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An *in vivo* Bioassay for the Screening and Testing on Endocrine Disrupters using Transgenic Mice

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Endocrine disrupter, a group of environmental agents, has results in adverse health effects on human and wildlife by mimicking or interfering with the actions of endogeneous hormone. Numerous industrial compounds has mostly estrogenic activity including steroid, phytoestrogen, and synthetic chemicals. A bioassay for the screening and testing model will be necessary to evaluate these compounds for their possible effect on the endocrine system. For this purpose, a hybrid gene combining *LacZ* coding sequence and human *CYP3A4* gene promoter was constructed. Since human *CYP3A4* gene promoter contains three copies of estrogen receptor binding site (ERE), one copy of progesterone receptor binding site (PRE) and glucocorticoid receptor binding site (GRE), this feature will be greatly suitable to use for the expression of *LacZ* gene under the control of *CYP3A4* promoter whose region are respond to estrogenic compound. In this study, the hybrid gene was transfected into the human liver HepG2 cells and transfectant was treated with estrogenic compounds. The b-galactosidase activity were then measured. To study in vivo system the hybrid gene were microinjected into the mouse fertilized embryo. From the DNA-PCR analysis, the *LacZ* reporter gene were identified from the 3 males and 5 females of offspring Thus, use of these mice will improve the assessment by contributing insights into the mechanism of how various chemicals can interact with steroidlike signalling pathways.

H201

Functional Screening of Plant Cell Death Suppressors using Yeast Genetic System

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Programmed cell death (PCD) in plants is the active process of cell death occurs during development and in response to environmental cues. However, little is known about the genes and molecular mechaisms regulating the PCD in plant cells. Recently, it has been demonstrated that expression of animal Bax, a death-promoting member of the Bcl-2 family proteins, in yeast and plant cells is lethal. These results indicate that some processes of PCD in animal, yeast, and plant may be shared. To identify genes involved in Bax-induced cell death in plants, we transformed yeast cells expressing Bax with several plant cDNA libraries and we selected for cells surviving after induction of Bax. So far, we have identified more than 10 kinds of cell death suppressor genes and found that all of the genes did not interfere with the production of the Bax protein in yeast as determined by immunoblot analysis. Among these suppressors, one of the clones (PBI1, plant bax inhibitor 1) was plant ascorbate peroxidase homologue. The expression of PBI1 in yeast confers resistance to cell death induced by H_2O_2. In plants, the PBI1 gene expression is greatly enhanced by H_2O_2 and UV stresses. When compared with control plants, the transgenic tobacco plants expressing antisensense RNA for PBI showed increased susceptibility to H_2O_2 stress. These results suggest that Bax toxicity may be due, at least in part, to the generation of oxidative

stress.

H202

Scale-up and Purification of Plant Chitinase induced from Hypersensitive Stress

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We induced chitinase from soybean, cabbage, corn under various condition and the chitinase was purified chitin-affinity chromatography and CM-cellulose chromatography. The optimal pH of purified chitinase under osmotic stress was 5.0±0.05 and that was highly activated at 10 mM Zn^{2+} ion. The inhibition rate was 86% at Phytophthora capsici. The induced chitinase by fungal elicitor has optimal pH of 6.0±0.2 and activation of high value at Fusarium oxysporium elicitor included 12% oilgosaccharide. The inhibition rate was 67.3% at Trichocherum viridet. The activity of expressive chitinase by abiotic stress was highly activated at salinity concentration of 50 mM. The inhibition rate was 72% at Fusarium oxysporium. Cabbage callus cell expressed high levels of the antifungal activity among the plant cell. The optimum time and pH of treated F. moniliforme elicitor for antifungal agent production were 48 hours to 168 hours and pH 5.8 ±0.05 in medium. Antifungal activity was detected in the crude extracellular solution, which was expressed as units/ml crude solution, was in descending order of 168 hours > 144 hours and 126 hours > 96 hours. The accumulation of antifungal activity during drought stress was confirmed by enzyme activity.

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Inactivation of Blood-Born Model Viruses in Low pH Intravenous Immunoglobulin

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Viral safety is a prerequisite for manufacturing clinical immunoglobulin solution which is prepared from human plasma pools. The study presented here was thus designed to evaluate the efficacy of incubation of final immunoglobulin solution at pH 3.86 for 14 days at 25° C for inactivation of several blood-born model viruses. Bovine viral diarrhoea virus (BVDV), Bovine herpes virus (BHV), Murine encephalomyocarditis virus (EMCV) and Porcine parvovirus (PPV) were selected for this study. Immunoglobulin solutions were spiked with the viruses and the amount of virus during incubation was kinetically quantified by 50% tissue culture infectious dose ($TCID_{50}$). BVDV titer was reduced from an initial titer of 5.75 $\log_{10}$ $TCID_{50}$ to undetectable level within 9 days of incubation. BHV titer was reduced from an initial titer of 8.61 $\log_{10}$ $TCID_{50}$ to undetectable level within 5 days of incubation. EMCV titer was reduced from an initial titer of 8.39 $\log_{10}$ $TCID_{50}$ to 3.20 $\log_{10}$ $TCID_{50}$ after 14 days of incubation. PPV titer was reduced from an initial titer of 6.28 $\log_{10}$ $TCID_{50}$ to 4.32 $\log_{10}$ $TCID_{50}$ after 14 days of incubation. The log clearance factors achieved during the low pH incubation were >3.86 for BVDV, >7.43 for BHV, 5.19 for EMCV, and 1.96 for PPV. These results indicate that low pH incubation process for immunoglobulin has sufficient virus reducing capacity to achieve a high margin of virus safety.

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