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Molecular cloning and overexpression of DGA1, an acyl-CoA-dependent diacylglycerol acyltransferase, in the oleaginous yeast Rhodosporidiobolus fluvialis DMKU-RK253

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ABSTRACT

Triacylglycerol (TAG) is a major component of lipid storage in yeast. The acyl CoA: diacylgycerol acyltransferase (DGAT) that catalyzes the final and rate-limiting step in the production of TAG is rather interesting. Consequently, cloning and analysis of the gene-encoding TAG synthase, diacylglycerol acyltransferase gene (DGA1), of the oleaginous yeast Rhodosporidiobolus fluvialis DMKU-RK253 were undertaken. Analysis of the deduced amino acid sequence of DGA1 from R. fluvialis DMKU-RK253 (RfDGA1) showed similarity with the acyl-CoA: diacylglycerol acyltransferase 2 (DGAT2) from other organisms. The cDNA of RfDGA1 was cloned into the yeast expression vector pYES2 and heterologously overexpressed in Saccharomyces cerevisiae. One of the transformants showed a 1.6-fold increase in lipid content compared with the wild-type strain harbouring the pYES2 empty vector. Furthermore, DGA1 overexpression in R. fluvialis DMKU-RK253 resulted in a 2.5-fold increase in lipid content when compared with the wild-type strain, and no significant differences in fatty acid composition were observed between RfDGA1-overexpressed and wild-type strains. Taken together, our results supported our hypothesis that the RfDGA1 is a genetic factor that can be used for the development of a strain with improved lipid accumulation capabilities.

Fusion proteins towards fungi and bacteria in plant protection

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ABSTRACT

In agriculture, although fungi are considered the foremost problem, infections by bacteria also cause significant economical losses. The presence of different diseases in crops often leads to a misuse of the proper therapeutic, or the combination of different diseases forces the use of more than one pesticide. This work concerns the development of a ‘super-Blad’: a chimeric protein consisting of Blad polypeptide, the active ingredient of a biological fungicide already on the market, and two selected peptides, SP10-5 and Sub5, proven to possess biological potential as antibacterial agents. The resulting chimeric protein obtained from the fusion of Blad with SP10-5 not only maintained strong antibacterial activity, especially against Xanthomonas spp. and Pseudomonas syringae, but was also able to retain the ability to inhibit the growth of both yeast and filamentous fungi. However, the antibacterial activity of Sub5 was considerably diminished when fused with Blad, which seems to indicate that not all fusion proteins behave equally. These newly designed drugs can be considered promising compounds for use in plant protection. A deeper and focused development of an appropriate formulation may result in a potent biopesticide that can replace, per se, two conventional chemistries with less impact on the environment.

Divalent cations increase the conjugation efficiency of the incompatibility P-7 group plasmid pCAR1 among different Pseudomonas hosts

Ayako Sakuda, Chiho Suzuki-Minakuchi, Kazuhiro Matsui, Yurika Takahashi, Kazunori Okada, Hisakazu Yamane, Masaki Shintani, Hideaki Nojiri

ABSTRACT

The incompatibility (Inc) P-7 group plasmid pCAR1 can be efficiently transferred among bacteria in artificial microcosms in the presence of divalent cations Ca2+ and Mg2+. One-on-one mating assays between Pseudomonas strains with different plasmids showed that the promotion of conjugation efficiency by divalent cations was exhibited in other plasmids, including pB10 and NAH7; however, this effect was larger in IncP-7 plasmids. The impact on pCAR1 conjugation differed according to donor–recipient pairs, and conjugation efficiency promotion was clearly detected between the donors P. resinovorans CA10dm4 and P. fluorescens Pf0-1 and the recipients P. putida KT2440 and CA10dm4. Transcriptome analyses showed that pCAR1 gene expression did not respond to cation changes, including the tra/trh genes involved in its transfer. However, the transcription of oprH genes, encoding putative outer-membrane proteins in both the donor and the recipient, were commonly upregulated under cation-limited conditions. The conjugation frequency of pCAR1 in the KT2440 oprH mutant was found not to respond to cations. This effect was partially recovered by complementation with the oprH gene, suggesting that OprH is involved in the increase of pCAR1 conjugation efficiency by divalent cations.

A phylogenetic and evolutionary analysis of antimycin biosynthesis

Rebecca Joynt, Ryan F. Seipke

ABSTRACT

Streptomyces species and other Actinobacteria are ubiquitous in diverse environments worldwide and are the source of, or inspiration for, the majority of antibiotics. The genomic era has enhanced biosynthetic understanding of these valuable chemical entities and has also provided a window into the diversity and distribution of natural product biosynthetic gene clusters. Antimycin is an inhibitor of mitochondrial cytochrome c reductase and more recently was shown to inhibit Bcl-2/Bcl-XL-related anti-apoptotic proteins commonly overproduced by cancerous cells. Here we identify 73 putative antimycin biosynthetic gene clusters (BGCs) in publicly available genome sequences of Actinobacteria and classify them based on the presence or absence of cluster-situated genes antP and antQ, which encode a kynureninase and a phosphopantetheinyl transferase (PPTase), respectively. The majority of BGCs possess either both antP and antQ (L-form) or neither (S-form), while a minority of them lack either antP or antQ (IQ- or IP-form, respectively). We also evaluate the biogeographical distribution and phylogenetic relationships of antimycin producers and BGCs. We show that antimycin BGCs occur on five of the seven continents and are frequently isolated from plants and other higher organisms. We also provide evidence for two distinct phylogenetic clades of antimycin producers and gene clusters, which delineate S-form from L- and I-form BGCs. Finally, our findings suggest that the ancestral antimycin producer harboured an L-form gene cluster which was primarily propagated by vertical transmission and subsequently diversified into S-, IQ- and IP-form biosynthetic pathways.

Impact of stress on the gut microbiome of free-ranging western lowland gorillas

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ABSTRACT

Exposure to stressors can negatively impact the mammalian gastrointestinal microbiome (GIM). Here, we used 454 pyrosequencing of 16S rRNA bacterial gene amplicons to evaluate the impact of physiological stress, as evidenced by faecal glucocorticoid metabolites (FGCM; ng/g), on the GIM composition of free-ranging western lowland gorillas (Gorilla gorilla gorilla). Although we found no relationship between GIM alpha diversity (H) and FGCM levels, we observed a significant relationship between the relative abundances of particular bacterial taxa and FGCM levels. Specifically, members of the family Anaerolineaceae (ρ=0.4, FDR q=0.01), genus Clostridium cluster XIVb (ρ=0.35, FDR q=0.02) and genus Oscillibacter (ρ=0.35, FDR q=0.02) were positively correlated with FGCM levels. Thus, while exposure to stressors appears to be associated with minor changes in the gorilla GIM, the consequences of these changes are unknown. Our results may have implications for conservation biology as well as for our overall understanding of factors influencing the non-human primate GIM.

ParA-like protein influences the distribution of multi-copy chromosomes in cyanobacterium Synechococcus elongatus PCC 7942

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ABSTRACT

While many bacteria, such as Escherichia coli and Bacillus subtilis, harbour a single-copy chromosome, freshwater cyanobacteria have multiple copies of each chromosome per cell. Although it has been reported that multi-copy chromosomes are evenly distributed along the major axis of the cell in cyanobacterium Synechococcus elongatus PCC 7942, the distribution mechanism of these chromosomes remains unclear. In S. elongatus, the carboxysome, a metabolic microcompartment for carbon fixation that is distributed in a similar manner to the multi-copy chromosomes, is regulated by ParA-like protein (hereafter ParA). To elucidate the role of ParA in the distribution of multi-copy chromosomes, we constructed and analysed ParA disruptant and overexpressing strains of S. elongatus. Our fluorescence in situ hybridization assay revealed that the parA disruptants displayed an aberrant distribution of their multi-copy chromosomes. In the parA disruptant the multiple origin and terminus foci, corresponding to the intracellular position of each chromosomal region, were aggregated, which was compensated by the expression of exogenous ParA from other genomic loci. The parA disruptant is sensitive to UV-C compared to the WT strain. Additionally, giant cells appeared under ParA overexpression at the late stage of growth indicating that excess ParA indirectly inhibits cell division. Screening of the ParA-interacting proteins by yeast two-hybrid analysis revealed four candidates that are involved in DNA repair and cell membrane biogenesis. These results suggest that ParA is involved in the pleiotropic cellular functions with these proteins, while parA is dispensable for cell viability in S. elongatus.

The cell wall of the filamentous anoxygenic phototrophic bacterium Oscillochloris trichoides

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ABSTRACT

The filamentous anoxygenic phototrophic bacterium Oscillochloris trichoides DG-6 has been studied, and it has been shown that there are no lipopolysaccharides on the cell surface. Fatty acids hydroxylated at the C3 position, amino sugars and phosphate-containing compounds characteristic of lipid A have also not been found. The genes encoding for proteins responsible for the synthesis of lipopolysaccharides and the genes for the transport system, usually localized in the outer membrane of Gram-negative bacteria, have not been detected in the genome. The rigid layer of the cell wall contains a peptidoglycan consisting of alanine, glutamine, ornithine and glycine, in the respective ratio 1.8:1.5:1.0:0.6. Thus, the investigated bacterium, Osc. trichoides, is a monoderm. The cell wall also contains a branched α-1,4-d-glucan with a repeating unit consisting of glucose residues linked by α-1→4 bonds (α-1→6 at the branching sites). Such polymers have not previously been reported in phototrophic bacteria.

Dissimilar pigment regulation in Serpula lacrymans and Paxillus involutus during inter-kingdom interactions

James P. Tauber, Ramses Gallegos-Monterrosa, Ákos T. Kovács, Ekaterina Shelest, Dirk Hoffmeister

ABSTRACT

Production of basidiomycete atromentin-derived pigments like variegatic acid (pulvinic acid-type) and involutin (diarylcyclopentenone) from the brown-rotter Serpula lacrymans and the ectomycorrhiza-forming Paxillus involutus, respectively, is induced by complex nutrition, and in the case of S. lacrymans, bacteria. Pigmentation in S. lacrymans was stimulated by 13 different bacteria and cell-wall-damaging enzymes (lytic enzymes and proteases), but not by lysozyme or mechanical damage. The use of protease inhibitors with Bacillus subtilis or heat-killed bacteria during co-culturing with S. lacrymans significantly reduced pigmentation indicating that enzymatic hyphal damage and/or released peptides, rather than mechanical injury, was the major cause of systemic pigment induction. Conversely, no significant pigmentation by bacteria was observed from P. involutus. We found additional putative transcriptional composite elements of atromentin synthetase genes in P. involutus and other ectomycorrhiza-forming species that were absent from S. lacrymans and other brown-rotters. Variegatic and its precursor xerocomic acid, but not involutin, in return inhibited swarming and colony biofilm spreading of Bacillus subtilis, but did not kill B. subtilis. We suggest that dissimilar pigment regulation by fungal lifestyle was a consequence of pigment bioactivity and additional promoter motifs. The focus on basidiomycete natural product gene induction and regulation will assist in future studies to determine global regulators, signalling pathways and associated transcription factors of basidiomycetes.

CAR gene cluster and transcript levels of carotenogenic genes in Rhodotorula mucilaginosa

Sara Landolfo, Giuseppe Ianiri, Salvatore Camiolo, Andrea Porceddu, Giuliana Mulas, Rossella Chessa, Giacomo Zara, Ilaria Mannazzu

ABSTRACT

A molecular approach was applied to the study of the carotenoid biosynthetic pathway of Rhodotorula mucilaginosa. At first, functional annotation of the genome of R. mucilaginosa C2.5t1 was carried out and gene ontology categories were assigned to 4033 predicted proteins. Then, a set of genes involved in different steps of carotenogenesis was identified and those coding for phytoene desaturase, phytoene synthase/lycopene cyclase and carotenoid dioxygenase (CAR genes) proved to be clustered within a region of ~10 kb. Quantitative PCR of the genes involved in carotenoid biosynthesis showed that genes coding for 3-hydroxy-3-methylglutharyl-CoA reductase and mevalonate kinase are induced during exponential phase while no clear trend of induction was observed for phytoene synthase/lycopene cyclase and phytoene dehydrogenase encoding genes. Thus, in R. mucilaginosa the induction of genes involved in the early steps of carotenoid biosynthesis is transient and accompanies the onset of carotenoid production, while that of CAR genes does not correlate with the amount of carotenoids produced. The transcript levels of genes coding for carotenoid dioxygenase, superoxide dismutase and catalase A increased during the accumulation of carotenoids, thus suggesting the activation of a mechanism aimed at the protection of cell structures from oxidative stress during carotenoid biosynthesis. The data presented herein, besides being suitable for the elucidation of the mechanisms that underlie carotenoid biosynthesis, will contribute to boosting the biotechnological potential of this yeast by improving the outcome of further research efforts aimed at also exploring other features of interest.

Expression of the small regulatory RNA gene mmgR is regulated negatively by AniA and positively by NtrC in Sinorhizobium meliloti 2011

Germán Ceizel Borella, Antonio Lagares Jr., Claudio Valverde

ABSTRACT

In the N2-fixing symbiont of alfalfa root nodules, Sinorhizobium meliloti 2011, the mmgR gene encodes a 77 nt small untranslated RNA (sRNA) that negatively regulates the accumulation of polyhydroxybutyrate (PHB) when the bacterium is grown under conditions of surplus carbon (C) in relation to nitrogen (N). We previously showed that the expression of mmgR is primarily controlled at the transcriptional level and that it depends on the cellular N status, although the regulatory mechanism and the factors involved were unknown. In this study, we provide experimental data supporting that: (a) mmgR is induced upon N limitation with the maximum expression found at the highest tested C/N molar ratio in the growth medium; (b) a conserved heptamer TTGTGCA located between the −35 and −10 mmgR promoter elements is necessary and sufficient for induction by N limitation; (c) induction of mmgR requires the N-status regulator NtrC; (d) under C limitation, mmgR transcription is repressed by AniA, a global regulator of C flow; (e) the mmgR promoter contains a conserved dyadic motif (TGC[N3]GCA) partially overlapping the heptamer TTGTGCA, which was also found in the promoters of the PHB-related genes phaP1, phaP2, phaZ and phaR (aniA) of S. meliloti and other alpha-proteobacteria. Taken together, these results suggest that the mmgR promoter would integrate signals from the metabolism of C and N through – at least – the global regulators NtrC and AniA, to provide an optimal level of the MmgR sRNA to fine-tune gene expression post-transcriptionally according to varying C and N availability.

Global mapping of MtrA-binding sites links MtrA to regulation of its targets in Mycobacterium tuberculosis

Ayan Chatterjee, Arun Kumar Sharma, Amar Chandra Mahatha, Srijon Kaushik Banerjee, Manish Kumar, Sudipto Saha, Joyoti Basu, Manikuntala Kundu

ABSTRACT

Mycobacterium tuberculosis employs two-component systems (TCSs) for survival within its host. The TCS MtrAB is conserved among mycobacteria. The response regulator MtrA is essential in M. tuberculosis. The genome-wide chromatin immunoprecipitation (ChIP) sequencing performed in this study suggested that MtrA binds upstream of at least 45 genes of M. tuberculosis, including those involved in cell wall remodelling, stress responses, persistence and regulation of transcription. It binds to the promoter regions and regulates the peptidoglycan hydrolases rpfA and rpfC, which are required for resuscitation from dormancy. It also regulates the expression of whiB4, a critical regulator of the oxidative stress response, and relF, one-half of the toxin–antitoxin locus relFG. We have identified a new consensus 9 bp loose motif for MtrA binding. Mutational changes in the consensus sequence greatly reduced the binding of MtrA to its newly identified targets. Importantly, we observed that overexpression of a gain-of-function mutant, MtrAY102C, enhanced expression of the aforesaid genes in M. tuberculosis isolated from macrophages, whereas expression of each of these targets was lower in M. tuberculosis overexpressing a phosphorylation-defective mutant, MtrAD56N. This result suggests that phosphorylated MtrA (MtrA-P) is required for the expression of its targets in macrophages. Our data have uncovered new MtrA targets that suggest that MtrA is required for a transcriptional response that likely enables M. tuberculosis to persist within its host and emerge out of dormancy when the conditions are favourable.

Recombinant expression and characterisation of the oxygen-sensitive 2-enoate reductase from Clostridium sporogenes

Pawel M. Mordaka, Stephen J. Hall, Nigel Minton, Gill Stephens

ABSTRACT

'Ene'-reductases have attracted significant attention for the preparation of chemical intermediates and biologically active products. To date, research has been focussed primarily on Old Yellow Enzyme-like proteins, due to their ease of handling, whereas 2-enoate reductases from clostridia have received much less attention, because of their oxygen sensitivity and a lack of suitable expression systems. A hypothetical 2-enoate reductase gene, fldZ, was identified in Clostridium sporogenes DSM 795. The encoded protein shares a high degree of homology to clostridial FMN- and FAD-dependent 2-enoate reductases, including the cinnamic acid reductase proposed to be involved in amino acid metabolism in proteolytic clostridia. The gene was cloned and overexpressed in Escherichia coli. Successful expression depended on the use of strictly anaerobic conditions for both growth and enzyme preparation, since FldZ was oxygen-sensitive. The enzyme reduced aromatic enoates, such as cinnamic acid or p-coumaric acid, but not short chain unsaturated aliphatic acids. The β,β-disubstituted nitroalkene, (E)-1-nitro-2-phenylpropene, was reduced to enantiopure (R)-1-nitro-2-phenylpropane with a yield of 90%. By contrast, the α, β-disubstituted nitroalkene, (E)-2-nitro-1-phenylpropene, was reduced with a moderate yield of 56% and poor enantioselectivity (16% ee for (S)-2-nitro-1-phenylpropane). The availability of an expression system for this recombinant clostridial 2-enoate reductase will facilitate future characterisation of this unusual class of 'ene'-reductases, and expand the biocatalytic toolbox available for enantioselective hydrogenation of carbon-carbon double bonds.

Increased productivity in poultry birds by sub-lethal dose of antibiotics is arbitrated by selective enrichment of gut microbiota, particularly short-chain fatty acid producers

Sohini Banerjee, Abhijit Sar, Arijit Misra, Srikanta Pal, Arindom Chakraborty, Bomba Dam

ABSTRACT

Antibiotics are widely used at sub-lethal concentrations as a feed supplement to enhance poultry productivity. To understand antibiotic-induced temporal changes in the structure and function of gut microbiota of chicken, two flocks were maintained for six weeks on a carbohydrate- and protein-rich diet. The feed in the conventional diet (CD) group was supplemented with sub-lethal doses of chlorotetracycline, virginiamycin and amoxicillin, while the organic diet (OD) had no such addition. Antibiotic-fed birds were more productive, with a lower feed conversion ratio (FCR). Their faecal samples also had higher total heterotrophic bacterial load and antibiotic resistance capability. Deep sequencing of 16S rDNA V1-V2 amplicons revealed Firmicutes as the most dominant phylum at all time points, with the predominant presence of Lactobacillales members in the OD group. The productivity indicator, i.e. higher Firmicutes: Bacteroidetes ratio, particularly in the late growth phase, was more marked in CD amplicon sequences, which was supported by culture-based enumerations on selective media. CD datasets also showed the prevalence of known butyrate-producing genera such as Faecalibacterium, Ruminococcus, Blautia, Coprococcus and Bacteroides, which correlates closely with their higher PICRUSt-based in silico predicted 'glycan biosynthesis and metabolism'-related Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologues. Semi-quantitative end-point PCR targeting of the butyryl-CoA: acetate CoA-transferase gene also confirmed butyrate producers as being late colonizers, particularly in antibiotic-fed birds in both the CD flocks and commercial rearing farms. Thus, antibiotics preferentially enrich bacterial populations, particularly short-chain fatty acid producers that can efficiently metabolize hitherto undigestable feed material such as glycans, thereby increasing the energy budget of the host and its productivity.

Glucose can be transported and utilized in Escherichia coli by an altered or overproduced N-acetylglucosamine phosphotransferase system (PTS)

Jacob Crigler, Laude Bannerman-Akwei, Ashley E. Cole, Mark A. Eiteman, Elliot Altman

ABSTRACT

Escherichia coli Δglk ΔmanZ ΔptsG glucose- strains that lack the glucose phosphotransferase system (PTS) and the mannose PTS as well as glucokinase have been widely used by researchers studying the PTS. In this study we show that both fast- and slow-growing spontaneous glucose+ revertants can be readily obtained from Δglk ΔmanZ ΔptsG glucose- strains. All of the fast-growing revertants either altered the N-acetylglucosamine PTS or caused its overproduction by inactivating the NagC repressor protein, which regulates the N-acetylglucosamine PTS, and these revertants could utilize either glucose or N-acetylglucosamine as a sole carbon source. When a ΔnagE deletion, which abolishes the N-acetylglucosamine PTS, was introduced into the Δglk ΔmanZ ΔptsG glucose- strains, fast-growing revertants could no longer be isolated. Based on our results and other studies, it is clear that the N-acetylglucosamine PTS is the most easily adaptable PTS for transporting and phosphorylating glucose, other than the glucose PTS and mannose PTS, which are the primary glucose transport systems. While the slow-growing glucose+revertants were not characterized, they were likely mutations that other researchers have observed before and affect other PTSs or sugar kinases.

Galactooligosaccharide supplementation provides protection against Citrobacter rodentium-induced colitis without limiting pathogen burden

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ABSTRACT

Many enteric pathogens, including Salmonella and enteropathogenic and enterohemorrhagic Escherichia coli, express adhesins that recognize and bind to carbohydrate moieties expressed on epithelial cells. An attractive strategy for inhibiting bacterial adherence employs molecules that mimic these epithelial binding sites. Prebiotic oligosaccharides are non-digestible, fermentable fibres capable of modulating the gut microbiota. Moreover, they may act as molecular decoys that competitively inhibit adherence of pathogens to host cells. In particular, galactooligosaccharides (GOS) and other prebiotic fibres have been shown to inhibit pathogen adherence to epithelial cells in vitro. In the present study, we determined the ability of prophylactic GOS administration to reduce enteric pathogen adherence both in vitro and in vivo as well as protect against intestinal inflammation. GOS supplementation significantly reduced the adherence of the epithelial-adherent murine bacterial pathogen Citrobacter rodentium in a dose-dependent manner to the surface of epithelial cells in vitro. A 1- to 2-log reduction in bacterial adherence was observed at the lowest and highest doses tested, respectively. However, mouse studies revealed that treatment with GOS neither reduced the adherence of C. rodentium to the distal colon nor decreased its dissemination to systemic organs. Despite the absence of adherence inhibition, colonic disease scores for GOS-treated, C. rodentium-infected mice were significantly lower than those of untreated C. rodentium-infected animals (P=0.028). Together, these data suggest that GOS has a direct protective effect in ameliorating disease severity following C. rodentium infection through an anti-adherence-independent mechanism.

Analysis of essential gene dynamics under antibiotic stress in Streptococcus sanguinis

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ABSTRACT

The paradoxical response of Streptococcus sanguinis to drugs prescribed for dental and clinical practices has complicated treatment guidelines and raised the need for further investigation. We conducted a high throughput study on concomitant transcriptome and proteome dynamics in a time course to assess S. sanguinis behaviour under a sub-inhibitory concentration of ampicillin. Temporal changes at the transcriptome and proteome level were monitored to cover essential genes and proteins over a physiological map of intricate pathways. Our findings revealed that translation was the functional category in S. sanguinis that was most enriched in essential proteins. Moreover, essential proteins in this category demonstrated the greatest conservation across 2774 bacterial proteomes, in comparison to other essential functional categories like cell wall biosynthesis and energy production. In comparison to non-essential proteins, essential proteins were less likely to contain 'degradation-prone' amino acids at their N-terminal position, suggesting a longer half-life. Despite the ampicillin-induced stress, the transcriptional up-regulation of amino acid-tRNA synthetases and proteomic elevation of amino acid biosynthesis enzymes favoured the enriched components of essential proteins revealing 'proteomic signatures' that can be used to bridge the genotype–phenotype gap of S. sanguinis under ampicillin stress. Furthermore, we identified a significant correlation between the levels of mRNA and protein for essential genes and detected essential protein-enriched pathways differentially regulated through a persistent stress response pattern at late time points. We propose that the current findings will help characterize a bacterial model to study the dynamics of essential genes and proteins under clinically relevant stress conditions.

Analysis of the Mycoplasma bovis lactate dehydrogenase reveals typical enzymatic activity despite the presence of an atypical catalytic site motif

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ABSTRACT

The lactate dehydrogenase (LDH) of Mycoplasma genitalium has been predicted to also act as a malate dehydrogenase (MDH), but there has been no experimental validation of this hypothesized dual function for any mollicute. Our analysis of the metabolite profile of Mycoplasma bovis using gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS) detected malate, suggesting that there may be MDH activity in M. bovis. To investigate whether the putative l-LDH enzyme of M. bovis has a dual function (MDH and LDH), we performed bioinformatic and functional biochemical analyses. Although the amino acid sequence and predicted structural analysis of M. bovis l-LDH revealed unusual residues within the catalytic site, suggesting that it may have the flexibility to possess a dual function, our biochemical studies using recombinant M. bovis -LDH did not detect any MDH activity. However, we did show that the enzyme has typical LDH activity that could be inhibited by both MDH substrates oxaloacetate (OAA) and malate, suggesting that these substrates may be able to bind to M. bovis LDH. Inhibition of the conversion of pyruvate to lactate by OAA may be one method the mycoplasma cell uses to reduce the potential for accumulation of intracellular lactate.

A novel mechanism of fluconazole: fungicidal activity through dose-dependent apoptotic responses in Candida albicans

Wonjong Lee, Dong Gun Lee

ABSTRACT

Fluconazole (FLC) is a well-known fungistatic agent that inhibits ergosterol biosynthesis. We showed that FLC exhibits dose-dependent fungicidal activity, and investigated the fungicidal mechanism of FLC on Candida albicans. To confirm the relationship between fungicidal activity and the inhibition of ergosterol, we assessed membrane dysfunctions via propidium iodide influx and potassium leakage, as well as morphological change. Interestingly, while membrane disruption was not observed at all tested concentrations of FLC, potassium efflux and cell shrinkage were observed at high dosages of FLC (HDF). Low-dosage FLC (LDF) treatment did not induce significant changes. Next, we examined whether the fungicidal activity of FLC was associated with apoptosis in C. albicans. FLC caused dose-dependent apoptotic responses, including phosphatidylserine externalization and DNA fragmentation. It was also involved in glutathione depletion followed by oxidative damage. In particular, unlike LDF, HDF leads to the disruption of mitochondrial homeostasis, including mitochondrial membrane depolarization and accumulation of calcium and reactive oxygen species. HDF-induced mitochondrial dysfunction promoted the release of cytochrome c from mitochondria to the cytosol, and activated intracellular metacaspase. In conclusion, the dose-dependent fungicidal activity of FLC was due to an apoptotic response in C. albicans.

Molecular characterization and regulation of operons for asparagine and aspartate uptake and utilization in Pseudomonas aeruginosa

Guoqing Li, Chung-Dar Lu

ABSTRACT

Pseudomonas aeruginosa can utilize proteogenic amino acids as the sole source of carbon and nitrogen. In particular, utilization of l-Asp and l-Asn is insensitive to carbon catabolite repression as strong growth remains in the mutants devoid of the essential CbrAB activators of most catabolic genes. Transcriptome analysis was conducted to identify genes for the catabolism, uptake and regulation of these two amino acids. Gene inactivation and growth phenotype analysis established two asparaginases AsnA and AsnB for the degradation of l-Asn to l-Asp, whereas only AnsB is required for the deamidation of d-Asn to d-Asp. While d-Asp is a dead-end product, conversion of l-Asp to fumarate is catalysed by an aspartase AspA as further evidenced by enzyme kinetics. The results of measuring promoter-lacZ expression in vivo and mobility shift assays in vitro demonstrated that asnR and aspR encode two transcriptional regulators in response to l-Asn and l-Asp, respectively, for the induction of the ansPA operon and the aspA gene. Exogenous l-Glu also caused induction of the aspA gene, most likely due to its conversion to l-Asp by the aspartate transaminase AspC. Expression of several transporters were found inducible by l-Asn and/or l-Asp, including AatJQMP for acid amino acids, DctA and DctPQM for C4-dicarboxylates, and PA5530 for C5-dicarboxylates. In summary, a complete pathway and regulation for l-Asn and l-Asp catabolism was established in this study. Cross induction of three transport systems for dicarboxylic acids may provide a physiological explanation for the insensitivity of l-Asn and l-Asp utilization to carbon catabolite repression.

Acinetobacter baumannii isolate BAL_212 from Vietnam produces the K57 capsular polysaccharide containing a rarely occurring amino sugar N-acetylviosamine

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ABSTRACT

The structures of capsular polysaccharides (CPSs) produced by different Acinetobacter baumannii strains have proven to be invaluable in confirming the role of specific genes in the synthesis of rare sugars through the correlation of genetic content at the CPS biosynthesis locus with sugars found in corresponding CPS structures. A module of four genes (rmlA, rmlB, vioA and vioB) was identified in the KL57 capsule biosynthesis gene cluster of A. baumannii isolate BAL_212 from Vietnam. These genes were predicted to direct the synthesis of 4-acetamido-4,6-dideoxy-d-glucose (N-acetylviosamine, d-Qui4NAc) and the K57 CPS was found to contain this monosaccharide. The K57 structure was determined and, in addition to d-Qui4NAc, included three N-acetylgalactosamine residues in the main chain, with a single glucose side branch. The KL57 gene cluster has not been found in any other A. baumannii genomes, but the rmlA-rmlB-vioA-vioB module is present in the KL119 gene cluster that would likely produce a d-Qui4NAc-containing CPS.

OpaR and RpoS are positive regulators of a virulence factor PrtA in Vibrio parahaemolyticus

San-Chi Chang, Chia-Yin Lee

ABSTRACT

PrtA is an extracellular serine protease of Vibrio parahaemolyticus and has haemolytic and cytotoxic activities. Many extracellular proteases have been shown to be required for nutrient intake and the infection mechanism of vibrios. In this study, we report that OpaR, a quorum sensing regulator, and RpoS, a general stress response regulator, play important roles in the PrtA regulation pathway. Extracellular protease activity was highest during the late-log growth of Vibrio parahaemolyticus no.93 (VP93). The absence of PrtA distinctly decreased the extracellular protease activity. Deletion of opaR or rpoS alone reduced PrtA-specific activity of VP93. Quantitative reverse-transcriptase PCR and Western blot analysis suggested that OpaR and RpoS promote PrtA expression at the transcriptional level and affect the amount of extracellular PrtA. A luciferase assay revealed that OpaR regulates prtA on the prtA promoter region. Electrophoretic mobility shift assays indicated that the purified His-OpaR was able to bind specifically to two sequences (PrtA-1 and PrtA-2) of the prtA promoter region. Footprinting analysis showed that OpaR regulates prtA by binding to the promoter region of prtA at positions –269 to –246 and –88 to –68 from the prtA translational start site. Together, the results suggest that PrtA was upregulated by two global regulators, OpaR and RpoS.

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Relationship of environmental disturbances and the infectious potential of fungi

Hazael Hernandez, Luis R. Martinez

ABSTRACT

Fungi are critical organisms for the environment and offer many benefits to modern society through their application in the pharmaceutical, beverage and food industries. In contrast, fungal pathogens are emerging threats to humans, animals, plants and insects with potential to cause devastating mortality, morbidity and economic loss. Outbreaks associated with anthropogenic alterations of the environment, including climate change-related events such as natural disasters, are responsible for human, animal and plant disease. Similarly, fungi and their metabolites also have a negative impact in agriculture, posing a serious threat to our food supplies. Here, we describe the existing knowledge and importance of understanding the relationship of fungi and the environment in the context of human, animal and plant disease. Our goal is to encourage communication between scientists and the general public to create informed awareness about the impact of fungi in their daily lives and their environment.

The DNases of pathogenic Lancefield streptococci

Alex Remmington, Claire E. Turner

ABSTRACT

DNases are abundant among the pathogenic streptococci, with most species harbouring genes for at least one. Despite their prevalence, however, the role for these extracellular enzymes is still relatively unclear. The DNases of the Lancefield group A Streptococcus, S. pyogenes are the best characterized, with a total of eight DNase genes identified so far. Six are known to be associated with integrated prophages. Two are chromosomally encoded, and one of these is cell-wall anchored. Homologues of both prophage-associated and chromosomally encoded S. pyogenes DNases have been identified in other streptococcal species, as well as other unique DNases. A major role identified for streptococcal DNases appears to be in the destruction of extracellular traps produced by immune cells, such as neutrophils, to ensnare bacteria and kill them. These traps are composed primarily of DNA which can be degraded by the secreted and cell-wall-anchored streptococcal DNases. DNases can also reduce TLR-9 signalling to dampen the immune response and produce cytotoxic deoxyadenosine to limit phagocytosis. Upper respiratory tract infection models of S. pyogenes have identified a role for DNases in potentiating infection and transmission, possibly by limiting the immune response or through some other unknown mechanism. Streptococcal DNases may also be involved in interacting with other microbial communities through communication, bacterial killing and disruption of competitive biofilms, or control of their own biofilm production. The contribution of DNases to pathogenesis may therefore be wide ranging and extend beyond direct interference with the host immune response.

Loving the poison: the methylcitrate cycle and bacterial pathogenesis

Stephen K. Dolan, Andre Wijaya, Stephen M. Geddis, David R. Spring, Rafael Silva-Rocha, Martin Welch

ABSTRACT

Propionate is an abundant catabolite in nature and represents a rich potential source of carbon for the organisms that can utilize it. However, propionate and propionate-derived catabolites are also toxic to cells, so propionate catabolism can alternatively be viewed as a detoxification mechanism. In this review, we summarize recent progress made in understanding how prokaryotes catabolize propionic acid, how these pathways are regulated and how they might be exploited to develop novel antibacterial interventions.

Stress responses, outer membrane permeability control and antimicrobial resistance in Enterobacteriaceae

Sushovan Dam, Jean-Marie Pagès, Muriel Masi

ABSTRACT

Bacteria have evolved several strategies to survive a myriad of harmful conditions in the environment and in hosts. In Gram-negative bacteria, responses to nutrient limitation, oxidative or nitrosative stress, envelope stress, exposure to antimicrobials and other growth-limiting stresses have been linked to the development of antimicrobial resistance. This results from the activation of protective changes to cell physiology (decreased outer membrane permeability), resistance transporters (drug efflux pumps), resistant lifestyles (biofilms, persistence) and/or resistance mutations (target mutations, production of antibiotic modification/degradation enzymes). In targeting and interfering with essential physiological mechanisms, antimicrobials themselves are considered as stresses to which protective responses have also evolved. In this review, we focus on envelope stress responses that affect the expression of outer membrane porins and their impact on antimicrobial resistance. We also discuss evidences that indicate the role of antimicrobials as signaling molecules in activating envelope stress responses.

Triggering the stringent response: signals responsible for activating (p)ppGpp synthesis in bacteria

Sophie E. Irving, Rebecca M. Corrigan

ABSTRACT

The stringent response is a conserved bacterial stress response mechanism that allows bacteria to respond to nutritional challenges. It is mediated by the alarmones pppGpp and ppGpp, nucleotides that are synthesized and hydrolyzed by members of the RSH superfamily. Whilst there are key differences in the binding targets for (p)ppGpp between Gram-negative and Gram-positive bacterial species, the transient accumulation of (p)ppGpp caused by nutritional stresses results in a global change in gene expression in all species. The RSH superfamily of enzymes is ubiquitous throughout the bacterial kingdom, and can be split into three main groups: the long-RSH enzymes; the small alarmone synthetases (SAS); and the small alarmone hydrolases (SAH). Despite the prevalence of these enzymes, there are important differences in the way in which they are regulated on a transcriptional and post-translational level. Here we provide an overview of the diverse regulatory mechanisms that are involved in governing this crucial signalling network. Understanding how the RSH superfamily members are regulated gives insights into the varied important biological roles for this signalling pathway across the bacteria.

DL-endopeptidases function as both cell wall hydrolases and poly-γ-glutamic acid hydrolases

Tatsuya Fukushima, Natsuki Uchida, Masatoshi Ide, Takeko Kodama, Junichi Sekiguchi

ABSTRACT

Biopolymers on the cell surface are very important for protecting microorganisms from environmental stresses, as well as storing nutrients and minerals. Synthesis of biopolymers is well studied, while studies on the modification and degradation processes of biopolymers are limited. One of these biopolymers, poly-γ-glutamic acid (γ-PGA), is produced by Bacillus species. Bacillus subtilis PgdS, possessing three NlpC/P60 domains, hydrolyses γ-PGA. Here, we have demonstrated that several dl-endopeptidases with an NlpC/P60 domain (LytE, LytF, CwlS, CwlO, and CwlT) in B. subtilis digest not only an amide bond of d-γ-glutamyl-diaminopimelic acid in peptidoglycans but also linkages of γ-PGA produced by B. subtilis. The hydrolase activity of dl-endopeptidases towards γ-PGA was inhibited by IseA, which also inhibits their hydrolase activity towards peptidoglycans, while the hydrolysis of PgdS towards γ-PGA was not inhibited. PgdS hydrolysed only the d-/l-Glu–d-Glu linkages of d-Glu-rich γ-PGA (d-Glu:l-Glu=7:3) and l-Glu-rich γ-PGA (d-Glu:l-Glu=1:9), indicating that PgdS can hydrolyse only restricted substrates. On the other hand, the dl-endopeptidases in B. subtilis cleaved d-/l-Glu–d-/l-Glu linkages of d-Glu-rich γ-PGA (d-Glu:l-Glu=7:3), indicating that these enzymes show different substrate specificities. Thus, the dl-endopeptidases digest γ-PGA more flexibly than PgdS, even though they are annotated as "dl-endopeptidase, digesting the d-γ-glutamyl-diaminopimelic acid linkage (d–l amino acid bond)".

Engineering Escherichia coli to grow constitutively on D-xylose using the carbon-efficient Weimberg pathway

Luca Rossoni, Reuben Carr, Scott Baxter, Roxann Cortis, Thomas Thorpe, Graham Eastham, Gill Stephens

ABSTRACT

Bio-production of fuels and chemicals from lignocellulosic C5 sugars usually requires the use of the pentose phosphate pathway (PPP) to produce pyruvate. Unfortunately, the oxidation of pyruvate to acetyl-coenzyme A results in the loss of 33% of the carbon as CO2, to the detriment of sustainability and process economics. To improve atom efficiency, we engineered Escherichia coli to utilize d-xylose constitutively using the Weimberg pathway, to allow direct production of 2-oxoglutarate without CO2 loss. After confirming enzyme expression in vitro, the pathway expression was optimized in vivo using a combinatorial approach, by screening a range of constitutive promoters whilst systematically varying the gene order. A PPP-deficient (ΔxylAB), 2-oxoglutarate auxotroph (Δicd) was used as the host strain, so that growth on d -xylose depended on the expression of the Weimberg pathway, and variants expressing Caulobacter crescentus xylXAB could be selected on minimal agar plates. The strains were isolated and high-throughput measurement of the growth rates on d-xylose was used to identify the fastest growing variant. This strain contained the pL promoter, with C. crescentus xylA at the first position in the synthetic operon, and grew at 42% of the rate on d-xylose compared to wild-type E. coli using the PPP. Remarkably, the biomass yield was improved by 53.5% compared with the wild-type upon restoration of icd activity. Therefore, the strain grows efficiently and constitutively on d-xylose, and offers great potential for use as a new host strain to engineer carbon-efficient production of fuels and chemicals via the Weimberg pathway.

Digestion of peptidoglycan near the cross-link is necessary for the growth of Bacillus subtilis

Masayuki Hashimoto, Hiroaki Matsushima, I. Putu Suparthana, Hiroshi Ogasawara, Hiroki Yamamoto, ChingHao Teng, Junichi Sekiguchi

ABSTRACT

Bacterial cells are covered with peptidoglycan (PG) layer(s), serving as the cellular exoskeleton. The PG sacculus changes its shape during cell growth, and thus both the synthesis and disassembly of PG are important for cell proliferation. In Bacillus subtilis, four dl-endopeptidases (DLEPases; LytE, LytF, CwlO and CwlS) are involved in the maintenance of cell morphology. The lytE cwlO double mutant exhibits synthetic lethality and defective cell elongation, while the lytE lytF cwlS triple mutant exhibits defective cell separation, albeit with septum formation. LytE is involved in both cell separation and elongation. We propose that DLEPases have varied roles in cell separation and elongation. To determine these roles, the catalytic domain of LytE was substituted with another catalytic domain that digests the other bonds in PG. By using the chimeric enzymes, we assessed the suppression of the synthetic lethality by the cell elongation defect and the disruption of chain morphology by the cell separation defect. All the constructed chimeric enzymes suppressed the cell separation defect, restoring the chain morphology. Digestion at any position of PG broke the linkage between two daughter cells, releasing them from each other. However, only d,d-endopeptidases suppressed the lack of DLEPase in the lytE cwlO double mutant. This indicated that the release of tension on the expanding PG sacculus is not the sole essential function of DLEPases. Considering that the structure of the digested PG is important for cell elongation, the digested product might be reused in the growth process in some way.

Colony analysis and deep learning uncover 5-hydroxyindole as an inhibitor of gliding motility and iridescence in Cellulophaga lytica

Maylis Chapelais-Baron, Isabelle Goubet, Renaud Péteri, Maria de Fatima Pereira, Tâm Mignot, Apolline Jabveneau, Eric Rosenfeld

ABSTRACT

Iridescence is an original type of colouration that is relatively widespread in nature but has been either incompletely described or entirely neglected in prokaryotes. Recently, we reported a brilliant ‘pointillistic’ iridescence in agar-grown colony biofilms of Cellulophaga lytica and some other marine Flavobacteria that exhibit gliding motility. Bacterial iridescence is created by a unique self-organization of sub-communities of cells, but the mechanisms underlying such living photonic crystals are unknown. In this study, we used Petri dish assays to screen a large panel of potential activators or inhibitors of C. lytica’s iridescence. Derivatives potentially interfering with quorum-sensing and other communication or biofilm formation processes were tested, as well as metabolic poisons or algal exoproducts. We identified an indole derivative, 5-hydroxyindole (5HI, 250 µM) which inhibited both gliding and iridescence at the colonial level. 5HI did not affect growth or cell respiration. At the microscopic level, phase-contrast imaging confirmed that 5HI inhibits the gliding motility of cells. Moreover, the lack of iridescence correlated with a perturbation of self-organization of the cell sub-communities in both the WT and a gliding-negative mutant. This effect was proved using recent advances in machine learning (deep neuronal networks). In addition to its effect on colony biofilms, 5HI was found to stimulate biofilm formation in microplates. Our data are compatible with possible roles of 5HI or marine analogues in the eco-biology of iridescent bacteria.

Identification of antigenic proteins from Mycobacterium avium subspecies paratuberculosis cell envelope by comparative proteomic analysis

Shanmugasundaram Karuppusamy, Lucy Mutharia, David Kelton, Niel Karrow, Gordon Kirby

ABSTRACT

Johne's disease (JD) is contagious, chronic granulomatous enteritis of ruminants caused by Mycobacterium avium subsp. paratuberculosis (MAP). The aim of this study was to identify antigenic proteins from the MAP cell envelope (i.e. cell wall and cytoplasmic membranes) by comparing MAP, M. avium subsp. hominissuis (MAH) and M. smegmatis (MS) cell envelope protein profiles using a proteomic approach. Composite two-dimensional (2D) difference gel electrophoresis images revealed 13 spots present only in the image of the MAP cell envelope proteins. Using serum from MAP-infected cattle, immunoblot analysis of 2D gels revealed that proteins in the 13 spots were antigenic. These proteins were identified by liquid chromatography tandem mass spectrometry as products of the following genes: sdhA, fadE25_2, mkl, citA, gapdh, fadE3_2, moxR1, mmp, purC, mdh, atpG, fbpB and desA2 as well as two proteins without gene names identified as transcriptional regulator (MAP0035) protein and hypothetical protein (MAP1233). Protein functions ranged from energy generation, cell wall biosynthesis, protein maturation, bacterial replication and invasion of epithelial cells, functions considered essential to MAP virulence and intracellular survival. Five MAP cell envelope proteins, i.e. SdhA, FadE25_2, FadE3_2, MAP0035 and DesA2 were recombinantly expressed, three of which, i.e. SdhA, FadE25_2 and DesA2, were of sufficient purity and yield to generate polyclonal antibodies. Immunoblot analysis revealed antibodies reacted specifically to the respective MAP cell envelope proteins with minimal cross-reactivity with MAH and MS cell envelope proteins. Identification and characterization of MAP-specific proteins and antibodies to those proteins may be useful in developing new diagnostic tests for JD diagnosis.

The YscE/YscG chaperone and YscF N-terminal sequences target YscF to the Yersinia pestis type III secretion apparatus

Clarice de Azevedo Souza, Kristian L. Richards, YoSon Park, Michael Schwartz, Julie Torruellas Garcia, Sara Schesser Bartra, Gregory V. Plano

ABSTRACT

The needle structures of type III secretion (T3S) systems are formed by the secretion and polymerization of a needle subunit protein, YscF in Yersinia pestis. A subset of T3S systems employ unique heterodimeric chaperones, YscE and YscG in Y. pestis, to prevent the polymerization of needle subunits within the bacterial cell. We demonstrate that the YscE/YscG chaperone is also required for stable YscF expression and for secretion of YscF. Overexpression of a functional maltose-binding protein (MBP)–YscG hybrid protein stabilized cytoplasmic YscF but YscF was not secreted in the absence of YscE. Furthermore, a YscE mutant protein was identified that functioned with YscG to stabilize cytosolic YscF; however, YscF was not secreted. These findings confirm a role for the YscE/YscG chaperone in YscF secretion and suggest that YscE may have a specific role in this process. Recent studies have shown that YscF deleted of its N-terminal 15 residues is still secreted and functional, suggesting that YscF may not require an N-terminal secretion signal. However, we demonstrate that YscF contains an N-terminal secretion signal and that a functional N-terminal signal is required for YscF secretion.

Anti-inflammatory effect of two Lactobacillus strains during infection with Gardnerella vaginalis and Candida albicans in a HeLa cell culture model

Carolina M. A. Santos, Maria Cecília V. Pires, Thiago L. Leão, Anna Karolina S. Silva, Lilian S. Miranda, Flaviano S. Martins, Aristóbolo M. Silva, Jacques R. Nicoli

ABSTRACT

Lactobacilli are the dominant bacteria of the vaginal tract of healthy women and they play a major role in the maintenance of mucosal homeostasis, preventing genital infections, such as bacterial vaginosis (BV) and vulvovaginal candidiasis (VVC). It is now known that one mechanism of this protection is the influence that lactobacilli can exert on host immune responses. In this context, we evaluated two Lactobacillus strains (L. plantarum 59 and L. fermentum 137) for their immunomodulatory properties in response to Gardnerella vaginalis (BV) or Candida albicans (VVC) infections in a HeLa cell infection model. G. vaginalis and C. albicans triggered the secretion of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-8) and the activation of NF-κB in HeLa cells, in contrast to L. plantarum 59 and L. fermentum 137. Treatments with the Lactobacillus strains or their cell-free supernatants before (pre-treatment) or after (post-treatment) the challenge with the pathogens resulted in decreased secretion of pro-inflammatory cytokines and decreased activation of NF-κB. The treatments with Lactobacillus strains not only decreased the secretion of IL-8, but also its expression, as confirmed by gene reporter luciferase assay, suggesting transcription-level control by lactobacilli. In conclusion, L. plantarum 59 and L. fermentum 137 were confirmed to have an anti-inflammatory effect against G. vaginalis and C. albicans and they were able to influence signalling in NF-κB pathway, making them interesting candidates as probiotics for the prevention or treatment of BV and VVC.

Optimization of triacylglycerol and starch production in Chlamydomonas debaryana NIES-2212 with regard to light intensity and CO2 concentration

Masakazu Toyoshima, Naoki Sato

ABSTRACT

Triacylglycerol (TAG) and starch produced by micro-algae are potential sources of biofuel. Our previous studies showed that the unicellular green alga, Chlamydomonas debaryana NIES-2212, which is a rare species of Chlamydomonas that possesses phosphatidylcholine (PC), is a seed organism for the development of biofuel producers. This alga accumulates large amounts of TAG and starch under completely photo-autotrophic conditions during stationary phase without nutrient deprivation. The present study was performed to optimize the growth conditions of this alga with regard to light intensity and CO2 concentration to improve the efficiency of TAG and starch production. The growth rate of C. debaryana was greater at higher light intensity, although there was no significant difference in the final cell density of the culture. The highest contents of TAG and starch, approximately 200 fmol cell–1 and 600 pg cell–1, respectively, were achieved with a light intensity of 200 μmol m–2 s–1 bubbled with air containing 5.0% CO2. These results suggest that optimization of light intensity and CO2 concentration can enhance the productivity of TAG and starch by C. debaryana NIES-2212.

Streptomyces coelicolor strains lacking polyprenol phosphate mannose synthase and protein O-mannosyl transferase are hyper-susceptible to multiple antibiotics

Robert Howlett, Nicholas Read, Anpu Varghese, Charles Kershaw, Y. Hancock, Margaret C. M. Smith

ABSTRACT

Polyprenol phosphate mannose (PPM) is a lipid-linked sugar donor used by extra-cytoplasmic glycosyl tranferases in bacteria. PPM is synthesiszed by polyprenol phosphate mannose synthase, Ppm1, and in most Actinobacteria is used as the sugar donor for protein O-mannosyl transferase, Pmt, in protein glycosylation. Ppm1 and Pmt have homologues in yeasts and humans, where they are required for protein O-mannosylation. Actinobacteria also use PPM for lipoglycan biosynthesis. Here we show that ppm1 mutants of Streptomyces coelicolor have increased susceptibility to a number of antibiotics that target cell wall biosynthesis. The pmt mutants also have mildly increased antibiotic susceptibilities, in particular to β-lactams and vancomycin. Despite normal induction of the vancomycin gene cluster, vanSRJKHAX, the pmt and ppm1 mutants remained highly vancomycin sensitive indicating that the mechanism of resistance is blocked post-transcriptionally. Differential RNA expression analysis indicated that catabolic pathways were downregulated and anabolic ones upregulated in the ppm1 mutant compared to the parent or complemented strains. Of note was the increase in expression of fatty acid biosynthetic genes in the ppm1- mutant. A change in lipid composition was confirmed using Raman spectroscopy, which showed that the ppm1 - mutant had a greater relative proportion of unsaturated fatty acids compared to the parent or the complemented mutant. Taken together, these data suggest that an inability to synthesize PPM (ppm1) and loss of the glycoproteome (pmt- mutant) can detrimentally affect membrane or cell envelope functions leading to loss of intrinsic and, in the case of vancomycin, acquired antibiotic resistance.

Cytochromes in anaerobic growth of Acidithiobacillus ferrooxidans

Paul R. Norris, Ludovic Laigle, Susan Slade

ABSTRACT

The mineral sulfide-oxidising Acidithiobacillus ferrooxidans has been extensively studied over many years but some fundamental aspects of its metabolism remain uncertain, particularly with regard to its anaerobic oxidation of sulfur. This label-free, liquid chromatography-electron spray ionisation-mass spectrometry-based proteomic analysis estimated relative protein abundance during aerobic and anaerobic growth of At. ferrooxidans. One of its two bc 1 complexes, that encoded by the petII operon, was strongly implicated in anaerobic ferric iron-coupled sulfur oxidation, probably in conjunction with two cytochromes. These two cytochromes are homologs of the Cyc2 and Cyc1 proteins that are involved in ferrous iron oxidation. The previously undetected cytochromes apparently associated with anaerobic growth in At. ferrooxidans appear to be absent in many other ferrous iron-oxidising acidophiles that can also reduce ferric iron, which suggests a diversity in the ferric-iron-coupled sulfur oxidation pathways. For aerobic growth of At. ferrooxidans, this analysis was consistent with the generally accepted mechanism for its oxidation of ferrous iron. Unexpectedly, proteins encoded by the petI operon were not abundant and generally not detected in the proteomic analyses of cells grown aerobically on sulfur, although there was some expression of genes of the petI and petII operons in these cells.

Accumulation of ornithine lipids in Vibrio cholerae under phosphate deprivation is dependent on VC0489 (OlsF) and PhoBR system

Livia C. Barbosa, Carolina L. Goulart, Marcela M. Avellar, Paulo M. Bisch, Wanda M. A. von Kruger

ABSTRACT

Ornithine lipids (OLs) are phosphorus-free lipids found in many bacteria grown under phosphate deprivation, a condition that activates the PhoBR system and leads to phosphate uptake and metabolism. Two OL synthesis pathways have already been described. One depends on OlsB and OlsA acyltransferases to add, respectively, the first and second acyl chains to an ornithine molecule. The other pathway is carried out by OlsF, a bifunctional enzyme responsible for both acylation steps. Although Vibrio cholerae lacks olsBA genes, an olsF homologue (vc0489) was identified in its genome. In this work we demonstrated that V. cholerae produces OLs and expresses vc0489 in response to phosphate depletion, in a PhoBR-dependent manner. In Escherichia coli, under similar condition, vc0489 expression leads to OL accumulation. These results indicate a strong connection between OL synthesis and VC0489 from V. cholerae and, for the first time, a direct regulation of an olsF homologue by the PhoBR system.

The fission yeast Schizosaccharomyces pombe Mtf2 is required for mitochondrial cox1 gene expression

Jinyu Liu, Yan Li, Jie Chen, Yirong Wang, Mengting Zou, Ruyue Su, Ying Huang

ABSTRACT

Mitochondrial gene expression is essential for adenosine triphosphate synthesis via oxidative phosphorylation, which is the universal energy currency of cells. Here, we report the identification and characterization of a homologue of Saccharomyces cerevisiae Mtf2 (also called Nam1) in Schizosaccharomyces pombe. The Δmtf2 mutant with the intron-containing mitochondrial DNA (mtDNA) exhibited impaired growth on a rich medium containing the non-fermentable carbon source glycerol, suggesting that mtf2 is involved in mitochondrial function. mtf2 deletion in a mitochondrial intron-containing background resulted in a barely detectable level of the cox1 mRNA and a reduction in the level of the cob1 mRNA, and severely impaired cox1 translation. In contrast, mtf2 deletion in a mitochondrial intron-less background did not affect the levels of cox1 and cob1 mRNAs. However, Cox1 synthesis could not be restored to the control level in the Δmtf2 mutant with intron-less mtDNA. Our results suggest that unlike its counterpart in S. cerevisiae which plays a general role in synthesis of mtDNA-encoded proteins, S. pombe Mtf2 primarily functions in cox1 translation and the effect of mtf2 deletion on splicing of introns in mtDNA is likely due to a deficiency in the synthesis of intron-encoded maturases.

cAMP-CRP acts as a key regulator for the viable but non-culturable state in Escherichia coli

Kazuki Nosho, Hiroko Fukushima, Takehiro Asai, Masahiro Nishio, Reiko Takamaru, Koseki Joseph Kobayashi-Kirschvink, Tetsuhiro Ogawa, Makoto Hidaka, Haruhiko Masaki

ABSTRACT

A variety of bacteria, including Escherichia coli, are known to enter the viable but non-culturable (VBNC) state under various stress conditions. During this state, cells lose colony-forming activities on conventional agar plates while retaining signs of viability. Diverse environmental stresses including starvation induce the VBNC state. However, little is known about the genetic mechanism inducing this state. Here, we aimed to reveal the genetic determinants of the VBNC state of E. coli. We hypothesized that the VBNC state is a process wherein specific gene products important for colony formation are depleted during the extended period of stress conditions. If so, higher expression of these genes would maintain colony-forming activities, thereby restraining cells from entering the VBNC state. From an E. coli plasmid-encoded ORF library, we identified genes that were responsible for maintaining high colony-forming activities after exposure to starvation condition. Among these, cpdA encoding cAMP phosphodiesterase exhibited higher performance in the maintenance of colony-forming activities. As cpdA overexpression decreases intracellular cAMP, cAMP or its complex with cAMP-receptor protein (CRP) may negatively regulate colony-forming activities under stress conditions. We confirmed this using deletion mutants lacking adenylate cyclase or CRP. These mutants fully maintained colony-forming activities even after a long period of starvation, while wild-type cells lost most of this activity. Thus, we concluded that the lack of cAMP-CRP effectively retains high colony-forming activities, indicating that cAMP-CRP acts as a positive regulator necessary for the induction of the VBNC state in E. coli.

Azotobacter vinelandii: the source of 100 years of discoveries and many more to come

Jesse D. Noar, Jose M. Bruno-Bárcena

ABSTRACT

Azotobacter vinelandii has been studied for over 100 years since its discovery as an aerobic nitrogen-fixing organism. This species has proved useful for the study of many different biological systems, including enzyme kinetics and the genetic code. It has been especially useful in working out the structures and mechanisms of different nitrogenase enzymes, how they can function in oxic environments and the interactions of nitrogen fixation with other aspects of metabolism. Interest in studying A. vinelandii has waned in recent decades, but this bacterium still possesses great potential for new discoveries in many fields and commercial applications. The species is of interest for research because of its genetic pliability and natural competence. Its features of particular interest to industry are its ability to produce multiple valuable polymers – bioplastic and alginate in particular; its nitrogen-fixing prowess, which could reduce the need for synthetic fertilizer in agriculture and industrial fermentations, via coculture; its production of potentially useful enzymes and metabolic pathways; and even its biofuel production abilities. This review summarizes the history and potential for future research using this versatile microbe.

Microbe Profile: Mycobacterium tuberculosis: Humanity's deadly microbial foe

Stephen V. Gordon, Tanya Parish

ABSTRACT

Mycobacterium tuberculosis is an expert and deadly pathogen, causing the disease tuberculosis (TB) in humans. It has several notable features: the ability to enter non-replicating states for long periods and cause latent infection; metabolic remodelling during chronic infection; a thick, waxy cell wall; slow growth rate in culture; and intrinsic drug resistance and antibiotic tolerance. As a pathogen, M. tuberculosis has a complex relationship with its host, is able to replicate inside macrophages, and expresses diverse immunomodulatory molecules. M. tuberculosis currently causes over 1.8 million deaths a year, making it the world’s most deadly human pathogen.

Optimizing host cell physiology and stress avoidance for the production of recombinant human tumour necrosis factor α in Escherichia coli

Tania Selas Castiñeiras, Steven G. Williams, Antony Hitchcock, Jeffrey A. Cole, Daniel C. Smith, Tim W. Overton

ABSTRACT

As high-level recombinant protein production (RPP) exerts a massive stress on the production host, an extensive literature on RPP optimization focuses on separating the growth phase from RPP production once sufficient biomass has been obtained. The aim of the current investigation was to optimize the benefits of the relatively neglected alternative strategy to achieve high-level RPP during growth by minimizing stress on the host. High yields of the biopharmaceutical recombinant human tumour necrosis factor alpha (rhTNFα) were obtained by fed-batch fermentation relevant to industrial production based upon parameters that most severely affected RPP in preliminary laboratory scale batch cultures. Decreasing the inducer concentration and growth temperature, but increasing the production period, were far more effective for increasing RPP yields than changing the growth phase at which production was induced. High yields of up to 5 g l−1 of rhTNFα were obtained with minimal plasmid loss, even in synthetic media that lack animal-derived components and are therefore fully compliant with regulatory requirements. Most of the product was soluble and biologically active. In summary, stress minimization was shown to be an effective way to optimize the production of rhTNFα. Data generated in shake-flask experiments allowed the design of intensified bioreactor cultures in which RPP and growth could be balanced, leading to higher yield of both rhTNFα and biomass than with previous fermentations. An additional benefit of this approach is avoidance of lysis during harvesting and downstream processing.

The impact of ERAD on recombinant protein secretion in Pichia pastoris (syn Komagataella spp.)

Richard J. Zahrl, Diethard Mattanovich, Brigitte Gasser

ABSTRACT

The yeast Pichia pastoris (syn. Komagataella spp.) is a popular cell factory for recombinant protein production. Yeasts in general provide a good starting point for cell factory engineering. They are intrinsically robust and easy to manipulate and cultivate. However, their secretory pathway is not evolutionarily adapted to high loads of secretory protein. In particular, more complex proteins, like the antibody fragment (Fab) used in this study, overwhelm the folding and secretion capacity. This triggers cellular stress responses, which may cause excessive intracellular degradation. Previous results have shown that, in fact, about 60% of the newly synthesized Fab is intracellularly degraded. Endoplasmic reticulum-associated protein degradation (ERAD) is one possible intracellular degradation pathway for proteins aimed for secretion. We therefore targeted ERAD for cell factory engineering and investigated the impact on recombinant protein secretion in P. pastoris. Three components of the ERAD-L complex, which is involved in the degradation of luminal proteins, and a protein involved in proteasomal degradation, were successfully disrupted in Fab-secreting P. pastoris. Contrary to expectation, the effect on secretion was marginal. In the course of more detailed investigation of the impact of ERAD, we took a closer look at the intracellular variants of the recombinant protein. This enabled us to further zero in on the issue of intracellular Fab degradation and exclude an overshooting ER quality control. We propose that a major fraction of the Fab is actually degraded before entering the secretory pathway.

Gene drive inhibition by the anti-CRISPR proteins AcrIIA2 and AcrIIA4 in Saccharomyces cerevisiae

Erianna M. Basgall, Samantha C. Goetting, Megan E. Goeckel, Rachael M. Giersch, Emily Roggenkamp, Madison N. Schrock, Megan Halloran, Gregory C. Finnigan

ABSTRACT

Given the widespread use and application of the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas gene editing system across many fields, a major focus has been the development, engineering and discovery of molecular means to precisely control and regulate the enzymatic function of the Cas9 nuclease. To date, a variety of Cas9 variants and fusion assemblies have been proposed to provide temporally inducible and spatially controlled editing functions. The discovery of a new class of 'anti-CRISPR' proteins, evolved from bacteriophage in response to the prokaryotic nuclease-based immune system, provides a new platform for control over genomic editing. One Cas9-based application of interest to the field of population control is that of the 'gene drive'. Here, we demonstrate use of the AcrIIA2 and AcrIIA4 proteins to inhibit active gene drive systems in budding yeast. Furthermore, an unbiased mutational scan reveals that titration of Cas9 inhibition may be possible by modification of the anti-CRISPR primary sequence.

The Gram-positive model organism Bacillus subtilis does not form microscopically detectable cardiolipin-specific lipid domains

Alex-Rose Pogmore, Kenneth H. Seistrup, Henrik Strahl

ABSTRACT

Rather than being homogenous diffusion-dominated structures, biological membranes can exhibit areas with distinct composition and characteristics, commonly termed as lipid domains. Arguably the most comprehensively studied examples in bacteria are domains formed by cardiolipin, which have been functionally linked to protein targeting, the cell division process and the mode of action of membrane-targeting antimicrobials. Cardiolipin domains were originally identified in the Gram-negative model organism Escherichia coli based on preferential staining by the fluorescent membrane dye nonylacridine orange (NAO), and later reported to also exist in other Gram-negative and -positive bacteria. Recently, the lipid-specificity of NAO has been questioned based on studies conducted in E. coli. This prompted us to reanalyse cardiolipin domains in the Gram-positive model organism Bacillus subtilis. Here we show that logarithmically growing B. subtilis does not form microscopically detectable cardiolipin-specific lipid domains, and that NAO is not a specific stain for cardiolipin in this organism.

A drag force interpolation model for capsule-shaped cells in fluid flows near a surface

Krister Wiklund, Hanqing Zhang, Tim Stangner, Bhupender Singh, Esther Bullitt, Magnus Andersson

ABSTRACT

We report an interpolation model to calculate the hydrodynamic force on tethered capsule-shaped cells in micro-fluidic flows near a surface. Our model is based on numerical solutions of the full Navier–Stokes equations for capsule-shaped objects considering their geometry, aspect ratio and orientation with respect to fluid flow. The model reproduced the results from computational fluid dynamic simulations, with an average error of <0.15% for objects with an aspect ratio up to 5, and the model exactly reproduced the Goldman approximation of spherical objects close to a surface. We estimated the hydrodynamic force imposed on tethered Escherichia coli cells using the interpolation model and approximate models found in the literature, for example, one that assumes that E. coli is ellipsoid shaped. We fitted the 2D-projected area of a capsule and ellipsoid to segmented E. coli cells. We found that even though an ellipsoidal shape is a reasonable approximation of the cell shape, the capsule gives 4.4% better agreement, a small difference that corresponds to 15% difference in hydrodynamic force. In addition, we showed that the new interpolation model provides a significantly better agreement compared to estimates from commonly used models and that it can be used as a fast and accurate substitute for complex and computationally heavy fluid dynamic simulations. This is useful when performing bacterial adhesion experiments in parallel-plate flow channels. We include a MATLAB script that can track cells in a video time-series and estimate the hydrodynamic force using our interpolation formula.

Characterization of the pixB gene in Xenorhabdus nematophila and discovery of a new gene family

John Lucas, Mary Goetsch, Matt Fischer, Steven Forst

ABSTRACT

Xenorhabdus nematophila are Gram-negative bacteria that engage in mutualistic associations with entomopathogenic nematodes. To reproduce, the nematodes invade insects and release X. nematophila into the haemolymph where it functions as an insect pathogen. In complex medium, X. nematophila cells produce two distinct types of intracellular crystalline inclusions, one composed of the methionine-rich PixA protein and the other composed of the PixB protein. Here we show that PixB crystalline inclusions were neither apparent in X. nematophila cells grown in medium that mimics insect haemolymph (Grace's medium) nor in cells grown directly in the insect haemocoel. The identified pixB gene was regulated by a conserved σ70 promoter while the pixA promoter was less well conserved. Expression of pixA and pixB under biological conditions was analysed using GFP promoter reporters. Microplate fluorescence detection and flow cytometry analyses revealed that pixB was expressed at high levels in Grace's medium and in insect haemolymph and at lower levels in complex medium, while pixA was expressed at lower levels under all conditions. Although pixB was highly expressed in Grace's medium, PixB crystalline inclusions were not present, suggesting that under biological conditions PixB production may be controlled post-transcriptionally. Although a pixB-minus strain was constructed, the function of PixB remains unresolved. The pixB gene was present in few Xenorhabdus species and pixB-type genes were identified in some Proteobacteria and Gram-positive species, while pixA was only present in Xenorhabdus species. Two conserved sequences were identified in PixB-type proteins that characterize this previously unrecognized gene family.

Shiga toxin 2 translocation across intestinal epithelium is linked to virulence of Shiga toxin-producing Escherichia coli in humans

Seav-Ly Tran, Claire Jenkins, Valérie Livrelli, Stephanie Schüller

ABSTRACT

Shiga toxin-producing Escherichia coli (STEC) are characterized by the release of potent Shiga toxins (Stx), which are associated with severe intestinal and renal disease. Although all STEC strains produce Stx, only a few serotypes cause infection in humans. To determine which virulence traits in vitro are linked to human disease in vivo, 13 Stx2a-producing STEC strains of seropathotype (SPT) A or B (associated with severe human intestinal disease and outbreaks) and 6 strains of SPT D or E (rarely or not linked to human disease) were evaluated in a microaerobic human colonic epithelial infection model. All SPT strains demonstrated similar growth, colonization of polarized T84 colon carcinoma cells and Stx release into the medium. In contrast, Stx translocation across the T84 cell monolayer was significantly lower in SPT group DE compared to SPT group AB strains. Further experiments showed that Stx penetration occurred via a transcellular pathway and was independent of bacterial type III secretion and attaching and effacing lesion formation. These results suggest that the extent of Stx transcytosis across the gut epithelium may represent an important indicator of STEC pathogenicity for humans.

Hydrophobins contribute to root colonization and stress responses in the rhizosphere-competent insect pathogenic fungus Beauveria bassiana

Soumya Moonjely, Nemat O. Keyhani, Michael J. Bidochka

ABSTRACT

The hyd1/hyd2 hydrophobins are important constituents of the conidial cell wall of the insect pathogenic fungus Beauveria bassiana. This fungus can also form intimate associations with several plant species. Here, we show that inactivation of two Class I hydrophobin genes, hyd1 or hyd2, significantly decreases the interaction of B. bassiana with bean roots. Curiously, the Δhyd1/Δhyd2 double mutant was less impaired in root association than Δhyd1 or Δhyd2. Loss of hyd genes affected growth rate, conidiation ability and oosporein production. Expression patterns for genes involved in conidiation, cell wall integrity, insect virulence, signal transduction, adhesion, hydrophobicity and oosporein production were screened in the deletion mutants grown in different conditions. Repression of the major MAP-Kinase signal transduction pathways (Slt2 MAPK pathway) was observed that was more pronounced in the single versus double hyd mutants under certain conditions. The Δhyd1/Δhyd2 double mutant showed up-regulation of the Hog1 MAPK and the Msn2 transcription factor under certain conditions when compared to the wild-type or single hyd mutants. The expression of the bad2 adhesin and the oosporein polyketide synthase 9 gene was severely reduced in all of the mutants. On the other hand, fewer changes were observed in the expression of key conidiation and cell wall integrity genes in hyd mutants compared to wild-type. Taken together, the data from this study indicated pleiotropic consequences of deletion of hyd1 and hyd2 on signalling and stress pathways as well as the ability of the fungus to form stable associations with plant roots.

Porphyromonas gingivalis hydrogen sulfide enhances methyl mercaptan-induced pathogenicity in mouse abscess formation

Suguru Nakamura, Koki Shioya, B. Yukihiro Hiraoka, Nao Suzuki, Tomonori Hoshino, Taku Fujiwara, Nobuo Yoshinari, Toshihiro Ansai, Akihiro Yoshida

ABSTRACT

Porphyromonas gingivalis produces hydrogen sulfide (H2S) from l-cysteine. However, the role of H2S produced by P. gingivalis in periodontal inflammation is unclear. In this study, we identified the enzyme that catalyses H2S production from l-cysteine and analysed the role of H2S using a mouse abscess model. The enzyme identified was identical to methionine γ-lyase (PG0343), which produces methyl mercaptan (CH3SH) from l-methionine. Therefore, we analysed H2S and CH3SH production by P. gingivalis W83 and a PG0343-deletion mutant (ΔPG0343) with/without l-cysteine and/or l-methionine. The results indicated that CH3SH is produced constitutively irrespective of the presence of l-methionine, while H2S was greatly increased by both P. gingivalis W83 and ΔPG0343 in the presence of l-cysteine. In contrast, CH3SH production by ΔPG0343 was absent irrespective of the presence of l-methionine, and H2S production was eliminated in the absence of l-cysteine. Thus, CH3SH and H2S production involves different substrates, l-methionine or l-cysteine, respectively. Based on these characteristics, we analysed the roles of CH3SH and H2S in abscess formation in mice by P. gingivalis W83 and ΔPG0343. Abscess formation by P. gingivalis W83, but not ΔPG0343, differed significantly in the presence and absence of l-cysteine. In addition, the presence of l-methionine did not affect the size of abscesses generated by P. gingivalis W83 and ΔPG0343. Therefore, we conclude that H2S produced by P. gingivalis does not induce inflammation; however, H2S enhances inflammation caused by CH3SH. Thus, these results suggest the H2S produced by P. gingivalis plays a supportive role in inflammation caused by methionine γ-lyase.

Identification of interactions among host and bacterial proteins and evaluation of their role early during Shigella flexneri infection

Kelly A. Miller, Anna Cristina Garza-Mayers, Yiuka Leung, Marcia B. Goldberg

ABSTRACT

Shigella species cause diarrhoea by invading and spreading through the epithelial layer of the human colon. The infection triggers innate immune responses in the host that the bacterium combats by translocating into the host cell cytosol via a type 3 secretion system bacterial effector proteins that interfere with host processes. We previously demonstrated that interaction of the Shigella type 3 secreted effector protein IcsB with the host protein Toca-1 inhibits the innate immune response microtubule-associated protein light-chain 3 (LC3)-associated phagocytosis, and that IcsB interaction with Toca-1 is required for inhibition of this host response. Here, we show that Toca-1 in vitro precipitated not only IcsB, but also the type 3 secreted proteins OspC3, IpgD and IpaB. OspC3 and IpgD precipitation with Toca-1 was dependent on IcsB. Early during infection, most of these proteins localized near intracellular Shigella. We examined whether interactions among these proteins restrict innate host cell responses other than LC3-associated phagocytosis. In infected cells, OspC3 blocks production and secretion of the mature pro-inflammatory cytokine IL-18; however, we found that interaction of OspC3 with IcsB, either directly or indirectly via Toca-1, was not required for OspC3-mediated restriction of IL-18 production. These results indicate that interactions of the host protein Toca-1 with a subset of type 3 effector proteins contribute to the established function of some, but not all involved, effector proteins.

Transcriptome analysis of a Pseudomonas aeruginosa sn-glycerol-3-phosphate dehydrogenase mutant reveals a disruption in bioenergetics

Jon Shuman, Tyler Xavier Giles, Leslie Carroll, Kenji Tabata, Austin Powers, Sang-Jin Suh, Laura Silo-Suh

ABSTRACT

Pseudomonas aeruginosa causes acute and chronic human infections and is the major cause of morbidity and mortality in cystic fibrosis (CF) patients. We previously determined that the sn-glycerol-3-phosphate dehydrogenase encoded by glpD plays a larger role in P. aeruginosa physiology beyond its role in glycerol metabolism. To better understand the effect of a glpD mutation on P. aeruginosa physiology we compared the transcriptomes of P. aeruginosa strain PAO1 and the PAO1ΔglpD mutant using RNA-seq analysis. We determined that a null mutation of glpD significantly altered amino acid metabolism in P. aeruginosa and affected the production of intermediates that are channelled into the tricarboxylic acid cycle. Moreover, the loss of glpD induced a general stress response mediated by RpoS in P. aeruginosa. Several other phenotypes observed for the P. aeruginosa glpD mutant include increased persister cell formation, reduced extracellular ATP accumulation and increased heat output. Taken together, these findings implicate sn-glycerol-3-phosphate dehydrogenase as a key player in energy metabolism in P. aeruginosa.

Release of nitric oxide by the Escherichia coli YtfE (RIC) protein and its reduction by the hybrid cluster protein in an integrated pathway to minimize cytoplasmic nitrosative stress

Basema Balasiny, Matthew D. Rolfe, Claire Vine, Charlene Bradley, Jeffrey Green, Jeff Cole

ABSTRACT

Synthesis of the Escherichia coli YtfE protein, also known as RIC, for the repair of damaged iron centres, is highly induced during anaerobic growth under conditions of nitrosative stress. How YtfE repairs nitrosative damage remains unclear. Contrary to previous reports, we show that strains defective in YtfE that lack the high-affinity NO reductase activity of the hybrid cluster protein (Hcp) are less sensitive to nitrosative stress than isogenic ytfE+ strains, which are extremely sensitive. Evidence that this sensitivity is due to YtfE-dependent release of NO into the cytoplasm includes: relief of growth inhibition by PTIO (2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide), which degrades NO; relief of nitrosative stress by deletion of narG encoding the nitrate reductase that is the major source of NO from nitrite; partial suppression of nitrosative stress due to loss of Hcp function by a further mutation in ytfE; YtfE-dependent loss of aconitase and fumarase activities in the absence of Hcp; and YtfE-dependent relief of NsrR repression of the hcp promoter in response to cytoplasmic NO. We suggest that a major role for YtfE is to reverse nitrosative damage by releasing, directly or indirectly, NO from nitrosylated proteins into the cytoplasm where the high-affinity NO reductase activity of Hcp ensures its reduction to N2O. If so, the concerted action of YtfE and Hcp would not only maintain the cytoplasmic concentration of NO in the low nM range, but also provide a rationalization for the coordinate regulation of Hcp and YtfE synthesis by NsrR.

Putative extracellular α-class carbonic anhydrase, EcaA, of Synechococcus elongatus PCC 7942 is an active enzyme: a sequel to an old story

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ABSTRACT

Carbonic anhydrase (CA) EcaA of Synechococcus elongatus PCC 7942 was previously characterized as a putative extracellular α-class CA, however, its activity was never verified. Here we show that EcaA possesses specific CA activity, which is inhibited by ethoxyzolamide. An active EcaA was expressed in heterologous bacterial system, which supports the formation of disulfide bonds, as a full-length protein (EcaA+L) and as a mature protein that lacks a leader peptide (EcaA-L). EcaA-L exhibited higher specific activity compared to EcaA+L. The recombinant EcaA, expressed in a bacterial system that does not support optimal disulfide bond formation, exhibited extremely low activity. This activity, however, could be enhanced by the thiol-oxidizing agent, diamide; while a disulfide bond-reducing agent, dithiothreitol, further inactivated the enzyme. Intact E. coli cells that overexpress EcaA+L possess a small amount of processed protein, EcaA-L, whereas the bulk of the full-length protein resides in the cytosol. This may indicate poor recognition of the EcaA leader peptide by protein export systems. S. elongatus possessed a relatively low level of ecaA mRNA, which varied insignificantly in response to changes in CO2 supply. However, the presence of protein in the cells is not obvious. This point to the physiological insignificance of EcaA in S. elongatus, at least under the applied experimental conditions.

Lack of glyoxylate shunt dysregulates iron homeostasis in Pseudomonas aeruginosa

Sunhee Ha, Bora Shin, Woojun Park

ABSTRACT

The aceA and glcB genes, encoding isocitrate lyase (ICL) and malate synthase, respectively, are not in an operon in many bacteria, including Pseudomonas aeruginosa, unlike in Escherichia coli. Here, we show that expression of aceA in P. aeruginosa is specifically upregulated under H2O2-induced oxidative stress and under iron-limiting conditions. In contrast, the addition of exogenous redox active compounds or antibiotics increases the expression of glcB. The transcriptional start sites of aceA under iron-limiting conditions and in the presence of iron were found to be identical by 5′ RACE. Interestingly, the enzymatic activities of ICL and isocitrate dehydrogenase had opposite responses under different iron conditions, suggesting that the glyoxylate shunt (GS) might be important under iron-limiting conditions. Remarkably, the intracellular iron concentration was lower while the iron demand was higher in the GS-activated cells growing on acetate compared to cells growing on glucose. Absence of GS dysregulated iron homeostasis led to changes in the cellular iron pool, with higher intracellular chelatable iron levels. In addition, GS mutants were found to have higher cytochrome c oxidase activity on iron-supplemented agar plates of minimal media, which promoted the growth of the GS mutants. However, deletion of the GS genes resulted in higher sensitivity to a high concentration of H2O2, presumably due to iron-mediated killing. In conclusion, the GS system appears to be tightly linked to iron homeostasis in the promotion of P. aeruginosa survival under oxidative stress.

Polyamines are required for normal growth in Sinorhizobium meliloti

Victor A. Becerra-Rivera, Ed Bergström, Jane Thomas-Oates, Michael F. Dunn

ABSTRACT

Polyamines (PAs) are ubiquitous polycations derived from basic l-amino acids whose physiological roles are still being defined. Their biosynthesis and functions in nitrogen-fixing rhizobia such as Sinorhizobium meliloti have not been extensively investigated. Thin layer chromatographic and mass spectrometric analyses showed that S. meliloti Rm8530 produces the PAs, putrescine (Put), spermidine (Spd) and homospermidine (HSpd), in their free forms and norspermidine (NSpd) in a form bound to macromolecules. The S. meliloti genome encodes two putative ornithine decarboxylases (ODC) for Put synthesis. Activity assays with the purified enzymes showed that ODC2 (SMc02983) decarboxylates both ornithine and lysine. ODC1 (SMa0680) decarboxylates only ornithine. An odc1 mutant was similar to the wild-type in ODC activity, PA production and growth. In comparison to the wild-type, an odc2 mutant had 45% as much ODC activity and its growth rates were reduced by 42, 14 and 44% under non-stress, salt stress or acid stress conditions, respectively. The odc2 mutant produced only trace levels of Put, Spd and HSpd. Wild-type phenotypes were restored when the mutant was grown in cultures supplemented with 1 mM Put or Spd or when the odc2 gene was introduced in trans. odc2 gene expression was increased under acid stress and reduced under salt stress and with exogenous Put or Spd. An odc1 odc2 double mutant had phenotypes similar to the odc2 mutant. These results indicate that ODC2 is the major enzyme for Put synthesis in S. meliloti and that PAs are required for normal growth in vitro.

Disruption of the GDP-mannose synthesis pathway in Streptomyces coelicolor results in antibiotic hyper-susceptible phenotypes

Robert Howlett, Katri Anttonen, Nicholas Read, Margaret C. M. Smith

ABSTRACT

Actinomycete bacteria use polyprenol phosphate mannose as a lipid linked sugar donor for extra-cytoplasmic glycosyl transferases that transfer mannose to cell envelope polymers, including glycoproteins and glycolipids. We showed recently that strains of Streptomyces coelicolor with mutations in the gene ppm1 encoding polyprenol phosphate mannose synthase were both resistant to phage φC31 and have greatly increased susceptibility to antibiotics that mostly act on cell wall biogenesis. Here we show that mutations in the genes encoding enzymes that act upstream of Ppm1 in the polyprenol phosphate mannose synthesis pathway can also confer phage resistance and antibiotic hyper-susceptibility. GDP-mannose is a substrate for Ppm1 and is synthesised by GDP-mannose pyrophosphorylase (GMP; ManC) which uses GTP and mannose-1-phosphate as substrates. Phosphomannomutase (PMM; ManB) converts mannose-6-phosphate to mannose-1-phosphate. S. coelicolor strains with knocked down GMP activity or with a mutation in sco3028 encoding PMM acquire phenotypes that resemble those of the ppm1 - mutants i.e. φC31 resistant and susceptible to antibiotics. Differences in the phenotypes of the strains were observed, however. While the ppm1 - strains have a small colony phenotype, the sco3028::Tn5062 mutants had an extremely small colony phenotype indicative of an even greater growth defect. Moreover we were unable to generate a strain in which GMP activity encoded by sco3039 and sco4238 is completely knocked out, indicating that GMP is also an important enzyme for growth. Possibly GDP-mannose is at a metabolic branch point that supplies alternative nucleotide sugar donors.

Three novel proteins co-localise with polyhydroxybutyrate (PHB) granules in Rhodospirillum rubrum S1

Tanja Narancic, Elisa Scollica, Gerard Cagney, Kevin E. O'Connor

ABSTRACT

Polyhydroxybutyrate (PHB), a biodegradable polymer accumulated by bacteria is deposited intracellularly in the form of inclusion bodies often called granules. The granules are supramolecular complexes harbouring a varied number of proteins on their surface, which have specific but incompletely characterised functions. By comparison with other organisms that produce biodegradable polymers, only two phasins have been described to date for Rhodosprillum rubrum, raising the possibility that more await discovery. Using a comparative proteomics strategy to compare the granules of wild-type R. rubrum with a PHB-negative mutant housing artificial PHB granules, we identified four potential PHB granules' associated proteins. These were: Q2RSI4, an uncharacterised protein; Q2RWU9, annotated as an extracellular solute-binding protein; Q2RQL4, annotated as basic membrane lipoprotein; and Q2RQ51, annotated as glucose-6-phosphate isomerase. In silico analysis revealed that Q2RSI4 harbours a Phasin_2 family domain and shares low identity with a single-strand DNA-binding protein from Sphaerochaeta coccoides. Fluorescence microscopy found that three proteins Q2RSI4, Q2EWU9 and Q2RQL4 co-localised with PHB granules. This work adds three potential new granule associated proteins to the repertoire of factors involved in bacterial storage granule formation, and confirms that proteomics screens are an effective strategy for discovery of novel granule associated proteins.

Differential requirements for processing and transport of short-chain versus long-chain O-acylcarnitines in Pseudomonas aeruginosa

Jamie A. Meadows, Graham G. Willsey, Matthew J. Wargo

ABSTRACT

The opportunistic pathogen Pseudomonas aeruginosa can metabolize carnitine and O-acylcarnitines, which are abundant in host muscle and other tissues. Acylcarnitines are metabolized to carnitine and a fatty acid. The liberated carnitine and its catabolic product, glycine betaine, can be used as osmoprotectants, to induce the secreted phospholipase C PlcH, and as sole carbon, nitrogen and energy sources. P. aeruginosa is incapable of de novo synthesis of carnitine and acylcarnitines, therefore they must be imported from an exogenous source. In this study, we present the first characterization of bacterial acylcarnitine transport. Short-chain acylcarnitines are imported by the ABC transporter CaiX-CbcWV. Medium- and long-chain acylcarnitines (MCACs and LCACs) are hydrolysed extracytoplasmically and the free carnitine is transported primarily through CaiX-CbcWV. These findings suggest that the periplasmic protein CaiX has a binding pocket that permits short acyl chains on its carnitine ligand and that there are one or more secreted hydrolases that cleave MCACs and LCACs. To identify the secreted hydrolase(s), we used a saturating genetic screen and transcriptomics followed by phenotypic analyses, but neither led to identification of a contributing hydrolase, supporting but not conclusively demonstrating redundancy for this activity.

Non-canonical Escherichia coli transcripts lacking a Shine–Dalgarno motif have very different translational efficiencies and do not form a coherent group

Petra Ludwig, Madeleine Huber, Matthias Lehr, Marius Wegener, Karolin Zerulla, Christian Lange, Joerg Soppa

ABSTRACT

Translation initiation in 50–70% of transcripts in Escherichia coli requires base pairing between the Shine–Dalgarno (SD) motif in the mRNA and the anti-SD motif at the 3′ end of the 16S rRNA. However, 30–50% of E. coli transcripts are non-canonical and are not preceded by an SD motif. The 5′ ends of 44 E. coli transcripts were determined, all of which contained a 5′-UTR (no leaderless transcripts), but only a minority contained an SD motif. The 5′-UTR lengths were compared with those listed in RegulonDB and reported in previous publications, and the identities and differences were obtained in all possible combinations. We aimed to quantify the translational efficiencies of non-canonical 5′-UTRs using GusA reporter gene assays and Northern blot analyses. Ten non-canonical 5′-UTRs and two control 5′-UTRs with an SD motif were cloned upstream of the gusA gene. The translational efficiencies were quantified under five different conditions (different growth rates via two different temperatures and two different carbon sources, and heat shock). The translational efficiencies of the non-canonical 5′-UTRs varied widely, from 5 to 384% of the positive control. In addition, the non-canonical transcripts did not exhibit a common regulatory pattern with changing environmental parameters. No correlation could be observed between the translational efficiencies of the non-canonical 5′-UTRs and their lengths, sequences, GC content, or predicted secondary structures. The introduction of an SD motif enhanced the translational efficiency of a poorly translated non-canonical transcript, while the efficiency of a well-translated non-canonical transcript remained unchanged. Taken together, the mechanisms of translation initiation at non-canonical transcripts in E. coli still need to be elucidated.

Transcriptional profiling of the clpX mutant in Bacillus anthracis reveals regulatory connection with the lrgAB operon

Kevin M. Claunch, Madeline Bush, Christopher R. Evans, Jacob A. Malmquist, Matthew C. Hale, Shauna M. McGillivray

ABSTRACT

ClpX functions as either an independent chaperone or a component of the ClpXP protease, a conserved intracellular protease that acts as a global regulator in the bacterial cell by degrading regulatory proteins, stress response proteins and rate-limiting enzymes. Previously, we found that loss of clpX in Bacillus anthracis Sterne leads to increased susceptibility to antimicrobial agents that target the cell envelope. The aim of this study was to identify genes within the regulatory network of clpX that contribute to antimicrobial resistance. Using microarray analysis, we found 119 genes that are highly differentially expressed in the ΔclpX mutant, with the majority involved in metabolic, transport or regulatory functions. Several of these differentially expressed genes, including glpF, sigM, mrsA, lrgA and lrgB, are associated with cell wall-active antibiotics in other bacterial species. We focused on lrgA and lrgB, which form the lrgAB operon and are downregulated in ΔclpX, because loss of lrgAB increases autolytic activity and penicillin susceptibility in Staphylococcus aureus. While we observed no changes in autolytic activity in either ΔclpX or ΔlrgAB B. anthracis Sterne, we find that both mutants have increased susceptibility to the antimicrobial peptide LL-37 and daptomycin. However, phenotypes between ΔclpX and ΔlrgAB are not identical as ΔclpX also displays increased susceptibility to penicillin and nisin but ΔlrgAB does not. Therefore, while decreased expression of lrgAB may be partially responsible for the increased antimicrobial susceptibility seen in the ΔclpX mutant, disruption of other pathways must also contribute to this phenotype.

Essentiality of WalRK for growth in Bacillus subtilis and its role during heat stress

Hiraku Takada, Yuh Shiwa, Yuta Takino, Natsuki Osaka, Shuhei Ueda, Satoru Watanabe, Taku Chibazakura, Masayuki Su'etsugu, Ryutaro Utsumi, Hirofumi Yoshikawa

ABSTRACT

WalRK is an essential two-component signal transduction system that plays a central role in coordinating cell wall synthesis and cell growth in Bacillus subtilis. However, the physiological role of WalRK and its essentiality for growth have not been elucidated. We investigated the behaviour of WalRK during heat stress and its essentiality for cell proliferation. We determined that the inactivation of the walHI genes which encode the negative modulator of WalK, resulted in growth defects and eventual cell lysis at high temperatures. Screening of suppressor mutations revealed that the inactivation of LytE, an dl-endopeptidase, restored the growth of the ΔwalHI mutant at high temperatures. Suppressor mutations that reduced heat induction arising from the walRK regulon were also mapped to the walK ORF. Therefore, we hypothesized that overactivation of LytE affects the phenotype of the ΔwalHI mutant. This hypothesis was corroborated by the overexpression of the negative regulator of LytE, IseA and PdaC, which rescued the growth of the ΔwalHI mutant at high temperatures. Elucidating the cause of the temperature sensitivity of the ΔwalHI mutant could explain the essentiality of WalRK. We proved that the constitutive expression of lytE or cwlO using a synthetic promoter uncouples these expressions from WalRK, and renders WalRK nonessential in the pdaC and iseA mutant backgrounds. We propose that the essentiality of WalRK is derived from the coordination of cell wall metabolism with cell growth by regulating dl-endopeptidase activity under various growth conditions.

Transcription factor VdCmr1 is required for pigment production, protection from UV irradiation, and regulates expression of melanin biosynthetic genes in Verticillium dahliae

Yonglin Wang, Xiaoping Hu, Yulin Fang, Amy Anchieta, Polly H. Goldman, Gustavo Hernandez, Steven J. Klosterman

ABSTRACT

Verticillium dahliae is a soilborne fungus that causes vascular wilt diseases on numerous plant species worldwide. The production of darkly melanized microsclerotia is crucial in the disease cycle of V. dahliae, as these structures allow for long-term survival in soil. Previously, transcriptomic and genomic analysis identified a cluster of genes in V. dahliae that encodes some dihydroxynaphthalene (DHN) melanin biosynthetic pathway homologues found in related fungi. In this study, we explored the roles of cluster-specific transcription factor VdCmr1, as well as two other genes within the cluster encoding a polyketide synthase (VdPKS1) and a laccase (VdLac1), enzymes at initial and endpoint steps in DHN melanin production. The results revealed that VdCmr1 and VdPKS1 are required for melanin production, but neither is required for microsclerotia production. None of the three genes were required for pathogenesis on tobacco and lettuce. Exposure of ΔVdCmr1 and wild-type strains to UV irradiation, or to high temperature (40 °C), revealed an approx. 50% reduction of survival in the ΔVdCmr1 strain, relative to the wild-type strain, in response to either condition. Expression profiles revealed that expression of some melanin biosynthetic genes are in part dependent on VdCmr1. Combined data indicate VdCmr1 is a key regulator of melanin biosynthesis, and that via regulation of melanogenesis, VdCmr1 affects survival of V. dahliae in response to abiotic threats. We conclude with a model showing regulation of VdCmr1 by a high osmolarity glycerol response (Hog)-type MAP kinase pathway.

In vitro characterization and identification of potential substrates of a low molecular weight protein tyrosine phosphatase in Streptococcus pneumoniae

Zuleeza Ahmad, Renato Morona, Alistair J. Standish

ABSTRACT

Streptococcus pneumoniae is a major human pathogen responsible for significant mortality and morbidity worldwide. Within the annotated genome of the pneumococcus lies a previously uncharacterized protein tyrosine phosphatase which shows homology to low molecular weight protein tyrosine phosphatases (LMWPTPs). LMWPTPs modulate many processes critical for the pathogenicity of a number of bacteria including capsular polysaccharide biosynthesis, stress response and persistence in host macrophages. Here, we demonstrate that Spd1837 is indeed a LMWPTP, by purifying the protein, and characterizing its phosphatase activity. Spd1837 showed specific tyrosine phosphatase activity, and it did not form higher order oligomers in contrast to many other LMWPTPs. Substrate-trapping assays using the wild-type and the phosphatase-deficient Spd1837 identified potential substrates/interacting proteins including major metabolic enzymes such as ATP-dependent-6-phosphofructokinase and Hpr kinase/phosphorylase. Given the tight association between the bacterial basic physiology and virulence, this study hopes to prompt further investigation of how the pneumococcus controls its metabolic flux via the LMWPTP Spd1837.

Role of CovR phosphorylation in gene transcription in Streptococcus mutans

Pratick Khara, Saswat Sourav Mohapatra, Indranil Biswas

ABSTRACT

Streptococcus mutans, the primary aetiological agent of dental caries, is one of the major bacteria of the human oral cavity. The pathogenicity of this bacterium is attributed not only to the expression of virulence factors, but also to its ability to respond and adapt rapidly to the ever-changing conditions of the oral cavity. The two-component signal transduction system (TCS) CovR/S plays a crucial role in virulence and stress response in many streptococci. Surprisingly, in S. mutans the response regulator CovR appears to be an orphan, as the cognate sensor kinase, CovS, is absent in all the strains. We found that acetyl phosphate, an intracellular phosphodonor molecule known to act in signalling, might play a role in CovR phosphorylation in vivo. We also found that in vitro, upon phosphorylation by potassium phosphoramide (a high-energy phophodonor) CovR formed a dimer and showed altered electrophoretic mobility. As expected, we found that the conserved aspartic acid residue at position 53 (D53) was the site of phosphorylation, since neither phosphorylation nor dimerization was seen when an alanine-substituted CovR mutant (D53A) was used. Surprisingly, we found that the ability of CovR to act as a transcriptional regulator does not depend upon its phosphorylation status, since the D53A mutant behaved similarly to the wild-type protein in both in vivo and in vitro DNA-binding assays. This unique phosphorylation-mediated inhibition of CovR function in S. mutans sheds light on an unconventional mechanism of the signal transduction pathway.

RgsA, an RpoS-dependent sRNA, negatively regulates rpoS expression in Pseudomonas aeruginosa

Pei Lu, Yifei Wang, Yangbo Hu, Shiyun Chen

ABSTRACT

As a master regulator, the alternative sigma factor RpoS coordinates the transcription of genes associated with protection against environmental stresses in bacteria. In Pseudomonas aeruginosa, RpoS is also involved in quorum sensing and virulence. The cellular RpoS level is regulated at multiple levels, whereas the post-transcriptional regulation of rpoS in P. aeruginosa remains unclear. To identify and characterize small regulatory RNAs (sRNAs) regulating RpoS in P. aeruginosa, an sRNA library expressing a total of 263 sRNAs was constructed to examine their regulatory roles on rpoS expression. Our results demonstrate that rpoS expression is repressed by the RpoS-dependent sRNA RgsA at the post-transcriptional level. Unlike OxyS, an sRNA previously known to repress rpoS expression under oxidative stress in Escherichia coli, RgsA represses rpoS expression during the exponential phase. This repression requires the RNA chaperone Hfq. Furthermore, the 71–77 conserved region of RgsA is necessary for full repression of rpoS expression, and the −25 to +27 region of rpoS mRNA is sufficient for RgsA-mediated rpoS repression. Together, our results not only add RgsA to the RpoS regulatory circuits but also highlight the complexity of interplay between sRNAs and transcriptional regulators in bacteria.
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Encystation: the most prevalent and underinvestigated differentiation pathway of eukaryotes

Pauline Schaap, Christina Schilde

ABSTRACT

Not long ago, protists were considered one of four eukaryote kingdoms, but recent gene-based phylogenies show that they contribute to all nine eukaryote subdomains. The former kingdoms of animals, plants and fungi are now relegated to lower ranks within subdomains. Most unicellular protists respond to adverse conditions by differentiating into dormant walled cysts. As cysts, they survive long periods of starvation, drought and other environmental threats, only to re-emerge when conditions improve. For protists pathogens, the resilience of their cysts can prevent successful treatment or eradication of the disease. In this context, effort has been directed towards understanding the molecular mechanisms that control encystation. We here firstly summarize the prevalence of encystation across protists and next focus on Amoebozoa, where most of the health-related issues occur. We review current data on processes and genes involved in encystation of the obligate parasite Entamoeba histolytica and the opportunistic pathogen Acanthamoeba. We show how the cAMP-mediated signalling pathway that controls spore and stalk cell encapsulation in Dictyostelium fruiting bodies could be retraced to a stress-induced pathway controlling encystation in solitary Amoebozoa. We highlight the conservation and prevalence of cAMP signalling genes in Amoebozoan genomes and the suprisingly large and varied repertoire of proteins for sensing and processing environmental signals in individual species.

The FlaG regulator is involved in length control of the polar flagella of Campylobacter jejuni

Tomoharu Inoue, Clive S. Barker, Hideyuki Matsunami, Shin-Ichi Aizawa, Fadel A. Samatey

ABSTRACT

Campylobacter jejuni cells have bipolar flagella. Both flagella have similar lengths of about one helical turn, or 3.53±0.52 μm. The flagellar filament is composed of two homologous flagellins: FlaA and FlaB. Mutant strains that express either FlaA or FlaB alone produce filaments that are shorter than those of the wild-type. It is reported that the flaG gene could affect filament length in some species of bacteria, but its function remains unknown. We introduced a flaG-deletion mutation into the C. jejuni wild-type strain and flaA- or flaB-deletion mutant strains, and observed their flagella by microscopy. The ΔflaG mutant cells produced long filaments of two helical turns in the wild-type background. The ΔflaAG double mutant cells produced very short FlaB filaments. On the other hand, ΔflaBG double mutant cells produced long FlaA filaments and their morphology was not helical but straight. Furthermore, FlaG was secreted, and a pulldown assay showed that sigma factor 28 was co-precipitated with purified polyhistidine-tagged FlaG. We conclude that FlaG controls flagella length by negatively regulating FlaA filament assembly and discuss the role of FlaA and FlaB flagellins in C. jejuni flagella formation.

Chitin-induced T6SS in Vibrio cholerae is dependent on ChiS activation

Rhishita Chourashi, Suman Das, Debarpan Dhar, Keinosuke Okamoto, Asish K. Mukhopadhyay, Nabendu Sekhar Chatterjee

ABSTRACT

Vibrio cholerae regularly colonizes the chitinous exoskeleton of crustacean shells in the aquatic region. The type 6 secretion system (T6SS) in V. cholerae is an interbacterial killing device. This system is thought to provide a competitive advantage to V. cholerae in a polymicrobial community of the aquatic region under nutrient-poor conditions. V. cholerae chitin sensing is known to be initiated by the activation of a two-component sensor histidine kinase ChiS in the presence of GlcNAc2 (N,N′-diacetylchitobiose) residues generated by the action of chitinases on chitin. It is known that T6SS in V. cholerae is generally induced by chitin. However, the effect of ChiS activation on T6SS is unknown. Here, we found that ChiS inactivation resulted in impaired bacterial killing and reduced expression of T6SS genes. Active ChiS positively affected T6SS-mediated natural transformation in V. cholerae. ChiS depletion or inactivation also resulted in reduced colonization on insoluble chitin surfaces. Therefore, we have shown that V. cholerae colonization on chitinous surfaces activates ChiS, which promotes T6SS-dependent bacterial killing and horizontal gene transfer. We also highlight the importance of chitinases in T6SS upregulation.

A simple mung bean infection model for studying the virulence of Pseudomonas aeruginosa

Sneha Garge, Sheyda Azimi, Stephen P. Diggle

ABSTRACT

Here we highlight the development of a simple and high-throughput mung bean model to study virulence in the opportunistic pathogen Pseudomonas aeruginosa. The model is easy to set up, and infection and virulence can be monitored for up to 10 days. In a first test of the model, we found that mung bean seedlings infected with PAO1 showed poor development of roots and high mortality rates compared to uninfected controls. We also found that a quorum-sensing (QS) mutant was significantly less virulent when compared with the PAO1 wild-type. Our work introduces a new tool for studying virulence in P. aeruginosa that will allow for high-throughput virulence studies of mutants and testing of the in vivo efficacy of new therapies at a time when new antimicrobial drugs are desperately needed.

A highly efficient genetic system for the identification of a harzianum B biosynthetic gene cluster in Trichoderma hypoxylon

Huan Liu, Gang Wang, Wei Li, Xingzhong Liu, Erwei Li, Wen-Bing Yin

ABSTRACT

Trichoderma hypoxylon is a fungicolous species which produces rich secondary metabolites. However, no genetic transformation method is available for further studies. Here, we developed a marker-less transformation system based on the complementation of an uridine/uracil biosynthetic gene by protoplast transformation. An uridine/uracil auxotrophic mutant of Δthpyr4 was obtained by using a positive screening protocol with 5′-fluoroorotic acid as a selective reagent. To improve the homologous integration rates, the orthologues of ku70 and lig4 which play critical roles in non-homologous end-joining recombination were disrupted. The resulting thlig4 mutant showed remarkable transformation rates of 89%, while no change was found in the thku70 deletion mutant compared with the WT strain. This suggests that thlig4 play a key role in the non-homologous recombination in this strain. Using this system, the biosynthetic gene cluster of trichothecene (tri) harzianum B was identified by deletion of the thtri5 in T. hypoxylon. Comparative genome analysis revealed that the trichothecene biosynthetic gene cluster in T. hypoxylon shared similar organizations with T. arundinaceum and T. brevicompactum, even though their encoded products are different in structures. Taken together, the highly efficient genetic system provides a convenient tool for studying the biosynthetic diversity and mining the novel natural product from the fungi.

GlnR and PhoP regulate β-glucosidases involved in cellulose digestion in response to nitrogen and phosphate availability

Ya Xu, Bang-Ce Ye

ABSTRACT

The limited catalytic efficiency of cellulose-degrading enzymes restricts cellulose digestion. We investigated the transcriptional regulation of genes encoding key cellulose degrading enzymes, namely β-glucosidases, in the industrial actinobacterium Saccharopolyspora erythraea. We observed that the expression of most β-glucosidase-encoding genes was controlled by the availability of nitrogen and phosphate via their respective global regulators, namely GlnR and PhoP. Electrophoretic mobility shift assay demonstrated that GlnR and PhoP bound directly to the promoters of β-glucosidase-encoding genes. Deletion of glnR resulted in lower transcript levels and activity of β-glucosidases, leading to decreased bacterial growth on cellulose. Overexpression of glnR and phoP or nitrogen/phosphate starvation increased the transcript levels and total activity of β-glucosidases. Moreover, GlnR/PhoP-mediated cellobiose utilization was also observed in Streptomyces coelicolor A3(2). These findings provide insights into the regulatory roles played by GlnR and PhoP in coordinating nitrogen/phosphate metabolism and carbohydrate utilization, and indicate potential strategies for cellulose fermentation in the production of bio-based chemicals by actinobacteria.

Pseudomonas aeruginosa PumA acts on an endogenous phenazine to promote self-resistance

Abigail J. Sporer, Christopher Beierschmitt, Anastasia Bendebury, Katherine E. Zink, Alexa Price-Whelan, Marisa C. Buzzeo, Laura M. Sanchez, Lars E. P. Dietrich

ABSTRACT

The activities of critical metabolic and regulatory proteins can be altered by exposure to natural or synthetic redox-cycling compounds. Many bacteria, therefore, possess mechanisms to transport or transform these small molecules. The opportunistic pathogen Pseudomonas aeruginosa PA14 synthesizes phenazines, redox-active antibiotics that are toxic to other organisms but have beneficial effects for their producer. Phenazines activate the redox-sensing transcription factor SoxR and thereby induce the transcription of a small regulon, including the operon mexGHI-opmD, which encodes an efflux pump that transports phenazines, and PA14_35160 (pumA), which encodes a putative monooxygenase. Here, we provide evidence that PumA contributes to phenazine resistance and normal biofilm development, particularly during exposure to or production of strongly oxidizing N-methylated phenazines. We show that phenazine resistance depends on the presence of residues that are conserved in the active sites of other putative and characterized monooxygenases found in the antibiotic producer Streptomyces coelicolor. We also show that during biofilm growth, PumA is required for the conversion of phenazine methosulfate to unique phenazine metabolites. Finally, we compare ΔmexGHI-opmD and ΔpumA strains in assays for colony biofilm morphogenesis and SoxR activation, and find that these deletions have opposing phenotypic effects. Our results suggest that, while MexGHI-OpmD-mediated efflux has the effect of making the cellular phenazine pool more reducing, PumA acts on cellular phenazines to make the pool more oxidizing. We present a model in which these two SoxR targets function simultaneously to control the biological activity of the P. aeruginosa phenazine pool.

Identification of Staphylococcus aureus genes involved in the formation of structured macrocolonies

Charlotte Wermser, Daniel Lopez

ABSTRACT

The human pathogen Staphylococcus aureus causes difficult-to-eradicate biofilm-associated infections that generally become chronic. Understanding the genetic regulation of biofilm formation in S. aureus is central to a precise definition of the conditions and genes involved in development of chronic biofilm-associated infections. Biofilm-related genes have been detected by comparing mutants using the classical submerged biofilm formation assay, in which cells adhere to the bottom of a well containing culture medium. We recently developed an alternative biofilm formation model for S. aureus, based on macrocolony formation on agar plates, comparable to an assay used to study biofilm formation in a few other bacterial species. As organism features are the result of environmental conditions as well as of genes, we used a genome-wide collection of transposon-mapped mutants in this macrocolony assay to seek S. aureus developmental genes and pathways not identified by the classical biofilm formation assay. We identified routes related to glucose and purine metabolism and clarified their regulatory link to macrocolony formation. Our study demonstrates that formation of microbial communities must be correlated to specific growth conditions, and the role of metabolism must be considered in S. aureus biofilm formation and thus, in the development of chronic infections.

EssC is a specificity determinant for Staphylococcus aureus type VII secretion

Franziska Jäger, Holger Kneuper, Tracy Palmer

ABSTRACT

The type VII protein secretion system (T7SS) is found in actinobacteria and firmicutes, and plays important roles in virulence and interbacterial competition. A membrane-bound ATPase protein, EssC in Staphylococcus aureus, lies at the heart of the secretion machinery. The EssC protein from S. aureus strains can be grouped into four variants (EssC1–EssC4) that display sequence variability in the C-terminal region. Here we show that the EssC2, EssC3 and EssC4 variants can be produced in a strain deleted for essC1, and that they are able to mediate secretion of EsxA, an essential component of the secretion apparatus. They are, however, unable to support secretion of the substrate protein EsxC, which is only encoded in essC1-specific strains. This finding indicates that EssC is a specificity determinant for T7 protein secretion. Our results support a model in which the C-terminal domain of EssC interacts with substrate proteins, whereas EsxA interacts elsewhere.

Small RNA Esr41 inversely regulates expression of LEE and flagellar genes in enterohaemorrhagic Escherichia coli

Naoki Sudo, Akiko Soma, Sunao Iyoda, Taku Oshima, Yui Ohto, Kenta Saito, Yasuhiko Sekine

ABSTRACT

Enterohaemorrhagic Escherichia coli (EHEC) is a life-threatening human pathogen worldwide. The locus of enterocyte effacement (LEE) in EHEC encodes a type three secretion system and effector proteins, all of which are essential for bacterial adherence to host cells. When LEE expression is activated, flagellar gene expression is down-regulated because bacterial flagella induce the immune responses of host cells at the infection stage. Therefore, this inverse regulation is also important for EHEC infection. We report here that a small regulatory RNA (sRNA), Esr41, mediates LEE repression and flagellar gene activation. Multiple copies of esr41 abolished LEE expression by down-regulating the expression of ler and pch, which encode positive regulators of LEE. This regulation led to reduced EHEC adhesion to host cells. Translational gene-reporter fusion experiments revealed that Esr41 regulates ler expression at a post-transcriptional level, and pch transcription, probably via an unknown target of Esr41. Esr41-mediated ler and pch repression was not observed in cells lacking hfq, which encodes an RNA-binding protein essential for most sRNA functions, indicating that Esr41 acts in an Hfq-dependent manner. We previously reported an increase in cell motility induced by Esr41. This motility enhancement was also observed in EHEC lacking ler, showing that Esr41-mediated enhancement of cell motility is in a ler-independent manner. In addition, Esr41 activated the expression of flagellar Class 3 genes by indirectly inducing the transcription of fliA, which encodes the sigma factor for flagellar synthesis. These results suggest that Esr41 plays important roles in the inverse regulation of LEE and flagellar gene expression.

RelA/DTD-mediated regulation of spore formation and toxin production by Clostridium perfringens type a strain SM101

Ryoichi Saito, Prabhat K. Talukdar, Saud S. Alanazi, Mahfuzur R. Sarker

ABSTRACT

RelA is a global regulator for stationary phase development in the model bacterium Bacillus subtilis. The relA gene forms a bicistronic operon with the downstream dtd gene. In this study, we evaluated the significance of RelA and DTD proteins in spore formation and toxin production by an important gastrointestinal pathogen Clostridium perfringens. Our β-glucuronidase assay showed that in C. perfringens strain SM101, relA forms a bicistronic operon with its downstream dtd gene, and the relA promoter is expressed during both vegetative and sporulation conditions. By constructing double relA dtd and single dtd mutants in C. perfringens SM101, we found that: (1) RelA is required for maintaining the efficient growth capacity of SM101 cells during vegetative conditions; (2) both RelA and DTD are required for spore formation and enterotoxin (CPE) production by SM101; (3) RelA/DTD activate CodY, which is known to activate spore formation and CPE production in SM101 by activating a key sporulation-specific σ factor F; (4) as expected, RelA/DTD activate sporulation-specific σ factors (σE, σF, σG and σK) by positively regulating Spo0A production; and finally (5) RelA, but not DTD, negatively regulates phospholipase C (PLC) production by repressing plc gene expression. Collectively, our results demonstrate that RelA modulates cellular physiology such as growth, spore formation and toxin production by C. perfringens type A strain SM101, although DTD also plays a role in these pleiotropic functions in coordination with RelA during sporulation. These findings have implications for the understanding of the mechanisms involved in the infectious cycle of C. perfringens.

The ClpY-ClpQ protease regulates multicellular development in Bacillus subtilis

Yiyang Yu, Fang Yan, Yinghao He, Yuxuan Qin, Yun Chen, Yunrong Chai, Jian-hua Guo

ABSTRACT

ATP-dependent proteases play essential roles in both protein quality control and the regulation of protein activities in bacteria. ClpYQ (also known as HslVU) is one of several highly conserved ATP-dependent proteases in bacteria. The regulation and biological function of ClpYQ have been well studied in Gram-negative bacteria, but are poorly understood in Gram-positive species. In this study, we showed that in the Gram-positive bacterium Bacillus subtilis, the ΔclpYQ deletion mutant formed early and robust biofilms, while swarming motility was severely impaired. Colonies of the ΔclpYQ mutant were also much less mucoid on agar plates, indicating the loss of the production of secreted γ-poly-dl-glutamic acid (γ-PGA). Global proteomic analysis using isobaric tags for relative and absolute quantification (iTRAQ) confirmed that a number of proteins involved in motility, chemotaxis and the production of γ-PGA were less abundant in the ΔclpYQ mutant. The results from both iTRAQ and Western immunoblotting showed that levels of the biofilm master repressor SinR were modestly reduced in the ΔclpYQ mutant, but probably significantly enough to alter biofilm regulation due to the ultrasensitivity of the expression of biofilm genes to SinR protein levels. Western immunoblotting also showed that the abundance of CodY, whose gene is clustered with clpYQ in the same operon, was not impacted on by ΔclpYQ. Lastly, our results suggested that, unlike in Escherichia coli, ClpYQ does not play an essential role in heat-shock response in both B. subtilis and Bacillus cereus. In conclusion, we propose that the ClpYQ protease is primarily involved in multicellular development in B. subtilis.

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Microbe Profile: Corynebacterium diphtheriae – an old foe always ready to seize opportunity

Paul A. Hoskisson

ABSTRACT

Corynebacterium diphtheriae is a globally important Gram-positive aerobic Actinobacterium capable of causing the toxin-mediated disease, diphtheria. Diphtheria was a major cause of childhood mortality prior to the introduction of the toxoid vaccine, yet it is capable of rapid resurgence following the breakdown of healthcare provision, vaccination or displacement of people. The mechanism and treatment of toxin-mediated disease is well understood, however there are key gaps in our knowledge on the basic biology of C. diphtheriae particularly relating to host colonisation, the nature of asymptomatic carriage, population genomics and host adaptation.

Metal induction of two metallothionein genes in the ectomycorrhizal fungus Suillus himalayensis and their role in metal tolerance

Tania Kalsotra, Shikha Khullar, Radhika Agnihotri, Mondem Sudhakara Reddy

ABSTRACT

Metallothioneins (MTs) are small proteins with highly conserved cysteine residues and are involved in metal homeostasis and metal detoxification. Two metallothionein genes ShMT1 and ShMT2 from the ectomycorrhizal fungus Suillus himalayensis were characterised for their potential role in heavy metal detoxification. The response of these MTs to the exogenous concentrations of copper and cadmium was studied by qPCR analysis. The exogenous copper but not the cadmium at the tested concentrations induced the expression of the MT genes. The functional role of ShMTs was validated by expressing the two genes through functional complementation in yeast mutant strain cup1Δ (copper-sensitive), ycf1Δ (cadmium- sensitive) and zrc1Δ (zinc-sensitive). The mutant strain successfully expressed the two genes resulting in wild-type phenotype restoration of copper, cadmium and zinc tolerance. The present study shows that the ectomycorrhizal fungus S. himalayensis encodes two metallothionein genes (ShMT1 and ShMT2) which are more inducible by copper than cadmium and could play an important role in their detoxification.

Polymorphisms in the Helicobacter pylori NY43 strain and its prophage-cured derivatives

Hiroaki Takeuchi, Mizuki Kira, Sayuri Konishi, Jumpei Uchiyama, Shigenobu Matsuzaki, Yoshihisa Matsumura

ABSTRACT

This study aimed to determine the characteristics of the Helicobacter pylori host NY43 strain and its prophage-cured derivative. H. pylori colonizing the human stomach cause many diseases. They show high genetic diversity, allowing the development of mutant strains that can form bacterial communities adapted to specific environmental conditions. Bacteriophage activities are associated with bacterial evolution, including pathogenicity development. Herein, we reported the complete genome sequence and genomic organization of two H. pylori prophages, KHP30 and KHP40; the effects of KHP30 on the behaviours of NY43 are not yet known. We showed that approximately 57% prophage-cured derivatives spontaneously appeared in the exponential phase during liquid culture, and the biological characteristics of these derivatives differed from those of the host NY43. KHP30 reinfected the cured derivatives, and the curing ratio was influenced by culture conditions. KHP30 was shown to promote the development of a flexible H. pylori community with variable characteristics.

The biofilm matrix polysaccharides cellulose and alginate both protect Pseudomonas putida mt-2 against reactive oxygen species generated under matric stress and copper exposure

Nanna B. Svenningsen, Esteban Martínez-García, Mette H. Nicolaisen, Victor de Lorenzo, Ole Nybroe

ABSTRACT

In natural environments most bacteria live in biofilms embedded in complex matrices of extracellular polymeric substances (EPS). This lifestyle is known to increase protection against environmental stress. Pseudomonas putida mt-2 harbours genes for the production of at least four different EPS polysaccharides, including alginate and cellulose. Little is known about the functional properties of cellulose, while alginate attenuates the accumulation of reactive oxygen species (ROS) caused by matric stress. By using mutants that are deficient in either alginate or cellulose production we show that even cellulose attenuates the accumulation of matric stress-induced ROS for cells in biofilms. Further, both cellulose and alginate attenuate ROS generated through exposure to copper. Interestingly, the two EPS polysaccharides protect cells in both liquid culture and in biofilms against ROS caused by matric stress, indicating that cellulose and alginate do not need to be produced as an integral part of the biofilm lifestyle to provide tolerance towards environmental stressors.

Antisense transcription in Pseudomonas aeruginosa

Denitsa Eckweiler, Susanne Häussler

ABSTRACT

A large number of antisense transcripts have been detected in diverse microbial genomes and considerable effort has been devoted to elucidating the functional role of antisense transcription. In this study, we reanalysed extensive RNA sequencing data from the opportunistic pathogen Pseudomonas aeruginosa and found that the majority of genes have a propensity for antisense transcription. Although antisense transcripts were found in more than 80% of the genes of the P. aeruginosa genome, the majority of sequencing reads were mapping sense and only a minority (<2%) were mapping antisense to genes. Similarly to the sense expression levels, the antisense expression levels varied under different environmental conditions, with the sense and antisense expression levels often being inversely regulated and modulated by the activity of alternative sigma factors. Environment-modulated antisense transcription showed a bias towards being antisense to genes within regions of genomic plasticity and to those encoding small regulatory RNAs. In the future, the validation and functional characterization of antisense transcripts, and novel transcripts that are antisense to small regulatory RNAs in particular, have the potential to contribute to our understanding of the various levels of transcriptional regulation and its dynamics in the bacterial pathogen P. aeruginosa.

Random sorting of Campylobacter jejuni phase variants due to a narrow bottleneck during colonization of broiler chickens

Joseph J. Wanford, Lea Lango-Scholey, Harald Nothaft, Yue Hu, Christine M. Szymanski, Christopher D. Bayliss

ABSTRACT

Phase variation (PV), involving stochastic switches in gene expression, is exploited by the human pathogen Campylobacter jejuni to adapt to different environmental and host niches. Phase-variable genes of C. jejuni modulate expression of multiple surface determinants, and hence may influence host colonization. Population bottlenecks can rapidly remove the diversity generated by PV, and strict single-cell bottlenecks can lead to propagation of PV states with highly divergent phenotypes. Using a combination of high-throughput fragment size analysis and comparison with in vivo and in silico bottleneck models, we have characterized a narrow population bottleneck during the experimental colonization of broiler chickens with C. jejuni strain 81-176. We identified high levels of variation in five PV genes in the inoculum, and subsequently, massively decreased population diversity following colonization. Each bird contained a dominant five-gene phasotype that was present in the inoculum indicative of random sorting through a narrow, non-selective bottleneck during colonization. These results are evidence of the potential for confounding effects of PV on in vivo studies of Campylobacter colonization factors and poultry vaccine studies. Our results are also an argument for population bottlenecks as mediators of stochastic variability in the propensity to survive through the food chain and cause clinical human disease.

Characterization of the small flavin-binding dodecin in the roseoflavin producer Streptomyces davawensis

Petra Ludwig, Daniel C. Sévin, Tobias Busche, Jörn Kalinowski, Florian Bourdeaux, Martin Grininger, Matthias Mack

ABSTRACT

Genes encoding dodecin proteins are present in almost 20% of archaeal and in more than 50% of bacterial genomes. Archaeal dodecins bind riboflavin (vitamin B2), are thought to play a role in flavin homeostasis and possibly also help to protect cells from radical or oxygenic stress. Bacterial dodecins were found to bind riboflavin-5′-phosphate (also called flavin mononucleotide or FMN) and coenzyme A, but their physiological function remained unknown. In this study, we set out to investigate the relevance of dodecins for flavin metabolism and oxidative stress management in the phylogenetically related bacteria Streptomyces coelicolor and Streptomyces davawensis. Additionally, we explored the role of dodecins with regard to resistance against the antibiotic roseoflavin, a riboflavin analogue produced by S. davawensis. Our results show that the dodecin of S. davawensis predominantly binds FMN and is neither involved in roseoflavin biosynthesis nor in roseoflavin resistance. In contrast to S. davawensis, growth of S. coelicolor was not reduced in the presence of plumbagin, a compound, which induces oxidative stress. Plumbagin treatment stimulated expression of the dodecin gene in S. davawensis but not in S. coelicolor. Deletion of the dodecin gene in S. davawensis generated a recombinant strain which, in contrast to the wild-type, was fully resistant to plumbagin. Subsequent metabolome analyses revealed that the S. davawensis dodecin deletion strain exhibited a very different stress response when compared to the wild-type indicating that dodecins broadly affect cellular physiology.

Insights into the initiation of chromosome II replication of the pressure-loving deep-sea bacterium Photobacterium profundum SS9

Kamila K. Myka, Peter McGlynn, Gail P. Ferguson

ABSTRACT

How DNA metabolism is adapted to survival of organisms such as the bacterium Photobacterium profundum SS9 at high pressure is unknown. Previously, a high pressure-sensitive P. profundum SS9 transposon mutant (FL31) was identified, with an insertion in a putative rctB gene. The Vibrio cholerae RctB protein is essential for replication initiation at the origin of chromosome II, oriCII. Using a plasmid-based system in E. coli we have identified the replication origin of chromosome II from P. profundum SS9 and have shown that the putative rctB gene, disrupted in FL31, is essential for oriCII function. Moreover, we found that a region corresponding to the V. cholerae oriCII incompatibility region (incII) exerts an inhibitory effect on P. profundum oriCII. The truncated rctB gene in FL31 confers insensitivity to incII inhibition, indicating that the C-terminus of RctB is important for the negative regulation of replication. The RctB proteins of V. cholerae and P. profundum are partially interchangeable, but full functionality is achieved only with the cognate origin. Our findings provide the first characterization of the replication origin of chromosome II in a deep-sea bacterium.

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The transmembrane segment of TagH is required for wall teichoic acid transport under heat stress in Bacillus subtilis

Takeshi Yamada, Mari Miyashita, Jun Kasahara, Tatsuhito Tanaka, Masayuki Hashimoto, Hiroki Yamamoto

ABSTRACT

Wall teichoic acids (WTAs) are anionic polymers that are covalently linked to peptidoglycan and play important roles in cell shape determination, cell division, autolysis, pathogenesis and antibiotic resistance in Gram-positive bacteria. In Bacillus subtilis, WTA is synthesized in the cytoplasm and translocated by an ABC transporter, TagGH. In this study, we found that the transmembrane segment of TagH is required for WTA transport under high temperatures. Cells expressing TagH302-FL (a construct fused to the 6×FLAG tag after the transmembrane segment, which lacks the C-terminal extracellular domain) grew normally at high temperatures, similar to those expressing the full-length TagH-FL fusion. In contrast, cells expressing TagH275-FL, which lacks both the transmembrane segment and the extracellular domain, exhibited a temperature-sensitive phenotype at temperatures above 49 °C and a growth defect at 50 °C. Interestingly, this growth defect was dissolved by an additional incubation at 37 °C. A similar temperature-sensitive phenotype was observed in cells expressing an N-terminal 6×FLAG tag fusion of TagH275. Immunofluorescence microscopy (IFM) indicated that TagG and TagH are localized on the cytoplasmic membrane in a patch-like manner. In addition, the C-terminal-truncated forms, TagH275-FL and TagH302-FL, were localized in similar patch-like patterns at 37 °C; only foci for TagH275-FL were remarkably reduced at high temperatures. Moreover, cell surface decoration with WTA was considerably reduced in cells harbouring TagH275-FL at high temperature, supporting the results of IFM observation. These results suggest that the transmembrane segment of TagH plays an important role in WTA export at high temperatures.

The role of inter-species interactions in Salinispora specialized metabolism

Nastassia V. Patin, Dimitrios J. Floros, Chambers C. Hughes, Pieter C. Dorrestein, Paul R. Jensen

ABSTRACT

Bacterial genome sequences consistently contain many more biosynthetic gene clusters encoding specialized metabolites than predicted by the compounds discovered from the respective strains. One hypothesis invoked to explain the cryptic nature of these gene clusters is that standard laboratory conditions do not provide the environmental cues needed to trigger gene expression. A potential source of such cues is other members of the bacterial community, which are logical targets for competitive interactions. In this study, we examined the effects of such interactions on specialized metabolism in the marine actinomycete Salinispora tropica. The results show that antibiotic activities and the concentration of some small molecules increase in the presence of co-occurring bacterial strains relative to monocultures. Some increases in antibiotic activity could be linked to nutrient depletion by the competitor as opposed to the production of a chemical cue. Other increases were correlated with the production of specific compounds by S. tropica. In particular, one interaction with a Vibrio sp. consistently induced antibiotic activity and was associated with parent ions that were unique to this interaction, although the associated compound could not be identified. This study provides insight into the metabolomic complexities of bacterial interactions and baseline information for future genome mining efforts.

Linkage of the Nit1C gene cluster to bacterial cyanide assimilation as a nitrogen source

Lauren B. Jones, Pallab Ghosh, Jung-Hyun Lee, Chia-Ni Chou, Daniel A. Kunz

ABSTRACT

A genetic linkage between a conserved gene cluster (Nit1C) and the ability of bacteria to utilize cyanide as the sole nitrogen source was demonstrated for nine different bacterial species. These included three strains whose cyanide nutritional ability has formerly been documented (Pseudomonas fluorescens Pf11764, Pseudomonas putida BCN3 and Klebsiella pneumoniae BCN33), and six not previously known to have this ability [Burkholderia (Paraburkholderia) xenovorans LB400, Paraburkholderia phymatum STM815, Paraburkholderia phytofirmans PsJN, Cupriavidus (Ralstonia) eutropha H16, Gluconoacetobacter diazotrophicus PA1 5 and Methylobacterium extorquens AM1]. For all bacteria, growth on or exposure to cyanide led to the induction of the canonical nitrilase (NitC) linked to the gene cluster, and in the case of Pf11764 in particular, transcript levels of cluster genes (nitBCDEFGH) were raised, and a nitC knock-out mutant failed to grow. Further studies demonstrated that the highly conserved nitB gene product was also significantly elevated. Collectively, these findings provide strong evidence for a genetic linkage between Nit1C and bacterial growth on cyanide, supporting use of the term cyanotrophy in describing what may represent a new nutritional paradigm in microbiology. A broader search of Nit1C genes in presently available genomes revealed its presence in 270 different bacteria, all contained within the domain Bacteria, including Gram-positive Firmicutes and Actinobacteria, and Gram-negative Proteobacteria and Cyanobacteria. Absence of the cluster in the Archaea is congruent with events that may have led to the inception of Nit1C occurring coincidentally with the first appearance of cyanogenic species on Earth, dating back 400–500 million years.

Promiscuity of methionine salvage pathway enzymes in Methanocaldococcus jannaschii

Danielle V. Miller, Benjamin J. Rauch, Kim Harich, Huimin Xu, John J. Perona, Robert H. White

ABSTRACT

The methionine salvage pathway (MSP) is critical for regeneration of S-adenosyl-l-methionine (SAM), a widely used cofactor involved in many essential metabolic reactions. The MSP has been completely elucidated in aerobic organisms, and found to rely on molecular oxygen. Since anaerobic organisms do not use O2, an alternative pathway(s) must be operating. We sought to evaluate whether the functions of two annotated MSP enzymes from Methanocaldococcus jannaschii, a methylthioinosine phosphorylase (MTIP) and a methylthioribose 1-phosphate isomerase (MTRI), are consistent with functioning in a modified anaerobic MSP (AnMSP). We show here that recombinant MTIP is active with six different purine nucleosides, consistent with its function as a general purine nucleoside phosphorylase for both AnMSP and purine salvage. Recombinant MTRI is active with both 5-methylthioribose 1-phosphate and 5-deoxyribose 1-phosphate as substrates, which are generated from phosphororolysis of 5′-methylthioinosine and 5′-deoxyinosine by MTIP, respectively. Together, these data suggest that MTIP and MTRI may function in a novel pathway for recycling the 5′-deoxyadenosine moiety of SAM in M. jannaschii. These enzymes may also enable biosynthesis of 6-deoxy-5-ketofructose 1-phosphate (DKFP), an essential intermediate in aromatic amino acid biosynthesis. Finally, we utilized a homocysteine auxotrophic strain of Methanosarcina acetivorans Δma1821-22Δoahs (HcyAux) to identify potential AnMSP intermediates in vivo. Growth recovery experiments of the M. acetivorans HcyAux were performed with known and proposed intermediates for the AnMSP. Only one metabolite, 2-keto-(4-methylthio) butyric acid, rescued growth of M. acetivorans HcyAux in the absence of homocysteine. This observation may indicate that AnMSP pathways substantially differ among methanogens from phylogenetically divergent genera.

Utilisation of 10-formyldihydrofolate as substrate by dihydrofolate reductase (DHFR) and 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) tranformylase/IMP cyclohydrolase (PurH) in Escherichia coli

Shivjee Sah, Riyaz Ahmad Shah, Ashwin Govindan, Rajagopal Varada, Kervin Rex, Umesh Varshney

ABSTRACT

Dihydrofolate reductase (DHFR) and 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase/IMP cyclohydrolase (PurH) play key roles in maintaining folate pools in cells, and are targets of antimicrobial and anticancer drugs. While the activities of bacterial DHFR and PurH on their classical substrates (DHF and 10-CHO-THF, respectively) are known, their activities and kinetic properties of utilisation of 10-CHO-DHF are unknown. We have determined the kinetic properties (k cat/K m) of conversion of 10-CHO-DHF to 10-CHO-THF by DHFR, and to DHF by PurH. We show that DHFR utilises 10-CHO-DHF about one third as efficiently as it utilises DHF. The 10-CHO-DHF is also utilised (as a formyl group donor) by PurH albeit slightly less efficiently than 10-CHO-THF. The utilisation of 10-CHO-DHF by DHFR is ~50 fold more efficient than its utilisation by PurH. A folate deficient Escherichia coli (ΔpabA) grows well when supplemented with adenine, glycine, thymine and methionine, the metabolites that arise from the one-carbon metabolic pathway. Notably, when the ΔpabA strain harboured a folate transporter, it grew in the presence of 10-CHO-DHF alone, suggesting that it (10-CHO-DHF) can enter one-carbon metabolic pathway to provide the required metabolites. Thus, our studies reveal that both DHFR and PurH could utilise 10-CHO-DHF for folate homeostasis in E. coli.

Electrostatic interactions are important for chaperone–client interaction in vivo

Changhan Lee, Hyunhee Kim, James C. A. Bardwell

ABSTRACT

It has long been thought that chaperones are primarily attracted to their clients through the hydrophobic effect. However, in in vitro studies on the interaction between the chaperone Spy and its substrate Im7, we recently showed that long-range electrostatic interactions also play a key role. Spy functions in the periplasm of Gram-negative bacteria, which is surrounded by a permeable outer membrane. The ionic conditions in the periplasm therefore closely mimic those in the media, which allowed us to vary the ionic strength of the in vivo folding environment. Using folding biosensors that link protein folding to antibiotic resistance, we were able to monitor Spy chaperone activity in Escherichia coli in vivo as a function of media salt concentration. The chaperone activity of Spy decreased when the ionic strength of the media was increased, strongly suggesting that electrostatic forces play a vital role in the action of Spy in vivo.

Deletion of gene encoding the nucleoid-associated protein H-NS unmasks hidden regulatory connections in El Tor biotype Vibrio cholerae

Raedeen Russell, Hongxia Wang, Jorge A. Benitez, Anisia J. Silva

ABSTRACT

Hypervirulent atypical El Tor biotype Vibrio cholerae O1 isolates harbour mutations in the DNA-binding domain of the nucleoid-associated protein H-NS and the receiver domain of the response regulator VieA. Here, we provide two examples in which inactivation of H-NS in El Tor biotype vibrios unmasks hidden regulatory connections. First, deletion of the helix-turn-helix domain of VieA in an hns mutant background diminished biofilm formation and exopolysaccharide gene expression, a function that phenotypically opposes its phosphodiesterase activity. Second, deletion of vieA in an hns mutant diminished the expression of σE, a virulence determinant that mediates the envelope stress response. Hns mutants were highly sensitive to envelope stressors compared to wild-type. However, deletion of vieA in the hns mutant restored or exceeded wild-type resistance. These findings suggest an evolutionary path for the emergence of hypervirulent strains starting from nucleotide sequence diversification affecting the interaction of H-NS with DNA.

Microbe Profile: Aspergillus fumigatus: a saprotrophic and opportunistic fungal pathogen

Wenxia Fang, Jean-Paul Latgé

ABSTRACT

Aspergillus fumigatus is a saprotrophic fungus that continuously disseminates spores (conidia) into the environment. It is also the most common and opportunistic aerial fungal pathogen, causing allergic and chronic lung pathologies including the fatal invasive aspergillosis in immunocompromised patients. The pathobiology of aspergillosis is complex and depends on the competence of the host immune system. Moreover, A. fumigatus has become a model to study unique features of fungi. This includes the fungal cell wall, which not only acts as a rigid skeleton for protection against hostile environments but also plays significant roles during infection by manipulating the host immune response.

Cell-wall dyes interfere with Cryptococcus neoformans melanin deposition

Ricardo Perez-Dulzaides, Emma Camacho, Radames J. B. Cordero, Arturo Casadevall

ABSTRACT

Melanization is an intrinsic characteristic of many fungal species, but details of this process are poorly understood because melanins are notoriously difficult pigments to study. While studying the binding of cell-wall dyes, Eosin Y or Uvitex, to melanized and non-melanized Cryptococcus neoformans cells we noted that melanization leads to reduced fluorescence intensity, suggesting that melanin interfered with dye binding to the cell wall. The growth of C. neoformans in melanizing conditions with either of the cell-wall dyes resulted in an increase in supernatant-associated melanin, consistent with blockage of melanin attachment to the cell wall. This effect provided the opportunity to characterize melanin released into culture supernatants. Released melanin particles appeared mostly as networked structures having dimensions consistent with previously described extracellular vesicles. Hence, dye binding to the cell wall created conditions that resembled the ‘leaky melanin’ phenotype described for certain cell-wall mutants. In agreement with earlier studies on fungal melanins biosynthesis, our observations are supportive of a model whereby C. neoformans melanization proceeds by the attachment of melanin nanoparticles to the cell wall through chitin, chitosan, and various glucans.

Evaluation of a novel outer membrane surface-exposed protein, LIC13341 of Leptospira, as an adhesin and serodiagnostic candidate marker for leptospirosis

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ABSTRACT

The outer membrane proteins of the pathogen are targeted to understand host–pathogen interactions and are central to the development of diagnostics. We report that Leptospira interrogans serovar Copenhageni strain Fiocruz L1-130 contains a gene LIC13341 that encodes a conserved outer membrane/periplasmic lipoprotein. The gene LIC13341 was cloned into expression vector pET28a and the recombinant LIC13341 (r-LIC13341) protein was purified from Escherichia coli BL21 (DE3) using affinity chromatography. The secondary structure of the purified r-LIC13341 protein featured a typical β-strand when observed by circular dichroism spectroscopy. Immunoblotting using antibodies raised against r-LIC13341 in BALB/c mice can detect LIC13341 expression in the Leptospira lysates and suggested that antigen LIC13341 is immunogenic. Phase separation and protease assays determined that LIC13341 is a surface-exposed outer membrane protein of Leptospira. The r-LIC13341 can bind to a wide spectrum of host extracellular matrices (ECMs). The specific adherence of Leptospira to laminin and hyaluronic acid of the ECM was competitively inhibited in the presence of r-LIC13341. The enzyme-linked immunosorbent assay and immunoblot performed using human or bovine leptospirosis serum (n=50) recognized r-LIC13341, suggesting that LIC13341 is expressed in diverse hosts during Leptospira infection. Thus, the present finding suggests that the Leptospira LIC13341 antigen is a versatile outer membrane adhesin of diagnostic importance.

Intergenic evolution during host adaptation increases expression of the metallophore pseudopaline in Pseudomonas aeruginosa

Grith Miriam Maigaard Hermansen, Morten Lindqvist Hansen, Seyed Mohammad Hossein Khademi, Lars Jelsbak

ABSTRACT

Regulating intracellular levels of biological metal ions is essential for all bacterial species, as they are needed for virulence and a range of metabolic processes. Zinc is the second most abundant metal ion in Pseudomonas aeruginosa, but little is known about its regulation. Recent studies have identified a novel operon, zrmABCD (also called cntOLMI), encoding a metallophore system (pseudopaline) involved in zinc acquisition. Expression of this operon has been implicated in human infections and is regulated by the transcriptional regulator Zur (Zn2+ uptake regulator). In this study, we show that the intergenic promoter region in front of zrmABCD is a target for recurrent adaptive mutations during chronic infection of cystic fibrosis (CF) patients. We characterize the inter- and intraclonal sequence polymorphisms found in the promoter region of the metallophore system and find that most alterations increase promoter activity. One of the evolved promoters displays a more than 10-fold increase compared to the ancestral strain due to the combined effect of an altered binding site of Zur and changes to the RpoD-binding motif. This specific evolved promoter responds differently to changes in metal ion concentrations in chelated medium. We have previously shown that P. aeruginosa evolves toward iron acquisition from haemoglobin during long-term CF infections. We hereby provide the second example of adaptive mutations targeting intergenic regions that affect metal ion uptake systems during CF infections, and the first involving zinc uptake. Our results suggest that the scarcity of metal ions (including iron and zinc) is an important evolutionary driver in CF host adaptation.

Comprehensive screening of antimicrobials to control phytoplasma diseases using an in vitro plant–phytoplasma co-culture system

Kazuyuki Tanno, Kensaku Maejima, Akio Miyazaki, Hiroaki Koinuma, Nozomu Iwabuchi, Yugo Kitazawa, Takamichi Nijo, Masayoshi Hashimoto, Yasuyuki Yamaji, Shigetou Namba

ABSTRACT

Phytoplasmas are plant-pathogenic bacteria that infect many important crops and cause serious economic losses worldwide. However, owing to an inability to culture phytoplasmas, screening of antimicrobials on media is difficult. The only antimicrobials being used to control phytoplasmas are tetracycline-class antibiotics. In this study, we developed an accurate and efficient screening method to evaluate the effects of antimicrobials using an in vitro plant–phytoplasma co-culture system. We tested 40 antimicrobials, in addition to tetracycline, and four of these (doxycycline, chloramphenicol, thiamphenicol and rifampicin) decreased the accumulation of ‘Candidatus (Ca.) Phytoplasma asteris'. The phytoplasma was eliminated from infected plants by the application of both tetracycline and rifampicin. We also compared nucleotide sequences of rRNAs and amino acid sequences of proteins targeted by antimicrobials between phytoplasmas and other bacteria. Since antimicrobial target sequences were conserved among various phytoplasma species, the antimicrobials that decreased accumulation of ‘Ca. P. asteris' may also have been effective against other phytoplasma species. These approaches will provide new strategies for phytoplasma disease management.

Helicobacter pylori nickel storage proteins: recognition and modulation of diverse metabolic targets

Zachary Saylor, Robert Maier

ABSTRACT

Nickel metabolism and trafficking in Helicobacter pylori is complex, perhaps more so than in any other pathogen. Along with nickel enzymes and their associated nickel-binding maturation machinery, H. pylori contain nickel storage proteins, Hpn and Hpnl. Through a combined crosslinking and enrichment approach, we show that Hpn/Hpnl interact with a wide array of partners; over 100 proteins were captured, including known nickel-enzyme maturation proteins, and other proteins outside known H. pylori nickel-associated proteins. The crosslinker binds to exposed amines, but there was no correlation between lysine content and the pulldown abundance of captured proteins. Phenotypic characterization of mutant strains (Δhpn, Δhpnl, or ΔhpnΔhpnl) was used to explore interactions. Nickel deprivation affected the hydrogenase activity of the ΔhpnΔhpnl strain much more severely than the wild-type (WT), whereas the activities of the single mutants were similar to WT. Leucyl aminopeptidase activity was affected in opposite ways in the mutant strains: Δhpn had a threefold decrease, while Δhpnl had a sevenfold increase, compared to the parent. Similar mutant strain analysis supported Hpn and Hpnl acting synergistically to suppress aliphatic amidase activity in a nickel-dependent manner. Recombinant amidase could bind a variety of divalent metals. Amidase activity was greatest in the mutant strains and was inhibited by exogenous nickel. The addition of pure storage protein to extracts from the mutants only restored the suppression of amidase activity for the mutant strain lacking that protein; both storage proteins are needed for amidase suppression. These results suggest that Hpn and Hpnl play more diverse roles than previously thought.

Microbe Profile: Saccharomyces eubayanus, the missing link to lager beer yeasts

José Paulo Sampaio

ABSTRACT

Saccharomyces eubayanus was described less than 10 years ago and its discovery settled the long-lasting debate on the origins of the cold-tolerant yeast responsible for lager beer fermentation. The largest share of the genetic diversity of S. eubayanus is located in South America, and strains of this species have not yet been found in Europe. One or more hybridization events between S. eubayanus and S. cerevisiae ale beer strains gave rise to S. pastorianus, the allopolyploid yeasts responsible for lager beer production worldwide. The identification of the missing progenitor of lager yeast opened new avenues for brewing yeast research. It allowed not only the selective breeding of new lager strains, but revealed also a wild yeast with interesting brewing abilities so that a beer solely fermented by S. eubayanus is currently on the market.

Broad-spectrum antimicrobial activity by Burkholderia cenocepacia TAtl-371, a strain isolated from the tomato rhizosphere

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ABSTRACT

The Burkholderia cepacia complex (Bcc) comprises a group of 24 species, many of which are opportunistic pathogens of immunocompromised patients and also are widely distributed in agricultural soils. Several Bcc strains synthesize strain-specific antagonistic compounds. In this study, the broad killing activity of B. cenocepacia TAtl-371, a Bcc strain isolated from the tomato rhizosphere, was characterized. This strain exhibits a remarkable antagonism against bacteria, yeast and fungi including other Bcc strains, multidrug-resistant human pathogens and plant pathogens. Genome analysis of strain TAtl-371 revealed several genes involved in the production of antagonistic compounds: siderophores, bacteriocins and hydrolytic enzymes. In pursuit of these activities, we observed growth inhibition of Candida glabrata and Paraburkholderia phenazinium that was dependent on the iron concentration in the medium, suggesting the involvement of siderophores. This strain also produces a previously described lectin-like bacteriocin (LlpA88) and here this was shown to inhibit only Bcc strains but no other bacteria. Moreover, a compound with an m/z 391.2845 with antagonistic activity against Tatumella terrea SHS 2008T was isolated from the TAtl-371 culture supernatant. This strain also contains a phage-tail-like bacteriocin (tailocin) and two chitinases, but the activity of these compounds was not detected. Nevertheless, the previous activities are not responsible for the whole antimicrobial spectrum of TAtl-371 seen on agar plates, suggesting the presence of other compounds yet to be found. In summary, we observed a diversified antimicrobial activity for strain TAtl-371 and believe it supports the biotechnological potential of this Bcc strain as a source of new antimicrobials.

Cinnamaldehyde disrupts biofilm formation and swarming motility of Pseudomonas aeruginosa

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ABSTRACT

Bacterial biofilms can cause serious health care complications associated with increased morbidity and mortality. There is an urge to discover and develop new biofilm inhibitors from natural products or by modifying natural compounds or understanding the modes of action of existing compounds. Cinnamaldehyde (CAD), one of the major components of cinnamon oil, has been demonstrated to act as an antimicrobial agent against a number of Gram-negative and Gram-positive pathogens, including Pseudomonas aeruginosa, Helicobacter pylori and Listeria monocytogenes. Despite the mechanism of action of CAD against the model organism P. aeruginosa being undefined, based on its antimicrobial properties, we hypothesized that it may disrupt preformed biofilms of P. aeruginosa. The minimum inhibitory concentration (MIC) of CAD for planktonic P. aeruginosa was determined to be 11.8 mM. Membrane depolarization assays demonstrated disruption of the transmembrane potential of P. aeruginosa. CAD at 5.9 mM (0.5 MIC) disrupted preformed biofilms by 75.6% and 3 mM CAD (0.25 MIC) reduced the intracellular concentrations of the secondary messenger, bis-(3′–5′)-cyclic dimeric guanosine monophosphate (c-di-GMP), which controls P. aeruginosa biofilm formation. The swarming motility of P. aeruginosa was also reduced by CAD in a concentration-dependent manner. Collectively, these findings show that sub-MICs of CAD can disrupt biofilms and other surface colonization phenotypes through the modulation of intracellular signalling processes.

Oxyresveratrol-induced DNA cleavage triggers apoptotic response in Candida albicans

Suhyun Kim, Dong Gun Lee

ABSTRACT

Oxyresveratrol is a naturally occurring phytoalexin produced by plants in response to infection. Biological activities of oxyresveratrol have been studied such as antioxidant, anticancer and anti-inflammation. However, further antimicrobial activity and its mechanism need to be investigated. This study exhibited growth inhibition against pathogenic fungi and investigated its mode of action. Oxyresveratrol inflicted cleavage on DNA, leading to G2/M phase arrest. DNA damage by oxyresveratrol was not the result of oxidative stress but it was triggered by direct binding to DNA. Oxyresveratrol-treated cells showed an apoptotic pathway characterized by phosphatidylserine exposure, apoptotic volume decrease and metacaspase activation. Mitochondria-associated apoptotic features also appeared. Oxyresveratrol-induced Ca2+ overload led to mitochondrial membrane depolarization and release of cytochrome c from mitochondria to cytosol. In conclusion, oxyresveratrol with DNA-binding affinity induces DNA cleavage, and eventually leads to mitochondria-mediated apoptosis in Candida albicans.

Isolation of colonization-defective Escherichia coli mutants reveals critical requirement for fatty acids in bacterial colony formation

Kazuki Nosho, Koji Yasuhara, Yuto Ikehata, Tomohiro Mii, Taichiro Ishige, Shunsuke Yajima, Makoto Hidaka, Tetsuhiro Ogawa, Haruhiko Masaki

ABSTRACT

Most bacterial cells in nature exhibit extremely low colony-forming activity, despite showing various signs of viability, impeding the isolation and utilization of many bacterial resources. However, the general causes responsible for this state of low colony formation are largely unknown. Because liquid cultivation typically yields more bacterial cell cultures than traditional solid cultivation, we hypothesized that colony formation requires one or more specific gene functions that are dispensable or less important for growth in liquid media. To verify our hypothesis and reveal the genetic background limiting colony formation among bacteria in nature, we isolated Escherichia coli mutants that had decreased frequencies of colony formation but could grow in liquid medium from a temperature-sensitive mutant collection. Mutations were identified in fabB, which is essential for the synthesis of long unsaturated fatty acids. We then constructed a fabB deletion mutant in a wild-type background. Detailed behavioural analysis of the mutant revealed that under fatty acid-limited conditions, colony formation on solid media was more sensitively and seriously impaired than growth in liquid media. Furthermore, growth under partial inhibition of fatty acid synthesis with cerulenin or triclosan brought about similar phenotypes, not only in E. coli but also in Bacillus subtilis and Corynebacterium glutamicum. These results indicate that fatty acids have a critical importance in colony formation and that depletion of fatty acids in the environment partly accounts for the low frequency of bacterial colony formation.

Mce2R/Rv0586 of Mycobacterium tuberculosis is the functional homologue of FadR E. coli

Suhail Yousuf, Rajendra Kumar Angara, Ajit Roy, Shailesh Kumar Gupta, Rohan Misra, Akash Ranjan

ABSTRACT

Lipid metabolism is critical to Mycobacterium tuberculosis survival and infection. Unlike Escherichia coli, which has a single FadR, the M. tuberculosis genome encodes five proteins of the FadR sub-family. While the role of E. coli FadR as a regulator of fatty acid metabolism is well known, the definitive functions of M. tuberculosis FadR proteins are still under investigation. An interesting question about the M. tuberculosis FadRs remains open: which one of these proteins is the functional homologue of E. coli FadR? To address this, we have applied two different approaches. The first one was the bioinformatics approach and the second one was the classical molecular genetic approach involving complementation studies. Surprisingly, the results of these two approaches did not agree. Among the five M. tuberculosis FadRs, Rv0494 shared the highest sequence similarity with FadR E. coli and Rv0586 was the second best match. However, only Rv0586, but not Rv0494, could complement E. coli ΔfadR, indicating that Rv0586 is the M. tuberculosis functional homologue of FadR E. coli . Further studies showed that both regulators, Rv0494 and Rv0586, show similar responsiveness to LCFA, and have conserved critical residues for DNA binding. However, analysis of the operator site indicated that the inter-palindromic distance required for DNA binding differs for the two regulators. The differences in the binding site selection helped in the success of Rv0586 binding to fadB upstream over Rv0494 and may have played a critical role in complementing E. coli ΔfadR. Further, for the first time, we report the lipid-responsive nature of Rv0586.

The Gram-negative phytopathogen Xanthomonas campestris pv. campestris employs a 5'UTR as a feedback controller to regulate methionine biosynthesis

Jian-Ling Zhang, Dan Wang, Yu-Wei Liang, Wan-Ying Zhong, Zhen-Hua Ming, Dong-Jie Tang, Ji-Liang Tang

ABSTRACT

The synthesis of methionine is critical for most bacteria. It is known that cellular methionine has a feedback effect on the expression of met genes involved in de novo methionine biosynthesis. Previous studies revealed that Gram-negative bacteria control met gene expression at the transcriptional level by regulator proteins, while most Gram-positive bacteria regulate met genes at post-transcriptional level by RNA regulators (riboregulators) located in the 5′UTR of met genes. However, despite its importance, the methionine biosynthesis pathway in the Gram-negative Xanthomonas genus that includes many important plant pathogens is completely uncharacterized. Here, we address this issue using the crucifer black rot pathogen Xanthomonas campestris pv. campestris (Xcc), a model bacterium in microbe–plant interaction studies. The work identified an operon (met) involved in de novo methionine biosynthesis in Xcc. Disruption of the operon resulted in defective growth in methionine-limited media and in planta. Western blot analysis revealed that the expression of the operon is dependent on methionine levels. Further molecular analyses demonstrated that the 5′UTR, but not the promoter of the operon, is involved in feedback regulation on operon expression in response to methionine availability, providing an example of a Gram-negative bacterium utilizing a 5′UTR region to control the expression of the genes involved in methionine biosynthesis.

Molecular determinants of Burkholderia pseudomallei BpeEF-OprC efflux pump expression

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ABSTRACT

Burkholderia pseudomallei, the cause of melioidosis, is intrinsically resistant to many antibiotics. Acquired multidrug resistance, including resistance to doxycycline and co-trimoxazole used for melioidosis eradication phase therapy, is mainly attributed to constitutive expression of the BpeEF-OprC efflux pump. Constitutive expression of this pump is caused by mutations affecting two highly similar LysR-type transcriptional regulators (LTTR), BpeT and BpeS, but their interaction with the regulatory region governing BpeEF-OprC expression has not yet been studied. The bpeE-bpeF-oprC genes are distally located in the llpE-bpeE-bpeF-oprC operon. The llpE gene encodes a putative lipase/esterase of unknown function. We show that in a bpeT mutant llpE is constitutively co-transcribed with bpeE-bpeF-oprC. As expected from previous studies with B. cenocepacia, deletion of llpE does not affect antibiotic efflux. Using transcriptional bpeE′-lacZ fusions, we demonstrate that the 188 bp bpeT-llpE intergenic region located between bpeT and the llpE-bpeE-bpeF-oprC operon contains regulatory elements needed for control of bpeT and llpE-bpeE-bpeF-oprC operon expression. By native polyacrylamide gel electrophoresis and electrophoretic mobility shift assays with purified recombinant BpeT and BpeS proteins, we show BpeT and BpeS form oligomers that share a 14 bp binding site overlapping the essential region required for llpE-bpeE-bpeF-oprC expression. The binding site contains the conserved T-N11-A LTTR box motif involved in binding of LysR proteins, which in concert with two other possible LTTR boxes may mediate BpeT and BpeS regulation of BpeEF-OprC expression. These studies form the basis for further investigation of BpeEF-OprC expression and regulation at the molecular level by yet unknown external stimuli.

A transcriptomic analysis of the mycobacteriophage D29 genome reveals the presence of novel stoperator-associated promoters in its right arm

Niketa Bhawsinghka, Arkajyoti Dutta, Jayanta Mukhopadhyay, Sujoy K. Das Gupta

ABSTRACT

Mycobacteriophage D29 is a lytic phage that infects various species of Mycobacterium including M. tuberculosis. Its genome has 77 genes distributed almost evenly between two converging operons designated as left and right. Transcription of the phage genome is negatively regulated by multiple copies of an operator-like element known as stoperator that acts by binding the phage repressor Gp71. The function of the D29 genes and their expression status are poorly understood and therefore we undertook a transcriptome analysis approach to address these issues. The results indicate that the average transcript intensity of the right arm genes was higher than of those on the left, at the early stage of infection. Moreover, the fold increase from early to the late stage was found to be less for the right arm genes than for the left. Both observations support the prediction that the right arm genes are expressed early whereas the left arm ones are expressed late. The analysis further revealed a break in the continuity of the right arm operon between 89, the first gene in it, and 88, the next. Gene 88 was found to be expressed from a newly identified promoter located between 88 and 89. Another new promoter was found upstream of 89. Thus, the promoter Pleft, identified earlier, is not the only one that drives expression of the right arm genes. All these promoters overlap with stoperators, with which they share a conserved sequence motif, TTGACA, commonly known as the −35 promoter element. We demonstrate mutually exclusive binding of RNA polymerase and Gp71 to the stoperator-promoters and conclude that stoperators can function as −35 promoter elements and that they can control gene expression not only negatively as was believed earlier but in many cases positively as well.

Characterisation of a monooxygenase in Shiraia bambusicola

Huaxiang Deng, Ruijie Gao, Xiangru Liao, Yujie Cai

ABSTRACT

A monooxygenase-encoding gene (Mono) is located in the hypocrellin gene cluster of Shiraia sp. SUPER-H168 and was targeted by a clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system. The ΔMono mutant abolished hypocrellin production, whereas the ΔMono complement mutant restored hypocrellin production. Relative expression levels of the Mono and its adjacent genes were abolished in the ΔMono mutant compared with the wild-type strain. These results indicate the essential role of Mono in hypocrellin biosynthesis. The Mono gene of Shiraia bambusicola was further expressed in Pichia pastoris and salicylate monooxygenase activity was detected, which suggested that this monooxygenase has the ability to catalyse decarboxylative hydroxylation. The relative growth ratio of the ΔMono mutant was significantly improved compared with the wild-type strain. In contrast to the wild-type strain, the ΔMono mutant also represented excellent oxidative stress tolerance after exposure to high concentrations of H2O2 (16 mM) based on the increasing activities of superoxide dismutase, catalase, and glutathione peroxidase. These results suggest that ΔMono mutants could be used as microbial cell factories to produce metabolites that will cause oxidative stress. This study also enhances our understanding of hypocrellin biosynthesis and opens an avenue for decoding the hypocrellin pathway.

Naturally occurring polymorphisms in the virulence regulator Rsp modulate Staphylococcus aureus survival in blood and antibiotic susceptibility

Aishwarya Krishna, Matthew T. G. Holden, Sharon J. Peacock, Andrew M. Edwards, Sivaramesh Wigneshweraraj

ABSTRACT

Nasal colonization by the pathogen Staphylococcus aureus is a risk factor for subsequent infection. Loss of function mutations in the gene encoding the virulence regulator Rsp are associated with the transition of S. aureus from a colonizing isolate to one that causes bacteraemia. Here, we report the identification of several novel activity-altering mutations in rsp detected in clinical isolates, including for the first time, mutations that enhance agr operon activity. We assessed how these mutations affected infection-relevant phenotypes and found loss and enhancement of function mutations to have contrasting effects on S. aureus survival in blood and antibiotic susceptibility. These findings add to the growing body of evidence that suggests S. aureus 'trades off' virulence for the acquisition of traits that benefit survival in the host, and indicates that infection severity and treatment options can be significantly affected by mutations in the virulence regulator rsp.

MarR family transcriptional regulator and subinhibitory antibiotics regulate type VI secretion gene clusters in Burkholderia pseudomallei

Liliana Losada, April A. Shea, David DeShazer

ABSTRACT

Burkholderia pseudomallei, the aetiological agent of melioidosis, is an inhabitant of soil and water in many tropical and subtropical regions worldwide. It possesses six distinct type VI secretion systems (T6SS-1 to T6SS-6), but little is known about most of them, as they are poorly expressed in laboratory culture media. A genetic screen was devised to locate a putative repressor of the T6SS-2 gene cluster and a MarR family transcriptional regulator, termed TctR, was identified. The inactivation of tctR resulted in a 50-fold increase in the expression of an hcp2–lacZ transcriptional fusion, indicating that TctR is a negative regulator of the T6SS-2 gene cluster. Surprisingly, the tctR mutation resulted in a significant decrease in the expression of an hcp6–lacZ transcriptional fusion. B. pseudomallei K96243 and a tctR mutant were grown to logarithmic phase in rich culture medium and RNA was isolated and sequenced in order to identify other genes regulated by TctR. The results identified seven gene clusters that were repressed by TctR, including T6SS-2, and three gene clusters that were significantly activated. A small molecule library consisting of 1120 structurally defined compounds was screened to identify a putative ligand (or ligands) that might bind TctR and derepress transcription of the T6SS-2 gene cluster. Seven compounds, six fluoroquinolones and one quinolone, activated the expression of hcp2–lacZ. Subinhibitory ciprofloxacin also increased the expression of the T6SS-3, T6SS-4 and T6SS-6 gene clusters. This study highlights the complex layers of regulatory control that B. pseudomallei utilizes to ensure that T6SS expression only occurs under very defined environmental conditions.

Changing the paradigm for hospital outbreak detection by leading with genomic surveillance of nosocomial pathogens

Sharon J. Peacock, Julian Parkhill, Nicholas M. Brown

ABSTRACT

The current paradigm for hospital outbreak detection and investigation is based on methodology first developed over 150 years ago. Daily surveillance to detect patients positive for pathogens of particular importance for nosocomial infection is supported by epidemiological investigation to determine their relationship in time and place, and to identify any other factor that could link them. The antibiotic resistance pattern is commonly used as a surrogate for bacterial relatedness, although this lacks sensitivity and specificity. Typing may be used to define bacterial relatedness, although routine methods lack sufficient discriminatory power to distinguish relatedness beyond the level of bacterial clones. Ultimately, the identification of an outbreak remains a predominately subjective process reliant on the intuition of experienced infection control professionals. Here, we propose a redesign of hospital outbreak detection and investigation in which bacterial species associated with nosocomial transmission and infection undergo routine prospective whole-genome sequencing. Further investigation is based on the probability that isolates are associated with an outbreak, which is based on the degree of genetic relatedness between isolates. Evidence is provided that supports this model based on studies of MRSA (methicillin-resistant Staphylococcus aureus), together with the benefits of a 'Sequence First' approach. The feasibility of implementation is discussed, together with residual barriers that need to be overcome prior to implementation.

Inactivation of invertase enhances sucrose production in the cyanobacterium Synechocystis sp. PCC 6803

Friedrich Kirsch, Quan Luo, Xuefeng Lu, Martin Hagemann

ABSTRACT

Sucrose is naturally synthesized by many cyanobacteria under high salt conditions, which can be applied to produce this widely used feedstock. To improve sucrose production with the moderate halo-tolerant cyanobacterium Synechocystis sp. PCC 6803, we identified and biochemically characterized the sucrose-degrading invertase. Inactivating the invertase encoding gene sll0626 (inv) significantly increased cellular sucrose levels; interestingly sucrose over-accumulation was also observed under NaCl-free conditions. The subsequent inactivation of inv in the mutant ΔggpS, which cannot synthesize the major compatible solute glucosylglycerol, resulted in further enhanced sucrose accumulation in the presence of 1.5% NaCl. Then, inv mutation was introduced into the previously obtained sucrose-producing strain WD25 (Du W, Liang F, Duan Y, Tan X, Lu X. Metab Eng 2013;19:17–25), which resulted in almost 40% higher sucrose accumulation. These findings show that invertase is an interesting target in obtaining efficient sucrose production in cyanobacterial host cells.

Genome sequence, metabolic properties and cyanobacterial attachment of Porphyrobacter sp. HT-58-2 isolated from a filamentous cyanobacterium–microbial consortium

Rebecca-Ayme Hughes, Xiaohe Jin, Yunlong Zhang, Ran Zhang, Sabrina Tran, Philip G. Williams, Jonathan S. Lindsey, Eric S. Miller

ABSTRACT

Tolyporphins are structurally diverse tetrapyrrole macrocycles produced by the cyanobacterial culture HT-58-2. Although tolyporphins were discovered over 25 years ago, little was known about the microbiology of the culture. The studies reported herein expand the description of the community of predominantly alphaproteobacteria associated with the filamentous HT-58-2 cyanobacterium and isolate a dominant bacterium, Porphyrobacter sp. HT-58-2, for which the complete genome is established and growth properties are examined. Fluorescence in situ hybridization (FISH) analysis of the cyanobacterium–microbial community with a probe targeting the 16S rRNA of Porphyrobacter sp. HT-58-2 showed fluorescence emanating from the cyanobacterial sheath. Although genes for the biosynthesis of bacteriochlorophyll a (BChl a) are present in the Porphyrobacter sp. HT-58-2 genome, the pigment was not detected under the conditions examined, implying the absence of phototrophic growth. Comparative analysis of four Porphyrobacter spp. genomes from worldwide collection sites showed significant collinear gene blocks, with two inversions and three deletion regions. Taken together, the results enrich our understanding of the HT-58-2 cyanobacterium–microbial culture.

Dynamics of acute Montipora white syndrome: bacterial communities of healthy and diseased M. capitata colonies during and after a disease outbreak

Silvia Beurmann, Blake Ushijima, Patrick Videau, Christina M. Svoboda, Apaala Chatterjee, Greta S. Aeby, Sean M. Callahan

ABSTRACT

Coral diseases contribute to the decline of coral reefs globally and threaten the health and future of coral reef communities. Acute Montipora white syndrome (aMWS) is a tissue loss disease that has led to the mortality of hundreds of Montipora capitata colonies in Kāneʻohe Bay, Hawaiʻi in recent years. This study describes the analysis of coral-associated bacterial communities using high-throughput sequencing generated by the PacBio RSII platform. Samples from three health states of M. capitata (healthy, healthy-diseased and diseased) were collected during an ongoing aMWS outbreak and a non-outbreak period and the bacterial communities were identified to determine whether a shift in community structure had occurred between the two periods. The bacterial communities associated with outbreak and non-outbreak samples were significantly different, and one major driver was a high abundance of operational taxonomic units (OTUs) identified as Escherichia spp. in the outbreak sequences. In silico bacterial source tracking suggested this OTU was likely from sewage contamination of livestock, rather than human, origin. The most abundant coliform OTU was a culturable E. fergusonii isolate, strain OCN300, however, it did not induce disease signs on healthy M. capitata colonies when used in laboratory infection trials. In addition, screening of the sequencing output found that the most abundant OTUs corresponded to previously described M. capitata pathogens. The synergistic combination of known coral pathogens, sewage contaminants and other stressors, such as fluctuating seawater temperatures and bacterial pathogens, have the potential to escalate the deterioration of coral reef ecosystems.

Basal levels of (p) ppGpp differentially affect the pathogenesis of infective endocarditis in Enterococcus faecalis

Cristina Colomer-Winter, Anthony O. Gaca, Olivia N. Chuang-Smith, José A. Lemos, Kristi L. Frank

ABSTRACT

The alarmone (p)ppGpp mediates the stringent response and has a recognized role in bacterial virulence. We previously reported a stringent response-like state in Enterococcus faecalis isolated from a rabbit foreign body abscess model and showed that E. faecalis mutants with varying levels of cellular (p)ppGpp [Δrel, ΔrelQ and the (p)ppGpp0 ΔrelΔrelQ] had differential abilities to persist within abscesses. In this study, we investigated whether (p)ppGpp contributes to the pathogenesis of E. faecalis infective endocarditis (IE), a biofilm infection of the heart valves. While the stringent response was not activated in heart valve-associated E. faecalis, deletion of the gene encoding the bifunctional (p)ppGpp synthetase/hydrolase Rel significantly impaired valve colonization. These results indicate that the presence of (p)ppGpp is dispensable for E. faecalis to cause IE, whereas the ability to regulate (p)ppGpp levels is critical for valve colonization. Next, we characterized how basal (p)ppGpp levels affect processes associated with IE pathogenesis. Despite being defective in binding to BSA-coated polystyrene surfaces, the Δrel strain bound to collagen- and fibronectin-coated surfaces and ex vivo porcine heart valves as well as the parent and ΔrelΔrelQ strains, ruling out the possibility that the impaired IE phenotype was due to an attachment defect. Moreover, differences in cellular (p)ppGpp levels did not affect extracellular gelatinase activity but significantly impaired enterococcal invasion of human coronary artery endothelial cells. Taken together, this study uncovers for the first time the fact that differences in basal (p)ppGpp levels, rather than the stringent response, differentially affect processes that contribute to the pathogenesis of IE.

Manganese is a Deinococcus radiodurans growth limiting factor in rich culture medium

Francesca Borsetti, Fabrizio Dal Piaz, Federico D'Alessio, Alessandra Stefan, Renato Brandimarti, Anindita Sarkar, Ankona Datta, Alejandro Montón Silva, Tanneke den Blaauwen, Mucchi Alberto, Enzo Spisni, Alejandro Hochkoeppler

ABSTRACT

To understand the effects triggered by Mn2+ on Deinococcus radiodurans, the proteome patterns associated with different growth phases were investigated. In particular, under physiological conditions we tested the growth rate and the biomass yield of D. radiodurans cultured in rich medium supplemented or not with MnCl2. The addition of 2.5–5.0 μM MnCl2 to the medium neither altered the growth rate nor the lag phase, but significantly increased the biomass yield. When higher MnCl2 concentrations were used (10–250 μM), biomass was again found to be positively affected, although we did observe a concentration-dependent lag phase increase. The in vivo concentration of Mn2+ was determined in cells grown in rich medium supplemented or not with 5 μM MnCl2. By atomic absorption spectroscopy, we estimated 0.2 and 0.75 mM Mn2+ concentrations in cells grown in control and enriched medium, respectively. We qualitatively confirmed this observation using a fluorescent turn-on sensor designed to selectively detect Mn2+ in vivo. Finally, we investigated the proteome composition of cells grown for 15 or 19 h in medium to which 5 μM MnCl2 was added, and we compared these proteomes with those of cells grown in the control medium. The presence of 5 μM MnCl2 in the culture medium was found to alter the pI of some proteins, suggesting that manganese affects post-translational modifications. Further, we observed that Mn2+ represses enzymes linked to nucleotide recycling, and triggers overexpression of proteases and enzymes linked to the metabolism of amino acids.

Mycobacterium smegmatis PrrAB two-component system influences triacylglycerol accumulation during ammonium stress

Jason D. Maarsingh, Shelley E. Haydel

ABSTRACT

The PrrAB two-component system is conserved across all sequenced mycobacterial species and is essential for viability in Mycobacterium tuberculosis, thus making it a promising drug target. The prrAB operon was successfully deleted in nonpathogenic Mycobacterium smegmatis, and the ΔprrAB mutant strain exhibited clumping in ammonium-limited medium and significantly reduced growth during ammonium and hypoxic stress. To assess the influence of M. tuberculosis PrrA overexpression, we constructed a recombinant M. smegmatis ΔprrAB mutant strain which overexpresses M. tuberculosis prrA. M. smegmatis prrAB and M. tuberculosis prrA complemented the M. smegmatis ΔprrAB deletion mutant in Middlebrook M7H9 and ammonium-limited media and during hypoxic and ammonium stress. Based on quantitative untargeted mass spectrometry-based lipidomics, triacylglycerol lipid species were significantly upregulated in the ΔprrAB mutant strain compared to the wild-type when cultured in ammonium-limited medium, revealing that M. smegmatis PrrAB influences triacylglycerol levels during ammonium stress. These results were qualitatively corroborated by thin-layer chromatography. Furthermore, the ΔprrAB mutant significantly upregulated expression of several genes (glpK, GPAT, WS/DGAT, accA3, accD4, accD6 and Ag85C) that participate in triacylglycerol and lipid biosynthetic pathways, thus corroborating the lipidomics analyses.

Genetics of biosynthesis and structure of the K53 capsular polysaccharide of Acinetobacter baumannii D23 made up of a disaccharide K unit

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ABSTRACT

The KL53 capsular polysaccharide (CPS) gene cluster of Acinetobacter baumannii D23 was sequenced, and includes a single gtr gene encoding the glycosyltransferase Gtr2, and the itrA1 gene for ItrA1 that is known to initiate CPS biosynthesis with d-QuiNAc4NAc. The K53 CPS was isolated and studied by one- and two-dimensional 1H and 13C nuclear magnetic resonance (NMR) spectroscopy before and after O-deacetylation. The disaccharide K unit of the CPS was established as →3)-α-d-GalpNAcA4Ac-(1→3)-β-d-QuipNAc4NAc-(1→, where GalNAcA and QuiNAc4NAc indicate 2-acetamido-2-deoxygalacturonic acid and 2,4-diacetamido-2,4,6-trideoxyglucose, respectively. This established the linkage formed by Gtr2. The degree of 4-O-acetylation of d-GalNAcA by Atr18, encoded at the KL53 locus, is ~55%.

New envelope stress factors involved in σE activation and conditional lethality of rpoE mutations in Salmonella enterica

Agustina Amar, Magdalena Pezzoni, Ramón A. Pizarro, Cristina S. Costa

ABSTRACT

Salmonella enterica serovar Typhimurium (S. typhimurium) can cause food- and water-borne illness with diverse clinical manifestations. One key factor for S. typhimurium pathogenesis is the alternative sigma factor σE, which is encoded by the rpoE gene and controls the transcription of genes required for outer-membrane integrity in response to alterations in the bacterial envelope. The canonical pathway for σE activation involves proteolysis of the antisigma factor RseA, which is triggered by unfolded outer-membrane porins (OMPs) and lipopolysaccharides (LPS) that have accumulated in the periplasm. This study reports new stress factors that are able to activate σE expression. We demonstrate that UVA radiation induces σE activity in a pathway that is dependent on the stringent response regulator ppGpp. Survival assays revealed that rpoE has a role in the defence against lethal UVA doses that is mediated by functions that are dependent on and independent of the alternative sigma factor RpoS. We also report that the envelope stress generated by phage infection requires a functional rpoE gene for optimal bacterial tolerance and that it is able to induce σE activity in an RseA-dependent fashion. σE activity is also induced by hypo-osmotic shock in the absence of osmoregulated periplasmic glucans (OPGs). It is known that the rpoE gene is not essential in S. typhimurium. However, we report here two cases of the conditional lethality of rpoE mutations in this micro-organism. We demonstrate that rpoE mutations are not tolerated in the absence of OPGs (at low to moderate osmolarity) or LPS O-antigen. The latter case resembles that of the prototypic Escherichia coli strain K12, which neither synthesizes a complete LPS nor tolerates null rpoE mutations.

FliW controls growth-phase expression of Campylobacter jejuni flagellar and non-flagellar proteins via the post-transcriptional regulator CsrA

Jiaqi Li, Connor J. Gulbronson, Marek Bogacz, David R. Hendrixson, Stuart A. Thompson

ABSTRACT

Campylobacter jejuni is an important human pathogen that causes 96 million cases of acute diarrheal disease worldwide each year. We have shown that C. jejuni CsrA is involved in the post-transcriptional regulation of more than 100 proteins, and altered expression of these proteins is presumably involved in the altered virulence-related phenotypes of a csrA mutant. Mutation of fliW results in C. jejuni cells that have greatly truncated flagella, are less motile, less able to form biofilms, and exhibit a reduced ability to colonize chicks. The loss of FliW results in the altered expression of 153 flagellar and non-flagellar proteins, the majority of which are members of the CsrA regulon. The number of proteins dysregulated in the fliW mutant was greater at mid-log phase (120 proteins) than at stationary phase (85 proteins); 52 proteins showed altered expression at both growth phases. Loss of FliW altered the growth-phase- and CsrA-mediated regulation of FlaA flagellin. FliW exerts these effects by binding to both FlaA and to CsrA, as evidenced by pull-down assays, protein-protein cross-linking, and size-exclusion chromatography. Taken together, these results show that CsrA-mediated regulation of both flagellar and non-flagellar proteins is modulated by direct binding of CsrA to the flagellar chaperone FliW. Changing FliW: CsrA stoichiometries at different growth phases allow C. jejuni to couple the expression of flagellar motility to metabolic and virulence characteristics.

Defining the regulatory mechanism of NikR, a nickel-responsive transcriptional regulator, in Brucella abortus

James A. Budnick, Evymarie Prado-Sanchez, Clayton C. Caswell

ABSTRACT

Metals are essential micronutrients for virtually all forms of life, but metal acquisition is a double-edged sword, because high concentrations of divalent cations can be toxic to the cell. Therefore, the genes involved in metal acquisition, storage and efflux are tightly regulated. The present study characterizes a nickel-responsive transcriptional regulator in the intracellular mammalian pathogen, Brucella abortus. Deletion of bab2_0432 (nikR) in B. abortus led to alterations in the nickel-responsive expression of the genes encoding the putative nickel importer NikABCDE and, moreover, NikR binds directly to a specific DNA sequence within the promoter region of nikA in a metal-dependent manner to control gene expression. While NikR is involved in controlling the expression of nikA, nikR is not required for the infection of macrophages or mice by B. abortus. Overall, this work characterizes the role of NikR in nickel-responsive gene expression, as well as the dispensability of nikR for Brucella virulence.

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Salmonella infection – prevention and treatment by antibiotics and probiotic yeasts: a review

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ABSTRACT

Global Salmonella infection, especially in developing countries, is a health and economic burden. The use of antibiotic drugs in treating the infection is proving less effective due to the alarming rise of antibiotic-resistant strains of Salmonella, the effects of antibiotics on normal gut microflora and antibiotic-associated diarrhoea, all of which bring a growing need for alternative treatments, including the use of probiotic micro-organisms. However, there are issues with probiotics, including their potential to be opportunistic pathogens and antibiotic-resistant carriers, and their antibiotic susceptibility if used as complementary therapy. Clinical trials, animal trials and in vitro investigations into the prophylactic and therapeutic efficacies of probiotics have demonstrated antagonistic properties against Salmonella and other enteropathogenic bacteria. Nonetheless, there is a need for further studies into the potential mechanisms, efficacy and mode of delivery of yeast probiotics in Salmonella infections. This review discusses Salmonella infections and treatment using antibiotics and probiotics.

Microbe Profile: Wolbachia: a sex selector, a viral protector and a target to treat filarial nematodes

Mark J. Taylor, Seth R. Bordenstein, Barton Slatko

ABSTRACT

Wolbachia is the most widespread genus of endosymbiotic bacteria in the animal world, infecting a diverse range of arthropods and nematodes. A broad spectrum of associations from parasitism to mutualism occur, with a tendency to drive reproductive manipulation or influence host fecundity to spread infection through host populations. These varied effects of Wolbachia are exploited for public health benefits. Notably, the protection of insect hosts from viruses is being tested as a potential control strategy for human arboviruses, and the mutualistic relationship with filarial nematodes makes Wolbachia a target for antibiotic therapy of human and veterinary nematode diseases.

Quantifying the parametric sensitivity of ethanol production by Scheffersomyces (Pichia) stipitis: development and verification of a method based on the principles of growth on mixtures of complementary substrates

Shraddha Maitra, Atul Narang

ABSTRACT

Under aerobic conditions, Crabtree-negative yeasts grow but do not ferment, and under anaerobic conditions, they ferment but do not grow. It is therefore believed that fermentation by these yeasts is sensitive to small variations of the operating parameters, e.g. dilution rate, mass transfer coefficient and oxygen solubility. However, this parametric sensitivity has never been quantified. Here, we present a method to quantify the parametric sensitivity of ethanol production in the Crabtree-negative yeast Scheffersomycesstipitis. The method is based on our experimental observation that S. stipitis cultures follow the principles of growth on mixtures of complementary substrates. Specifically, if a chemostat operating at fixed, and is fed with progressively increasing glucose feed concentrations, the culture passes through three regimes. (1) At low, the culture is carbon-limited and no ethanol is produced. (2) At high, the culture is oxygen-limited and ethanol is produced, but unused glucose is lost with the effluent. (3) At intermediate, both glucose and oxygen are limiting, and ethanol is produced without loss of glucose. Ethanol must therefore be produced in this dual-limited regime. The dual-limited regime can be predicted by simple unstructured models. It is characterized by the relation, where and denote the g of glucose consumed per g of oxygen during carbon- and oxygen-limited growth. Hence, the parametric sensitivity of fermentation by Crabtree-negative yeasts can be improved by targeting the yields and.

Enlargement of Deinococcus grandis spheroplasts requires Mg2+ or Ca2+

Koki Nishino, Yusuke Morita, Sawako Takahashi, Mai Okumura, Shusaku Shiratani, Kosuke Umemura, Issay Narumi, Chie Kondo, Ryosuke Ochiai, Taku Oshima, Hiromi Nishida

ABSTRACT

While the cell wall strictly controls cell size and morphology in bacteria, spheroplasts lack cell walls and can become enlarged in growth medium under optimal conditions. Optimal conditions depend on the bacterial species. We frequently observed extreme enlargement of spheroplasts of the radiation-resistant bacterium Deinococcus grandis in Difco Marine Broth 2216, but not in TGY broth (a commonly used growth medium for Deinococcus). Thorough investigation of media components showed that the presence of Mg2+ or Ca2+ promoted extreme spheroplast enlargement, synthesizing the outer membrane. Our findings strongly suggest that Mg2+ or Ca2+ enlarges spheroplasts, which could change the lipid composition of the spheroplast membrane.

Development of a replicating plasmid based on the native oriC in Mycoplasma pneumoniae

Cedric Blötz, Carole Lartigue, Yanina Valverde Timana, Estelle Ruiz, Bernhard Paetzold, Julia Busse, Jörg Stülke

ABSTRACT

Bacteria of the genus Mycoplasma have recently attracted considerable interest as model organisms in synthetic and systems biology. In particular, Mycoplasma pneumoniae is one of the most intensively studied organisms in the field of systems biology. However, the genetic manipulation of these bacteria is often difficult due to the lack of efficient genetic systems and some intrinsic peculiarities such as an aberrant genetic code. One major disadvantage in working with M. pneumoniae is the lack of replicating plasmids that can be used for the complementation of mutants and the expression of proteins. In this study, we have analysed the genomic region around the gene encoding the replication initiation protein, DnaA, and detected putative binding sites for DnaA (DnaA boxes) that are, however, less conserved than in other bacteria. The construction of several plasmids encompassing this region allowed the selection of plasmid pGP2756 that is stably inherited and that can be used for genetic experiments, as shown by the complementation assays with the glpQ gene encoding the glycerophosphoryl diester phosphodiesterase. Plasmid-borne complementation of the glpQ mutant restored the formation of hydrogen peroxide when bacteria were cultivated in the presence of glycerol phosphocholine. Interestingly, the replicating plasmid can also be used in the close relative, Mycoplasma genitalium but not in more distantly related members of the genus Mycoplasma. Thus, plasmid pGP2756 is a valuable tool for the genetic analysis of M. pneumoniae and M. genitalium.

Comparative transcriptome analyses of magainin I-susceptible and -resistant Escherichia coli strains

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ABSTRACT

Antimicrobial peptides (AMPs) have attracted considerable attention because of their multiple and complex mechanisms of action toward resistant bacteria. However, reports have increasingly highlighted how bacteria can escape AMP administration. Here, the molecular mechanisms involved in Escherichia coli resistance to magainin I were investigated through comparative transcriptomics. Sub-inhibitory concentrations of magainin I were used to generate four experimental groups, including magainin I-susceptible E. coli, in the absence (C) and presence of magainin I (CM); and magainin I-resistant E. coli in the absence (R) and presence of magainin I (RM). The total RNA from each sample was extracted; cDNA libraries were constructed and further submitted for Illumina MiSeq sequencing. After RNA-seq data pre-processing and functional annotation, a total of 103 differentially expressed genes (DEGs) were identified, mainly related to bacterial metabolism. Moreover, down-regulation of cell motility and chaperone-related genes was observed in CM and RM, whereas cell communication, acid tolerance and multidrug efflux pump genes (ABC transporter, major facilitator and resistance-nodulation cell division superfamilies) were up-regulated in these same groups. DEGs from the C and R groups are related to basal levels of expression of homeostasis-related genes compared to CM and RM, suggesting that the presence of magainin I is required to change the transcriptomics panel in both C and R E. coli strains. These findings show the complexity of E. coli resistance to magainin I through the rearrangement of several metabolic pathways involved in bacterial physiology and drug response, also providing information on the development of novel antimicrobial strategies targeting resistance-related transcripts and proteins herein described.

Utilization of macrophage extracellular trap nucleotides by Mycoplasma hyopneumoniae

Clair R. Henthorn, F. Chris Minion, Orhan Sahin

ABSTRACT

Mycoplasma hyopneumoniae is the causative agent of enzootic pneumonia in swine, an important disease worldwide. It has finite biosynthetic capabilities, including a deficit in de novo nucleotide synthesis. The source(s) for nucleotides in vivo are unknown, but mycoplasmas are known to carry membrane-bound nucleases thought to participate in the acquisition of nucleotides from host genomic DNA. Recent research has demonstrated that neutrophils can produce extracellular traps (NETs), chromatin NETs decorated with granular proteins to interact with and eliminate pathogens. We hypothesized that M. hyopneumoniae could utilize its membrane nuclease to obtain nucleotides from extracellular traps to construct its own DNA. Using the human monocytic cell line THP-1, we induced macrophage extracellular traps (METs), which are structurally similar to NETs. The thymidine analogue ethynyl deoxyuridine (EdU) was incorporated into THP-1 DNA and METs were induced. When incubated with M. hyopneumoniae, METs were degraded and the modified nucleotide label could be co-localized within M. hyopneumoniae DNA. When the nucleases were inhibited, MET degradation and nucleotide transfer were also inhibited. Controls confirmed that the EdU originated directly from the METs and not from free nucleotides arising from intracellular pools released during extrusion of the chromosomal DNA. M. hyopneumoniae incorporated labelled nucleotides more efficiently when ‘fed’ on METs than from free nucleotides in the medium, suggesting a tight linkage between nuclease degradation of DNA and nucleotide transport. These results strongly suggest that M. hyopneumoniae could degrade extracellular traps formed in vivo during infection and incorporate those host nucleotides into its own DNA.

Nitric oxide controls c-di-GMP turnover in Dinoroseobacter shibae

Patricia Bedrunka, Fabien Olbrisch, Martina Rüger, Susanne Zehner, Nicole Frankenberg-Dinkel

ABSTRACT

The ubiquitous bacterial second messenger bis-(3′-5′)-cyclic dimeric GMP (c-di-GMP) is involved in the regulation of numerous processes including biofilm formation, motility, virulence, cell cycle and differentiation. In this study, we searched the genome of the ecologically important marine alphaproteobacterium Dinoroseobacter shibae DFL12T for genes encoding putative c-di-GMP-modulating enzymes. Overall, D. shibae was found to possess two diguanylate cyclases (Dshi_2814 and Dshi_2820) as well as two c-di-GMP-specific phosphodiesterases (Dhi_0329 and Dshi_3065). Recombinant expression and purification followed by enzymatic analysis revealed that all four proteins exhibit their proposed activity. Furthermore, adjacent to Dshi_2814 we identified a gene encoding a heme nitric oxide/oxygen binding (H-NOX) protein. These proteins are often found in association with c-di-GMP signal transduction pathways and modulate their function through binding of diatomic gases such as nitric oxide. Here, we demonstrate that H-NOX constitutes a functional unit together with the diguanylate cyclase Dshi_2814. NO-bound H-NOX strongly inhibits DGC activity. Based on these results, and with respect to previously published data including micro-array analysis, we propose an interlinkage of c-di-GMP signalling with cell–cell communication and differentiation in D. shibae.

The monofunctional cobalamin biosynthesis enzyme precorrin-3B synthase (CobZRR) is essential for anaerobic photosynthesis in Rhodospirillum rubrum but not for aerobic dark metabolism

Robin Ghosh, Erik Roth, Khaled Abou-Aisha, Rudolf Saegesser, Caroline Autenrieth

ABSTRACT

The in vivo physiological role of the gene cobZ, which encodes precorrin-3B synthase, which catalyzes the initial porphyrin ring contraction step of cobalamin biosynthesis via the cob pathway, has been demonstrated here for the first time. Cobalamin is known to be essential for an early step of bacteriochlorophyll biosynthesis in anoxygenic purple bacteria. The cobZ (cobZRR) gene of the purple bacterium Rhodospirillum rubrum was localized to a 23.5 kb insert of chromosomal DNA contained on the cosmid pSC4. pSC4 complemented several mutants of bacteriochlorophyll and carotenoid biosynthesis, due to the presence of the bchCX and crtCDEF genes at one end of the cosmid insert, flanking cobZRR . A second gene, citB/tcuB, immediately downstream of cobZRR, shows homologies to both a tricarballylate oxidoreductase (tcuB) and a gene (citB) involved in signal transduction during citrate uptake. CobZRR shows extensive homology to the N-terminal domain of the bifunctional CobZ from Rhodobacter capsulatus, and the R. rubrum citB/tcuB gene is homologous to the CobZ C-terminal domain. A mutant, SERGK25, containing a terminatorless kanamycin interposon inserted into cobZRR , could not grow by anaerobic photosynthesis, but grew normally under dark, aerobic and microaerophilic conditions with succinate and fructose as carbon sources. The anaerobic in vivo activity of CobZ indicates that it does not require oxygen as a substrate. The mutant excreted large amounts of protoporphyrin IX-monomethylester, a brown precursor of bacteriochlorophyll biosynthesis. The mutant was complemented either by the cobZRR gene in trans, or when exogenous cobalamin was added to the medium. A deletion mutant of tcuB/citB did not exhibit the cob phenotype. Thus, a role for tcuB/citB in cobalamin biosynthesis could not be confirmed.

Disrupting folate metabolism reduces the capacity of bacteria in exponential growth to develop persisters to antibiotics

Jasmine Morgan, Matthew Smith, Mark T. Mc Auley, J. Enrique Salcedo-Sora

ABSTRACT

Bacteria can survive high doses of antibiotics through stochastic phenotypic diversification. We present initial evidence that folate metabolism could be involved with the formation of persisters. The aberrant expression of the folate enzyme gene fau seems to reduce the incidence of persisters to antibiotics. Folate-impaired bacteria had a lower generation rate for persisters to the antibiotics ampicillin and ofloxacin. Persister bacteria were detectable from the outset of the exponential growth phase in the complex media. Gene expression analyses tentatively showed distinctive profiles in exponential growth at times when bacteria persisters were observed. Levels of persisters were assessed in bacteria with altered, genetically and pharmacologically, folate metabolism. This work shows that by disrupting folate biosynthesis and usage, bacterial tolerance to antibiotics seems to be diminished. Based on these findings there is a possibility that bacteriostatic antibiotics such as anti-folates could have a role to play in clinical settings where the incidence of antibiotic persisters seems to drive recalcitrant infections.

Taurine dioxygenase (tauD)-independent taurine assimilation in Escherichia coli

Masanobu Nishikawa, Lianhua Shen, Ken'ichi Ogawa

ABSTRACT

On the basis of previous studies on taurine assimilation in Escherichia coli, TauD, an iron- and α-ketoglutarate-dependent taurine dioxygenase, has been regarded as an indispensable factor for assimilation. However, we found that tauD-deficient strains did not lose their taurine assimilation ability when there was no deletion of ssuD, which encodes a reduced flavin mononucleotide [FMNH(2)]-dependent alkanesulfonate monooxygenase, which is responsible for the desulfonation of alkanesulfonates. There were no significant differences in lag phase time, growth rate and final growth yield between the tauD-deficient strain and the tauD wild-type strain. Iron increased the growth rate and final growth yield of the ssuD mutant, but not those of the tauD mutant. The double deletion of tauD and ssuD resulted in the loss of the ability to assimilate taurine. When ssuD was artificially expressed in the double-deletion mutant, the mutant recovered its taurine assimilation ability. These findings indicate that there is another taurine assimilation pathway that is dependent on ssuD but independent of tauD.

A novel regulatory factor affecting the transcription of methionine biosynthesis genes in Escherichia coli experiencing sustained nitrogen starvation

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ABSTRACT

The initial adaptive transcriptional response to nitrogen (N) starvation in Escherichia coli involves large-scale alterations to the transcriptome mediated by the transcriptional activator, NtrC. One of these NtrC-activated genes is yeaG, which encodes a conserved bacterial kinase. Although it is known that YeaG is required for optimal survival under sustained N starvation, the molecular basis by which YeaG benefits N starved E. coli remains elusive. By combining transcriptomics with targeted metabolomics analyses, we demonstrate that the methionine biosynthesis pathway becomes transcriptionally dysregulated in ΔyeaG bacteria experiencing sustained N starvation. It appears the ability of MetJ, the master transcriptional repressor of methionine biosynthesis genes, to effectively repress transcription of genes under its control is compromised in ΔyeaG bacteria under sustained N starvation, resulting in transcriptional derepression of MetJ-regulated genes. Although the aberrant biosynthesis does not appear to be a contributing factor for the compromised viability of ΔyeaG bacteria experiencing sustained N starvation, this study identifies YeaG as a novel regulatory factor in E. coli affecting the transcription of methionine biosynthesis genes under sustained N starvation.

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Cysteine biosynthesis in Neisseria species

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ABSTRACT

The principal mechanism of reducing sulfur into organic compounds is via the synthesis of l-cysteine. Cysteine is used for protein and glutathione synthesis, as well as being the primary sulfur source for a variety of other molecules, such as biotin, coenzyme A, lipoic acid and more. Glutathione and other cysteine derivatives are important for protection against the oxidative stress that pathogenic bacteria such as Neisseria gonorrhoeae and Neisseria meningitidis encounter during infection. With the alarming rise of antibiotic-resistant strains of N. gonorrhoeae, the development of inhibitors for the future treatment of this disease is critical, and targeting cysteine biosynthesis enzymes could be a promising approach for this. Little is known about the transport of sulfate and thiosulfate and subsequent sulfate reduction and incorporation into cysteine in Neisseria species. In this review we investigate cysteine biosynthesis within Neisseria species and examine the differences between species and with other bacteria. Neisseria species exhibit different arrangements of cysteine biosynthesis genes and have slight differences in how they assimilate sulfate and synthesize cysteine, while, most interestingly, N. gonorrhoeae by virtue of a genome deletion, lacks the ability to reduce sulfate to bisulfide for incorporation into cysteine, and as such uses the thiosulfate uptake pathway for the synthesis of cysteine.

Use of Lactococcus lactis as a production system for peptides and enzymes encoded by a Lantibiotic gene cluster from Bifidobacterium longum

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ABSTRACT

Bifidobacterium longum DJO10A was previously demonstrated to be able to produce a broad-spectrum lantibiotic, but production in media was very limited and only periodically on solid media. Given the difficulty of obtaining these lantibiotic peptides using B. longum DJO10A due to its tightly controlled production, genes predicted to be required for its production and immunity were designed and codon optimized according to the preferred codon used by Lactococcus lactis. Since the lanR1 gene within this lantibiotic gene cluster was the only one without a characterized analogue from other lantibiotic gene clusters, its annotation was re-examined as it was previously suggested to be a regulatory protein. Lack of DNA binding motifs did not support this, and one current analysis suggested a high likelihood of it interacting with LanD. Therefore, gene lanR1 together with lanADMIT were codon optimized and synthesized. Those genes were then cloned into an efficient dual-plasmid nisin-controlled expression system in L. lactis. The addition of the lanR1 gene exhibited toxicity in E. coli, specifically causing a shorter cell size as observed by SEM. No toxicity was observed in L. lactis. While this production system did not result in the production of a bioactive lantibiotic by L. lactis, it did successfully produce all the peptides and enzymes encoded by the original lantibiotic gene cluster from B. longum, as confirmed by LC-MS. This will now facilitate efforts into determining the proper conditions required for these enzymes to produce a bioactive lantibiotic.

Escherichia coli mutation rates and spectra with combinations of environmental limitations

Ram P. Maharjan, Thomas Ferenci

ABSTRACT

Micro-organisms often face multiple stresses in natural habitats. Individual stresses are well known to influence mutation rates and the spectra of mutational types, but the extent to which multiple stresses affect the genetic variation in populations is unknown. Here we investigate pair-wise combinations of nutritional stresses in Escherichia coli to determine their effect on mutation rates and mutational types. Environmental interactions modified both the rate and spectrum of mutations in double-limited environments, but the effects were not additive or synergistic relative to single stresses. Generally, bacteria in the mixed environments behaved as if one of the two single-stress stimuli was more dominant and the genetic variation seen with every dual limitation was intermediate between known patterns with individual stresses. The composition of mutational types with double stresses was also intermediate between individual stress patterns. At least with mutations, the single stressor results available are reasonable indicators of stress-induced genetic variation in multifaceted natural habitats. With the influence of 11 conditions available on mutational patterns, we can now also see the clustering of mutational types as a function of these environments.

Phosphate and carbohydrate facilitate the formation of filamentous Salmonella enterica during osmotic stress

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ABSTRACT

Salmonella enterica is a human pathogen that can produce filamentous cells in response to environmental stress. The molecular mediators and biosynthetic pathways that contribute to the formation of filamentous cells (>10 μm in length) during osmotic stress are mostly unknown. The comparison of filamentous and non-filamentous cells in this study was aided by the use of a filtration step to separate cell types. Osmotic stress caused an efflux of phosphate from cells, and the addition of phosphate and a carbohydrate to Luria broth with 7% NaCl (LB-7NaCl) significantly increased the proportion of filamentous cells in the population (58%). In addition to direct measurements of intracellular and extracellular phosphate concentrations, the relative abundance of the iraP transcript that is induced by phosphate limitation was monitored. Non-filamentous cells had a greater relative abundance of iraP transcript than filamentous cells. IraP also affects the stability of RpoS, which regulates the general stress regulon, and was detected in non-filamentous cells but not filamentous cells. Markers of metabolic pathways for the production of acetyl-CoA (pflB, encoding for pyruvate formate lyase) and fatty acids (fabH) that are essential to membrane biosynthesis were found in greater abundance in filamentous cells than non-filamentous cells. There were no differences in the DNA, protein and biomass levels in filamentous and non-filamentous cells after 48 h of incubation, although the filamentous cells produced significantly ($P<0.05$) more acetate. This study found that phosphate and carbohydrate enhanced the formation of filamentous cells during osmotic stress, and there were differences in key regulatory elements and markers of metabolic pathways in filamentous and non-filamentous S. enterica.

Effect of limitation of iron and manganese on microalgae growth in fresh water

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ABSTRACT

Eutrophication is caused by the rapid growth of microalgae. Iron and manganese are important micronutrients for microalgae growth. However, the effect of the limitation of iron and manganese on microalgae growth in fresh water has not been well understood. In this study, natural mixed algae, Anabaena flosaquae and Scenedesmus quadricanda, were cultivated under different quotas of iron and manganese to reveal the effect of the limitation of iron and manganese on the growth of microalgae in fresh water. The results showed that the growth rate of algae is influenced more by iron than by manganese. However, the effect of manganese cannot be overlooked: when the initial manganese quota was replete, i.e. 0.6–0.8 mg l^{-1}, manganese was able to relieve the effect of iron limitation on microalgae growth in fresh water. We further found that the microalgae showed an uptake preference for iron over manganese. Iron had a competitive effect on manganese uptake, while manganese had less impact on iron uptake by microalgae. The information obtained in the current study is useful for the provision of water quality warnings and for the control of microalgae bloom in fresh water.

Automated detection of bacterial growth on 96-well plates for high-throughput drug susceptibility testing of Mycobacterium tuberculosis

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ABSTRACT

M. tuberculosis grows slowly and is challenging to work with experimentally compared with many other bacteria. Although microtitre plates have the potential to enable high-throughput phenotypic testing of M. tuberculosis, they can be difficult to read and interpret. Here we present a software package, the Automated Mycobacterial Growth Detection Algorithm (AMyGDA), that measures how much M. tuberculosis is growing in each well of a 96-well microtitre plate. The plate used here has serial dilutions of 14 anti-tuberculosis drugs, thereby permitting the MICs to be elucidated. The three participating laboratories each inoculated 38 96-well plates with 15 known M. tuberculosis strains (including the standard H37Rv reference strain) and, after 2 weeks' incubation, measured the MICs for all 14 drugs on each plate and took a photograph. By analysing the images, we demonstrate that AMyGDA is reproducible, and that the MICs measured are comparable to those measured by a laboratory scientist. The AMyGDA software will be used by the Comprehensive Resistance Prediction for Tuberculosis: an International Consortium (CRyPTIC) to measure the drug susceptibility profile of a large number (>30000) of samples of M. tuberculosis from patients over the next few years.

Initial stages of endophytic colonization by Metarhizium involves rhizoplane colonization

Larissa Barelli, Camila C. Moreira, Michael J. Bidochka

ABSTRACT

Here we assessed the time course of rhizoplane colonization by the endophytic insect pathogenic fungus Metarhizium robertsii. We describe a method of quantifying root colonization of bean plants by M. robertsii using quantitative polymerase chain reaction (qPCR). Results of this method were compared to the standard plate count method using colony-forming units (c.f.u.). Both the c.f.u. and qPCR methods were used to monitor the time-course of haricot bean (Phaseolus vulgaris) colonization by a strain of M. robertsii that expresses the green fluorescent protein (ARSEF 2575-GFP) for colony verification. There was a strong correlation between the results of the c.f.u. and qPCR methods, indicating that both methods are well suited for the determination of colonization of P. vulgaris roots by M. robertsii. Primers for a catalase gene (cat) amplified DNA from M. robertsii, M. brunneum and M. guizhouense. Primers for a nitrogen response-regulator (nrr) additionally detected M. acridum and M. flavoviride, whereas Metarhizium perilipin-like protein (mpl) primers were specific to M. robertsii alone. However, cat was the only target that specifically amplified Metarhizium in experiments utilizing non-sterile soil. Endophytic colonization of P. vulgaris at 60 days post-inoculation with M. robertsii was detected from surface-sterilized roots with more sensitivity using our qPCR technique over the c.f.u. method. Our results suggest that there is a prolonged period of rhizoplane colonization by Metarhizium with transient, low-level endophytic colonization of the root system of P. vulgaris that persists for the entirety of the plant life cycle.

Metabolic constraints on Magnaporthe biotrophy: loss of de novo asparagine biosynthesis aborts invasive hyphal growth in the first infected rice cell

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ABSTRACT

The blast fungus Magnaporthe oryzae devastates global rice yields and is an emerging threat to wheat. Determining the metabolic strategies underlying M. oryzae growth in host cells could lead to the development of new plant protection approaches against blast. Here, we targeted asparagine synthetase (encoded by ASN1), which is required for the terminal step in asparagine production from aspartate and glutamine, the sole pathway to de novo asparagine biosynthesis in M. oryzae. Consequently, the Δasn1 mutant strains could not grow on minimal media without asparagine supplementation. Spores harvested from supplemented plates could form appressoria and penetrate rice leaf surfaces, but biotrophic growth was aborted and the Δasn1 strains were nonpathogenic. This work provides strong genetic evidence that de novo asparagine biosynthesis, and not acquisition from the host, is a critical and potentially exploitable metabolic strategy employed by M. oryzae in order to successfully colonize rice cells.

The apolipoprotein N-acyl transferase Lnt is dispensable for growth in Acinetobacter species

Celena M. Gwin, Natalia Prakash, J Christian Belisario, Lubaina Haider, Marlene L. Rosen, Luis R. Martinez, Nathan W. Rigel

ABSTRACT

Directing the flow of protein traffic is a critical task faced by all cellular organisms. In Gram-negative bacteria, this traffic includes lipoproteins. Lipoproteins are synthesized as precursors in the cytoplasm and receive their acyl modifications upon export across the inner membrane. The third and final acyl chain is added by Lnt, which until recently was thought to be essential in all Gram-negatives. In this report, we show that Acinetobacter species can also tolerate a complete loss-of-function mutation in lnt. Absence of a fully functional Lnt impairs modification of lipoproteins, increases outer membrane permeability and susceptibility to antibiotics, and alters normal cellular morphology. In addition, we show that loss of lnt triggers a global transcriptional response to this added cellular stress. Taken together, our findings provide new insights on and support the growing revisions to the Gram-negative lipoprotein biogenesis paradigm.

Anti-Pseudomonas activity of 3-nitro-4-phenylfuroxan

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ABSTRACT

Pseudomonas aeruginosa is a microorganism that is well adapted to both clinical and industrial settings, where it can form adherent communities that are difficult to eradicate. New anti-Pseudomonas compounds and strategies are necessary, as the current antimicrobial approaches for the inhibition of biofilm formation and, above all, the eradication of formed biofilms are ineffective. Compounds that belong to the furoxan family, which are well-known NO donors, have recently been shown to display anti-Pseudomonas activity. The present study investigates three furoxan compounds that are substituted at the hetero-ring with electron-withdrawing groups (NO2, CN and CONH2) for their effects on P. aeruginosa PAO1 growth and biofilm formation/dispersal. Of the furoxans tested, only 3-nitro-4-phenylfuroxan (KN455) inhibited the growth of suspended P. aeruginosa PAO1 cultures. Furthermore, KN455 inhibited the formation of both younger and older biofilms with very high yields and thus proved itself to be toxic to planktonic subpopulations. It also displayed moderate eradicating power. The activity of KN455 does not appear to be related to its capacity to release small amounts of NO. Interestingly, the isomer 4-nitro-3-phenylfuroxan (KN454), included for comparison, displayed a comparable antibiofilm rate, but did not show the same antimicrobial activity against suspended cells and planktonic subpopulations. While hypotheses as to the mechanism of action have been formulated, further investigations are necessary to shed light onto the antimicrobial activity of this furoxan.

Deletion of MSMEG_1350 in Mycobacterium smegmatis causes loss of epoxy-mycolic acids, fitness alteration at low temperature and resistance to a set of mycobacteriophages

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ABSTRACT

Mycobacterium smegmatis is intrinsically resistant to thiacetazone, an anti-tubercular thiourea; however we report here that it causes a mild inhibition in growth in liquid medium. Since mycolic acid biosynthesis was affected, we cloned and expressed Mycobacterium smegmatis mycolic acid methyltransferases, postulated as targets for thiacetazone in other mycobacterial species. During this analysis we identified MSMEG_1350 as the methyltransferase involved in epoxy mycolic acid synthesis since its deletion led to their total loss. Phenotypic characterization of the mutant strain showed colony morphology alterations at all temperatures, reduced growth and a slightly increased susceptibility to SDS, lipophilic and large hydrophilic drugs at 20 °C with little effect at 37 °C. No changes were detected between parental and mutant strains in biofilm formation, sliding motility or sedimentation rate. Intriguingly, we found that several mycobacteriophages severely decreased their ability to form plaques in the mutant strain. Taken together our results prove that, in spite of being a minor component of the mycolic acid pool, epoxy-mycolates are required for a proper assembly and functioning of the cell envelope. Further studies are warranted to decipher the role of epoxy-mycolates in the M. smegmatis cell envelope.

***The End