Antifreeze Proteins: A Boon to Agriculture, Food and Medicine

Researchers at the Queen's University in Canada have succeeded in isolating antifreeze proteins from the spruce budworm (Choristoneura fumiferana) and common mealworm beetle (Tenebrio molitor), representing major advances in insect biology. They found that the effect of the antifreeze proteins of these insects are fourfold higher than that of fish at a concentration of 10–20 mg/ml, and 10–100 fold higher at lower concentrations.

Many fish, insects and plants contain antifreeze proteins which are crucial for their survival at extreme temperatures. Cold marine fish produce at least four different types of antifreeze glycoproteins (AFGPs) and antifreeze proteins (AFPs), while cold tolerant plants, such as winter rye, produce antifreeze proteins that resemble pathogenesis-related proteins. Research into fish antifreeze proteins was carried out as early as the 1960s. Many marine fish living in polar and north temperate seas contain antifreeze proteins that depress the freezing point of fish blood and prevent it from freezing by inhibiting the growth of ice crystals. Researchers are using recombinant DNA technology to study their structure and mechanism of action.

At the Queen's University the isolation, by a group of researchers, of an insect gene with a DNA code which produces antifreeze proteins, points to significant breakthroughs in the areas of agriculture, the frozen food industry and medicine. An important aspect of the research at the Queen's University is the cloning of the insect gene in order to introduce it into microorganisms for large-scale production of the antifreeze proteins.

Just as in fish and insects, the survival of biological materials is under threat during freezing. Antifreeze proteins not only lower the temperature at which samples freeze, they also lessen the cell damage by keeping the ice crystals small. Basically, antifreeze proteins work by binding ice crystals and inhibiting ice crystals formation. Owing to these two unique properties, antifreeze proteins are being evaluated for applications in medicine, including the long-term storage of blood cells, sperms and embryos, and preservation of organs for transplantation.

Antifreeze proteins also have applications in agriculture. They may help in the development of frost protection and frost-resistant crops, lengthening the growing season for farmers, and even extending the northern limits of crop-growing boundaries.

Antifreeze proteins will also be of benefit to the frozen food industry. By lowering the temperature for crystal formation, the antifreeze protein reduces the size of the ice crystals formed in food, thereby lessening the damage to the food. It also allows frozen foods to be stored at slightly lower temperatures, which saves energy.
Rising Hepatitis C Cases Spark New Research

The Atlanta-based Centers for Disease Control and Prevention (CDC) estimate that some 4 million people in the US are infected with the hepatitis C virus (HCV), while the World Health Organization puts the global incidence at 170 million.

Hepatitis C infection usually takes 10 to 20 years before it begins to be manifested. So although approximately 85% of people with acute HCV will go on to develop chronic hepatitis, most carriers do not know they have the disease until much later. In about 20% of the carriers, hepatitis infection will progress to cirrhosis of the liver within two decades. In up to 20% of those who develop cirrhosis, hepatocellular carcinoma (HCC) will follow, which is usually fatal within months of diagnosis.

Until HCV was cloned and sequenced in 1989, hepatitis C was simply lumped in with ‘non-A, non-B’ hepatitis types. Given that there is currently no broadly useful vaccine for control of the disease, there is fear of a silent epidemic building up that has the potential to overtake HIV in terms of the number of people affected.

In March last year, at the Consensus Development Conference on the Management of Hepatitis C organized by the National Institutes of Health, the conference’s consensus statement declared that there is "an urgent need for effective antiviral therapeutics capable of inhibiting virus replication and stopping or delaying the progression of liver disease."

Currently, three recombinant forms of interferon, a natural antiviral glycoprotein, are used in the treatment of hepatitis C, but these are ineffective in the majority of patients. Recently, Schering-Plough announced that based on clinical trials it has conducted, treating patients with a combination of injected interferon and oral ribavirin produces a tenfold increase in effectiveness of eradicating the virus. A number of biotechnology and pharmaceutical companies involved in HIV drugs development are turning their efforts toward development of similar compounds for HCV treatment.

Indian Lab Develops New Antibiotic

An Indian research laboratory has developed a new antibiotic which inhibits the growth of some gram negative and gram positive bacteria. It was also found to exhibit anti-fungal properties. The antibiotic was produced from the ‘Orrisan’ horse-shoe crab, and has been named Orlisin. Its effects are quite similar to those of tetracycline. With further studies, Orlisin could be used in various treatments. The ‘Orrisan’ horse-shoe crab has been living along the Orissa state coast for almost 350 million years.

Plant Glucosides a Popular R&D Area in China

The Kunming Institute of Botany, Academia Sinica, has been conducting R&D on plant glucosides since the mid-1970s. These are important secondary products of metabolism which are the active components in many drug plants. Water soluble glucosides are especially important components in traditional Chinese drugs. Many plant glucosides are now being used in the manufacture of natural drugs and cosmetics and as ingredients in food.

At a symposium on plant glucosides held in Kunming (昆明) on 12 August 1997, participants agreed that research in this area will increase in popularity in the field of biotechnology and influence the modernization of traditional Chinese drug production. Experts also believe that, with the rapid development of techniques and methods of compound isolation, analysis and structure determination, the time needed to study the structure and function of plant glucosides can be significantly reduced.

Meanwhile, research on plant glucosides has helped to promote R&D on other local plants, especially those that may become potential drug resources. It has also driven the development of related industries. Products which are doing well in the market include preparations of ginseng and panax pseudo-ginseng made from ginseng saponins and preparations of ginkgo leaves using flavone glucosides as raw materials.
New Methods to Treat Artery Blockage

Dr. Jeff Isner, of St. Elizabeth’s Medical Center and Tufts University School of Medicine, with his team, has successfully used gene therapy to treat patients with obstructed blood vessels in their legs. The team has injected DNA for vascular endothelial growth factor directly into skeletal muscle near a blocked artery in the leg. The growth factor stimulates growth of blood vessels around the affected part, which then provides an alternate route for blood to flow and reach the lower leg. The results were reported at the 70th annual scientific meeting of the American Heart Association held in Orlando, Florida.

Patients were given two injections of the DNA, about four weeks apart. The treatment prompted growth of new blood vessels within two to three weeks. Following this, three patients who had been scheduled for amputations did not have to go through with them. For another six patients, the excruciating pain they had been experiencing lessened. Only one patient did not respond to the treatment.

Patients with severe blockages in their leg arteries cannot be treated with drugs, because there are none which are effective for their condition. Eventually, they may have to resort to amputation as the lack of blood flow will lead to infections and gangrene. Many patients have bypass surgery, but this may not work in the long term.

Another team, headed by Michael Mann and Victor J. Dzau of Brigham and Women’s Hospital and Harvard Medical School in Boston, used a technique to turn off the genes that promote cell growth in the inner lining of veins used to bypass clogged leg arteries. Dr. Mann’s team placed a vein to be used for bypass in a special bath of genetic material about ten minutes before surgery. The bath contained segments of DNA (transcription factor decoys) which block the activity of genes necessary to promote cell growth in the lining of the vessel. By doing this, the researchers hoped to reach a high percentage of cells in the lining to switch off the genes’ function. The treatment seemed to be effective in four out of five patients treated, and their vessels were free from obstruction nine months after treatment. This finding was also reported during the meeting.

Although the positive results reported by both groups were only based on a handful of patients, many felt that it represented real progress in the treatment of vascular diseases using gene therapy.

Industrial Production of Phytase in China

Professor Fan Yunliu (范雲六) from the Research Center for Biological Technology of the Chinese Academy of Agricultural Sciences (CAAS) has collaborated with Dr. Yao Bin (姚斌) from the Institute of Fodder (CAAS) to study the phytase gene. They have genetically engineered the gene and reported significant progress. Recently, they managed to clone the phytase gene in Aspergillus niger, and introduce it to the yeast chromosome after molecular modification. They reported significant expression whereby phytase was secreted on the culture medium. Following this, a fermenter with a capacity of more than 5000 cm² was used and an output of 500 000 units/ml (5 mg phytase protein/ml fermented liquid) was recorded. According to the scientists, this is more than 3000 times that of the original phytase mold, and more than 50 times the output of ‘engineering mold’ of phytase so far reported. The Chinese experts have characterized the phytase produced as follows:

• **High output**
  The output is many times higher than that reported so far.

• **Stable expression**
  This is in favor of industrial production.

• **Two-functions**
  The phytase produced is suitable to be used in the fodder industry, and also provides a large amount of edible protein.

They have also cited many other favorable factors such as the advanced fermentation technology used, and low production costs which are in favor of industrial production. According to the scientists, the phytase gene engineering not only plays an important role in overcoming the protein shortage in the fodder industry, but is also a new way of mass producing phytase.

The enzyme phytase decomposes inositol-6-phosphate into phosphoric acid and inositol. Some animals do not produce this enzyme therefore their systems will not be able to utilize phosphorus (from phosphoric acid) from plants. To overcome this shortcoming, phytase can be added to fodder. So far, China obtains its phytase supply from Germany and Holland. With the recent progress in phytase production China will be able to produce phytase for the local market at a more competitive price.
First Human Vaccine for ‘Bird Flu’ Developed

Protein Sciences Corp., a biotechnology company and the world leader in making complex proteins, has successfully developed the first experimental human vaccine against the H5N1 virus. The vaccine will be used by the US National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), in clinical trials. The company has already delivered 1000 doses of the new vaccine to NIH. The clinical trial will be conducted on laboratory and healthcare workers who are at risk from exposure to the virus in the US and other countries. The H5N1 virus has already claimed the lives of several victims in Hong Kong. The virus originated in birds, and it is not known how it is passed to humans.

The vaccine, which is genetically engineered, is based on highly purified hemagglutinin protein from the H5 subtype produced using the company’s patented vaccine technology. The gene for the H5 hemagglutinin was cloned from genetic material of the virus which infected the first ‘bird flu’ victim in Hong Kong, a three-year-old boy who died in May 1997. The cloned gene was later engineered into a production process which uses baculovirus and insect cell fermentation (BEVS). Protein Sciences, which holds the patents for BEVS technology, has previously developed vaccines against common influenza viruses and conducted clinical trials in collaboration with NIH. These vaccines were effective in protecting humans and were also safe.

Using the company’s unique technology, the vaccine can be produced very quickly, within a couple of weeks. Conventional vaccine preparation takes months or even years.

Vaccine Effective on Chickens

Although the new H5N1 vaccine has not been tested in humans yet, a similar avian vaccine tested on chickens proved effective. The study was successfully completed by the US Department of Agriculture. Chickens which were injected with the vaccine had full protection from the H5N1 virus.

Protein Sciences Corp. previously tested its recombinant H5N2 and H7 avian influenza vaccines successfully. It has approached officials in China for approval of its H5 vaccines. The availability of such vaccines could prevent widespread occurrences of avian flu. Economic losses in the poultry industry could be reduced. The recent ‘bird flu’ incident in Hong Kong caution every nation to take the necessary measures, including vaccination, to prevent such incidences from occurring.

The US Patent and Trademark Office has issued a ‘Notice of Allowance’ covering all claims in the company’s application for a patent on influenza hemagglutinin vaccine produced using BEVS technology for human and animal use, including the new H5N1 influenza vaccine.

The purified H5 hemagglutinin is available for research purposes.

Interested parties may order it at http://www.proteinsciences.com

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Micropropagation Without Tube

Li Changxiao (李長濤), an associate professor and head of the Ningxia Hui Autonomous Region Institute of Biological Engineering, has succeeded in breeding more than 40 species of plants, including ginko, using micropropagation technology without a tube. The new technology can be applied not only for micropropagation of economic plants, but also for transgenic plants.

This technique involves treating leaves and other small buds with a special liquid before planting. After 4 to 11 days, the plant is fully grown. This differs from the traditional method which involves cutting and grafting. Conventional micropropagation has its limits because of the complex technology involved, and requires a large investment. However, the micropropagation technique without a tube maintains all the advantages of tissue culture and conventional nursery. It may even be more efficient than tube micropropagation. It has great use for the clonal propagation of plants.

Li Changxiao has adopted this technology for many plants on a large scale of production. He has successfully propagated 42 types of economic plants, including the Chinese lycium, which has been planted on an area of 1 million mu.