Special Instructions

Cultivation of Methanogens

Methanogens are a diverse group of strict anaerobes which are widely distributed in nature and can be found in a variety of permanently anoxic habitats like flooded soils, sediments, sewage-sludge digestors or the digestive tract of certain animals. All known methanogens are affiliated to the Archaea and extremely sensitive to oxygen. The hallmark feature of methanogens is the reduction of C-1 compounds (e. g., CO_2, methanol, formate, or N-methyl groups) to methane (CH_4). Some enzymes and cofactors are unique for this metabolic pathway and therefore only found in methanogens. The coenzyme F420 involved in methanogenesis causes an intense autofluorescence of cells under excitation by shortwave UV light. This autofluorescence is a diagnostic feature and can be used to check cultures of methanogens for contaminants by epifluorescence microscopy.

A detailed description of the cultivation of ***Methanosarcina barkeri*** **DSM 800^T** follows below to exemplify the recommended handling of methanogens. *M. barkeri* was one of the earliest species of methanogens isolated in axenic culture. It is metabolically very versatile compared to other methanogens and can use also acetate as carbon and energy source (Bryant and Boone, 1987).

M. barkeri DSM 800^T is cultured in DSMZ medium 120a and delivered in 5 ml aliquots in Hungate tubes. After receipt check a sample of the culture by phase-contrast microscopy. To do this remove aseptically an aliquot of the culture with an 1 ml syringe which was made anoxic as described in the DSMZ Technical Information Cultivation of anaerobes (https://www.dsmz.de/fileadmin/Bereiche/Microbiology/Dateien/Kultivierungshinweise/Kultivierungshinweise_neu_CD/englAnaerob_rev_01.pdf)
Under the microscope the cells of this strain appear as nonmotile, large, irregular shaped spheroid bodies which normally occur as packages of several cells. Occasionally, some *Methanosarcina* strains tend to form large aggregates of up to 1000 µm in diameter, which are visible to the naked eye. A phase-contrast micrograph of *Methanosarcina barkeri* is shown in Fig. 1A, whereas the epifluorescence micrograph in Fig. 1B illustrates the typical blue-green autofluorescence of these cells.

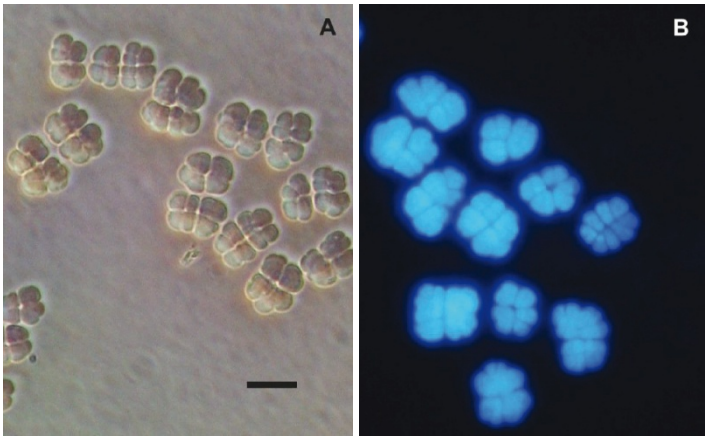

Fig. 1 Phase-contrast (A) and epifluorescence micrograph (B) of DSM 800^T. Bar, 10 µm.

Prepare medium 120a recommended for strain DSM 800^T as indicated on the DSMZ web site http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium120a.pdf

This methanogen grows well with methanol as substrate and therefore a supply of the culture with H_2/CO_2 gas mixture is not necessary.

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Note: Methanogens other than *Methanosarcina* spp. that grow only with H_2/CO_2 as substrate are supplied in media prepared under H_2/CO_2. Vials of these strains are overpressurized to 0.5 to 1 bar with a gas mixture of 80% H_2 and 20% CO_2. For the cultivation of these strains it is necessary to supply fresh gas mixture in regular intervals to avoid underpressure by the consumption of H_2/CO_2 and to remove the produced CH_4.

Prior to inoculation reduce medium 120a, which contains the redox indicator resazurin, by injection of the reducing agents sulfide and cysteine. Wait until the medium has become completely colorless (**redox potential below -110 mV**). Then, transfer 0.25 ml of a late logarithmic phase culture of DSM 800^T to 5 ml of freshly prepared medium 120a. Incubate at 30 - 37 °C for up to one week. Growth and methane production (gas bubbles) become visible within 48 hours of incubation.

Caution: *Methanosarcina* species that grow on methanol can produce a considerable amount of gas according to the formula: 4 CH_3OH -> 3 CH_4 + CO_2 + 2 H_2O
Similar to other microorganisms that can produce overpressure during growth in closed vials, *Methanosarcina* spp. should be incubated in culture vessels (e.g. Balch tubes) that resist substantial pressure and are filled only up to 25% with liquid medium. In addition, fast growing cultures should be vented at least on a daily basis to avoid overpressure. Wear protective goggles during handling of cultures that produce overpressure!

In liquid cultures growth is frequently flocculent and starts with the formation of **large cell aggregates** that settle down at the bottom of the vial. It is not necessary to agitate tubes during the incubation. *Methanosarcina barkeri* strains normally grow well in laboratory batch cultures and a turbidity above OD_{600nm} 0.5 is frequently reached at the end of the growth phase.
Cultures of DSM 800^T and related strains grown to the early stationary phase are stable at 4 - 10 °C for up to four weeks.

Literature

Bryant, M. P., and Boone, D. R. 1987. Emended description of strain MS^T (DSM 800^T), the type strain of *Methanosarcina barkeri*. *Int. J. Syst. Bacteriol.* **37**, 169-170.

Notes

1. Abbreviations (excl. chemicals, reagents and measuring units):
   fig. = figure
   OD = optical density

2. Red colored information indicates an important subject regarding to the content given herein.

3. The information contained herein is offered for informational purposes only and is based on the present state of our knowledge. Recipients of our microorganisms must take responsibility for observing existing laws and regulations. DSMZ does accept no responsibility for the accuracy, sufficiency, reliability or for any loss or injury resulting from the use of the information.

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