The relationship between initial adhesion and biofilm formation by drinking water bacteria

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In the majority of ecosystems, microbial cells grow in association with surfaces leading to the formation of highly structured sessile microbial communities, called biofilms. Intentional and unintentional biofilms concern a broad range of areas; however, they require particular attention in the industrial/environmental and biomedical areas. Drinking water systems are known to harbour biofilms, even though these environments are oligotrophic and often contain a disinfectant. In drinking water distribution systems, microbial adhesion will initiate biofilm formation, exacerbating contamination of drinking water, reducing the aesthetic quality of potable water, increasing the corrosion rate of pipes and reducing microbiological safety through increased survival of pathogens. The development of a biofilm is believed to occur in a sequential process that includes transport of microorganisms to surfaces, initial microbial attachment, formation of microcolonies, extracellular polymeric substances (EPS) production and biofilm maturation.

This study was performed in order to assess the relationship between the initial cell attachment process and the subsequent biofilm development using drinking water isolated bacteria belonging to six different genera and representing more than 80 % of the total genera isolated and identified. The bacteria used were *Acinetobacter calcoaceticus*, *Burkholderia cepacia*, *Methylobacterium* sp., *Mycobacterium mucogenicum*, *Sphingomonas capsulata* and *Staphylococcus* sp. Some of this isolates (*B. cepacia*, *M. mucogenicum* and *Staphylococcus* sp.) are recognized as problematic opportunistic bacteria. Adhesion was allowed to occur for 2 h, while biofilms were developed during 72 h. Polystyrene (PS) surface was used as substrata. In both cases, bacteria were characterized in terms of mass and metabolic activity using specific dyes, crystal violet (CV) and sodium 3,3'-[1[(phenylamino)carbonyl]-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene sulfonic acid hydrate (XTT), respectively. It was found that the tested bacteria adhered and formed biofilms on the selected surface, showing signs of metabolic activity. *Staphylococcus* sp. adhered strongly to PS, while *Sph. capsulata* adhered in a small extent. Analyzing bacteria adhesion, using an increasing gradation, it is found the following sequence: *Sph. capsulata* < *M. mucogenicum* < *B. cepacia* < *Methylobacterium* sp. < *A. calcoaceticus* < *Staphylococcus* sp. Biofilm mass results revealed that *Methylobacterium* sp. formed biofilms with a higher mass amount and *B. cepacia* developed low mass amount biofilms. An increasing gradation for biofilm formation ability provides the following sequence: *B. cepacia* < *Sph. capsulata* < *Staphylococcus* sp. < *A. calcoaceticus* < *M. mucogenicum* < *Methylobacterium* sp. In some cases (*A. calcoaceticus* and *Staphylococcus* sp.), the metabolic activity was determined to be inversely related to biomass increase, in terms of cell number or biofilm. The results demonstrate that assays evaluating initial adherence to PS cannot predict the best biofilm-forming drinking water bacteria and final biofilm characteristics, proposing that other events following adhesion (cell-cell interactions, bacteria biochemical and genetic characteristics, EPS production and other biofilm-related features) may play a remarkable role on biofilm formation and differentiation.

Keywords: bacterial adhesion; biofilms; drinking water bacteria