



# MaCuMBA

Marine Microorganisms: Cultivation Methods for Improving their  
Biotechnological Applications

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## *Deliverable D2.19*

**Protocols for media and isolation of bacteria by chemotaxis**

**Organisation name of lead contractor:** DSMZ

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Dissemination Level	
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## Summary

In marine environment between 20 and 70% of the bacteria are motile. Direct video microscopic observation has revealed that motile bacteria in natural samples are chemotactically active (Fenchel, 2002). Developing chemotaxis assays may provide information about the behaviour of a significant proportion of the marine bacterial community thereby allowing the identification of potential growth substrates. Another advantage of this method is to collect potentially new bacteria and use them directly for cultivation or genome sequencing attempts.

In chemotaxis assays, glass capillaries are loaded with defined substrate solutions, inserted in a suspension of motile organisms, and the accumulation of cells in the capillary is monitored by direct or indirect methods. Three different protocols were described in this deliverable. Only DMSZ was involved in this deliverable.

The microscopic chemotaxis assay was especially developed for short-term chemotaxis measurements of aerobic microorganisms. This technique permits a rapid screening of a limited number of chemoattractants. The Meplats bottles chemotaxis assay was developed to 1) analyse slow-moving microorganisms in natural samples, 2) test a larger number of chemoattractant and 3) to collect a larger number of bacteria. Finally, the PVC rack chemotaxis assay was developed to study bacteria directly within aquatic ecosystem. This technique will permit to do isolation trials when the environmental conditions can not be reproduced easily in laboratory.

All these protocols will be applied in the deliverable D2.20 “Collection of >100 novel microorganisms using chemotaxis”. We will have fresh samples in September 2013 to test these chemotaxis protocols.

### Reference:

Fenchel, 2002. Microbial behavior in a heterogeneous world. *Science*, 296 (5570), 1068:1071.

### Protocols for media and isolation of bacteria by chemotaxis

#### 1. The microscopic chemotaxis chamber

This chemotaxis assay is prepared by using small coverslips as spacers between microscope slide and lid. The “chamber” is created by sealing the two short and one of the long edges with a paraffin/mineral oil mixture.

The capillaries used for this assay can be a flat rectangular capillary that permits a direct light microscopic examination of its contents. However, if direct microscopy of the content is not an issue, conventional round glass capillaries may be used.

The microscopic chamber is then filled slowly with the sample or the culture aliquot with a Pasteur pipette. The capillaries were filled by capillary force with a solution of test substrates previously prepared (final concentration 0.5-10 mM) and sealed at one end with plasticine. The capillaries are then inserted in the microscopic chamber in order to have their open end extend into the sample.

2. *The Meplats bottles*

Metplats bottles are modified for chemotaxis experiments by drilling 10 holes of 3 mm diameter through one of the large sidewall.

The bottle is then filled with the sample or the culture sample. After loading with test compounds as described in section1, each capillary is inserted into a hole until its end extends into the sample. The capillaries are then fixed in each hole with plasticine.

3. *The PVC rack*

A solid PVC rack (50x5x3 cm) is drilled with 15 holes in its top. The holes are spaced 3 cm apart in order to avoid interference of individual chemotaxis assays.

After loading with test compounds, each capillary is inserted into a hole in order to have its open end upward. The two ends of the rack are then attached to a one-meter steel wire and connected to a calibrated steel wire which in turn is connected to a buoy. This chemotaxis assay can be positioned directly in sea or lake water, with a vertical precision of about 5 cm.

*General*

For each of these chemotaxis assays, the open end of the capillaries can be sealed by plasticine for microscopic observation (specially for the flat capillaries). The capillary content can be harvested using small disposable capillary plastic pipette tips and used for further microscopic studies, biomolecular analysis, or cultivation trials.