

NEWS RELEASE

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Scientists Discover That Enzyme Degrades Mad Cow Disease Prion

FOR IMMEDIATE RELEASE

Research by North Carolina State University scientists, in conjunction with scientists from the Netherlands and BioResource International, an NC State spin-off biotechnology company, has shown that, under proper conditions, an enzyme can fully degrade the prion – or protein particle – believed to be responsible for mad cow disease and other related animal and human diseases.

These transmissible prions – believed to be the cause of bovine spongiform encephalopathy (BSE), the technical name for mad cow disease, as well as the human and sheep versions, called Creutzfeldt-Jakob disease and scrapie, respectively – are highly resistant to degradation, says Dr. Jason Shih, professor of biotechnology and poultry science at NC State. But the new research, which tested the effects of a bacterial enzyme keratinase on brain tissues from cows with BSE and sheep with scrapie, showed that, when the tissue was pretreated and in the presence of a detergent, the enzyme fully degraded the prion, rendering it undetectable.

The research was published in the Dec. 1 edition of *The Journal of Infectious Diseases*.

Shih's colleagues in the research study included first author Jan Langeveld, Dick Van de Wiel, Jan Garssen and Alex Bossers from the Central Institute for Animal Disease Control in Lelystad, The Netherlands; and Giles Shih and Jeng-Jie Wang from BioResource International, which is located on NC State's Centennial Campus.

The researchers now plan another study to test the effectiveness of the enzyme on the treated BSE prions in mice. The two-year study begins in January 2004 and is funded with \$190,000 from the National Cattleman's Beef Association.

"Our work has been done in vitro, or in test tubes, and we've reduced the prion to undetectable levels," Jason Shih says. "Our work with mice will show whether these undetectable levels of prion are indeed non-infectious."

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Jason Shih will also test keratinase's effectiveness in decontaminating equipment that processes animal by-products. Many scientists believe that mad cow disease is spread by healthy animals eating feed containing by-products from BSE-infected animals. Using keratinase to gobble up harmful prions on the processing equipment would go a long way in reducing the risk of spreading BSEs like mad cow disease, Shih believes.

This study to optimize the degradation process is funded for two years with \$180,000 from the Food and Drug Administration. Shih says in lieu of using actual BSE materials, which are quite dangerous to work with, researchers will use a surrogate protein produced from yeast that has similar physical and chemical properties, but is non-pathogenic.

Shih hit upon the idea of using keratinase to degrade prions based on his more than two decades of work as a poultry scientist looking for ways to manage poultry waste. He discovered that a bacteria, *Bacillus licheniformis* strain PWD-1, could degrade chicken feathers. Shih isolated and characterized the bacterial enzyme keratinase, and then isolated and sequenced the gene that encodes keratinase. By fermentation technology, he was able to develop a way to produce mass quantities of the enzyme, and did studies that proved many valuable applications of the enzyme.

Shih found that keratinase can be added to chicken feed to increase digestibility and the efficiency of the feed; that is, chickens who eat feed with the enzyme grow to optimal weight quicker and need less feed to grow to that optimal weight. The enzyme thus can provide the same benefit in feed that antibiotics currently provide. Animal producers are looking for safer substitutes to antibiotics, and Shih believes that keratinase can serve that purpose.

Soon, it will become clear whether keratinase can also help prevent mad cow and other harmful diseases caused by prions.

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Note to editors: An abstract of *The Journal of Infectious Diseases* paper follows.

“Enzymatic Degradation of Prion Protein in Brain Stem from Infected Cattle and Sheep”

Authors: Jan P.M. Langeveld, Dick F.M. Van de Wiel, G. Jan Garssen and Alex Bossers, Central Institute for Animal Disease Control, Lelystad, The Netherlands; Jeng-Jie Wang and Giles Shih, BioResource International; and Jason C.H. Shih, Department of Poultry Science, North Carolina State University

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Abstract: Prions – infectious agents involved in transmissible spongiform encephalopathies – normally survive proteolytic and mild protein-destructive processes. Using bacterial keratinase produced by *Bacillus licheniformis* strain PWD-1, we tested conditions to accomplish the full degradation of prion protein (PrP) in brain-stem tissue from animals with bovine spongiform encephalopathy and scrapie. The detection of PrP^{Sc}, the disease-associated isoform of PrP, in homogenates was done by Western blotting and various antibodies. The results indicated that only in the presence of detergents did heat pretreatment at >100°C allow the extensive enzymatic breakdown of PrP^{Sc} to a state where it is immunochemically undetectable. Proteinase K and 2

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other subtilisin proteases, but not trypsin and pepsin, were also effective. This enzymatic process could lead to the development of a method for the decontamination of medical and laboratory equipment. The ultimate effectiveness of this method of prion inactivation has to be tested in mouse bioassays.