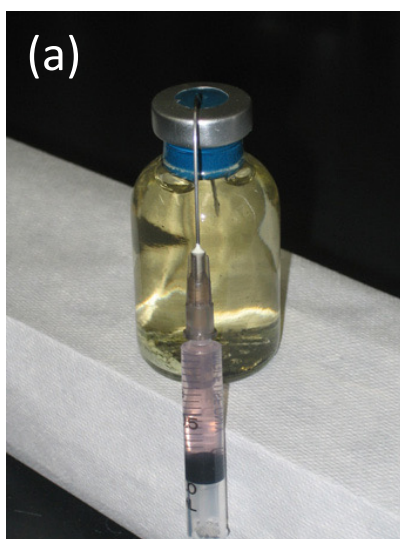


Cultivation of an obligately piezophilic, strictly anaerobic hyperthermophile

JCM makes an obligately piezophilic, strictly anaerobic hyperthermophile, *Pyrococcus yayanosii* JCM 16557^T* accessible to the community. Below, we show how this strain is cultivated at JCM:

1. Prepare JCM Medium No. 811 according to the medium recipe. Autoclave the medium without sulfur powder and the reducing agents.
2. Aseptically put sulfur powder into a 10~20 ml vial, then seal with a butyl rubber stopper and replace the gas phase with a nitrogen gas.
3. Using a syringe, fill the autoclaved medium in the vial almost completely (leave bubbles if they are small).
4. Reduce the medium, then inoculate a culture in the medium. Separately, take approximately 0.5 ml of the medium by a syringe with a needle and stab the butyl rubber stopper with the needle. If necessary, vent the needle and/or trim the syringe so that they can be contained in the pressure cylinder of the high-pressure incubation system. (Fig. a).
5. Fill water into the cylinder and put the inoculated culture with the syringe into the cylinder. Attach the lid to the cylinder.
6. Check the assembled high-pressure incubation system for leaks. Then, place the system in an incubator operated at 90-98°C for cultivation. The pressure in the cylinder will be raised as temperature of the system raises. If the pressure is likely to go beyond the growth pressure range, adjust pressure by the pressure bulb. (*P. yayanosii*, optimum pressure 58 MPa, maximum pressure 120 MPa, at 98°C).
7. After the cultivation, cool down the inoculation system gradually. Then, remove the culture bottle from the inoculation system.



Example of the cultivation of *Pyrococcus yayanosii* JCM 16557^T. (a) A culture of the strain that was placed in the cylinder of the high-pressure inoculation system. (b) An apparatus of the high-pressure inoculation system placed in a high-temperature incubator. The pressure gauge is not durable at high temperature: therefore, it is separated from the system by the pipework.

*Birrien *et al. Int. J. Syst. Evol. Microbiol.* **61**: 2827-2831, 2011.

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