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ACINETOBACTER CALCOACETICUS PLAYS A BRIDGING FUNCTION IN DRINKING WATER BIOFILM FORMATION

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Biofilm formation in drinking water distribution systems improves drinking water contamination by reducing the microbiological safety through the increased survival of pathogens. The knowledge of the main mechanisms promoting drinking water biofilm formation is of great interest as it can contribute to their understanding and control. The development of microbial biofilm communities results from a series of processes, including initial surface-association and adherence, subsequent multiplication of the constituent organisms, adherence of other species; and production of extracellular polymeric substances. Many of these events are well described. Bacterial surface properties, coaggregation and coadhesion along with inter-species relationships are processes that are believed to play a determinant role in the formation of multispecies biofilms in drinking water distribution systems. Nevertheless, the function of coaggregation in the initial development of drinking water biofilms and the role of each bacterium in biofilm community still remain unclear. The purpose of the present study was to investigate the intergeneric coaggregation of six heterotrophic bacteria (*Acinetobacter calcoaceticus*, *Burkholderia cepacia, Methylobacterium sp., Mycobacterium mucogenicum, Sphingomonas capsulata* and *Staphylococcus sp.*), identified by 16S rRNA gene sequencing and isolated from a laboratorial drinking water distribution system, by a visual coaggregation assay, scanning electron microscopy and epifluorescence microscopy using DAPI staining. The surface-associated molecules involved in coaggregation process were also investigated by heat and protease treatment, and by sugar reversal tests. The role of the isolated *A. calcoaceticus* strain as bridging organism in drinking water biofilms was assessed by multispecies biofilms experiments, through a strain exclusion process, using a microtiter plate technique by means of CV staining to assess biofilm mass.

A. calcoaceticus, was found not only to autoaggregate, but also to coaggregate with four of the five other isolates (the exception being *Methylobacterium* sp.) to different degrees as assessed by the visual assay, highlighting a possible bridging function in a biofilm consortium. In its absence, no coaggregation was found. Microscopic observations revealed a higher degree of interaction for all the aggregates than did the visual assay. Heat and protease reversed autoaggregation and coaggregation, suggesting that interactions were lectin-saccharide mediated. The bridging function of *A. calcoaceticus* was evidenced by multispecies biofilm studies, through a strain exclusion process.

The overall results demonstrated that *A. calcoaceticus* plays a bridging function in drinking water biofilm formation. This bacterium coaggregates with almost all other tested bacteria and their presence in a multispecies community represents a colonization advantage. Probably, this bacterium may facilitate the association of the other species which do not coaggregate directly each other, increasing the opportunity for metabolic cooperation. The presence and absence of *A. calcoaceticus* in multispecies biofilms can, therefore, enhance or decrease, respectively, biofilm formation by drinking water bacteria.