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Biofilms of foodborne pathogenic bacteria: how important are they?

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ABSTRACT

Biofilms are recognised as an important mode of life in bacteria. All species of foodborne bacterial pathogens are known to form biofilms *in vitro* under the right growth conditions. This fact is often extrapolated to claim that biofilms are critical to the transmission of foodborne pathogens, particularly during processing. While this may be the case little direct *in situ* evidence, with some exceptions, is available to confirm this. This is because there are a number of difficulties in studying pathogen biofilms in food processing facilities. The reasons for these issues are discussed by comparison to work in the medical biofilm area, and by using species such as *Listeria monocytogenes* and *Campylobacter jejuni* as examples. A range of potential solutions and avenues for future research are presented.

Keywords: biofilms, *Campylobacter*, foodborne pathogens, food processing, industry, *in situ*, *Listeria,* pathogenic *E. coli*.

Introduction

Biofilms are generally defined as a community of microorganisms attached to a surface or interface, and to each other, and encased in a matrix that they have produced. The matrix consists of extracellular polysaccharides, proteins and extracellular DNA and provides protection from the environment, a nutrient trap and facilitates interaction between cells.[1] Estimates suggest that upward of 40% of prokaryotic life exist in biofilms confirming the importance of this mode of life and the need to understand it better in a range of contexts.[2]

It is widely asserted that biofilms are critical to the ability of bacterial foodborne pathogens, such as *Listeria monocytogenes*, *Salmonella enterica*, pathogenic *Escherichia coli* and thermophilic *Campylobacter*, to move through the food system (particularly during processing) and cause human disease.[3] For this reason, studies investigating biofilm formation by single strains or collections of foodborne pathogenic bacteria in model systems, some mimicking those seen in food processing, are legion (without identifying specific papers a brief search of any database will confirm this). A high proportion of these studies use a micro-titre plate-based (polystyrene) crystal violet assay to quantify the biofilms, sometimes under a range of incubation conditions. In these cases the bacterial strains used (or most of them) demonstrate an ability to form biofilms in the model system. Often conclusions are drawn about their capability (or potential capability) to use biofilms to survive, persist and transmit in food-related environments. Rightly the caveat that further investigations need to be conducted, ideally *in situ* during food production, before any strong conclusions can be drawn is sometimes included. Unfortunately, aside from providing evidence of the wide distribution of the biofilm formation trait, these studies often contribute very little to our understanding of the role of foodborne pathogen biofilms in the food system. The reasons these studies fail in this regard, the difficulties in conducting more relevant studies and possible solutions to this are discussed below.

Why is it difficult to study foodborne pathogenic biofilms *in situ*?

In primary or further processing food facilities, particularly those processing high risk foods, the presence of pathogens is generally monitored for daily using swabs and/or product samples. For example, *Listeria monocytogenes* is monitored for in a range of small goods, dairy, fish, and poultry production facilities. In many of these which produce ready-to-eat largely untreated foods, such as fresh fish, the concerns around biofilms of

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this (and other) pathogens are clearly greater than in others, such as dairy. If positives are encountered corrective actions are taken which may entail stopping production, tracing back potential contamination in raw ingredients and implementing extensive additional cleaning protocols.[4] Product recalls may also ensue. Often these actions will resolve the problem and production will begin again. In the case of *L. monocytogenes*, only if the problem persists and the same strain reoccurs will consideration be given to the potential of a biofilm reinfecting the plant. In most cases tracing the source of the potential biofilms is difficult and doing so requires dismantling equipment, stopping production and specialist testing. In many cases the strain of concern cannot be traced, and *L. monocytogenes* biofilms cannot be confirmed.[5] In the case of other pathogens the situation may be more difficult with single persistent strains not generally the only cause of contamination. A further complication is apparent in that a wide range of potential bacteria can and do form biofilms in processing facilities, including on floors and walls, and in drains, which create a lot 'noise' in locating the pathogen of concern.

A comparison to what may be entailed in establishing the presence of biofilms on a medical catheter, implants or on teeth can give us insights into the issues with food-processing facility related biofilms. Generally, an infection in a patient alerts clinical staff to a potential issue. In the case of catheters or teeth, for example, they may be removed providing a ready source of material to investigate *in situ* biofilms using microscopy and disruptive sampling for molecular techniques. In the case of implants the relatively sterile interior of the human body often means only a single strain or species will form biofilms and cause issues and this strain can be isolated. These approaches can establish without a doubt that a biofilm is contributing to their persistence.[6] In food production only a limited number of foods, such as heat processed dairy products, may provide similar scenarios. However, while heat resistant spore forming spoilage bacteria, such as *Geobacillus*, can be categorically shown to form biofilms in dairy processing pipes,[7] pathogenic bacteria are generally not a major issue in these systems and are controlled by the heat.

The above scenarios highlight the key issues in establishing a role for biofilm formation by foodborne pathogenic bacteria *in situ* during processing. Namely, (1) the availability of samples of equipment and infrastructure to investigate the categorical presence of pathogen biofilms; (2) the complexity of the microbiological populations during processing and the ability to identify pathogens among other species; and (3) the difficulty of establishing if pathogens are part of mixed-species biofilms or simply adhering to them as they might to other surfaces.

What are the potential solutions to conducting more relevant studies?

To establish the importance of biofilms in bacterial foodborne pathogen transmission, and particularly during food processing, some systematic changes in the way they are investigated are required.

The first of these changes is conducting *in vitro* studies which provide information on the ability of pathogens to form biofilms under conditions more relevant to food-related environments. For example, numerous studies examine biofilm formation by *Campylobacter jejuni* at 37°C or 42°C in microaerobic environments in monoculture using micro-titre plates and draw conclusions about their importance in processing. This is not really useful as *C. jejuni* is very unlikely to encounter these conditions in the processing environment.[8] Studies in air, at ambient processing temperatures, and together with other bacteria that form biofilms suggest that *C. jejuni* is far more likely a 'passenger' on surfaces and other biofilms than an active biofilm former or participant in the community.[9]

The second is the wider introduction of in-processing biofilm sampling equipment. Some studies have been conducted by adhering, for example, stainless steel slides onto equipment or in drains which are then removed at particular times. A more satisfactory approach is the design of equipment and infrastructure with removable and replaceable areas or sections which can be routinely monitored as 'sentinels' for the presence of biofilm formation and in particular pathogens forming or associated with the biofilms. The potential for in-line real-time monitoring of biofilms through digital means is a reality in some plants and situations but generally does not indicate the presence or absence of pathogens.[10]

The third is the development of markers for biofilm formation. Differentiating cells that are simply present or transitory from cells that have been growing in a biofilm is critical to understanding the broader role of biofilm formation in foodborne pathogen transmission. This is an area which is receiving a lot of attention in the medical biofilm sphere and in which little work has been conducted in the foodborne pathogen space. The presence of extracellular molecular components produced only in the biofilm matrix, including polysaccharides or extracellular DNA, using mass spectroscopy or other methods, for example, may represent a way to assess if cells are part of, or have been recently associated with, biofilms. Other potential options may include the presence or absence of flagella that are switched on or off in a biofilm. This approach is in its infancy but is likely to grow in importance as techniques for detecting molecules evolve.[11]

The fourth approach is to develop a better understanding of the relationship between foodborne pathogenic bacteria and non-pathogenic microbes that are strong biofilm formers. As indicated above, biofilms in most food processing facilities are unlikely to be monocultures and the complex biofilms that form in drains, for example, may provide environments that allow pathogen biofilm formation. An example of one such group of organisms of wide interest in this context are the pseudomonads. Psychrotrophic *Pseudomonas* species can form extensive biofilms on surfaces and on food themselves. They may also provide environments conducive to the survival of foodborne pathogens such as *C. jejuni*.[9] However, what is not clear is how they interact with pathogens at a physical and molecular level and how this impacts their survival. The advent of 'omics' technologies and more sensitive molecular detection techniques will allow a better understanding of these interactions and provide possible mechanisms to manipulate them to the positive.[12]

Conclusion

In short, the answer posed to the question in the title is that in most cases, with some exception, we don't know. What we do know is that, unsurprisingly, most if not all foodborne bacterial pathogens can form biofilms and occur in processing facilities. We need to move on from re-establishing this to work on understanding if and where biofilms play a role in individual pathogen/food processing combinations. This requires not only a better understanding of biofilms *in situ* but also closer cooperation with industry, both of which have their own challenges.

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Biography

Gary Dykes is an Honorary Professor in the School of Agriculture and Food Sciences at the University of Queensland and runs his own consulting business. His research interests in survival, persistence and control of foodborne pathogenic bacteria with a focus on surface attachment and biofilm formation by *Campylobacter* and *Salmonella*.