Panel II Summary
Field Experience in Using Microorganisms as Bioindicators, Biosensors or Biomonitors

Michael Schechtman,[1]

We had very interesting and wide-ranging presentations and discussions from four distinguished speakers, Dr. Katsuji Watanabe from the Kyushu National Agricultutal Experiment Station, Kumamoto, Japan, Dr. Fenny Dane, Auburn University, Auburn, Alabama, USA, Dr. Scott Lohrke, University College, Cork, Ireland, and Dr. Marco Nuti, Università di Padova, Padova, Italy. These discussions focused on the ecological features and population composition of various soil and rhizosphere environments. These environments were studied via examination of indigenous microbial populations or with the use of genetically modified microbes both as tools and as specific objects of study in themselves. This summary will only briefly indicate what the topics of study themselves were, but will attempt to indicate in more detail several common themes that emerged in the presentations or in the ensuing discussions.

To briefly recapitulate the topics of the presentations:

Dr. Watanabe studied the presence and distribution of soil proteases that may be important in carbon and nitrogen cycling in the soil. A wide variety of soil proteases were found, of bacterial and fungal origins, and the presence of particular types of soil proteases of particular origin depended on the soil treatment (e.g., fallow or cultivated land, with or without slurry application).

The second presentation was by Dr. Dane,who used a lux-marked derivative of the plant pathogen *X. campestris* pathovar *campestris* (which infects crucifers), and employed the associated bioluminescence as a tool for studying plant-pathogen interactions, as well as the environmental survival of the marked microorganism.

The third presentation was from Dr. Lohrke, who assessed persistence, survival, and a variety of environmental parameters when a genetically marked *Pseudomonas fluorescens* strain that may be useful for biocontrol of fungal *Pytium* infections was used, initially, in experiments on sugar beet. United States Department of Agriculture

The final presentation was from Dr. Nuti, using genetically modified and marked strains of the biofertilizing or biostimulating microorganisms *Rhizobium legumi-nosarum* and *Azospirillum brasilliense* and examining both efficacy and stimulatory effects as well as effects on a variety of other environmental parameters.

What were some common themes that emerged from some or all of these presenta-

[1] Biotechnology, Biologics, and Environment Protection USDA-APHIS,4700 River Road, Unit 147, Riverdalc. MD 20737-1237, USA.

tions, or in the discussions? There were several of these, I believe, and they vary as to whether they are specifically or directly related to defined biosafety issues, or were more general scientific considerations or conclusions.

The first theme I would note is one of optimism and increasing familiarity. The experiments presented demonstrated first off that progress is being made with environmental releases of microorganisms, that they can be successfully monitored in the environment and familiarity with them gained, and that, despite considerable variability in the types of results observed, no adverse or unexpected effects were noted in these trials. It needs to be further mentioned, however, that the aim of producing new microbial products for environmental uses is to produce organisms that will in fact have impacts -- desirable ones. There needs to be the realization that there is a conceptual distinction between environmental effect and risk. Effects, and the potential for them, need to be carefully evaluated, but an effect in and of itself is not necessarily a risk.

After that essentially general and positive note, I will switch to more technical themes. The first is that the complexity of the receiving environments for these organisms can, and frequently does, lead to lack of reproducibility of observed effects. Variations in resident microbial populations in terms of both indigenous composition or persistence of marked organisms were noted, for example, when the land was put to different uses (e.g., fallow vs. cultivation); when there were uncontrollable changes in external disease pressure; and under different temperature or moisture conditions. Such variations were seen, not surprisingly, in all four studies, but I think that from the biosafety perspective it highlights the importance of having baseline information about the natural range of microbial community responses to such environmental changes as a necessary adjunct for evaluating the significance of any result that is observed in a field experiment utilizing a genetically modified microorganism. This would include having some idea of the magnitude of changes to microbial flora that are brought about in the course of normal agricultural practices-- tillage, fertilizer addition, pesticide and herbicide application, etc.

Third, there is the issue of persistence. Among the released genetically modified microorganisms discussed, none appeared to be capable of survival at high soil titers over the long term-- they all declined to low or undetectable levels during the course of the experiments. Several caveats to this conclusion should be noted, though. One was the real possibility that some of the microorganisms could have entered a viable but nonculturable state and might have been missed. Second, the immediate level of persistence was again strongly dependent on the local environmental conditions so that, for example, the introduced *X. campestris* strain persisted on host plants far longer through winter trials than through summer ones. Also, it was pointed out that these test strains have not been modified to enhance their survival or competitiveness, and the observed lack of persistence may not be generalizable to other strains; indeed, in the case of some organisms that may be designed for certain biocontrol uses, a lack of persistence may not always even be desirable.

A related scientific conclusion, though neither a biosafety issue *per se* nor a conclu-

sion unique to these presentations, related to the considerable complexity of the microbial populations under examination in field situations. In Dr. Watanabe's studies, the spectrum of microbes producing proteolytic activities varied greatly according to soil treatment, though families of proteases seemed to be evident. When Dr. Lohrke looked at effects of introducing a marked *P. fluorescens* strain on populations of related organisms in soil, the level of detectable variation in the indigenous populations was very high, perhaps higher than might have been expected. Similarly, the importance of adaptation to particular niches for survival and persistence was pointed out in Dr. Nuti's results, which noted considerable difference in colonizing ability between local indigenous *Rhizobium* and non-indigenous strains. I think, though, that it is an important and open question of how to tie the immense scientific interest in this complexity and variability-- topics that are intimately tied themselves with "product-oriented" requirements for efficacy and reproducibility of environmental effects-- to questions that are central in the "need to know" sense for biosafety.

This distinction was further clarified by one commenter during the discussion period, who inquired the following: given the ubiquity of antibiotic resistance markers in soil microorganisms, why or when should we be concerned about the use of these markers in fleld trials? Rather than overstep my role as moderator and attempt to provide a personal answer to this important question, I would simply note that this is precisely the sort of question we need to take a hard look at. For field trials using microorganisms, in which there are so many environmental parameters that are of interest and may be investigated, and which investigators may attempt to control in their experiments or in their construct design, we must be very clear-minded and critical in making distinctions between information and/or testing requirements that are of the "nice to know" or "nice to do" variety, and those that are really essential for biosafety.

Finally, one last matter arose that relates to another matter that has been tied to biosafety concerns, and that is the issue of horizontal gene transfer. None of the experiments presented uncovered any evidence of this phenomenon taking place. It should be noted, though, that it is not clear that one can extrapolate from these cases in any general way, and moreover, even if horizontal gene transfer had been observed between any bacterial species in these experiments, that observation would in itself probably have elicited neither great surprise nor immediate predictions of new risks.

Overall, what we heard were very positive results; no unexpected pitfalls in terms of biosafety regarding these organisms were evident. It is clear that bioindicators and biomonitors will be effectively used in the environment (though more research is of course needed to ensure efficacy), and from these discussions I believe future developments will be very promising.