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Editorial

Role of Biofilm and Protozoa in the Ecology of Pathogenic Bacteria of Food and Environmental Origin

Patrizia Messi*

Department of Life Sciences, University of Modena and Reggio Emilia, Italy

***Corresponding Author**: Patrizia Messi, Department of Life Sciences, University of Modena and Reggio Emilia, Italy.

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The biology of the most environmental and food-borne pathogens is well known, but the mechanisms that allow them to adapt and survive in different environmental conditions are not well understood yet. Among these, biofilm formation and internalization in protozoa are two important strategies that make bacteria more resistant than their planktonic counterpart to environmental stress conditions, such as those that may occur during the water sanitization process and food production cycles. Biofilm is a functional consortium of microorganisms attached to a surface and enclosed in a matrix predominantly made up of polysaccharide material [21]. Microbial biofilms are characterized by an enhanced resistance to sanitizers, disinfectants and antimicrobial agents, and are responsible for the increased environmental spread of the entrapped bacteria [9]. Some studies have shown the persistence of some water-borne and food-borne pathogens on drinking water systems and on food contact surfaces [16,41]. In both cases, multispecies biofilms, often thicker and more stable than monospecies, may increase the possibility for entrapped pathogens to better survive [8,29]. The persistence and accumulation of germs, as biofilms, could be a source of subsequent contamination, leading to an easier transmission of pathogens to humans. Another problem is that inside the biofilm bacteria, protected from esopolysaccharide (EPS) matrix, exchange more easily the resistance factors and other biological characters responsible for virulence and biological advantage [24,27]. It is well established that the resistance to antibiotics [11], to biocides and heavy metals are biological characteristics highly expressed and transferable in biofilms. Biofilm formation is a complex and dynamic process that involves a series of steps. Firstly, the adsorption/attachment of cells to a surface with weak chemical bounds and, successively, the adhesion become more tight due to the presence of fimbriae, flagella, and to the secretion of exopolymer [18,42]. Other microorganisms are then entrapped and acquire the ability adhere to substrates. The last colonizers of this microbial structure are protozoa and, in natural communities, where microorganisms live in high cell density (as it happens in biofilms), these predators can easily feed on the bacterial entrapped in the community. It is known that, even if the predator-prey relation represents the normal feeding way of amoebae, bacteria can establish a positive interaction that allow them to survive and even multiply within the intracellular environment of certain protozoa. These bacteria may avoid digestion by escaping from fagosoma before fusion with lysosomes or resisting to the cellular mechanisms of digestion in phagosomes, and escape into the host cell cytoplasm where they can multiply [6,13]. Some protozoa such *A. castellanii, A. polyphaga* and *T. pyriformis* produce small vesicles containing viable bacteria, therefore representing a risk of infection to man by inhalation and of environmental and food contamination. *Acanthamoeba* spp. in particular, may act as environmental reservoir or "Trojan horse" of bacterial pathogens and this behaviour has been suggested to be a key step in the capacity of many bacteria to produce human infections [5]. For *Legionella pneumophila* it is well documented the contribute of protozoa for its survival and virulence, especially free-living amoebae. Rowbotham [30] was the frst to describe the ability of *L. pneumophila* to multiply intracellularly within protozoa and the host–parasite interaction has been shown to be crucial in the pathogenesis and ecology of this pathogen. Other water bacteria such as *Burkholderia cepacia, Burkholderia pseudomallei, Aeromonas* spp., *Pseudomonas aeruginosa* and other pigmented *Pseudomonas* species, frequently endowed with virulence characteristics responsible for environmental selective pressure [2,20,22,26,28], have been reported to survive within protozoa. There is also growing evidence that protozoan grazing is one of the critical ecological factors controlling the abundance of several food-borne pathogens in the environment, microorganisms that can be transmitted to human populations and determine outbreaks and epidemics [1,10,12,15,19,32,35,36]. Important food-borne pathogens like *Shigella* spp., enterohemorrhagic *Escherichia coli* and *Campylobacter jejuni* require a very low infective dose [4,7], on the contrary, other pathogens, as *Vibrio cholera*, require a large number of cells to successfully infect a host [33]. The latter can benefit from a biological reservoir in which to grow to high concentrations, and free-living amoebae may play an important role as reservoirs, vectors, and an effective hosts, where the pathogen can easily increase its microbial load. Other widespread and important food-borne bacteria, as *Salmonella enterica, Yersinia enterocolitica, Listeria monocytogenes, Staphylococcus aureus* are capable to survive and multiply in protozoal hosts [3,15,17]. In all cases, deriving endosymbiotic relationship is beneficial, allowing both partners to survive and take advantage by using nutrients (catabolites excreted, remains of dead bacteria, etc.), developing new characteristics and adaptating to new environments [14]. Free-living amoeba, such as *Acanthamoeba* spp., are ubiquitous and are frequently isolated from natural aquatic systems, soil, reptiles, and human intestine [34,38]. They have also been isolated from spinaches and fresh salad vegetables [12,31], from meat-cutting plants [39,40] and fish [37]. So, given the documented environmental distribution of protozoa and the ability to act as host and to protect certain environmental and food-borne pathogens, the association with free-living amoebae, besides other strategies such biofilm formation, may be another way by which some bacteria may persist in water distribution systems and food processing plants and may contaminate water and food, despite of sanitization procedures. The role of protozoa as a reservoir for the maintenance of pathogenic bacteria and as possible vectors for the transmission of human and animal disease is plenty recognized, but information about the interaction between these organisms and their amoebal hosts at a cellular level is limited. Some of the bacteria that survive and replicate within protozoa are also facultative or obligate intracellular pathogens of human macrophages. There is some evidence to support the notion that infection of amoebae and infection of macrophages has a common molecular basis, and the ability of some bacterial species to cause human disease might be a consequence of an evolutionary selection for intracellular growth and survival within environmental protozoa [5,25]. Adaptation of bacteria to survive within amoebae may also contribute to their virulence, making these pathogens capable of infecting human cells. The relationship between virulence and intra-amoebal replication capability was demonstrated by Molmeret., *et al.* [23], who studied the growth kinetics within *Acanthamoeba* of two clinical isolates of the same *L. pneumophila* strain. A peculiar variation in growth rate was observed, as the isolate recovered from the dead patient grew rapidly in amoeba, while that isolated from a surviving patient failed to grow at all, results also supported and confirmed by molecular analysis. In conclusion, the diffusion of bacterial pathogens and their transmission to humans are a biological processes influenced by many factors, including the physiology of the microorganism and the mechanisms used for surviving in an often extra-cellular hostile environment. Actually, the researcher's interest is focused on the importance of microbial community causing the persistence of pathogens in the environment, since within structures like biofilm or within protozoan hosts can take place a series of biological interactions which may have significant implications for human health. Biofilm and free-living protozoa are now recognized as critical sources for the dissemination of various pathogens by providing an idoneous shelter for multiplication. Therefore, to implement new knowledge on the role of biofilm and protozoa in the diffusion of pathogens and to better understand the role of such interactions in the epidemiology of some infectious diseases, it is advisable to investigate the mechanisms that promote or hinder the formation of biofilm on several environments. For new future prospectives in developing new and more efficient sanitation/preservation strategies, studies on the interaction between pathogens of

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