USDA Research Kernels

Our Latest Research Results – April 2014

Selective Chromogenic and Fluorogenic Peptide Substrates for the Assay of Cysteine Peptidases in Complex Mixtures

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Submitted to: Analytical Biochemistry
Cysteine peptidases are enzymes that are critical to most life processes. For our research, these peptidases are important in the digestion of dietary protein in many stored product beetles. The currently available commercial substrates used to analyze these enzymes have problems, including low solubility, lack of sensitivity and selectivity, and spontaneous hydrolysis. Therefore, we designed new substrates for cysteine peptidases that address many of these problems. The new substrates were demonstrated to have improved solubility and were selective for a specific group of cysteine peptidases. These substrates were superior in the analysis of cysteine peptidases in stored product beetles. The new substrates will be useful in cases where specificity is needed in the analysis of cysteine peptidases in complex systems.
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Molecular Markers Linked to Genes Important for Hard Winter Wheat Production and Marketing in the U.S. Great Plains

Authors: S. Liu, J.C. Rudd, G. Bai, S.D. Haley, A. Ibrahim, Q. Xiu
Submitted to: Crop Science
Wheat diseases and insects as well as abiotic stress can significantly reduce wheat grain yield and end-use quality in the Great Plains. Many resistance genes to these stresses and associated DNA markers to these genes have been identified. Because direct evaluation of wheat resistance to all these stresses is difficult, use of DNA markers to predict presence of these important genes in breeding materials can be a powerful tool for effective selection of these genes in breeding. This study reviewed the availability of DNA markers associated with various genes that are important in US hard winter wheat (HWW) and validated usefulness of some of these markers using a set of diverse wheat lines developed by breeding programs in the Great Plains. These important genes covered in this study include genes conferring resistance to greenbug (7), Russian wheat aphid (4), Hessian fly (9), wheat curl mite (4), leaf, stem and stripe rusts (26), wheat streak mosaic virus WSMV (2), and pre-harvesting sprouting (1); end-use quality genes including high (3) and low (13) molecular weight glutenin subunits, gliadin (3), polyphenol oxidase (2), granule-bound starch synthase (3), and puroindoline (2); and genes for other traits such as plant height (12) and photoperiod sensitivity (1). We found that most markers available are diagnostic in only a few genetic backgrounds. Only these markers that were developed from the gene sequences or alien fragments are highly diagnostic across various backgrounds. This study provides useful information for breeders to use DNA markers effectively in their breeding program.
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A Glycoprotein Subunit Vaccine Elicits a Strong Rift Valley Fever Virus Neutralizing Antibody Response in a Natural Host Species, the Sheep

Authors: B. Faburay, M. Lebedev, D.S. McVey, W. Wilson, I. Morozov, A. Young, J.A. Richt
Submitted to: Vaccine
Vaccination approaches to control mosquito-borne virus Rift Valley fever have limitations due to safety concerns. The virus causes severe to lethal disease in domestic ruminants and man and is endemic in Sub-Saharan Africa. There also are concerns that the virus could be accidentally introduced into non-endemic countries. This paper describes the evaluation of a new, safe subunit vaccine candidate using recombinantly produced viral proteins.
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Efficacy of a Recombinant Rift Valley Fever Virus MP-12 with NSm Deletion as a Vaccine Candidate in Sheep

Authors: H.M. Weingartl, C.K. Nfon, S. Zhang, P. Marszal, W.C. Wilson, J.C. Morrill, G.E. Bettinger, C.J. Peters
Submitted to: Vaccine
Rift Valley fever virus (RVFV), a mosquito-borne virus in the *Bunyaviridae* family and *Phlebovirus genus*, causes RVF, a disease of ruminants and man, endemic in Sub-Saharan African countries. However, outbreaks in Yemen and Saudi Arabia demonstrate the ability for RVFV to spread into virgin territory and thus the need

exists to develop safe and efficacious vaccines that can be used outside the endemic zones. Commercial RVFV vaccines are available but have limitations that prevent their use in disease-free countries. Consequently, there are ongoing efforts to develop and/or improve RVFV vaccines with global acceptability. In this study a previously developed MP-12-derived vaccine candidate with a large deletion of the NSm gene in the pre Gn region of the M segment (arMP-12-ΔNSm21/384) developed by T. Ikegami, that was already shown to be safe in pregnant sheep causing neither abortion nor fetal malformation was further evaluated. This vaccine was tested for protection of sheep from viremia and fever following challenge with virulent RVFV ZH501 strain. A single vaccination with arMP-12-ΔNSm21/384 fully protected sheep when challenged four weeks post vaccination, thereby demonstrating that this vaccine is efficacious in protecting these animals from RVFV infection.

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Temporospatial Fate of Bacteria and Immune Effector Expression in House Flies (*Musca domestica* L.) Fed GFP-*E. coli* O157:H7

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Submitted to: Medical and Veterinary Entomology

House flies harbor and transmit a variety of human gastrointestinal pathogens including *E. coli* O157:H7. Interactions between ingested bacteria and the fly gut directly impact bacterial persistence, survival and ultimately their ability to transmit pathogens to humans and other animals. In this study, we assessed the fate of GFP-expressing *E. coli* O157:H7 (GFP-ECO157) in house flies along with fly antimicrobial responses for 12 h post-ingestion, in order to get a better idea of the location and persistence of bacteria harbored inside of flies as well as their defense responses to bacteria. In flies fed GFP-ECO157, culture and microscopy revealed a steady decrease in bacterial load over 12 h, which was likely attributable to the combined effects of immobilization, lysis and excretion by peristalsis. However, viable bacteria were observed in both the crop and rectum of flies, which implies that house flies can putatively transmit this pathogen in excreta. Quantitative gene expression analysis of antimicrobial peptides (AMP) and the bacteria-digesting enzyme *lysozyme* showed minimal upregulation in both the gut and carcass of house flies fed GFP-ECO157. However, these genes were upregulated in fly heads and salivary glands, and effector proteins were detected in the gut of some flies. Collectively, these data indicate that house flies can serve as reservoirs of *E. coli* O157:H7 for up to 12 h, and factors in addition to AMPs and lysozyme may contribute to bacteria destruction in the gut.

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Registration of 'Antero' Wheat

Submitted to: Journal of Plant Registrations

Authors: S.D. Haley, J.J. Johnson, F.B. Peairs, J.A. Stromberger, E.E. Hudson-Arns, S.A. Seifert, V.A. Valdez, R.A. Kottke, J.B. Rudolph, G. Bai, X. Chen, R.L. Bowden, Y. Jin, J.A. Kolmer, M.S. Chen, B.W. Seabourn, F.E. Dowell.

'Antero' (PI 667743) is a hard white winter wheat released by the Colorado Agricultural Experiment Station through a marketing agreement with the Colorado Wheat Research Foundation. Antero was selected from the cross KS01HW152-1/'TAM 111' made in 2003 at Fort Collins, CO. Antero was selected as an F3:4 line in July 2007 and assigned experimental line number CO07W245. Antero was released because of its superior grain yield under non-irrigated and irrigated production conditions in eastern Colorado, its resistance to stripe rust and stem rust, and its milling quality attributes. The name Antero was chosen in recognition of Mount Antero.

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Complete Sequence Analysis of Bluetongue Virus Serotype 2 (BTV-2) Isolates Including One Novel to Western North America

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Submitted to: Journal of Veterinary Diagnostic Investigation

Bluetongue is a disease of domestic and wild ruminants that can be fatal and is caused by an insect-transmitted virus. There are four types of the virus throughout the United States (US), while one type was previously only detected in the southeastern US. Genetic analysis performed in this study suggest co-circulation of viruses in the southeastern US, and supports the previous finding that the western isolate is related to recent southeastern strains. This study further supports the need for an ongoing entomologic and livestock surveillance program for this economically important livestock disease.

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