Review paper
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BACTERIAL PERSISTENCE AND TRANSIENT SURVIVAL ON OPEN SURFACES

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Abstract

It is now well admitted that cleaning and disinfection (C&D) do not allow the removal of all micro-organisms from open surfaces even when very aggressive procedures are applied and even on very hygienic materials as stainless steel. We observed in a meat site on a stainless steel piece of equipment after C&D that one to 10 CFU/cm^2 were detected on agar medium while viable but not culturable cells (detected by ethidium monoazide real time qPCR) ranged from 10^3 to 10^4cells/cm^2 and total cells (detected by qPCR) ranged from 10^3 to 10^5 cells/cm^2.When considering pure bacterial cultures, a subpopulation of bacteria adheres on surfaces and from this subpopulation a fraction resists to disinfection or shows high attachment strengths. However, those laboratory findings were obtained with low concentration of cleaning and disinfecting products without mechanical action as applied on processing surfaces. By contrast, when applying industrial procedure on materials experimentally contaminated, it is difficult to find surviving CFUs on surfaces.

Such conflicting observations allow raising several hypotheses to help understanding why cells are not eliminated from food processing surfaces in food industry premises or equipment. Here we will discuss two possible phenomena: persistence and transient survival.

Key words: *Cleaning, disinfection, bacterial persistence, harborage sites*.

1. Introduction

It is now well admitted that cleaning and disinfection (C&D) do not allow the removal of all micro-organisms from open surfaces (Gibson *et al*. [1], Mettler and Carpentier [2]).

Overall industrial C&D allows around 1 decimal reduction of the aerobic mesophilic culturable bacteria (Gibson *et al*. [1]) and this raises the question of whether bacteria remaining after C&D are persistent or transient survivors. We will here first describe laboratory studies aiming at understanding the behavior of micro-organisms on surfaces. Field results giving some element to answer our question will then be presented.

2. Behavior of microorganisms on surfaces

Population heterogeneity

When a bacterial suspension comes into contact with a surface, only some of the cells are able to adhere. For instance, after deposition of a bacterial suspension of *Listeria monocytogenes* on a glass slide the number of attached cells stopped increasing after 2.5 hours and then accounted for only 1% of the suspended cells (Sommer [3]). When an attached community of bacterial cells is submitted to C&D only a few of them are able to resist (Figure 1). This fraction of surviving cells is smaller when disinfectant concentration increases and is greater for old attached communities (Frank and Koffi [4]). Such fractions also exist in bacterial suspensions but are markedly smaller which is why attached cells are considered to be more resistant than suspended cells including cells suspended after detachment. This minority subpopulation consists of cells which are referred to as "persister cells" by Lewis [5]. Persister cells are phenotypic variants which appear frequently but in an unpredictable manner, which means a great variability between results from independent experiments for the number of cells surviving disinfection. Consequently there has to be a sufficiently high number of initial attached cells for there to be any chance of finding "persister cells" after disinfection when applied at the recommended concentrations (Marouani-Gadri *et al*. [6]). The initial number of cells, necessary to ensure any certainty of some cells surviving, varies greatly according to

the bacterial species and strains and depends on the attachment strength and intrinsic resistance to disinfection of the microbial strain considered. Heterogeneity of subpopulation is also observed when considering attachment strength of bacteria as illustrated by Figure 2. We showed that culturable cells surviving disinfection appear to be both highly resistant and highly "sticky" to surfaces (Midelet *et al*. [7]). All those results show that studying the behavior of a suspended bacterial population, as done for instance by the European suspension test to assess the efficacy of disinfectants, gives a view quite different from what is really happening on industrial surfaces.

Experimental achievement of a bacterial persistence

When submitting a surface contaminated once with a bacterial culture to a daily chemical treatment with cleaning and disinfecting products and a daily soiling with organic matter, it is possible to make the bacterial strain persist on the surface (Marouani-Gadri *et al*. [6] and Peneau *et al*. [8]). Such studies have been conducted with C&D product(s) applied at concentration lower than those recommended and without mechanical action to model what happens in hard-to reach places. As regards pathogenic bacteria such as *E. coli* O157:H7, *Salmonella*, *L. monocytogene,* the microbial load first begins to decrease but after a few days in some conditions (sufficient initial bacterial load, sufficient temperature) bacterial counts does increase. This is the result of an increase in resistance to bactericidal concentration of disinfectant and of bacterial adaptation allowing growth to resume and to produce more cells than the amount removed by the chemical treatment applied (Carpentier and Cerf [9]).

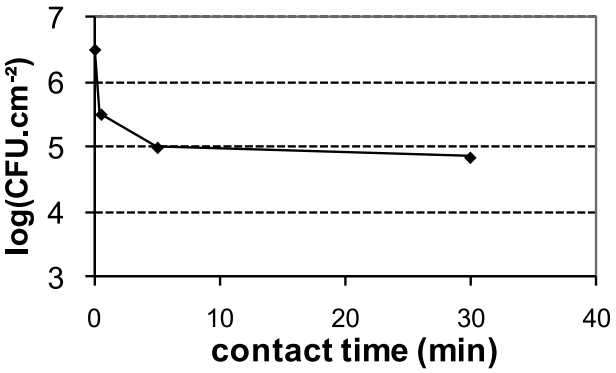

Figure 1. Survival of *Listeria monocytogenes* attached cells submitted to 0.001% didecyl-dimethyl-ammonium chloride (Carpentier and Chassaing, unpublished results)

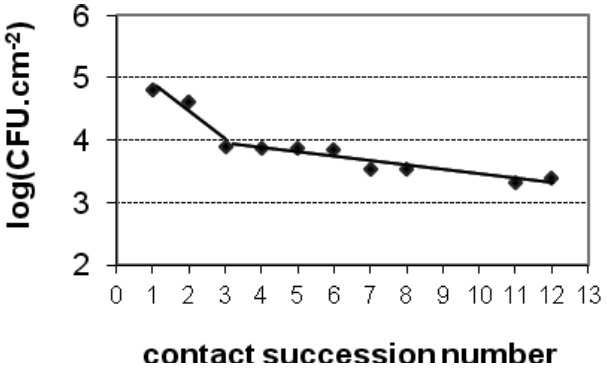

Figure 2. Detachment by contact with an agar medium of a *Pseudomonas fluorescens* biofilm (Midelet *et al*. [7])

Field study

Three surveys of the bacterial ecosystem of food contact surfaces in a cutting room of a bovine meat processing site were conducted in 2009 and 2010. After very aggressive C&D procedures, we counted 1 to 10 CFU/cm^2 on stainless steel and a mean of 400 CFU/cm^2 on a conveyor belt made of polyvinyl chloride. Three hundred and twenty isolates were identified showing a diversity of more than 50 genera and confirming the dominance, already described in the literature, of *Staphylococcus* and *Pseudomonas* genera among culturable bacteria. Isolates from those two genera were then sub-typed by Rep-PCR to detect potentially persistent strains. Thirty six rep-PCR types were identified among the 41 *Pseudomonas* isolates but no types were found at more than one survey, suggesting that none of them were persistent in the processing plant. By contrast, among the 37 *Staphylococcus* isolates 18 rep-PCR types were identified. Three types were found in two surveys either at the second and third survey or at the first and second survey or at the first and third survey. This suggests that only the latter strain among those isolated persists on a long term basis in the processing plant. This study shows that dominant bacteria from the processing surfaces of the meat plant studied are not an "in house" flora as previously thought and that transient survival is there far more common than persistence.

3. Conclusions

Presence of bacteria after C&D is likely due to the presence of a low number of cells that are highly resistant to detachment and to disinfectants. However, when C&D are correctly applied, those cells are progressively eliminated and persistence does not occur frequently. Therefore we assume that the population that resides on surfaces is likely composed of strains that are constantly renewed.

4. References

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