Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biotechnology Advances 21 (2003) 585–598
BIOTECHNOLOGY ADVANCES
www.elsevier.com/locate/biotechadv

Producing drugs from marine sponges

El Hassan Belarbi [a,*], Antonio Contreras Gómez [a], Yusuf Chisti [b], Francisco García Camacho [a], Emilio Molina Grima [a]

[a] *Department of Chemical Engineering, University of Almería, 04071 Almería, Spain*
[b] *Institute of Technology and Engineering, PN 456, Massey University, Private Bag 11 222, Palmerston North 5320, New Zealand*

Abstract

Marine sponges are potential sources of many unique metabolites, including cytotoxic and anticancer compounds. Natural sponge populations are insufficient or inaccessible for producing commercial quantities of metabolites of interest. This review focuses on methods of producing sponge biomass to overcome supply limitations. Production techniques discussed include aquaculture in the sea, the controlled environments of aquariums, and culture of sponge cells and primmorphs. Cultivation in the sea and aquariums are currently the only practicable and relatively inexpensive methods of producing significant quantities of sponge biomass. In the future, metabolite production from cultured sponge cells and primmorphs may become feasible. Obtaining a consistent biomass yield in aquariums requires attention to many factors that are discussed in this work.
© 2003 Elsevier Inc. All rights reserved.

Keywords: Sponges; Sponge aquaculture; Marine biotechnology; Bioactives

1. Introduction

The potential of marine life as a source of novel molecules is immense and has been barely investigated. Because of their longer evolutionary history, marine organisms likely posses a greater molecular diversity than do their terrestrial counterparts. In comparison with the other lifeforms, bioactive compounds have been detected especially frequently in sponges. Sponges (phylum *Porifera*) are most primitive of the multicelled animals that have existed for 700–800 million years. Of the approximately 15,000

* Corresponding author. Tel.: +34-950-015566; fax: +34-950-015484.
E-mail address: ebelarbi@ual.es (E.H. Belarbi).

0734-9750/03/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0734-9750(03)00100-9

sponge species, most occur in marine environments. Only about 1% of the species inhabits freshwater.

Sponges produce toxins and other compounds to repel and deter predators (Uriz et al., 1996a; Pawlik et al., 2002), compete for space with other sessile species (Porter and Targett, 1988; Davis et al., 1991; Becerro et al., 1997), and for communication and protection against infection. Of the investigated marine sponge species, >10% has exhibited cytotoxic activity (Zhang et al., 2003) suggesting production of potential medicinals. Potentially therapeutic compounds identified in sponges include anticancer agents and immunomodulators. Some sponges seem to produce potentially useful antifouling agents (Armstrong et al., 1999).

Although many bioactives have been discovered in sponges (Garson, 1994; Uriz et al., 1996b; Osinga et al., 1998; Munro et al., 1999; Pomponi, 1999; Faulkner, 2000; Sepcic, 2000; Richelle-Maurer et al., 2003), only a few of these compounds have been commercialized. Concentrations of the desired bioactives in sponges are generally low, e.g. 0.4% of dry weight, but concentrations as high as 12% have been recorded for some metabolites (Unson et al., 1994).

Sponges can attain an enormous size (Fig. 1), but they grow slowly (e.g. a biomass doubling time of months to over a year) and the growth rate depends a lot on the species and culture conditions. In nature, growth varies with season and this is partly linked with seasonal variations in the feed quantity and quality. In most cases, the natural sponge population is too small or too inaccessible for commercial harvest (Pomponi, 1999);

Fig. 1. A large barrel sponge. Courtesy of Jonathan Bird, Oceanic Research Group.

however, at least one compound, manoalide, is recovered from the sponge *Luffariella variabilis* harvested in the wild (Pomponi, 1999).

Sponges often have associated symbiotic microbial populations (Lee et al., 2001; Richelle-Maurer et al., 2003). Symbionts include archaea, bacteria, cyanobacteria, and microalgae. In some cases, these microorganisms and not sponge cells are the likely source of the secondary metabolites of interest (Bewley and Faulkner, 1998; Lee et al., 2001; Proksch et al., 2002). For example, the polybrominated biphenyl ether antibiotics isolated from the sponge *Dysidea herbacea* are really produced by the endosymbiotic cyanobacterium *Oscillatoria spongeliae* (Osinga et al., 1998). Work on isolation and cultivation of sponge symbionts and the nature of symbiotic relationships have been reviewed elsewhere (Lee et al., 2001). Fungi associated with marine sponges are also known to produce many bioactive agents (Holler et al., 2000).

This review focuses on the strategies for producing sponge biomass for the recovery of bioactive agents. Sponges can be cultivated from cuttings taken from a parent and 'planted' in the sea or the better-controlled environments of aquariums. In addition, culture of sponge cells and various types of cell aggregates provides an alternative method for producing sponge metabolites. These methods are discussed here.

2. Sea-based aquaculture

Cultivation of sponge in the sea from cuttings (explants) was first established over a century ago for producing bath sponge. This technology has reemerged and is being advanced for producing sponge-sourced metabolites (Verdenal and Vacelet, 1990; Adams et al., 1995; Battershill and Page, 1996; Duckworth et al., 1997; Müller et al., 1999a; Munro et al., 1999). This "sponge farm" approach can be used at various levels of sophistication and can include the use of temperature-controlled chambers provided with supplemental feed and flow of oxygenated sea water. Sponge species that have been cultivated in the sea include *Latrunculia brevis*, *Lissodendoryx nsp*, *Mycale mrryi*, *Polimastia croceus*, and *Raspailia agminata*. Sponges grown in the sea have been shown to produce the metabolites of interest (Battershill and Page, 1996; Müller et al., 1999a; Munro et al., 1999).

Although marine aquaculture is being developed to produce sponge biomass inexpensively, aquaculture in the sea has significant limitations. The culture conditions cannot be controlled for sustained rapid growth and the productivity is susceptible to vagaries of weather. In addition, sponge farms are vulnerable to disease and infestations of parasites. Warmer periods can be particularly troublesome, as sponges succumb to pathogens more readily during warm periods. The historical development of sponge aquaculture has been reviewed by Osinga et al. (1999a).

3. Contained cultivation in aquariums

Culture in fully contained aquariums (Pennec et al., 2003) can provide superior control of production conditions. The culture requirements of a sponge depend primarily on the

natural habitat from which it originated. Some habitats naturally experience significant environmental fluctuations (e.g. variable currents in estuarine habitats) and the endemic species are better adapted to tolerating the fluctuations. In contrast, other sponges respond adversely to small changes in the environment.

Aquaculture from explants is the preferred method of cultivation. Explants generally grow more rapidly than do whole sponge transplants (Kinne, 1977). The techniques for explant preparation have been described by Simpson (1963) and others. Explants should be cut from a healthy sponge submerged in relatively cold water. The parent should be free from parasites and other encrusting growths. Minimum time should elapse between collection from the wild and the cutting of explants. Between collection and processing, the sponge should be held in cold water (e.g. 4–6 °C). A sharp sterile scalpel should be used for cutting explants. Explants should be cut in such a way that each cutting includes a part of the exterior surface of the parent sponge, i.e. the surface covered with the skin cells (Osinga et al., 1999a). Larger explants having a high proportion of intact skin survive better than small explants (Duckworth et al., 1997). Squeezing the cutting can damage the tissue (Osinga et al., 1999a).

Antibiotic added to water during cutting can prevent future infections. After the explants are cut, the water should be changed to prevent possible poisoning of the explants by substances released during the cutting. Explants can be strung on nylon ropes for cultivation in the sea. In the relatively confined environment of aquariums, any cuttings that begin to decay should be removed as soon as possible to prevent poisoning. Care should be taken to prevent inadvertent co-introduction of larval forms of sponge predators with the explants.

Explant culture has been described for the Mediterranean sponge *Chondrosia reniformis* (Nickel and Brümmer, 2003), the cold water boreal sponge *Geodia barretti* (Hoffmann et al., 2003), *Geodia cydonium* (Müller et al., 1999a), *Pseudosuberites andrewsi* (Osinga et al., 1999a,b, 2003), *C. reniformis* (Nickel and Brümmer, 2003), *Ephydatia fluviatilis* (Francis et al., 1990), and many other morphologically distinct sponge species (Battershill and Page, 1996; Duckworth et al., 1997). The mass of growing explants can be estimated from measurement of the projected area (Ayling, 1983; Osinga et al., 1999b) and direct weighing under water (Osinga et al., 1999b). Growth morphology is known to be influenced by the intensity of the prevailing water current (Kaandorp, 1999).

Many sponges do not survive exposure to air. Removing the sponge from water drains the pores and channels (the aquiferous system) in the sponge body and fills them with air. When the sponge is returned to water, the channels of the aquiferous system remain blocked with air and the circulation of water is not reestablished. Therefore, all collection and manipulations should be performed under water.

In a closed cultivation system, it may be possible to achieve proliferation by inducing the sponge to produce larvae. Depending on the species, release of larvae may be induced by an increase in temperature, exposure to light, and changes in velocity of the prevailing current (Osinga et al., 1999a). In addition, sponge culture can be initiated from buds and gemmules (i.e. reduction bodies) that form when the sponge encounters an unfavorable environment (Osinga et al., 1999a).

An aquarium bioreactor for sponge cultivation should be sufficiently mixed for suspending the feed particles and providing uniform temperature and oxygen levels.

Sedimentation of food and other organic matter should be prevented so that anaerobic zones do not develop because of bacterial activity. The water flow should remove any secreted metabolites and excrement. These and other considerations relevant to contained culture in aquariums are discussed in the following sections.

3.1. Feeds and feeding

Sponges are sessile filter feeders. Water laden with nutrient particles is drawn through small pores or ostia on the outer wall of the sponge and expelled through larger openings called oscula (Fig. 2). This network of channels and chambers constitutes the ''aquiferous'' system of the sponge. The flow is unidirectional and is driven by movement of cilia on choanocytes, the cells lining the expanded chambers in aquiferous channels. The pumping action of the aquiferous system is easily demonstrated by squirting a nontoxic dye at the base of the sponge and observing the colored stream emerge from the oscula (Fig. 3). The pumping rate can be quantitatively determined using the uptake of particles as an indicator (Turon et al., 1997).

Choanocytes trap and internalize the food particles. Ingested feed particles have been observed in choanocytes within 2 h of feeding (Osinga et al., 1999b). To enter and flow through the intake pores to reach the flagellated choanocytes, food particles must be generally <50 μm in size. Choanocytes filter out and ingest even the tiniest particles. Ingested particles that remain undigested are released back into the outflowing water. Bacteria-sized particles are filtered out most efficiently.

Sponges feed nonselectively. All kinds of plankton, bacteria, decaying organic particles, and dissolved organic matter may be used as food (Pile et al., 1996, 1997). Because the uptake of particulates is nonselective, inert detritus such as clay are also taken up. Movement of nonfood inerts into the aquiferous system can significantly reduce its ability to pump water (Gerrodette and Flechsig, 1979). A reduced pumping in turn means a low nutrient intake and consequently a reduced growth rate. Sponge farms and aquariums should be designed to prevent occurrence of inert suspended particles in the culture environment.

In aquariums and other closed systems, bacteria (e.g. *Escherichia coli*), yeasts, and microalgae (e.g. *Chlorella sorokiana*, *Rhodomonas* sp., *Nannochloropsis* sp., *Phaeodacty-*

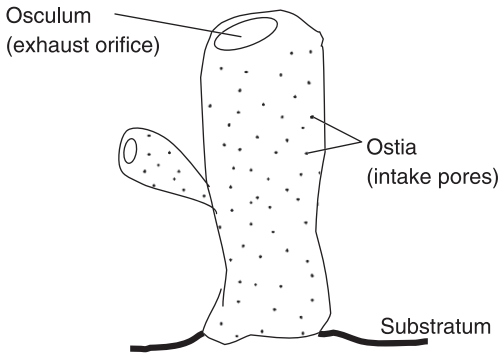

Fig. 2. Intake and exhaust orifices of the aquiferous system of a tube sponge.

Fig. 3. A dye squirted around the base of a purple tube sponge colors the jet emerging from the osculum at the top of the sponge. Courtesy of Jonathan Bird, Oceanic Research Group.

lum tricornutum; *Chlamydomonas reinhardtii*) have been used as sponge feed (Poirrier et al., 1981; Francis et al., 1990; Imsiecke, 1994; Thomassen and Riisgård, 1995; Osinga et al., 1999b, 2003; Sipkema et al., 2003; Nickel and Brümmer, 2003; Zhang et al., 2003). Not all feeds are equally satisfactory. For example, the tropical sponge *P. andrewsi* grew distinctly better when fed on the microalga *P. tricornutum* compared to when the feed was *Nannochloropsis* sp., another microalga (Osinga et al., 2003). Food quantity and nutritional quality are important considerations that have not been studied to any depth. Studies suggest that the often reported difficulties in sustaining sponge growth in aquariums may have to do with an unsatisfactory quality of food and not its insufficiency (Osinga et al., 2001). Approaches for selecting suitable aquaculture feeds have been outlined by Osinga et al. (1999a). There is some evidence that sponges can absorb dissolved organic nutrients present in the water (Osinga et al., 2001, 2003; Belarbi et al., 2003). Uptake of dissolved amino acids by the sponge *Cliona celata* has been documented (Ferguson, 1982).

3.2. The need for silica

A great number of marine sponges require silica for building the needle-like spicules that constitute a part of the sponges' skeletal support. Spicules are synthesized by

specialized cells (sclerocytes) that deposit the dissolved silica taken in with the water on protein filaments (Bergquist, 1978). Lack of dissolved silica can easily limit sponge growth. Indeed, the extinction of many sponge species during the Cretaceous period is associated with a decrease of the available silica in the oceans because of the emergence of diatoms that have efficient systems for capturing silica (Maldonado et al., 1999).

A minimum concentration of dissolved silica must be maintained in the culture medium to prevent a limitation. The silica consumption of the sponge *Halichondri panicea* follows Michaelis–Menten kinetics and depends on the availability of other nutrients (Reincke and Barthel, 1997). Methods for determining silica uptake rate have been described by Frøhlich and Barthel (1997). Dissolved silica can be supplied as sodium metasilicate (Na_2SiO_3) (Osinga et al., 1998, 1999a) and sodium fluorosilicate (Na_2SiF_6) (Reincke and Barthel, 1997). Osinga et al. (1998, 1999a,b) used a 0.25-mM concentration of $Na_2SiO_3 \cdot 9H_2O$ for maintaining *P. andrewsi* in an aquarium.

Natural diet of sponges includes many diatoms such as *P. tricornutum* that have a siliceous exoskeleton. In principle, therefore, some of the silica needed by the sponge could be supplied via the diatoms, but it is unclear if this silica can be used to form spicules. Use of a diatom feed has enhanced growth of the sponge *P. andrewsi* relative to using other microalgal feeds (Osinga et al., 2003), but this effect has not been conclusively linked to the presence of a siliceous exoskeleton in diatoms.

3.3. Salinity, pH, and temperature

Marine sponges should be cultured at the salinity of seawater (35 ‰ wt/wt dissolved salts). A hypersaline environment tends to dehydrate the sponge cells whereas a lower than normal salinity could lead to dilution of the intracellular content. Salinities of up to 46 ‰ are tolerated by species such as *Hippospongia lache* (Osinga et al., 1999a) but salinities of less than 26 ‰ can be lethal (Osinga et al., 1999a). The normal cultivation pH for marine sponges is between pH 7.8 and 8.4, i.e. the pH of seawater (Brown et al., 1992).

Sponges are sensitive to temperature. In nature, most sponges experience only a slow seasonal change in temperature and are not adapted for too rapid or too big a fluctuation in ambient temperature. A decrease in temperature is generally better tolerated than a temperature rise (Osinga et al., 1999a). Too high a temperature crashes the culture. Massive die-offs of the Mediterranean sponge *Crambe crambe* were recorded in France and Italy during the summer of 1999 after a prolonged increase in temperature of up to 24 °C. A high temperature normally stimulates sexual reproduction in sponges. To prevent diversion of metabolic energy from biomass generation to sexual reproduction, the culture environment should maintain a temperature slightly lower than the summer temperature of the normal sponge habitat.

3.4. Dissolved oxygen

Sponges require oxygen. Oxygen is absorbed from the water flowing through the aquiferous system. Oxygen consumption ranges from 0.2 to 25 μmol O_2 h^{-1} per cubic centimeter of sponge volume (Osinga et al., 1999a). Oxygen consumption rates for specific marine sponges have been compiled by Osinga et al. (1999a). Some sponge

species are adversely affected by less than a minimum level of dissolved oxygen being available. Oxygen is generally provided by aerating the water by bubbling before it is fed to the aquarium or bubbling air within a confined volume of the aquarium.

3.5. Effects of light

Many tropical sponges harbor photosynthetic endosymbionts that require light for survival. Compounds produced by these symbionts provide nutrients for the sponge (Sarà, 1971). As a consequence, the net primary productivity of some sponges is dependent on the availability of light and light intensity can strongly influence the geographic distribution of sponge species (Wilkinson, 1983). Sponges with a high concentration of photosynthetic endosymbionts require less organic food for energy.

Although light promotes the growth of sponges with photosynthetic endosymbionts, light is not always beneficial. Growth inhibition by light has been documented (Wilkinson and Vacelet, 1979) and appears to be associated with the sponges' sensitivity to ultraviolet radiation (Wilkinson and Vacelet, 1979; Osinga et al., 1999a). Generally, sponges should be grown in the dark unless a species harboring photosynthetic symbionts is being cultivated.

3.6. Waste removal

In closed culture systems, the bioactive and cytotoxic agents produced by the sponge can rapidly build up to inhibitory levels. Similarly, metabolic wastes (mainly ammonia) accumulate rapidly. Metabolically produced ammonia is toxic to most aquatic animals in low concentrations. Ammonia concentrations as low as 60 μM can kill half the exposed population of many marine invertebrates. Therefore, ammonia must be continuously removed from a closed culture system. Accumulation of ammonia is likely a severe problem in high-density cultivation systems, but there is little information on the rate of generation of ammonia and ammonia tolerance of sponges. Toxicity of ammonia is pH dependent. The ammonium ion (NH_4^+) does not readily permeate the cell and is therefore much less toxic than NH_3. The relative concentration of these two forms depends on pH.

Accumulation of inhibitors may not pose a problem if the water is used on a once through basis. If, however, some or all of the aquarium's water is recycled, metabolic wastes and other inhibitory excreted metabolites must be removed from the recycle flow. Wastes can be removed by passing the water through a biofilter containing a naturally developed microbial population. Such biofilters are commonly used in fish tanks and other aquaculture systems. A biofilter is likely to be ineffective for removing any toxic secondary metabolites that may be excreted by the sponge, but adsorption methods can be devised to remove such compounds.

4. Cell and primmorph culture

Sponge cells suspended in a nutrient broth may be potentially induced to produce metabolites of interest, but this has not been demonstrated on any significant scale. Most sponge cells are totipotent, i.e. individual cells can regenerate the whole sponge, and

therefore cell culture may be a way of initiating a homogeneous sponge population that is free of contaminants. This totipotent capability of sponge cells was demonstrated as early as 1907 (Wilson, 1907).

Primary cultures of sponge cells are produced by dispersing the largely undifferentiated cell mass that constitutes the sponge (Pomponi and Willoughby, 1994; Ilan et al., 1996; Müller et al., 1999b; Zhang et al., 2003; Richelle-Maurer et al., 2003; De Rosa et al., 2003). Sponge cells are easily dissociated by agitating and rubbing small explants in artificial seawater that is free of Ca^{2+} and Mg^{2+}. The crude suspension is filtered through a wire mesh screen or cheese cloth to remove debris and provide a suspension of cells in the filtrate. A small amount of EDTA (e.g. 10 mM) added to the water helps in dissociating the cells by chelating any multivalent metal ions. Sponge cells vary significantly in size depending on the species. Dissociated cells of *Stylotella agminata* tend to be 5–10 μm in size (Zhang et al., 2003). Sponges such as *Suberites domuncula* have larger cells of 20–60 μm.

Although primary sponge cells in suspension can be induced to divide using phytohemaglutinin (a mitosis inducing lectin), proliferation ceases after a few division cycles (Pomponi et al., 1997). An inability to maintain cell division and thus establish continuous cell lines remains a major hurdle to using cultivated cells for metabolite production.

No continuous cell lines appear to have been established from sponges (Leys, 1997; Rinkevich, 1999) even though many attempts have been made (Ilan et al., 1996; Pomponi and Willoughby, 1994; Pomponi et al., 1997). Primary in vitro cultures of adult sponge cells have been maintained for nearly 6 months (Ilan et al., 1996) and cultures derived from sponge embryo have survived for almost twice as long as adult cell cultures (Rinkevich, 1999). In nature, sponges can live for over 1500 years. This longevity is consistent with the observed high telomerase activity (a genetic indicator of proliferative potential) of most sponges. The failure of disaggregated cells to thrive for long is probably associated with the apparent need for cell–cell contact for maintaining a proliferation capability. Cellular assemblies of *S. domuncula* and *G. cydonium* have been shown to have a high telomerase activity, indicating a high proliferation capacity (Koziol et al., 1998). Telomerase activity was lost rapidly on disaggregating the cells (Koziol et al., 1998). The inability to maintain cell division in dissociated cells may be overcome if cancerous cells of sponge can be found or cancer can be induced into available cells. Alternatively, it may be possible to fuse normal sponge cells with immortal cells of other marine invertebrates to produce hybridomas capable of continuous growth.

Sponge cells in suspension can be difficult to distinguish from other contaminating cells. In the past, protozoa and thraustochytrides have been cultivated under the mistaken impression that they were sponge cells (Klautau et al., 1994; Custodio et al., 1995; Rinkevich, 1999). In view of the ease of contamination, authenticity of cultured cells should be regularly verified using DNA methods or by analyzing for the presence of specific metabolites (Pomponi et al., 1997). Axenic sponge cells have been obtained in a few cases (Wijffels et al., 2001). Some primary cells have been shown to produce metabolites of interest both before and after induced cell division (Pomponi et al., 1997).

If freely suspended continuous cultures of sponge cells can be established and shown to produce the desired metabolites, the cells could be grown in bioreactors of the kind now used for animal cells (Spier, 2000). Many of the same bioreactor design issues that are relevant for current commercial animal cell cultures would need addressing for sponge

cells (Chisti, 2000, 2001). Culture of sponge cells anchored on commercial microcarriers has been achieved for at least one sponge species. Immobilization of sponge cells in artificial matrices (e.g. agarose beads) has been attempted and some proliferation has been observed at least over the short term (Wijffels et al., 2001).

Primmorphs are an organized, usually spherical, clump of cells produced by primary cells in suspension culture. A culture of primary cells will generally form aggregates within hours and primmorphs within days (Zhang et al., 2003). Depending on the sponge, primmorphs can range in size from 40 μm to 3 mm. Cross sections through primmorphs reveal an organized structure with an outer unicellular epithelium-like layer of pinacocytes and central core made primarily of spherulous cells (Müller et al., 1999b). The primmorph structure is discussed further by Wijffels et al. (2001) and Sipkema et al. (2003). Primmorphs survive extended starvation. Unlike dissociated sponge cells, primmorphs retain telomerase activity. If formed in the presence of sponge symbionts, the symbiotic microorganisms will be included in the primmorphs. In contrast, cellular detritus and nonsymbiotic microorganisms that may be present in suspension are excluded from primmorphs during formation by the aggregating sponge cells. Metabolites characteristic of adult sponge have been shown to be produced in some primmorph cultures (Müller et al., 2000).

Primmorphs have been generated from many sponges including *S. agminata* (Zhang et al., 2003), *Ircinia muscarum*, *S. domuncula* (Custodio et al., 1998; Müller et al., 1999b; Pennec et al., 2003; Sipkema et al., 2003), *Dysidea avara* (Müller et al., 2000), *G. cydonium* (Sipkema et al., 2003), *Axinalla polypoides* (Sipkema et al., 2003), *H. panicea* (Sipkema et al., 2003), *Stylissa massa* (Wijffels et al., 2001; Sipkema et al., 2003), *Halicolana oculata* (Sipkema et al., 2003), and *P. andrewsi* (Sipkema et al., 2003). Extended culture of dissociated cells and primmorphs invariably requires the use of antibiotics for suppressing bacterial contamination, especially in rich media. Also, unless antibiotics are used, intracellular symbionts can attack moribund sponge. Antibiotics such as penicillin, streptomycin, and rifamycin that do not affect eukaryotes are generally effective in controlling bacterial contamination. These antibiotics do not inhibit fungal growth and proliferation of fungi can be a recurring problem in sponge cell and primmorph culture. Cell cultures may need to be supplemented with antifungal agents such as amphotericin. Certain compounds and antibiotic cocktails can prevent the formation of primmorphs (Sipkema et al., 2003).

Culture of cells and primmorphs requires more elaborate media than simple seawater. A medium for maintaining primary cells was reported by Pomponi and Willoughby (1994). It consisted of a commercial medium for culturing animal cells supplemented with artificial seawater (without Ca^{2+} and Mg^{2+}) and buffered to pH 7.0 with phosphate buffer. The medium contained 5% (vol/vol) fetal bovine serum. Attempts are being made to further improve the culture media available for growing sponge cells (Willoughby and Pomponi, 2000).

5. Concluding remarks

Sponges and sponge symbionts produce numerous unique metabolites of potential commercial value. Producing many of these metabolites would require large quantities of

sponge biomass that cannot be sustainably harvested from natural populations. Production of cultivated sponge biomass from sea-based farms is feasible, but productivity is variable. Biomass production in controlled environments of aquariums has the potential to provide consistent yields, but many aspects of aquarium cultivation remain unknown for most sponges. Culture of sponge cells and, more likely, primmorphs can become a future source of metabolites; however, cell and primmorph cultures are not feasible at present for producing large amounts of biomass.

Major questions remain concerning the production of sponge-sourced bioactives: can methods be developed for culturing healthy sponge without its endosymbionts? Can endosymbiotic bacteria be cultured in the absence of live sponge tissue and cells, to produce metabolites of interest? Studies are needed of sponge nutrition and how nutrition can influence growth and metabolite production. What might be the influence of precursor feeding? All these and many other questions remain to be answered.

Acknowledgements

This work was supported by the Ministerio de Ciencia y Tecnología (REN2001-2312-C03-03/MAR), Spain.

References

Adams C, Stevely JM, Sweat D. Economic feasibility of small-scale sponge farming in Pohnpei, Federated Sates of Micronesia. J World Aquac Soc 1995;26:132–42.

Armstrong E, McKenzie JD, Goldsworthy GT. Aquaculture of sponges on scallops for natural products research and antifouling. J Biotechnol 1999;70:163–74.

Ayling AL. Growth and regeneration rates in thinly encrusting Demospongiae from temperate waters. Biol Bull 1983;165:343–52.

Battershill CN, Page MJ. Sponge aquaculture for drug production. Aquac Update 1996;5–6 [Spring].

Becerro MA, Turon X, Uriz MJ. Multiple functions for secondary metabolites in encrusting marine invertebrates. J Chem Ecol 1997;23:1527–47.

Belarbi EH, Ramírez Domínguez M, Cerón García MC, Contreras Gómez A, García Camacho F, Molina Grima E. Cultivation of explants of the marine sponge *Crambe crambe* in closed systems. Biomol Eng 2003;20:333–7.

Bergquist PR. Sponges. London: Hutchinson; 1978.

Bewley CA, Faulkner DJ. Lithistid sponges: star performers or hosts to the stars? Angew Chem, Int Ed 1998; 37:2162–78.

Brown J, Colling A, Park O, Phillips J, Rothery O, Wright J. Seawater, its composition, properties and behaviour. Oxford: Pergamon; 1992.

Chisti Y. Animal-cell damage in sparged bioreactors. Trends Biotechnol 2000;18:420–32.

Chisti Y. Hydrodynamic damage to animal cells. Crit Rev Biotechnol 2001;21:67–110.

Custodio MR, Imsiecke G, Borojevic R, Rinkevich B, Rogerson A, Müller WEG. Evolution of cell adhesion systems: evidence for Arg-Gly-Asp mediated adhesion in the protozoan *Neoparamoeba aestuarina*. J Eukaryot Microbiol 1995;42:721–4.

Custodio MR, Prokic I, Steffen R, Koziol C, Borojevic R, Brümmer F, et al. Primmorphs generated from dissociated cells of sponge *Suberites domuncula*: a model system for studies of cell proliferation and cell death. Mech Ageing Dev 1998;105:45–59.

Davis AR, Butler AJ, van Altena I. Settlement behaviour of ascidian larvae: preliminary evidence for inhibition by sponge allelochemicals. Mar Ecol, Prog Ser 1991;72:117–23.

De Rosa S, De Caro S, Iodice C, Tommonaro G, Stefanov K, Popov S. Development in primary cell culture of demosponges. J Biotechnol 2003;100:119–25.

Duckworth AR, Battershill CN, Bergquist PR. Influence of explant procedures and environmental factors on culture success of thee sponges. Aquaculture 1997;156:251–67.

Faulkner DJ. Marine pharmacology. Antonie Van Leeuwenhoek Int J Gen Mol Microbiol 2000;77:135–45.

Ferguson JC. A comparative study of the net benefits derived from the uptake and release of free amino acids by marine invertebrates. Biol Bull Mar Biol Lab Woods Hole 1982;162:1–17.

Francis JC, Bart L, Poirrier MA. Effect of medium pH on the growth rate of *Ephydatia fluviatilis* in laboratory culture. In: Rützler K, editor. New perspectives in sponge biology. Washington (DC): Smithsonian Institution; 1990. p. 485–90.

Frøhlich H, Barthel D. Silica uptake of the marine sponge *Halichondria panicea* in Kiel Bight. Mar Biol 1997;128:115–25.

Garson M. The biosynthesis of sponge secondary metabolites: why it is important. In: van Soest RWM, van Kempen TMG, Braekman JC, editors. Sponges in time and space. Rotterdam: AA Balkema; 1994. p. 427–40.

Gerrodette T, Flechsig AO. Sediment-induced reduction in the pumping rate of the tropical sponge *Verongia lacunosa*. Mar Biol 1979;55:103–10.

Hoffmann F, Rapp HT, Zöller T, Reitner J. Growth and regeneration in cultivated fragments of the boreal deep water sponge *Geodia barretti* Bowerbank, 1858 (Geodiidae, Tetractinellida, Demospongiae). J Biotechnol 2003;100:109–18.

Holler U, Wright AD, Matthee GF, Konig GM, Draeger S, Aust HJ, et al. Fungi from marine sponges: diversity, biological activity and secondary metabolites. Mycol Res 2000;104:1354–65.

Ilan M, Contini H, Carmeli S, Rinkevich B. Progress towards cell cultures from a marine sponge that produces bioactive compounds. J Mar Biotechnol 1996;4:145–9.

Imsiecke G. Ingestion and digestion of *Chlamydomonas reinhardtii* (Volvocales) by the freshwater sponge *Spongilla lacustris* (Spongillidae). In: van Kempen TMG, Braekman JC, editors. Sponges in time and space. Rotterdam: AA Balkema; 1994. p. 371–6.

Kaandorp JA. Morphological analysis of growth forms of branching marine sessile organisms along environmental gradients. Mar Biol 1999;134:295–306.

Kinne O. Cultivation of animals—research cultivation: 3. Porifera. In: Kinne O, editor. Marine ecology, vol. 3, part 2. London: Wiley; 1977. p. 627–41.

Klautau M, Custodio MR, Borojevic R. In vitro culture of primary cell lines from marine sponges. In: van Soest, RWM, van Kempen TMG, Braekman JC, editors. Sponges in time and space. Rotterdam: AA Balkema; 1994. p. 401–6.

Koziol C, Borojevic R, Steffen R, Müller WEG. Sponges (Porifera) model systems to study the shift from immortal to senescent somatic cells: the telomerase activity in somatic cells. Mech Ageing Dev 1998;100: 107–20.

Lee YK, Lee JH, Lee HK. Microbial symbiosis in marine sponges. J Microbiol 2001;39:254–64.

Leys SP. Sponge cell culture: a comparative evaluation of adhesion to a native tissue extract and other culture substrates. Tissue Cell 1997;29:77–87.

Maldonado M, Carmona MC, Uriz MJ, Cruzado A. Decline in Mesozoic reef-building sponges explained by silicon limitation. Nature 1999;401:785–8.

Müller WEG, Wimmer W, Schatton W, Bohm M, Batel R, Filic Z. Initiation of an aquaculture of sponges for the sustainable production of bioactive metabolites in open systems: example, *Geodia cydonium*. Mar Biotechnol 1999a;1:569–79.

Müller WEG, Wiens M, Batel R, Steffen R, Schröder HC, Borojevic R, et al. Establishment of a primary cell culture from a sponge: primmorphs from *Suberites domuncula*. Mar Ecol, Prog Ser 1999b;178:205–19.

Müller WEG, Böhm M, Batel R, De Rosa S, Tommonaro G, Müller IM, et al. Application of cell culture for the production of bioactive compounds from sponges: synthesis of avarol by primmorphs from *Dysidea avara*. J Nat Prod 2000;63:1077–81.

Munro MHG, Blunt JW, Dumdei EJ, Hickford SJH, Lill RE, Li S, et al. The discovery and development of marine compounds with pharmaceutical potential. J Biotechnol 1999;70:15–25.

Nickel M, Brümmer F. In vitro sponge fragment culture of *Chondrosia reniformis* (Nardo, 1847). J Biotechnol 2003;100:147–59.

Osinga R, Tramper J, Wijffels RH. Cultivation of marine sponges for metabolite production: applications for biotechnology? Trends Biotechnol 1998;16:130–4.

Osinga R, Tramper J, Wijiffels RH. Cultivation of marine sponges. Mar Biotechnol 1999a;1:509–32.

Osinga R, de Beukelaer PB, Meijer EM, Tramper J, Wijffels RH. Growth of the sponge *Pseudosuberites* (aff.) *andrewsi* in a closed system. J Biotechnol 1999b;70:155–61.

Osinga R, Kleijn R, Groenendijk E, Niesink P, Tramper J, Wijffels RH. Development of in vivo sponge cultures: particle feeding by the tropical sponge *Pseudosuberites* aff. *andrewsi*. Mar Biotechnol 2001;3:544–54.

Osinga R, Belarbi EH, Molina Grima E, Tramper J, Wijffels RH. Progress towards a controlled culture of the marine sponge *Pseudosuberites andrewsi* in a bioreactor. J Biotechnol 2003;100:141–6.

Pawlik JR, McFall G, Zea S. Does the odor from sponges of the genus *Ircinia* protect them from fish predators? J Chem Ecol 2002;28:1103–15.

Pennec GL, Perovic S, Ammar MSA, Grebenjuk VA, Steffen R, Brümmer F, et al. Cultivation of primmorphs from the marine sponge *Suberites domuncula*: morphogenetic potential of silicon and iron. J Biotechnol 2003;100:93–108.

Pile AJ, Patterson MR, Witman JD. In situ grazing on plankton < 10 μm by the boreal sponge *Mycale lingua*. Mar Ecol, Prog Ser 1996;141:95–102.

Pile AJ, Patterson MR, Savarese M, Chernyk VI, Fialkov VA. Trophic effects of sponge feeding within Lake Baikal's zone. 2. Sponge abundance, diet, feeding efficiency and carbon flux. Limnol Oceanogr 1997;42: 178–84.

Poirrier MA, Francis JC, La Biche RA. A continuous-flow system for growing fresh-water sponges in the laboratory. Hydrobiologia 1981;79:255–9.

Pomponi SA. The bioprocess—technological potential of the sea. J Biotechnol 1999;70:5–13.

Pomponi SA, Willoughby R. Sponge cell culture for production of bioactive metabolites. In: van Soest RWM, van Kempen TMG, Braekman JC, editors. Sponges in time and space. Rotterdam: AA Balkema; 1994. p. 395–400.

Pomponi SA, Willoughby R, Kraighn ME, Wright AE. Development of techniques for in vitro production of bioactive natural products from marine sponges. In: Maramorosh K, Mitsuhashi J, editors. Invertebrate cell culture. Novel directions and biotechnology applications. Enfield (NH): Science Publisher; 1997. p. 231–7.

Porter JM, Targett WM. Allelochemical interactions between sponges and corals. Biol Bull 1988;175:230–9.

Proksch P, Edrada RA, Ebel R. Drugs from the sea—current status and microbiological implications. Appl Microbiol Biotechnol 2002;59:125–34.

Reincke T, Barthel D. Silica uptake kinetics of *Halichondria panicea* in Kiel Bight. Mar Biol 1997;129:591–3.

Richelle-Maurer E, Gomez R, Braekman J-C, van de Vyver G, van Soest RWM, Devijver C. Primary cultures from the marine sponge *Xestospongia muta* (Petrosiidae, Haplosclerida). J Biotechnol 2003;100:169–76.

Rinkevich B. Cell cultures from marine invertebrates: obstacles, new approaches and recent improvements. J Biotechnol 1999;70:133–53.

Sarà M. Ultrastructural aspects of the symbiosis between two species of the genus *Aphanocapsa* (Cyanophyceae) and *Ircinia variabilis* (Demospongiae). Mar Biol 1971;11:214–21.

Sepcic K. Bioactive alkylpyridinium compounds from marine sponges. J Toxicol, Toxin Rev 2000;19:139–60.

Simpson TL. The biology of the marine sponge *Microciona prolifera* (Ellis and Solander): I. A study of cellular function and differentiation. J Exp Zool 1963;154:135–47.

Sipkema D, van Wielink R, van Lammeren AAM, Tramper J, Osinga R, Wijffels RH. Primmorphs from seven marine sponges: formation and structure. J Biotechnol 2003;100:127–39.

Spier RE, editor. Encyclopedia of cell technology, vols. 1 and 2. New York: Wiley; 2000. p. 1–1249.

Thomassen S, Riisgård HU. Growth and energetics of the sponge *Halichondria panicea*. Mar Ecol, Prog Ser 1995;128:239–46.

Turon X, Galera J, Uriz MJ. Clearance rates and aquiferous systems in two sponges with contrasting life-history strategies. J Exp Zool 1997;278:22–36.

Unson MD, Holland ND, Faulkner DJ. A brominated secondary metabolite synthesized by cyanobacterial symbiont of a marine sponge and accumulation of the crystalline metabolite in the sponge tissue. Mar Biol 1994;119:1–11.

Uriz MJ, Turon X, Becerro MA, Galera J. Feeding deterrence in sponges. The role of toxicity, physical defenses, energetic contents, and life-history stage. J Exp Mar Biol Ecol 1996a;205:187–204.

Uriz MJ, Becerro MA, Tur JM, Turon X. Location of toxicity within the Mediterranean sponge *Crambe crambe* (Demospongiae: Poecilosclerida). Mar Biol 1996b;124:583–90.

Verdenal B, Vacelet J. Sponge culture on vertical ropes in the northwestern Mediterranean Sea. In: Rützler K, editor. New perspectives in sponge biology. Washington: Smithsonian Institution Press; 1990. p. 416–24.

Wijffels RH, Osinga R, Pomponi S, Tramper J. Marine sponges as biocatalysts. In: Cabral JMS, Mota M, Tramper J, editors. Multiphase bioreactor design. London: Taylor & Francis; 2001. p. 477–93.

Wilkinson CR. Net primary productivity in coral reef sponges. Science 1983;219:410–2.

Wilkinson CR, Vacelet J. Transplantation of marine sponges to different conditions of light and current. J Exp Mar Biol Ecol 1979;37:91–104.

Willoughby R, Pomponi SA. Quantitative assessment of marine sponge cells in vitro: development of improved growth medium. In Vitro Cell Dev Biol, Anim 2000;36:194–200.

Wilson HV. On some phenomena of coalescence regeneration in sponges. J Exp Zool 1907;5:245–58.

Zhang W, Zhang X, Cao X, Xu J, Zhao Q, Yu X, et al. Optimizing the formation of in vitro sponge primmorphs from the Chinese sponge *Stylotella agminata* (Ridley). J Biotechnol 2003;100:161–8.