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## **COMPLEX AUTOCHTHONOUS BIOFILM COMMUNITY LIMITS THE INVASION OF A PURE CULTURE OF *AQUABACTERIUM* SP. AT VARIOUS CONCENTRATIONS**

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Invasion and colonization of an autochthonous biofilm community by invading microbes might alter the overall ecosystem functioning. Such changes could be positive when a desired process is wanted to be enhanced (e.g. bioaugmentation), or negative if a pathogen implements in beneficial biofilms. The success of invasion appears to depend on several factors such as competition for resources, diversity of the autochthonous community, the number of invaders and predation.

The aim of this study is to test the invasion of mature and early stage complex biofilms by various concentrations of an allochthonous microbial strain, *Aquabacterium* sp., and the putative effects on the autochthonous biofilm community structure. A mature biofilm was developed on coupons attached to stainless rings inserted into two bubble columns reactors (5 L) which were operated in continuous mode for 35 days with a fixed hydraulic retention time of 65 minutes. After 15 days of operation, additional clean coupons were inserted as virgin surfaces for the development of early stage biofilm. Simultaneously, a suspension of *Aquabacterium* sp. (500 ml) was injected to one of the two reactors. The second reactor was used as a control without the addition of an invading population. The experiment was done twice using two concentrations of *Aquabacterium* sp. (OD = 0.04 and OD = 0.5) corresponding to the injection of a total number of cells equal to  $1.6 \times 10^{10}$  cells and  $2 \times 10^{11}$ , respectively. The capacity of *Aquabacterium* sp. to attach and remain in the biofilm was then monitored by quantitative PCR using primers targeting the 16S rRNA gene. In the two experiments, *Aquabacterium* sp. was detected for 20 days in both mature and early stage biofilms. However, the number of invaders decreased sharply over time showing that the presence

of *Aquabacterium* sp. in the biofilm was only transient while it was displaced from the biofilm or taken up by predators. The overall microbial community structure of the biofilm was not significantly affected by the addition of *Aquabacterium* sp., as similar PCR-SSCP profiles were observed before and after the addition of the invaders, and in comparison with the control reactor.

In conclusion, this study suggests that the number of invaders does not influence the colonization success. Also, the competition for space and nutrients of the highly diverse biofilm, even at an early stage, appears to limit the invasion of *Aquabacterium* sp..