Useful methods: Cefotaxime: a useful antibiotic for duckweed culture management

In 1 collection

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Duckweed

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We use this protocol and it's working

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ABSTRACT

This protocol describes about cefotaxime a useful antibiotic for duckweed culture management. It contains protocols from the The International Steering Committee on Duckweed Research and Application (ISCDRA) Newsletter. A complete list of these news letters can be found here.

ATTACHMENTS

383-845.pdf

GUIDELINES

Biology is complicated! This expression of amazement is exemplified by the deceivingly simple duckweed plants and the complex task of caring for a living collection of over 800 clones (or strains) in the Rutgers Duckweed Stock Cooperative (RDSC). In order to maintain a germplasm stock for duckweed that will have consistent and reliable characteristics and performance, we endeavor to keep them as gnotobiotic cultures that are not complicated by bacteria or fungal endophytes. While this can be done for the majority of clones in our collection, some strains and species can be particularly recalcitrant to purging their resident microbes. These will require sequential sterilization using various concentrations of sodium hypochlorite (the active ingredient in bleach) that can take a lot of patience, dexterity and time. Once a gnotobiotic culture has been achieved, however, duckweed clones can also easily become "reinfected" with bacterial or fungal endophytes during their subculture. In many cases, while the infection per se often does not cause an overt pathological phenotype in appearance at first, the compromised strains are more prone to turn yellow in their fronds upon long term culture as well as displaying slow growth and death in later stages. To counter some of these challenges, we have found that the antibiotic cefotaxime is a very useful agent in helping us to manage the large collection of duckweed strains in the RDSC (see picture to the right). We mainly utilize this antibiotic to help remove difficult bacteria from duckweed during

the sterilization phase, and to suppress reinfection of gnotobiotic duckweed plants by new bacteria strains.

Some important characteristics of cefotaxime are thus important to appreciate by the user. Cefotaxime is a β-lactam antibiotic, related to penicillin, and can inhibit both Gram-negative and Gram-positive bacteria. However, it is important to note that it is apparently not active against Pseudomonas and Enterococcus species. Like Penicillin, cefotaxime inhibits bacteria cell wall biosynthesis that eventually causes lysis of the bacteria. In addition, it can inhibit cell division in cyanobacteria as well as organellar division in glaucophytes and bryophytes. Interestingly, cefotaxime has very low toxicity in vascular plants and is thus often used in plant tissue culture. For duckweed cultures, we typically apply cefotaxime at a final concentration of 100 mg/L in order to destroy or inhibit multiplication of any remaining bacteria in bleach-treated duckweed tissues during their recovery from the harsh treatment. We also routinely maintain our strains in the RDSC on multiple types of medium for long term (~3 month) storage, a couple of which contains cefotaxime. These are 0.5X Schenk and Hildebrandt (SH) salts, cefotaxime, ± 0.1%(W/V) sucrose. However, we note that for some strains in the Wolffia and Wolffiella genera, heightened sensitivity to cefotaxime may occur and lower concentrations of the antibiotic could be needed.

For the more recalcitrantly infected duckweed clones, cefotaxime-containing agar plates could be one way to help purge the resident microbes using a dilution-by-division approach. In this method, we will spot a few clusters of bleach-treated duckweed fronds onto an SH plate with cefotaxime (SHcef plates) and let the fronds regenerate from the protected meristems (see example in picture below for fronds from a Lemna species). These will be transferred to new SH-cef plates after 2 to 3 weeks and wait for new clusters to form. Fronds from the edge of the new clusters are then plated onto another fresh SH-cef plate and grown again to a cluster before

repeating this process one more time. Finally, new fronds at the edge of the clusters are transferred onto SH-sucrose plates to promote more rapid plant growth. When new clusters are formed, fronds are then checked for bacteria presence by plating on LB and TSB agar plates. In this time-consuming approach, we reason that as the plant divides in the presence of cefotaxime, the remaining bacteria present deep within the meristem pocket(s) of the duckweed will be sequentially diluted since their division will be inhibited even if they are recalcitrant against lysis. Thus, after several rounds of subculturing with this procedure, one may be able to obtain gnotobiotic fronds located away from the original mother frond, which could still contain dormant bacteria. It should be noted that cefotaxime is not very stable at 25oC, decreasing in activity by ~20% after 5 days (1). Thus, transfer to fresh plates will be necessary after 2 to 3 weeks under most plant culture conditions in order to maintain its efficacy.

In summary, we hope this Discussion topic is of interest to the general community as well as helpful to duckweed researchers and application specialists for maintaining their own culture collections. For convenience, we have appended at the end of this article a detailed protocol that we use to include cefotaxime in our culturing media.

References:

1. Behin S, Punitha ISR, and Krishnan S (2012) Physical and Chemical Stability Studies on Cefotaxime and its Dosage Forms by Stability Indicating HPTLC Method. Int. J. Pharma. Chem. and Biol. Sci. 2(4): 517-523. ISSN: 2249-9504

Preparation of Cefotaxime Stock Solution

1

Note

https://www.goldbio.com/documents/1036/Cefotaxime+Stock+Solution.pdf

Add 1 g of cefotaxime (GoldBio; Catalog # C-104) to 10 mL sterile H_2O. Dissolve completely.

2 Filter sterilize solution using 0.22 µm syringe filter.

3 Aliquot into 1-mL centrifuge tubes.

4 Store at -20 °C until use.

Preparing Agar Media With Cefotaxime

1h

5 Autoclave agar media at 122 °C for 00:30:00. 30m

6 Let agar media cool until it's warm to the touch.

7 Thaw cefotaxime stock solution.

8 Add 500 µL cefotaxime stock solution (100 mg/mL stock; 1,000x) to 500 mL agar media for a final concentration of 100 undetermined.

9 Pour plates. Plates are left Overnight in laminar flow hood to solidify and dry. 30m

10 Store plates the following day at 4 °C until use.

Preparing Liquid Media With Cefotaxime 1h

11 Thaw cefotaxime stock solution.

12 Add 500 µL cefotaxime stock solution (100 mg/mL stock; 1,000x) to 500 mL liquid media for a final concentration of 100 undetermined .