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Growth of diatom *Amphora* sp. cultured on agar plates by streak plate technique

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Abstract Agar plate culturing is critical in a wide variety of scientific research, yet growing diatoms is challenging. The optimal agar concentration for *Amphora* sp. growth on f/2 medium and the duration of *Amphora* sp. survival on agar plates was identified. This study tested the simple approach for detecting cell colony growth on an agar plate by growing *Amphora* sp. in an F/2 medium with varying agar concentrations (0,4 percent, 0,6 percent, 0,7 percent). Following the discovery of the optimal agar concentration for *Amphora* sp. growth, the research continued with the regeneration of *Amphora* sp. from the old stock agar plate to the new plate of agar using the streak plate method. After *Amphora* sp. was cultured for four weeks, six weeks and eight weeks, regeneration was carried out on the new plates. *Amphora* sp. grew well on F/2 medium with a 0,4% agar concentration compared with other agar concentrations. The colony that appears became an indicator. *Amphora* sp. regeneration also showed that *Amphora* sp. was still growing well in new plates, all *Amphora* sp. from stock that was four weeks, six weeks, and eight weeks old, as indicated by the existence of multiple colonies and no contamination.

Keywords: Diatom, Plate culture, Agar concentrations, Streak method, Colony

Introduction

Microalgae are photosynthetic microorganisms found in almost all bodies of water (Alam *et al*., 2019). They have been utilized as a source for the production of biofuel, bioethanol (Alam *et al*., 2017); (Aziz *et al.*, 2017), animal feed (Shang *et al*., 2018); (Sirakov *et al.*, 2018), nutraceuticals, food additives (Suleria *et al.*, 2015) and cosmetics (Ariede *et al*., 2017). Microalgae are also being examined for their medical potential; their pigments have been found to have anti-inflammatory, antibacterial, antifungal, anticancer, and antioxidant activities (El Gamal, 2010).

Diatoms are thought to contribute up to 25% of the world's primary productivity of biomass (Scala and Bowler, 2001). Microalgae require only a

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few simple nutrients (light, water, CO2 and minerals) for growth and photosynthesis (Chtourou *et al*., 2015). Diatoms, include *Amphora* sp., are a species of microalgae that contribute significantly to global carbon fixation (Kimura and Tomaru, 2013).

Amphora sp. is a benthic diatom that is regularly found in a wide variety of surface coastal waters. Its growth rate and nutritional value have been extensively studied (De la Peña, 2007). Since they are frequently mass-cultured and settled onto plates as a diet for juvenile grazing abalones, these photosynthetic microorganisms play a critical role in seawater aquaculture as feed and settlement inducers (Avendaño-Herrera and Riquelme, 2007; Capinpin Jr, 2007; Van Colen *et al*., 2009).

The study of how diatoms grow is essential for the existence of the diatom collection. This collection is a stock that is constantly being prepared for scientific studies and various applications. They are essential for building the knowledge base on biodiversity. They act as safe depositaries of biological materials, contributing significantly to acquiring taxonomic, physiological, genetic, morphological, chemical, and ontogenetic information. (de Oliveira Lourenço, 2020). Numerous researchers have investigated the biology and ecology of diatoms using a variety of experimental techniques. Various microalgae have been observed growing in solid media. *Chaetoceros tenuissimus* cells, which are challenging to grow on standard plates using conventional methods, survived when seeded using the decantation approach on a plate medium with fine grooves (Kimura and Tomaru, 2013). Another research result, *Skeletonema marinoi* can grow on F/2 media with 2% agar (Kourtchenko *et al.*, 2018).

The capacity to grow on solid media is required for a large number of molecular and screening procedures. In molecular and microbiology, an agar-based solid medium is widely utilized for various applications, including cell culture, screening, selection, and storage (Kourtchenko *et al*., 2018). Storage methods include agar media, liquid, and cryopreservation. One benefit of implementing solid media over liquid media is that it can remain longer, minimizing mutations in existing microalgae collections.

In the 1950s, agar plate cultivation methods for microalgae were established. Single-cell isolation by streak plates is one of the world's critical global methods for microalgae and pure culture separation. This procedure is easy to grasp and reliable and can be used at the laboratory level (Alam *et al*., 2019). The objectives of the research were to optimize the percentage of agar in culture medium of *Amphora* sp. and to assess the survival ability of *Amphora* sp. in agar plate.

Materials and methods

Collection of microalgae

Diatom samples (*Amphora* sp.) were obtained during the MBE National Cheng Kung University laboratory's sample collection. This sample collection is an *Amphora* sp. sample that is continuously rejuvenated utilizing the dot method on an agar plate (F/2 media, 1% agar) every month. This stock from *Amphora* sp. is stored at 24 ℃ in a storage cabinet with a 3000 lux illumination.

Preparation of agar plate

The preparation of agar plate media began with the preparation of seawater with a salinity of 28ppt. F/2 medium containing NaNo3, NaH2PO4H2O, Na2SiO39H2O, and Trace Metal were utilized. Agar plates are prepared by dissolving 0,4%, 0,6%, and 0,8% agar in F/2 media. These plates are autoclaved for 15 minutes at 121 ℃. The plates will then be allowed to cool before the vitamin is added, and a 10 ml warm agar medium will be added to the plates. These plates will be allowed to cool, inverted to prevent drying, and at least 72 hrs before streaking (Parvin *et al.*, 2007).

Streak plate technique

In axenic conditions, microalgae will be allowed to streak through micropipette tips in plates. A micropipette tip was loaded with a sample and streaked across the agar surface. Samples were placed on medium F/2 agar plates with agar concentrations of 0,4%, 0,6%, and 0,8%, three times each. After streaking, agar plates were incubated for at least seven days to allow colonies to form. Before plating with 0,4%, 0,6%, or 0,8% agar, a single species' cell growth, and purity were checked under a microscope.

Incubation of Amphora sp.

Amphora sp. samples on agar plates with f/2 media with different agar concentrations were placed in an incubator with a temperature of 24 ℃. In this incubator, a lamp with irradiation of 3000 lux was placed. Observations were made after incubation for seven days.

Monitoring Amphora sp growth

The appearance of a colony indicated the growth of microalgae culture. The way was to observe the colony directly with the naked eye and a

microscope instrument. The existence of colonies on the agar plate and the purity of the developing *Amphora* sp. cells were determined through observation.

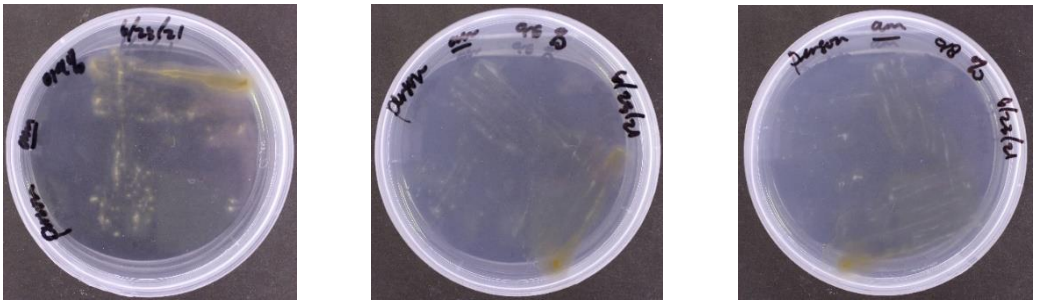
Amphora sp. survival on F/2 media

After establishing the optimal agar concentration at f/2 media for Amphora growth, regeneration was conducted on grown *Amphora* sp. stock. Regeneration was performed on stock aged four weeks, six weeks, and eight weeks to assess the survival ability of *Amphora* sp. on the agar plate. The streak method was utilized, which entails three repetitions of transferring one part of *Amphora* sp. to a new plate with f/2 medium, 0.4 percent agar. The colony growth was monitored one week after inoculation.

Results

Growth of Amphora sp. in F/2 medium at various agar concentrations

Amphora sp. growth was observed from three f/2 media with different agar concentrations (Figure 1). One week after *Amphora* sp. was inoculated on the agar plate, observations were made.



F/2 0,4% F/2 0,6% F/2 0,8%

Figure 1. *Amphora* sp. growth after one week was cultured on f/2 media with varying concentrations of agar

Amphora sp. grew differently on each agar plate when agar concentrations were varied. Numerous colonies appeared on f/2 media containing 0.4 percent agar. Several colonies were also observed in F/2 media with 0.6 percent and 0.8 percent agar concentrations, but not as many as in 0.4 percent agar media. The higher the agar content in f/2 media, the fewer *Amphora* sp. colonies grew. Additionally, *Amphora* sp. colonies exhibited the

same characteristics. It demonstrated that the colonies that form were all of the same species and that no contamination had occurred.

Survival ability of Amphora sp. after culturing 4 weeks, 6 weeks and 8 weeks

Further observations were conducted on *Amphora* sp. cultured on f/2 medium with 4% agar. This *Amphora* sp. sample was regenerated at weeks 4, 6, and 8 (Figure 2).

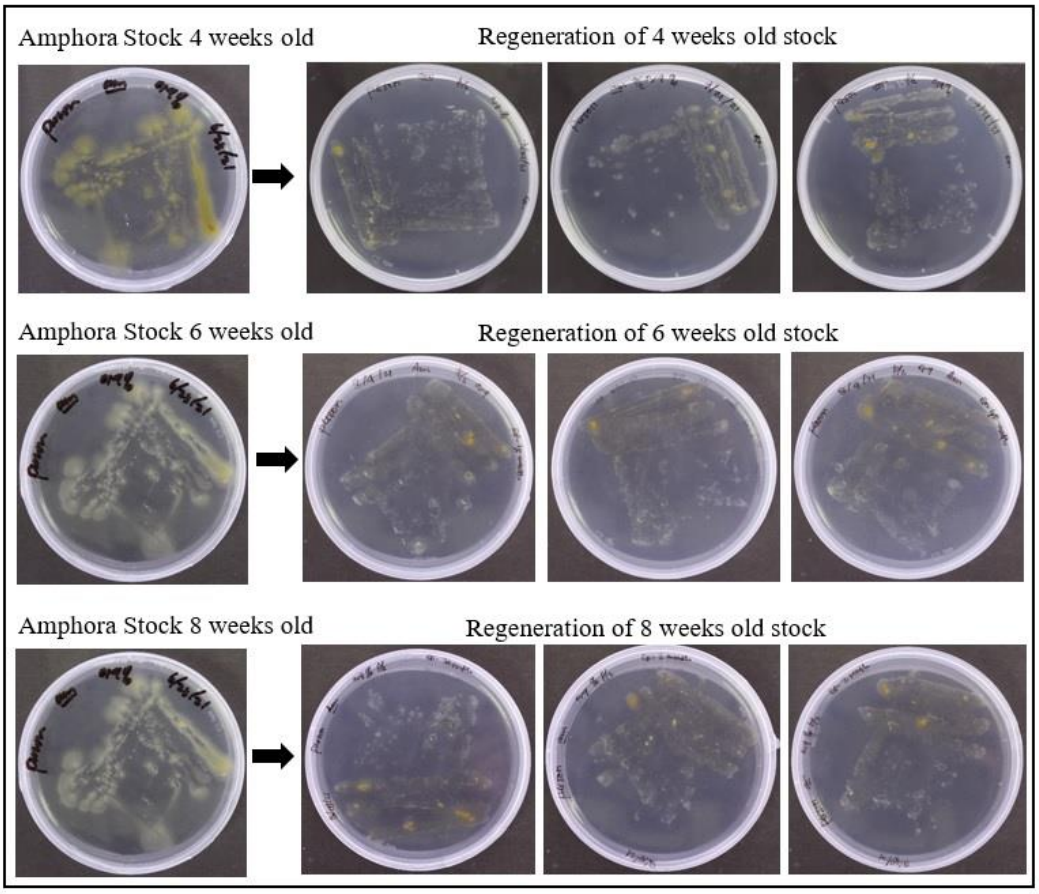


Figure 2. *Amphora* sp. regeneration occurs at weeks 4, 6, and 8

Observations were made for *Amphora* sp. growing on f/2 medium with 4% agar concentration. This concentration was based on prior findings showing the highest number of *Amphora* colonies appeared on this media.

Regeneration was performed at 4, 6, and 8 weeks of the same *Amphora* sp. sample. The colonies that formed after one week of inoculation onto the new agar plate were inspected. The regeneration of 4-week-old *Amphora* sp. colonies revealed that they had still developed on the new agar plates. *Amphora* sp. colonies were also grown after regeneration of *Amphora* sp. for six and eight weeks. No contamination was detected in any of the developed colonies whether from the regeneration of *Amphora* sp. in 4, 6, or 8 weeks.

Discussion

Microalgae preservation is critical in a series of microalgae research studies since they are an object of study that must be kept in good condition. The growth of diatoms on agar plates was observed in this study since prior research indicated that diatoms can grow on this medium. The study demonstrated that *Amphora* sp. colonies grew on each f/2 medium with agar concentrations of 0,4%, 0,6%, and 0,8%. This finding is also identical with (Kimura and Tomaru, 2013) , which produced algae on solid medium but used a different microalgae species. The decantation method demonstrated that *Chaetoceros tenuissimus* cells proliferated consistently on the surface of a dimpled plate according to the design of each pocket. (Andersen, 2005) examined the effects of various culture media on a variety of microalgal species. However, there is no universally applicable plate culture method for cultivating algae. Additionally, research on algal plate culture techniques has involved the use of soft agar media (Nagasaki and Imai, 1994) (Lakeman *et al.*, 2007). They discovered that such media are beneficial for a variety of algal species, but one significant disadvantage is that algal cells are lodged in the low-melting point agar gel. Cell embedding is a complicated operation that requires experience to do well.

Additionally, the agar plate method has advantages over the liquid media method, including greater durability. Microalgae culture on liquid media requires maintaining freshly prepared optimal conditions for 4–15 days (de Oliveira Louren ço, 2020), depending on the strain's growth rate, whereas it can last up to one month on agar media. The more frequently regeneration is performed, the greater the likelihood of mutation and contamination. Along with the agar plate and liquid media culture methods, another method is cryopreservation (Brand *et al.*, 2013). However, the treatment of *Amphora* sp. using this method has not been widely published.

The cultivation of algae varies considerably, based not only on the unique organism but also on the intended application of the culture. The streak plate method was used in this investigation. Since the microalgae

colonies will grow individually, this strategy can help to minimize contamination. Contamination will be easier to detect and re-purify. *Chlamydomonas*, *Pavlova*, *Synura*, and *Tetraselmis* are all examples of flagellate algae that grow well on agar; nevertheless, certain algae, including flagellates (such as *Heterosigma*, *Pelagomonas*, and *Peridinium*), do not grow well on agar. On agar, Coccoid, cryptophyte, and chlorarachniophyte cells can all grow normally (Parvin *et al*., 2007). F/2 media with varying agar concentrations were utilized. Regeneration has been attempted previously using 1% agar, but colonies remain rare. Thus, the percentage is to be lowered in this investigation. According to (Parvin *et al*., 2007), certain algae require trace agar concentrations between 0.3% and 0.6%.

Colonies that form are visible to the naked eye. However, to observe microalgae cells in greater detail, a microscope was used. The cell diameter of *Amphora* sp. is 10 µm (de Viçose *et al*., 2012). It was confined to colony observation, and confirmation of the absence of contamination in this study. The results were expected to serve as the foundation for selecting a suitable medium for microalgae storage. *Amphora* sp. may survive on agar plates for up to 8 weeks. This could be related to Amphora's slow growth rate. In a recent work, we discovered that when *Amphora* sp. was cultivated at 27 ℃ for three weeks under autotrophic circumstances, it had not yet reached the log phase, but another variety of diatom (*Phaeodactylum Tricornutum*) reached the log phase on day 12.

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