

### Cultivation of Acidophiles

Acidophiles are adapted to low pH values and show maximum growth rates below pH 5. Many acidophiles are also thermophilic and can be found in thermal vents or hot springs that are acidic. Mesophilic acidophiles, on the other hand, are frequently found in mine drainage water or soils of low pH.

A large number of acidophilic strains do not survive lyophilization and hence are delivered as actively growing cultures from the DSMZ. Cultures grown to stationary phase are usually not very stable and lyse rapidly in spent media. This can be explained by the fact that cells need continuously metabolic energy to maintain a neutral intracellular pH against a steep gradient of excess extracellular protons. Therefore, it is important to transfer the obtained cultures into freshly prepared media immediately upon receipt.

A majority of the fastidious acidophilic strains are aerobic autotrophs and grow either by the oxidation of ferrous iron or sulfur compounds. In order to exemplify the handling of these acidophiles the cultivation of two distinct strains is described in detail: *Acidithiobacillus caldus* DSM 8584<sup>T</sup> grows with elemental sulfur and *Leptospirillum ferrooxidans* DSM 2705<sup>T</sup> with ferrous iron. Additional information on the cultivation of heterotrophic acidophiles, like e.g. *Sulfolobus* spp., can be found in the DSMZ Special Instructions *Hyperthermophiles*.

#### ***Acidithiobacillus caldus* DSM 8584<sup>T</sup>**

The genus *Acidithiobacillus* represents a separate class within the *Proteobacteria*, designated as *Acidithiobacillia*. Cells are Gram-negative, small rods (approx. 0.5 x 2.0 µm) that are motile by means of polar flagella. While all members of the genus *Acidithiobacillus* can use sulfur or reduced sulfur compounds for autotrophic growth, some strains can utilize also ferrous iron or hydrogen as energy source.

You will receive from the DSMZ an ampoule with a freeze dried sample of strain DSM 8584<sup>T</sup>, the type strain of *A. caldus*. Prepare medium 150a recommended for this strain according to the instructions given on the DSMZ web site: [http://www.dsmz.de/microorganisms/medium/pdf/DSMZ\\_Medium150a.pdf](http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium150a.pdf).

**Note: Elemental sulfur melts and then aggregates during autoclaving at 121 °C thereby making it unusable by microorganisms. Hence, it is important to sterilize the sulfur powder separately from the liquid medium. This can be achieved by filling screw cap tubes (18 x 100 mm, borosilicate glass) with sulfur powder (approx. 2/3 volume) to which 1 or 2 drops of distilled water is added. The screw cap should not be tightly closed. The tubes are heated in a water bath to 90-100 °C for 3 hours on each of 3 successive days. The sterilized sulfur can be stored at room temperature in the dark for several months.**

Open the ampoule carefully as described in *Handling of cultures and opening of ampoules with freeze-dried material*. Add approx. 0.5 ml of the freshly prepared medium to the freeze dried pellet and resuspend it. Transfer the cell suspension by using a sterile Pasteur pipette to 5 ml medium in screw cap tubes (16 x 160 mm, borosilicate glass). Prepare 1:10 and 1:100 dilutions in two other tubes and incubate all tubes in a slanted position at 45 °C. Growth should become evident after 2-5 days. As a result of growth most of the sulfur gets coated by bacteria and sinks to the bottom of the tube. In contrast, the sulfur remains at the liquid-air-interface in freshly prepared sterile medium. Fully grown cultures are not very stable and should be weekly transferred in fresh medium. Young cultures can be stored at 4 °C for up to 14 days.

For the cultivation of larger volumes prepare medium in screw cap Erlenmeyer flasks (e. g., 30 ml medium in a 200 ml flask) and incubate with gentle shaking on a rotary shaker. Use 5-10% (v/v) of a culture grown to the late logarithmic phase as inoculum.

### *Leptospirillum ferrooxidans* DSM 2705<sup>T</sup>

Members of the genus *Leptospirillum* belong to the class *Nitrospira* and play an important role in the bacterial leaching of sulfidic minerals in acidic environments. Cells are Gram-negative and have a vibroid or spiral-shaped morphology. They are motile by means of a single polar flagellum.

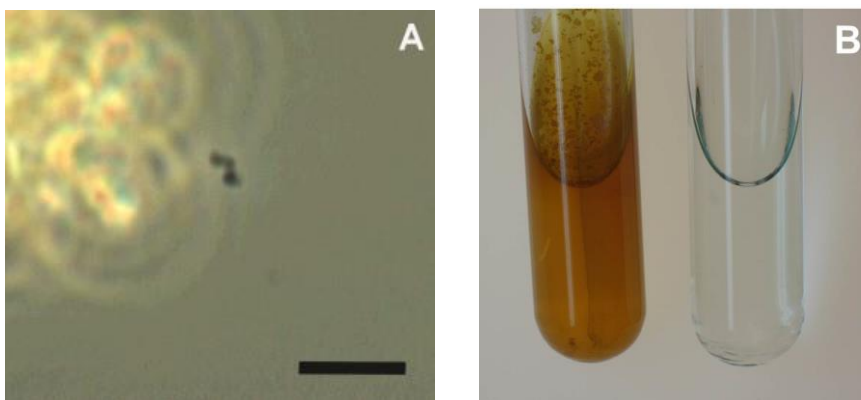
Leptospirilli are strict autotrophs that can only use ferrous iron for growth. Different from *Acidithiobacillus* strains they are not able to utilize elemental sulfur. In addition, they have generally lower pH optima (pH 1.3-2.0) than members of the genus *Acidithiobacillus*.

*L. ferrooxidans* DSM 2705<sup>T</sup> is cultured in DSM medium 882 and delivered in 5 ml aliquots in screw cap tubes. The density of cells in cultures grown to stationary phase is too low to produce a visible turbidity. Hence, after receipt check a sample of the culture for viable cells by phase-contrast microscopy. Under the microscope you will normally find only several motile vibroid cells per field of view besides numerous large deposits of iron oxide (Fig. 1A). Prepare medium 882 recommended for strain DSM 2705<sup>T</sup> as indicated on the DSMZ web site:

[http://www.dsmz.de/microorganisms/medium/pdf/DSMZ\\_Medium882.pdf](http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium882.pdf).

**Note: In our experience *Leptospirillum* strains are extremely sensitive to traces of organic substances, which may significantly inhibit growth. To avoid a contamination of medium 882 with organic compounds use only absolutely clean glass vials, chemicals of high purity and double-distilled or MilliQ water for medium preparation.**

Screw cap tubes with 5 ml of freshly prepared medium are inoculated with 5-10% of the original culture obtained from the DSMZ. Prepare 1:10 and 1:100 dilutions in two other tubes and incubate all tubes in a slanted position at 30°C without shaking. The purpose of the dilution series is to deplete organic materials, which may have accumulated during culture growth (via cell leakage and lysis) and may inhibit growth. Hence, it is only necessary to prepare dilution series, if cultures are used as inoculum that have already reached late stationary phase. Growth becomes visible after 3-10 days by a change of the color of the medium to rusty-brown as shown in Fig. 1B.



**Fig 1** Growth of *Leptospirillum ferrooxidans* DSM 2705<sup>T</sup> in liquid culture.

(A) Phase-contrast micrograph of a single cell along with iron oxide deposits. Bar = 5 µm.

(B) Stationary phase culture in DSMZ medium 882 (left) and uninoculated medium (right).

Young, active cultures of *Leptospirillum* strains remain active at 15°C for at least 2 weeks. The viability of cultures can be increased to several months by adding of a sterile suspension of pyrite to stationary-phase ferrous iron-grown cultures (Johnson, 2001).

### Literature

Johnson, D. B. 2001. *Genus II. Leptospirillum Hippe 2000, 503<sup>VP</sup> (ex Markosyan 1972, 26)*, pp. 453-457. In Garrity et al. (eds.), *Bergey's Manual of Systematic Bacteriology*, 2<sup>nd</sup> ed., vol. 1. Springer, New York.

### Notes

1. Abbreviations (excl. chemicals, reagents and measuring units):  
approx.= approximately  
fig= figure
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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