

## Radioisotope Research Facility

---

Kunihiko Fukuda, *Professor and Director*

Yukio Yoshizawa, *Assistant Professor*

### General Summary

The Radioisotope Research Facility was established to support medical and biological research with radioisotopes. We have supported researchers by suggesting methods and practical techniques for experiments. Lectures and training sessions were held for researchers and for medical students and graduate students who are starting to work with radioisotopes. In 2010, 28 researchers from 9 departments and 12 students of 2 curricula used the laboratory in this facility. Major nuclides used for experiments were  $^{32}\text{P}$ ,  $^{51}\text{Cr}$ ,  $^{125}\text{I}$ ,  $^{35}\text{S}$ , and  $^3\text{H}$ .

### Research Activities

#### *The active site of exfoliative toxin A of Staphylococcus aureus*

Exfoliative toxin A (ETA) of *Staphylococcus aureus* is the causative agent of staphylococcal scalded-skin syndrome (SSSS) and disrupts desmosomes to half desmosomes in neonatal mouse epidermal tissue injected with ET and in patients with SSSS. We have previously reported that the nitration of tyrosine (Tyr) residues causes ETA to lose all toxicity. To determine the biologically active site of ETA, we prepared mutant ETA using oligonucleotide primers designed to introduce mutations with the polymerase chain reaction. Substitution of serine (Ser)-195, which is believed to be the active center of the serine protease, and other 16 Ser residues had no effect on ETA activity or immunoreactivity with anti-ETA rabbit serum. On the other hand, substitution of Tyr-17-18 and Tyr-225-232 destroyed both activity and immunoreactivity.

The promoter region of the *eta* gene consists of -35 TTGTTT, -10 TATAAT, and S-D sequence GGATGA, and an inverted sequence is present between the -10 and the S-D sequences. There is an open reading frame of 1,382 bp adjacent to the *eta* promoter. Without this gene, ETA production decreases markedly. A gel mobility shift assay was performed with the gene product ETAexp and a DNA fragment of 381 bp including the *eta* promoter sequence. Results indicate that ETAexp bound to the inverted sequence and activated the transcription of the *eta* gene.

#### *Development of techniques for determining radioactivity*

Radon gas emanates from the ground directly or is brought by groundwater and mineral water to the surface of the earth and released into the atmosphere. Radon is the second leading cause after smoking of lung cancer in the general population. A reference level of 300 Bq/m<sup>3</sup> was proposed to minimize the health hazard due to indoor radon exposure (International Commission on Radiological Protection, 2009). Liquid scintillation counting has been a standard method for measuring radon in air and water. The popularity of this method is due to the high solubility of radon in scintillation solvents, such as

toluene and xylene. These solvents, however, could not satisfy safety requirements because of their volatile nature and low flash points. We have used silicone oil as scintillation solvent to overcome these undesirable properties. The open-vial method was applied to measure radon in air by Horiuchi and Murakami in 1983. The toluene scintillator used in their study evaporated during the exposure, and correction of the toluene quantity was required. This correction was unnecessary when the silicone oil scintillator was applied for the open-vial method. No evaporation of silicone oil was observed during 48 hours' exposure. The open-vial method using the silicone oil scintillator was sensitive enough to detect radon at a level of 160 Bq/m<sup>3</sup>.

*Study of resistance mechanisms in radiation-resistant organisms*

Tardigrades show remarkable adaptability under extreme environmental conditions, such as high radiation, high temperature, and high pressure. Activated sludge was obtained from the Ariake Water Reclamation Center of the Tokyo Metropolitan Government and used to collect tardigrades. Tardigrades isolated from activated sludge were identified with 18S-ribosomal DNA as *Isohypsibius*. *Isohypsibius* from activated sludge assume a barrel-like form, but not the complete 'tun' (that means barrel shaped tardigrade) under drying conditions. We examined the effects of radiation on the tardigrade with <sup>60</sup>Co irradiation at the Takasaki Advanced Radiation Research Institute. *Isohypsibius* tardigrades exposed to radiation showed a moderately higher survival rate than did those not irradiated.