Cultivation protocol of *Clostridium stercorarium*

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We received the freeze dried ***Clostridium stercorariumculture*** **(DSM No.8532)** delivered from DSMZ in ampoules.

To cultivate *C. stercorarium*, culture medium should be prepared first. The recipe for *C. stercorarium* is shown as below:

KH_2PO_4	0.50 g
$K_2HPO_4 \cdot 3\ H_2O$	1.00 g
Urea	2.00 g
$MgCl_2 \cdot 6\ H_2O$	0.50 g
$CaCl_2 \cdot 2\ H_2O$	0.05 g
$FeSO_4 \cdot 7\ H_2O$	1.25 mg
Morpholinopropane sulfonic acid	10.00 g
Resazurin	1.00 mg
Yeast extract	6.00 g
Cellobiose	5.00 g
Cysteine-$HCl \cdot H_2O$	1.00 g
Distilled water	1000.00 ml
Adjust pH to 7.2	

The ingredients except **cellobiose** were mixed well by swirling and autoclaved at **121°C for 25 min** while cellobiose was sterilized separately by filtration. The liquid medium (or broth) could be used for growing bacteria in test tubes.

Broth with about **1.5% agar** added was poured into **Petri dishes** and let each plate cool until totally solid. The plates could be used to isolate pure culture in the following step.(Figure 1)

Figure 1

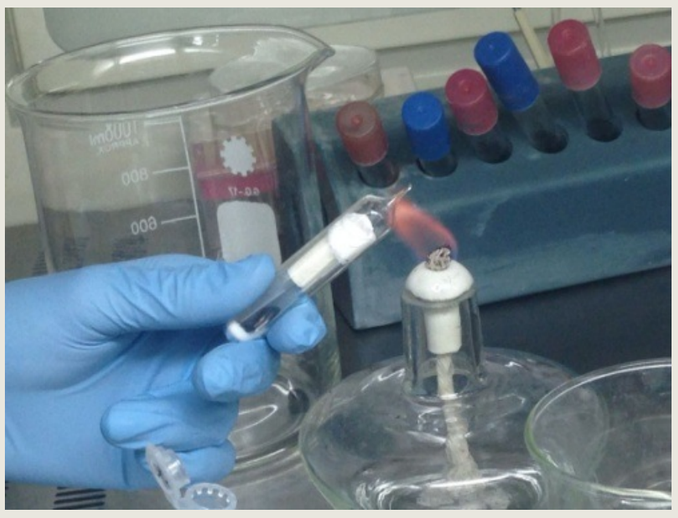

Figure 2

When the medium was ready, we opened the ampoules carefully and **rehydrated** the dried cultures near a flame to keep it under sterile conditions.(Figure 2)

Then, **0.5ml** culture medium was added in the vial to allow the pellet to rehydrate for **30 minutes**. The whole content was mixed gently and transferred to **a 15ml tube with 5ml liquid medium**. The bacteria were cultivated at **60℃ overnight** in an anaerobic bag.

The following day, a small sample from the *Clostridium stercorarium* inoculums was spread on the surface of the plate to separate single colonies by using spread plate method (**no dilution**). The plates were put into an anaerobic bag and cultured at **60°C for 2 days**.

However, we did not get single colony because of the high density of colonies at first. Then, **10-3**, **10-4** and **10-5** dilutions of the original broth were respectively spread on the plates. Finally, single colonies were successfully isolated from these plates and could be applied for further study.