Abilities of *Alcanivorax borkumensis* to form biofilms and to degrade plastics

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Introduction

Because of its indispensable role in human life, plastic production increases every year. Most of plastic wastes are released in landfill or in the sea. These synthetic polymers have large ecological and health impacts. Nevertheless, plastics are colonized by microorganisms like bacteria, fungi and diatoms. This distinct environmental niche is called "plastisphere". In addition, it has been shown that a small part of these microorganisms are able to degrade plastics to sustain their growth. In this research, bacteria from plastic samples (Mediterranean Sea, Corsica) are cultivated with plastics in liquid medium with low concentration of carbon in a way to select bacteria able to degrade 4 types of plastics: Low Density PolyEthylene (LDPE), PolyEthylene Terephthalate (PET), PolyVinyl Chloride (PVC) and PolyStyrene (PS). Only one species characterises the majority of bacterial community on LDPE biofilms, this bacterium corresponds to *Alcanivorax borkumensis*, This genus is known to be able to grow on alkanes as sole source of carbon. Biofilm formation is the first essential step for plastic degradation. So, the ability of *A. borkumensis* to form a biofilm on 2 types of plastic (PET and PS) is studied and these biofilms are characterised according to the nature of the plastics and two sources of carbon (pyruvate or hexadecane).

Methods

Plastic samples → 

Mediterranean Sea, Corsica

→ 

Liquid medium
0.05% yeast extract +
1 type of plastic film
(PVC, LDPE, PET or PS)
140 rpm

→ 
Biofilm development

→ Biofilm structure analysis and study of signs of degradation (SEM)

→ Bacterial communities study (DGGE) and sequencing

Enrichment culture analysis

Only one species characterises the majority of bacterial community on LDPE biofilms, except one sample that contains another species. The first species corresponds to the bacterium *Alcanivorax borkumensis* and the second to *Microbulbifer* sp. (Fig.1). Moreover, some holes and cracks are observed on LDPE (Fig.2).

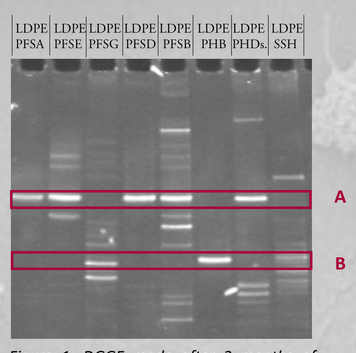

Figure 1: DGGE made after 2 months of enrichment culture on the LDPE. Boxes show bands corresponding to (A) Alcanivorax borkumensis and (B) Microbulbifer sp. which are enriched in the majority of biofilms on the LDPE.

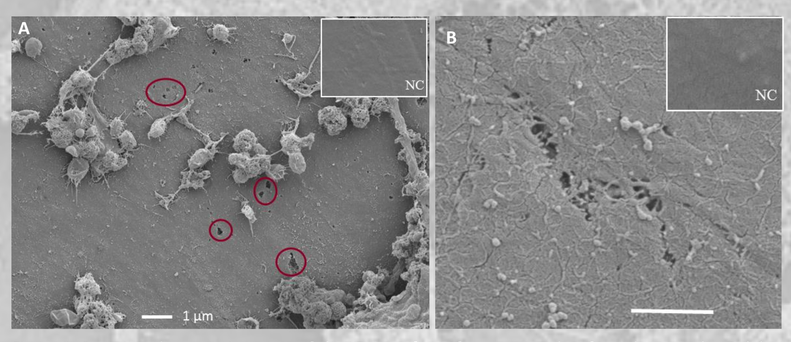

Figure 2: Scanning electron microscopy of the LDPE surface after two months of enrichment culture. (A) Biofilms on plastics, (B) plastic lacking biofilm. Holes and cracks present on the surface of the plastic are highlighted by circles, NC: Negative Control.

Growth curve of *Alcanivorax borkumensis*

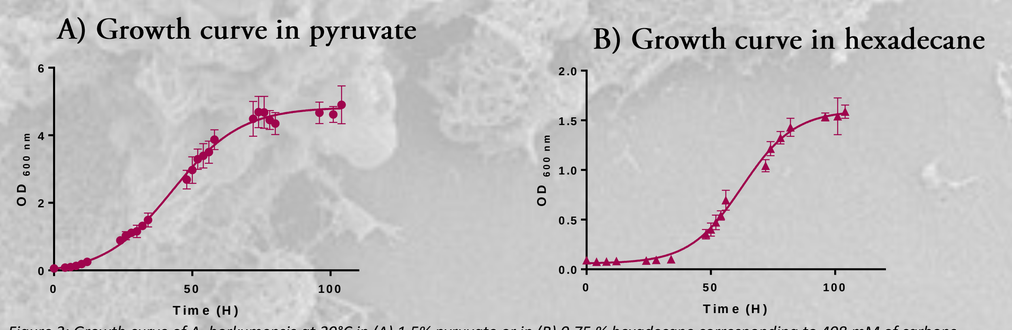

Figure 3: Growth curve of A. borkumensis at 30°C in (A) 1.5% pyruvate or in (B) 0.75 % hexadecane corresponding to 408 mM of carbons.

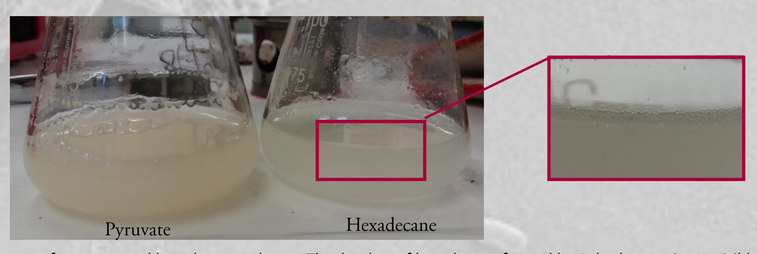

Figure 4: Pictures of pyruvate and hexadecane cultures. The droplets of hexadecane formed by A. borkumensis are visible.

A. borkumensis was isolated from enrichment culture. This bacterium was able to grow on hexadecane as sole source of carbon (Fig.3) and produced biosurfactant to interact with alkanes (Fig.4).

Quantification of biofilm formation on PS and PET

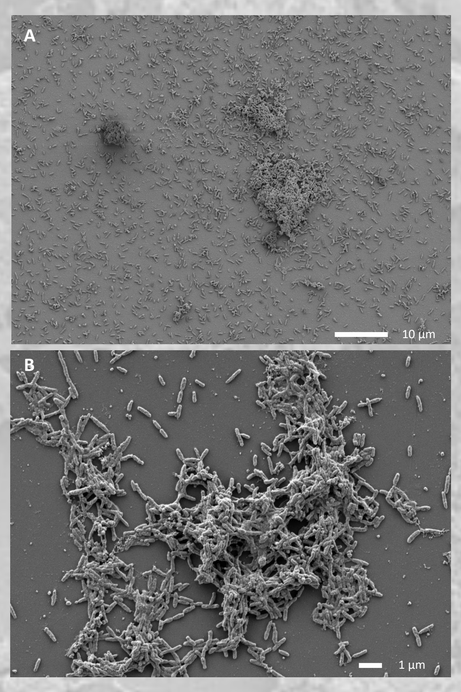

Figure 5: Scanning electron microscopy on biofilm (A) after 144H in hexadecane and (B) after 96H in pyruvate on PET. No differences in biofilm structure were observed in both media and on both plastics.

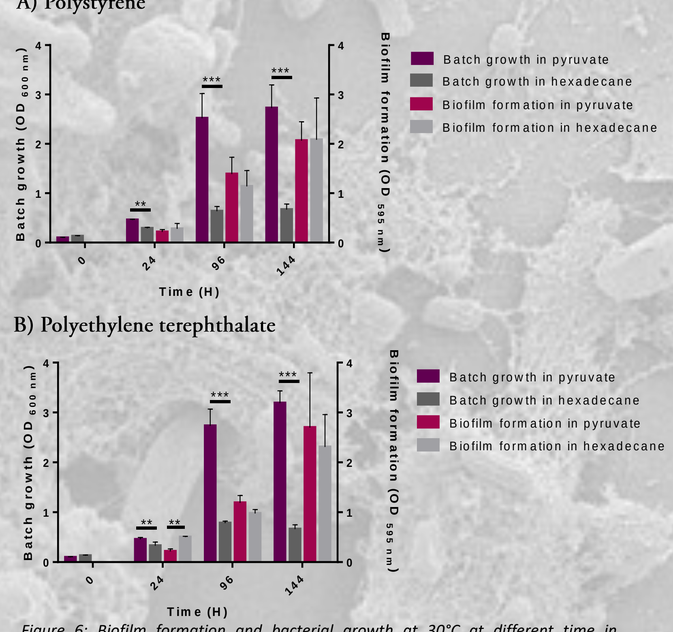

*Figure 6: Biofilm formation and bacterial growth at 30°C at different time in hexadecane or in pyruvate medium, (A) with polyethylene terephthalate films or (B) polystyrene films . The biofilm formation was assessed by crystal violet method at OD 595 nm and the batch growth was assessed by the OD 600 nm of the supernatant of the culture. (T-test; *, P < 0.05; **, P < 0.01; ***, P < 0.001)*

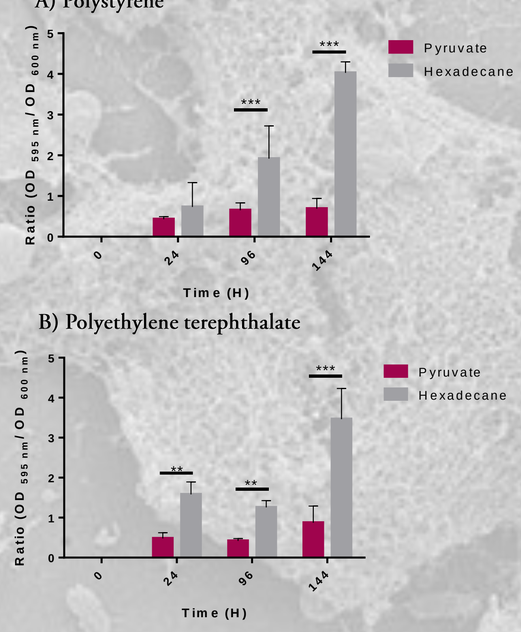

*Figure 7 : Ratio of biofilm formation to batch growth on (A) polystyrene and on (B) Polyethylene terephthalate. (T-test; *, P < 0.05; **, P < 0.01; ***, P < 0.001)*

A. borkumensis is able to form biofilm on plastic in presence of the 2 studied sources of carbon (Fig.5 and Fig.6). However, this bacterium grows more in the supernatant in the pyruvate medium than hexadecane (Fig.6). The ratio of the formation of biofilm on the bacteria present in the supernatant highlights the proportion of bacteria present in biofilm. Hexadecane medium significantly promotes biofilm formation on both types of plastics (Fig.7).

Conclusion

These two microorganisms are potential candidates for LDPE degradation. *Alcanivorax borkumensis* and *Microbulbifer sp.* are very interesting because they are known to degrade hydrocarbons and to degrade complex polymers respectively. The first one is able to form biofilms on plastic. The hexadecane medium promotes the formation of biofilm. Now, biofilm formation on LDPE and PVC will also be studied. Then, the ability of this bacterium to degrade the 4 types of plastics (PET, PVC, LDPE and PS) with or without pretreatment of UVs will be tested.

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