A NEW PHYTASE ENZYME TO BENEFIT THE ENVIRONMENT, NATURAL RESOURCES, AND NUTRITION

Food-producing animals such as swine, poultry, and pre-ruminant calves do not have the necessary enzyme in their gastrointestinal tracts that would enable them to digest phytate-phosphorus, the major form of phosphorus in plant feeds. Consequently, the unutilized feed phosphorus ends up in manure, causing environmental pollution. Many areas such as Chesapeake Bay and Onslow, North Carolina, suffer from environmental problems caused by this kind of animal waste.

At the same time, inorganic phosphorus is supplemented in diets for these simple-stomached animals to meet their nutrient requirements. This supplementation represents the third largest expense of animal diet. A nonrenewable natural resource, the easily accessible inorganic phosphorus deposit on earth will be exhausted in approximately 80 years at current extraction and usage rate.

Phytase, a phosphohydrolytic enzyme, initiates the removal of phosphate from phytate (myo-inositol hexakisphosphate). Xingen Lei, Animal Science, and his research lab, with strong support from the Cornell Center for Advanced Technology in Biotechnology, have developed a novel bacterial AppA2 phytase and an efficient yeast expression system to produce the needed enzyme. Because the Cornell enzyme has a high turnover rate, acidic pH optimum, and strong resistance to pepsin, it overcomes two of the three limitations of the current commercial phytases.

environmental pollution, the depletion of a nonrenewable resource, and a significant expense for an animal nutrient diet supplement all result from the lack of an enzyme that would enable food-producing animals to digest phytate-phosphorus, the major form of phosphorus in plant feeds.

Robert Barker/CU

Undergraduate researcher Sarah Rice '06 (l.) and Ph.D. student Wanming Zhang at a phytase group meeting in Lei's lab.

Commercial phytases have been developed and supplemented in the diets of swine, poultry, and fish in a number of countries—Europe, North America, and Asia—to reduce phosphorus pollution of animal waste. These phytases have three major constraints: 1) suboptimal catalytic efficiency; 2) high susceptibility to proteolysis in the stomach; and 3) inadequate stability to resist heat inactivation during feed pelleting.

As tested by leading scientists in peer institutions, the Cornell phytase is three- to fourfold more effective in swine and poultry diets than the commercial phytases. Based on these exceptional attributes, Phytex (Maine), a newly established company to market Cornell phytase technology, has chosen this enzyme to launch its first product worldwide.

To make the enzyme resist heat inactivation by feed pelleting, Lei's lab has taken four independent and collaborative approaches to improve its thermostability. First, the researchers applied the powerful directed evolution method to generate heat-stable mutants and developed a "high-throughput" 96-well plate screening system, using a yeast expression system. Random mutageneses were assembled by site-directed recombination with target selection.

Next, they determined the crystal structural basis for the heat stability of different phytases and prepared a number of heat-stable mutants by site-directed mutagenesis. This part of the research is in collaboration with two leading phytase scientists from the USDA and structure biologists in Cornell's Department of Molecular and Genetic Biology. They tested various expression systems to enhance phytase thermostability by post-translational modifications. Lei's next endeavor was to collaborate with Phytex in developing a chemical coating to protect the phytase from heat inactivation.

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Xingen Lei, Animal Science

Lei's continuing research leads to developments that will enable animal producers to comply with the Clean Water Act. This will help cut 90,000 tons of phosphorus excretion per year from animal waste, reduce the feed cost of inorganic phosphorus supplementation by $150 million per year, preserve the nonrenewable inorganic phosphorus deposits in the U.S., and produce a more competitive phytase for the $500 million world market potential.

(l. to r.) Wanming Zhang, Ph.D. student, Xingen Lei, Animal Science, Karl Roneker, Cornell Swine Farm manager, and Koji Yasuda '05 convene at the phytase research meeting.

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Jiming Li, research associate, presents research plan for engineering novel phytases using genomics approaches.

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Graduate student Angela Pagano, Animal Science, presents data on the function and fate of supplemental phytases in the digestive tracts of pigs.

Robert Barker/CU

Taewan Kim, postdoctoral associate, offers his research view to Lei's group.

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Ph.D. student Moonsoo Kim, Food Science, presents her research progress in generating new phytases by error-prone PCR.

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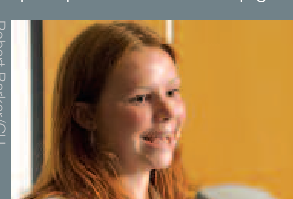
Ph.D. student Wanming Zhang presents her data on phytase protein purification.

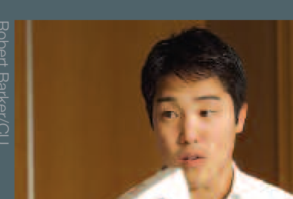
Robert Barker/CU

Sarah Rice '06, Biological Science, Music, and Pre-med, presents her data on the effectiveness of engineered phytases in improving the utilization of phytate-phosphorus in diets for pigs.



Robert Barker/CU

Koji Yasuda '05, Animal Science and Pre-vet, presents his results on using pigs as a model for fighting against global mineral deficiencies in humans.



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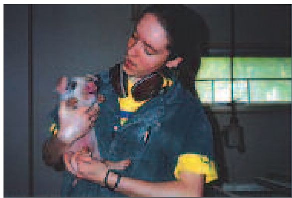

Sarah Crowe '03, now at the University of Minnesota pursuing a D.V.M. and M.S. degree in public health

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Robert Barker/CU

Taewan Kim, postdoctoral associate

With these efforts, Lei hopes to develop a heat-stable phytase that will overcome the major limitation in current phytase application. If this is accomplished, animal producers will be able to comply with the Clean Water Act. This will help cut 90,000 tons of phosphorus excretion per year from animal waste, reduce the feed cost of inorganic phosphorus supplementation by $150 million per year, and preserve the nonrenewable inorganic phosphorus deposits in the U.S. A heat-stable phytase will enable Phytex to produce a more competitive phytase for the $500 million world market potential.

An effective and heat-stable phytase may also help the fight against nutritional deficiency of iron and zinc, a health problem that affects approximately 30-50 percent of the world population. In developing countries, the staple foods are primarily of plant origin. The high abundance of phytate in these foods significantly reduces absorption of dietary iron and zinc by chelating with these elements and rendering them unavailable for digestion. Using young anemic pigs as an in vivo model, Lei's lab has demonstrated that supplemental dietary phytase can effectively release phytate-bound iron in corn and soy for hemoglobin synthesis. Other researchers have shown a similar effect of phytase in humans.

phytex (maine), a newly established company to market the cornell phytase technology, has chosen this enzyme to launch its first product worldwide.

At the present time, Lei's group is collaborating with plant scientists in China to transfer a phytase gene in staple crops, such as rice. Their goal is to provide an adequate amount of phytase in regular food to alleviate or eradicate iron and zinc deficiencies in those high-risk subjects.

In addition to external funding, strong publications, valuable patents, and fruitful collaborations, this project has provided excellent educational opportunities at Cornell. Six postdoctoral fellows, five graduate students, and 10 undergraduate students have received research training through this project. The undergraduate researchers majored in animal science, biological science, and other disciplines. They participated in laboratory and animal experiments by taking research credits, conducting honors thesis research, and participating in summer internships.

A number of undergraduate researchers have become the first-author or co-author of publications in peer-reviewed journals and have moved on to graduate or professional schools. Several recent examples are Jessica Thornton, '00 (D.V.M. program at London Royal Veterinary College), Obdulio Piloto, '01 (Ph.D. program in molecular medicine at Johns Hopkins University), Jessica Gentile, '02 (D.V.M. program at Kansas State University), and Sarah Crowe, '03 (D.V.M. and M.S. in public health program at University of Minnesota). Currently, two Cornell undergraduate researchers, Sarah Rice (double-majoring in biological science and music) and Koji Yasuda (majoring in animal science) are involved in the research.

Xingen Lei
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