Academic Prizes and Awards

1. The Award of The Phytopathological Society of Japan in 2004

Studies on fungicide resistance in phytopathogenic fungi

The Phytopathological Society of Japan presented its award to Dr. Hideo Ishii in 2004, for his outstanding research activities on fungicide resistance in phytopathogenic fungi.

Ishii has been at the forefront of research on fungicide resistance for nearly three decades. In 1975, Japanese pear trees were severely damaged by scab (*Venturia nashicola*) in many areas despite frequent applications of benzimidazole fungicides. This prompted Dr. Ishii to start studies on fungicide resistance, and since then he has continued close involvement in this research subject.

He demonstrated the fungicide resistance in *V. nashicola* and other pathogens. Through surveys on epidemiological characteristics of resistant fungal isolates in the field, he suggested strategies, such as applications of alternating benzimidazole fungicides or their mixing with unrelated fungicides would delay but not stop the build-up of resistance.

Ishii was the first to demonstrate that benzimidazole resistance controlled by a single major gene. To confirm this, he developed methods to artificially cross *V. nashicola*. Ascospore analysis suggested that the manifestations of three different levels of benzimidazole resistance, i.e. high, intermediate, and weak resistance were due to three allelic mutations in a single gene on a chromosome and that each level was controlled by one of the multiple alleles. He also showed that the increased sensitivity of highly benzimidazole-resistant isolates to *N*-phenylcarbamate and *N*-phenylformamidoxime compounds was controlled by a single major gene.

He elucidated the biochemical mechanism of benzimidazole resistance and negative cross-resistance to *N*-phenylcarbamates and *N*-phenylformamidoximes. In *V. nashicola*, *Botrytis cinerea* (grey mold), and *Gibberella fujikuroi* (Bakanae disease on rice), the binding of ^{14}C-carbendazim, the benzimidazole fungicide, to tubulin-like proteins was much lower in benzimidazole-resistant isolates than in sensitive isolates, suggesting that a decreased affinity of the fungicide to the target protein is a major factor of resistance. So far, this was the first report that the mode of inheritance of benzimidazole resistance was discussed with biochemical characterizations of resistance using isolates of phytopathogenic fungi collected from the field. Most benzimidazole-resistant isolates collected from the field exhibited codon changes either at positions 198 or 200 in β-tubulin genes. The substitution of glutamic acid (GAG) at codon 198 by alanine (GCG) resulted in high resistance to benzimidazoles. This point mutation was further associated with the negative cross-resistance to diethofencarb, the *N*-phenylcarbamate fungicide, and DCPF (*N*-(3,5-dichloro-4-propynyloxyphenyl)-*N*'-methoxyformamidine) as well.

He developed PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) methods for identifying resistant isolates. Allele-specific PCR (ASPCR) primers were also designed and used for the diagnosis of resistance. SSCP (single-strand DNA conformation polymorphism) analysis was first introduced in the diagnosis of fungicide resistance.

Currently, strobilurin fungicides (QoIs) are the most important group of fungicides as they are highly effective against a wide variety of fungal pathogens on various crops. Unfortunately, however, QoIs are also known to possess high risk for resistance development in target pathogens. Ishii detected QoI-resistant isolates of downy mildew and Corynespora leaf spot on cucumber in an early stage of resistance development, and contributed to diminishing the occurrence of resistance in practice.

He found a single point mutation, i.e. one base change at codon 143, in the fungicide-targeted cytochrome *b* gene from resistant isolates of cucurbit powdery mildew, cucumber downy mildew, and others. Substitution of glycine at 143 in cytochrome *b* by alanine resulted in high resistance to QoIs strongly indicating that resistance to QoIs in field isolates of plant pathogens due to a target site alteration.

To develop rapid testing methods for QoI resistance, PCR-RFLP was successfully employed and this method is currently used for resistance monitoring in practice. When the use of QoI fungicides was stopped, intracellular selection of the normal wild-type DNA might have occurred in multi-copy mitochondrial DNA of resistant isolates resulting in a decrease in the proportion of mutated DNA.

2. The Award of The Phytopathological Society of Japan in 2004

Population Biology of Individualistic Plant Pathogenic Fungi and its Application to Disease Control

Dr. Naoyuki Matsumoto, Department of Biological Safety, won the award of the Phytopathological Society of Japan in 2004. His interest has been focused on population biology of "individualistic" plant pathogenic fungi. The population structure of these fungi was found to reflect their ecological strategy and environmental conditions. He has more recently presented the prospect of the use of fungal viruses to control root diseases of fruit trees caused by *Helicobasidium mompa* and *Rosellinia necatrix* as an application of fungal population biology.

(1) Detecting individuals

Field isolates of *Typhula incarnata*, *T. ishikariensis*, *Sclerotium rolfsii*, *H. mompa*, and *R. necatrix* were assigned to mycelial compatibility groups (MCGs) based on the presence or absence of a dark demarcation line produced between colonies in paired culture. MCG designation was used to distinguish individual strains. MCGs existed in the field, as a rule, in patches spatially separated from each other. The genetic identity of each MCG was confirmed by molecular markers with two exceptions where different genotypes constituted a single MCG.

(2) Predominant MCG of *Typhula ishikariensis* biotype A

Recent climatic change characterized by warm winters and deep snow cover in eastern Hokkaido favored the occurrence of a snow mold fungus, *T. ishikariensis* biotype A, on alfalfa. *T. ishikariensis* biotype B had originally predominated in this region, and the fungus does not attack dicots. As a consequence, alfalfa had practically been free from snow mold. Surveys in damaged fields in eastern Hokkaido on the population structure of biotype A elucidated the ubiquity of a certain MCG by the founder effect.

(3) Factors affecting population structure of *Typhula* spp.

The difference in life history strategy between *T. incarnata* and *T. ishikariensis* was reflected in the difference in population structure. Highly heterogeneous populations of *T. incarnata* is ascribed to its high birth rate and high mortality of genets, resulting in consistently complex population structure regardless of the extent of habitat disturbance. *T. ishikariensis*, on the other hand, is characterized by low birth rate and low mortality of genets due to its uninfectious basidiospores and to persistent sclerotia. Its life-history strategy is consequently liable to be affected by environmental conditions.

(4) Application of population biology to biocontrol

Dr. Matsumoto directed a biocontrol project on root diseases of fruit trees caused by *H. mompa* and *R. necatrix* using fungal viruses as an application of population biology. Field observations indicate that the population structure of both pathogens is prone to the spread of viral antagonists. The simple population structure of the pathogens, along with the high economic significance of fruit trees, favor the exploitation of fungal viruses. Results from the project revealed their prospect as biocontrol agents of root diseases of fruit trees caused by *H. mompa* and *R. necatrix.*

3. The Award of The Phytopathological Society of Japan in 2004

Molecular biological studies of Ralstonia solanacearum and related plant pathogenic bacteria

Dr. Kenichi Tsuchiya, Department of Biological Safety, won the Award of The Phytopathological Society of Japan in 2004. In connection with recent changes in agricultural systematics and global trade as well as the public demand for sustainable agriculture, effective detection and discrimination methods are necessary not only for elucidating the genetic diversity of foreign strains and of indigenous pathogenic strains but also to assess beneficial traits and potential risks of biocontrol agents. For these purposes, he conducted molecular biological studies of *Ralstonia solanacearum* (Rs) and related plant pathogenic bacteria to characterize their phenotypic and genetic properties as summarized below:

(1) Molecular biological studies of the strains of *Ralstonia solanacearum*

Rs is one of the most important pathogenic bacteria that causes wilt diseases of plants worldwide. Known to be a heterogeneous species, it has been classified according to a binary system of race and biovar.

Japanese strains were divided into four pathogenic groups according to race. Groups I to III were pathogenic to solanaceous plants such as tomato and eggplant, which corresponded to race 1. Group IV was pathogenic to mainly potato, and corresponded to race 3. In biovar determination based on utilization of carbohydrates,

strains were divided into N2, 3 and 4.

Based on 16S rDNA sequences, Japanese group 1 of *Rs* was found to be closely related to Asian and Australian biovars 3, 4 and 5, whereas Japanese group 2 was homogeneous with Indonesian biovars 2 and N2. Similarly, by rep-PCR analysis using REP (repetitive extragenic palindromic), ERIC (enterobacterial repetitive intergenic consensus) and BOX primers, Japanese strains consisted of two main groups: one with all race 1, and the other with only race 3 strains.

(2) Serological and molecular characterization of the phytopathogenic bacteria

Monoclonal antibodies (MABs) were produced against *Rs* and bacterial spots of tomato and pepper (*Xcv*) and of mango (*Xcm*). By using the MABs, serological relationships of Japanese *Xcv*s with worldwide strains have been demonstrated for the first time. All the Japanese *Xcm* strains turned out to be group I strains among those so far reported in the world. Thirteen MABs selected against *Rs,* were divided into groups based on specific reactivity to strains from tomato (race 1), potato (race 3) or ginger (race 4).

By PCR-RFLP (polymerase chain reaction-based restriction fragment length polymorphism) analysis of 16S rDNA, strains of soft rot bacteria *Erwinia carotovora* subsp. *carotovora* (*Ecc*) isolated in Japan, Korea and Thailand were differentiated into two groups. Most strains from Korea and Japan belonged to the same group. *Ecc* strains isolated from mulberry in Japan were unique, and may belong to a new subspecies of *Ecc*.

(3) Practical detection and molecular characterization of biocontrol agents

By combining ELISA (enzyme-linked immunosorbent assay) using specific antisera with selective media, certain species of pseudomonads were efficiently detected from rhizosphere soil. PCR-based investigation for antibiotics-related genes from bacterial isolates was successful. Antibiotic substances such as pyrrolnitrin (Prn) and 2,4-diacetylphloroglucinol were confirmed by TLC (thin layer chromatography), HPLC (high performance liquid chromatography) and so on.

The *Burkholderia cepacia* complex (Bcc) can control certain plant diseases. Combining a simple direct ELISA with a two-step incubation method on a selective medium, Bcc was specifically detected from soil. One MAB established was specific to serovar group A, which encompasses the majority of natural strains from the environment. Bcc strains derived from clinical sources were assigned to several Genomovars (Gvs.), whereas the majority of those from environmental sources belonged to Gv.I. All *esmR* (epidemic strain marker gene) positive strains belonged to Gv.III, whereas most *prnC* positive strains belonged to Gv.I.

4. The Japanese Society of Soil Science and Plant Nutrition Award for the Excellent Paper on Soil Science and Plant Nutrition

Uptake response of crops to potassium from Andosol and potassium-breaking minerals applied to Andosol

This paper published in the *Japanese Journal of Soil Science and Plant Nutrition* was awarded the Excellent Paper Award by the Japanese Society of Soil Science and Plant Nutrition. Ms. Megumi Sugiyama, senior author of this paper, is a researcher of the Soil Biochemistry Unit, Heavy Metal Group. In their paper, she and co-author Dr. Noriharu Ae, former leader of the same unit, elucidated a new aspect of potassium (K) uptake of plants from soil: if a crop has the ability to take up potassium (K) from the firmly held K minerals (non-exchangeable K) in soil, silicate (SiO_2), one of the major mineral constituents, is expected to be released from the soil and/or to be absorbed by the crop. To confirm this hypothesis, pot experiments were conducted using Andosol which is poor in K supplying power. They found that K uptake by crops differed among crop species, and that when the K uptake was large, the sum of crude silicate in the shoot of crop and available silicate (2.5% acetic acid extractable Si) in the soil after harvesting was larger than the amount of available silicate in the soil of un-planted pots. This supports the above hypothesis that crops absorb “non-exchangeable K” from soil minerals in association with the solublization of silicate mineral.