Alcanivorax borkumensis, a key player for the low-density polyethylene degradation

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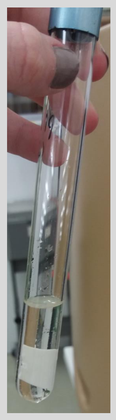
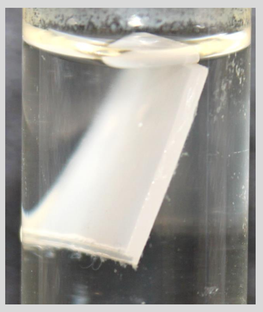
Introduction

Plastic production has increased every year over the last century. Most of these synthetic polymers are released either in landfills or in the sea. Once released in the environment, synthetic polymers are rapidly colonized by microorganisms such as fungi, diatoms or bacteria, which form a biofilm. Consequently, the term "plastisphere" is used to describe the environmental niche formed by these plastics. The bacterial communities of the plastisphere seemingly include bacteria capable of degrading synthetic polymers. However, the rate of degradation is very slow and the development of plastic-associated communities is still poorly understood. Previous work conducted by our lab described that *Alcanivorax borkumensis,* hydrocarbon-degrading bacterium, was strongly selected on Low-Density PolyEthylene (LDPE) after 2 months of enrichment culture as potential LDPE degraders. The aim of this study is to quantify the ability of this bacterium to form biofilm on plastics and its capacity to degrade synthetic polymer.

Methods

Plastic samples → 

Mediterranean Sea, Corsica →  Liquid medium 0.05% yeast extract + 1 type of plastic film (LDPE, PET or PS) 140 rpm →  Selection of *Alcanivorax borkumensis* on LDPE → Quantification of plastic biofilm (PET, LDPE, PS and PVC) and planktonic growth by crystal violet assays and by turbidity respectively; Quantification of the LDPE degradation by weight loss method

Results

• Biofilm formation by *A. borkumensis*

The capacity of *A. borkumensis* to form biofilm on 4 plastics (PET, PS, LDPE and PVC) was tested with 2 different carbon sources: pyruvate or hexadecane (Fig.1). In presence of pyruvate, only a small percentage of bacteria are in biofilm (+/- 10%) and there are no difference between plastics (Fig.1B,E). In hexadecane, since 96H of culture, this bacterium clearly displays a higher affinity for the LDPE (more than 70% of bacteria in biofilm) than the others (20%) (Fig.1A,E), supporting our previous observations that showed its specific enrichment after two months on LDPE.

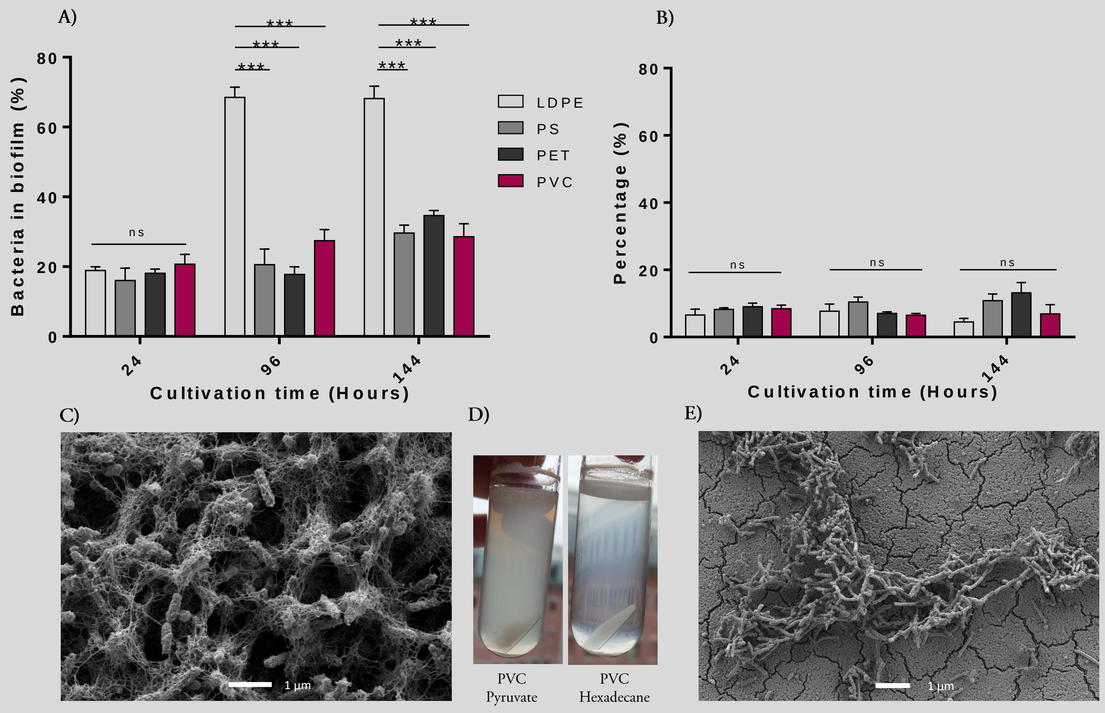


Figure 1: Biofilm quantification of *A. borkumensis* on plastics (PET, LDPE, PS and PVC) by crystal violet assay (OD_{595nm}), (A) Percentage of bacteria in biofilm with hexadecane (0.75%), (B) Percentage of bacteria in biofilm with pyruvate (1.5%), (C) Biofilm in hexadecane medium on LDPE at 96H, (D) biofilm formation on PVC in hexadecane or pyruvate medium after 96H of culture, (E) Biofilm in pyruvate medium on LDPE at 96H.

• LDPE degradation

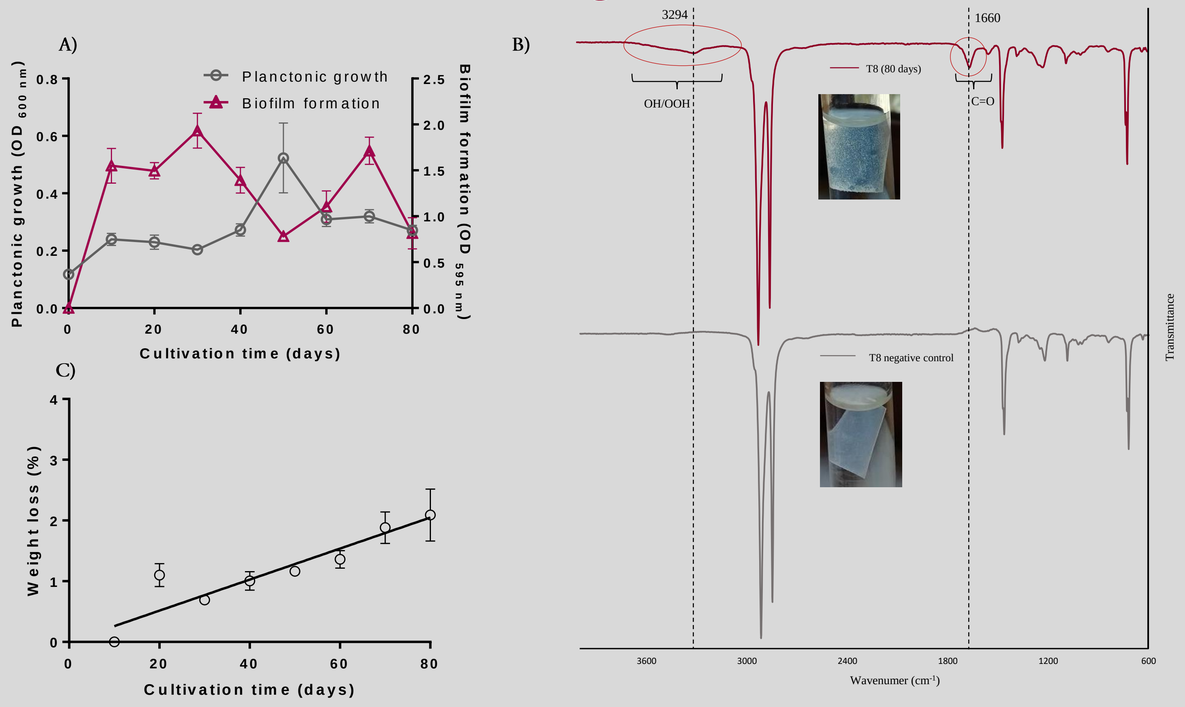


Figure 2: LDPE degradation by *Alcanivorax borkumensis* with 0.05% yeast extract, (A) Planctonic growth (turbidity OD_{600nm}) and biofilm formation (crystal violet assays OD_{595nm}) on 80 days , (B) ATR-FTIR spectra of LDPE films after 80 days of culture in 0,05% yeast extract, the negative control was immerged in medium without bacteria (C) percentage of weight loss of the LDPE on 80 days of culture,

Simultaneously to the biofilm formation, the ability of *Alcanivorax borkumensis* for the LDPE degradation was monitored by weight loss method (Fig.2A,C). Interestingly, preliminary results showed a significant plastic weight loss of 2.1% after 80 days in medium containing 0.05% yeast extract and the LDPE films. Moreover, ATR-FTIR spectra revealed the formation of C=O and OOH/OH functional groups resulting from the polymer degradation, showing the ability of this bacterium to degrade LDPE (Fig.2B).

Conclusion

Our results clearly showed that *Alcanivorax borkumensis* forms large biofilms on plastic surface, with a strong affinity for the LDPE. In presence of alkanes, *Alcanivorax* sp. is known to modify its cell membrane hydrophobicity, produce biosurfactants in order to form biofilm and interact with them. Moreover, it seems that this bacterium was able to degrade LDPE, with 2.1 % of weight loss in presence of 0.05% yeast. ATR-FTIR spectra confirm the degradation by the formation of oxydative groups. Optimal conditions must be found to improve this degradation. If all studies revealed its ability to biomineralize petroleum hydrocarbon, we showed for the first time its capacity to degrade a solid structure based into alkane such as petroleum-based plastic LDPE. The mechanism of a partial depolymerization of LDPE - a crucial step for its degradation by *Alcanivorax borkumensis* - is unknown and needs to be further investigated.

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