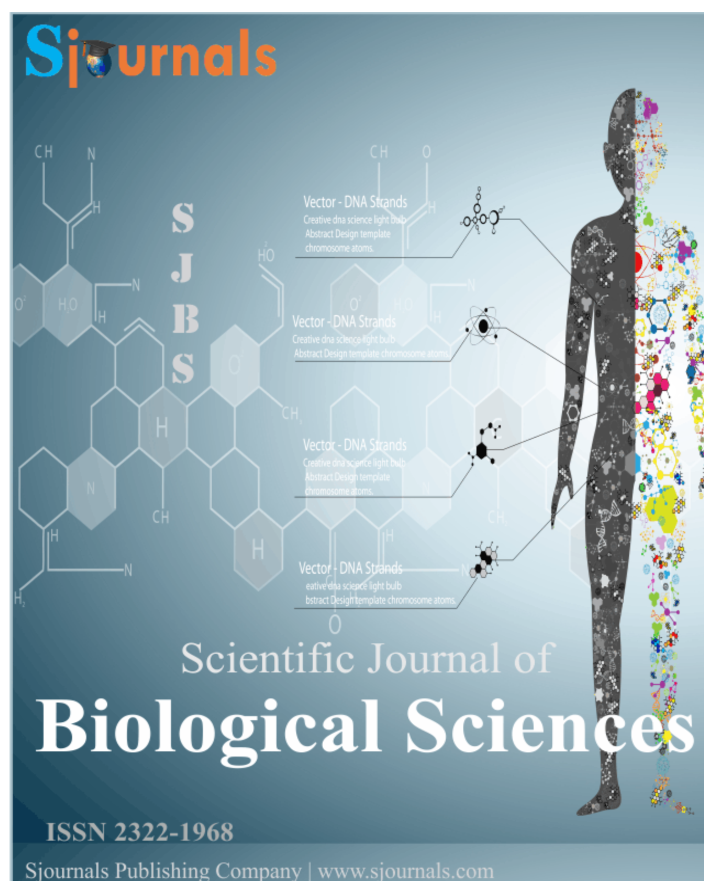
Provided for non-commercial research and education use.

Not for reproduction, distribution or commercial use.



This article was published in an Sjournals journal. The attached copy is furnished to the author for non-commercial research and education use, including for instruction at the authors institution, sharing with colleagues and providing to institution administration.

Other uses, including reproduction and distribution, or selling or licensing copied, or posting to personal, institutional or third party websites are prohibited.

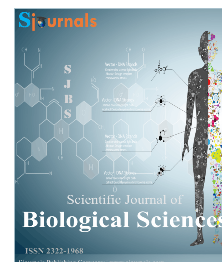
In most cases authors are permitted to post their version of the article (e.g. in Word or Text form) to their personal website or institutional repository. Authors requiring further information regarding Sjournals´s archiving and manuscript policies encouraged to visit:

http://www.sjournals.com

© 2018 Sjournals Publishing Company

Sjournals
Scientific Journals
Scientific Journal of Biological Sciences (2018) 7(1) 242-246
ISSN 2322-1968
doi: 10.14196/sjbs.v7i1.2570

CODEN (USA): SJBSBH



Contents lists available at Sjournals

Scientific Journal of **Biological Sciences**

Journal homepage: www.sjournals.com

Review article

The procedures of microalgal isolation from nature

Yasamin Pesaran Afsharian*
Department of Chemical Engineering, Islamic Azad University, Tabriz, Iran.

*Corresponding author: yasamin.afshar@yahoo.com

ARTICLE INFO

Article history,
Received 14 December 2017
Accepted 15 January 2018
Available online 22 January 2018
iThenticate screening 16 December 2017
English editing 14 January 2018
Quality control 21 January 2018

Keywords,
Microalgae
Resources
Sampling
Enrichment
Isolation

ABSTRACT

The application of microalgae in various industries like feed, food, cosmetic and pharmaceutical has accentuated the necessity of their isolation from nature. In some cases, experimental verifications are halted because of using inappropriate sampling or isolation methods. These limitations can be conquered by choosing more practical approaches. This study would assist the selection of more utilitarian isolation and sampling techniques in the base of sample types.

© 2018 Sjournals. All rights reserved.

1. Introduction

Healthy microalgae fulfill a greater lifespan and significant ability to make high quality products. This makes sense in that opting accurate sampling and isolation methods along with choosing suitable culture media can maintain the algal health and result sufficient products and prosperous experimental data. Obtaining a high quality algal biomass takes into account factors such as light, water temperature, nutrient concentration, salinity, and pH (Brennan and Owende, 2010; Mutanda et al., 2011). These elements must be adjusted to microalgae natural habitats in all of experimental stages.

For coastal marine algae, for instance, temperature and salinity are vital survival factors, while for oceanic phytoplankters water quality and metal toxicity are additional requirements. Among variety of algal species, soil algae can grow successfully in different laboratory conditions (Robert and Masanobu, 2005); furthermore, some

microalgae species would die after one or more subculturing as a result of lacking particular supplements, and some others' survival may be connected to the presence of other living organisms. The second means of getting victorious isolation is the elimination of weeds; since weeds are isolated and cultured more easier than target species, they would be in a sharp outcompete with focused cells (Robert and Masanobu, 2005); so that, omitting them definitely leads to improving target cell growth. Among other isolation methods, serial dilution, single-cell isolation, and streaking are widely used techniques.

The purpose of this study is to describe isolation steps besides introducing successful sampling techniques in order to declining experimental errors. Flourishing microalgae isolation is done followed by the underlying steps:

2. Microalgae isolation steps

2.1. The selection of microalgae sources

Diverse aquatic and continental habitats can be chosen as microalgae sources. References was introduced as satisfactory conductors of detecting renown target species, and ingenuity is proposed to discover unknown microalgal species from the nature.

2.2. Sampling

NC Department of Environmental Quality (North Carolina. June 2016) introduced three algal sample types: filamentous, periphyton, and phytoplankton. Filamentous algae are in long strands and can be picked up easily by hand, periphyton grow on rock, soil, or sand and can be collected by scraping, chipping or brushing, and phytoplankton are found in the water column and sampled with syringe, water-sampling bottles, or plankton-nets.

Collection method, time, good sterile technique, and preservation condition (Robert and Masanobu, 2005; Poonam et al., 2015) are essential involved factors in microalgae collection. Appropriate collection method is necessary for preventing demised or damaged cells. What's more, successful growth of isolated cells is closely related to algal condition or health at the time of the collection. Another aspect of time is necessity of rapid and constant transportation, particularly for organisms dying quickly within a few hours after sampling. Effective instrument sterilization declines the possibility of unwanted contaminants which can interrupt the isolation of unknown species. To manage the carrying well, plastic bottles were reported as the ideal way for transporting phytoplankton; the bottle must be washed out with the sample water and, then, filled without any bubble; it is better to cover the bottle in a paper or a wet toweling to isolate the sample during shipment; concentrated samples like dense bloom have to be diluted with filtered sample water before carrying (Maria and Carmelo, 2016). It is important to filtering samples immediately after sampling to remove zooplankters and other unwanted organisms (Robert and Masanobu, 2005).

Lastly, for mimicking the environmental condition in laboratory, it is vital to keep a record of biotic and abiotic factors of the sampling site (Mutanda et al., 2011). The location of the sampling site should be documented for reference and resampling.

2.3. Sample enrichment

Enrichment process provides, simultaneously, a suitable environment for growth and reproduction of target species, and maybe inhibitory one for unwanted organisms (Pringsheim, 1950). For preparation of specific physical and chemical conditions, it is important to have a basic knowledge of microalgal taxonomic and their natural habitat (Poonam et al., 2015).

Nutritional tension can wipe out target species immediately after sampling; however, this would not interrupt the algae survival in nature, because of recycling physiological action of other organisms (Robert and Masanobu, 2005). This nutritional limitation is deleted by enrichment process in laboratories. In some senses, unenriched samples are preferred when the target species is rare in contrast with weedy organisms (Robert and Masanobu, 2005). Enrichment monitoring is proposed to assess time for some species multiplying quickly after enriching but die suddenly and organisms reported to appear in the sample weeks or even months after sampling (Robert and Masanobu, 2005).

Soil-water extracts (Pringsheim, 1950; Pringsheim, 1912), macronutrients (Andersen, 2005), metals traces (Mutanda et al., 2011), and sea foam (Harold and Schlichting, 1971) were mentioned as the most common algal enrichments. According to the biomass concentration, a minimum amount of supplements should be added at

sequential stages over different time spans (Robert and Masanobu, 2005). pH adjustment is suggested to obtain bacteria-free media where the addition of supplements can instigate bacterial growth (Poonam et al., 2015).

2.4. Isolation

2.4.1. Direct isolation

The main purpose of direct isolation is single cells, colonies or filaments transference from enriched media onto sterilized agar medium or into a decontaminated broth. In direct isolation technique, the first step is providing the proper culture medium. The main reason is that dissimilar algal species have different chemical preferences. Weedy microalgae such as chlorella and most of the diatoms grow well in both poor and supplemented medium as opposed to many others preferring only weak culture medium (Robert and Masanobu, 2005).

Algae species with tiny body structure have some limitations in the selection of isolation method. Tiny freshwater species can often be grown on agar, while oceanic ones prefer dilution technique with the use of poor medium without any particulates. Solid state isolation, liquid state isolation, and single-cell isolation are three common direct isolation techniques (He et al., 2012).

2.4.1.1. Solid state isolation

Streaking can isolate a pure strain by making axenic cultures. In this process, samples are streaked onto agar surface via a loaded loop. After streaking, the incubation could be lasted from a few days for soil and freshwater algae up to several months for oceanic species. Microalgal colonies are distinguishable by means of their specific morphological characteristics like size, shape, and color (José, 2015). The isolated colony can be further subcultured onto plates or into broth by the use of a micropipette or nichrome or loop. Streaking mentioned as a prosperous technique for most algal strains, especially coccoids, diatoms, soil microalgae, filamentous algae, and small cyanobacteria. An atomizer can be utilized to scatter small droplets onto agar surface under sterilized condition (Pfau et al., 1971).

Agar pour technique is used to isolate algae preferring to grow into agar rather than agar surface (Toledo and Palenik, 1997). Although it is possible to maintain cells by continuous agar pour technique, this method is only used to isolate cells, in corporation with other manners. Removing epiphytes from filamentous algae is done via dragging through agar. This process often cuts off the epiphytes from the filament, and the cleaned filament can then be subcultured.

2.4.1.2. Liquid state isolation

Serial dilution results in a geometric progression of a solute concentration in a logarithmic fashion. This protocol results in reducing the microorganisms' concentration in medium, and it is effective for dominant organisms and most often to discover new species. This stepwise method is usually done prior to establishing a single-cell isolation. Its main purpose is to deposit one cell into a test tube, flask, or plate (Throndsen, 1978). The estimation of dilution sets and the number of anticipated cells in the enriched culture are directly related (Poonam et al., 2015). A small volume of the enriched sample is assumed to have a single cell (Robert and Masanobu, 2005). For unknown cell numbers, making repeated serial dilutions of 1: 10, and between five and six repetitions is proposed as a practical strategy (Robert and Masanobu, 2005). The success of the technique depends on the accuracy of the measured amount of cell culture during the transference from one medium to another.

Culture medium, distilled water, seawater, filtered water from the sample site, or some combination of these can be used as dilution (Robert and Masanobu, 2005). Weedy organisms need full- strength medium in contrast with fastidious species requiring very dilute culture media. In some cases, it is necessary to add some supplements into isolation tubes; moreover, isolates should be incubated at different temperature or light regimes.

2.4.1.3. Single-cell isolation

This method deposits a single cell into a sterilized medium under microscopic observation by means of prepared micropipette. Sterile seawater, pond water, or other culture media can be used as droplets. Single-cell isolation is employed in two different ways (Robert and Masanobu, 2005): first by picking up the cells via micropipette, and second by deleting unwanted cells surrounding the target cells.

Single-cell technique is mostly used for the separation of phytoplankton (Maria and Carmelo, 2016) and sand

dwelling algae. Loose flagellates and attached algae are two common types of sand adhering species. Micropipettes are utilized to collect free flagellates from water surface, and strong adhering algae from coverslips (Round, 1981). For preventing from shear stress, the prepared micropipette tip should be in an appropriate size. Too small tips result in cell damage, and too large openings, will make the cell gathering difficult because of accumulating unwanted materials. Viable cells are identified by some signs of damages like cessation of swimming in flagellates, different light refraction in diatoms, and Leakage of protoplasm (Robert and Masanobu, 2005).

Traditional single-cell isolation is time-consuming and requires sterilized requirements. An automated method is a rapid and more efficient one (Montero et al., 2011); however, the instruments are costly and need responsible experts.

2.4.2. Other isolation methods

Some of the other isolation techniques comprises: centrifugation and settling, UV irradiation, filtration, addition of chemicals (Lee and Shen, 2004), and phototaxis (Andersen, 2005). Gravity separation, centrifugation and settling, is famous for condensing target species rather than single cell separation. For achieving single target cells, it must be coupled with other isolation methods. Centrifugation results loose pellets from large dinoflagellates and provides decanting for concentrating smaller cells. Cell damaging as a consequence of shear stress is the sole limitation of centrifugation. Settling is more helpful for nonswimming weighty cells. Density gradient centrifugation is another effective tactic for microalgae separation from mixture of organisms (Whitelam et al., 1983), or isolation from other algal species (Poonam et al., 2015).

As most microalgae species are more resistant to ultraviolet light than other single cell organisms (Yuan et al., 2013), UV irradiation before streaking can lead to isolated algal colonies. Vacuum membrane filtration followed by sonication is employed to filamentous algae separation from bacteria (Yuan et al., 2013). Antibiotics, such as Imipenem, Nystatin and Cycloheximide, are used to eliminate prokaryotic or eukaryotic contaminants from culture media (Schwartz et al., 1990). The addition of germanium dioxide in enrichment cultures can stop diatom growth if they are not desired (Lewin, 1966). Phototaxis is influential for isolation of flagellates (Guillard, 1995) which are powerful swimmers and phototactic. In this method, an intense light source is used to isolate cells via a prepared micropipette (Guillard, 1973).

3. Conclusion

This work overviewed usual microalgal sampling and isolation approaches, sampling techniques are chosen according to previous successful researches in well-known species, and creative selection is used for unfamiliar organisms. Isolation methods must be selected according to the microalgal taxonomy, and experimental parameters should be fixed with their environmental conditions. Direct isolation techniques are the main isolation methods, and result isolated individual cells or colonies, while other isolation techniques must be employed incorporation with these methods. Lastly, utilizing full-automatic equipment is a prospect in organism isolation science and can manage time and monetary expenses; in addition, mechanized sampling and isolation methods are beneficial for human health especially in evaluating harmful algal species like cyanobacteria.

References

Andersen, R.A., 2005. Algal culturing techniques. Elsevier/Academic Press.

Brennan, L., Owende, P., 2010. Biofuels from microalgaeda review of technologies for production, processing, and extractions of biofuels and co-products. Renew. Sustain. Energ. Rev., 14, 557-577.

Guillard, R.R.L., 1973. Methods for microflagellates and nannoplankton. In: Stein, J.R. (Ed.), Handbook of phycological methods: Culture methods and growth measurements. Cambridge University Press, Cambridge, 69-85.

Guillard, R.R.L., 1995. Culture methods. In: Hallegraeff, G.M., Anderson, D.M., Cembella, A.D. (Eds.), Manual on harmful marine microalgae. IOC Manuals and Guides No. 33. UNESCO, Paris, 45-62.

Harold, E., Schlichting, J.R., 1971. A preliminary study of the algae and protozoa in seafoam. Bot. Mar., 14, 24-28.

He, M.L., Li, L., Liu, J.G., 2012. Isolation of wild microalgae from natural water bodies for high hydrogen producing strains. Int. J. Hydrogen Energ., 37, 4046-4056.

Hoppenrath, M., 2000. Morphology and taxonomy of six marine sand-dwelling Amphidiniopsis species

(Dinophyceae, Peridiniales), four of them new, from the German Bight, North Sea. Phycologia., 39, 482-497.
José C.M. Pires, 2015. Mass production of microalgae. In: Se, K.K. (Ed.), Handbook of marine microalgae. Elsevier Inc., 55-68.
Lee, Y.-K., Shen, H., 2004. Basic culturing techniques. In: Richmond, A. (Ed.), Handbook of microalgal culture: Biotechnology and Applied Phycology. Blackwell Publishing, Australia, 40-56.
Lewin, J., 1966. Silicon metabolism in diatoms. V. germanium dioxide, a specific inhibitor of diatom growth. Phycologia., 6, 1-12.
Maria, N.T., Carmelo, R.T., 2016. Microalgae current research and applications. Caister Academic Press, U.K.
Montero, M.F., Aristizabal, M., Reina, G.G., 2011. Isolation of high-lipid content strains of the marine microalga Tetraselmis suecica for biodiesel production by flow cytometry and single-cell sorting. J. Appl. Phycol., 23, 1053-1057.
Mutanda, T., Ramesh, D., Karthikeyan, S., Kumari, S., Anandraj, A., Bux, F., 2011. Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production. Bioresour. Technol., 102, 57-70.
Mutanda, T., Ramesh, D., Karthikeyan, S., Kumari, S., Anandraj, A., Bux, F., 2011. Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production. Bioresour. Technol., 102, 57-70.
Pfau, J., Werthmiller, K., Senger, H., 1971. Permanent automatic synchronization of microalgae achieved by photoelectrically controlled dilution. Arch. Microbiol., 75, 338.
Poonam, S., Sanjay, K.G., Abhishek, G., Ismail, R., Faizal, B., 2015. Microalgae isolation and basic culturing techniques. In: Se, K.K. (Ed.), Handbook of marine microalgae. Elsevier Inc., London, 43-54.
Pringsheim, E.G., 1912. Die kultur von algen in agar. Beitr. Biol. Pfl., 11, 305–332.
Pringsheim, E.G., 1950. The soil-water culture technique for growing algae. In: Brunel, J., Prescott, G.W., Tiffany, L.H. (Eds.), The culturing of algae. Charles F. Kettering Foundation, Dayton, Ohio, 19-26.
Robert, A.A., Masanobu, K., 2005. Traditional microalgae isolation techniques. In: Robert, A.A. (Ed.), Algal culturing techniques. Elsevier Inc., California, 83-100.
Round, F.E., 1981. The ecology of algae. Cambridge University Press, Cambridge.
Schwartz, R.E., Hirsch, C.F., Sesin, D.F., Flor, J.E., Chartrain, M., Fromtling, R.E., Harris, G.H., Salvatore, M.J., Liesch, J.M., Yudin, K., 1990. Pharmaceuticals from cultured algae. J. Ind. Microl., 5, 113-124.
Throndsen, J., 1978. The dilution-culture method. In: Sournia, A. (Ed.), Phytoplankton manual. UNESCO, Paris, 218-224.
Toledo, G., Palenik, B., 1997. Synechococcus diversity in the California current as seen by RNA polymerase (rpoC1) gene sequences of isolated strains. Appl. Environ. Microbiol., 63, 4298-303.
Whitelam, G.C., Lanaras, T., Codd, G.A., 1983. Rapid separation of microalgae by density gradient centrifugation in percoll. Br. Phycol. J., 18, 23.
Yuan, K.L., Wei, C., Hui, S., Danxiang, H., Yantao, L., Howland, D.T.J., Jerilyn, A.T., Qiang, H., 2013. Basic culturing and analytical measurement techniques. In: Amos, R., Qiang, H. (Eds.), Handbook of microalgal culture. John Wiley and Sons, Ltd., 37-68.

How to cite this article: Pesaran Afsharian, Y., 2018. The procedures of microalgal isolation from nature. Scientific Journal of Biological Sciences, 7(1), 242-246.

Submit your next manuscript to Sjournals Central and take full advantage of:
- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in DOAJ, and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at www.sjournals.com

Sjournals
where the scientific revolution begins