Cultivation and preservation of *Uab amorphum*

Uab amorphum SRT547（=JCM 39082）is a planctomycete bacterium which is able to engulf other bacteria and small eukaryotic cells through a phagocytosis-like mechanism*. At JCM, the strain is maintained and delivered to users as a co-culture with a prey bacterium *Altermonas macleodii*.

Cultivation

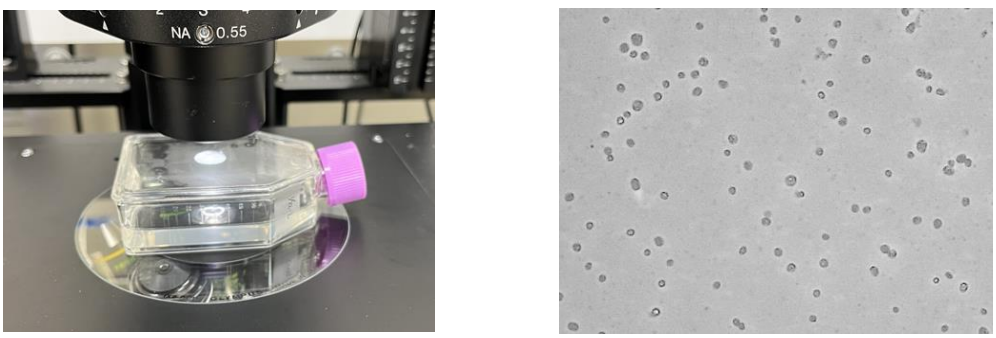
1. Prepare JCM medium No. 1273 according to the recipe described, and autoclave. After cooling, aseptically distribute the medium in tissue culture bottles (e.g., 20 ml culture medium in a 25 cm^2 surface area bottle with a filter cap).
2. Just before inoculation, shake an inoculating co-culture mildly and transfer a cell-suspension into a fresh culture medium (ca. 5% inoculum). Statically cultivate at 25°C. Generally, growth occurs within a week. It grows fully in 2-3 weeks, then the cell-number decreases.

Microscopic observation and PCR detection

1. It is highly recommended to observe *Uab* cells under an inverted microscope, because most of *Uab* cells collect at the bottom of the culture.
2. It is also possible to confirm the presence of *Uab* cells in the culture by the 16S rRNA gene PCR testing. We use the following specific PCR primers to detect it:
   PhgoF3: 5'-TTCCATGCAAGTCGAGCGAG
   PhgoR2: 5'-GGAACACATTCACCGCAGTAT

Preservation

1. A grown culture can be stocked at 4°C for only a limited time. Therefore, it is recommended to preserve the culture by freezing at an early occasion. It can be preserved at -80°C in the growth medium containing 10% trehalose or 10% glycerol.
2. On reviving from the frozen stock culture, it needs to remove the cryoprotectant (i.e., trehalose or glycerol) from the inoculum as it stimulates growth of the prey bacteria only. Therefore, after thawing the stock culture, centrifuge and remove the supernatant. Then, suspend in a fresh culture medium.



Observation under an inverted microscope (Right: larger cells are *Uab* cells)

*Shiratori, T. *et al.* Phagocytosis-like cell engulfment by a planctomycete bacterium. *Nat. Commun.* **10**: 5529, 2019.