IBS International Biofilm Summit

In Collaboration with CIBUSTEC

FEATURED SPEAKERS 2019

Frank BOELAERT

Biological Hazards and Contaminants (BIOCONTAM) Unit, European Food Safety Authority (IT)

MONITORING OF ZOONOSES AND FOOD-BORNE OUTBREAKS IN THE EUROPEAN UNION

During 2017, campylobacteriosis was the commonest reported zoonosis in the European Union (EU) and its EU trend for confirmed human cases increasing since 2008 stabilised during 2013–2017. The decreasing EU trend for confirmed human salmonellosis cases since 2008 ended during 2013–2017, and the proportion of human *Salmonella* Enteritidis cases increased. Sixteen Member States (MS) met all *Salmonella* reduction targets for poultry, whereas 12 MS failed meeting at least one. The EU flock prevalence of target *Salmonella* serovars in breeding hens, laying hens, broilers and fattening turkeys decreased or remained stable compared to 2016, and slightly increased in breeding turkeys. The notification rate of human listeriosis further increased in 2017, despite *Listeria* seldom exceeding the EU food safety limit in ready-to-eat food. The decreasing EU trend for confirmed yersiniosis cases since 2008 stabilised during 2013–2017. The number of confirmed shiga toxin-producing *Escherichia coli* (STEC) infections in humans was stable. A total of 5,079 food-borne (including waterborne) outbreaks were reported. *Salmonella* was the commonest detected agent with S. Enteritidis causing one out of seven outbreaks, followed by other bacteria, bacterial toxins and viruses. The agent was unknown in 37.6% of all outbreaks. *Salmonella* in eggs and *Salmonella* in meat and meat products were the highest risk agent/food pairs.

Romain BRIANDET

Research Director, INRA Micalis (FR)

BIOFILM CONTROL: FROM CHEMICAL TO BIOLOGICAL ARSENAL?

Microbial life abounds on surfaces in natural, medical and industrial environments. A solid substrate, water and some nutrients are sufficient to allow the construction of a microbial fortress, a so-called biofilm. A better understanding of the functioning of these microbial communities is a challenging and crucial issue, as it constitutes a prerequisite to the optimization of control strategies. Survival properties developed by these surface-associated ecosystems are beginning to be deciphered in the context of rudimentary axenic laboratory biofilms. Gelatinous organic matrices consisting of complex mixtures of self-produced biopolymers ensure the cohesion of these biological structures and contribute to their tolerance to chemical biocides. There is also growing evidence that surface-associated microbial pathogens can be controlled by guided biofilm ecology approaches in the fields (biocontrol), the processing units (bioprotection) and food matrices (biopreservation).

Keywords: biofilm architecture, multispecies interactions, biocontrol, bioprotection, bioconservation, real-time confocal imaging.

Trond MORETRO

Research Scientist,
NOFIMA AS (NO)

THE USE OF DNA SEQUENCING TECHNOLOGIES FOR INVESTIGATION OF CONTAMINATION ROUTES IN THE FOOD INDUSTRY; OPPORTUNITIES AND BOTTLENECKS

It has been shown that certain types of *Listeria monocytogenes* can persist in the food industry for years, and although less studied this is likely also the case for potential food spoilage bacteria. In order to reveal contamination routes and thus be able to introduce targeted control measures, there is a need for typing methods with high resolution.

The last years there have been a rapid development in the methodology for typing of microorganisms with sequence based methods. Whole genome sequencing (WGS) is now increasingly being used by food safety- and public health authorities in outbreak investigations. WGS is a transformational technology, and has superior sensitivity and accuracy compared with traditional methods used in surveillance, source tracking and investigations of foodborne outbreaks.

It is likely that the food industry in the not so far future will be met with expectations of implementing WGS technology from authorities and customers. In order to unlock the full potential of WGS for food safety and public health, data must be shared and compared. Even if WGS methodology is now available, there are still technical, legal and other regulatory barriers that must be solved to enable a large scale implementation of whole genome sequencing by the food industry.

Examples of the use of sequencing technologies in outbreak investigations and in source tracking of *Listeria* in the food industry will be presented.

Koen DE REU- ILVO

Senior researcher,
Research Institute for Agriculture, Fisheries and Food (BE)

OCCURRENCE, CHARACTERIZATION AND SPOILAGE POTENTIAL OF BIOFILMS IN DIFFERENT FOOD COMPANIES

Introduction: The importance and role of biofilms in persistent infections with spoilage organisms and pathogenic bacteria is still insufficiently known. Research about sampling, detection and characterization of biofilms in the food industry can help to provide new insights in this issue.

Purpose: The aim of this study was to sample biofilms in different food companies and to characterize the microbial population and matrix components of these presumptive biofilms.

Methods: Surfaces in 8 food companies were sampled after cleaning and disinfection. On the samples, different microbiological enumerations were performed and the dominant bacteria were identified. Also, the biofilm matrix components proteins, carbohydrates and uronic acids were determined. Finally the collected dominant bacteria from the biofilms in the food companies were tested under lab conditions on their biofilm forming capacities using a biofilm model based on microtiter plates.

Significance: Detection and characterization of biofilms in the concerned food companies gave useful insights in the potential to cause food spoilage and foodborne infections and offered a basis for the development of more efficient cleaning and disinfection procedures.

H.P. STEENACKERS

MICA Lab,
Centre of Microbial
and Plant Genetics,
KU Leuven (BE)

COMPETITIVE INTER-SPECIES INTERACTIONS UNDERLIE THE INCREASED ANTIMICROBIAL TOLERANCE IN MULTISPECIES BREWERY BIOFILMS

Genetic diversity often enhances the tolerance of microbial communities against antimicrobial treatment. However the sociobiology underlying this antimicrobial tolerance remains largely unexplored. Here we analyze how inter-species interactions can increase antimicrobial tolerance. We apply our approach to 17 industrially relevant multispecies biofilm models, based on species isolated from 58 contaminating biofilms in three breweries. Sulfathiazole was used as antimicrobial agent because it showed the highest activity out of 22 biofilm inhibitors tested. Our analysis reveals that competitive interactions dominate among species within brewery biofilms. We show that antimicrobial treatment can reduce the level of competition and therefore cause a subset of species to bloom. The result is a 1.2-42.7-fold lower percentage inhibition of these species and increased overall tolerance. In addition, we show that the presence of *Raoultella* can also directly enhance the inherent tolerance of *Pseudomonas* to antimicrobial treatment, either because the species protect each other or because they induce specific tolerance phenotypes as a response to competitors. Overall, our study emphasizes that the dominance of competitive interactions is central to the enhanced antimicrobial tolerance of the multispecies biofilms, and that the activity of antimicrobials against multispecies biofilms cannot be predicted based on their effect against monocultures.

Pier Sandro COCCONCELLI

Università Cattolica
del Sacro Cuore (IT)

FACTOR AFFECTING THE BIOFILMS IN THE FOOD PROCESSING PLANTS

The formation of microbial biofilms on surfaces of food processing plants is a complex process influenced by different factors: i) the characteristics of abiotic surfaces, ii) the physicochemical conditions of the niche (e.g. temperature, pressure, flow rate in pipelines), iii) the food matrix (e.g. pH, protein and sugar content) and iv) the microbial species making the biofilm. This complexity limits the possibility to predict and counteract the biofilm formation in processed food industry and, in particular, in closed circuits.
During the last years we have focused on the study of biofilm formation by pathogenic and spoilage bacteria, such as *Listeria, Bacillus cereus, Lactobacillus fructivorans* and *Streptococcus thermophilus*. The interaction between surfaces, food matrix and bacteria were investigated in dynamic conditions using flow cells. Particularly, in *Listeria* the role of the *luxS* gene in biofilm formation and the survival to sanitation treatments was investigated on different abiotic surfaces. The ability of *B. cereus* to colonize the stainless steel and to complete the sporulation cycle and the subsequent germination process were studied in the presence of different food matrixes. When the ability of *L. fructivorans*, an acid resistant species responsible of spoilage in acid sauces such as mayonnaise, to colonize stainless steel pipelines was studied, food components (proteins and lipid) were shown to affect biofilm formation and survival. *S. thermophilus*, a thermophilic bacterium which can colonize the milk pasteurization plant, is able to make biofilm on stainless steel in a two steps process where first, milk proteins adhere to the abiotic surface and then the bacterial cell attach to caseins. Mutant selection and genomic studies demonstrated that the PrtS proteinase, a key enzyme for fast growth on milk, plays a key role in the biofilm formation.

Gun WIRTANEN

Seinäjoki University of Applied Sciences (FI)

BIOFILM RISKS IN FOOD PROCESSING

Microbes prefer to stick to surfaces instead of swimming in solutions, which also applies for microbes in the food processing. There are both good and bad microbes in the surrounding. Therefore, it is to remember that any microbe in the wrong place is bad for the products produced. In case, the biofilm forming microbes are of pathogenic origin and the products in contact with the contaminated surface have gone through all heat-treatment procedures the contamination can lead to severe foodborne outbreaks. The number of human listeriosis cases in EU has increased from about 1900 confirmed cases in 2013 to about 2500 in both 2016 and 2017. This despite that *Listeria monocytogenes* in ready-to-eat foods (RTEs) seldom exceeded the safety limit (≤ 100 CFU/g), when packaged food leaves the food factory. Common RTEs contaminated with *L. monocytogenes* are fish and meat products, various types of cheese, vegetable, fruit and salads. The number of infections caused by Shiga toxin-producing *Escherichia coli* (STEC) has annually been around 6000 cases in 2013-2017. Foods commonly contaminated with STEC are meat and milk products, vegetables, fruits and juices. Not all reported cases of pathogens in processed foods can be connected to biofilms, but in case the contamination in the process is recurring, the reason is mostly due to biofilms in unhygienic process solutions. Important tools in preventing biofilm formation in wet processes are good design, including hygienic design, choice of surface materials and building of process lines, and manufacturing practices, e.g. maintenance, cleaning and disinfection procedures in the process. The process lines and equipment must further be properly maintained. They should also be accessible, cleanable and drainable. This presentation will deal with biofilm formation – of both spoilage microbes and pathogens - on surfaces and how to avoid biofilms using above-mentioned measures.

Pedro RODRIGUEZ LOPEZ

Postdoc Researcher, Department of Food and Drug, University of Parma (IT)

WHEN UNIONS MAKES FORCE: FIGHTING BACTERIAL COMMUNITIES HARBOURING PATHOGENS IN THE FOOD INDUSTRY

As in human societies, bacteria are able to associate in the environment of the food industry forming multispecies biofilms. These structures allow certain microorganisms, using different mechanisms, to collaborate among them consequently developing special features such as an increased tolerance to antimicrobials or even the capacity to remain invisible to detection systems. This is especially relevant when such organisms are foodborne pathogens such as *Salmonella sp.* or *Listeria monocytogenes.*

To overcome this issue of concern, over the last years, food microbiologists' research has permitted to develop various advances in the field of food safety by gaining further insight into the factors that affect the adhesion and maturation, and the generation of antimicrobial tolerance among multispecies biofilms.

The presentation will be aimed to give a general perspective about the recent advances in the study and methodology for environmental pathogen control in the food industry.

Erdogan CEYLAN

Director of Research, Mérieux NutriSciences (US)

ENDEMIC POPULATION OF LISTERIA MONOCYTOGENES THROUGH FORMATION OF BIOFILM AND ITS PERSISTENCE IN FOOD-PROCESSING ENVIRONMENT

The number of outbreaks and recalls associated with *Listeria monocytogenes* is increasing despite the implementation of food safety and sanitation programs in the food industry. Poor sanitation practices are often cited as the root cause of *L. monocytogenes* contamination. Due to its ability to resist harsh environmental conditions and to form biofilm, *L. monocytogenes* can persist in food processing facilities and equipment surfaces for many years. Once formed, biofilms are hard to eliminate and act as a potential source of transmission of foodborne pathogens. Persistent strains of the pathogen are not restricted to a particular food sector or product. These strains may adapt to the inhospitable conditions, and develop resistance to sanitizers, disinfectants, UV light and desiccation, or persist in harborage sites that are not effectively disinfected. *L. monocytogenes* can become endemic within processing environments by suboptimal application of biofilm eradication practices. Persistent strains are the major reason for the contamination of food products after they are processed.

John LAROCHELLE

CEO, Environmental Infection Prevention (US)

LATEST INNOVATIONS RELATED TO BIOFILM DETECTION AND ELIMINATION

There is an understanding that biofilms are omnipresent in the environment. However, there is an underappreciation of where they can develop, and what the harms of these niche environments can have for those present. Efforts towards understanding biofilm development in our environment has increased in recent years and has led to new strategies to mitigate this challenge. There is an increasing focus on floors, drains and sink traps, given their role in pathogen transfer and role in colonization. Sinks have especially been highlighted, as they have been shown to be a significant reservoir for multidrug resistant organisms, and are at the nexus between dirty and clean; a perfect situation for the transfer of pathogens back to the patient. Current research efforts by The Infection Prevention Strategy (TIPS) and InfectionControl.tips in collaboration with William Osler Healthcare in Canada are identifying significant pathogens and decontamination methods with that address the blight of multidrug-resistant organisms. Many technologies, including ultraviolet irradiation, chemical disinfectant and infection control-based engineering are showing much promise in the realm of pathogen reduction and prevention. We will navigate the available infection control options, as well future technologies that show promise to mitigating this threat.

THE ROLE OF ENZYMATIC CLEANING IN ENVIRONMENTAL PATHOGEN CONTROL

John BUTTS

President, FoodSafetyByDesign LLC (US)

"Microbiologically Clean" is the key operative phrase for pathogen control. Experience teaches up this is not just for contact surfaces. Microbiologically clean refers to the exposed product environment. Vectors are continuously moving organisms of concern as well as spoilage bacteria along pathways towards product and contact surfaces. Experience also tells us if we start dirty, the process will continue to operate in that state. Incidental contamination of a transfer point along a pathway can be wiped clean over time. This is not necessarily true when transfer points bear biofilm formation. Biofilm is the "gift that keeps on giving". Unfortunately, the food industry is not blessed with an abundance of equipment and facilities that would have high scores from a sanitary design perspective. Legacy equipment and facilities dot our landscape. These legacy assets must be cleaned and sanitized to the "microbiologically clean state" during each sanitation cycle. The task is complicated with difficult to disassemble equipment and inaccessible facility areas. An additional complication is a shortage of sanitation labor in manufacturing facility areas. Enzymatic cleaning helps manufactures over come some of the risk associated with these ongoing business challenges.

BIOFILM CONTROL: FROM CHEMICAL TO BIOLOGICAL ARSENAL?

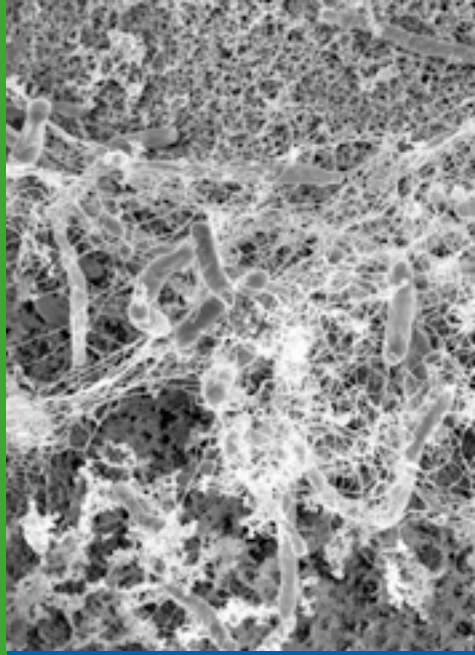
Chris GRIFFITH

Hygiene Consultant and Emeritus Professor at University of Wales (GB)

Foodborne diseases, although theoretically easily preventable, remain a major global health and economic problem. Analysis of causative risk factors indicate that cross contamination, although likely to be underestimated, is a major concern. Factors influencing cross contamination, including the role of surface contamination are examined. Microbial survival and the concept of "transient and resident organisms" are explored and the importance and influence of biofilms discussed. Although a precursor to biofilm control there is no single perfect solution to surface testing or biofilm detection and individual and integrated approaches are compared. This is linked to "what information you may want, or need, to know ".Several case studies are analysed and the implications for the Italian food industry are discussed

IBS International Biofilm Summit

In Collaboration with CIBUSTEC



PARMA

OCTOBER 23 - 24

2019