

## **PreS2 mutant of hepatitis B virus as early marker of hepatocellular carcinoma**

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Hepatitis B virus (HBV) is a significant cause of serious liver diseases, including chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). Recent studies have indicated that mutations in the preS2 region of the HBV surface gene are involved in carcinogenesis during chronic infection and thus may serve as clinical indicators of increased risk for disease progression. However, methods previously used to investigate preS2 mutations were time-consuming and not especially sensitive. Consequently, fragment length polymorphism analysis (FLPA) was developed as a novel molecular technique to screen patient serum samples for preS2 mutations with high throughput. In this study, FLPA was used to characterize the prevalence and distribution of preS2 variants among chronic HBV carriers and then evaluated for effectiveness as a clinical tool. The frequencies of preS2 mutations were found to be significantly higher in HBV carriers with severe liver disease than in asymptomatic carriers. Compared to other methods, FLPA was shown to be a more sensitive and convenient assay for identifying subpoulations of viral variants. Thus, this study demonstrates the potential clinical utility of FLPA screening protocols for preS2 mutants to aid in the early detection of HCC, as this remains one of the best ways to improve patient prognosis.

## **Identification of an inducible gene necessary for lactose metabolism in the oligotrophic bacterium *Caulobacter crescentus***

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To gain insight into the metabolic capabilities of common environmental microbes, we have identified a novel gene required for lactose catabolism in the oligotrophic, aquatic bacterium *Caulobacter crescentus*. Phenotype microarray analysis revealed that this *lac* gene is involved in the metabolism of at least two other carbon sources. We modified the *Escherichia coli* beta-galactosidase assay specifically to quantify the enzymatic activity of the *Caulobacter lac* gene product; our assays indicate that the activity is inducible by lactose and requires cell envelope integrity. To assess regulation of expression in more detail, we fused the *E. coli lacZ* reporter gene to the promoter region of the *Caulobacter lac* gene. Our results suggest that transcription of the *lac* gene is regulated by lactose and that this regulation depends on the presence of the *lac* gene product. Identification of an inducible lactose metabolism system demonstrates that *Caulobacter* has evolved to scavenge for rare nutrients.

## **Characterization of eight Gram-positive, yellow-pigmented bacteria with a description of *Citrococcus barcroftii* sp. nov. from the White Mountains**

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Seven yellow-pigmented, Gram-positive bacterial species were collected by undergraduate students from a variety of habitats and subsequently characterized according to morphology, nucleotide sequence and metabolism. The cultures were maintained on nutrient agar at room temperature. The cultural characteristics of colonies were documented photographically, and the morphology of individual cells was determined using Gram stain and nigrosin indirect staining methods. The chromosomal DNA from each isolate was extracted and 16S r-RNA was amplified using PCR. Nucleotide sequences were compared against gene bank entries using the NCBI BLAST. Metabolism was characterized using enzymatic testing limited to; TSI, SIM, MR-VP, bile esculine and urea hydrolysis, catalase activity, citrate utilization and aerobic acid formation from glucose, sucrose, mannitol, arabinose, rhamnose, lactose, sorbitol and inositol. Seven isolates were identified as *Microbacterium testaceum*, *Micobacterium foliorum*, *Brevibacterium aureum*, *Argococcus jenensis*, *Micrococcus luteus* and *Kocuria paulustris*. The eighth culture did not match documented strains, and *Citrococcus barcroftii* was proposed as a novel species.

## **Production of a murine monoclonal antibody against the human $\alpha 4\beta 7$ gut-homing integrin receptor**

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This study describes the production of a murine monoclonal antibody (mAb) against the human  $\alpha 4\beta 7$  gut-homing integrin receptor found on both T and B lymphocytes. The recruitment of lymphocytes via the  $\alpha 4\beta 7$  receptor to the gut has been associated with chronic inflammatory bowel disease (IBD). There is currently only one anti- $\alpha 4\beta 7$  mAb in existence, but it is not commercially available. Studies involving this receptor are in need of such an antibody. Two different immunization strategies were employed in generating the desired polyclonal antibodies in mice: the first involved injecting murine leukemia B cells virally-transfected with  $\beta 7$  while the second strategy utilized a novel technique where  $\beta 7$  knock-out mice were immunized with  $\beta 7$  wild-type murine splenocytes with naturally occurring cell-surface  $\beta 7$ . As a final boost, mice were injected intraperitoneally or intravenously with human peripheral blood mononuclear cells (hPBMC), with a rationale of boosting epitopes shared between murine and human  $\beta 7$ . Immune sera and supernatants of several clones generated by hybridoma technology were tested using ELISA and flow cytometric screening assays, evaluating the staining pattern of human peripheral T cells vs. a mouse T cell line over-expressing  $\beta 7$  molecules (TK1). The  $\alpha 4\beta 7$  monoclonal antibody produced can be used to identify lymphocyte subsets that migrate to the gut.

## **Rapid detection of *Burkholderia cepacia* complex in cystic fibrosis sputum using LightCycler PCR**

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*Burkholderia cepacia* complex (Bcc) consists of 9 different genomovars, which are major pathogens in cystic fibrosis (CF) patients and a major cause of death and transplant rejection. Standard culture for *Burkholderia cepacia* is slow, laborious, fairly expensive, and potentially insensitive. Creation of a sensitive Real-Time PCR method may be a better screening method for these patients, and will provide a rapid response on empirical therapy on acute respiratory conditions. This test can also be used to confirm the identification of suspicious colonies with high specificity. The developed test was able to detect all reference strain isolates, and was negative against a panel of 30 respiratory-related organisms. When tested against patient samples it showed no false positives, and the specificity of the test proved to be equal to the conventional method, 100%. The PCR method also showed a detection level of 5.4 pg/mL for the target DNA. After testing 850 sputum samples, the overall sensitivity of the culture method compared to the PCR method was only 65%. In comparison to the conventional culture method, the developed LightCycler PCR method showed higher sensitivity, equal specificity, more cost effectiveness, and a much better turn-around-time. This study supports PCR method as an effective diagnostic procedure for screening Bcc in CF patients.

**Identification of three Gram negative bacteria: *Roseomonas mucosa*, *Acinetobacter radioresistens*, *Chelatococcus paloroseus* sp. nov.**

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Three different types of Gram negative bacteria were isolated from three different sources: a hand-washing exercise plate, a blood agar plate exposed to a contact lens, and laboratory tap water. The isolates were investigated using phenotypic methods and partial phylogenetic analysis of their 16S r-RNA gene sequences. Comparisons of the nucleotide sequences to those available in public databases through the NCBI BLAST showed the isolates to be phylogenetically related to *Roseomonas mucosa*, *Acinetobacter radioresistens* and *Chelatococcus asaccharovorans* respectively. The 16S r-RNA gene sequences from the hand-wash isolate showed 98% similarity to those obtained from *Roseomonas mucosa* (AF538712) but shared a high degree of similarity with those from *Roseomonas gilardii* and *Roseomonas terpenica* as well. DNA from the contact lens showed 100% similarity with samples obtained from *Acinetobacter radioresistens*. Although the nucleotide sequences from the water isolate showed 98% similarity to those of *Chelatococcus asaccharovorans* (AJ871433), the culture was unable to utilize nitrolotriacetate (NTA), lactate or citrate as sole source of carbon, and was unable to hydrolyze urea or produce cytochrome C. These features plus unique cultural characteristics prompted the proposal of *Chelatococcus paloroseus* as a novel species.

## **Uncultivated environmental prokaryotic model to study human disease-associated bacteria**

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Several uncultivated prokaryotes have become candidates as potential emerging human pathogens. The diverse TM7 candidate phylum of the Bacteria Domain, for example, has no cultivated species and has been associated with human periodontitis, an oral disease that affects nearly 18% of the population in industrialized countries. TM7 bacteria have also been detected in a broad range of environmental habitats. In this study we investigated the diversity of TM7 bacteria in environmental samples and analyzed their relatedness to human TM7. Our methodology involved prokaryotic genomic DNA extracted from sludge, soil, and arthropod feces; PCR amplification with TM7-specific primers targeting the 16S rDNA gene; and the cloning and sequencing of a ~1,200 bp PCR product. Sequences with TM7 as the top BLAST hit to human TM7 established phylogenetically using ARB software. We also quantified total TM7 and total Bacteria 16S rDNA gene copy numbers via TaqMan. Lastly, TM7 single cells were identified via Fluorescent In Situ Hybridization (FISH). Bioinformatic data analysis showed the presence of TM7 sequences with 98% similarity and sequence coverage with a TM7 clone previously associated with human oral microbial communities. TaqMan results indicated the abundance of TM7 ranged from 0.04 to 18% of the total sludge prokaryotic community among samples and that sludge samples had 50 times higher abundance of TM7 than previously reported TaqMan values in human oral samples. Cell morphology determined via FISH agreed with previously described morphologies, including oral TM7. Our long-term goal is to use environmental models to better understand the role of uncultivable bacteria, such as TM7, in humans.

## **Antibacterial activity of herbal extracts used in Native American traditional medicine.**

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The rise in antibiotic-resistant bacteria poses a threat to human health. There is therefore a need to find new, effective alternative antibacterial agents, including those derived from plants. According to Native American folklore various common plants were used for their medicinal value but their efficacy has not been tested. We hypothesized that these plants have antibacterial properties. *Passiflora incarnata*, *Taraxacum officinale*, *Dipsacus sativus*, *C. pycnocephalus*, *Sonchus oleraceus*, and *Ranunculus californicus* were evaluated for their antibacterial activity against gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* bacteria. Ethanolic, methanolic, acetone, and aqueous crude extracts were screened using the disk-diffusion method.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts that showed inhibition were determined. The MIC of the methanolic and acetone extracts of *T. officinale* leaf against *S. aureus* is 0.25 g/mL, and 0.5 g/mL against *E. coli*. The MIC of the acetone extract of *C. pycnocephalus* leaf is 0.25 g/mL against *S. aureus* and 0.5 g/mL against *E. coli*. The acetone extract of *D. sativus* flower was bactericidal against *S. aureus* (MBC=4.38 g/mL) and the ethanolic extract of *R. californicus* flower was bactericidal against *E. coli* (MBC=26 mg/mL). Subsequent screening with paper chromatography has confirmed antimicrobial activity of *T. officinale* and *D. sativus* against *E. coli*, and determined the R<sub>f</sub> value of their effective compounds. Nuclear magnetic resonance spectrometry and high performance liquid chromatography are currently being used to analyze and identify the antimicrobial compounds in the extracts.

## **Application of high-throughput sequencing to haloarchaeal genomics and systems biology**

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Next-generation DNA sequencing technology provides access to novel approaches in studying microbial genomics and systems biology. We are using the massively parallel DNA sequencing capacity of the Illumina's Genome Analyzer II to explore the genomics and transcriptomics of the haloarchaea. Preliminary studies have shown that chromatin immunoprecipitation combined with sequencing (ChIP-seq) provides a promising method for high throughput analysis of transcription factor networks in *Halobacterium salinarum* NRC-1. Similar to the successful applications of ChIP-seq in eukaryotic systems, our study pioneers the application of this high-resolution, whole-genome mapping technique in prokaryotes. Another related study is examining the potential for using high-throughput sequencing to map origins of DNA replication in the haloarchaea. This method hinges on the formation of multiple nested replication forks at a single origin of replication during rapid growth. This process, which allows rapid genome replication, creates a situation where more gene copies are found near the origin of replication than at the terminus. These "gene dosage gradients" will be quantified by mapping short sequence "tags" from rapidly growing cultures to identify genomic regions with peaks in sequence coverage. We expect that these peaks in the coverage map will accurately pinpoint active replication origins.

## **Detection of *Neisseria gonorrhoeae* isolates with mosaic *penA* genes and potential cephalosporin resistance in San Francisco, California**

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Isolates of *Neisseria gonorrhoeae* with resistance to oral third-generation cephalosporins have been detected in Asia, Australia and parts of Europe. Such isolates have been characterized and found to possess multiple alterations in *penA* genes when compared with the *penA* genes of cephalosporin-susceptible isolates. These alterations consist of multiple sequences with homology to other *Neisseria* species indicating the possibility that these altered *penA* genes, termed "mosaic," arose through recombination of *N. gonorrhoeae* with other *Neisseria* species. We sought to determine whether mosaic *penA* alleles could be detected in *N. gonorrhoeae* isolates found in San Francisco. Using a real-time PCR assay designed to detect the mosaic *penA* allele, we screened 54 available *N. gonorrhoeae* isolates from male patients with urethritis who visited the municipal STD clinic from January-October of 2008. Of these, five (9.3%) tested positive for the mosaic *penA* gene. We subsequently sequenced the entire *penA* gene of these isolates. Three of the mosaic *penA* alleles were nearly identical to those previously found in cephalosporin-resistant strains of *N. gonorrhoeae* in Asia, whereas two of the isolates had novel *penA* alleles with fewer mosaic alterations. As determined by Etest, these mosaic isolates had cefpodoxime MICs that were 5.8 to 62.5 times higher than cephalosporin susceptible, non-mosaic isolates from the same community. These data indicate that strains of *N. gonorrhoeae* possessing a mosaic *penA* gene are present in San Francisco. The ability to monitor and characterize such strains might be very important, given the already widespread resistance of *N. gonorrhoeae* to multiple other antimicrobial agents.