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*Abeoforma whisleri*_culture method

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1 *Works for me* This protocol is published without a DOI.

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Growing medium

1 BD Difco Marine Broth 2216 • Marine Agar 2216
https://legacy.bd.com/europe/regulatory/Assets/IFU/Difco_BBL/212185.pdf

Growth conditions

2 Temperature 17ºC

Cryopreservation and recovery (from liquid cultures)

3 **Cryopreservation** (10%DMSO in 1M sorbitol or 10%(glycerol 60%) in sorbitol 1M)

1. Pre-grow the culture in appropriate growing medium until almost saturated but not too old.

2. Mix 1.35mL of the culture with 150 ul of DMSO in 1M sorbitol or (glycerol 60%) in sorbitol 1M in the screw-top cryovials.

3. Transfer the cryovials to the Freezing container, Nalgene®Mr. Frosty unit. Place it in the -80ºC freezer and leave it overnight.

4. Remove cryovials from the Freezing container and store in appropriate bobes for -80ºC.

Recovery

1. Fill a flask with 10mL fresh Growing medium.

2. Take out the frozen stock from the freezer, carry it to the culture hood in a cryo box to prevent it from thawing.

3. With a pipette tip (or a loop), take a chunk of the frozen stock and resuspend it in the culture flask (~10uL is probably sufficient). Take the frozen stock back to the -80ºC as rapidly as possible.

4. Let it recover for at least 3-4 days and proceed with another transfer in a fresh medium even if the cells are not completely grown it will help to dilute the remaining DMSO from the cryopreservation, the time will vary depending on the amount of the initial inoculum and the culture type.

*NOTE: because of DMSO some precipitates may appear in the culture.
All protists must be stored in liquid nitrogen for long-term storage. Although storage at -80°C is sometimes sufficient for a temporary alternative.