

Poultry Pathogen *Models for Predictive Microbiology*

PEGGY GREB (K11896-1)



In a modeling study to predict growth of *Salmonella* on chicken, food technologist Thomas Oscar uses an automated counting system to count pathogen colonies (the black dots) on an agar medium.

Food technologist Thomas P. Oscar wants to make poultry as pathogen-free as possible. His research focuses on modeling growth and survival of *Salmonella* and *Campylobacter*—the two most prevalent bacterial food pathogens—on chicken, the most consumed meat product in the United States.

Oscar and technician Jacquelyn B. Ludwig are a two-person team at a research laboratory on Maryland's Eastern Shore. The ARS Poultry Food Safety Research Laboratory is part of the Microbial Food Safety Research Unit of the Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania. But it's physically located on the University of Maryland Eastern Shore (UMES) campus in Princess Anne—in the heart of the Delmarva Peninsula, one of the country's leading poultry-producing regions.

Their research is part of a growing field—known as predictive microbiology—that forecasts the behavior

of foodborne pathogens in response to environmental conditions encountered in food production and processing operations. Models predict how long it takes for pathogens to grow under certain conditions and how fast they grow once they start. Models help processors make proper decisions about food safety and help regulatory agencies and scientists learn more about the conditions in which pathogens, such as *Salmonella*, thrive.

An estimated 1.4 million cases of salmonellosis food poisoning occur annually in the United States, according to the Centers for Disease Control and Prevention, and about 10 percent of those cases are from poultry. Most people recover

without treatment, but some get diarrhea so severe that

they have to be hospitalized. If the infection spreads from the intestines to the bloodstream,

it can cause death unless the patient is treated promptly

with antibiotics. The elderly, infants, and those with impaired immune systems are most at risk.

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Real-World Competition

“One way to better assess food safety,” Oscar says, “is to develop pathogen models that take into account real-life competition from other microbes present in food. These models would be more realistic, make better predictions, and help better ensure that the food industry doesn't needlessly dispose of safe food.”

Previously, Oscar says, models were often developed in broth with no other microbes present. Researchers thought this would allow them to accurately predict pathogen behavior in food. Recent findings by Oscar and ERRC colleagues Mark L. Tamplin, microbiologist, and Andy Hwang, food technologist, indicate that this is not always the case.

The ARS researchers will produce more realistic models by using a system Oscar developed to rate performance of current models. Called the “acceptable prediction zone method,” it establishes criteria for verifying and validating models as having acceptable or unacceptable predictive records, classifies them to show which are best, and then pinpoints any changes that are needed. Models are evaluated under specific conditions to find which ones meet established standards and demonstrate a level of performance that could be used with confidence in the food industry to predict food safety.

Oscar thinks most current broth models are “overly fail-safe,” meaning they predict much higher pathogen numbers than would be present in real food with microbial competition. Still, he says, it's better to over-predict the growth rate of a pathogen than to under-predict it. His laboratory is working on two promising techniques to improve the models.

One method is to introduce a jellyfish gene into *Salmonella* to make the bacteria fluoresce, or glow, so they'll be easier to detect among other microorganisms on raw poultry. At first, the foreign DNA slowed *Salmonella* growth, making the models inaccurate. But Oscar and colleagues Dwayne Boucaud, assistant professor and microbiologist at UMES,

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Technician Jacquelyn Ludwig grinds chicken in preparation for modeling experiments of *Salmonella*.

and Kalpana Dulal, a graduate student, are working on a new way of introducing the foreign DNA that will allow normal growth.

Oscar has had more immediate success with the second approach. He and his colleagues found that a strain of antibiotic-resistant *Salmonella* can be a valuable tool for modeling pathogen growth. *Salmonella typhimurium* DT104 is known to be resistant to five different antibiotics. The researchers put four of those five antibiotics in an agar medium they were using to determine the amount of *Salmonella* in a food sample. The antibiotics killed off competing microorganisms on the agar

plates and left just the *Salmonella*. The scientists were able to follow the surviving *Salmonella* and model its growth on raw poultry meat, showing how it behaves in a real food environment. The DT104 strain helped confirm their theory that models developed in the absence of microbial competition do not accurately predict pathogen behavior in foods that contain other spoilage organisms.

“One of the biggest hurdles we face in developing models in food with other microorganisms has been not having a valid marker pathogen strain that we could follow in the presence of other microorganisms,” Oscar says. “The DT104 strain, which exists in nature, has allowed us to jump over this hurdle and holds great promise for developing better *Salmonella* models for the food industry.”

Oscar’s study doesn’t end with *Salmonella*. Along with Kisun Yoon, assistant professor at UMES, and graduate student Candace Burnette, he has new findings on *Campylobacter*, the leading cause of bacterial food poisoning worldwide.

The three scientists recently found that

C. jejuni died faster at room temperatures than at refrigeration temperatures. The data collected in the study was used to develop one of the first predictive models for *C. jejuni*. The new model will be included in ERRC’s Pathogen Modeling Program, a package of models available on the World Wide Web at www.arserrc.gov/mfs/pathogen.htm. The availability of these and other food safety models should accelerate use of models by food industries and researchers interested in the field of predictive microbiology.—By **Jim Core**, ARS.

This research is part of Food Safety (Animal and Plant Products), an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

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Graduate student Kalpana Dulal (left) and microbiologist Dwayne Boucaud, of the University of Maryland Eastern Shore, examine expression of green fluorescent protein by *Salmonella* on agar medium.