Marine organisms represent a rather unexplored source of new activity and biological functions of molecules for biotechnologies. The research in chemical ecology of the marine environment leads to looking at metabolites of recognition or defence produced by these models. Marine bacteria belonging to the Pseudoalteromonas genus of the Gammaproteobacteria class are often found in association with marine eukaryotes, and their ability to produce a variety of biological activities attracted a particular attention. The marine Pseudoalteromonas sp. 3J6 and D41 were selected for their capacity to inhibit the biofilm formation of other bacteria. The study of antibiofilm metabolites synthesised by marine bacteria 3J6 and D41 biofilms can lead to the development of new anti-fouling compounds or applications in surface hygiene. The main originality of these compounds is to be specifically antibiofilm, they have no activity against planktonic bacteria.

A major objective of this project is to screen the activity spectrum and to characterise and identify these original compounds produced by the bacteria 3J6 and D41.

**Investigating the antibiofilm activity spectrum**

Two different approaches were experimentally used in biofilm studies:

- one method that quantifies the total biomass, the thickness, the architecture and the viability of a biofilm involving growth under flow conditions (Flowcell system, Fig.1), staining by Syto 61, Sytox and with observations in Confocal Laser Scanning Microscopy (Fig.2 and 3).

- another that allows the determination of the total biomass involving staining by crystal violet in a 96-wells microplate in static conditions (Fig.4).

The molecules produced by the bacteria 3J6 and D41 had a wide spectrum of activity since it affected all the Gram negative marine strains tested except other Pseudoalteromonas strains (Fig. 5). Biofilm biovolumes of the sensitive strains were reduced 3 to 530 fold and the percentages of non viable cells were increased 3 to 225 fold.

Interestingly, molecules produced by the bacteria 3J6 also impaired biofilm formation by three strains belonging to the human pathogenic species *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Escherichia coli*.

**Perspective : Identify the antibiofilm compounds**

The development of techniques and methods for molecules purification will be an essential step. The purification scheme chooses differents extractions (Fig. 6) and chromatography techniques (Fig. 7) with differents selectivities (hydrophobicity, size exclusion and ion exchange chromatography). All separation methods will be coupled with a MALDI-TOF-Mass Spectrometer (Fig. 8).

**Conclusion**

The anti-biofilm molecule produced by *Pseudoalteromonas* sp. 3J6 and D41 presents the originality of differing from other classical anti-bacterial compounds by its biofilm specific activity. Further experiments has been undertaken to purify and identify the anti-biofilm molecules secreted by these bacteria.