Cultivate the "uncultivable": Using the ichip for the isolation of TBT-degrading bacteria

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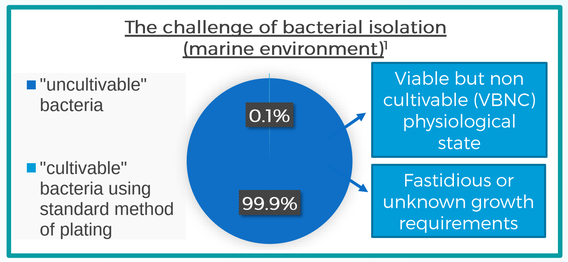
Abstract

Only around 0,1 of bacteria from the marine environment can be grown on standard agar plates. In bioremediation, bacterial isolation is necessary to better understand the mechanisms of contaminants biodegradation. An innovative techniques called the ichip was tested for the isolation of tributyltin (TBT)-degrading bacteria. Using the ichip increased the cultivability of sediment bacteria from both untouched sediment and sediment spiked with TBT and incubated for 3 months at 20°C by a factor of respectively 5,5 and 9,5, It also facilitated the isolation of TBT-resistant bacteria.

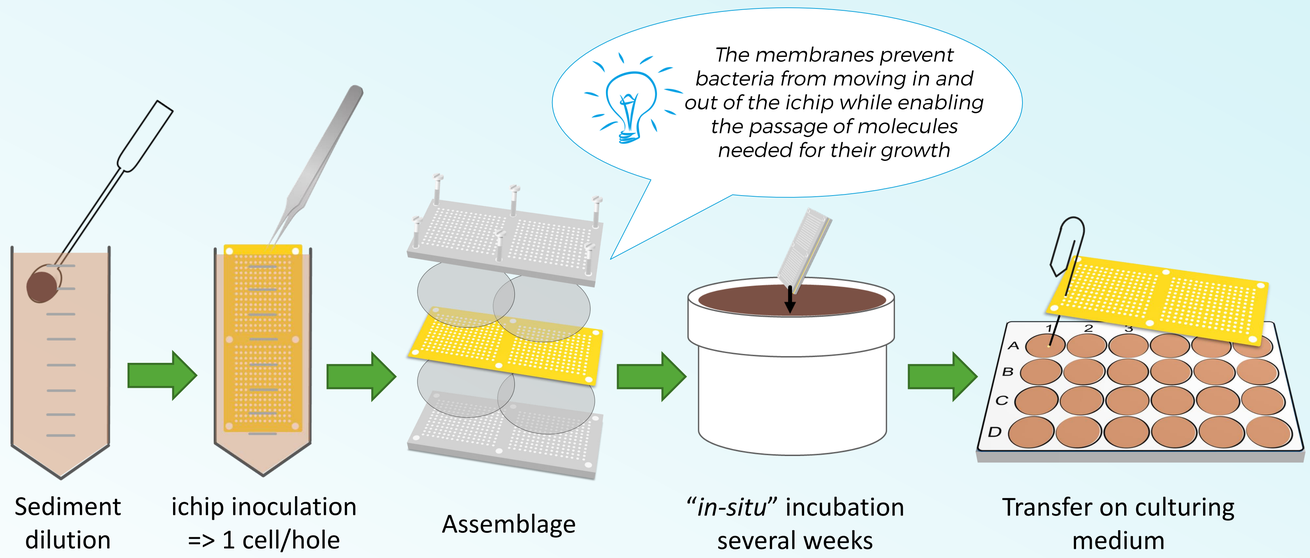
Introduction

Microbial cultivation and isolation contributes to the understanding of microbial community functioning. It has an interest in the field of bioremediation. Studying pure cultures of bacteria capable of contaminant degradation enables us to better understand the kinetics, the pathways and the conditions for biodegradation. It has a direct implication for the optimization of bioremediation technologies, whether to select the best bacteria in a bioaugmentation approach or to understand the best conditions of degradation in a biostimulation one.

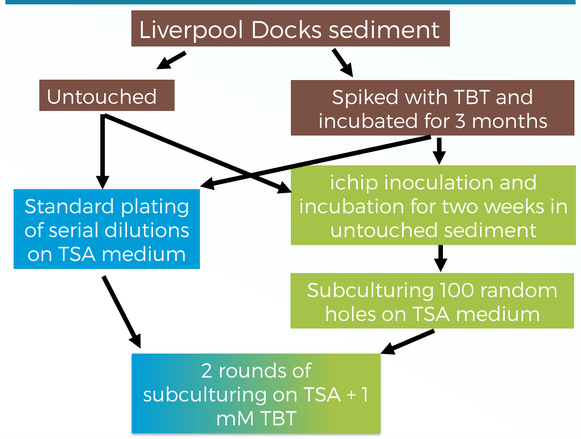


Some techniques were developed to enhance the success of isolation. Notably, the techniques which uses a step during which the isolated cells are being put in contact with their native environment were proved efficient to adapt certain species for growth under laboratory conditions. This study investigates the success of one of these techniques involving the use of the ichip[2] for the isolation of TBT-degrading bacteria.

The journey through bacterial isolation using an ichip

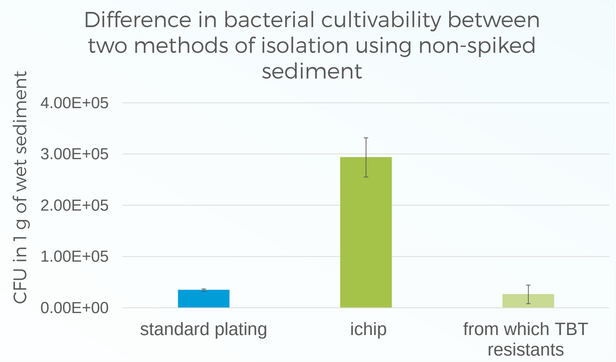


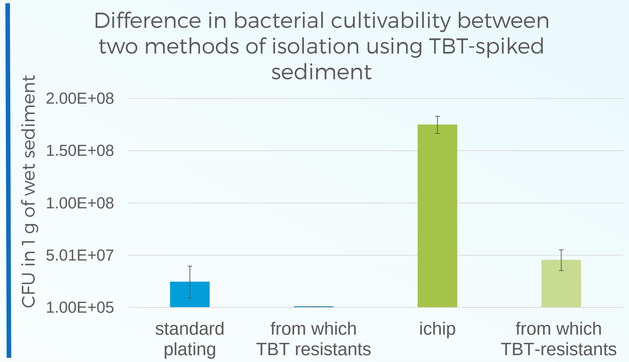
Material and Methods



Results

Difference in abundance of cultivable bacteria when using the ichip compared to standard plating method for the isolation of sediment bacteria





Discussion

For both sediment, performing a round of growth using an ichip increased greatly the number of bacteria retrieved from the environment. Putting the bacteria back into their environment constitutes an intermediary step during which they can adapt to growth on synthetic medium.

The cultivability increased by a factor of 5,5 for untouched sediment compared to 9,5 for sediment spiked with TBT and incubated at 20°C. This is easily explained by the fact that spiking and incubation act as an enrichment step, it will boost microbial growth and select for the TBT-resistant community. The bacteria are therefore already in a replicating state and more likely to be cultivable. It also explains why there is a higher fraction of TBT-resistant among the isolates for TBT-spiked sediment.

Although these results are already valuable for the target isolation of contaminant degrading bacteria, it would be interesting to investigate the diversity of the isolates obtained with the ichip. The ichip was previously to study seawater and soil bacterial communities and showed that a higher number of new taxa could be isolated using the ichip[2]. It is reasonable to think that the same outcome could be obtained in the present study that focuses on sediment bacteria.

Conclusion and Perspectives

The ichip was proved successful to significantly increase the number of bacteria that can be isolated from sediment. The TBT-resistant isolates obtained can subsequently be suggested to further characterization. Their TBT-degradation abilities can be compared for a potential use in bioremediation, in a bioaugmentation approach. They can be identified to potentially reveal species that were never cultivated before, or species that were not known for TBT-degradation ability. Their genome can also be analysed to better understand the mechanisms of TBT-resistance and degradation.

The ichip is therefore a useful tool to increase the efficiency of bacterial isolation in numerous context. In bioremediation, it is of particular interest and could be used to study bacteria involved in the biodegradation of many other contaminants of interest.

References

[1] Hahn, M.W., Koll, U., Schmidt, J., 2019. Isolation and Cultivation of Bacteria, in: Hurst, C.J. (Ed.), The Structure and Function of Aquatic Microbial Communities, Advances in Environmental Microbiology. Springer International Publishing, Cham, pp. 313–351.

[2] Nichols, D. et al. Use of Ichip for High-Throughput In Situ Cultivation of "Uncultivable" Microbial Species. Appl. Environ. Microbiol. 76, 2445–2450 (2010).

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Introduction

Microbial cultivation and isolation contributes to the understanding of microbial community functioning. It has an interest in many fields, including the field of bioremediation. Studying pure cultures of bacteria capable of contaminant (*e.g.* TBT) degradation enables us to better understand the kinetics, the pathways and the conditions for biodegradation. It has a direct implication for the optimization of bioremediation technologies, whether to select the best bacteria in a bioaugmentation approach or to understand the best conditions of degradation in a biostimulation one.

However, isolation of bacteria has always been a challenge, the vast majority of the total environmental bacteria can't be grown using standard methods of isolation, due to many factors such as the need for specific growing conditions, nutrients of interactions. Some techniques were nevertheless successfully developed to increase environmental bacterial cultivability and enhance the success of isolation. Notably, the techniques which uses a step during which the isolated cells are being put contact with their native environment was proved efficient to adapt certain species for the growth under laboratory conditions.

The present study investigates the success of one of these techniques involving the use of the ichip[2] for the isolation of TBT-degrading bacteria.

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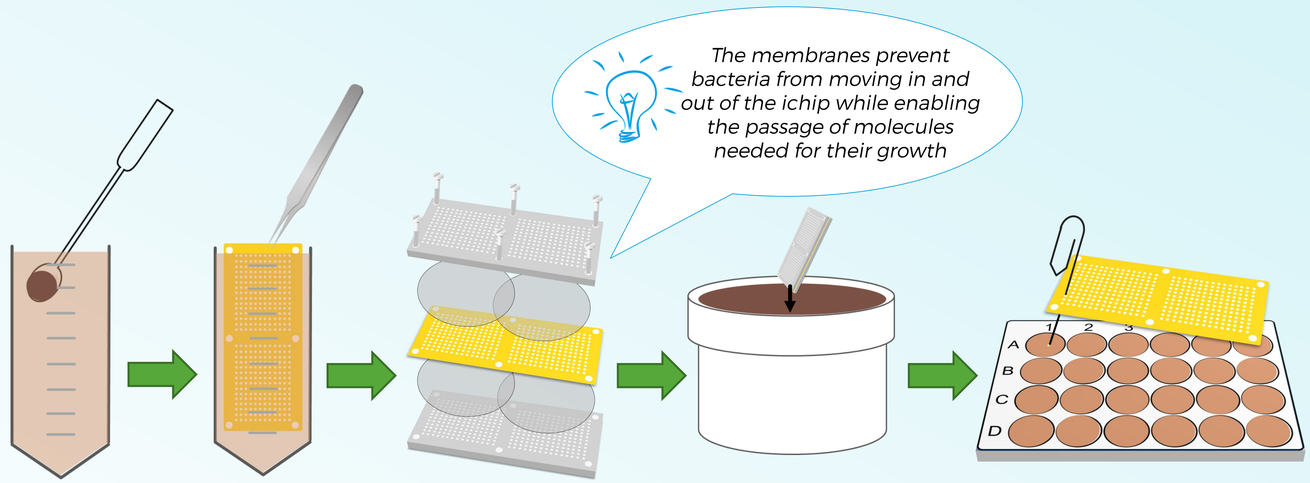

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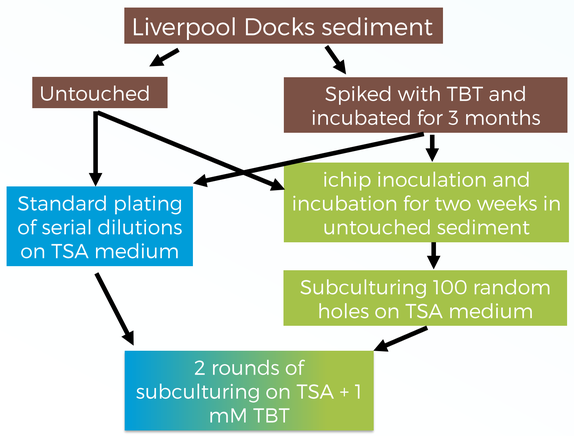

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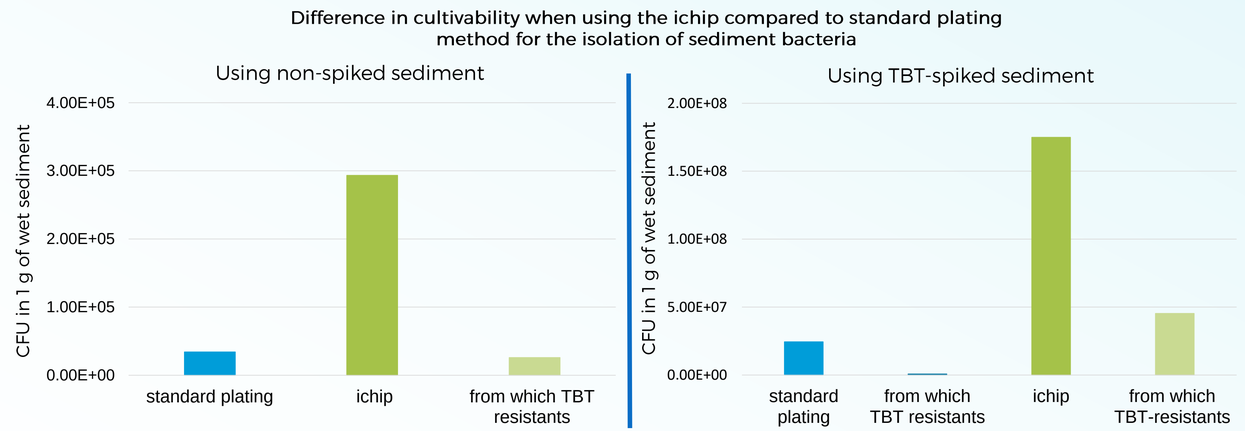

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References

[1] Jannasch, H.W., Jones, G.E., 1959. Bacterial Populations in Sea Water as Determined by Different Methods of Enumeration1. Limnology and Oceanography 4, 128–139. https://doi.org/10/cqtsf5
[2] Nichols, D. et al. Use of Ichip for High-Throughput In Situ Cultivation of "Uncultivable" Microbial Species. Appl. Environ. Microbiol. 76, 2445–2450 (2010).