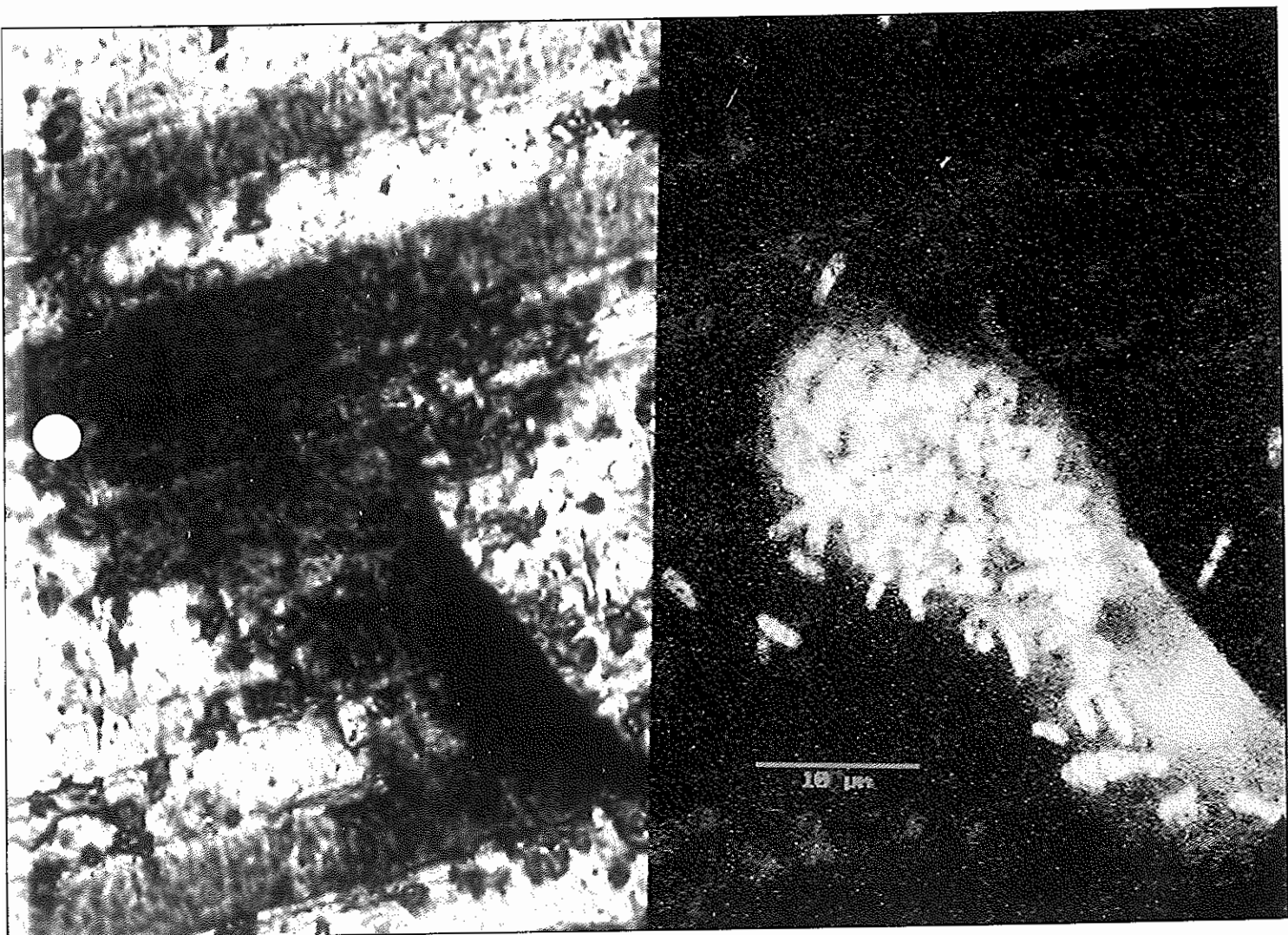


# ASM News

Volume 58  
Number 10  
October 1992

American Society  
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Tropical Disease Research Priorities Reassessed  
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# ASM News

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*Cover: Scanning confocal laser microscopic, color-transformed, epifluorescence image of a copper coupon after 7 days of exposure to an unidentified culture of a copper-corroding bacterium. This image, when superimposed on a reflected white light image of the same area of the coupon, indicates that the bacteria preferentially colonize depressions in the metal surface (see p. 546).*



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# Centers, New Technologies Focus on Biofilm Heterogeneity

*New research explores the structure and activity of biofilms*

G. G. GEESEY, W. G. CHARACKLIS, AND J. W. COSTERTON

The nonrandom distribution of microbial cells in biofilms carries important consequences for industry, medical practice, and ecosystems. For industry, several costly microbiological problems, including fouling, corrosion, and product contamination, can be traced to biofilm phenomena. To better understand and combat these problems, researchers increasingly are taking new approaches. In particular, they are adapting new imaging tools and developing new mathematical models for analyzing systems to probe the heterogeneous microbial populations found in biofilms.

Not long ago, most research aimed at understanding biofilms was conducted by individual investigators working with only a few colleagues. More recently, some of these investigators have been coalescing to form multidisciplinary research centers—a strategy deemed necessary because of the complexity of the questions and the growing number of approaches researchers find themselves taking to address those questions.

Several recently formed centers now devote substantial energy to biofilm research, including The Center for Interfacial Microbial Process Engineering at Montana State University, Bozeman; The Center for Environmental Biotechnology at the University of Tennessee, Knoxville; and the Center for Applied Biosurfaces Engineering Research at the University of Calgary, Calgary, Alberta, Canada. In addition to these centers, others, such as the Center for Marine Biotechnology at the University of Maryland, support

substantial, if not dedicated, research programs on biofilms.

At the Center for Interfacial Microbial Process Engineering, founded by William Characklis at Montana State University, researchers are developing mathematical expressions to describe the distribution of bacterial cells that colonize metal surfaces. The results are then used to predict biofilm accumulation and activity in conduits and porous media. The predictive capabilities are intended to improve the competitiveness of U.S. industry by reducing costs associated with periodic plant shutdowns to clean or replace failed equipment or with removing undesirable microbial metabolites from the final product during manufacture.

The Center for Environmental Biotechnology at the University of Tennessee is directed by Gary Saylor and David White. Researchers there employ molecular genetics and analytical chemical techniques to probe complex associations between different microbial species growing in biofilms on surfaces. The focus is on activities that are responsible for degradation of toxic compounds in the environment and on microbially influenced corrosion.

Research at the Center for Applied Biosurfaces Engineering and Research at the University of Calgary, which is under the direction of Bill Costerton, focuses on the development of novel approaches to control biofilm growth and activity, including bioelectric technology to permeabilize the biofilm matrix to nutrients, biocides, and antibiotics. Doug Caldwell at the University of Saskatoon, who collaborates with Costerton, has developed a way to establish and maintain specific microbial associations within biofilms grown in the laboratory that mimic those associations found in nature.

Early studies of bacterial adhesion and biofilm formation utilized laboratory strains unaccustomed to the natural environmental pressures that promote attachment and growth on surfaces. Adhesion experi-

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### New Forms of Microscopy Providing Insights

Even with such modifications to classical microbiological culture techniques, other barriers have blocked the study of biofilms. For example, the conventional imaging tools of phase-contrast and electron microscopy (transmission electron microscopy and scanning electron microscopy) limit our ability to resolve the heterogeneous microbial populations within biofilms. We could see that the cells were anchored to the surface, but we knew little about the features of the surface that stimulated bacterial adhesion. We could see that there was an extracellular polymeric matrix, but it was either blurred or badly distorted when dehydrated, because in its native state it contains 99% water. We also imagined that the biofilm cells were randomly distributed in a homogeneous matrix of unknown dimensions.

These perceptions are now rapidly being replaced by examining clearer images of undisturbed biofilms with the introduction of confocal scanning laser microscopy (CSLM), environmental scanning electron microscopy, and atomic force microscopy.

CSLM is providing new insight into the surface features that promote attachment. Superimposed reflected and fluorescent CSLM images reveal selective colonization of intergranular boundaries and depressions on the surface of some metal alloys by specific bacteria. The three-dimensional mapping capabilities of CSLM also are unveiling the true distribution of cells within biofilms. For example, the matrix accounts for 75 to 95% of the volume of the biofilm, and bacterial

cells usually grow in discrete microcolonies within it. The bacterial component is patchy, and penetration probes (dextrans) reveal that the matrix is concentrated around the bacterial microcolonies but is diffuse in water channels that traverse the biofilm. CSLM also shows that biofilm bacteria (e.g., methanogens) achieve optimal physiological efficiency if they grow to produce large monospecies microcolonies surrounded by metabolically cooperative species (e.g., acetogens) of bacteria. Mixed-species biofilms of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* contain regions where each species occurs independently and other regions where they exist in intimate association.

Through the use of recently developed microsensor technology, we know that the activities of bacteria within the biofilm have a profound impact on a local environment. For example, microsensors reveal strong oxygen gradients within biofilms of oxygen-consuming bacteria. By combining CSLM and microsensor technology, we can relate heterogeneities in bacterial densities and activities to chemical heterogeneities within biofilms.

A major challenge is to express in mathematical terms these and other microbial and chemical distributions on surfaces and within biofilms. If this can be achieved, it may be possible to develop models of microscale phenomena such as microbe-influenced pitting corrosion. Computer simulation models (e.g., BIOSIM) are being used to describe the relationship between nutrients and biocide loading and biofilm accumulation in conduits and porous media at the mesoscale. Incorporation of microscale heterogeneity will contribute to more effective modeling efforts at the mesoscale, particularly in transport processes and biofilm accumulation. Microscale and mesoscale phenomena may even be important to macroscale modeling of oil field souring.

New imaging and probe technologies are profoundly altering our mental image of bacterial biofilms. Previously, we thought of bacterial cells distributed in a chemically and physically homogeneous matrix of uncertain dimensions. We now view biofilms as assemblages of microcolonies of bacteria, which may establish metabolic cooperation with cells of adjacent microcolonies, embedded in a matrix exhibiting both chemical and structural heterogeneity, a matrix that forms a barrier to toxic substances yet allows transport of nutrients for cell growth and survival. As in most biological systems, order is transcendent, and apparent randomness only betrays our previous ignorance. □

# ASM News

## ICAAC Continues as Premier Infectious Disease Meeting

From relatively modest beginnings over 30 years ago, ASM's Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) has become the preeminent infectious disease meeting in the world. The 32nd ICAAC, convening this year in Anaheim, Calif., is expected to draw a record-breaking 12,000-plus attendees, including about 4,000 international registrants. For the meeting this year, the Program Committee reviewed approximately 3,000 abstracts and scheduled 1,700 scientific presentations, more than 10 times the number at the first ICAAC.

"From a meeting that was first held in one room, ICAAC has become recognized as the premier infectious disease meeting," said Clyde Thornsberry, chair of the ICAAC Program Committee. ASM established a Committee on Interscience Conferences in 1960 and sponsored the first official ICAAC in 1961. At that meeting, 828 people attended and 158 papers were presented. Attendance was 2,400 in 1975 and reached almost 8,000 by 1985.

Exhibits were first held at the 1973 meeting, where there were 27 exhibiting companies in 31 booths. At the 1992 meeting, 148 companies will be exhibiting in 518 booths.

"ICAAC is a cutting-edge meeting that attracts world-class scientists and physicians. The Program Committee selects only new and innovative research for presentation. We often receive abstracts that are

perfectly good and publishable but are rejected because the information has been previously presented at ICAAC. We also don't accept research that has been presented elsewhere," Thornsberry said.

Two new features will be added this year, he pointed out, a special session updating recent information on AIDS and another on late-breaking research developments (see box).

The focus on AIDS is only one of the ways ICAAC has changed since its inception. Robert Moellering, ICAAC program chair (1980-1982) and current editor in chief of *Antimicrobial Agents and Chemotherapy*, noted that as the meeting has grown, the scope of the program has become even broader, going beyond

the emphasis on antimicrobial agents to include epidemiology and clinical microbiology.

"The antiviral area has grown considerably, as have the areas of immunology and host defenses," Moellering said.

"However, one of the reasons for the popularity of ICAAC," Moellering said, "is that the meeting encompasses a specific area—infectious diseases. It is perceived internationally as a quality meeting, unlike meetings where every paper is accepted."

"The program committee has been selective and has maintained high quality, and the rate of rejection hasn't changed much. However, the Program Committee has struck a nice balance and has also

### ICAAC Innovations

The ICAAC Program Committee has added two special sessions at this year's meeting in Anaheim, one on recent findings in AIDS and another on late-breaking developments occurring after the deadline for abstract submissions.

"AIDS: 1992—The Experts Discuss Recent Findings" will take place on Tuesday, 13 October, in Pacific Ballroom C of the Anaheim Hilton from noon to 1:30 p.m. This roundtable will focus on the latest discoveries in AIDS research, discussing the findings that have been reported at recent meetings as well as in the press. This will be an interactive session, not formal presentations.

Experts will include invited guests from the Centers for Disease Control, the National Institutes of Health, and the World Health Organization and will be moderated by ICAAC Committee members Richard Whitley and Robert Schooley.

The "Late Breaker Session" will be held on Wednesday, 14 October, from noon to 1:30 p.m. in the California Pavilion of the Anaheim Hilton. The program will be limited to a small number of presentations on new and highly significant developments. Only new abstracts and not those previously submitted will be selected. Final selection for the session will be made approximately 1 week before the meeting.

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This need comes at a time when the global environment and politics are in a state of flux, Godal says. "The environment is a major element in diseases, and we often have a limited window of opportunity." Large-scale climate changes and fluctuating local practices are among the many matters that need to be faced. Meanwhile, however, global politics also present new opportunities and a major change in world temperament that are likely to affect such disease control research efforts. The disappearance of the Cold War, for example, represents an unparalleled opportunity for WHO and national health officials, who no longer need to cope with what was once a highly distracting set of international sensitivities when they plan research or public health measures in many settings.

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### New Forms of Microscopy Providing Insights

Even with such modifications to classical microbiological culture techniques, other barriers have blocked the study of biofilms. For example, the conventional imaging tools of phase-contrast and electron microscopy (transmission electron microscopy and scanning electron microscopy) limit our ability to resolve the heterogeneous microbial populations within biofilms. We could see that the cells were anchored to the surface, but we knew little about the features of the surface that stimulated bacterial adhesion. We could see that there was an extracellular polymeric matrix, but it was either blurred or badly distorted when dehydrated, because in its native state it contains 99% water. We also imagined that the biofilm cells were randomly distributed in a homogeneous matrix of unknown dimensions.

These perceptions are now rapidly being replaced by examining clearer images of undisturbed biofilms with the introduction of confocal scanning laser microscopy (CSLM), environmental scanning electron microscopy, and atomic force microscopy.

CSLM is providing new insight into the surface features that promote attachment. Superimposed reflected and fluorescent CSLM images reveal selective colonization of intergranular boundaries and depressions on the surface of some metal alloys by specific bacteria. The three-dimensional mapping capabilities of CSLM also are unveiling the true distribution of cells within biofilms. For example, the matrix accounts for 75 to 95% of the volume of the biofilm, and bacterial

cells usually grow in discrete microcolonies within it. The bacterial component is patchy, and penetration probes (dextrans) reveal that the matrix is concentrated around the bacterial microcolonies but is diffuse in water channels that traverse the biofilm. CSLM also shows that biofilm bacteria (e.g., methanogens) achieve optimal physiological efficiency if they grow to produce large monospecies microcolonies surrounded by metabolically cooperative species (e.g., acetogens) of bacteria. Mixed-species biofilms of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* contain regions where each species occurs independently and other regions where they exist in intimate association.

Through the use of recently developed microsensors technology, we know that the activities of bacteria within the biofilm have a profound impact on a local environment. For example, microsensors reveal strong oxygen gradients within biofilms of oxygen-consuming bacteria. By combining CSLM and microsensors technology, we can relate heterogeneities in bacterial densities and activities to chemical heterogeneities within biofilms.

A major challenge is to express in mathematical terms these and other microbial and chemical distributions on surfaces and within biofilms. If this can be achieved, it may be possible to develop models of microscale phenomena such as microbe-influenced pitting corrosion. Computer simulation models (e.g., BIOSIM) are being used to describe the relationship between nutrients and biocide loading and biofilm accumulation in conduits and porous media at the mesoscale. Incorporation of microscale heterogeneity will contribute to more effective modeling efforts at the mesoscale, particularly in transport processes and biofilm accumulation. Microscale and mesoscale phenomena may even be important to macroscale modeling of oil field souring.

New imaging and probe technologies are profoundly altering our mental image of bacterial biofilms. Previously, we thought of bacterial cells distributed in a chemically and physically homogeneous matrix of uncertain dimensions. We now view biofilms as assemblages of microcolonies of bacteria, which may establish metabolic cooperation with cells of adjacent microcolonies, embedded in a matrix exhibiting both chemical and structural heterogeneity, a matrix that forms a barrier to toxic substances yet allows transport of nutrients for cell growth and survival. As in most biological systems, order is transcendent, and apparent randomness only betrays our previous ignorance. □