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‘IN-SILICO’ CHEMOTAXONOMY; A NEW TOOL FOR MICROBIAL SYSTEMATICS?

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Characterizations of novel microorganisms require a polyphasic approach that include phylogenetic, biochemical and chemotaxonomic investigations. The analysis of various cellular components such as polar lipid, fatty acid, and peptidoglycan types may be used in order to better differentiate between nearest neighbors. These chemotaxonomic traits help augment the data obtained from physiological tests to achieve a complete profile in microbial taxonomy. However, the costly and time consuming nature of these tests coupled with the lack of curated databases to compare the data accurately makes reproducibility difficult. Furthermore, few laboratories now have the skills to perform extensive chemotaxonomic studies which are required by many peer-reviewed journals and the description of novel taxa. A newly described bacterium from our laboratory recovered from the human gut, *Ezakiella peruensis* will serve as a model for a proposed study for “in-silico” chemotaxonomy. Polar lipids, respiratory quinones and peptidoglycan will be analyzed via traditional methods and the data will be compared to its genome. We hypothesize that embracing the information contained in the genome and identifying genes responsible for the metabolic pathways that lead to the production of important taxonomic and diagnostic cell-wall and plasma-membrane components, can be a powerful tool in identification and classification systems. This “in-silico” chemotaxonomy will compliment existing laboratory protocols using chemical and physiological tests to aid in the identification of microorganisms.

EFFECT OF EXTRINSIC REWARDS AND SOCIAL COMPARISON ON INHIBITORY CONTROL IN ADULTS WITH ADHD

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Multiple studies show that motivation can improve inhibitory control in children with ADHD, but there is little evidence demonstrating similar effects in adult populations. We attempted to understand the effects of tangible rewards (like money) and upward social comparisons on behavior inhibition in adults with ADHD. False information regarding the performance of other participants was used to elicit an upwards social comparison. In this study, 46 participants were prescreened for ADHD symptoms, randomly assigned to one of three monetary reward conditions (none, low, and high), and assessed for correct inhibition using a Stop-Signal Task (SST). In a second session, they performed the SST after being informed of what other participants had scored (i.e. false feedback). Participants showed strongest inhibitory control in the low-reward/feedback condition, and weakest inhibitory control in the no-reward/feedback condition. Overall, participants exhibited better inhibitory control when monetary rewards were higher. Unexpectedly, false feedback did not appear to improve inhibitory control, with the exception of the low-reward/feedback condition. There was some evidence of ceiling effects in the data, so further investigation is needed to examine the relationship between rewards, social comparison, and inhibitory control.

EFFECTS OF β -FNA ON TAK-1 PHOSPHORYLATION IN NORMAL HUMAN ASTROCYTES

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Introduction: Neuroinflammation is a component of many neurological disorders including CNS infections, neurodegenerative diseases, depression and other mental health disorders.

β -funaltrexamine (β -FNA) shows promise as an effective means to combat neuroinflammation. More specifically, β -FNA inhibits interleukin-1 β (IL-1 β)-induced chemokine expression in normal human astrocytes (NHA). β -FNA is best characterized as a *mu*-opioid receptor (MOR) antagonist but, the anti-inflammatory actions identified thus far are MOR-independent. Our objective was to better understand the mechanism by which β -FNA inhibits inflammatory signaling by assessing its effects on the activation (phosphorylation) of transforming growth factor beta-activated kinase 1 (TAK-1).

Methods: NHA were maintained in cell culture with media replenished every 48-72 h until 80% confluence was reached. Cells were exposed to IL-1 β (3 ng/ml), β -FNA (10 μ M), IL-1 β + β -FNA or untreated for 30-120 minutes. Western Blot analysis was used to measure phosphorylated TAK-1 (p-TAK-1), TAK-1 and β -tubulin expression in whole cell lysates.

Results: A trend toward increased p-TAK-1 in IL-1 β treated cells was observed, but did not reach the level of statistical significance. Similarly, β -FNA tended to decrease IL-1 β -induced TAK-1 phosphorylation, but again not to the level of significance.

Conclusion: β -FNA may decrease TAK-1 activation by interrupting phosphorylation mechanisms, but further experiments are required, likely with modified experimental conditions (e.g., increased IL-1 β concentration and earlier time points).

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PRODUCING RECOMBINANT PROTEINS OF EMCV FOR ASSAYS

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The purpose of this research project is to produce a safe, inexpensive recombinant protein assay to detect antibodies against encephalomyocarditis virus (EMCV). EMCV is a cardio virus present in rodents and known to spread to larger animals proving lethal for hippos and elephants. By procuring effective recombinant proteins we will eliminate the need for the current infected cell protein assay allowing for a more effective, inexpensive, and safer alternative to detect the presence of EMCV. The first phase of the project involved the PCR amplification of EMCV primers MV012/MV013 and MV014/MV015. Thereon, using the cloning and verification of PCR Product into pTOPO as an intermediary phase, the objective was to insert the DNA into pBAD/His A plasmid vector for protein expression. The penultimate phase involved expression of pBAD/His A proteins using an arabinose promoter. Lastly, the final phase of the project involved running an ELISA of the yielded recombinant proteins to determine their effectiveness as an assay. Overall we were successfully able to yield PCR product, clone DNA into pTOPO for replicating and verifying the DNA sequence, and clone the product into pBAD/His A vector as our goal remains to optimize protein expression and purification methods and repeat our ELISAs.

SECURITY METRICS AND DATA LOSS

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We address the issue of security metrics as a technique for improving overall security and explore some of the problems with its use. Solutions are explored in the context of a small-scale approach for computer security evaluation, versus the contemporary application to modeling and improving complex systems.

CULTIVATION STUDIES ON THE GASTROINTESTINAL TRACT FROM AN INDIGENOUS PERUVIAN COMMUNITY YIELDS SEVERAL NOVEL BACTERIAL TAXA *

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While the literature contains many examples of studies focused on the human gut microbiome of individuals from western populations, indigenous populations with a “non-western” diet and lifestyles are underrepresented. In order to truly determine if there is a core human microbiome, individuals with a variety of diets and geographic regions also need to be included in these investigations. The primary purpose of this study is to test the hypothesis that traditional communities from remote regions harbor novel microorganisms influenced by diet, health, and environmental conditions. We used rRNA-based road maps generated in our laboratories to target previously uncultivated bacterial groups to investigate their phylogenetic, physiological, biochemical, and chemotaxonomic properties. Freshly voided fecal samples were collected from members of the Afro-Peruvian community of Cruz Verde in Tambo de Mora, region Ica, in Peru. Multiple enrichments using an array of substrates were constructed and inoculated with 1 ml of fecal slurry. All isolates recovered from the enrichments were maintained on blood agar plates and were screened using 16s gene sequence analysis. A number of isolates yielded relatively low sequence similarity values to those in DNA databases; phylogenetic tree topologies demonstrated that a number of isolates belonged to a group of organisms known as the anaerobic Gram-positive cocci. The nearest relatives included members of the genera *Peptoniphilus*, *Finegoldia*, *Gallicola* and *Parvimonas*. To date, our studies have identified two novel genera and a new species belonging to the genus *Peptoniphilus* recovered from a single individual. Our investigations demonstrate that remote indigenous communities harbor novel microbial taxa and further studies employing culture-based approaches of human gut microbiomes of diverse communities are encouraged to augment the insights provided by molecular investigations. Cultivation and characterization of novel organisms from these unique communities will help to gain a deeper understanding of ecological and functional diversity of the gastrointestinal tract of indigenous communities.

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