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Health & Welfare

Ascidian, sponge culture supplies bioactive metabolites

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Feeding regimes tested at Harbor Branch Oceanographic Institute

Nylon mesh provides a good substratum and allows growth on both sides, which promotes biomass production in this colony of *Ecteinascidia turbinata*.

In Australian testing, good growth rates and increased biosynthesis of target compounds were achieved in cultured *E. turbinata* and *A. corrugate*, marine species whose metabolites have antitumor properties. The results suggested that in-vitro culture is a viable method for supplying some ascidian and sponge metabolites for drug development and production.

Several ascidian and sponge species contain biologically active metabolites that have pharmaceutical potential as drugs. Since most of these bioactive metabolites are found at low concentrations within the ascidians or sponges, large amounts of biomass are required for drug development or production. Because large-scale harvesting would likely be environmentally destructive and expensive, alternative supply methods are needed.

In vitro culture

One possible method of supplying sufficient and sustainable quantities of bioactive metabolite is in vitro culture, whereby the ascidians or sponges are grown under controlled conditions in tanks on land. However, for in vitro culture to succeed, it is vital to develop appropriate feeding regimes that promote growth and biosynthesis production of the target metabolites.

Both ascidians and sponges filter suspended particles of generally less than 20-µm size. In 2002-2003 research at Harbor Branch Oceanographic Institute in Florida, USA, the author based feeding regimes on the natural concentrations of suspended particles that his test organisms experience in the wild.

Mangrove ascidian

The mangrove ascidian *Ecteinascidia turbinata* produces ecteinascidins, metabolites that have strong antitumor properties. Currently undergoing clinical trials, this species is a colonial ascidian consisting of individual zooids held together by a common stolon, a rootlike structure that also attaches the colony to the substratum.

In the author's tests, colonies of approximately 25 zooids were attached to nylon mesh and placed in aquariums containing 15 l of filtered seawater that was changed daily. The colonies were fed three microalgae – *Chaetoceros gracilis*, *Isochrysis galbana* and *Nannochloropsis* sp. – either individually or in combination, at a concentration of 80,000 cells per millileter, a natural concentration (1 NC).

Growth was greatest overall for colonies fed a monospecific diet of *I. galbana* and a multispecific diet of *C. gracilis* and *I. galbana*, probably because the two diets best met the nutritional requirements of *E. turbinata*. To determine the optimal food concentration for in vitro culture, colonies were fed both diets at one, two, and four times the natural concentration. After four weeks, *E. turbinata* fed the "natural-level" diets had grown little, while colonies fed the higher concentrations doubled in size.

This clearly showed the phenomenal growth rates that can be obtained with in vitro culture when good diets are used. Another promising finding was that in vitro culture can promote target metabolite biosynthesis, with farmed *E. turbinata* colonies containing four times the normal concentration of ecteinascidins.

Caribbean sponge

Found on Caribbean coral reefs, the sponge (*Axinella corrugata)* produces stevensine, a metabolite with antitumor properties. In research by the author, individual *A. corrugata* were collected and cut into smaller pieces or explants and placed into aquariums containing filtered seawater.

For eight weeks, the explants were fed a multispecific diet of bacteria, yeast, and microalgae at four different concentrations: 1 NC (200,000 bacteria per millileter with 23,000 microalgae and yeast per millileter), 3 NC, 5 NC, and 5 + 1 NC (5 NC of bacteria and 1 NC of microalgae and yeast).

The growth of *A. corrugata* varied greatly among the food concentrations. Explants fed the 3 NC diet increased in weight by 22 percent (Fig. 1). In contrast, explants fed the 1 NC diet did not grow, while explants fed the higher-concentration diets lost weight.

These results indicated that growth of *A. corrugata* increases as food concentration increases until a threshold is reached, at which high cell concentration has a negative impact. Reduced growth in the explants that received high-concentration diets may have resulted from the additional cells blocking the sponges' aquiferous systems, the structures that transport water throughout the sponges.

The concentration of food also greatly affected metabolite biosynthesis in *A. corrugata*. Explants fed the 3 NC diet had double the normal concentration of stevensine (Fig. 2). In contrast, stevensine concentration in explants fed the other diets was similar to that of wild sponges.

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