the contribution of Prs proteins to PRPP synthetase activity.

Identification and characterization of chemosensors for d-malate, unnatural enantiomer of malate, in Ralstonia pseudosolanacearum

Mattana Tunchai, Akiko Hida, Shota Oku, Yutaka Nakashimada, Takahisa Tajima, Junichi Kato

ABSTRACT

Ralstonia pseudosolanacearum Ps29 is attracted by nonmetabolizable d-malate, an unnatural enantiomer. Screening of a complete collection of single-mcp-gene deletion mutants of Ps29 revealed that the RSc1156 homologue is a chemosensor for d-malate. An RSc1156 homologue deletion mutant of Ps29 showed decreased but significant responses to d-malate, suggesting the existence of another d-malate chemosensor. McpM previously had been identified as a chemosensor for l-malate. We constructed an RSc1156 homologue mcpM double deletion mutant and noted that this mutant failed to respond to d-malate; thus, the RSc1156 homologue and McpM are the major chemosensors for d-malate in this organism. To further characterize the ligand specificities of the RSc1156 homologue and McpM, we constructed a Ps29 derivative (designated K18) harbouring deletions in 18 individual mcp genes, including mcpM and RSc1156. K18 harbouring the RSc1156 homologue responded strongly to l-tartrate and d-malate and moderately to d-tartrate, but not to l-malate or succinate. K18 harbouring mcpM responded strongly to l-malate and d-tartrate and moderately to succinate, fumarate and d-malate. Ps29 utilizes l-malate and l-tartrate, but not d-malate. We therefore concluded that l-tartrate and l-malate are natural ligands of the RSc1156 homologue and McpM, respectively, and that chemotaxis toward d-malate is a fortuitous response by the RSc1156 homologue and McpM in Ps29. We propose re-designation of the RSc1156 homologue as McpT. In tomato plant infection assays, the mcpT deletion mutant of highly virulent R. pseudosolanacearum MAFF106611 was as infectious as wild-type MAFF106611, suggesting that McpT-mediated chemotaxis does not play an important role in tomato plant infection.

Cross-regulation between two common ancestral response regulators, HprR and CusR, in Escherichia coli

Hiroyuki Urano, Myu Yoshida, Ayano Ogawa, Kaneyoshi Yamamoto, Akira Ishihama, Hiroshi Ogasawara

ABSTRACT

The uncharacterized two-component system YedVW of Escherichia coli is involved in stress response to hydrogen peroxide. To identify the H2O2-sensing role of YedV, a set of single Cys-to-Ala substitution mutants were constructed. One particular mutant with C165A substitution in the membrane domain rendered YedV inactive in H2O2-dependent transcription of its regulatory target hiuH. We then proposed to rename YedVW to HprSR (hydrogen peroxide response sensor/regulator). One unique characteristic of HprR is the overlapping of its recognition sequence with that of the Cu (II)-response two-component system regulator CusR. Towards understanding this unique regulation system, in this study we analysed the interplay between HprR and CusR with respect to transcription of hiuH, a regulatory target of HprR, and cusC, a target of CusR. Under low protein concentrations in vitro and in vivo, two regulators recognize and transcribe both hiuH and cusC promoters, albeit at different efficiency, apparently in a collaborative fashion. This is a new type of transcription regulation of the common target genes in response to different external signals. Upon increase in protein concentrations, however, HprR and CusR compete with each other in transcription of the common targets, thereby exhibiting a competitive interplay.

Identification of new regulatory genes involved in the pathogenic functions of the rice-pathogenic bacterium Burkholderia glumae

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ABSTRACT

Burkholderia glumae is an emerging plant-pathogenic bacterium that causes disease in rice in several of the major rice-producing areas throughout the world. In the southern United States, B. glumae is the major causal agent of bacterial panicle blight of rice and has caused severe yield losses in recent decades. Despite its importance, few management options are available for diseases caused by B. glumae, and knowledge of how this pathogen causes disease is limited. In an effort to identify novel factors that contribute to the pathogenicity of B. glumae, random mutagenesis using the miniTn5gus transposon was performed on two strains of B. glumae. Resultant mutants were screened in the laboratory for altered phenotypes in various known or putative virulence factors, including toxoflavin, lipase and extracellular polysaccharides. Mutants that exhibited altered phenotypes compared to their parent strain were selected and subsequently characterized using a PCR-based method to identify the approximate location of the transposon insertion. Altogether, approximately 20000 random mutants were screened and 51 different genes were identified as having potential involvement in the production of toxoflavin, lipase and/or extracellular polysaccharide. Especially, two regulatory genes, ntpR and tepR, encoding a LysR-type transcriptional regulator and a σ54-dependent response regulator, respectively, were discovered in this study as new negative regulatory factors for the production of toxoflavin, the major phytotoxin synthesized by B. glumae and involved in bacterial pathogenesis.

Suppressor analysis of eepR mutant defects reveals coordinate regulation of secondary metabolites and serralysin biosynthesis by EepR and HexS

Robert M. Q Shanks, Nicholas A Stella, Roni M Lahr, Marissa A Aston, Kimberly M Brothers, Jake D Callaghan, Cihad Sigindere, Xinyu Liu

ABSTRACT

The EepR transcription factor positively regulates secondary metabolites and tissue-damaging metalloproteases. To gain insight into mechanisms by which EepR regulates pigment and co-regulated factors, genetic suppressor analysis was performed. Suppressor mutations that restored pigment to the non-pigmented ΔeepR mutant mapped to the hexS ORF. Mutation of hexS also restored haemolysis, swarming motility and protease production to the eepR mutant. HexS is a known direct and negative regulator of secondary metabolites in Serratia marcescens and is a LysR family regulator and an orthologue of LrhA. Here, we demonstrate that HexS directly controls eepR and the serralysin gene prtS. EepR was shown to directly regulate eepR expression but indirectly regulate hexS expression. Together, these data indicate that EepR and HexS oppose each other in controlling stationary phase-associated molecules and enzymes.

Volume 163, Issue 3, March 2017

Oenococcus oeni: Queen of the cellar, nightmare of geneticists

Cosette Grandvalet

ABSTRACT

Oenococcus oeni is a wine-associated lactic acid bacterium (LAB) responsible mostly for wine malolactic fermentation (MLF). This fastidious bacterium (auxotrophic for many amino acids and slow growing) possesses remarkable adaptability to harsh physicochemical conditions and can reprogramme its metabolic pathways to enhance its survival in wine. Thus, O. oeni is an instructive bacterial model for investigating stress response mechanisms in LAB. However, the lack of appropriate techniques to modify the O. oeni genome has hampered molecular studies of this species. The application of recent advances in molecular genetics promises to provide a better understanding of the regulation of stress responses in this species in the future.

Ethanol, glycogen and glucosylglycerol represent competing carbon pools in ethanol-producing cells of Synechocystis sp. PCC 6803 under high-salt conditions

Nadin Pade, Stefan Mikkat, Martin Hagemann

ABSTRACT

Cyanobacteria are photoautotrophic micro-organisms, which are increasingly being used as microbial cell factories to produce, for example, ethanol directly from solar energy and CO2. Here, we analysed the effects of different salt concentrations on an ethanol-producing strain of Synechocystis sp. PCC 6803 that overexpresses the pyruvate decarboxylase (pdc) from Zymomonas mobilis and the native alcohol dehydrogenase (adhA). Moderate salinities of 2% NaCl had no negative impact on ethanol production, whereas the addition of 4% NaCl resulted in significantly decreased ethanol yields compared to low-salt conditions. Proteomic analysis identified a defined set of proteins with increased abundances in ethanol-producing cells. Among them, we found strong up-regulation of α-1,4 glucan phosphorylase (GlgP, Slr1367) in the producer strain, which consistently resulted in a massive depletion of glycogen pools in these cells regardless of the salinity. The salt-induced accumulation of the compatible solute glucosylglycerol was not affected by the ethanol production. Glycogen and probably compatible solutes could present competing pools with respect to organic carbon, explaining the decreased ethanol production at the highest salinity.

MazEF toxin-antitoxin proteins alter Escherichia coli cell morphology and infrastructure during persister formation and regrowth

Junho Cho, Anita Nicole Carr, Lisa Whitworth, Brent Johnson, Kevin Scott Wilson

ABSTRACT

When exposed to antibiotics, many bacteria respond by activating intracellular 'toxin' proteins, which arrest cell growth and induce formation of persister cells that survive antibiotics. After antibiotics are removed, persisters can regrow by synthesizing 'antitoxin' proteins that sequester toxin proteins. In Escherichia coli, MazE antitoxin sequesters the activity of MazF toxin, which extensively cleaves cellular RNAs. Although the functions of MazEF proteins are well characterized, there is surprisingly little known about their effects on cell structure. Here, using a combination of microscopy techniques, we visualized the effects of MazEF and three bactericidal antibiotics on E. coli cell morphology and infrastructure. When ectopically expressed in E. coli, MazF temporarily stalled cell growth and induced persister formation, but only mildly elevated DNA mutagenesis. Viewed by electron microscopy, MazF-expressing persister cells were arrested in cell growth and division. Their chromosomal DNAs were compacted into thread-like structures. Their ribosomes were excluded from their nucleoids. After exposure to ciprofloxacin, persister regrowth was activated by MazE. Cell division remained inhibited while cells became extraordinarily elongated, then divided multiple times during stationary growth phase. This extreme filamentation during persister regrowth was unique to ciprofloxacin-treated persisters, likely caused by inhibition of cell division during regrowth, and was not observed with kanamycin-treated persisters.

Identification of Naegleria fowleri proteins linked to primary amoebic meningoencephalitis

Melissa Jamerson, Jacqueline A Schmoyer, Jay Park, Francine Marciano-Cabral, Guy A Cabral

ABSTRACT

Naegleria fowleri (N. fowleri) causes primary amoebic meningoencephalitis, a rapidly fatal disease of the central nervous system. N. fowleri can exist in cyst, flagellate or amoebic forms, depending on environmental conditions. The amoebic form can invade the brain following introduction into the nasal passages. When applied intranasally to a mouse model, cultured N. fowleri amoebae exhibit low virulence. However, upon serial passage in mouse brain, the amoebae acquire a highly virulent state. In the present study, a proteomics approach was applied to the identification of N. fowleri amoeba proteins whose expression was associated with the highly virulent state in mice. Mice were inoculated intranasally with axenically cultured amoebae or with mouse-passaged amoebae. Examination by light and electron microscopy revealed no morphological differences. However, mouse-passaged amoebae were more virulent in mice as indicated by exhibiting a two log10 titre decrease in median infective dose 50 (ID50). Scatter plot analysis of amoebic lysates revealed a subset of proteins, the expression of which was associated with highly virulent amoebae. MS-MS indicated that this subset contained proteins that shared homology with those linked to cytoskeletal rearrangement and the invasion process. Invasion assays were performed in the presence of a select inhibitor to expand on the findings. The collective results suggest that N. fowleri gene products linked to cytoskeletal rearrangement and invasion may be candidate targets in the management of primary amoebic meningoencephalitis.

DNA double-strand break repair is involved in desiccation resistance of Sinorhizobium meliloti, but is not essential for its symbiotic interaction with Medicago truncatula

Pierre Dupuy, Benjamin Gourion, Laurent Sauviac, Claude Bruand

ABSTRACT

The soil bacterium Sinorhizobium meliloti, a nitrogen-fixing symbiont of legume plants, is exposed to numerous stress conditions in nature, some of which cause the formation of harmful DNA double-strand breaks (DSBs). In particular, the reactive oxygen species (ROS) and the reactive nitrogen species (RNS) produced during symbiosis, and the desiccation occurring in dry soils, are conditions which induce DSBs. Two major systems of DSB repair are known in S. meliloti: homologous recombination (HR) and non-homologous end-joining (NHEJ). However, their role in the resistance to ROS, RNS and desiccation has never been examined in this bacterial species, and the importance of DSB repair in the symbiotic interaction has not been properly evaluated. Here, we constructed S. meliloti strains deficient in HR (by deleting the recA gene) or in NHEJ (by deleting the four ku genes) or both. Interestingly, we observed that ku and/or recA genes are involved in S. meliloti resistance to ROS and RNS. Nevertheless, an S. meliloti strain deficient in both HR and NHEJ was not altered in its ability to establish and maintain an efficient nitrogen-fixing symbiosis with Medicago truncatula, showing that rhizobial DSB repair is not essential for this process. This result suggests either that DSB formation in S. meliloti is efficiently prevented during symbiosis or that DSBs are not detrimental for symbiosis efficiency. In contrast, we found for the first time that both recA and ku genes are involved in S. meliloti resistance to desiccation, suggesting that DSB repair could be important for rhizobium persistence in the soil.

Proteome analysis reveals differential expression of proteins involved in triacylglycerol accumulation by Rhodococcus jostii RHA1 after addition of methyl viologen

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ABSTRACT

Rhodococcus jostii RHA1 is able to degrade toxic compounds and accumulate high amounts of triacylglycerols (TAG) upon nitrogen starvation. These NADPH-dependent processes are essential for the adaptation of rhodococci to fluctuating environmental conditions. In this study, we used an MS-based, label-free and quantitative proteomic approach to better understand the integral response of R. jostii RHA1 to the presence of methyl viologen (MV) in relation to the synthesis and accumulation of TAG. The addition of MV promoted a decrease of TAG accumulation in comparison to cells cultivated under nitrogen-limiting conditions in the absence of this pro-oxidant. Proteomic analyses revealed that the abundance of key proteins of fatty acid biosynthesis, the Kennedy pathway, glyceroneogenesis and methylmalonyl-CoA pathway, among others, decreased in the presence of MV. In contrast, some proteins involved in lipolysis and β-oxidation of fatty acids were upregulated. Some metabolic pathways linked to the synthesis of NADPH remained activated during oxidative stress as well as under nitrogen starvation conditions. Additionally, exposure to MV resulted in the activation of complete antioxidant machinery comprising superoxide dismutases, catalases, mycothiol biosynthesis, mycothione reductase and alkyl hydroperoxide reductases, among others. Our study suggests that oxidative stress response affects TAG accumulation under nitrogen-limiting conditions through programmed molecular mechanisms when both stresses occur simultaneously.

The KL24 gene cluster and a genomic island encoding a Wzy polymerase contribute genes needed for synthesis of the K24 capsular polysaccharide by the multiply antibiotic resistant Acinetobacter baumannii isolate RCH51

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ABSTRACT

The whole-genome sequence of the multiply antibiotic resistant Acinetobacter baumannii isolate RCH51 belonging to sequence type ST103 (Institut Pasteur scheme) revealed that the set of genes at the capsule locus, KL24, includes four genes predicted to direct the synthesis of 3-acetamido-3,6-dideoxy-d-galactose (d-Fuc3NAc), and this sugar was found in the capsular polysaccharide (CPS). One of these genes, fdtE, encodes a novel bifunctional protein with an N-terminal FdtA 3,4-ketoisomerase domain and a C-terminal acetyltransferase domain. KL24 lacks a gene encoding a Wzy polymerase to link the oligosaccharide K units to form the CPS found associated with isolate RCH51, and a wzy gene was found in a small genomic island (GI) near the cpn60 gene. This GI is in precisely the same location as another GI carrying wzy and atr genes recently found in several A. baumannii isolates, but it does not otherwise resemble it. The CPS isolated from RCH51, studied by sugar analysis and 1D and 2D 1H and 13C NMR spectroscopy, revealed that the K unit has a branched pentasaccharide structure made up of Gal, GalNAc and GlcNAc residues with d-Fuc3NAc as a side branch, and the K units are linked via a β-d -GlcpNAc-(1→3)-β-d-Galp linkage formed by the Wzy encoded by the GI. The functions of the glycosyltransferases encoded by KL24 were assigned to formation of specific bonds. A correspondence between the order of the genes in KL24 and other KL and the order of the linkages they form was noted, and this may be useful in future predictions of glycosyltransferase specificities.

Growth inhibition of fungus Phycomyces blakesleeanus by anion channel inhibitors anthracene-9-carboxylic and niflumic acid attained through decrease in cellular respiration and energy metabolites

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ABSTRACT

Increasing resistance of fungal strains to known fungicides has prompted identification of new candidates for fungicides among substances previously used for other purposes. We have tested the effects of known anion channel inhibitors anthracene-9-carboxylic acid (A9C) and niflumic acid (NFA) on growth, energy metabolism and anionic current of mycelium of fungus Phycomyces blakesleeanus. Both inhibitors significantly decreased growth and respiration of mycelium, but complete inhibition was only achieved by 100 and 500 μM NFA for growth and respiration, respectively. A9C had no effect on respiration of human NCI-H460 cell line and very little effect on cucumber root sprout clippings, which nominates this inhibitor for further investigation as a potential new fungicide. Effects of A9C and NFA on respiration of isolated mitochondria of P. blakesleeanus were significantly smaller, which indicates that their inhibitory effect on respiration of mycelium is indirect. NMR spectroscopy showed that both A9C and NFA decrease the levels of ATP and polyphosphates in the mycelium of P. blakesleeanus, but only A9C caused intracellular acidification. Outwardly rectifying, fast inactivating instantaneous anionic current (ORIC) was also reduced to 33±5 and 21±3% of its pre-treatment size by A9C and NFA, respectively, but only in the absence of ATP. It can be assumed from our results that the regulation of ORIC is tightly linked to cellular energy metabolism in P. blakesleeanus, and the decrease in ATP and polyphosphate levels could be a direct cause of growth inhibition.

Mycobacterial phenolic glycolipid synthesis is regulated by cAMP-dependent lysine acylation of FadD22

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ABSTRACT

The mycobacterial cell envelope is unique in its chemical composition, and has an important role to play in pathogenesis. Phthiocerol dimycocerosates (PDIMs) and glycosylated phenolphthiocerol dimycocerosates, also known as phenolic glycolipids (PGLs), contribute significantly to the virulence of Mycobacterium tuberculosis. FadD22 is essential for PGL biosynthesis. We have recently shown in vitro that FadD22 is a substrate for lysine acylation by a unique cAMP-dependent, protein lysine acyltransferase found only in mycobacteria. The lysine residue that is acylated is at the active site of FadD22. Therefore, acylation is likely to inhibit FadD22 activity and reduce PGL biosynthesis. Here, we show accumulation of PGLs in a strain of M. bovis BCG deleted for the gene encoding the cAMP-dependent acyltransferase, katbcg , with no change seen in PDIM synthesis. Complementation using KATbcg mutants that are deficient in cAMP-binding or acyltransferase activity shows that PGL accumulation is regulated by cAMP-dependent protein acylation in vivo. Expression of FadD22 and KATbcg mutants in Mycobacterium smegmatis confirmed that FadD22 is a substrate for lysine acylation by KATbcg. We have therefore described a mechanism by which cAMP can regulate mycobacterial virulence as a result of the ability of this second messenger to modulate critical cell wall components that affect the host immune response.

Evidence that pneumococcal WalK is regulated by StkP through protein–protein interaction

Gro Anita Stamsås, Daniel Straume, Zhian Salehian, Leiv Sigve Håvarstein

ABSTRACT

WalRK is the only two-component regulatory system essential for viability in Streptococcus pneumoniae. Despite its importance, the biological role of this system is not well understood. However, previous studies have shown that it has a crucial role in controlling pneumococcal cell division. Considerable efforts have been made to understand how the WalRK system is regulated, but no signal(s) sensed by the WalK histidine kinase has been identified so far. Here, we provide evidence that the serine/threonine protein kinase StkP modulates the activity of WalK through direct protein–protein interaction, suggesting that this interaction is one of the signals sensed by WalK. In most low-G+C content Gram-positive bacteria, WalK orthologues are attached to the cytoplasmic membrane via two transmembrane segments separated by a large extracellular loop believed to function as a sensor domain. In contrast, members of the genus Streptococcus have WalK histidine kinases that are anchored to the cytoplasmic membrane by a single transmembrane segment. It has been a long-standing question whether this segment only serves as a membrane anchor or if it also functions as a signal-sensing domain. Our data strongly support the latter, i.e. that the transmembrane segment senses signals that regulate the activity of WalK.

Identification of new members of the Escherichia coli K-12 MG1655 SlyA regulon

Thomas D Curran, Fatima Abacha, Stephen P Hibberd, Matthew D Rolfe, Melissa M Lacey, Jeffrey Green

ABSTRACT

SlyA is a member of the MarR family of bacterial transcriptional regulators. Previously, SlyA has been shown to directly regulate only two operons in Escherichia coli K-12 MG1655, fimB and hlyE (clyA). In both cases, SlyA activates gene expression by antagonizing repression by the nucleoid-associated protein H-NS. Here, the transcript profiles of aerobic glucose-limited steady-state chemostat cultures of E. coli K-12 MG1655, slyA mutant and slyA over-expression strains are reported. The transcript profile of the slyA mutant was not significantly different from that of the parent; however, that of the slyA expression strain was significantly different from that of the vector control. Transcripts representing 27 operons were increased in abundance, whereas 3 were decreased. Of the 30 differentially regulated operons, 24 have previously been associated with sites of H-NS binding, suggesting that antagonism of H-NS repression is a common feature of SlyA-mediated transcription regulation. Direct binding of SlyA to DNA located upstream of a selection of these targets permitted the identification of new operons likely to be directly regulated by SlyA. Transcripts of four operons coding for cryptic adhesins exhibited enhanced expression, and this was consistent with enhanced biofilm formation associated with the SlyA over-producing strain.

Transcriptional analysis of mating and pre-infection stages of the anther smut, Microbotryum lychnidis-dioicae

Su San Toh, Zehua Chen, David J Schultz, Christina A Cuomo, Michael H Perlin

ABSTRACT

Microbotryum lychnidis-dioicae is an obligate biotrophic parasite of the wildflower species Silene latifolia. This dikaryotic fungus, commonly known as an anther smut, requires that haploid, yeast-like sporidia of opposite mating types fuse and differentiates into dikaryotic hyphae that penetrate host tissue as part of the fungal life cycle. Mating occurs under conditions of cool temperatures and limited nutrients. Further development requires host cues or chemical mimics, including a variety of lipids, e.g. phytols. To identify global changes in transcription associated with developmental shifts, RNA-Seq was conducted at several in vitro stages of fungal propagation, i.e. haploid cells grown independently on rich and nutrient-limited media, mated cells on nutrient-limited media as well as a time course of such mated cells exposed to phytol. Comparison of haploid cells grown under rich and nutrient-limited conditions identified classes of genes probably associated with general nutrient availability, including components of the RNAi machinery. Some gene enrichment patterns comparing the nutrient-limited and mated transcriptomes suggested gene expression changes associated with the mating programme (e.g. homeodomain binding proteins, secreted proteins, proteins unique to M. lychnidis-dioicae¸ multicopper oxidases and RhoGEFs). Analysis for phytol treatment compared with mated cells alone allowed identification of genes likely to be involved in the dikaryotic switch (e.g. oligopeptide transporters). Gene categories of particular note in all three conditions included those in the major facilitator superfamily, proteins containing PFAM domains of the secretory lipase family as well as proteins predicted to be secreted, many of which have the hallmarks of fungal effectors with potential roles in pathogenicity.

Temporal upregulation of host surface receptors provides a window of opportunity for bacterial adhesion and disease

Rajendra Kc, Shakti D Shukla, Eugene H Walters, Ronan F O'Toole

ABSTRACT

Host surface receptors provide bacteria with a foothold from which to attach, colonize and, in some cases, invade tissue and elicit human disease. In this review, we discuss several key host receptors and cognate adhesins that function in bacterial pathogenesis. In particular, we examine the elevated expression of host surface receptors such as CEACAM-1, CEACAM-6, ICAM-1 and PAFR in response to specific stimuli. We explore how upregulated receptors, in turn, expose the host to a range of bacterial infections in the respiratory tract. It is apparent that exploitation of receptor induction for bacterial adherence is not unique to one body system, but is also observed in the central nervous, gastrointestinal and urogenital systems. Prokaryotic pathogens which utilize this mechanism for their infectivity include Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis and Escherichia coli. A number of approaches have been used, in both in vitro and in vivo experimental models, to inhibit bacterial attachment to temporally expressed host receptors. Some of these novel strategies may advance future targeted interventions for the prevention and treatment of bacterial disease.

Pb2+ tolerance by Frankia sp. strain EAN1pec involves surface-binding

Teal Furnholm, Medhat Rehan, Jessica Wishart, Louis S Tisa

ABSTRACT

Several Frankia strains have been shown to be lead-resistant. The mechanism of lead resistance was investigated for Frankia sp. strain EAN1pec. Analysis of the cultures by scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDAX) and Fourier transforming infrared spectroscopy (FTIR) demonstrated that Frankia sp. strain EAN1pec undergoes surface modifications and binds high quantities of Pb +2 . Both labelled and unlabelled shotgun proteomics approaches were used to determine changes in Frankia sp. strain EAN1pec protein expression in response to lead and zinc. Pb 2+ specifically induced changes in exopolysaccharides, the stringent response, and the phosphate (pho) regulon. Two metal transporters (a Cu2+-ATPase and cation diffusion facilitator), as well as several hypothetical transporters, were also upregulated and may be involved in metal export. The exported Pb2+ may be precipitated at the cell surface by an upregulated polyphosphate kinase, undecaprenyl diphosphate synthase and inorganic diphosphatase. A variety of metal chaperones for ensuring correct cofactor placement were also upregulated with both Pb+2 and Zn+2 stress. Thus, this Pb+2 resistance mechanism is similar to other characterized systems. The cumulative interplay of these many mechanisms may explain the extraordinary resilience of Frankia sp. strain EAN1pec to Pb+2. A potential transcription factor (DUF156) binding site was identified in association with several proteins identified as upregulated with heavy metals. This site was also discovered, for the first time, in thousands of other organisms across two kingdoms.

Functional amyloids in Streptococcus mutans, their use as targets of biofilm inhibition and initial characterization of SMU_63c

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ABSTRACT

Amyloids have been identified as functional components of the extracellular matrix of bacterial biofilms. Streptococcus mutans is an established aetiologic agent of dental caries and a biofilm dweller. In addition to the previously identified amyloidogenic adhesin P1 (also known as AgI/II, PAc), we show that the naturally occurring antigen A derivative of S. mutans wall-associated protein A (WapA) and the secreted protein SMU_63c can also form amyloid fibrils. P1, WapA and SMU_63c were found to significantly influence biofilm development and architecture, and all three proteins were shown by immunogold electron microscopy to reside within the fibrillar extracellular matrix of the biofilms. We also showed that SMU_63c functions as a negative regulator of biofilm cell density and genetic competence. In addition, the naturally occurring C-terminal cleavage product of P1, C123 (also known as AgII), was shown to represent the amyloidogenic moiety of this protein. Thus, P1 and WapA both represent sortase substrates that are processed to amyloidogenic truncation derivatives. Our current results suggest a novel mechanism by which certain cell surface adhesins are processed and contribute to the amyloidogenic capability of S. mutans. We further demonstrate that the polyphenolic small molecules tannic acid and epigallocatechin-3-gallate, and the benzoquinone derivative AA-861, which all inhibit amyloid fibrillization of C123 and antigen A in vitro, also inhibit S. mutans biofilm formation via P1- and WapA-dependent mechanisms, indicating that these proteins serve as therapeutic targets of anti-amyloid compounds.

A chitinase is required for Xylella fastidiosa colonization of its insect and plant hosts

Fabien Labroussaa, Michael Ionescu, Adam R Zeilinger, Steven E Lindow, Rodrigo P. P Almeida

ABSTRACT

Xylella fastidiosa colonizes the xylem network of host plant species as well as the foregut of its required insect vectors to ensure efficient propagation. Disease management strategies remain inefficient due to a limited comprehension of the mechanisms governing both insect and plant colonization. It was previously shown that X. fastidiosa has a functional chitinase (ChiA), and that chitin likely serves as a carbon source for this bacterium. We expand on that research, showing that a chiA mutant strain is unable to grow on chitin as the sole carbon source. Quantitative PCR assays allowed us to detect bacterial cells in the foregut of vectors after pathogen acquisition; populations of the wild-type and complemented mutant strain were both significantly larger than the chiA mutant strain 10 days, but not 3 days, post acquisition. These results indicate that adhesion of the chiA mutant strain to vectors may not be impaired, but that cell multiplication is limited. The mutant was also affected in its transmission by vectors to plants. In addition, the chiA mutant strain was unable to colonize host plants, suggesting that the enzyme has other substrates associated with plant colonization. Lastly, ChiA requires other X. fastidiosa protein(s) for its in vitro chitinolytic activity. The observation that the chiA mutant strain is not able to colonize plants warrants future attention to be paid to the substrates for this enzyme.

Variable virulence phenotype of Xenorhabdus bovienii (γ-Proteobacteria: Enterobacteriaceae) in the absence of their vector hosts

John G McMullen II, Rebecca McQuade, Jean-Claude Ogier, Sylvie Pagès, Sophie Gaudriault, S Patricia Stock

ABSTRACT

Xenorhabdus bovienii bacteria have a dual lifestyle: they are mutualistic symbionts to many species of Steinernema nematodes and are pathogens to a wide array of insects. Previous studies have shown that virulence of X. bovienii–Steinernema spp. pairs decreases when the nematodes associate with non-cognate bacterial strains. However, the virulence of the X. bovienii strains alone has not been fully investigated. In this study, we characterized the virulence of nine X. bovienii strains in Galleria mellonella and Spodoptera littoralis and performed a comparative genomic analysis to correlate observed phenotypes with strain genotypes. Two X. bovienii strains were found to be highly virulent against the tested insect hosts, while three strains displayed attenuated insect virulence. Comparative genomic analyses revealed the presence of several clusters present only in virulent strains, including a predicted type VI secretion system (T6SS). We performed intra-species-competition assays, and showed that the virulent T6SS+ strains generally outcompeted the less virulent T6SS− strains. Thus, we speculate that the T6SS in X. bovienii may be another addition to the arsenal of antibacterial mechanisms expressed by these bacteria in an insect, where it could potentially play three key roles: (1) competition against the insect host microbiota; (2) protection of the insect cadaver from necrotrophic microbial competitors; and (3) outcompeting other Xenorhabdus species and/or strains when co-infections occur.

Volatile organic compounds produced by a soil-isolate, Bacillus subtilis FA26 induce adverse ultra-structural changes to the cells of Clavibacter michiganensis ssp. sepedonicus, the causal agent of bacterial ring rot of potato

Faheem Uddin Rajer, Huijun Wu, Yongli Xie, Shanshan Xie, Waseem Raza, Hafiz Abdul Samad Tahir, Xuewen Gao

ABSTRACT

Rhizobacterial volatile organic compounds (VOCs) play an important role in the suppression of soil-borne phytopathogens. In this study, the VOCs produced by a soil-isolate, Bacillus subtilis FA26, were evaluated in vitro for their antibacterial activity against Clavibacter michiganensis ssp. sepedonicus (Cms), the causal agent of bacterial ring rot of potato. The VOCs emitted by FA26 inhibited the growth of Cms significantly compared with the control. Scanning and transmission electron microscopy analyses revealed distorted colony morphology and a wide range of abnormalities in Cms cells exposed to the VOCs of FA26. Varying the inoculation strategy and inoculum size showed that the production and activity of the antibacterial VOCs of FA26 were dependent on the culture conditions. Headspace solid-phase microextraction/gas chromatography–mass spectrometry analyses revealed that FA26 produced 11 VOCs. Four VOCs (benzaldehyde, nonanal, benzothiazole and acetophenone) were associated with the antibacterial activity against Cms. The results suggested that the VOCs produced by FA26 could control the causal agent of bacterial ring rot of potato. This information will increase our understanding of the microbial interactions mediated by VOCs in nature and aid the development of safer strategies for controlling plant disease.

Vacuolar H+-ATPase subunit Vma1p functions as the molecular ligand in the vacuole-targeting fungicidal activity of polymyxin B

Maki Iida, Keiichi Yamada, Yoshiya Nango, Yoshihiro Yamaguchi, Akira Ogita, Ken-ichi Fujita, Toshio Tanaka

ABSTRACT

Polymyxin B (PMB) is a cationic cyclic peptide that can selectively inhibit the growth of Gram-negative bacteria by disrupting the outer membrane permeability barrier through binding to lipopolysaccharide (LPS). Here, a fluorescent PMB derivative (PMB-Ds) was applied to visually confirm the vacuole as a direct lethal target of PMB against fungal cells, which lack LPS. PMB-Ds could be visualized in the normal rounded vacuolar membrane of Saccharomyces cerevisiae cells, suggesting the presence of a molecular ligand assisting the vacuole-targeting mobilization of the peptide in the organism. Vma1p, a cytoplasmic subunit constituent of the yeast vacuolar-type ATPase, was identified as one of the PMB-binding proteins by means of mass spectrometry. Mutant cells carrying a deletion of Vma1p but not those with deletions in two separate PMB-binding proteins were shown to be resistant to the vacuolar membrane disruptive action of PMB. Furthermore, the mutant cells were resistant to PMB lethality even when treated with PMB in combination with allicin, an allyl sulfur compound, which can selectively enhance the vacuole-targeting fungicidal activity of the peptide. In contrast, the parent cells were not made resistant to the vacuolar membrane disruptive action of PMB even if cells were pre-treated with bafilomycin A1, a specific inhibitor of the yeast vacuolar-type H+-ATPase. However, the parent cells were rendered more resistant to PMB consequent to Vma1p-GFP localization in the cytoplasm. These findings suggested a role for Vma1p in the vacuole-targeting fungicidal activity of PMB comparable to that of LPS in the outer membrane of Gram-negative bacteria.

Unravelling the biosynthesis of pyriculol in the rice blast fungus Magnaporthe oryzae

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ABSTRACT

Pyriculol was isolated from the rice blast fungus Magnaporthe oryzae and found to induce lesion formation on rice leaves. These findings suggest that it could be involved in virulence. The gene MoPKS19 was identified to encode a polyketide synthase essential for the production of the polyketide pyriculol in the rice blast fungus M. oryzae. The transcript abundance of MoPKS19 correlates with the biosynthesis rate of pyriculol in a time-dependent manner. Furthermore, gene inactivation of MoPKS19 resulted in a mutant unable to produce pyriculol, pyriculariol and their dihydro derivatives. Inactivation of a putative oxidase-encoding gene MoC19OXR1, which was found to be located in the genome close to MoPKS19, resulted in a mutant exclusively producing dihydropyriculol and dihydropyriculariol. By contrast, overexpression of MoC19OXR1 resulted in a mutant strain only producing pyriculol. The MoPKS19 cluster, furthermore, comprises two transcription factors MoC19TRF1 and MoC19TRF2, which were both found individually to act as negative regulators repressing gene expression of MoPKS19. Additionally, extracts of ΔMopks19 and ΔMoC19oxr1 made from axenic cultures failed to induce lesions on rice leaves compared to extracts of the wild-type strain. Consequently, pyriculol and its isomer pyriculariol appear to be the only lesion-inducing secondary metabolites produced by M. oryzae wild-type (MoWT) under these culture conditions. Interestingly, the mutants unable to produce pyriculol and pyriculariol were as pathogenic as MoWT, demonstrating that pyriculol is not required for infection.

Environment-directed activation of the Escherichia coli flhDC operon by transposons

Zhongge Zhang, Chika Kukita, M. Zafri Humayun, Milton H Saier Jr

ABSTRACT

The flagellar system in Escherichia coli K12 is expressed under the control of the flhDC-encoded master regulator FlhDC. Transposition of insertion sequence (IS) elements to the upstream flhDC promoter region up-regulates transcription of this operon, resulting in a more rapid motility. Wang and Wood (ISME J 2011;5:1517–1525) provided evidence that insertion of IS5 into upstream activating sites occurs at higher rates in semi-solid agar media in which swarming behaviour is allowed as compared with liquid or solid media where swarming cannot occur. We confirm this conclusion and show that three IS elements, IS1, IS3 and IS5, transpose to multiple upstream sites within a 370 bp region of the flhDC operon control region. Hot spots for IS insertion correlate with positions of stress-induced DNA duplex destabilization (SIDD). We show that IS insertion occurs at maximal rates in 0.24% agar, with rates decreasing dramatically with increasing or decreasing agar concentrations. In mixed cultures, we show that these mutations preferentially arise from the wild-type parent at frequencies of up to 3×10−3 cell−1 day−1 when the inoculated parental and co-existing IS-activated mutant cells are entering the stationary growth phase. We rigorously show that the apparent increased mutation frequencies cannot be accounted for by increased swimming or by increased growth under the selective conditions used. Thus, our data are consistent with the possibility that appropriate environmental conditions, namely those that permit but hinder flagellar rotation, result in the activation of a mutational pathway that involves IS element insertion upstream of the flhDC operon.

RNase E and RNase J are needed for S-adenosylmethionine homeostasis in Sinorhizobium meliloti

Kathrin Baumgardt, Hendrik Melior, Ramakanth Madhugiri, Sebastian Thalmann, Adam Schikora, Matthew McIntosh, Anke Becker, Elena Evguenieva-Hackenberg

ABSTRACT

The ribonucleases (RNases) E and J play major roles in E. coli and Bacillus subtilis, respectively, and co-exist in Sinorhizobium meliloti. We analysed S. meliloti 2011 mutants with mini-Tn5 insertions in the corresponding genes rne and rnj and found many overlapping effects. We observed similar changes in mRNA levels, including lower mRNA levels of the motility and chemotaxis related genes flaA, flgB and cheR and higher levels of ndvA (important for glucan export). The acyl-homoserine lactone (AHL) levels were also higher during exponential growth in both RNase mutants, despite no increase in the expression of the sinI AHL synthase gene. Furthermore, several RNAs from both mutants migrated aberrantly in denaturing gels at 300 V but not under stronger denaturing conditions at 1300 V. The similarities between the two mutants could be explained by increased levels of the key methyl donor S-adenosylmethionine (SAM), since this may result in faster AHL synthesis leading to higher AHL accumulation as well as in uncontrolled methylation of macromolecules including RNA, which may strengthen RNA secondary structures. Indeed, we found that in both mutants the N 6-methyladenosine content was increased almost threefold and the SAM level was increased at least sevenfold. Complementation by induced ectopic expression of the respective RNase restored the AHL and SAM levels in each of the mutants. In summary, our data show that both RNase E and RNase J are needed for SAM homeostasis in S. meliloti.

Regulation of the Escherichia coli ydhY-T operon in the presence of alternative electron acceptors

Naji Awad A Al Ibrahim, Jeffrey Green

ABSTRACT

The Escherichia coli K-12 ydhY-T operon, coding for a predicted oxidoreductase complex, is activated under anaerobic conditions and repressed in the presence of nitrate or nitrite. Anaerobic activation is mediated by the transcription factor FNR, and nitrate/nitrite repression is mediated by NarXL and NarQP. In vitro transcription reactions revealed that the DNA upstream of ydhY-T contains sufficient information for RNA polymerase alone to initiate transcription from five locations. FNR severely inhibited synthesis of two of these transcripts (located upstream of, and within, the FNR binding site) and activated the FNR-dependent promoter previously identified in vivo. Enhanced expression of ydhY-T in an hns mutant was consistent with the location of ydhY-T within a promoter island and the FNR-independent transcription observed in vitro. FNR-dependent transcription in vitro was decreased in the presence of NarL~P. DNaseI footprinting indicated that FNR and NarL~P simultaneously bound at the ydhY-T promoter region and that NarL~P-mediated repression was due to occupation of the 7-2-7 site located downstream of the FNR-dependent promoter. Expression of ydhY-T during the anaerobic growth cycle was repressed when nitrate was present but less so in the presence of nitrite. In vivo transcription measurements indicated that the alternative electron acceptors, DMSO and fumarate, could also lower ydhY-T expression, whereas trimethylamine-N-oxide (TMAO) permitted high expression. Therefore, expression of ydhY-T is subject to complex regulation in response to electron acceptor availability that involves at least three transcription factors, FNR (anaerobic activation), NarL~P (nitrate repression) and H-NS (repression in the absence of an antagonist; e.g. FNR).

Mutual interaction enables the mycobacterial plasmid pAL5000 origin binding protein RepB to recruit RepA, the plasmid replicase, to the origin

Soniya Chatterjee, Madhu Manti Patra, Sourabh Samaddar, Arnab Basu, Sujoy K Das Gupta

ABSTRACT

The Mycobacterium fortuitum plasmid, pAL5000, is the most-studied member of a family of plasmids that are found in Actinobacteria. Its replication is brought about by the combined action of two plasmid-encoded replication proteins, RepA and RepB. RepB has earlier been shown to be a sigma factor homologue that possesses origin-binding activity. The mechanism by which RepA functions, and its relationship with RepB, if any, has not been explored yet. In this study, we show that RepA shares a common catalytic domain, with proteins belonging to the primase-polymerase and DNA polymerase X families. We demonstrate that RepA is functionally a DNA polymerase and that mutations that alter two conserved aspartic acid residues present within the catalytic core lead to inactivation of plasmid replication. Replication of pAL5000 was shown not to depend on the host primase, and thus it is most likely that RepA is responsible for the priming act. We further demonstrate that RepA and RepB function as a pair and that the functional cooperation between the two requires physical contact. The C-terminal domain of RepA, which is structurally a helical bundle, is responsible for unwinding the origin in a site-specific manner and also for the establishment of contacts with RepB. The results presented show that RepB functions by recruiting RepA to the origin in much the same way as sigma factors recruit RNA polymerase core enzyme to promoters.

Genome amplification and promoter mutation expand the range of csgD-dependent biofilm responses in an STEC population

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ABSTRACT

Expression of the major biofilm components of E. coli, curli fimbriae and cellulose, requires the CsgD transcription factor. A complex regulatory network allows environmental control of csgD transcription and biofilm formation. However, most clinical serotype O157:H7 strains contain prophage insertions in the csgD regulator, mlrA, or mutations in other regulators that restrict csgD expression. These barriers can be circumvented by certain compensating mutations that restore higher csgD expression. One mechanism is via csgD promoter mutations that switch sigma factor utilization. Biofilm-forming variants utilizing RpoD rather than RpoS have been identified in glycerol freezer stocks of the non-biofilm-forming food-borne outbreak strain, ATCC 43894. In this study we used whole genome sequencing and RNA-seq to study genotypic and transcriptomic differences between those strains. In addition to defining the consequences of the csgD promoter switch and identifying new csgD-controlled genes, we discovered a region of genome amplification in our laboratory stock of 43894 (designated 43894OW) that contributed to the regulation of csgD-dependent properties.

Volume 163, Issue 5, May 2017

Systematics of haloarchaea and biotechnological potential of their hydrolytic enzymes

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ABSTRACT

Halophilic archaea, also referred to as haloarchaea, dominate hypersaline environments. To survive under such extreme conditions, haloarchaea and their enzymes have evolved to function optimally in environments with high salt concentrations and, sometimes, with extreme pH and temperatures. These features make haloarchaea attractive sources of a wide variety of biotechnological products, such as hydrolytic enzymes, with numerous potential applications in biotechnology. The unique trait of haloarchaeal enzymes, haloenzymes, to sustain activity under hypersaline conditions has extended the range of already-available biocatalysts and industrial processes in which high salt concentrations inhibit the activity of regular enzymes. In addition to their halostable properties, haloenzymes can also withstand other conditions such as extreme pH and temperature. In spite of these benefits, the industrial potential of these natural catalysts remains largely unexplored, with only a few characterized extracellular hydrolases. Because of the applied impact of haloarchaea and their specific ability to live in the presence of high salt concentrations, studies on their systematics have intensified in recent years, identifying many new genera and species. This review summarizes the current status of the haloarchaeal genera and species, and discusses the properties of haloenzymes and their potential industrial applications.

Microbe Profile: Akkermansia muciniphila: a conserved intestinal symbiont that acts as the gatekeeper of our mucosa

Willem M de Vos

ABSTRACT

Akkermansia muciniphila is an abundant inhabitant of the intestinal tract of humans and many other animals. It is the sole intestinal representative of the verrucomicrobia in human stools and depleted in adults suffering from obesity, diabetes and several other diseases. A. muciniphila degrades intestinal mucin into mainly propionic and acetic acid, and lives in symbiosis with its host, marked by signalling to immune and metabolic pathways, priming trophic chains and likely providing competitive exclusion at the host–microbe interface. Since its recent discovery, A. muciniphila has increasingly been studied and recognized as a true intestinal symbiont promoting beneficial interactions in the intestinal tract.

Expanding the substrates for a bacterial hydrogenlyase reaction

Ciaran M Lamont, Ciarán L Kelly, Constanze Pinske, Grant Buchanan, Tracy Palmer, Frank Sargent

ABSTRACT

Escherichia coli produces enzymes dedicated to hydrogen metabolism under anaerobic conditions. In particular, a formate hydrogenlyase (FHL) enzyme is responsible for the majority of hydrogen gas produced under fermentative conditions. FHL comprises a formate dehydrogenase (encoded by fdhF) linked directly to [NiFe]-hydrogenase-3 (Hyd-3), and formate is the only natural substrate known for proton reduction by this hydrogenase. In this work, the possibility of engineering an alternative electron donor for hydrogen production has been explored. Rational design and genetic engineering led to the construction of a fusion between Thermotoga maritima ferredoxin (Fd) and Hyd-3. The Fd-Hyd-3 fusion was found to evolve hydrogen when co-produced with T. maritima pyruvate::ferredoxin oxidoreductase (PFOR), which links pyruvate oxidation to the reduction of ferredoxin. Analysis of the key organic acids produced during fermentation suggested that the PFOR/Fd-Hyd-3 fusion system successfully diverted pyruvate onto a new pathway towards hydrogen production.

Chlamydia trachomatis induces an upregulation of molecular biomarkers podoplanin, Wilms' tumour gene 1, osteopontin and inflammatory cytokines in human mesothelial cells

Anna De Filippis, Elisabetta Buommino, Marina Di Domenico, Antonia Feola, Raffaella Brunetti-Pierri, Antonietta Rizzo

ABSTRACT

Chlamydia trachomatis is the most prevalent infection of the genital tract in women worldwide. C. trachomatis has a tendency to cause persistent infection and induce a state of chronic inflammation, which has been reported to play a role in carcinogenesis. We report that persistent C. trachomatis infection increases the expression of inflammatory tumour cytokines and upregulates molecular biomarkers such as podoplanin, Wilms' tumour gene 1 and osteopontin in primary cultures of mesothelial cells (Mes1) and human mesothelioma cells (NCI). Infection experiments showed that Mes1 and NCI supported the growth of C. trachomatis in vitro, and at an m.o.i. of 4, the inclusion-forming units/cell showed many intracellular inclusion bodies after 3 days of infection. However, after 7 days of incubation, increased proliferative and invasive activity was also observed in Mes1 cells, which was more evident after 14 days of incubation. ELISA analysis revealed an increase in vascular endothelial growth factor, IL-6, IL-8, and TNF-α release in Mes1 cells infected for a longer period (14 days). Finally, real-time PCR analysis revealed a strong induction of podoplanin, Wilms' tumour gene 1 and osteopontin gene expression in infected Mes1 cells. The aim of the present study was to investigate the inflammatory response elicited by C. trachomatis persistent infection and the role played by inflammation in cell proliferation, secretion of proinflammatory cytokines and molecular biomarkers of cancer. The results of this study suggest that increased molecular biomarkers of cancer by persistent inflammation from C. trachomatis infection might support cellular transformation, thus increasing the risk of cancer.

Biofilm community succession: a neutral perspective

Stephen Woodcock, William T Sloan

ABSTRACT

Although biofilms represent one of the dominant forms of life in aqueous environments, our understanding of the assembly and development of their microbial communities remains relatively poor. In recent years, several studies have addressed this and have extended the concepts of succession theory in classical ecology into microbial systems. From these datasets, niche-based conceptual models have been developed explaining observed biodiversity patterns and their dynamics. These models have not, however, been formulated mathematically and so remain untested. Here, we further develop spatially resolved neutral community models and demonstrate that these can also explain these patterns and offer alternative explanations of microbial succession. The success of neutral models suggests that stochastic effects alone may have a much greater influence on microbial community succession than previously acknowledged. Furthermore, such models are much more readily parameterised and can be used as the foundation of more complex and realistic models of microbial community succession.

Impact of temperature on Marinobacter hydrocarbonoclasticus SP17 morphology and biofilm structure during growth on alkanes

Priscilla Branchu, Alexis Canette, Sara Medina Fernandez, Julie Mounier, Thierry Meylheuc, Romain Briandet, Régis Grimaud, Murielle Naïtali

ABSTRACT

Alkanes are widespread pollutants found in soil, freshwater and marine environments. Marinobacter hydrocarbonoclasticus (Mh) strain SP17 is a marine bacterium able to use many hydrophobic organic compounds, including alkanes, through the production of biofilms that allow their poor solubility to be overcome. This study pointed out that temperature is an environmental factor that strongly affects the biofilm formation and morphology of Mh on the model alkanes, hexadecane and paraffin. We showed that Mh biofilm formation and accumulation of intracytoplasmic inclusions are higher on solid alkanes (hexadecane at 10 °C and paraffin at 10 °C and 30 °C) than on liquid alkane (hexadecane at 30 °C) or soluble substrate (lactate at both temperatures). We also found that Mh produces more extracellular polymeric substances at 30 °C than at 10 °C on alkanes and none on lactate. We observed that bacterial length is significantly higher at 10 °C than at 30 °C on lactate and hexadecane. On paraffin, at 30 °C, the cell morphology is markedly altered by large rounded or irregularly shaped cytoplasmic inclusions. Altogether, the results showed that Mh is able to adapt and use alkanes as a carbon source, even at low temperature.

Physical contact and carbon transfer between a lichen-forming Trebouxia alga and a novel Alphaproteobacterium

Mieko Kono, Hideyuki Tanabe, Yoshihito Ohmura, Yoko Satta, Yohey Terai

ABSTRACT

Recent progress in molecular techniques has begun to alter traditional recognition of lichens as symbiotic organisms comprised of a fungus and photosynthetic partners (green algae and/or cyanobacteria). Diverse organisms, especially various non-photosynthetic bacteria, are now indicated to be integral components of lichen symbiosis. Although lichen-associated bacteria are inferred to have functions that could support the symbiosis, little is known about their physical and nutritional interaction with fungi and algae. In the present study, we identified specific interaction between a lichen-forming alga and a novel bacterium. Trebouxia alga was isolated from a lichen, Usnea hakonensis, and kept as a strain for 8 years. Although no visible bacterial colonies were observed in this culture, high-throughput sequencing of DNA isolated from the culture revealed that the strain is composed of a Trebouxia alga and an Alphaproteobacterium species. In situ hybridization showed that bacterial cells were localized on the surface of the algal cells. Physiological assays revealed that the bacterium was able to use ribitol, glucose and mannitol, all of which are known to exist abundantly in lichens. It was resistant to three antibiotics. Bacteria closely related to this species were also identified in lichen specimens, indicating that U. hakonensis may commonly associate with this group of bacteria. These features of the novel bacterium suggest that it may be involved in carbon cycling of U. hakonensis as a member of lichen symbiosis and less likely to have become associated with the alga after isolation from lichen.

Functional characterization of the collagen-binding protein DIP2093 and its influence on host–pathogen interaction and arthritogenic potential of Corynebacterium diphtheriae

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ABSTRACT

Corynebacterium diphtheriae is typically recognized as the etiological agent of diphtheria, a toxaemic infection of the respiratory tract; however, both non-toxigenic and toxigenic strains are increasingly isolated from cases of invasive infections. The molecular mechanisms responsible for bacterial colonization and dissemination to host tissues remain only partially understood. In this report, we investigated the role of DIP2093, described as a putative adhesin of the serine-aspartate repeat (Sdr) protein family in host–pathogen interactions of C. diphtheriae wild-type strain NCTC13129. Compared to the parental strain, a DIP2093 mutant RN generated in this study was attenuated in its ability to bind to type I collagen, to adhere to and invade epithelial cells, as well as to survive within macrophages. Furthermore, DIP2093 mutant strain RN had a less detrimental impact on the viability of Caenorhabditis elegans as well as in the clinical severity of arthritis in mice. In conclusion, DIP2093 functions as a microbial surface component recognizing adhesive matrix molecules, and may be included among the factors that contribute to the pathogenicity of C. diphtheriae strains, independently of toxin production.

Commensal-derived OMVs elicit a mild proinflammatory response in intestinal epithelial cells

Daniel A Patten, Enas Hussein, Scott P Davies, Paul N Humphreys, Andrew Collett

ABSTRACT

Under normal physiological conditions, the intestinal immunity remains largely hyporesponsive to the commensal microbiota, yet also retains the inherent ability to rapidly respond to pathogenic antigens. However, immunomodulatory activities of extracellular products from commensal bacteria have been little studied, with previous investigations generally utilizing the live bacterium to study microbiota–epithelial interactions. In this study, we demonstrate that extracellular products of a commensal bacterium, Escherichia coli C25, elicit a moderate release of proinflammatory IL-8 and stimulate transcriptional up-regulation of Toll-like receptors (TLRs) in intestinal epithelial cell lines HT29-19A and Caco-2. Additionally, we show that removal of outer membrane vesicles (OMVs) reduces the proinflammatory effect of secreted products from E. coli C25. Furthermore, we show that isolated OMVs have a dose-dependent proinflammatory effect on intestinal epithelial cells (IECs). Interestingly, a relatively high concentration (40 μg ml−1 protein) of OMVs had no significant regulatory effects on TLR mRNA expression in both cell lines. Finally, we also demonstrate that pre-incubation with E. coli C25-derived OMVs subsequently inhibited the internalization of the bacterium itself in both cell lines. Taken together, our results suggest that commensal-derived extracellular products, in particular OMVs, could significantly contribute to intestinal homeostasis. We also demonstrate a unique interaction between commensal-derived OMVs and host cells.

Effect of subinhibitory concentrations of tigecycline and ciprofloxacin on the expression of biofilm-associated genes and biofilm structure of Staphylococcus epidermidis

Ewa Szczuka, Lucyna Jabłońska, Adam Kaznowski

ABSTRACT

Staphylococcus epidermidis is a leading cause of foreign body-associated infections. This is related to the bacterium's ability to form biofilms on synthetic materials. Bacteria within a biofilm may be exposed to subinhibitory concentrations (sub-MICs) of antibiotics because of an agent's limited penetration into the biofilm core. Here, we investigated the effect of sub-MICs of tigecycline and ciprofloxacin on the expression of biofilm-associated genes, i.e. icaA, altE and sigB, and the biofilm structure of five clinical isolates of S. epidermidis. For most tested isolates, the expression of these genes increased after exposure to 0.25 MIC and 0.5 MIC tigecycline. A slight decrease in icaAmRNA levels was observed only in two isolates in the presence of 0.25 MIC tigecycline. The effect of ciprofloxacin exposure was isolate-dependent. At 0.5 MIC, ciprofloxacin induced an increase of sigB and icaAmRNA levels in three of the five tested isolates. At the same time, expression of the altE gene increased in all isolates (from 1.3-fold to 42-fold, depending on the strain). Confocal laser scanning microscopy analysis indicated that sub-MIC ciprofloxacin decreased biofilm formation, whereas tigecycline stimulated this process. Our data suggest that sub-MIC tigecycline may have bearing on the outcome of infections.

K+ modulates genetic competence and the stress regulon of Streptococcus mutans

Gursonika Binepal, Iwona B Wenderska, Paula Crowley, Richard N Besingi, Dilani B Senadheera, L Jeannine Brady, Dennis G Cvitkovitch

ABSTRACT

Potassium (K+) is the most abundant cation in dental plaque fluid. Previously, we reported the link between K+ transport via Trk2 in Streptococcus mutans and its two critical virulence attributes: acid tolerance and surface adhesion. Herein, we build further on the intimate link between K+ levels and S. mutans biology. High (>25 mM) versus low (≤5 mM) K+ concentrations in the growth medium affected conformational epitopes of cell surface-localized adhesin P1. At low K+, the expression of stress response elements gcrR and codY, cell-adhesion-associated genes such as spaP and metabolism-associated genes such as bglP was induced at stationary phase ($P<0.05$), suggesting that K+-mediated regulation is growth phase-dependent and stress-sensitive. Production of the newly discovered secretory protein encoded by SMU_63 c was strongly dependent on the availability of K+ and growth phase. This protein is a newly discovered regulator of genetic competence and biofilm cell density. Thus, the influence of K+ on DNA transformation efficiency was also examined. Compared with 25 mM K+ concentration, the presence of low K+ reduced the transformation frequency by 100-fold. Genetic transformation was abolished in a strain lacking a Trk2 system under all K+ concentrations tested. Consistent with these findings, repression of competence-associated genes, comS and comX, was observed under low environmental K+ conditions and in the strain lacking Trk2. Taken together, these results highlight a pivotal role for environmental K+ as a regulatory cation that modulates stress responses and genetic transformation in S. mutans.

Activity and functional properties of the isocitrate lyase in the cyanobacterium Cyanothece sp. PCC 7424

Marianne Gründel, Henning Knoop, Ralf Steuer

ABSTRACT

Cyanobacteria are ubiquitous photoautotrophs that assimilate atmospheric CO2 as their main source of carbon. Several cyanobacteria are known to be facultative heterotrophs that are able to grow on diverse carbon sources. For selected strains, assimilation of organic acids and mixotrophic growth on acetate has been reported for decades. However, evidence for the existence of a functional glyoxylate shunt in cyanobacteria has long been contradictory and unclear. Genes coding for isocitrate lyase (ICL) and malate synthase were recently identified in two strains of the genus Cyanothece, and the existence of the complete glyoxylate shunt was verified in a strain of Chlorogloeopsis fritschii. Here, we report that the gene PCC7424_4054 of the strain Cyanothece sp. PCC 7424 encodes an enzymatically active protein that catalyses the reaction of ICL, an enzyme that is specific for the glyoxylate shunt. We demonstrate that ICL activity is induced under alternating day/night cycles and acetate-supplemented cultures exhibit enhanced growth. In contrast, growth under constant light did not result in any detectable ICL activity or enhanced growth of acetate-supplemented cultures. Furthermore, our results indicate that, despite the presence of a glyoxylate shunt, acetate does not support continued heterotrophic growth and cell proliferation. The functional validation of the ICL is supplemented with a bioinformatics analysis of enzymes that co-occur with the glyoxylate shunt. We hypothesize that the glyoxylate shunt in Cyanothece sp. PCC 7424, and possibly other nitrogen-fixing cyanobacteria, is an adaptation to a specific ecological niche and supports assimilation of nitrogen or organic compounds during the night phase.

Non-essential MCM-related proteins mediate a response to DNA damage in the archaeon Methanococcus maripaludis

Alison D Walters, James P. J Chong

ABSTRACT

The single minichromosome maintenance (MCM) protein found in most archaea has been widely studied as a simplified model for the MCM complex that forms the catalytic core of the eukaryotic replicative helicase. Organisms of the order Methanococcales are unusual in possessing multiple MCM homologues. The Methanococcus maripaludis S2 genome encodes four MCM homologues, McmA–McmD. DNA helicase assays reveal that the unwinding activity of the three MCM-like proteins is highly variable despite sequence similarities and suggests additional motifs that influence MCM function are yet to be identified. While the gene encoding McmA could not be deleted, strains harbouring individual deletions of genes encoding each of the other MCMs display phenotypes consistent with these proteins modulating DNA damage responses. M. maripaludis S2 is the first archaeon in which MCM proteins have been shown to influence the DNA damage response.

High intracellular c-di-GMP levels antagonize quorum sensing and virulence gene expression in Burkholderia cenocepacia H111

Nadine Schmid, Angela Suppiger, Elisabeth Steiner, Gabriella Pessi, Volkhard Kaever, Mustafa Fazli, Tim Tolker-Nielsen, Urs Jenal, Leo Eberl

ABSTRACT

The opportunistic human pathogen Burkholderia cenocepacia H111 uses two chemically distinct signal molecules for controlling gene expression in a cell density-dependent manner: N-acyl-homoserine lactones (AHLs) and cis-2-dodecenoic acid (BDSF). Binding of BDSF to its cognate receptor RpfR lowers the intracellular c-di-GMP level, which in turn leads to differential expression of target genes. In this study we analysed the transcriptional profile of B. cenocepacia H111 upon artificially altering the cellular c-di-GMP level. One hundred and eleven genes were shown to be differentially expressed, 96 of which were downregulated at a high c-di-GMP concentration. Our analysis revealed that the BDSF, AHL and c-di-GMP regulons overlap for the regulation of 24 genes and that a high c-di-GMP level suppresses expression of AHL-regulated genes. Phenotypic analyses confirmed changes in the expression of virulence factors, the production of AHL signal molecules and the biosynthesis of different biofilm matrix components upon altered c-di-GMP levels. We also demonstrate that the intracellular c-di-GMP level determines the virulence of B. cenocepacia to Caenorhabditis elegans and Galleria mellonella.

Quorum sensing and RsaM regulons of the rice pathogen Pseudomonas fuscovaginae

Gordana Uzelac, Hitendra Kumar Patel, Giulia Devescovi, Danilo Licastro, Vittorio Venturi

ABSTRACT

Pseudomonas fuscovaginae (Pfv) is an emerging plant pathogen causing sheath brown rot in rice, as well as diseases in other gramineae food crops including maize, sorghum and wheat. Pfv possesses two conserved N-acyl homoserine lactone (AHL) quorum sensing (QS) systems called PfvI/R and PfsI/R, which are repressed by RsaL and RsaM, respectively. The two systems are not hierarchically organized and are involved in plant virulence. In this study the AHL QS PfsI/R, PfvI/R and RsaM regulons were determined by transcriptomic analysis. The PfsI/R system regulates 98 genes, whereas 26 genes are regulated by the PfvI/R AHL QS system; only two genes are regulated by both systems. RsaM, on the other hand, regulates over 400 genes: 206 are negatively regulated and 260 are positively regulated. More than half of the genes controlled by the PfsI/R system and 65% by the PfvI/R system are also part of the RsaM regulon; this is due to RsaM being involved in the regulation of both systems. It is concluded that the two QS systems regulate a unique set of genes and that RsaM is a global regulator mediating the expression of different genes through the two QS systems as well as genes independently of QS.

A conserved hexanucleotide motif is important in UV-inducible promoters in Sulfolobus acidocaldarius

Thuong Ngoc Le, Alexander Wagner, Sonja-Verena Albers

ABSTRACT

Upon DNA damage, Sulfolobales exhibit a global gene regulatory response resulting in the expression of DNA transfer and repair proteins and the repression of the cell division machinery. Because the archaeal DNA damage response is still poorly understood, we investigated the promoters of the highly induced ups operon. Ups pili are involved in cellular aggregation and DNA exchange between cells. With LacS reporter gene assays we identified a conserved, non-palindromic hexanucleotide motif upstream of the ups core promoter elements to be essential for promoter activity. Substitution of this cis regulatory motif in the ups promoters resulted in abolishment of cellular aggregation and reduced DNA transfer. By screening the Sulfolobus acidocaldarius genome we identified a total of 214 genes harbouring the hexanucleotide motif in their respective promoter regions. Many of these genes were previously found to be regulated upon UV light treatment. Given the fact that the identified motif is conserved among S. acidocaldarius and Sulfolobus tokodaii promoters, we speculate that a common regulatory mechanism is present in these two species in response to DNA-damaging conditions.

Malonate degradation in Acinetobacter baylyi ADP1: operon organization and regulation by MdcR

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ABSTRACT

Transcriptional regulators in the LysR or GntR families are typically encoded in the genomic neighbourhood of bacterial genes for malonate degradation. While these arrangements have been evaluated using bioinformatics methods, experimental studies demonstrating co-transcription of predicted operons were lacking. Here, transcriptional regulation was characterized for a cluster of mdc genes that enable a soil bacterium, Acinetobacter baylyi ADP1, to use malonate as a carbon source. Despite previous assumptions that the mdc-gene set forms one operon, our studies revealed distinct promoters in two different regions of a nine-gene cluster. Furthermore, a single promoter is insufficient to account for transcription of mdcR, a regulatory gene that is convergent to other mdc genes. MdcR, a LysR-type transcriptional regulator, was shown to bind specifically to a site where it can activate mdc-gene transcription. Although mdcR deletion prevented growth on malonate, a 1 nt substitution in the promoter of mdcA enabled MdcR-independent growth on this carbon source. Regulation was characterized by methods including transcriptional fusions, quantitative reverse transcription PCR, reverse transcription PCR, 5′-rapid amplification of cDNA ends and gel shift assays. Moreover, a new technique was developed for transcriptional characterization of low-copy mRNA by increasing the DNA copy number of specific chromosomal regions. MdcR was shown to respond to malonate, in the absence of its catabolism. These studies contribute to ongoing characterization of the structure and function of a set of 44 LysR-type transcriptional regulators in A. baylyi ADP1.

Phylogenetic distribution of the euryarchaeal archaellum regulator EarA and complementation of a Methanococcus maripaludis ΔearA mutant with heterologous earA homologues

Yan Ding, Alison Berezuk, Cezar M Khursigara, Ken F Jarrell

ABSTRACT

Archaella are the swimming organelles in the Archaea. Recently, the first archaellum regulator in the Euryarchaeota, EarA Mma , was identified in Methanococcus maripaludis, one of the model organisms used for archaellum studies. EarA Mma binds to 6 bp consensus sequences upstream of the fla promoter to activate the transcription of the fla operon, which encodes most of the proteins required for archaella synthesis. In this study, synteny analysis showed that earA homologues are widely distributed in the phylum of Euryarchaeota, with the notable exception of extreme halophiles. We classified Euryarchaeota species containing earA homologues into five classes based on the genomic location of the earA genes relative to fla and chemotaxis operons. EarA homologues from Methanococcus vannielii, Methanothermococcus thermolithotrophicus and Methanocaldococcus jannaschii successfully complemented the function of EarA Mma in a ΔearAMma mutant, demonstrated by the restoration of FlaB2 expression in Western blot analysis and the appearance of archaella on the cell surface in complemented cells. Furthermore, the 6 bp consensus sequence was also found in the fla promoter region in these methanogens, indicating that the EarA homologues ly use a similar mechanism to activate transcription of the fla operons in their own hosts. Attempts to demonstrate complementation of the function of EarAMma in a ΔearAMma mutant by the EarA homologue of Pyrococcus furiosus were unsuccessful, despite the presence of a copy of the 6 bp consensus EarA-binding sequence upstream of the fla promoter in the P. furiosus genome.

Deactivation of the autotrophic sulfate assimilation pathway substantially reduces high-level β-lactam antibiotic biosynthesis and arthrospore formation in a production strain from Acremonium chrysogenum

Dominik Terfehr, Ulrich Kück

ABSTRACT

The filamentous ascomycete Acremonium chrysogenum is the only industrial producer of the β-lactam antibiotic cephalosporin C. Synthesis of all β-lactam antibiotics starts with the three amino acids l-α-aminoadipic acid, l-cysteine and l-valine condensing to form the δ-(l-α-aminoadipyl)-l-cysteinyl-d-valine tripeptide. The availability of building blocks is essential in every biosynthetic process and is therefore one of the most important parameters required for optimal biosynthetic production. Synthesis of l-cysteine is feasible by various biosynthetic pathways in all euascomycetes, and sequencing of the Acr. chrysogenum genome has shown that a full set of sulfur-metabolizing genes is present. In principle, two pathways are effective: an autotrophic one, where the sulfur atom is taken from assimilated sulfide to synthesize either l-cysteine or l-homocysteine, and a reverse transsulfuration pathway, where l-methionine is the sulfur donor. Previous research with production strains has focused on reverse transsulfuration, and concluded that both l-methionine and reverse transsulfuration are essential for high-level cephalosporin C synthesis. Here, we conducted molecular genetic analysis with A3/2, another production strain, to investigate the autotrophic pathway. Strains lacking either cysteine synthase or homocysteine synthase, enzymes of the autotrophic pathway, are still autotrophic for sulfur. However, deletion of both genes results in sulfur amino acid auxotrophic mutants exhibiting delayed biomass production and drastically reduced cephalosporin C synthesis. Furthermore, both single- and double-deletion strains are more sensitive to oxidative stress and form fewer arthrospores. Our findings provide evidence that autotrophic sulfur assimilation is essential for growth and cephalosporin C biosynthesis in production strain A3/2 from Acr. chrysogenum.

CamOptimus: a tool for exploiting complex adaptive evolution to optimize experiments and processes in biotechnology

Ayca Cankorur-Cetinkaya, Joao M. L. Dias, Jana Kludas, Nigel K. H. Slater, Juho Rousu, Stephen G. Oliver, Duygu Dikicioglu

ABSTRACT

Multiple interacting factors affect the performance of engineered biological systems in synthetic biology projects. The complexity of these biological systems means that experimental design should often be treated as a multiparametric optimization problem. However, the available methodologies are either impractical, due to a combinatorial explosion in the number of experiments to be performed, or are inaccessible to most experimentalists due to the lack of publicly available, user-friendly software. Although evolutionary algorithms may be employed as alternative approaches to optimize experimental design, the lack of simple-to-use software again restricts their use to specialist practitioners. In addition, the lack of subsidiary approaches to further investigate critical factors and their interactions prevents the full analysis and exploitation of the biotechnological system. We have addressed these problems and, here, provide a simple-to-use and freely available graphical user interface to empower a broad range of experimental biologists to employ complex evolutionary algorithms to optimize their experimental designs. Our approach exploits a Genetic Algorithm to discover the subspace containing the optimal combination of parameters, and Symbolic Regression to construct a model to evaluate the sensitivity of the experiment to each parameter under investigation. We demonstrate the utility of this method using an example in which the culture conditions for the microbial production of a bioactive human protein are optimized. CamOptimus is available through: (https://doi.org/10.17863/CAM.10257).

An endophytic Fusarium sp. isolated from Monarda citriodora produces the industrially important plant-like volatile organic compound hexanal

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ABSTRACT

An endophytic fungus, MC_25L, has been isolated from the leaves of MonardacitriodoraCerv. ex Lag., a medicinal and aromatic herb from the northwestern Himalayas. It produces a fruity fragrance while growing on potato dextrose agar, suggesting that it is producing volatile organic compounds (VOCs). The endophyte inhibited the growth of plant pathogens such asSclerotiniasp. and Aspergillusflavus by virtue of VOCs. Identification of MC_25L based on morphological and microscopic features, as well as ITS-based rDNA sequence analysis, revealed that it is a Fusariumsp. GC–MS analysis revealed that this endophyte produces a unique array of VOCs, in particular hexanal, p-fluoroanisole, pentafluoropropionic acid 2-ethylhexyl, (5E)-5-ethyl-2-methyl-5-hepten-3-one, 2-butyl-2-hexanol, (7E)-2-methyl-7-hexadecene and acoradiene. Three major compounds were hexanal, (5E)-5-ethyl-2-methyl-5-hepten-3-one and acoradiene, and they account for around 84.57% of the total VOCs. Moreover, of interest was the presence of hexanal, which has applications in the food and cosmetic industries, as well as in mycofumigation. This is the first report of a fungal endophyte producing the industrially important plant-like VOC hexanal. Hexanal is also active biologically. Thus this study indicates that Fusariumsp. (MC_25L) is a potential candidate for the up-scaling of hexanal.

Fine structure analysis of lipopolysaccharides in bacteriophage-resistant Pseudomonas aeruginosa PAO1 mutants

Libera Latino, Martine Caroff, Christine Pourcel

ABSTRACT

Pseudomonas aeruginosa lipopolysaccharides (LPS) serve as primary receptors for many bacteriophages and, consequently, their biosynthesis is frequently affected in phage-resistant mutants. We previously isolated phage-resistant PAO1 mutants using three different phages, and showed that they were affected in the synthesis of LPS. Here we have investigated in detail the effect of mutations in seven genes involved in different steps of the production of core and oligosaccharide chains. The band profile of purified LPS was analysed by PAGE, and we further characterized the O-chains and core structures by MALDI mass spectrometry (MS). Mild LPS extraction conditions and native LPS MS analyses helped unveil lipid A molecular species with three phosphate residues in the close vicinity of the already highly charged inner-core region. No other MS direct analysis has allowed this peculiarity to be demonstrated for native lipid A high-molecular-weight molecular species, in normal growth conditions and without involving separation techniques. The present results shed light on the possible interactions between the phages and the LPS structures in the early phase of infection.

A chromosome 4 trisomy contributes to increased fluconazole resistance in a clinical isolate of Candida albicans

Matthew Z. Anderson, Amrita Saha, Abid Haseeb, Richard J. Bennett

ABSTRACT

Candida albicans is an important opportunistic fungal pathogen capable of causing both mucosal and disseminated disease. Infections are often treated with fluconazole, a front-line antifungal drug that targets the biosynthesis of ergosterol, a major component of the fungal cell membrane. Resistance to fluconazole can arise through a variety of mechanisms, including gain-of-function mutations, loss of heterozygosity events and aneuploidy. The clinical isolate P60002 was found to be highly resistant to azole-class drugs, yet lacked mutations or chromosomal rearrangements known to be associated with azole resistance. Transcription profiling suggested that increased expression of two putative drug efflux pumps, CDR11 and QDR1, might confer azole resistance. However, ectopic expression of the P60002 alleles of these genes in a drug-susceptible strain did not increase fluconazole resistance. We next examined whether the presence of three copies of chromosome 4 (Chr4) or chromosome 6 (Chr6) contributed to azole resistance in P60002. We established that Chr4 trisomy contributes significantly to fluconazole resistance, whereas Chr6 trisomy has no discernible effect on resistance. In contrast, a Chr4 trisomy did not increase fluconazole resistance when present in the standard SC5314 strain background. These results establish a link between Chr4 trisomy and elevated fluconazole resistance, and demonstrate the impact of genetic background on drug resistance phenotypes in C. albicans.

Glucose consumption in carbohydrate mixtures by phosphotransferase-system mutants of Escherichia coli

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ABSTRACT

Escherichia coli lacking the glucose phosphotransferase system (PTS), mannose PTS and glucokinase are supposedly unable to grow on glucose as the sole carbon source (Curtis SJ, Epstein W. J Bacteriol 1975;122:1189–1199). We report that W ptsG manZ glk (ALS1406) grows slowly on glucose in media containing glucose with a second carbon source: ALS1406 metabolizes glucose after that other carbon source, including arabinose, fructose, glycerol, succinate or xylose, is exhausted. Galactose is an exception to this rule, as ALS1406 simultaneously consumes both galactose and glucose. The ability of ALS1406 to metabolize glucose in a xylose–glucose mixture was unchanged by an additional knockout in any single gene involved in carbohydrate transport and utilization, including agp (periplasmic glucose-1-phosphatase), galP (galactose permease), xylA (xylose isomerase), alsK (allose kinase), crr (glucose PTS enzyme IIA), galK (galactose kinase), mak (mannokinase), malE (maltose transporter), malX (maltose PTS enzyme IIBC), mglB (methyl-galactose transporter subunit), nagE (N-acetyl glucosamine PTS enzyme IICBA), nanK (N-acetyl mannosamine kinase) or pgm (phosphoglucose mutase). Glucose metabolism was only blocked by the deletion of two metabolic genes, pgi (phosphoglucose isomerase) and zwf (glucose-6-phosphate 1-dehydrogenase), which prevents the entry of glucose-6-phosphate into the pentose phosphate and Embden–Meyerhof–Parnas pathways. Carbon-limited steady-state studies demonstrated that xylose must be sub-saturating for glucose to be metabolized, while nitrogen-limited studies showed that xylose is partly converted to glucose when xylose is in excess. Under transient conditions, ALS1406 converts almost 25% (mass) xylose into glucose as a result of reversible transketolase and transaldolase and the re-entry of carbon into the pentose phosphate pathway via glucose-6-phosphate 1-dehydrogenase.

Differential effects of isc operon mutations on the biosynthesis and activity of key anaerobic metalloenzymes in Escherichia coli

Monique Jaroschinsky, Constanze Pinske, R. Gary Sawers

ABSTRACT

Escherichia coli has two machineries for the synthesis of FeS clusters, namely Isc (iron–sulfur cluster) and Suf (sulfur formation). The Isc machinery, encoded by the iscRSUA-hscBA-fdx-iscXoperon, plays a crucial role in the biogenesis of FeS clusters for the oxidoreductases of aerobic metabolism. Less is known, however, about the role of ISC in the maturation of key multi-subunit metalloenzymes of anaerobic metabolism. Here, we determined the contribution of each iscoperon gene product towards the functionality of the major anaerobic oxidoreductases in E. coli, including three [NiFe]-hydrogenases (Hyd), two respiratory formate dehydrogenases (FDH) and nitrate reductase (NAR). Mutants lacking the cysteine desulfurase, IscS, lacked activity of all six enzymes, as well as the activity of fumaratereductase, and this was due to deficiencies in enzyme biosynthesis, maturation or FeS cluster insertion into electron-transfer components. Notably, based on anaerobic growth characteristics and metabolite patterns, the activity of the radical-S-adenosylmethionine enzyme pyruvate formate-lyase activase was independent of IscS, suggesting that FeS biogenesis for this ancient enzyme has different requirements. Mutants lacking either the scaffold protein IscU, the ferredoxin Fdx or the chaperones HscA or HscB had similar enzyme phenotypes: five of the oxidoreductases were essentially inactive, with the exception being the Hyd-3 enzyme, which formed part of the H2-producing formate hydrogenlyase (FHL) complex. Neither the frataxin-homologue CyaY nor the IscX protein was essential for synthesis of the three Hyd enzymes. Thus, while IscS is essential for H2 production in E. coli, the other ISC components are non-essential.

Diversity of the auxotrophic requirements in natural isolates of Escherichia coli

Odile Bouvet, Emmanuelle Bourdelier, Jeremy Glodt, Olivier Clermont, Erick Denamur

ABSTRACT

Isolates of Escherichia coli, except Shigella, are generally prototrophic; they do not require any growth factors to grow in mineral medium. However, a nicotinic acid requirement is common among B2 phylogroup STc95 O18 E. coli clone strains. Nicotinic acid is a precursor of nicotinamide adenine dinucleotide (NAD), an essential molecule that plays central role in cellular metabolism. The defect in NAD synthesis of these strains is due to alterations in de novo biosynthesis pathway nadB gene. Here, by studying growth on minimal medium with glycolytic (glucose) or gluconeogenic (pyruvate or succinate) substrates as the carbon supply in a large panel of E. coli natural isolates representative of the species diversity, we identify an absolute nicotinic acid requirement in non-STc95 strains due in one case to a nadA inactivation. The growth on glucose medium of some extraintestinal pathogenic E. coli strains belonging to various non-O18 B2 phylogroup STc95 clones is restored either by aspartate or nicotinate, demonstrating that the nicotinic acid requirement can also be due to intracellular aspartate depletion. The auxotrophic requirements depend on the carbon source available in the environment. Moreover, some strains prototrophic in glucose medium become auxotrophic in succinate medium, and conversely, some strainsauxotrophic in glucose medium become prototrophic in succinate medium. Finally, a partial depletion of intracellular aspartate can be observed in some prototrophic strains belonging to various phylogroups. The observed more or less significant depletion according to isolates may be due to differences in tricarboxylic acid cycle enzyme activities. These metabolic defects could be involved in the adaptation of E. coli to its various niches.

DksA-HapR-RpoS axis regulates haemagglutinin protease production in Vibrio cholerae

Pallabi Basu, Ritesh Ranjan Pal, Shreya Dasgupta, Rupak K. Bhadra

ABSTRACT

DksA acts as a co-factor for the intracellular small signalling molecule ppGpp during the stringent response. We recently reported that the expression of the haemagglutinin protease (HAP), which is needed for shedding of the cholera pathogen Vibrio cholerae during the late phase of infection, is significantly downregulated in V. cholerae ΔdksA mutant (ΔdksAVc) cells. So far, it has been shown that HAP production by V. cholerae cells is critically regulated by HapR and also by RpoS. Here, we provide evidence that V. cholerae DksA (DksAVc) positively regulates HapR at both the transcriptional and post-transcriptional levels. We show that in ΔdksAVc cells the CsrB/C/D sRNAs, required for the maintenance of intracellular levels of hapR transcripts during the stationary growth, are distinctly downregulated. Moreover, the expression of exponential phase regulatory protein Fis, a known negative regulator of HapR, was found to continue even during the stationary phase in ΔdksAVc cells compared to that of wild-type strain, suggesting another layer of complex regulation of HapR by DksAVc. Extensive reporter construct-based and quantitative reverse-transcriptase PCR (qRT-PCR) analyses supported that RpoS is distinctly downregulated at the post-transcriptional/translational levels in stationary phase-grown ΔdksAVc cells. Since HAP expression through HapR and RpoS is stationary phase-specific in V. cholerae, it appears that DksAVc is also a critical stationary phase regulator for fine tuning of the expression of HAP. Moreover, experimental evidence provided in this study clearly supports that DksAVc is sitting at the top of the hierarchy of regulation of expression of HAP in V. cholerae.

Phage exposure causes dynamic shifts in the expression states of specific phase-variable genes of Campylobacter jejuni

Jack Aidley, Martine C. Holst Sørensen, Christopher D. Bayliss, Lone Brøndsted

ABSTRACT

Phase variation (PV) creates phenotypic heterogeneity at high frequencies and in a reversible manner. This phenomenon allows bacteria to adapt to a variety of different environments and selective pressures. In Campylobacter jejuni this reversible adaptive process is mediated by mutations in homopolymeric G/C tracts. Many C. jejuni-specific phages are dependent on phase-variable surface structures for successful infection. We previously identified the capsular polysaccharide (CPS) moiety, MeOPN-GalfNAc, as a receptor for phage F336 and showed that phase-variable expression of the transferase for this CPS modification, cj1421, and two other phase-variable CPS genes generated phage resistance in C. jejuni. Here we investigate the population dynamics of C. jejuni NCTC11168 when exposed to phage F336 in vitro using a newly described method – the 28-locus-CJ11168 PV analysis. Dynamic switching was observed in the ON/OFF states of three phase-variable CPS genes, cj1421, cj1422 and cj1426, during phage F336 exposure, with the dominant phage-resistant phasotype differing between cultures. Although loss of the phage receptor was predominately observed, several other PV events also led to phage resistance, a phenomenon that increases the chance of phage-resistant subpopulations being present in any growing culture. No other PV genes were affected and exposure to phage F336 resulted in a highly specific response, only selecting for phase variants of cj1421, cj1422 and cj1426. In summary, C. jejuni may benefit from modification of the surface in multiple ways to inhibit or reduce phage binding, thereby ensuring the survival of the population when exposed to phages.

The protein Slr1143 is an active diguanylate cyclase in Synechocystis sp. PCC 6803 and interacts with the photoreceptor Cph2

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ABSTRACT

Cyclic-di-GMP is an ubiquitous second messenger in bacteria. Several c-di-GMP receptor proteins have been identified to date, and downstream signalling pathways are often mediated through protein–protein interactions. The photoreceptor Cph2 from the cyanobacterium Synechocystis sp. PCC 6803 comprises three domains related to c-di-GMP metabolism: two GGDEF and one EAL domain. It has been shown that the C-terminal GGDEF domain acts as blue-light triggered c-di-GMP producer thereby inhibiting motility of the cells in blue light. The specific function of the other two c-di-GMP related domains remained unclear. In this study, we test knockout mutants of potential interaction partners of Cph2 for altered phototactic behaviour. Whereas wild-type cells are non-motile under high-intensity red light of 640 nm, the mutant Δslr1143 displays positive phototaxis. This phenotype can be complemented by overexpression of full-length Slr1143, which also results in an increased cellular c-di-GMP concentration. However, the non-motile phenotype of wild-type cells under high-intensity red light appears not to be due to an elevated cellular c-di-GMP content. Using co-precipitation and yeast two-hybrid assays, we demonstrate that the GGDEF domain of Slr1143 interacts with the EAL and the GGDEF domains of Cph2. However, under the test conditions, the interaction of the two proteins is not light-dependent. We conclude that Slr1143 is a new Cph2-interacting regulatory factor which modulates motility under red light and accordingly we propose Cip1 (Cph2-interacting protein 1) as a new designation for this gene product.

Volume 163, Issue 7, July 2017

Amphotericin B induces apoptosis-like programmed cell death in Naegleria fowleri and Naegleria gruberi

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ABSTRACT

Naegleria fowleri and Naegleria gruberi belong to the free-living amoebae group. It is widely known that the non-pathogenic species N. gruberi is usually employed as a model to describe molecular pathways in this genus, mainly because its genome has been recently described. However, N. fowleri is an aetiological agent of primary amoebic meningoencephalitis, an acute and fatal disease. Currently, the most widely used drug for its treatment is amphotericin B (AmB). It was previously reported that AmB has an amoebicidal effect in both N. fowleri and N. gruberi trophozoites by inducing morphological changes that resemble programmed cell death (PCD). PCD is a mechanism that activates morphological, biochemical and genetic changes. However, PCD has not yet been characterized in the genus Naegleria. The aim of the present work was to evaluate the typical markers to describe PCD in both amoebae. These results showed that treated trophozoites displayed several parameters of apoptosis-like PCD in both species. We observed ultrastructural changes, an increase in reactive oxygen species, phosphatidylserine externalization and a decrease in intracellular potassium, while DNA degradation was evaluated using the TUNEL assay and agarose gels, and all of these parameters are related to PCD. Finally, we analysed the expression of apoptosis-related genes, such as sir2 and atg8, in N. gruberi. Taken together, our results showed that AmB induces the morphological, biochemical and genetic changes of apoptosis-like PCD in the genus Naegleria.

Effective identification of Lactobacillus casei group species: genome-based selection of the gene mutL as the target of a novel multiplex PCR assay

Benedetta Bottari, Giovanna E. Felis, Elisa Salvetti, Anna Castioni, Ilenia Campedelli, Sandra Torriani, Valentina Bernini, Monica Gatti

ABSTRACT

Lactobacillus casei, Lactobacillus paracasei and Lactobacillus rhamnosus form a closely related taxonomic group (the L. casei group) within the facultatively heterofermentative lactobacilli. Strains of these species have been used for a long time as probiotics in a wide range of products, and they represent the dominant species of nonstarter lactic acid bacteria in ripened cheeses, where they contribute to flavour development. The close genetic relationship among those species, as well as the similarity of biochemical properties of the strains, hinders the development of an adequate selective method to identify these bacteria. Despite this being a hot topic, as demonstrated by the large amount of literature about it, the results of different proposed identification methods are often ambiguous and unsatisfactory. The aim of this study was to develop a more robust species-specific identification assay for differentiating the species of the L. casei group. A taxonomy-driven comparative genomic analysis was carried out to select the potential target genes whose similarity could better reflect genome-wide diversity. The gene mutL appeared to be the most promising one and, therefore, a novel species-specific multiplex PCR assay was developed to rapidly and effectively distinguish L. casei, L. paracasei and L. rhamnosus strains. The analysis of a collection of 76 wild dairy isolates, previously identified as members of the L. casei group combining the results of multiple approaches, revealed that the novel designed primers, especially in combination with already existing ones, were able to improve the discrimination power at the species level and reveal previously undiscovered intraspecific biodiversity.

Mutational analysis of the MS2 lysis protein L

Karthik R. Chamakura, Garrett B. Edwards, Ry Young

ABSTRACT

Small single-stranded nucleic acid phages effect lysis by expressing a single protein, the amurin, lacking muralytic enzymatic activity. Three amurins have been shown to act like ‘protein antibiotics’ by inhibiting cell-wall biosynthesis. However, the L lysis protein of the canonical ssRNA phage MS2, a 75 aa polypeptide, causes lysis by an unknown mechanism without affecting net peptidoglycan synthesis. To identify residues important for lytic function, randomly mutagenized alleles of L were generated, cloned into an inducible plasmid and the transformants were selected on agar containing the inducer. From a total of 396 clones, 67 were unique single base-pair changes that rendered L non-functional, of which 44 were missense mutants and 23 were nonsense mutants. Most of the non-functional missense alleles that accumulated in levels comparable to the wild-type allele are localized in the C-terminal half of L, clustered in and around an LS dipeptide sequence. The LS motif was used to align L genes from ssRNA phages lacking any sequence similarity to MS2 or to each other. This alignment revealed a conserved domain structure, in terms of charge, hydrophobic character and predicted helical content. None of the missense mutants affected membrane-association of L. Several of the L mutations in the central domains were highly conservative and recessive, suggesting a defect in a heterotypic protein–protein interaction, rather than in direct disruption of the bilayer structure, as had been previously proposed for L.

Proline utilization system is required for infection by the pathogenic α-proteobacterium Brucella abortus

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ABSTRACT

Proline utilization (Put) systems have been described in a number of bacteria; however, the importance and functionality of the Put system in the intracellular pathogen Brucellaabortus has not been explored. Generally, bacterial Put systems are composed of the bifunctional enzyme proline dehydrogenase PutA and its transcriptional activator PutR. Here, we demonstrate that the genes putA (bab2_0518) and putR (bab2_0517) are critical for the chronic infection of mice by B. abortus, but putA and putR are not required for the survival and replication of the bacteria in naive macrophages. Additionally, in vitro experiments revealed that putR is necessary for the ability of the bacteria to withstand oxidative stress, as a ΔputR deletion strain is hypersensitive to hydrogen peroxide exposure. Quantitative reverse transcription-PCR and putA-lacZ transcriptional reporter studies revealed that PutR acts as a transcriptional activator of putA in Brucella, and electrophoretic mobility shift assays confirmed that PutR binds directly to the putA promoter region. Biochemical analyses demonstrated that a purified recombinant B. abortus PutA protein possesses quintessential proline dehydrogenase activity, as PutA is capable of catalysing the conversion of proline to glutamate. Altogether, these data are the first to reveal that the Put system plays a significant role in the ability of B. abortus to replicate and survive within its host, as well as to describe the genetic regulation and biochemical activity of the Put system in Brucella.

Metarhizium robertsii produces indole-3-acetic acid, which promotes root growth in Arabidopsis and enhances virulence to insects

Xinggang Liao, Brian Lovett, Weiguo Fang, Raymond J. St Leger

ABSTRACT

The plant root colonizing insect-pathogenic fungus Metarhizium robertsii has been shown to boost plant growth, but little is known about the responsible mechanisms. Here we show that M. robertsii promotes lateral root growth and root hair development of Arabidopsis seedlings in part through an auxin [indole-3-acetic acid (IAA)]-dependent mechanism. M. robertsii, or its auxin-containing culture filtrate promoted root proliferation, activated IAA-regulated gene expression and rescued the root hair defect of the IAA-deficient rhd6 Arabidopsis mutant. Substrate feeding assays suggest that M. robertsii possesses tryptamine (TAM) and indole-3-acetamide tryptophan (Trp)-dependent auxin biosynthetic pathways. Deletion of Mrtdc impaired M. robertsii IAA production by blocking conversion of Trp to TAM but the reduction was not sufficient to affect plant growth enhancement. We also show that M. robertsii secretes IAA on insect cuticle. ΔMrtdc produced fewer infection structures and was less virulent to insects than the wild-type, whereas M. robertsii spores harvested from culture media containing IAA were more virulent. Furthermore, exogenous application of IAA increased appressorial formation and virulence. Together, these results suggest that auxins play an important role in the ability of M. robertsii to promote plant growth, and the endogenous pathways for IAA production may also be involved in regulating entomopathogenicity. Auxins were also produced by other Metarhizium species and the endophytic insect pathogen Beauveria bassiana suggesting that interplay between plant- and fungal-derived auxins has important implications for plant–microbe–insect interactions.

Ralstonia solanacearum novel E3 ubiquitin ligase (NEL) effectors RipAW and RipAR suppress pattern-triggered immunity in plants

Masahito Nakano, Kenji Oda, Takafumi Mukaihara

ABSTRACT

Ralstonia solanacearum is the causal agent of bacterial wilt in solanaceous crops. This pathogen injects more than 70 effector proteins into host plant cells via the Hrp type III secretion system to cause a successful infection. However, the function of these effectors in plant cells, especially in the suppression of plant immunity, remains largely unknown. In this study, we characterized two Ralstonia solanacearum effectors, RipAW and RipAR, which share homology with the IpaH family of effectors from animal and plant pathogenic bacteria, that have a novel E3 ubiquitin ligase (NEL) domain. Recombinant RipAW and RipAR show E3 ubiquitin ligase activity in vitro. RipAW and RipAR localized to the cytoplasm of plant cells and significantly suppressed pattern-triggered immunity (PTI) responses such as the production of reactive oxygen species and the expression of defence-related genes when expressed in leaves of Nicotiana benthamiana. Mutation in the conserved cysteine residue in the NEL domain of RipAW completely abolished the E3 ubiquitin ligase activity in vitro and the ability to suppress PTI responses in plant leaves. These results indicate that RipAW suppresses plant PTI responses through the E3 ubiquitin ligase activity. Unlike other members of the IpaH family of effectors, RipAW and RipAR had no leucine-rich repeat motifs in their amino acid sequences. A conserved C-terminal region of RipAW is indispensable for PTI suppression. Transgenic Arabidopsis plants expressing RipAW and RipAR showed increased disease susceptibility, suggesting that RipAW and RipAR contribute to bacterial virulence in plants.

Characterization of Vsr endonucleases from Neisseria meningitidis

Milena Bażlekowa, Monika Adamczyk-Popławska, Agnieszka Kwiatek

ABSTRACT

DNA methylation is a common modification occurring in all living organisms. 5-methylcytosine, which is produced in a reaction catalysed by C5-methyltransferases, can spontaneously undergo deamination to thymine, leading to the formation of T:G mismatches and C→T transitions. In Escherichia coli K-12, such mismatches are corrected by the Very Short Patch (VSP) repair system, with Vsr endonuclease as the key enzyme. Neisseria meningitidis possesses genes that encode DNA methyltransferases, including C5-methyltransferases. We report on the mutagenic potential of the meningococcal C5-methyltransferases M.NmeDI and M.NmeAI resulting from deamination of 5-methylcytosine. N. meningitidis strains also possess genes encoding potential Vsr endonucleases. Phylogenetic analysis of meningococcal Vsr endonucleases indicates that they belong to two phylogenetically distinct groups (type I or type II Vsr endonucleases). N. meningitidis serogroup C (FAM18) is a representative of meningococcal strains that carry two Vsr endonuclease genes (V.Nme18IIP and V.Nme18VIP). The V.Nme18VIP (type II) endonuclease cut DNA containing T:G mismatches in all tested nucleotide contexts. V.Nme18IIP (type I) is not active in vitro, but the change of Tyr69 to His69 in the amino acid sequence of the protein restores its endonucleolytic activity. The presence of tyrosine in position 69 is a characteristic feature of type I meningococcal Vsr proteins, while type II Vsr endonucleases possess His69. In addition to the T:G mismatches, V.Nme18VIP and V.Nme18IIPY69H recognize and digest DNA with T:T or U:G mispairs. Thus, for the first time, we demonstrate that the VSP repair system may have a wider significance and broader substrate specificity than DNA lesions that only result from 5-methylcytosine deamination.

DprA from Neisseria meningitidis: properties and role in natural competence for transformation

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ABSTRACT

DNA processing chain A (DprA) is a DNA-binding protein that is ubiquitous in bacteria and expressed in some archaea. DprA is active in many bacterial species that are competent for transformation of DNA, but its role in Neisseriameningitidis (Nm) is not well characterized. An Nm mutant lacking DprA was constructed, and the phenotypes of the wild-type and ΔdprA mutant were compared. The salient feature of the phenotype of dprA null cells is the total lack of competence for genetic transformation shown by all of the donor DNA substrates tested in this study. Here, Nm wild-type and dprA null cells appeared to be equally resistant to genotoxic stress. The gene encoding DprANm was cloned and overexpressed, and the biological activities of DprANm were further investigated. DprANm binds ssDNA more strongly than dsDNA, but lacks DNA uptake sequence-specific DNA binding. DprANm dimerization and interaction with the C-terminal part of the single-stranded binding protein SSBNmwere demonstrated. dprA is co-expressed with smg, a downstream gene of unknown function, and the gene encoding topoisomerase 1, topA.

Molecular and biochemical characteristics of the inulosucrase HugO from Streptomyces viridochromogenes DSM40736 (Tü494)

Hans-Jörg Frasch, Sander S. van Leeuwen, Lubbert Dijkhuizen

ABSTRACT

Polyfructans are synthesized from sucrose by plants (mostly inulin) and by both Gram-negative and Gram-positive bacteria (mostly levan). In the phylum Actinobacteria only levan synthesis by Actinomyces species has been reported. We have identified a putative fructansucrase gene (hugO) in Streptomyces viridochromogenes DSM40736 (Tü494). HugO was heterologously expressed and biochemically characterized. HPSEC-MALLS and 2D-1H-13C nuclear magnetic resonance (NMR) spectroscopy analysis showed that the fructan polymer produced in vitro has an Molecular Weight of 2.5*107 Da and is an inulin that is mainly composed of (β2–1)-linked fructose units. This is the first report of a fructansucrase from Streptomyces and an inulosucrase from Actinobacteria. Database searches showed that fructansucrases clearly occur more widely in streptomycetes. Analysis of the active site of HugO and other actinobacterial Gram-positive fructansucrases revealed that their +1 substrate-binding sites are conserved, but are most similar to those in Gram-negative fructansucrases. HugO also resembles Gram-negative fructansucrases in not requiring calcium ions for activity. The origin and properties of HugO and other actinobacterial fructansucrases thus clearly differ from those of previously characterized Gram-positive fructansucrases.

Cold-stress response during the stationary-growth phase of Antarctic and temperate-climate Penicillium strains

Jeni G. Miteva-Staleva, Ekaterina T. Krumova, Spassen V. Vassilev, Maria B. Angelova

ABSTRACT

Cold-induced oxidative stress during the aging of three Penicillium strains (two Antarctic and one from a temperate region) in stationary culture was documented and demonstrated a significant increase in the protein carbonyl content, the accumulation of glycogen and trehalose, and an increase in the activities of antioxidant enzymes (superoxide dismutase and catalase). The cell response to a temperature downshift depends on the degree of stress and the temperature characteristics of the strains. Our data give further support for the role of oxidative stress in the aging of fungi in stationary cultures. Comparing the present results for the stationary growth phase with our previous results for the exponential growth phase was informative concerning the relationship between the cold-stress response and age-related changes in the tested strains. Unlike the young cells, stationary-phase cultures demonstrated a more pronounced level of oxidative damage, as well as decreased antioxidant defence.

Genomic and physiological characterization of a laboratory-isolated Acinetobacter schindleri ACE strain that quickly and efficiently catabolizes acetate

Juan-Carlos Sigala, Brisa Paola Suárez, Alvaro R. Lara, Sylvie Le Borgne, Patricia Bustos, Rosa Isela Santamaría, Víctor González, Alfredo Martinez

ABSTRACT

An Acinetobacter strain, designated ACE, was isolated in the laboratory. Phylogenetic tests and average nucleotide identity value comparisons suggested that ACE belongs to the species Acinetobacter schindleri. We report for the first time the complete genome sequence of an A. schindleri strain, which consists of a single circular chromosome of 3 001 209 bp with an overall DNA G+C content of 42.9 mol% and six plasmids that account for 266 844 bp of extrachromosomal material. The presence or absence of genes related to carbon catabolism and antibiotic resistance were in agreement with the phenotypic characterization of ACE. This strain grew faster and with a higher biomass yield on acetate than the reference strain Acinetobacter baylyi ADP1. However, ACE did not use aromatic compounds and was unable to grow on common carbon sources, such as glucose, xylose, glycerol or citrate. The gluconeogenic and the catechol pathways are complete in ACE, but compounds that are converted to protocatechuate did not sustain growth since some genes of this pathway are missing. Likewise, this strain could not grow on glucose because it lacks the genes of the Entner–Doudoroff pathway. Minimal inhibitory concentration data showed that ACE was susceptible to most of the antimicrobial agents recommended for the clinical treatment of Acinetobacter spp. Some genes related to a possible human–microbe interaction were found in the ACE genome. ACE is likely to have a low pathogenic risk, as is the case with other A. schindleri strains. These results provide a valuable reference for broadening the knowledge of the biology of Acinetobacter.

Mutations in MmpL3 alter membrane potential, hydrophobicity and antibiotic susceptibility in Mycobacterium smegmatis

Matthew B. McNeil, Devon Dennison, Tanya Parish

ABSTRACT

MmpL3 is a promising target for novel anti-tubercular agents, with numerous compound series identified as MmpL3 inhibitors. Despite this, there is an incomplete understanding of MmpL3 function. Here we show that Mycobacterium smegmatis MmpL3 mutant strains had an altered cell wall hydrophobicity, disrupted membrane potential and growth defects in liquid media. Compensatory mutations that restored normal growth also returned membrane potential to wild-type. M. smegmatis MmpL3 mutant strains were resistant to two anti-tubercular agents, SQ109 and AU1235, but were more sensitive to rifampicin, erythromycin and ampicillin. Exposure of M. smegmatis to AU1235 affected the cell wall composition and increased the potency of rifampicin. However, MmpL3 mutants did not prevent the dissipation of membrane potential following exposure to SQ109. These results demonstrate that in M. smegmatis, MmpL3 contributes to a number of important phenotypes such as membrane potential, cell wall composition, antibiotic susceptibility and fitness.

Role of the inner-membrane histidine kinase RcsC and outer-membrane lipoprotein RcsF in the activation of the Rcs phosphorelay signal transduction system in Escherichia coli

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ABSTRACT

The Rcs phosphorelay signal transduction system of Escherichia coli controls genes for capsule production and many other envelope-related functions and is implicated in biofilm formation. The outer-membrane lipoprotein RcsF is an essential component of the Rcs system. Mislocalization of RcsF to the periplasm or the cytoplasmic membrane leads to high activation of the Rcs system, suggesting that RcsF functions by interacting with the cytoplasmic membrane component(s) of the system in activating the system. This is consistent with the result reported by Cho et al. (Cell 159, 1652–1664, 2014) showing that RcsF interacts with the periplasmic domain (YrfFperi) of the inner-membrane protein YrfF (IgaA in Salmonella enterica serovar Typhimurium), which is a negative regulator of the Rcs system. In this study we show that RcsF also interacts with the periplasmic domain of the innermembrane-localized histidine kinase RcsC (RcsCperi). RcsCperi, which was secreted to the periplasm by fusion to maltose-binding protein, titrated RcsF's activating effect. A bimolecular fluorescence complementation experiment showed interaction of RcsF with RcsCperi, as well as with YrfFperi. We conclude that RcsF interacts with the periplasmically exposed region of RcsC, as well as with that of YrfF.

Post-transcriptional regulation of target genes by the sRNA FnrS in Neisseria gonorrhoeae

Pooja Tanwer, Susanne Bauer, Elisabeth Heinrichs, Gurudutta Panda, Daman Saluja, Thomas Rudel, Dagmar Beier

ABSTRACT

Small non-coding RNAs (sRNAs) are well-established post-transcriptional regulators of gene expression in bacteria that respond to a variety of environmental stimuli. They usually act by base-pairing with their target mRNAs, which is commonly facilitated by the RNA chaperone Hfq. In this study we initiated the analysis of the sRNA FnrS of Neisseria gonorrhoeae, which is induced under anaerobic conditions. We identified four putative FnrS target genes using bioinformatics approaches and validated these target genes using translational reporter gene fusions in both Escherichia coli and N. gonorrhoeae, thereby demonstrating their downregulation by direct base-pairing between the respective mRNA and FnrS. We demonstrate deregulation of target mRNAs upon deletion of fnrS and provide evidence that the isc gene cluster required for iron–sulfur cluster biosynthesis, which harbours iscS, which is a direct target of FnrS, is coordinately downregulated by the sRNA. By mutational analysis we show that, surprisingly, three distinct regions of FnrS are employed for interaction with different target genes.

D-methionine interferes with non-typeable Haemophilus influenzae peptidoglycan synthesis during growth and biofilm formation

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ABSTRACT

Non-typeable Haemophilus influenzae (NTHi) is an opportunistic pathogen that plays a major role in a number of respiratory tract infections, including otitis media, cystic fibrosis and chronic obstructive pulmonary disease. Biofilm formation has been implicated in both NTHi colonization and disease, and is responsible for the increased tolerance of this pathogen towards antibiotic treatment. Targeting metabolic pathways that are important in NTHi biofilm formation represents a potential strategy to combat this antibiotic recalcitrance. A previous investigation demonstrated increased expression of a putative d-methionine uptake protein following exposure of NTHi biofilms to the ubiquitous signalling molecule, nitric oxide. We therefore hypothesized that treatment with exogenous d-methionine would impact on NTHi biofilm formation and increase antibiotic sensitivity. Treatment of NTHi during the process of biofilm formation resulted in a reduction in biofilm viability, increased biomass, changes in the overall biofilm architecture and the adoption of an amorphous cellular morphology. Quantitative proteomic analyses identified 124 proteins that were differentially expressed following d-methionine treatment, of which 51 (41%) were involved in metabolic and transport processes. Nine proteins involved in peptidoglycan synthesis and cell division showed significantly increased expression. Furthermore, d-methionine treatment augmented the efficacy of azithromycin treatment and highlighted the potential of d-methionine as an adjunctive therapeutic approach for NTHi biofilm-associated infections.

Two-component system CbrA/CbrB controls alginate production in Azotobacter vinelandii

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ABSTRACT

Azotobacter vinelandii, belonging to the Pseudomonadaceae family, is a free-living bacterium that has been considered to be a good source for the production of bacterial polymers such as alginate. In A. vinelandii the synthesis of this polymer is regulated by the Gac/Rsm post-transcriptional regulatory system, in which the RsmA protein binds to the mRNA of the biosynthetic algD gene, inhibiting translation. In several Pseudomonas spp. the two-component system CbrA/CbrB has been described to control a variety of metabolic and behavioural traits needed for adaptation to changing environmental conditions. In this work, we show that the A. vinelandii CbrA/CbrB two-component system negatively affects alginate synthesis, a function that has not been described in Pseudomonas aeruginosa or any other Pseudomonas species. CbrA/CbrB was found to control the expression of some alginate biosynthetic genes, mainly algD translation. In agreement with this result, the CbrA/CbrB system was necessary for optimal rsmA expression levels. CbrA/CbrB was also required for maximum accumulation of the sigma factor RpoS. This last effect could explain the positive effect of CbrA/CbrB on rsmA expression, as we also showed that one of the promoters driving rsmA transcription was RpoS-dependent. However, although inactivation of rpoS increased alginate production by almost 100%, a cbrA mutation increased the synthesis of this polymer by up to 500%, implying the existence of additional CbrA/CbrB regulatory pathways for the control of alginate production. The control exerted by CbrA/CbrB on the expression of the RsmA protein indicates the central role of this system in regulating carbon metabolism in A. vinelandii.

Systems and synthetic biology perspective of the versatile plant-pathogenic and polysaccharide-producing bacterium Xanthomonas campestris

Sarah Schatschneider, Jessica Schneider, Jochen Blom, Fabien Létisse, Karsten Niehaus, Alexander Goesmann, Frank-Jörg Vorhölter

ABSTRACT

Bacteria of the genus Xanthomonas are a major group of plant pathogens. They are hazardous to important crops and closely related to human pathogens. Being collectively a major focus of molecular phytopathology, an increasing number of diverse and intricate mechanisms are emerging by which they communicate, interfere with host signalling and keep competition at bay. Interestingly, they are also biotechnologically relevant polysaccharide producers. Systems biotechnology techniques have revealed their central metabolism and a growing number of remarkable features. Traditional analyses of Xanthomonas metabolism missed the Embden–Meyerhof–Parnas pathway (glycolysis) as being a route by which energy and molecular building blocks are derived from glucose. As a consequence of the emerging full picture of their metabolism process, xanthomonads were discovered to have three alternative catabolic pathways and they use an unusual and reversible phosphofructokinase as a key enzyme. In this review, we summarize the synthetic and systems biology methods and the bioinformatics tools applied to reconstruct their metabolic network and reveal the dynamic fluxes within their complex carbohydrate metabolism. This is based on insights from omics disciplines; in particular, genomics, transcriptomics, proteomics and metabolomics. Analysis of high-throughput omics data facilitates the reconstruction of organism-specific large- and genome-scale metabolic networks. Reconstructed metabolic networks are fundamental to the formulation of metabolic models that facilitate the simulation of actual metabolic activities under specific environmental conditions.

Microbe Profile: Candida albicans: a shape-changing, opportunistic pathogenic fungus of humans

Neil A. R. Gow, Bhawna Yadav

ABSTRACT

Candida albicans is normally a harmless commensal of human beings, but it can cause superficial infections of the mucosa (oral/vaginal thrush) in healthy individuals and (rarely) infections of the skin or nails. It can also become invasive, causing life-threatening systemic and bloodstream infections in immunocompromised hosts, where the mortality rate can be as high as 50%. It is the most common cause of serious fungal infection and is a common cause of nosocomial infections in hospitals. Some strains have been recognized that are resistant to azoles or echinocandins, which are the first-line antifungals for treatment of C. albicans infections.

Development of a CRISPR/Cas9-mediated gene-editing tool in Streptomyces rimosus

Haiyan Jia, Longmei Zhang, Tongtong Wang, Jin Han, Hui Tang, Liping Zhang

ABSTRACT

Clustered regularly interspaced short palindromic repeats, associated proteins (CRISPR/Cas), has been developed into a powerful, targeted genome-editing tool in a wide variety of species. Here, we report an extensive investigation of the type II CRISPR/Cas9 system for targeted gene editing in Streptomyces rimosus. S. rimosus is used in the production of the antibiotic oxytetracycline, and its genome differs greatly from other species of the genus Streptomyces in the conserved chromosome terminal and core regions, which is of major production and scientific research value. The genes zwf2 and devB were chosen as target genes, and were edited separately via single-site mutations, double-site mutations and gene fragment disruptions. The single-site mutation guided by sgRNA-1 or sgRNA-2, respectively, involved GG changing to CA, GC changing to AT, and GG changing to CC. The double-site mutations guided by sgRNA-1 and sgRNA-2 included deletions and/or point mutations. Consistently, all mutations occurred in the gRNA sequence regions. Deletion mutations were characterized by the absence of eight bases, including three bases upstream of the PAM (protospacer adjacent motif) sequence, the PAM sequence itself and two bases downstream of the PAM sequence. A mutant (zwf2 − devB −) with a high yield of oxytetracycline was successfully obtained, whose oxytetracycline level was increased by 36.8% compared to the original strain. These results confirm that CRISPR/Cas9 can successfully serve as a useful targeted genome editing system in S. rimosus.

Cdc42 activation state affects its localization and protein levels in fission yeast

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ABSTRACT

Rho GTPases control polarized cell growth and are well-known regulators of exocytic and endocytic processes. Cdc42 is an essential GTPase, conserved from yeast to humans, that is critical for cell polarization. Cdc42 is negatively regulated by the GTPase-activating proteins (GAPs) and the GDP dissociation inhibitors (GDIs), and positively regulated by guanine nucleotide exchange factors (GEFs). Cdc42 GTPase can be found in a GTP- or GDP-bound state, which determines the ability to bind downstream effector proteins and activate signalling pathways. Only GTP-bound Cdc42 is active. In this study we have analysed the localization of the different nucleotide-bound states of Cdc42 in Schizosaccharomyces pombe: the wild-type Cdc42 protein that cycles between an active and inactive form, the Cdc42G12V form that is permanently bound to GTP and the Cdc42T17N form that is constitutively inactive. Our results indicate that Cdc42 localizes to several membrane compartments in the cell and this localization is mediated by its C-terminal prenylation. Constitutively active Cdc42 localizes mainly to the plasma membrane and concentrates at the growing tips where it is considerably less dynamic than wild-type or GDP-bound Cdc42. Additionally we show that the activation state of Cdc42 also participates in the regulation of its protein levels mediated by endocytosis and by the exocyst complex.

Dissecting the protein architecture of DNA-binding transcription factors in bacteria and archaea

Nancy Rivera-Gómez, Mario Alberto Martínez-Núñez, Nina Pastor, Katya Rodriguez-Vazquez, Ernesto Perez-Rueda

ABSTRACT

Gene regulation at the transcriptional level is a central process in all organisms where DNA-binding transcription factors play a fundamental role. This class of proteins binds specifically at DNA sequences, activating or repressing gene expression as a function of the cell's metabolic status, operator context and ligand-binding status, among other factors, through the DNA-binding domain (DBD). In addition, TFs may contain partner domains (PaDos), which are involved in ligand binding and protein–protein interactions. In this work, we systematically evaluated the distribution, abundance and domain organization of DNA-binding TFs in 799 non-redundant bacterial and archaeal genomes. We found that the distributions of the DBDs and their corresponding PaDos correlated with the size of the genome. We also identified specific combinations between the DBDs and their corresponding PaDos. Within each class of DBDs there are differences in the actual angle formed at the dimerization interface, responding to the presence/absence of ligands and/or crystallization conditions, setting the orientation of the resulting helices and wings facing the DNA. Our results highlight the importance of PaDos as central elements that enhance the diversity of regulatory functions in all bacterial and archaeal organisms, and our results also demonstrate the role of PaDos in sensing diverse signal compounds. The highly specific interactions between DBDs and PaDos observed in this work, together with our structural analysis highlighting the difficulty in predicting both inter-domain geometry and quaternary structure, suggest that these systems appeared once and evolved with diverse duplication events in all the analysed organisms.

Interactions between host immune response and antigenic variation that control Borrelia burgdorferi population dynamics

Wei Zhou, Dustin Brisson

ABSTRACT

The population dynamics of pathogens within hosts result from interactions between host immune responses and mechanisms of the pathogen to evade or resist immune responses. Vertebrate hosts have evolved adaptive immune responses to eliminate the infection, while many pathogens evade immune clearance through altering surface antigens. Such interactions can result in a characteristic pattern of pathogen population dynamics within hosts consisting of population growth after infection, rapid population decline following specific immune responses, followed by persistence at low densities during a chronic infection stage. Despite the medical importance of chronic infections, little is known about the conditions of the interactions between variable antigens and the adaptive immune system that cause the characteristic pathogen population dynamics. Using the vls antigenic variation system of the Lyme disease pathogen, Borrelia burgdorferi, as a model system, we investigated conditions of the interaction between the antigenic variation system and the adaptive immune response that can explain the within-host population dynamics of B. burgdorferi using mathematical modelling. This characteristic population dynamic pattern can be explained by models that assume a variable immune removal rate of antibody-bound B. burgdorferi. However, models with a constant immune removal rate could reproduce the rapid population decline of B. burgdorferi populations but not their long-term persistence within hosts using parameter values determined by fitting empirical data. The model predictions, along with the assumptions about the interactions between B. burgdorferi and the immune response, can be tested experimentally to estimate the likelihood that each mechanism affects B. burgdorferi population dynamics in real infections.

Bacterial communities in the small intestine respond differently to those in the caecum and colon in mice fed low- and high-fat diets

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ABSTRACT

Bacterial communities in the mouse caecum and faeces are known to be altered by changes in dietary fat. The microbiota of the mouse small intestine, by contrast, has not been extensively profiled and it is unclear whether small intestinal bacterial communities shift with dietary fat levels. We compared the microbiota in the small intestine, caecum and colon in mice fed a low-fat (LF) or high-fat (HF) diet using 16S rRNA gene sequencing. The relative abundance of major phyla in the small intestine, Bacteriodetes, Firmicutes and Proteobacteria, was similar to that in the caecum and colon; the relative abundance of Verrucomicrobia was significantly reduced in the small intestine compared to the large intestine. Several genera were uniquely detected in the small intestine and included the aerotolerant anaerobe, Lactobacillus spp. The most abundant genera in the small intestine were accounted for by anaerobic bacteria and were identical to those identified in the large intestine. An HF diet was associated with significant weight gain and adiposity and with changes in the bacterial communities throughout the intestine, with changes in the small intestine differing from those in the caecum and colon. Prominent Gram-negative bacteria including genera of the phylum Bacteroidetes and a genus of Proteobacteria significantly changed in the large intestine. The mechanistic links between these changes and the development of obesity, perhaps involving metabolic endotoxemia, remain to be determined.

Pneumococcal neuraminidase activates TGF-β signalling

Nina Gratz, Lip Nam Loh, Beth Mann, Geli Gao, Robert Carter, Jason Rosch, Elaine I. Tuomanen

ABSTRACT

Neuraminidase A (NanA) is an important virulence factor that is anchored to the pneumococcal cell wall and cleaves sialic acid on host substrates. We noted that a secreted allele of NanA was over-represented in invasive pneumococcal isolates and promoted the development of meningitis when swapped into the genome of non-meningitis isolates replacing cell wall-anchored NanA. Both forms of recombinant NanA directly activated transforming growth factor (TGF)-β, increased SMAD signalling and promoted loss of endothelial tight junction ZO-1. However, in assays using whole bacteria, only the cell-bound NanA decreased expression of ZO-1 and showed NanA dependence of bacterial invasion of endothelial cells. We conclude that NanA secretion versus retention on the cell surface does not influence neurotropism of clinical isolates. However, we describe a new NanA-TGF-β signalling axis that leads to decreased blood-brain barrier integrity and enhances bacterial invasion.

The Pseudomonas aeruginosa dnaK gene is involved in bacterial translocation across the intestinal epithelial cell barrier

Jun Okuda, Satoshi Yamane, Syouya Nagata, Chinami Kunikata, Chigusa Suezawa, Masashi Yasuda

ABSTRACT

Pseudomonas aeruginosa can penetrate through polarized epithelial cell monolayers produced by the human adenocarcinoma cell line Caco-2. We previously identified genes associated with bacterial translocation through Caco-2 cell monolayers by analysing transposon insertion mutants with dramatically reduced penetration activity relative to that of the wild-type P. aeruginosa PAO1 strain. In this study, we focused on the dnaK mutant because the association between this gene and penetration activity is unknown. Inactivation of dnaK caused significant repression of bacterial penetration through Caco-2 cell monolayers, with decreased swimming, swarming and twitching motilities; bacterial adherence; and fly mortality rate; as well as dramatic repression of type III effector secretion and production of elastase and exotoxin A. However, type IV pilus protein PilA expression was not affected. These results suggest that dnaK is associated with bacterial motility and adherence, which are mediated by flagella and pili, and with toxin secretion, which plays a key role in the penetration of P. aeruginosa through Caco-2 cell monolayers. Inactivation of P. aeruginosa dnaK function may interfere with bacterial translocation and prevent septicaemia caused by P. aeruginosa.

Investigation of the Fim1 putative pilus locus of Streptococcus equi subspecies equi

Karen Frances Steward, Carl Robinson, Duncan J. Maskell, Chiara Nenci, Andrew Stephen Waller

ABSTRACT

The Gram-positive bacterium Streptococcus equi subspecies equi (S. equi) is the causative agent of strangles, among the most frequently diagnosed infectious diseases of horses worldwide. Genome analysis of S. equi strain 4047 (Se4047) identified a putative operon, Fim1, with similarity to the pilus loci of other Gram-positive bacteria. The Fim1 locus was present in all strains of S. equi and its close relative S. equi subspecies zooepidemicus (S. zooepidemicus) that have been studied to date. In this study we provide evidence that the putative structural pilus proteins, SEQ_0936 and CNE, are produced on the cell surface during in vitro growth and in vivo infection. Although the proteins encoded within the Fim1 locus are not essential for attachment or biofilm formation, over-transcription of SEQ_0936 and CNE enhanced attachment to equine tissue in vitro. Our data suggest that whilst the Fim1 locus does not produce a polymerized pilus structure, the products of the Fim1 locus may fulfil an adhesive function. The putative pilus-associated regulator, tetR, which contains a nonsense mutation in S. equi, was able to regulate transcription of the Fim1 locus following repair and over-transcription, confirming its predicted role in the operon.

Desulfovibrio DA2_CueO is a novel multicopper oxidase with cuprous, ferrous and phenol oxidase activity

Stefano Mancini, Ranjeet Kumar, Veena Mishra, Marc Solioz

ABSTRACT

Desulfovibrio sp. A2 is a novel Gram-negative sulfate-reducing bacterium that was isolated from sediments of the Norilsk mining/smelting area in Russia. The organism possesses a monocistronic operon encoding a 71 kDa periplasmic multicopperoxidase, which we call DA2_CueO. Histidine-tagged DA2_CueO expressed from a plasmid in Escherichia coli and purified by Ni–NTA affinity chromatography oxidizes Cu+ and Fe2+, and exhibits phenol oxidase activity with 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid), 2,3-dihydroxybenzoic acid and 2,6-dimethoxyphenol as substrates, using O2 as the oxidant. When expressed in an E. coli cueO knock-out strain, DA2_CueO exhibits phenol oxidase activity in vivo and enhances the copper tolerance of the strain. These findings indicate that the DA2_CueO gene of Desulfovibrio sp. A2 encodes a multicopperoxidase with a role in metal ion resistance. The enzyme displays some novel structural features, which are discussed.

Comparative proteomic profiles reveal characteristic Mycobacterium tuberculosis proteins induced by cholesterol during dormancy conditions

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ABSTRACT

Cholesterol has been reported to play an important role during Mycobacterium tuberculosis infection and during its dormant state inside the host. We present the determination of proteomic profiles of M. tuberculosis H37Rv in the presence of cholesterol as the sole carbon source under exponential growth and in two in vitro dormancy phases (NRP1 and NRP2). Using 2D-PAGE, we detected that M. tuberculosis expressed a high diversity of proteins in both exponential and non-replicative phases. We also found that cholesterol was involved in the overexpression of some proteins related to sulfur metabolism (CysA2), electron transport (FixB), cell wall synthesis (Ald), iron storage (BfrB), protein synthesis (Tig and EF-Tu) and dormancy maintenance (HspX and TB 31.7). According to our results we propose that proteins Ald, BfrB, FadA5 and TB31.7 are likely to play a fundamental role during in vitro dormancy of M. tuberculosis in the presence of cholesterol, helping to counteract its intracellular hostile microenvironment.

Characterization of the interaction between the small RNA-encoded peptide SR1P and GapA from Bacillus subtilis

Matthias Gimpel, Caroline Maiwald, Christoph Wiedemann, Matthias Görlach, Sabine Brantl

ABSTRACT

Small regulatory RNAs (sRNAs) are the most prominent post-transcriptional regulators in all kingdoms of life. A few of them, e.g. SR1 from Bacillus subtilis, are dual-function sRNAs. SR1 acts as a base-pairing sRNA in arginine catabolism and as an mRNA encoding the small peptide SR1P in RNA degradation. Both functions of SR1 are highly conserved among 23 species of Bacillales. Here, we investigate the interaction between SR1P and GapA by a combination of in vivo and in vitro methods. De novo prediction of the structure of SR1P yielded five models, one of which was consistent with experimental circular dichroism spectroscopy data of a purified, synthetic peptide. Based on this model structure and a comparison between the 23 SR1P homologues, a series of SR1P mutants was constructed and analysed by Northern blotting and co-elution experiments. The known crystal structure of Geobacillus stearothermophilus GapA was used to model SR1P onto this structure. The hypothetical SR1P binding pocket, composed of two α-helices at both termini of GapA, was investigated by constructing and assaying a number of GapA mutants in the presence and absence of wild-type or mutated SR1P. Almost all residues of SR1P located in the two highly conserved motifs are implicated in the interaction with GapA. A critical lysine residue (K332) in the C-terminal α-helix 14 of GapA corroborated the predicted binding pocket.

Indigenous lactobacilli strains of food and human sources reverse enteropathogenic E. coli O26:H11-induced damage in intestinal epithelial cell lines: effect on redistribution of tight junction proteins

Ruchi Jariwala, Hemanti Mandal, Tamishraha Bagchi

ABSTRACT

The aim of the study was to investigate the neutralizing effect of lactobacilli isolated from indigenous food and human sources on enteropathogenic Escherichia coli (EPEC) O26:H11-induced epithelial barrier dysfunction in vitro. This was assessed by transepithelial electrical resistance (TEER) and permeability assays using intestinal cell lines, HT-29 and Caco-2. Furthermore, the expression and distribution of tight junction (TJ) proteins were analysed by qRT-PCR and immunofluorescence assay, respectively. The nine strains used in the study were from different species viz. Lactobacillus fermentum, L actobacillus helveticus, L actobacillus salivarius and L actobacillus plantarum. All strains were able to reverse the decrease in TEER and corresponding increase in permeability across E. coli-infected monolayers. Maximum reversal was observed after 18 h [up to 93.8±2.0% by L. rhamnosus GG followed by L. fermentum IIs11.2 (92.6±2.2%) and L. plantarum GRI-2 (91.9±0.9%)] of lactobacilli exposure following EPEC O26:H11 infection. All strains were able to redistribute the TJ proteins to the cell periphery either partially or completely. Moreover, L. helveticus FA-7 was also able to significantly increase the mRNA expression of ZO-1 and claudin-1 (2.5-fold and 3.0-fold, respectively; $P<0.05$). The rapid reversal observed by these strains could be mostly because of the redistribution rather than increased mRNA expression of TJ proteins. In conclusion, L. helveticus FA-7, L. fermentum FA-1 and L. plantarum GRI-2 were good in all the aspects studied, and the other strains were good in some aspects. L. helveticus FA-7, L. fermentum FA-1 and L. plantarum GRI-2 can therefore be used for potential therapeutic purpose against intestinal epithelial dysfunction.

The effect of Hypomyces perniciosus on the mycelia and basidiomes of Agaricus bisporus

Chunlan Zhang, Makoto Kakishima, Jize Xu, Qi Wang, Yu Li

ABSTRACT

Hypomyces perniciosus has been reported as a destructive pathogen of Agaricus bisporus. Previous research suggested that the pathogenesis may not only be perpetuated by H. perniciosus, but also by bacteria. Clarification of the interaction between A. bisporus and H. perniciosus is a prerequisite for the development of effective control measures against wet bubble disease. Here, the effects of H. perniciosus on A. bisporus mycelia are examined in dual culture on agar media and in open-ended test tubes. During disease development, the putative causal agents and cytology of wet bubble-diseased mushrooms were followed microscopically. The interaction between H. perniciosus and the basidiome of A. bisporus was also studied using dual-cultured H. perniciosus and basidiome tissues. Dual-cultured mycelia from both fungi showed that growth continued even after contact was made, without any observable antagonistic lines or cytoplasmic changes of A. bisporus mycelia. Hypomyces perniciosus could be isolated from diseased basidiomes any time after inoculation, but bacteria were only recovered after the basidiomes of A. bisporus had been killed by H. perniciosus. Dual culture of the basidiome tissue of A. bisporus and H. perniciosus on agar media established that H. perniciosus can independently and rapidly degrade the basidiomes of A. bisporus. We conclude that H. perniciosus has no pathogenic activity on the mycelial stage of A. bisporus, but it can destroy A. bisporus basidiomes in the absence of bacteria. Wet bubble disease is evidently not caused by bacteria, but by the fungus, although bacteria likely participate in the disease after invasion by the fungus.

Characterization of feedback-resistant mevalonate kinases from the methanogenic archaeons Methanosaeta concilii and Methanocella paludicola

Ekaterina Kazieva, Yoko Yamamoto, Yoshinori Tajima, Keiichi Yokoyama, Joanna Katashkina, Yousuke Nishio

ABSTRACT

The inhibition of mevalonate kinase (MVK) by downstream metabolites is an important mechanism in the regulation of isoprenoid production in a broad range of organisms. The first feedback-resistant MVK was previously discovered in the methanogenic archaeon Methanosarcinamazei. Here, we report the cloning, expression, purification, kinetic characterization and inhibition analysis of MVKs from two other methanogens, Methanosaetaconcilii and Methanocellapaludicola. Similar to the M. mazei MVK, these enzymes were not inhibited by diphosphomevalonate (DPM), dimethylallyl diphosphate (DMAPP), isopentenyldiphosphate (IPP), geranylpyrophosphate (GPP) or farnesylpyrophosphate (FPP). However, they exhibited significantly higher affinity to mevalonate and higher catalytic efficiency than the previously characterized enzyme.

Discovery of a novel lantibiotic nisin O from Blautia obeum A2-162, isolated from the human gastrointestinal tract

Diane Hatziioanou, Cristina Gherghisan-Filip, Gerhard Saalbach, Nikki Horn, Udo Wegmann, Sylvia H. Duncan, Harry J. Flint, Melinda J. Mayer, Arjan Narbad

ABSTRACT

A novel lanC-like sequence was identified from the dominant human gut bacterium Blautia obeum strain A2-162. This sequence was extended to reveal a putative lantibiotic operon with biosynthetic and transport genes, two sets of regulatory genes, immunity genes, three identical copies of a nisin-like lanA gene with an unusual leader peptide, and a fourth putative lanA gene. Comparison with other nisin clusters showed that the closest relationship was to nisin U. B. obeum A2-162 demonstrated antimicrobial activity against Clostridium perfringens when grown on solid medium in the presence of trypsin. Fusions of predicted nsoA structural sequences with the nisin A leader were expressed in Lactococcus lactis containing the nisin A operon without nisA. Expression of the nisA leader sequence fused to the predicted structural nsoA1 produced a growth defect in L. lactis that was dependent upon the presence of biosynthetic genes, but failed to produce antimicrobial activity. Insertion of the nso cluster into L. lactis MG1614 gave an increased immunity to nisin A, but this was not replicated by the expression of nsoI. Nisin A induction of L. lactis containing the nso cluster and nisRK genes allowed detection of the NsoA1 pre-peptide by Western hybridization. When this heterologous producer was grown with nisin induction on solid medium, antimicrobial activity was demonstrated in the presence of trypsin against C. perfringens, Clostridium difficile and L. lactis. This research adds to evidence that lantibiotic production may be an important trait of gut bacteria and could lead to the development of novel treatments for intestinal diseases.

Involvement of signal peptidase I in Streptococcus sanguinis biofilm formation

Jessica Aynapudi, Fadi El-Rami, Xiuchun Ge, Victoria Stone, Bin Zhu, Todd Kitten, Ping Xu

ABSTRACT

Biofilm accounts for 65–80% of microbial infections in humans. Considerable evidence links biofilm formation by oral microbiota to oral disease and consequently systemic infections. Streptococcus sanguinis, a Gram-positive bacterium, is one of the most abundant species of the oral microbiota and it contributes to biofilm development in the oral cavity. Due to its altered biofilm formation, we investigated a biofilm mutant, ΔSSA_0351, that is deficient in type I signal peptidase (SPase) in this study. Although the growth curve of the ΔSSA_0351 mutant showed no significant difference from that of the wild-type strain SK36, biofilm assays using both microtitre plate assay and confocal laser scanning microscopy (CLSM) confirmed a sharp reduction in biofilm formation in the mutant compared to the wild-type strain and the paralogous mutant ΔSSA_0849. Scanning electron microscopy (SEM) revealed remarkable differences in the cell surface morphologies and chain length of the ΔSSA_0351 mutant compared with those of the wild-type strain. Transcriptomic and proteomic assays using RNA sequencing and mass spectrometry, respectively, were conducted on the ΔSSA_0351 mutant to evaluate the functional impact of SPase on biofilm formation. Subsequently, bioinformatics analysis revealed a number of proteins that were differentially regulated in the ΔSSA_0351 mutant, narrowing down the list of SPase substrates involved in biofilm formation to lactate dehydrogenase (SSA_1221) and a short-chain dehydrogenase (SSA_0291). With further experimentation, this list defined the link between SSA_0351-encoded SPase, cell wall biosynthesis and biofilm formation.

The glucosylglycerol-degrading enzyme GghA is involved in acclimation to fluctuating salinities by the cyanobacterium Synechocystis sp. strain PCC 6803

Friedrich Kirsch, Nadin Pade, Stephan Klähn, Wolfgang R. Hess, Martin Hagemann

ABSTRACT

The ggpS gene, which encodes the key enzyme for the synthesis of the compatible solute glucosylglycerol (GG), has a promoter region that overlaps with the upstream-located gene slr1670 in the cyanobacterium Synechocystissp. PCC 6803. Like ggpS, the slr1670 gene is salt-induced and encodes a putative glucosylhydrolase. A mutant strain with a slr1670 deletion was generated and found to be unable to adapt the internal GG concentrations in response to changes in external salinities. Whereas cells of the wild-type reduced the internal pool of GG when exposed to gradual and abrupt hypo-osmotic treatments, or when the compatible solute trehalose was added to the growth medium, the internal GG pool of Δslr1670 mutant cells remained unchanged. These findings indicated that the protein Slr1670 is involved in GG breakdown. The biochemical activity of this GG-hydrolase enzyme was verified using recombinant Slr1670 protein, which split GG into glucose and glycerol. These results validate that Slr1670, which was named GghA, acts as a GG hydrolase. GghA is involved in GG turnover in fluctuating salinities, and similar proteins are found in the genomes of other GG-synthesizing cyanobacteria.

Iron responsive-like elements in the parasite Entamoeba histolytica

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ABSTRACT

In Entamoeba histolytica, iron modulates virulence and gene expression via unknown regulatory mechanisms. The existence of a posttranscriptional iron regulatory system parallel with the iron-responsive element (IRE)/iron regulatory protein (IRP) system in the protozoan Trichomonas vaginalis has recently been reported. Due to their evolutionary closeness and the importance of iron for growth and virulence in these protozoa, we hypothesized the existence of an IRE/IRP-like mechanism in E. histolytica. To determine the presence of IRE-like elements in some mRNAs from this parasite, we performed in silico analyses of the 5′- and 3′-UTRs of mRNAs encoding virulence factors and cytoskeleton, ribosomal and metabolism proteins. The Zuker mfold software predicted IRE-like secondary structures in 52 of the 135 mRNAs analysed. However, only nine structures shared sequence similarity with the apical loop sequence (CAGUGN) of the previously reported human IRE-ferritin, whereas the GUU/UUG protozoan-specific motif was detected in 23 stem–loop structures. A new motif, AUU/AUUU, was also observed in 23 structures, suggesting the possible existence of an amoeba-specific motif. Additionally, cross-linking and RNA electrophoretic mobility shift assays showed specific RNA–protein interactions, using as a model two amoebic IRE-like elements from iron-regulated mRNAs and HeLa, T. vaginalis and E. histolytica cytoplasmic proteins. Our data suggest the presence of a posttranscriptional iron regulatory IRE/IRP-like mechanism in E. histolytica.

Pseudomonas aeruginosa gbdR gene is transcribed from a σ54-dependent promoter under the control of NtrC/CbrB, IHF and BetI

Diego Germán Sánchez, Emiliano David Primo, María Teresa Damiani, Angela Teresita Lisa

ABSTRACT

Pseudomonasaeruginosa uses choline as a source of carbon and nitrogen, and also for the synthesis of glycine betaine, an osmoprotectant under stress conditions such as drought and salinity. The transcription factor GbdR is the specific regulator of choline metabolism and it belongs to the Arac/XylS family of transcriptional regulators. Despite the link between choline catabolism and bacterial pathogenicity, gbdR regulation has not been explored in detail. In the present work, we describe how gbdR transcription can be initiated from a σ54-dependent promoter. gbdR transcription can be activated by NtrC in the absence of a preferential nitrogen source, by CbrB in the absence of a preferential carbon source, and by the integration host factor favouring DNA bending. In addition, we found that BetI negatively regulates gbdR expression in the absence of choline. We identified two overlapping BetI binding sites in the gbdR promoter sequence, providing an additional example of σ54-promoter down-regulation. Based on our findings, we propose a model for gdbR regulation and its impact on choline metabolism.

The Rhodobacter capsulatus gene transfer agent is induced by nutrient depletion and the RNAP omega subunit

Alexander B. Westbye, Zoe O'Neill, Tegan Schellenberg-Beaver, J. Thomas Beatty

ABSTRACT

Small bacteriophage-like particles called gene transfer agents (GTAs) that mediate DNA transfer between cells are produced by a variety of prokaryotes. The model GTA, produced by the alphaproteobacterium Rhodobacter capsulatus (RcGTA), is controlled by several cellular regulators, and production is induced upon entry into the stationary phase. We report that RcGTA production and gene transfer are stimulated by nutrient depletion. Cells depleted of organic carbon or blocked for amino acid biosynthesis increased RcGTA production and release from cells. Furthermore, cells lacking the sole RelA-SpoT homologue produced decreased levels of RcGTA, and the RNA polymerase omega (ω) subunit was required for appreciable production of RcGTA.

The length of poly(C) stretch in the Bordetella pertussis Pfim3 promoter determines the vag or vrg function of the fim3 gene

Nao Otsuka, Nicole Guiso, Valérie Bouchez

ABSTRACT

Bordetella pertussis, a human pathogenic bacterium, produces either one or two types of serologically distinct fimbriae, Fim2 and Fim3, as virulence factors. The expression of fim2 and fim3 is regulated by the BvgAS two-component system and the length of poly(C) stretches in Pfim promoters. In the Bvg+ phase, B. pertussis virulence-activated genes (vags) are up-regulated and virulence-repressed genes (vrgs) are down-regulated. Previous studies have shown that fim2 is a vag, but there is no consensus on fim3 regulation. We examined the regulation of fimbrial expression in B. pertussis clinical isolates. Our findings indicate that fim2 is a vag, while fim3 is a vag when Pfim3 poly(C)>13C, and a vrg when poly(C)≤13C. Although increased fim3 expression was observed in the Bvg– phase in isolates with Pfim3 poly(C)≤13C, Fim3 production was not detected, suggesting post-transcriptional regulation of fim3 expression. These findings provide an insight into the regulation of fimbrial expression in B. pertussis.

Volume 163, Issue 10, October 2017

Virulence determinants of Moraxella catarrhalis: distribution and considerations for vaccine development

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ABSTRACT

Moraxella catarrhalis is a human-restricted opportunistic bacterial pathogen of the respiratory mucosa. It frequently colonizes the nasopharynx asymptomatically, but is also an important causative agent of otitis media (OM) in children, and plays a significant role in acute exacerbations of chronic obstructive pulmonary disease (COPD) in adults. As the current treatment options for M. catarrhalis infection in OM and exacerbations of COPD are often ineffective, the development of an efficacious vaccine is warranted. However, no vaccine candidates for M. catarrhalis have progressed to clinical trials, and information regarding the distribution of M. catarrhalis virulence factors and vaccine candidates is inconsistent in the literature. It is largely unknown if virulence is associated with particular strains or subpopulations of M. catarrhalis, or if differences in clinical manifestation can be attributed to the heterogeneous expression of specific M. catarrhalis virulence factors in the circulating population. Further investigation of the distribution of M. catarrhalis virulence factors in the context of carriage and disease is required so that vaccine development may be targeted at relevant antigens that are conserved among disease-causing strains. The challenge of determining which of the proposed M. catarrhalis virulence factors are relevant to human disease is amplified by the lack of a standardized M. catarrhalis typing system to facilitate direct comparisons of worldwide isolates. Here we summarize and evaluate proposed relationships between M. catarrhalis subpopulations and specific virulence factors in the context of colonization and disease, as well as the current methods used to infer these associations.

Colworth prize lecture 2016: exploiting new biological targets from a whole-cell phenotypic screening campaign for TB drug discovery

Patrick Joseph Moynihan, Gurdyal S. Besra

ABSTRACT

Mycobacterium tuberculosis is the aetiological agent of tuberculosis (TB) and is the leading bacterial cause of mortality and morbidity in the world. One third of the world's population is infected with TB, and in conjunction with HIV represents a serious problem that urgently needs addressing. TB is a disease of poverty and mostly affects young adults in their productive years, primarily in the developing world. The most recent report from the World Health Organisation states that 8 million new cases of TB were reported and that ~1.5 million people died from TB. The efficacy of treatment is threatened by the emergence of multi-drug and extensively drug-resistant strains of M. tuberculosis. It can be argued that, globally, M. tuberculosis is the single most important infectious agent affecting mankind. Our research aims to establish an academic-industrial partnership with the goal of discovering new drug targets and hit-to-lead new chemical entities for TB drug discovery.

PfmA, a novel quorum-quenching N-acylhomoserine lactone acylase from Pseudoalteromonas flavipulchra

Na Liu, Min Yu, Youbin Zhao, Jingguang Cheng, Ke An, Xiao-Hua Zhang

ABSTRACT

Many bacteria, such as Proteobacteria, Cyanobacteria and Bacteroidetes, use N-acylhomoserine lactones (AHLs) as quorum-sensing (QS) signal molecules for communication. Enzymatic degradation of AHLs, such as AHL acylase and AHL lactonase, can degrade AHLs (quorum quenching, QQ) to attenuate or disarm the virulence of pathogens. QQ is confirmed to be common in marine bacterial communities. Many genes encoding AHL acylases are found in marine bacteria and metagenomic collections, but only a few of these have been characterized in detail. We have reported that the marine bacterium Pseudoalteromonas flavipulchra JG1 can degrade AHLs. In the present study, a novel AHL acylase PfmA, which can degrade AHLs with acyl chains longer than 10 carbons, was identified from strain JG1. Ultra-performance liquid chromatography (UPLC) and electrospray ionization mass spectrometry (ESI-MS) analysis demonstrated that PfmA functions as an AHL acylase, which hydrolysed the amide bond of AHL. The purified PfmA of P. flavipulchra JG1 showed optimum activity at 30 °C and pH 7.0. PfmA belongs to the N-terminal nucleophile (Ntn) hydrolase superfamily and showed homology to a member of penicillin amidases, but PfmA can degrade ampicillin but not penicillin G. The residue Ser256 in PfmA is the active site according to site-directed mutagenesis. Furthermore, PfmA reduced AHL accumulation and the production of virulence factors in Vibrio anguillarum VIB72 and Pseudomonas aeruginosa PAO1, and attenuated the virulence of P. aeruginosa to increase Artemia survival, which suggested that PfmA can be considered as a therapeutic agent to control AHL-mediated pathogenicity.

GalK-based suicide vector mediated allelic exchange in Mycobacterium abscessus

Stacy A. Gregoire, Joel Byam, Martin S. Pavelka Jr

ABSTRACT

Mycobacterium abscessus is a fast-growing environmental organism and an important emerging pathogen. It is highly resistant to many antibiotics and undergoes a smooth to rough colony morphology change that appears to be important for pathogenesis. Smooth environmental strains have a glycopeptidolipid (GPL) on the surface, while certain types of clinical strains are often rough and lack this GPL, due to mutations in biosynthetic genes or the mmpL4b transporter gene. We report here the development and evaluation of an allelic exchange system for unmarked alleles in M. abscessus ATCC19977, using a suicide vector bearing the E. coli galK gene and 2-deoxygalactose counterselection. We describe here two variant galK suicide vectors, and demonstrate their utility in constructing a variety of mutants with deletion alleles of the mmpL4b GPL transporter gene, the mbtH GPL biosynthesis gene, the known β-lactamase gene MAB_2875 and a putative β-lactamase gene, MAB_2833. We also show that a novel allele of the E. coli aacC4 gene, conferring apramycin resistance (aacC41), can be used as a selectable marker in M. abscessus ATCC19977 at single copy.

Identification and heterologous expression of the kocurin biosynthetic gene cluster

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ABSTRACT

The antibiotically bioactive thiopeptide compound kocurin was identified in extracts from a newly isolated Kocuria rosea strain. The axenic strain was retrieved from a soil sample of the intertidal area at the Paracas National Park, Peru. The genetic basis of this promising natural product with activity against methicillin-resistant Staphylococcus aureus (MRSA) strains was revealed by comparative genome analysis of this new isolate and other reported thiopeptide producer strains. The functionality of the predicted gene locus was experimentally proven by heterologous expression in Streptomyces coelicolor M1146. Expression of the gene cluster under the control of a constitutive promoter enabled the transgenic strain to produce kocurin in selected media. The kocurin biosynthetic gene cluster comprises nine open reading frames and spans around 12 kbp of the genome.

The MtrAB two-component system controls antibiotic production in streptomyces coelicolor A3 (2)

Nicolle F. Som, Daniel Heine, Neil Holmes, Felicity Knowles, Govind Chandra, Ryan F. Seipke, Paul A. Hoskisson, Barrie Wilkinson, Matthew I. Hutchings

ABSTRACT

MtrAB is a highly conserved two-component system implicated in the regulation of cell division in the Actinobacteria. It coordinates DNA replication with cell division in the unicellular Mycobacterium tuberculosis and links antibiotic production to sporulation in the filamentous Streptomyces venezuelae. Chloramphenicol biosynthesis is directly regulated by MtrA in S. venezuelae and deletion of mtrB constitutively activates MtrA and results in constitutive over-production of chloramphenicol. Here we report that in Streptomyces coelicolor, MtrA binds to sites upstream of developmental genes and the genes encoding ActII-1, ActII-4 and RedZ, which are cluster-situated regulators of the antibiotics actinorhodin (Act) and undecylprodigiosin (Red). Consistent with this, deletion of mtrB switches on the production of Act, Red and streptorubin B, a product of the Red pathway. Thus, we propose that MtrA is a key regulator that links antibiotic production to development and can be used to upregulate antibiotic production in distantly related streptomycetes.

Identification of genes involved in galactooligosaccharide utilization in Bifidobacterium breve strain YIT 4014T

Hidetsugu Sotoya, Akira Shigehisa, Taeko Hara, Hoshitaka Matsumoto, Hiroshi Hatano, Takahiro Matsuki

ABSTRACT

Galactooligosaccharides (GOS) are mixed oligosaccharides that are mainly composed of galactosyllactoses (GLs), which include 3′-GL, 4′-GL, and 6′-GL. Data from numerous in vitro and in vivo studies have shown that GOS selectively stimulate the growth of bifidobacteria. Previously, we identified the gene locus responsible for 4′-GL utilization, but the selective routes of uptake and catabolism of 3′- and 6′-GL remain to be elucidated. In this study, we used differential transcriptomics to identify the utilization pathways of these GLs within the Bifidobacterium breve YIT 4014T strain. We found that the BBBR_RS 2305–2320 gene locus, which includes a solute-binding protein (SBP) of an ATP-binding cassette (ABC) transporter and β-galactosidase, were up-regulated during 3′- and 6′-GL utilization. The substrate specificities of these proteins were further investigated, revealing that β-galactosidase hydrolyzed both 3′-GL and 6′-GL efficiently. Our surface plasmon resonance results indicated that the SBP bound strongly to 6′-GL, but bound less tightly to 3′-GL. Therefore, we looked for the other SBPs for 3′-GL and found that the BBBR_RS08090 SBP may participate in 3′-GL transportation. We also investigated the distribution of these genes in 17 bifidobacterial strains, including 9 B. breve strains, and found that the β-galactosidase genes were present in most bifidobacteria. Homologues of two ABC transporter SBP genes were found in all B. breve strains and in some bifidobacteria that are commonly present in the human gut microbiota. These results provide insights into the ability of human-resident bifidobacteria to utilize the main component of GOS in the gastrointestinal tract.

Cadmium ion inhibition of quorum signalling in Chromobacterium violaceum

Starla G. Thornhill, Manish Kumar, Leticia M. Vega, Robert J. C. McLean

ABSTRACT

Single-celled bacteria are capable of acting as a community by sensing and responding to population density via quorum signalling. Quorum signalling in Chromobacterium violaceum, mediated by the luxI/R homologue, cviI/R, regulates a variety of phenotypes including violacein pigmentation, virulence and biofilm formation. A number of biological and organic molecules have been described as quorum signalling inhibitors but, to date, metal-based inhibitors have not been widely tested. In this study, we show that quorum sensing is inhibited in C. violaceum in the presence of sub-lethal concentrations of cadmium salts. Notable Cd2+-inhibition was seen against pigmentation, motility, chitinase production and biofilm formation. Cd-inhibition of quorum-signalling genes occurred at the level of transcription. There was no direct inhibition of chitinase activity by Cd2+ at the concentrations tested. Addition of the cognate quorum signals, N-hexanoyl homoserine lactone or N-decanoyl homoserine lactone, even at concentrations in excess of physiological levels, did not reverse the inhibition, suggesting that Cd-inhibition of quorum signaling is irreversible. This study represents the first description of heavy metal-based quorum inhibition in C. violaceum.

Expression of matrix metalloproteinases in Naegleria fowleri and their role in invasion of the central nervous system

Charlton Lam, Melissa Jamerson, Guy Cabral, Ana Maris Carlesso, Francine Marciano-Cabral

ABSTRACT

Naegleria fowleri is a free-living amoeba found in freshwater lakes and ponds and is the causative agent of primary amoebic meningoencephalitis (PAM), a rapidly fatal disease of the central nervous system (CNS). PAM occurs when amoebae attach to the nasal epithelium and invade the CNS, a process that involves binding to, and degradation of, extracellular matrix (ECM) components. This degradation is mediated by matrix metalloproteinases (MMPs), enzymes that have been described in other pathogenic protozoa, and that have been linked to their increased motility and invasive capability. These enzymes also are upregulated in tumorigenic cells and have been implicated in metastasis of certain tumours. In the present study, in vitro experiments linked MMPs functionally to the degradation of the ECM. Gelatin zymography demonstrated enzyme activity in N. fowleri whole cell lysates, conditioned media and media collected from invasion assays. Western immunoblotting indicated the presence of the metalloproteinases MMP-2 (gelatinase A), MMP-9 (gelatinase B) and MMP-14 [membrane type-1 matrix metalloproteinase (MT1-MMP)]. Highly virulent mouse-passaged amoebae expressed higher levels of MMPs than weakly virulent axenically grown amoebae. The functional relevance of MMPs in media was indicated through the use of the MMP inhibitor, 1,10-phenanthroline. The collective in vitro results suggest that MMPs play a critical role in vivo in invasion of the CNS and that these enzymes may be amenable targets for limiting PAM.

Binding host proteins to the M protein contributes to the mortality associated with influenza–Streptococcus pyogenes superinfections

Andrea L. Herrera, Kuta Suso, Stephanie Allison, Abby Simon, Evelyn Schlenker, Victor C. Huber, Michael S. Chaussee

ABSTRACT

The mortality associated with influenza A virus (IAV) is often due to the development of secondary bacterial infections known as superinfections. The group A streptococcus (GAS) is a relatively uncommon cause of IAV superinfections, but the mortality of these infections is high. We used a murine model to determine whether the surface-localized GAS M protein contributes to the outcome of IAV–GAS superinfections. A comparison between wild-type GAS and an M protein mutant strain (emm3) showed that the M3 protein was essential to virulence. To determine whether the binding, or recruitment, of host proteins to the bacterial surface contributed to virulence, GAS was suspended with BALF collected from mice that had recovered from a sub-lethal infection with IAV. Following intranasal inoculation of naïve mice, the mortality associated with the wild-type strain, but not the emm3 mutant strain, was greater compared to mice inoculated with GAS suspended with either BALF from uninfected mice or PBS. Further analyses showed that both albumin and fibrinogen (Fg) were more abundant in the respiratory tract 8 days after IAV infection, that M3 bound both proteins to the bacterial surface, and that suspension of GAS with either protein increased GAS virulence in the absence of antecedent IAV infection. Overall, the results showed that M3 is essential to the virulence of GAS in an IAV superinfection and suggested that increased abundance of albumin and Fg in the respiratory tract following IAV infection enhanced host susceptibility to secondary GAS infection.

Marsupial and monotreme cathelicidins display antimicrobial activity, including against methicillin-resistant Staphylococcus aureus

Emma Peel, Yuanyuan Cheng, Julianne T. Djordjevic, Michael Kuhn, Tania Sorrell, Katherine Belov

ABSTRACT

With the growing demand for new antibiotics to combat increasing multi-drug resistance, a family of antimicrobial peptides known as cathelicidins has emerged as potential candidates. Expansions in cathelicidin-encoding genes in marsupials and monotremes are of specific interest as the peptides they encode have evolved to protect immunologically naive young in the harsh conditions of the pouch and burrow. Our previous work demonstrated that some marsupial and monotreme cathelicidins have broad-spectrum antibacterial activity and kill resistant bacteria, but the activity of many cathelicidins is unknown. To investigate associations between peptide antimicrobial activity and physiochemical properties, we tested 15 cathelicidin mature peptides from tammar wallaby, grey short-tailed opossum, platypus and echidna for antimicrobial activity against a range of bacterial and fungal clinical isolates. One opossum cathelicidin ModoCath4, tammar wallaby MaeuCath7 and echidna Taac-CATH1 had broad-spectrum antibacterial activity and killed methicillin-resistant Staphylococcus aureus. However, antimicrobial activity was reduced in the presence of serum or whole blood, and non-specific toxicity was observed at high concentrations. The active peptides were highly charged, potentially increasing binding to microbial surfaces, and contained amphipathic helical structures, which may facilitate membrane permeabilisation. Peptide sequence homology, net charge, amphipathicity and alpha helical content did not correlate with antimicrobial activity. However active peptides contained a significantly higher percentage of cationic residues than inactive ones, which may be used to predict active peptides in future work. Along with previous studies, our results indicate that marsupial and monotreme cathelicidins show potential for development as novel therapeutics to combat increasing antimicrobial resistance.

Alternative oxidase impacts ganoderic acid biosynthesis by regulating intracellular ROS levels in Ganoderma lucidum

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ABSTRACT

The alternative oxidase (AOX), which forms a branch of the mitochondrial respiratory electron transport pathway, functions to sustain electron flux and alleviate reactive oxygen species (ROS) production. In this article, a homologous AOX gene was identified in Ganoderma lucidum. The coding sequence of the AOX gene in G. lucidum contains 1038 nucleotides and encodes a protein of 39.48 kDa. RNA interference (RNAi) was used to study the function of AOX in G. lucidum, and two silenced strains (AOXi6 and AOXi21) were obtained, showing significant decreases of approximately 60 and 50%, respectively, in alternative pathway respiratory efficiency compared to WT. The content of ganoderic acid (GA) in the mutant strains AOXi6 and AOXi21 showed significant increases of approximately 42 and 44%, respectively, compared to WT. Elevated contents of intermediate metabolites in GA biosynthesis and elevated transcription levels of corresponding genes were also observed in the mutant strains AOXi6 and AOXi21. In addition, the intracellular ROS content in strains AOXi6 and AOXi21 was significantly increased, by approximately 1.75- and 1.93-fold, respectively, compared with WT. Furthermore, adding N-acetyl-l-cysteine (NAC), a ROS scavenger, significantly depressed the intracellular ROS content and GA accumulation in AOX-silenced strains. These results indicate that AOX affects GA biosynthesis by regulating intracellular ROS levels. Our research revealed the important role of AOX in the secondary metabolism of G. lucidum.

A manganese photosensitive tricarbonyl molecule [Mn(CO)3(tpa-κ3 N)]Br enhances antibiotic efficacy in a multi-drug-resistant Escherichia coli

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ABSTRACT

Carbon monoxide-releasing molecules (CORMs) are a promising class of new antimicrobials, with multiple modes of action that are distinct from those of standard antibiotics. The relentless increase in antimicrobial resistance, exacerbated by a lack of new antibiotics, necessitates a better understanding of how such novel agents act and might be used synergistically with established antibiotics. This work aimed to understand the mechanism(s) underlying synergy between a manganese-based photoactivated carbon monoxide-releasing molecule (PhotoCORM), [Mn(CO)3(tpa-κ3 N)]Br [tpa=tris(2-pyridylmethyl)amine], and various classes of antibiotics in their activities towards Escherichia coli EC958, a multi-drug-resistant uropathogen. The title compound acts synergistically with polymyxins [polymyxin B and colistin (polymyxin E)] by damaging the bacterial cytoplasmic membrane. [Mn(CO)3(tpa-κ3 N)]Br also potentiates the action of doxycycline, resulting in reduced expression of tetA, which encodes a tetracycline efflux pump. We show that, like tetracyclines, the breakdown products of [Mn(CO)3(tpa-κ3 N)]Br activation chelate iron and trigger an iron starvation response, which we propose to be a further basis for the synergies observed. Conversely, media supplemented with excess iron abrogated the inhibition of growth by doxycycline and the title compound. In conclusion, multiple factors contribute to the ability of this PhotoCORM to increase the efficacy of antibiotics in the polymyxin and tetracycline families. We propose that light-activated carbon monoxide release is not the sole basis of the antimicrobial activities of [Mn(CO)3(tpa-κ3 N)]Br.

Pseudomonas putida F1 uses energy taxis to sense hydroxycinnamic acids

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ABSTRACT

Soil bacteria such as pseudomonads are widely studied due to their diverse metabolic capabilities, particularly the ability to degrade both naturally occurring and xenobiotic aromatic compounds. Chemotaxis, the directed movement of cells in response to chemical gradients, is common in motile soil bacteria and the wide range of chemicals detected often mirrors the metabolic diversity observed. Pseudomonas putida F1 is a soil isolate capable of chemotaxis toward, and degradation of, numerous aromatic compounds. We showed that P. putida F1 is capable of degrading members of a class of naturally occurring aromatic compounds known as hydroxycinnamic acids, which are components of lignin and are ubiquitous in the soil environment. We also demonstrated the ability of P. putida F1 to sense three hydroxycinnamic acids: p-coumaric, caffeic and ferulic acids. The chemotaxis response to hydroxycinnamic acids was induced during growth in the presence of hydroxycinnamic acids and was negatively regulated by HcaR, the repressor of the hydroxycinnamic acid catabolic genes. Chemotaxis to the three hydroxycinnamic acids was dependent on catabolism, as a mutant lacking the gene encoding feruloyl-CoA synthetase (Fcs), which catalyzes the first step in hydroxycinnamic acid degradation, was unable to respond chemotactically toward p-coumaric, caffeic, or ferulic acids. We tested whether an energy taxis mutant could detect hydroxycinnamic acids and determined that hydroxycinnamic acid sensing is mediated by the energy taxis receptor Aer2.

Enterococcus faecalis and pathogenic streptococci inactivate daptomycin by releasing phospholipids

Elizabeth V. K. Ledger, Vera Pader, Andrew M. Edwards

ABSTRACT

Daptomycin is a lipopeptide antibiotic with activity against Gram-positive bacteria. We showed previously that Staphylococcus aureus can survive daptomycin exposure by releasing membrane phospholipids that inactivate the antibiotic. To determine whether other pathogens possess this defence mechanism, phospholipid release and daptomycin activity were measured after incubation of Staphylococcus epidermidis, group A or B streptococci, Streptococcus gordonii or Enterococcus faecalis with the antibiotic. All bacteria released phospholipids in response to daptomycin, which resulted in at least partial inactivation of the antibiotic. However, E. faecalis showed the highest levels of lipid release and daptomycin inactivation. As shown previously for S. aureus, phospholipid release by E. faecalis was inhibited by the lipid biosynthesis inhibitor platensimycin. In conclusion, several pathogenic Gram-positive bacteria, including E. faecalis, inactivate daptomycin by releasing phospholipids, which may contribute to the failure of daptomycin to resolve infections caused by these pathogens.

Profiling glucose-induced selective inhibition of disaccharide catabolism in Bacillus megaterium QM B1551 by stable isotope labelling

Tracy Youngster, Julie A. Wushensky, Ludmilla Aristilde

ABSTRACT

We investigated the co-catabolism of carbohydrate mixtures in Bacillus megaterium QM B1551 using a 13C-assisted metabolomics profiling approach. Specifically, we monitored the ability of B. megaterium to achieve the simultaneous catabolism of glucose and a common disaccharide – cellobiose, maltose, or sucrose. Growth experiments indicated that each disaccharide alone can serve as a sole carbon source for B. megaterium, in accordance with the genetic analysis of this bacterium, which predicted diverse metabolic capabilities. However, following growth on 13C-labelled glucose and each unlabelled disaccharide, the labelling patterns of the intracellular metabolites in glycolysis and the pentose phosphate pathway revealed a hierarchy in disaccharide catabolism: (i) complete inhibition of cellobiose catabolism, (ii) minimal catabolism of maltose and (iii) unbiased catabolism of sucrose. The labelling of amino acids confirmed this selective assimilation of each substrate in biomass precursors. This study highlights the fact that B. megaterium exhibits a mixed-carbohydrate utilization that is different from that of B. subtilis, the most studied model Bacillus species.

Escherichia coli type III secretion system 2 regulator EtrA promotes virulence of avian pathogenic Escherichia coli

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ABSTRACT

The Escherichia coli type III secretion system 2 (ETT2) is found in most E. coli strains, including pathogenic and commensal strains. Although many ETT2 gene clusters carry multiple genetic mutations or deletions, ETT2 is known to be involved in bacterial virulence. In enterohaemorrhagic E. coli (EHEC), ETT2 affects adhesion through the regulator EtrA, which regulates transcription and secretion of the type III secretion system (T3SS) encoded by the locus of enterocyte effacement (LEE). To date, no studies have been conducted on the role of EtrA in the virulence of avian pathogenic E. coli (APEC), which harbours only ETT2. Thus, we constructed etrA mutant and complemented strains of APEC and evaluated their phenotypes and pathogenicities. We found that the etrA gene deletion significantly reduced bacterial survival in macrophages, and proliferation and virulence in ducks. In addition, the etrA gene deletion reduced expression of the APEC fimbriae genes. Upregulation of genes encoding the pro-inflammatory cytokines interleukin (IL)-1β and IL-8 was also observed in HD-11 macrophages infected with the etrA gene mutant strain compared to the wild-type strain. Furthermore, the altered capacities of the mutant strain were restored by genetic complementation. Our observations demonstrate that the ETT2 regulator EtrA contributes to the virulence of APEC.

Volume 163, Issue 11, November 2017

Purified citritin in combination with vancomycin inhibits VRE in vitro and in vivo

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ABSTRACT

Gram-positive pathogens such as methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus faecium (VRE) have been frequently associated with bacterial resistance mechanisms. These mechanisms, in turn, restrict a range of therapeutic opportunities for the treatment of infections caused by these micro-organisms. Faced with this problem, the present study aims to isolate and characterize molecules with antimicrobial activity derived from the fungus Penicillium citrinum isolated from Cerrado soil. Furthermore, we also tested possible antibacterial potential alone and in combination with commercial antimicrobial agents. In this context, citrinin was isolated and characterized by nuclear magnetic resonance and electrospray ionization. Functional analyses showed MIC of 128 μg ml−1 against S. aureus ATCC 25923, E. faecalis ATCC 29212 and a clinical isolate of vancomycin-resistant E. faecium (VRE01). However, for a clinical strain of methicillin-resistant S. aureus (MRSA01), the MIC was 256 μg ml−1. In order to avoid such high concentrations and reduce the collateral effects, additive effects were evidenced by combining citrinin with cefoxitin against MRSA01 (FIC index=0.5) and also citrinin with vancomycin toward VRE01 (FIC index=0.5). In vivo studies with BALB/c-tipe mice (MRSA assay) demonstrated a clinical ineffectiveness of cefoxitin associated with citrinin (9.8 mg kg−1 of cefoxitin +0.2 mg kg−1 of citrinin), with this combination being inefficient to increase animal survival. However, the combination used in the treatment of VRE (23.5 mg kg−1 of citrinin +1.5 mg kg−1 of vancomycin) sepsis model was extremely promising, leading to an animal survival rate of 80 percent. In summary, our data show, for the first time, the possible successful use of citrinin associated with vancomycin for pathogenic bacteria control.

Markerless deletion of putative alanine dehydrogenase genes in Bacillus licheniformis using a codBA-based counterselection technique

David Kostner, Michael Rachinger, Wolfgang Liebl, Armin Ehrenreich

ABSTRACT

Bacillus licheniformis strains are used for the large-scale production of industrial exoenzymes from proteinaceous substrates, but details of the amino acid metabolism involved are largely unknown. In this study, two chromosomal genes putatively involved in amino acid metabolism of B. licheniformis were deleted to clarify their role. For this, a convenient counterselection system for markerless in-frame deletions was developed for B. licheniformis. A deletion plasmid containing up- and downstream DNA segments of the chromosomal deletion target was conjugated to B. licheniformis and integrated into the genome by homologous recombination. Thereafter, the counterselection was done by using a codBA cassette. The presence of cytosine deaminase and cytosine permease exerted a conditionally lethal phenotype on B. licheniformis cells in the presence of the cytosine analogue 5-fluorocytosine. Thereby clones were selected that lost the integrated vector sequence and the anticipated deletion target after a second recombination step. This method allows the construction of markerless mutants in Bacillus strains in iterative cycles. B. licheniformis MW3 derivatives lacking either one of the ORFs BL03009 or BL00190, encoding a putative alanine dehydrogenase and a similar putative enzyme, respectively, retained the ability to grow in minimal medium supplemented with alanine as the carbon source. In the double deletion mutant MW3 ΔBL03009 ΔBL00190, however, growth on alanine was completely abolished. These data indicate that the two encoded enzymes are paralogues fulfilling mutually replaceable functions in alanine utilization, and suggest that in B. licheniformis MW3 alanine utilization is initiated by direct oxidative transamination to pyruvate and ammonium.

N-acetylglucosamine affects Cryptococcus neoformans cell-wall composition and melanin architecture

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ABSTRACT

Cryptococcus neoformans is an environmental fungus that belongs to the phylum Basidiomycetes and is a major pathogen in immunocompromised patients. The ability of C. neoformans to produce melanin pigments represents its second most important virulence factor, after the presence of a polysaccharide capsule. Both the capsule and melanin are closely associated with the fungal cell wall, a complex structure that is essential for maintaining cell morphology and viability under conditions of stress. The amino sugar N-acetylglucosamine (GlcNAc) is a key constituent of the cell-wall chitin and is used for both N-linked glycosylation and GPI anchor synthesis. Recent studies have suggested additional roles for GlcNAc as an activator and mediator of cellular signalling in fungal and plant cells. Furthermore, chitin and chitosan polysaccharides interact with melanin pigments in the cell wall and have been found to be essential for melanization. Despite the importance of melanin, its molecular structure remains unresolved; however, we previously obtained critical insights using advanced nuclear magnetic resonance (NMR) and imaging techniques. In this study, we investigated the effect of GlcNAc supplementation on cryptococcal cell-wall composition and melanization. C. neoformans was able to metabolize GlcNAc as a sole source of carbon and nitrogen, indicating a capacity to use a component of a highly abundant polymer in the biospherenutritionally. C. neoformans cells grown with GlcNAc manifested changes in the chitosan cell-wall content, cell-wall thickness and capsule size. Supplementing cultures with isotopically 15N-labelled GlcNAc demonstrated that the exogenous monomer serves as a building block for chitin/chitosan and is incorporated into the cell wall. The altered chitin-to-chitosan ratio had no negative effects on the mother–daughter cell separation; growth with GlcNAc affected the fungal cell-wall scaffold, resulting in increased melanin deposition and assembly. In summary, GlcNAc supplementation had pleiotropic effects on cell-wall and melanin architectures, and thus established its capacity to perturb these structures, a property that could prove useful for metabolic tracking studies.

Response to photo-oxidative stress of Pseudomonas aeruginosa PAO1 mutants impaired in different functions

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ABSTRACT

Clinicians often have to deal with infections that are difficult to control because they are caused by superbugs resistant to many antibiotics. Alternatives to antibiotic treatment include antimicrobial photodynamic therapy (aPDT). The photodynamic process causes bacterial death, inducing oxidative stress through the photoactivation of photosensitizer molecules in the presence of oxygen. No PDT-resistant bacteria have been selected to date, thus the response to photo-oxidative stress in non-phototrophic bacteria needs further investigation. The opportunistic pathogen Pseudomonas aeruginosa, in particular, has been shown to be more tolerant to PDT than other micro-organisms. In order to find any genetic determinants involved in PDT-tolerance, a panel of transposon mutants of P. aeruginosa PAO1 involved in the quorum sensing signalling system and membrane cytoplasmic transport were photoinactivated as part of this study. Two pseudomonas quinolone signalling (PQS) knock-out mutants, pqsH - and pqsC -, were as PDT-sensitive as the PAO1 wild-type strains. Two PQS hyperproducer variants, pqsA - and rsaL -, were shown to be more tolerant to photo-oxidative stress than the wild-type strain. In the pqsA - mutant, the hyperpigmentation due to the presence of phenazines could protect cells against PDT stress, while in rsaL - no pigmentation was detectable. Furthermore, a mutant impaired in an ATP-binding cassette transport involved in maintaining the asymmetry of the outer membrane was significantly more tolerant to photo-oxidative stress than the wild-type strain. These observations support the involvement of quorum sensing and the importance of the bacterial cell envelope when dealing with photo-oxidative stress induced by photodynamic treatment.

Pseudomonas phage inhibition of Candida albicans

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ABSTRACT

Pseudomonas aeruginosa (Pa) and Candida albicans (Ca) are major bacterial and fungal pathogens in immunocompromised hosts, and notably in the airways of cystic fibrosis patients. The bacteriophages of Pa physically alter biofilms, and were recently shown to inhibit the biofilms of Aspergillus fumigatus. To understand the range of this viral–fungal interaction, we studied Pa phages Pf4 and Pf1, and their interactions with Ca biofilm formation and preformed Ca biofilm. Both forms of Ca biofilm development, as well as planktonic Ca growth, were inhibited by either phage. The inhibition of biofilm was reversed by the addition of iron, suggesting that the mechanism of phage action on Ca involves denial of iron. Birefringence studies on added phage showed an ordered structure of binding to Ca. Electron microscopic observations indicated phage aggregation in the biofilm extracellular matrix. Bacteriophage–fungal interactions may be a general feature with several pathogens in the fungal kingdom.

Ultrastructural and microbial analyses of cellulose degradation in leaf-cutter ant colonies

Rolando Daniel Moreira-Soto, Ethel Sanchez, Cameron R. Currie, Adrian A. Pinto-Tomás

ABSTRACT

Leaf-cutter ants (Atta and Acromyrmex) use fresh leaves to cultivate a mutualistic fungus (Leucoagaricus gongylophorus) for food in underground gardens. A new ant queen propagates the cultivar by taking a small fragment of fungus from her parent colony on her nuptial flight and uses it to begin her own colony. Recent research has shown that the ants' fungus gardens are colonized by symbiotic bacteria that perform important functions related to nitrogen fixation and have been implicated in contributing to plant biomass degradation. Here, we combine bacterial culturing in several media for counts and identification using the 16S rRNA gene with electron microscopy to investigate the process of cellulose degradation in the fungus garden and refuse dumps, and to assess the potential role of symbiotic bacteria. We show through electron microscopy that plant cell walls are visibly degraded in the bottom section of fungus gardens and refuse dumps, and that bacteria are more abundant in these sections. We also consistently isolated cellulolytic bacteria from all sections of fungus gardens. Finally, we show by culture-dependent and electron microscopy analysis that the fungus garden pellets carried by recently mated queens are colonized by fungus garden-associated bacteria. Taken together, our results indicate that cellulose is degraded in fungus gardens, and that fungus garden bacteria that may contribute to this deconstruction are vertically transmitted by new queens.

Stenotrophomonas maltophilia produces an EntC-dependent catecholate siderophore that is distinct from enterobactin

Megan Y. Nas, Nicholas P. Cianciotto

ABSTRACT

Stenotrophomonas maltophilia, a Gram-negative, multi-drug-resistant bacterium, is increasingly recognized as a key opportunistic pathogen. Thus, we embarked upon an investigation of S. maltophilia iron acquisition. To begin, we determined that the genome of strain K279a is predicted to encode a complete siderophore system, including a biosynthesis pathway, an outer-membrane receptor for ferrisiderophore, and other import and export machinery. Compatible with these data, K279a and other clinical isolates of S. maltophilia secreted a siderophore-like activity when grown at 25–37 °C in low-iron media, as demonstrated by a chrome azurol S assay, which detects iron chelation, and Arnow and Rioux assays, which detect catecholate structures. Importantly, these supernatants rescued the growth of iron-starved S. maltophilia, documenting the presence of a biologically active siderophore. A mutation in one of the predicted biosynthesis genes (entC) abolished production of the siderophore and impaired bacterial growth in low-iron conditions. Inactivation of the putative receptor gene (fepA) prevented the utilization of siderophore-containing supernatants for growth in low-iron conditions. Although the biosynthesis and import loci showed some similarity to those of enterobactin, a well-known catecholate made by enteric bacteria, the siderophore of K279a was unable to rescue the growth of an enterobactin-utilizing indicator strain, and conversely iron-starved S. maltophilia could not use purified enterobactin. Furthermore, the S. maltophilia siderophore displayed patterns of solubility in organic compounds and mobility upon thin-layer chromatography that were distinct from those of enterobactin and its derivative, salmochelin. Together, these data demonstrate that S. maltophilia secretes a novel catecholate siderophore.

Phosphoglycerate kinase acts as a futile cycle at high temperature

Theresa Kouril, Johann J. Eicher, Bettina Siebers, Jacky L. Snoep

ABSTRACT

In (hyper) thermophilic organisms metabolic processes have to be adapted to function optimally at high temperature. We compared the gluconeogenic conversion of 3-phosphoglycerate via 1,3-bisphosphoglycerate to glyceraldehyde-3-phosphate at 30 °C and at 70 °C. At 30 °C it was possible to produce 1,3-bisphosphoglycerate from 3-phosphoglycerate with phosphoglycerate kinase, but at 70 °C, 1,3-bisphosphoglycerate was dephosphorylated rapidly to 3-phosphoglycerate, effectively turning the phosphoglycerate kinase into a futile cycle. When phosphoglycerate kinase was incubated together with glyceraldehyde 3-phosphate dehydrogenase it was possible to convert 3-phosphoglycerate to glyceraldehyde 3-phosphate, both at 30 °C and at 70 °C, however, at 70 °C only low concentrations of product were observed due to thermal instability of glyceraldehyde 3-phosphate. Thus, thermolabile intermediates challenge central metabolic reactions and require special adaptation strategies for life at high temperature.

Pseudomonas aeruginosa variants obtained from veterinary clinical samples reveal a role for cyclic di-GMP in biofilm formation and colony morphology

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ABSTRACT

Overuse of antibiotics is contributing to an emerging antimicrobial resistance crisis. To better understand how bacteria adapt tolerance and resist antibiotic treatment, Pseudomonas aeruginosa isolates obtained from infection sites sampled from companion animals were collected and evaluated for phenotypic differences. Selected pairs of clonal isolates were obtained from individual infection samples and were assessed for antibiotic susceptibility, cyclic di-GMP levels, biofilm production, motility and genetic-relatedness. A total of 18 samples from equine, feline and canine origin were characterized. A sample from canine otitis media produced a phenotypically heterogeneous pair of P. aeruginosa isolates, 42121A and 42121B, which during growth on culture medium respectively exhibited hyper dye-binding small colony morphology and wild-type phenotypes. Antibiotic susceptibility to gentamicin and ciprofloxacin also differed between this pair of clonal isolates. Sequence analysis of gyrA, a gene known to be involved in ciprofloxacin resistance, indicated that 42121A and 42121B both contained mutations that confer ciprofloxacin resistance, but this did not explain the differences in ciprofloxacin resistance that were observed. Cyclic di-GMP levels also varied between this pair of isolates and were shown to contribute to the observed colony morphology variation and ability to form a biofilm. Our results demonstrate the role of cyclic di-GMP in generating the observed morphological phenotypes that are known to contribute to biofilm-mediated antibiotic tolerance. The generation of phenotypic diversity may go unnoticed during standard diagnostic evaluation, which potentially impacts the therapeutic strategy chosen to treat the corresponding infection and may contribute to the spread of antibiotic resistance.

Exogenous polyunsaturated fatty acids (PUFAs) alter phospholipid composition, membrane permeability, biofilm formation and motility in Acinetobacter baumannii

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ABSTRACT

Acinetobacter baumannii is a ubiquitous multidrug-resistant bacteria that is found on a variety of surfaces, including skin, hair and soil. During the past decade, A. baumannii has emerged as a significant cause of nosocomial infections in the United States. Recent studies have highlighted the ability of some bacteria to utilize a wide variety of fatty acids as a membrane remodelling strategy. Considering this, we hypothesized that fatty acids may have an effect on the emerging pathogen A. baumannii. Thin-layer chromatography indicated structural alterations to major phospholipids. Liquid chromatography/mass spectrometry confirmed the assimilation of numerous exogenous polyunsaturated fatty acids (PUFAs) into the phospholipid species of A. baumannii. The incorporation of fatty acids affected several bacterial phenotypes, including membrane permeability, biofilm formation, surface motility and antimicrobial peptide resistance.

The O-antigen structure of bacterium Comamonas aquatica CJG

Xiqian Wang, Anna N. Kondakova, Yutong Zhu, Yuriy A. Knirel, Aidong Han

ABSTRACT

Genus Comamonas is a group of bacteria that are able to degrade a variety of environmental waste. Comamonas aquatica CJG (C. aquatica) in this genus is able to absorb low-density lipoprotein but not high-density lipoprotein of human serum. Using 1H and 13C NMR spectroscopy, we found that the O-polysaccharide (O-antigen) of this bacterium is comprised of a disaccharide repeat (O-unit) of d-glucose and 2-O-acetyl-l-rhamnose, which is shared by Serratia marcescens O6. The O-antigen gene cluster of C. aquatica, which is located between coaX and tnp4 genes, contains rhamnose synthesis genes, glycosyl and acetyl transferase genes, and ATP-binding cassette transporter genes, and therefore is consistent with the O-antigen structure determined here.

The amino-terminal domain of ELL transcription elongation factor is essential for ELL function in Schizosaccharomyces pombe

Kumari Sweta, Preeti Dabas, Kamal Jain, Nimisha Sharma

ABSTRACT

Transcriptional elongation is a critical step for regulating expression of protein-coding genes. Multiple transcription elongation factors have been identified in vitro, but the physiological roles of many of them are still not clearly understood. The ELL (Eleven nineteen Lysine rich Leukemia) family of transcription elongation factors are conserved from fission yeast to humans. Schizosaccharomyces pombe contains a single ELL homolog (SpELL) that is not essential for its survival. Therefore to gain insights into the in vivo cellular functions of SpELL, we identified phenotypes associated with deletion of ell1 in S. pombe. Our results demonstrate that SpELL is required for normal growth of S. pombe cells. Furthermore, cells lacking ell1 + exhibit a decrease in survival when exposed to DNA-damaging conditions, but their growth is not affected under environmental stress conditions. ELL orthologs in different organisms contain three conserved domains, an amino-terminal domain, a middle domain and a carboxyl-terminal domain. We also carried out an in vivo functional mapping of these conserved domains within S. pombe ELL and uncovered a critical role for its amino-terminus in regulating all its cellular functions, including growth under different conditions, transcriptional elongation potential and interaction with S. pombe EAF. Taken together our results suggest that the domain organization of ELL proteins is conserved across species, but the in vivo functions as well as the relationship between the various domains and roles of ELL show species-specific differences.

VerZ, a Zn (II) 2Cys6 DNA-binding protein, regulates the biosynthesis of verticillin in Clonostachys rogersoniana

Zhe Guo, Tianchao Hao, Ying Wang, Yuanyuan Pan, Fengxia Ren, Xingzhong Liu, Yongsheng Che, Gang Liu

ABSTRACT

Verticillins are the dimeric epipolythiodioxopiperazines (ETPs) produced by the fungus Clonostachys rogersoniana. Despite their profound biological effects, they are commonly produced in rice medium as complex mixtures that are difficult to separate, limiting further study and evaluation for this class of metabolites. Therefore, there is an urgent need to understand the regulation of verticillin biosynthesis. Recently, we cloned the biosynthetic gene cluster of verticillin (ver), and identified the only regulatory gene verZ in this cluster. The deduced product of verZ contains a basic Zn(II)2Cys6 DNA-binding domain. Disruption of verZ significantly reduced the production of 11′-deoxyverticillin A (C42) and decreased the transcriptional level of the verticillin biosynthetic genes. To further reveal its function, a recombinant gene encoding the DNA-binding domain of VerZ was expressed in E. coli and the His6-tagged VerZbd was purified to homogeneity by Ni-NTA chromatography. Electrophoretic mobility shift assays (EMSAs) showed that VerZbd bound specifically to the promoter regions of the verticillin biosynthetic genes. Bioinformatic analysis of the VerZbd-binding regions revealed a conserved palindromic sequence of (T/C)(C/A)(G/T)GN3CC(G/T)(A/G)(G/C). Base substitution of the conserved sequence completely abolished the binding activity of VerZbd to its targets. These results suggested that VerZ controls verticillin production through directly activating transcription of the biosynthetic genes in C. rogersoniana.

Whole genome transcriptomics reveals global effects including up-regulation of Francisella pathogenicity island gene expression during active stringent response in the highly virulent Francisella tularensis subsp. tularensis SCHU S4

Amber L. Murch, Paul J. Skipp, Peter L. Roach, Petra C. F. Oyston

ABSTRACT

During conditions of nutrient limitation bacteria undergo a series of global gene expression changes to survive conditions of amino acid and fatty acid starvation. Rapid reallocation of cellular resources is brought about by gene expression changes coordinated by the signalling nucleotides' guanosine tetraphosphate or pentaphosphate, collectively termed (p)ppGpp and is known as the stringent response. The stringent response has been implicated in bacterial virulence, with elevated (p)ppGpp levels being associated with increased virulence gene expression. This has been observed in the highly pathogenic Francisella tularensis sub spp. tularensis SCHU S4, the causative agent of tularaemia. Here, we aimed to artificially induce the stringent response by culturing F. tularensis in the presence of the amino acid analogue l-serine hydroxamate. Serine hydroxamate competitively inhibits tRNAser aminoacylation, causing an accumulation of uncharged tRNA. The uncharged tRNA enters the A site on the translating bacterial ribosome and causes ribosome stalling, in turn stimulating the production of (p)ppGpp and activation of the stringent response. Using the essential virulence gene iglC, which is encoded on the Francisella pathogenicity island (FPI) as a marker of active stringent response, we optimized the culture conditions required for the investigation of virulence gene expression under conditions of nutrient limitation. We subsequently used whole genome RNA-seq to show how F. tularensis alters gene expression on a global scale during active stringent response. Key findings included up-regulation of genes involved in virulence, stress responses and metabolism, and down-regulation of genes involved in metabolite transport and cell division. F. tularensis is a highly virulent intracellular pathogen capable of causing debilitating or fatal disease at extremely low infectious doses. However, virulence mechanisms are still poorly understood. The stringent response is widely recognized as a diverse and complex bacterial stress response implicated in virulence. This work describes the global gene expression profile of F. tularensis SCHU S4 under active stringent response for the first time. Herein we provide evidence for an association of active stringent response with FPI virulence gene expression. Our results further the understanding of the molecular basis of virulence and regulation thereof in F. tularensis. These results also support research into genes involved in (p)ppGpp production and polyphosphate biosynthesis and their applicability as targets for novel antimicrobials.

The Agrobacterium tumefaciens CheY-like protein ClaR regulates biofilm formation

Nathan Feirer, DohHyun Kim, Jing Xu, Nico Fernandez, Christopher M. Waters, Clay Fuqua

ABSTRACT

The switch from a motile, planktonic existence to an attached biofilm is a major bacterial lifestyle transition that is often mediated by complex regulatory pathways. In this report, we describe a CheY-like protein required for control of the motile-to-sessile switch in the plant pathogen Agrobacterium tumefaciens. This regulator, which we have designated ClaR, possesses two distinct CheY-like receiver (REC) domains and is involved in the negative regulation of biofilm formation, through production of the unipolar polysaccharide (UPP) adhesin and cellulose. The ClaR REC domains share predicted structural homology with characterized REC domains and contain the majority of active site residues known to be essential for protein phosphorylation. REC1 is missing the conserved aspartate (N72) residue and although present in REC 2 (D193), it is not required for ClaR-dependent regulation suggesting that phosphorylation, which modulates the activity of many CheY-like proteins, appears not to be essential for ClaR activity. We also show that ClaR-dependent negative regulation of attachment is diminished significantly in mutants for PruA and PruR, proteins known to be involved in a pterin-mediated attachment regulation pathway. In A. tumefaciens, pterins are required for control of the intracellular signal cyclic diguanylate monophosphate through the DcpA regulator, but our findings suggest that pterin-dependent ClaR control of attachment can function independently from DcpA, including dampening of c-di-GMP levels. This report of a novel CheY-type biofilm regulator in A. tumefaciens thus also adds significant details to the role of pterin-mediated signalling.

Unravelling the transcriptional regulation of Saccharomyces cerevisiae UGA genes: the dual role of transcription factor Leu3

Marcos Palavecino-Ruiz, Mariana Bermudez-Moretti, Susana Correa-Garcia

ABSTRACT

Yeast cells can use γ-aminobutyric acid (GABA), a non-protein amino acid, as a nitrogen source that is mainly imported by the permease Uga4 and catabolized by the enzymes GABA transaminase and succinate-semialdehyde dehydrogenase, encoded by the UGA1 and UGA2 genes, respectively. The three UGA genes are inducible by GABA and subject to nitrogen catabolite repression. Hence, their regulation occurs through two mechanisms, one dependent on the inducer and the other on nitrogen source quality. The aim of this work was to better understand the molecular mechanisms of transcription factors acting on different regulatory elements present in UGA promoters, such as Uga3, Dal81, Leu3 and the GATA factors, and to establish the mechanism of the concerted action between them. We found that Gat1 plays an important role in the induction of UGA4 transcription by GABA and that Gzf3 has an effect in cells grown in a poor nitrogen source such as proline and that this effect is positive on UGA4 expression. We also found that Gln3 and Dal80 affect the interaction of Uga3 and Dal81 on UGA promoters. Moreover, our results indicated that the repressing activity of Leu3 on UGA4 and UGA1 occurs through Dal80 since we demonstrated that Leu3 facilitates Dal80 interaction with DNA. However, when the expression of GATA factors is null or negligible, Leu3 functions as an activator.

Nitrogen regulator GlnR directly controls transcription of genes encoding lysine deacetylases in Actinobacteria

Ying Xu, Di You, Bang-Ce Ye

ABSTRACT

N-Lysine acetylation is a dynamic, reversible and regulatory post-translational modification (PTM) in prokaryotes, which integrates and coordinates metabolisms responding to environmental clues. However, the molecular mechanism underlying the signalling pathway from nutrient sensing to protein acetylation remains incompletely understood in micro-organisms. Here we found that global nitrogen regulator GlnR directly controls transcription of genes encoding lysine deacetylases in Actinobacteria. Electrophoretic mobility shift assays and real-time PCR (RT-PCR) in three Actinobacteria species (Saccharopolyspora erythraea, Streptomyces coelicolor and Mycobacterium smegmatis) revealed that GlnR regulator protein is able to interact with the promoter regions of these genes and activate their transcription. Furthermore, it was demonstrated that cellular acetylation status (acetylome) is modulated by extracellular nitrogen availability. Our results present an example of the novel complete signal transduction mechanism of regulating protein deacetylation through a nutrient-sensing pleiotropic regulator in response to nutrient availability.

The nitrogen-regulated response regulator NrrA is a conserved regulator of glycogen catabolism in β-cyanobacteria

Shigeki Ehira, Yuka Shimmori, Satoru Watanabe, Hiroaki Kato, Hirofumi Yoshikawa, Masayuki Ohmori

ABSTRACT

Cyanobacteria acclimatize to nitrogen deprivation by changing cellular metabolism. The nitrogen-regulated response regulator A (NrrA) is involved in regulation of carbon metabolism in response to nitrogen deprivation. However, it has not been elucidated whether these regulatory functions of NrrA are particular to a few model strains or are general among diverse cyanobacteria. In this study, we showed that regulation and functions of NrrA were highly conserved among β-cyanobacteria, which included physiologically and ecologically diverse strains. All β-cyanobacteria had the nrrA gene, while it was absent in α-cyanobacteria. The canonical NtcA-dependent promoter sequence was found upstream of the nrrA genes in most β-cyanobacteria, and its expression was indeed induced by nitrogen deprivation. Biochemical and physiological analyses of NrrA from phylogenetically distinct cyanobacteria indicated that regulation of NrrA activity and NrrA functions, namely activation of glycogen catabolism, were also common to β-cyanobacteria. These results support the conclusion that NrrA plays an important role in acclimatization to nitrogen deprivation, and that activation of glycogen catabolism is a primitive response to nitrogen deprivation in β-cyanobacteria.

The sibling sRNAs NgncR_162 and NgncR_163 of Neisseria gonorrhoeae participate in the expression control of metabolic, transport and regulatory proteins

Susanne Bauer, Jonas Helmreich, Marie Zachary, Marc Kaethner, Elisabeth Heinrichs, Thomas Rudel, Dagmar Beier

ABSTRACT

Neisseria gonorrhoeae is the causative agent of gonorrhoea, the second most common bacterial sexually transmitted disease. Riboregulation mediated by small regulatory RNAs (sRNAs) is increasingly recognized as an important means of gene expression control in this human-restricted pathogen. sRNAs act at the post-transcriptional level by base-pairing with their target mRNAs which affects translation initiation and/or mRNA stability. In this study we initiated the characterization of a pair of highly conserved sRNAs of N. gonorrhoeae which exhibit redundant functions in the control of a common set of target genes. The identified targets of the sibling sRNAs NgncR_162 and NgncR_163 participate in basic metabolic processes including the methylcitrate and citrate cycle, aa uptake and degradation, and also in transcription regulation. Our data indicate that the sibling sRNAs control their targets via direct base-pairing between the same single-stranded domain(s) of the sRNA and the ribosome binding site in the 5′-untranslated region of the mRNA.

Volume 163, Issue 12, December 2017

Multi-copy single-stranded DNA in Escherichia coli

Xianxing Xie, Ruifu Yang

ABSTRACT

Multi-copy single-stranded DNA (msDNA) is composed of covalently bound single-stranded DNA and RNA, and synthesized by retron-encoded reverse transcriptase. msDNA-synthesizing systems are thought to be a recent acquisition by Escherichia coli because, to date, only seven types of msDNA, which differ markedly in their primary nucleotide sequences, have been found in a small subset of E. coli strains. The wide use of E. coli in molecular research means that it is important to understand more about these stable, covalently bound, single-stranded DNA or RNA compounds. The present review provides insights into the molecular biosynthesis, distribution and function of E. coli msDNA to raise awareness about these special molecules.

Guardians of the mycobacterial genome: A review on DNA repair systems in Mycobacterium tuberculosis

Amandeep Singh

ABSTRACT

The genomic integrity of Mycobacterium tuberculosis is continuously threatened by the harsh survival conditions inside host macrophages, due to immune and antibiotic stresses. Faithful genome maintenance and repair must be accomplished under stress for the bacillus to survive in the host, necessitating a robust DNA repair system. The importance of DNA repair systems in pathogenesis is well established. Previous examination of the M. tuberculosis genome revealed homologues of almost all the major DNA repair systems, i.e. nucleotide excision repair (NER), base excision repair (BER), homologous recombination (HR) and non-homologous end joining (NHEJ). However, recent developments in the field have pointed to the presence of novel proteins and pathways in mycobacteria. Homologues of archeal mismatch repair proteins were recently reported in mycobacteria, a pathway previously thought to be absent. RecBCD, the major nuclease-helicase enzymes involved in HR in E. coli, were implicated in the single-strand annealing (SSA) pathway. Novel roles of archeo-eukaryotic primase (AEP) polymerases, previously thought to be exclusive to NHEJ, have been reported in BER. Many new proteins with a probable role in DNA repair have also been discovered. It is now realized that the DNA repair systems in M. tuberculosis are highly evolved and have redundant backup mechanisms to mend the damage. This review is an attempt to summarize our current understanding of the DNA repair systems in M. tuberculosis.

Borrelia burgdorferi glycosaminoglycan-binding proteins: a potential target for new therapeutics against Lyme disease

Yi-Pin Lin, Lingyun Li, Fuming Zhang, Robert J. Linhardt

ABSTRACT

The spirochete bacterium Borrelia burgdorferi sensu lato is the causative agent of Lyme disease, the most common vector-borne disease in Europe and the United States. The spirochetes can be transmitted to humans via ticks, and then spread to different tissues, leading to arthritis, carditis and neuroborreliosis. Although antibiotics have commonly been used to treat infected individuals, some treated patients do not respond to antibiotics and experience persistent, long-term arthritis. Thus, there is a need to investigate alternative therapeutics against Lyme disease. The spirochete bacterium colonization is partly attributed to the binding of the bacterial outer-surface proteins to the glycosaminoglycan (GAG) chains of host proteoglycans. Blocking the binding of these proteins to GAGs is a potential strategy to prevent infection. In this review, we have summarized the recent reports of B. burgdorferi sensu lato GAG-binding proteins and discussed the potential use of synthetic and semi-synthetic compounds, including GAG analogues, to block pathogen interaction with GAGs. Such information should motivate the discovery and development of novel GAG analogues as new therapeutics for Lyme disease. New therapeutic approaches should eventually reduce the burden of Lyme disease and improve human health.

Endophytic fungal production rates of volatile organic compounds are highest under microaerophilic conditions

Heidi R. Schoen, Walter Berk Knighton, Brent M. Peyton

ABSTRACT

Volatile organic compound (VOC) production from an endophytic fungus was quantified at four oxygen concentrations (0, 1, 13 and 21%) throughout culture growth phases. The filamentous fungus, a Nodulisporium sp. (designated TI-13), was grown in a solid-state reactor with an agricultural byproduct, beet pulp, as the solid substrate. The VOCs, with potential applications as biofuels, natural flavour compounds and bioactive mixtures, were measured with a recently introduced platinum catalyst and proton transfer reaction mass spectrometry quantification system. The highest-specific production rates of carbon number four and higher VOCs were observed under microaerophilic conditions, which is the expected environment within the plant host. Specific production rates of two ester compounds increased by at least 19 times under microaerophilic conditions compared with those under any other oxygen concentration studied. Total VOC production, including small molecules such as ethanol and acetaldehyde, increased by 23 times when compared between aerobic and anoxic conditions, predominately due to increased production of ethanol. Additionally, total specific production for all 21 compounds quantified was highest under reduced oxygen conditions.

Chemotaxis to self-generated AI-2 promotes biofilm formation in Escherichia coli

Sneha Jani, Andrew L. Seely, George L. Peabody V, Arul Jayaraman, Michael D. Manson

ABSTRACT

Responses to the interspecies quorum-sensing signal autoinducer-2 (AI-2) regulate the patterns of gene expression that promote biofilm development. Escherichia coli also senses AI-2 as a chemoattractant, a response that requires the periplasmic AI-2-binding protein LsrB and the chemoreceptor Tsr. Here, we confirm, as previously observed, that under static conditions highly motile E. coli cells self-aggregate and form surface-adherent structures more readily than cells lacking LsrB and Tsr, or than ΔluxS cells unable to produce AI-2. This difference is observed both at 37 and 30 °C. Cells deleted for the genes encoding the lsrACDBFG operon repressor (ΔlsrR), or the AI-2 kinase (ΔlsrK), or an AI-2 uptake channel protein (ΔlsrC), or an AI-2 metabolism enzyme (ΔlsrG) are also defective in biofilm formation. The Δtsr and ΔlsrB cells are totally defective in AI-2 chemotaxis, whereas the other mutants show normal or near-normal chemotaxis to external gradients of AI-2. These data demonstrate that chemotaxis to external AI-2 is necessary but not sufficient to induce the full range of density-dependent behaviours that are required for optimal biofilm formation. We also demonstrate that, compared to other binding-protein-dependent chemotaxis systems in E. coli, low levels (on the order of ~250 molecules of periplasmic LsrB per wild-type cell and as low as ~50 molecules per cell in some mutants) are adequate for a strong chemotaxis response to external gradients of AI-2.

Herbicide ingredients change Salmonella enterica sv. Typhimurium and Escherichia coli antibiotic responses

Brigitta Kurenbach, Paddy S. Gibson, Amy M. Hill, Adam S. Bitzer, Mark W. Silby, William Godsoe, Jack A. Heinemann

ABSTRACT

Herbicides are frequently released into both rural and urban environments. Commercial herbicide formulations induce adaptive changes in the way bacteria respond to antibiotics. Salmonella enterica sv. Typhimurium and Escherichia coli were exposed to common co-formulants of formulations, and S. enterica sv. Typhimurium was exposed to active ingredients dicamba, 2,4-D and glyphosate to determine what ingredients of the commercial formulations caused this effect. Co-formulants Tween80 and carboxymethyl cellulose induced changes in response, but the pattern of the responses differed from the active ingredients, and effect sizes were smaller. A commercial wetting agent did not affect antibiotic responses. Active ingredients induced changes in antibiotic responses similar to those caused by complete formulations. This occurred at or below recommended application concentrations. Targeted deletion of efflux pump genes largely neutralized the adaptive response in the cases of increased survival in antibiotics, indicating that the biochemistry of induced resistance was the same for formulations and specific ingredients. We found that glyphosate, dicamba, and 2,4-D, as well as co-formulants in commercial herbicides, induced a change in susceptibility of the potentially pathogenic bacteria E. coli and S. enterica to multiple antibiotics. This was measured using the efficiency of plating (EOP), the relative survival of the bacteria when exposed to herbicide and antibiotic, or just antibiotic, compared to survival on permissive media. This work will help to inform the use of non-medicinal chemical agents that induce changes in antibiotic responses.

Co-expression and purification of the RadA recombinase with the RadB paralog from Haloferax volcanii yields heteromeric ring-like structures

Bushra B. Patoli, Jody A. Winter, Atif A. Patoli, Robin M. Delahay, Karen A. Bunting

ABSTRACT

The study of archaeal proteins and the processes to which they contribute poses particular challenges due to the often extreme environments in which they function. DNA recombination, replication and repair proteins of the halophilic euryarchaeon, Haloferax volcanii (Hvo) are of particular interest as they tend to resemble eukaryotic counterparts in both structure and activity, and genetic tools are available to facilitate their analysis. In the present study, we show using bioinformatics approaches that the Hvo RecA-like protein RadA is structurally similar to other recombinases although is distinguished by a unique acidic insertion loop. To facilitate expression of Hvo RadA a co-expression approach was used, providing its lone paralog, RadB, as a binding partner. At present, structural and biochemical characterization of Hvo RadA is lacking. Here, we describe for the first time co-expression of Hvo RadA with RadB and purification of these proteins as a complex under in vitro conditions. Purification procedures were performed under high salt concentration (>1 M sodium chloride) to maintain the solubility of the proteins. Quantitative densitometry analysis of the co-expressed and co-purified RadAB complex estimated the ratio of RadA to RadB to be 4:1, which suggests that the proteins interact with a specific stoichiometry. Based on a combination of analyses, including size exclusion chromatography, Western blot and electron microscopy observations, we suggest that RadA multimerizes into a ring-like structure in the absence of DNA and nucleoside co-factor.

Complementation of a metK-deficient E. coli strain with heterologous AdoMet synthetase genes

Gwenn G. Parungao, Mojun Zhao, Qinzhe Wang, Stephen P. Zano, Ronald E. Viola, Robert M. Blumenthal

ABSTRACT

S-adenosyl-l-methionine (AdoMet) is an essential metabolite, playing a wide variety of metabolic roles. The enzyme that produces AdoMet from l-methionine and ATP (methionine adenosyltransferase, MAT) is thus an attractive target for anti-cancer and antimicrobial agents. It would be very useful to have a system that allows rapid identification of species-specific inhibitors of this essential enzyme. A previously generated E. coli strain, lacking MAT (ΔmetK) but containing a heterologous AdoMet transporter, was successfully complemented with heterologous metK genes from several bacterial pathogens, as well as with MAT genes from a fungal pathogen and Homo sapiens. The nine tested genes, which vary in both sequence and kinetic properties, all complemented strain MOB1490 well in rich medium. When these strains were grown in glucose minimal medium, growth delays or defects were observed with some specific metK genes, defects that were dramatically reduced if l-methionine was added to the medium.

Dual-label flow cytometry-based host cell adhesion assay to ascertain the prospect of probiotic Lactobacillus plantarum in niche-specific antibacterial therapy

Sandipan Mukherjee, Aiyagari Ramesh

ABSTRACT

Host cell adhesion assays that provide quantitative insight on the potential of lactic acid bacteria (LAB) to inhibit adhesion of intestinal pathogens can be leveraged for the development of niche-specific anti-adhesion therapy. Herein, we report a dual-colour flow cytometry (FCM) analysis to assess the ability of probiotic Lactobacillus plantarum strains to impede adhesion of Enterococcus faecalis, Listeria monocytogenes and Staphylococcus aureus onto HT-29 cells. FCM in conjunction with a hierarchical cluster analysis could discern the anti-adhesion potential of L. plantarum strains, wherein the efficacy of L. plantarum DF9 was on a par with the probiotic L. rhamnosus GG. Combination of FCM with principal component analysis illustrated the relative influence of LAB strains on adhesion parameters k d and e m of the pathogen and identified probiotic LAB suitable for anti-adhesion intervention. The analytical merit of the FCM analysis was captured in host cell adhesion assays that measured relative elimination of adhered LAB vis-à-vis pathogens, on exposure to either LAB bacteriocins or therapeutic antibiotics. It is envisaged that the dual-colour FCM-based adhesion assay described herein would enable a fundamental understanding of the host cell adhesion process and stimulate interest in probiotic LAB as safe anti-adhesion therapeutic agents against gastrointestinal pathogens.

Amphibian skin defences show variation in ability to inhibit growth of Batrachochytrium dendrobatidis isolates from the Global Panzootic Lineage

Rachael Ellen Antwis, Ché Weldon

ABSTRACT

The fungal pathogen Batrachochytrium dendrobatidis has caused declines and extinctions in hundreds of amphibian species across the world. Virulence varies among and within lineages; the Global Panzootic Lineage (GPL) is the most pathogenic, although there is also variation in lethality among GPL isolates. Amphibians have a number of defences against pathogens, and skin products including the microbiota and host peptides have considerable influence over disease progression. Here we demonstrate that the collective skin products (the mucosome) of two amphibian species show significant variation in their ability to inhibit different globally distributed isolates of GPL. This may in part explain the variation in disease susceptibility of hosts to different strains of B. dendrobatidis. More work is required to identify particular traits associated with mucosomes that confer broad-spectrum inhibition across GPL in order to facilitate the development of prophylaxis and/or treatments for chytridiomycosis in situ.

Haem-iron plays a key role in the regulation of the Ess/type VII secretion system of Staphylococcus aureus RN6390

M. Guillermina Casabona, Holger Kneuper, Daniela Alferes de Lima, Catriona P. Harkins, Martin Zoltner, Erik Hjerde, Matthew T. G. Holden, Tracy Palmer

ABSTRACT

The Staphylococcus aureus type VII protein secretion system (T7SS) plays important roles in virulence and intra-species competition. Here we show that the T7SS in strain RN6390 is activated by supplementing the growth medium with haemoglobin, and its cofactor haemin (haem B). Transcript analysis and secretion assays suggest that activation by haemin occurs at a transcriptional and a post-translational level. Loss of T7 secretion activity by deletion of essC results in upregulation of genes required for iron acquisition. Taken together these findings suggest that the T7SS plays a role in iron homeostasis in at least some S. aureus strains.

Functional analysis of the EsaB component of the Staphylococcus aureus Type VII secretion system

M. Guillermina Casabona, Grant Buchanan, Martin Zoltner, Catriona P. Harkins, Matthew T. G. Holden, Tracy Palmer

ABSTRACT

Type VII secretion systems (T7SS) are found in many bacteria and secrete proteins involved in virulence and bacterial competition. In Staphylococcus aureus the small ubiquitin-like EsaB protein has been previously implicated as having a regulatory role in the production of the EsxC substrate. Here we show that in the S. aureus RN6390 strain, EsaB does not genetically regulate production of any T7 substrates or components, but is indispensable for secretion activity. Consistent with EsaB being an essential component of the T7SS, loss of either EsaB or EssC are associated with upregulation of a common set of iron acquisition genes. However, a further subset of genes were dysregulated only in the absence of EsaB. Quantitative western blotting indicates that EsaB is present at very low levels in cells. Substitution of a highly conserved threonine for alanine or arginine resulted in a loss of EsaB activity and destabilisation of the protein. Taken together our findings show that EsaB is essential for T7SS activity in RN6390.

Aeropyrum pernix membrane topology of protein VKOR promotes protein disulfide bond formation in two subcellular compartments

Stijntje Hibender, Cristina Landeta, Mehmet Berkmen, Jon Beckwith, Dana Boyd

ABSTRACT

Disulfide bonds confer stability and activity to proteins. Bioinformatic approaches allow predictions of which organisms make protein disulfide bonds and in which subcellular compartments disulfide bond formation takes place. Such an analysis, along with biochemical and protein structural data, suggests that many of the extremophile Crenarachaea make protein disulfide bonds in both the cytoplasm and the cell envelope. We have sought to determine the oxidative folding pathways in the sequenced genomes of the Crenarchaea, by seeking homologues of the enzymes known to be involved in disulfide bond formation in bacteria. Some Crenarchaea have two homologues of the cytoplasmic membrane protein VKOR, a protein required in many bacteria for the oxidation of bacterial DsbAs. We show that the two VKORs of Aeropyrum pernix assume opposite orientations in the cytoplasmic membrane, when expressed in E. coli. One has its active cysteines oriented toward the E. coli periplasm (ApVKORo) and the other toward the cytoplasm (ApVKORi). Furthermore, the ApVKORo promotes disulfide bond formation in the E. coli cell envelope, while the ApVKORi promotes disulfide bond formation in the E. coli cytoplasm via a co-expressed archaeal protein ApPDO. Amongst the VKORs from different archaeal species, the pairs of VKORs in each species are much more closely related to each other than to the VKORs of the other species. The results suggest two independent occurrences of the evolution of the two topologically inverted VKORs in archaea. Our results suggest a mechanistic basis for the formation of disulfide bonds in the cytoplasm of Crenarchaea.

Involvement of many chemotaxis sensors in negative chemotaxis to ethanol in Ralstonia pseudosolanacearum Ps29

Shota Oku, Akiko Hida, Tunchai Mattana, Takahisa Tajima, Yutaka Nakashimada, Junichi Kato

ABSTRACT

Ralstonia pseudosolanacearum Ps29 showed repellent responses to alcohols including methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 1,3-propanediol and prenol. R. pseudosolanacearum Ps29 possesses 22 putative chemoreceptors known as methyl-accepting chemotaxis proteins (MCPs). To identify a MCP involved in negative chemotaxis to ethanol, we measured ethanol chemotaxis of a complete collection of single mcp gene deletion mutants of R. pseudosolanacearum Ps29. However, all the mutants showed repellent responses to ethanol comparable to that of the wild-type strain. We constructed a stepwise- and multiple-mcp gene deletion mutant collection of R. pseudosolanacearum Ps29. Analysis of the collection found that an 18-mcp-knockout mutant (strain POC18) failed to respond to ethanol. Complementation analysis using POC18 as the host strain found that introduction of mcpA, mcpT, mcp09, mcpM, mcp15 and mcp19 restored the ability of POC18 to respond to ethanol. However, unexpectedly, strain POC10II, harbouring unmarked deletions in 10 mcp genes including mcpA, mcpT, mcp09, mcpM, mcp15 and mcp19 showed repellent responses to ethanol comparable to that of wild-type Ps29. We hypothesised that multiple mcp mutations in POC18 led to a shortage of MCPs required for formation of functional chemoreceptor arrays. When pPS16 (encoding McpP involved in phosphate chemotaxis) was introduced into POC18, POC18(pPS16) did not respond to phosphate. This result supports the hypothesis. But, genetic analysis revealed that MCPs (Mcp07, Mcp13, Mcp20 and Mcp21) are not essential for ethanol chemotaxis. Thus, we conclude that many and unspecified MCPs are involved in negative chemotaxis to ethanol in R. pseudosolanacearum Ps29.

Synthesis of N-acetyl-d-quinovosamine in Rhizobium etli CE3 is completed after its 4-keto-precursor is linked to a carrier lipid

Tiezheng Li, K. Dale Noel

ABSTRACT

Bacterial O-antigens are synthesized on lipid carriers before being transferred to lipopolysaccharide core structures. Rhizobium etli CE3 lipopolysaccharide is a model for understanding O-antigen biological function. CE3 O-antigen structure and genetics are known. However, proposed enzymology for CE3 O-antigen synthesis has been examined very little in vitro, and even the sugar added to begin the synthesis is uncertain. A model based on mutagenesis studies predicts that 2-acetamido-2,6-dideoxy-d-glucose (QuiNAc) is the first O-antigen sugar and that genes wreV, wreQ and wreU direct QuiNAc synthesis and O-antigen initiation. Previously, synthesis of UDP-QuiNAc was shown to occur in vitro with a WreV orthologue (4,6-hexose dehydratase) and WreQ (4-reductase), but the WreQ catalysis in this conventional deoxyhexose-synthesis pathway was very slow. This seeming deficiency was explained in the present study after WreU transferase activity was examined in vitro. Results fit the prediction that WreU transfers sugar-1-phosphate to bactoprenyl phosphate (BpP) to initiate O-antigen synthesis. Interestingly, WreU demonstrated much higher activity using the product of the WreV catalysis [UDP-4-keto-6-deoxy-GlcNAc (UDP-KdgNAc)] as the sugar-phosphate donor than using UDP-QuiNAc. Furthermore, the WreQ catalysis with WreU-generated BpPP-KdgNAc as the substrate was orders of magnitude faster than with UDP-KdgNAc. The inferred product BpPP-QuiNAc reacted as an acceptor substrate in an in vitro assay for addition of the second O-antigen sugar, mannose. These results imply a novel pathway for 6-deoxyhexose synthesis that may be commonly utilized by bacteria when QuiNAc is the first sugar of a polysaccharide or oligosaccharide repeat unit: UDP-GlcNAc → UDP-KdgNAc → BpPP-KdgNAc → BpPP-QuiNAc.

MtlR negatively regulates mannitol utilization by Vibrio cholerae

Tanner Byer, Jessica Wang, Mark G. Zhang, Naomi Vather, Anna Blachman, Bryan Visser, Jane M. Liu

ABSTRACT

The phosphoenopyruvate: carbohydrate phosphotransferase system (PTS) enables Vibrio cholerae – and other bacteria – to recognize and transport exogenous carbon sources for energy, including the six-carbon sugar alcohol, mannitol. The mannitol-specific PTS transporter is encoded by mtlA and its expression is expected to be regulated by the putative repressor encoded by the mtlR gene. Here, we show that mtlR overexpression inhibits V. cholerae growth in medium supplied with mannitol as the sole carbon source and represses MtlA-mediated biofilm formation. We demonstrate that when V. cholerae is grown in non-mannitol medium, knocking out mtlR leads to both increased MtlA protein and mtlA mRNA levels, with these increases being especially pronounced in non-glucose sugars. We propose that in non-mannitol, non-glucose growth conditions, MtlR is a major regulator of mtlA transcription. Surprisingly, with regard to mtlR expression, transcript and protein levels are highest in mannitol medium, conditions where mtlA expression should not be repressed. We further show that MtlR levels increase during growth of the bacteria and linger in cells switched from mannitol to non-mannitol medium. Our data suggests an expression paradigm for mtlA where MtlR acts as a transcriptional repressor responsible for calibrating MtlA levels during environmental transitions.

Involvement of the ytfK gene from the PhoB regulon in stationary-phase H 2 O 2 stress tolerance in Escherichia coli

Yumi Iwadate, Jun-ichi Kato

ABSTRACT

The Escherichia coli PhoB-PhoR two-component system responds to phosphate starvation and induces the expression of many genes. Previous studies suggested that phosphate starvation induces oxidative stress, but the involvement of the PhoB regulon in oxidative stress tolerance has not been clarified. Here, we showed that ytfK, one of the PhoB regulon genes, is involved in cell tolerance to a redox-cycling drug, menadione, and H2O2 in stationary-phase cells. A ytfK deletion mutant was sensitive to H2O2 when the cells were grown anaerobically or micro-aerobically in the presence of nitrate. Genetic analysis suggested that the ytfK gene has a functional relationship with the oxyR and fur genes, among the oxyR regulon, at least, a catalase-encoding katG gene and peroxidase-encoding ahpCF genes. Overproduction of YtfK resulted in a KatG-dependent decrease of H2O2 concentration in the cell suspension, suggesting that katG is one of the targets of YtfK. Using a katG′-lacZ reporter fusion, we showed that YtfK enhances the transcription of katG although it was not clarified whether YtfK functions directly or not. We also showed that ytfK disruption results in reduced viability of stationary-phase cells under phosphate starvation. These results indicated that YtfK is involved in H2O2 tolerance by stimulating directly or indirectly the transcription of at least the catalase gene, and that this system plays an important role in cellular survival during phosphate starvation.

Identification of novel small RNAs in Burkholderia cenocepacia KC-01 expressed under iron limitation and oxidative stress conditions

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ABSTRACT

Small RNA (sRNA)-mediated regulation of gene expression is a major tool to understand bacterial responses to environmental changes. In particular, pathogenic bacteria employ sRNAs to adapt to the host environment and establish infection. Members of the Burkholderia cepacia complex, normally present in soil microbiota, cause nosocomial lung infection especially in hospitalized cystic fibrosis patients. We sequenced the draft genome of Burkholderia cenocepacia KC-01, isolated from the coastal saline soil, and identified several potential sRNAs in silico. Expression of seven small RNAs (Bc_KC_sr1–7) was subsequently confirmed. Two sRNAs (Bc_KC_sr1 and Bc_KC_sr2) were upregulated in response to iron depletion by 2,2'-bipyridyl and another two (Bc_KC_sr3 and Bc_KC_sr4) responded to the presence of 60 μM H2O2 in the culture media. Bc_Kc_sr5, 6 and 7 remained unchanged under these conditions. Expression of Bc_KC_sr2, 3 and 4 also altered with a change in temperature and incubation time. A search in the Rfam and BSRD databases identified Bc_Kc_sr4 as candidate738 in B. pseudomallei D286 and assigned Bc_Kc_sr5 and 6 as tmRNA and 6S RNA, respectively. The novel sRNAs were conserved in Burkholderiaceae but did not have any homologue in other genera. Bc_KC_sr1 and 4 were transcribed independently while the rest were part of the 3′ UTR of their upstream genes. TargetRNA2 predicted that these sRNAs could target a host of cellular messages with very high stringency. Intriguingly, regions surrounding the translation initiation site for several enzymes involved in Fe–S cluster and siderophore biosynthesis, ROS homeostasis, porins, transcription and translation regulators, were among the suggested putative binding sites for these sRNAs.

Ethylene production in Synechocystis sp. PCC 6803 promotes phototactic movement

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ABSTRACT

Ethylene is a gaseous signal sensed by plants and bacteria. Heterologous expression of the ethylene-forming enzyme (EFE) from Pseudomonas syringae in cyanobacteria leads to the production of ethylene under photoautotrophic conditions. The recent characterization of an ethylene-responsive signalling pathway affecting phototaxis in the cyanobacterium Synechocystis sp. PCC 6803 implied that biotechnologically relevant ethylene synthesis may induce regulatory processes that are not related to changes in metabolism. Here, we provide data that indicate that endogenously produced ethylene accelerates the movement of cells towards light. Microarray analysis demonstrates that ethylene mainly deactivates transcription from the csiR1/lsiR promoter, which is under the control of the two-component system consisting of the ethylene- and UV-A-sensing histidine kinase UirS and the DNA-binding response regulator UirR. Surprisingly, ethylene production triggers a very specific transcriptional response and only a few other smaller transcriptional changes are detected in the microarray analysis.

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