Biotechnological potential of marine sponges

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Marine sponges (Porifera) have attracted significant attention from various scientific disciplines. Remarkable traces, left by sponges in the fossil records have been studied by paleontologists and evolutionary biologists. As sponges produce various novel chemical molecules, they have been a goldmine to chemists and also found their way into biotechnological applications. Microbiologists became fascinated by these unique animals with the discovery that sponges contain an abundance of unusual microorganisms, having potential for drug discovery. Cell biologists are investigating these simple animals to understand their basic organization on the cellular and skeletal level. Since the last few years, molecular biologists are working on sponge genome, which will contribute to the understanding of the evolution of molecular mechanism of metazoan genes and diseases. This review aims to provide an overview of the different interdisciplinary approaches involved in the exploration of marine sponges for their biotechnological potential.

MARINE sponges (Porifera) are the oldest metazoan group, having an outstanding importance as a living fossil[1]. There are approximately 8000 described species of sponges and perhaps twice as many un-described species[2]. They are grouped into three classes, the Hexactinellida (glass sponges), the Calcarea (calcarious sponges) and the Demospongiae. The latter class contains the vast majority of extant sponges living today. Sponges inhabit every type of marine environment, from polar seas to temperate and tropical waters and also thrive and prosper at all depths. They show an amazing variety of shapes, sizes and colours (Figure 1). Giant barrel sponges can reach up to 70 inches in height, while another tiny encrusting sponge may only be half of an inch long. Sponges are sessile organisms. However, due to their cellular plasticity, many sponges reorganize their bodies continuously and move during this process very slowly[3]. Except for the free-swimming larval stage (by means of cilia), sponges pass their whole subsequent existence fixed to a suitable substratum.

Sponges contain certain unifying attributes that are characteristic for the phylum Porifera. The organization of the sponge body is very simple. Epithelial cells (pinacocytes) line the outer surface and internal system of openings, channels and chambers, through which water is pumped continuously by flagellated cells called choanocytes. These simplest animals are very efficient filter-feeders. It has been estimated that some of them are able to filter their own body volume of water every 5 seconds[4]. This water current supplies food particles and oxygen and removes metabolic waste products. The major part of sponge biomass consists of a gelatinous matrix containing free-floating, non-differentiated cells. This part of the sponge body is called mesohyle. The mesohyle also contains the skeletal elements of the sponge body; spicules (needle-like structures made of either silicon or calcium carbonate) and spongin (collagenous fibres).

Chemical ecology

After reading all this information regarding sponges, the question will arise: How does this delicate looking simple sea creature protect itself against predators and pathogens in the marine environment? While answering this interesting ecological question, researchers found that sponges have defensive chemical weapons (secondary metabolites) for their protection. Intensive evolutionary pressure from competitors, that threaten by overgrowth, poisoning, infection or predation have armed sponges with an arsenal of potent chemical defence agents. Investigations in sponge chemical ecology have revealed that the secondary metabolites not only play various roles in the metabolism of the producer but also in their strategies in the given environment. The diversity of secondary metabolites produced in sponges has been highlighted in several reviews[5,6]. They range from derivatives of amino acids and nucleosides to macrolides, porphyrins, terpenoids to aliphatic cyclic peroxides and sterols. There is evidence documenting the role of sponge metabolites in chemical defence against predators[7–9] and epibionts[10–14]. Several studies show that sponges are rich in terpenoids and steroids, which are thought to function in antipredation, space competition and control of epibiont overgrowth[15]. The studies on sponge chemical ecology include three different aspects. First, diversity of chemical compounds produced by sponges; second, potential functions of these metabolites in nature and finally, the strategies for their use for human benefit[16].

Chemical ecology of sponges relates very closely to biotechnology by applying the epibiotic chemical defense of these organisms for the development of ecofriendly antifouling compounds or strategies. Biofouling causes deterioration and instability of structures and devices submerged in the sea, thus causing heavy economic losses.

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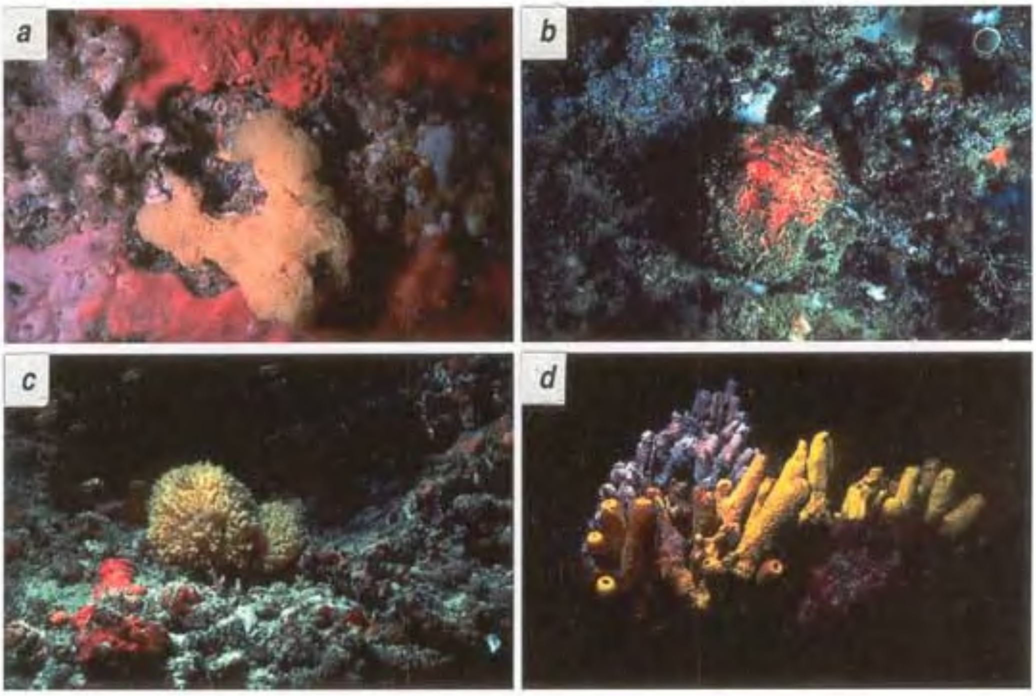


Figure 1 ***a–d.*** Different sponge species. ***a***, The calcareous sponge *Clathrina coreacea*; ***b***, The siliceous sponge *Tethya limski*; ***c***, *Tethya lyncurium*, a close relative of the red sponge; ***d***, The yellow horny sponge *Verongia aerophoba* and the pink *Dysidea avara*, both sponges contain bioactive compounds that have been extensively studied.

Antifouling protocols at present rely to a great extent on the application of toxic biocides, which have severe environmental concerns. The alternative efforts to develop eco-friendly antifouling compounds underline the significance of selective marine organisms like sponges. As these organisms prevent the settlement and growth of other organisms on their surfaces, efforts are underway to explore this strategy for application in antifouling research. In a series of experiments, it has been proved that marine sponges contain some bioactive metabolites, which have tremendous antifouling potential[17–24].

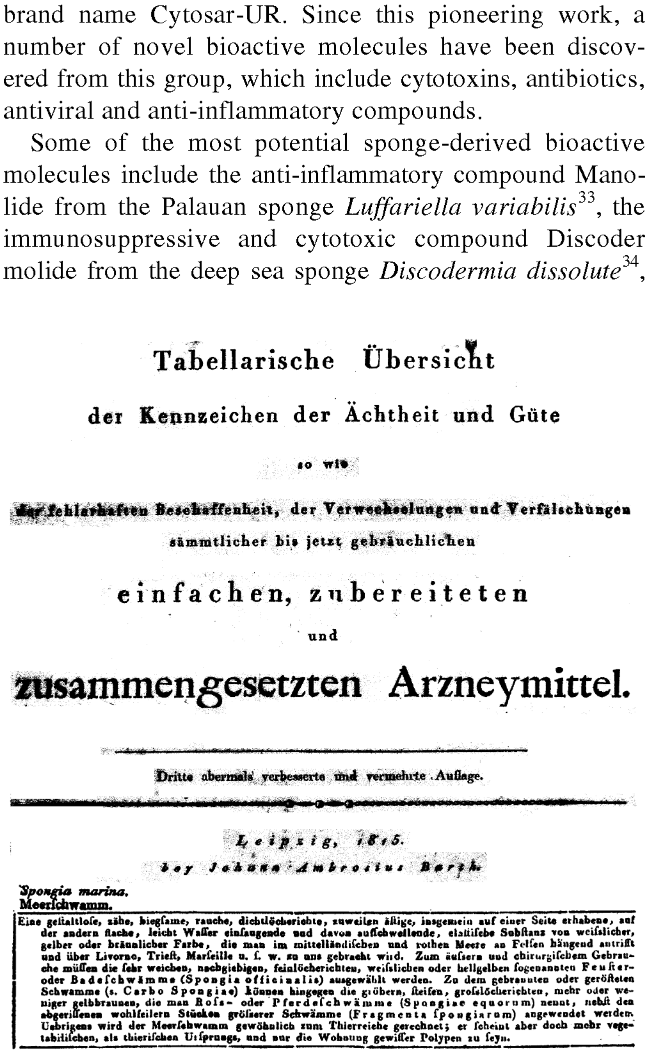
Drug discovery

Most medicines come from natural resources and scientists are still exploring the organisms of tropical rainforest for potentially valuable medical products. Historical records show that humans have become aware of the venomous nature of some sea creatures for at least 4000 years[25]. In the 19th and early 20th centuries, cod liver oil was used as food supplement. However, it was only in middle of the 20th century that scientists began to systematically probe oceans for medicines. So far, more than 10,000 bioactive molecules have been discovered from marine sources, with hundreds of new compounds still being discovered every year[26].

In terrestrial environment, plants are the richest sources of natural products. However in marine environment, this leading position is taken by invertebrates such as sponges, molluscs, bryozoans, tunicates, etc. They not only produce a great number of marine natural products currently known but also show the largest chemical diversity of natural products, including alkaloids, peptides, terpenes, polyketides, etc. Interestingly, out of 13 marine natural products (or analogues derived from them) that are currently in clinical trials as new drug candidates, 12 are derived from invertebrates[27].

Among marine invertebrates, Porifera (sponges) remain the most prolific phylum, concerning novel pharmacologically active compounds[28]. It has been known for centuries (Ebermaier 1815; Figure 2) that sponges contain bioactive compounds that are of potential medical importance. Richter[29] in 1907 outlined that the active component of the roasted bath sponge, used by Roger against struma, is iodine. The work on sponge natural products was systematically started by Bergmann and Feeney[30], who isolated three nucleosides from the Caribbean sponge *Cryptotethya crypta* Laubenfels, 1949. Antiviral properties of these nucleosides were demonstrated later[31] and initiated the synthesis of analogues which led to the first antiviral compound Ara-A (active against Herpes virus) and anti-tumour compound Ara C (effective in acute lymphoid leukaemia). The compounds Ara-A and Ara-C are the only marine invertebrate-related compounds in clinical use[32]. Arabinosyl Cytosine (Ara-C) is currently sold by the Pharmacia & Upjohn Company under the brand name Cytosar-UR. Since this pioneering work, a number of novel bioactive molecules have been discovered from this group, which include cytotoxins, antibiotics, antiviral and anti-inflammatory compounds.

Some of the most potential sponge-derived bioactive molecules include the anti-inflammatory compound Manolide from the Palauan sponge *Luffariella variabilis*[33], the immunosuppressive and cytotoxic compound Discoder molide from the deep sea sponge *Discodermia dissolute*[34],


Tabellarische Übersicht

der Kennzeichen der Ächtheit und Güte

so wie

der fehlerhaften Beschaffenheit, der Verwechselungen und Verfälschungen

sämmtlicher bis jetzt gebräuchlichen

einfachen, zubereiteten

und

zusammengesetzten Arzneymittel.

Dritte abermals verbesserte und vermehrte Auflage.

Leipzig, 1815.

bey Johann Ambrosius Barth.

Spongia marina.
Meerschwamm.

Eine gestaltlose, zähe, biegsame, rauche, dichtlöcherichte, zuweilen ästige, insgemein auf einer Seite erhabene, auf der andern flache, leicht Wasser einsaugende und davon aufschwellende, elastische Substanz von weisslicher, gelber oder bräunlicher Farbe, die man im mittelländischen und rothen Meere an Felsen hängend antrifft und über Livorno, Triest, Marseille u. s. w. zu uns gebracht wird. Zum äussern und chirurgischem Gebrauche müssen die sehr weichen, nachgiebigen, feinlöcherichten, weisslichen oder hellgelben sogenannten Fenster- oder Badeschwämme (Spongia officinalis) ausgewählt werden. Zu dem gebrannten oder gerösteten Schwamme (s. Carbo Spongiae) können hingegen die gröbern, steifen, grosslöcherichten, mehr oder weniger gelbbraunen, die man Ross- oder Pferdeschwämme (Spongiae equorum) nennt, nebst den abgerissenen wohlfeilern Stücken grösserer Schwämme (Fragmenta spongiarum) angewendet werden. Uebrigens wird der Meerschwamm gewöhnlich zum Thierreiche gerechnet; er scheint aber doch mehr vegetabilischen, als thierischen Ursprungs, und nur die Wohnung gewisser Polypen zu seyn.

Figure 2. Remarks by Ebermaier (1815) in his drug index where he mentioned the medical application of the roasted bath sponge as well as of the horse sponge in a topical and surgical way. At the end of the description he indicated that sponges should be classified to plants.

Table 1. Some of the sponge-derived bioactive products, which are currently in market or in clinical phases

Product	Application area	Status
Ara-A	Antiviral	Market
Ara-C	Anticancer	Market
Manoalide	Molecular probe: phospholipase A2 inhibitor	Market
IPL512602 (Steroid)	Anti-inflammatory/ asthma	Clinical phase II
Manoalide	Anti-inflammatory/ psoriasis	Clinical phase I
KRN 7000 (α-Galactosylceramide)	Anticancer	Clinical phase I
LAF389 (Amino acid derivative)	Anticancer	Clinical Phase I
Discodermolide (Polyketide)	Anticancer	Clinical phase I
HTI286 (Tripeptide)	Anticancer	Clinical phase I

anti-angiogenic brominated compound aeroplysina-1 from a sponge *Aplisina aerophoba*[35], tubulin polymerizing compound dictyostatin-1, a polyketide from *Spongia* sp.[36] etc. Some of the sponge-derived bioactive products, which are currently in market or clinical stages are listed in Table 1.

Microbial symbionts

Marine organisms are well known to have the specific relationship with numerous microorganisms and sponges are no exception to this. The sponge–microbial association is a topic of research since a long time[37]. In the feeding process, bacteria from surrounding seawater are continuously swirled in by the sponge-driven currents and most of these bacteria are retained in the sponge body. Interestingly, bacterial density in sponges is attributed to the temporal variations in the surrounding environment[14] and also linked to the irrigation system of the sponge[38]. Apart from this, it has been proved that some bacteria permanently reside in the sponge mesohyle, pointing to a close interaction between the host and associated bacteria[39–41]. It has been estimated that in some sponge species, as much as 40% of the animal biomass must be attributed to the bacteria, an amount that exceeds the bacterial population of seawater by two to four orders of magnitude[40].

The biology of bacterium–sponge relationship has elicited considerable interest among researchers, as marine sponges have been considered as a rich reservoir of bioactive compounds. The role of sponge-associated microorganisms in the synthesis of compounds of biological interest is the subject of scientific debate, as in some cases, these bacteria are reported to produce bioactive metabolites[38,42]. The role of sponge-associated bacteria in the host ebibiotic defense is also highlighted in a recent investigation[43] (Figure 3). There are some conflicts about the origin of compounds in sponges, i.e. surfactin-like depsipeptides were originally isolated from a marine sponge. However, it has later been shown that the bacterium, *Bacillus pumilus*, which is associated with the sponge, is the producer of these compounds[44]. Similar was the case with okadaic acid, a cytotoxic compound, which is now commercially available for biochemical research. Recently, our group has isolated a compound, 2-methylthio-1,4-napthoquinone, from an unknown bacterium, associated with marine sponge *Dysidea avara*, which showed strong anti-angiogenic and antimicrobial properties[45]. All these observations highlight the importance of sponge–bacteria association not only for biodiversity research but also for biotechnology.

Like with bacteria, sponges also exhibit an association with marine fungi, which produce bioactive substances. In the course of this project, Sorbicillactone A, one novel-type alkaloid was reported from sponge (*Ircinia fasciculata*) associated fungi *Penicillium chrysogenum*. This compound showed promising activities in several mammalian and viral systems and qualified for therapeutic human trials[46].

Polyketide biosynthetic genes from bacteria and fungi have been cloned, sequenced and expressed in heterologous hosts. Polyketide synthases (PKSs) are a class of enzymes that are involved in the biosynthesis of secondary metabolites such as erythromycin, rapamycin, tetracycline, lovastatin and resveratrol[47]. Some sponge-associated bacteria with antimicrobial assets are also detected to have polyketide synthase gene clusters and our group is presently working in this area.

Supply problem and cell culture

The metabolites having bioactive potential are often produced only in trace amounts by the sponges or their associated microorganisms. Several researchers stressed the fact that extremely large sponge biomass is needed for the commercial production of sponge metabolites; these amounts cannot be harvested from the sea every time. For example, in order to obtain approximately 1 g of the promising anticancer drug ET 743, close to 1 metric tonne (wet weight) of its natural source *E. turbinate* has to be harvested and extracted[48]. Supply problems still hamper the development of many promising metabolites of sponge origin and have stimulated research on alternative methods for sponge metabolite production. In this regard, three promising approaches are in practice, which comprise chemical synthesis, laboratory cell culture and aquaculture. Chemical synthesis is the most direct way to produce sponge metabolites in large quantities. However, developing a chemical production process can be very expensive, especially when complex novel molecules are concerned. This realization has given new impulse to research on methods for sponge culture either in laboratory or in the field.

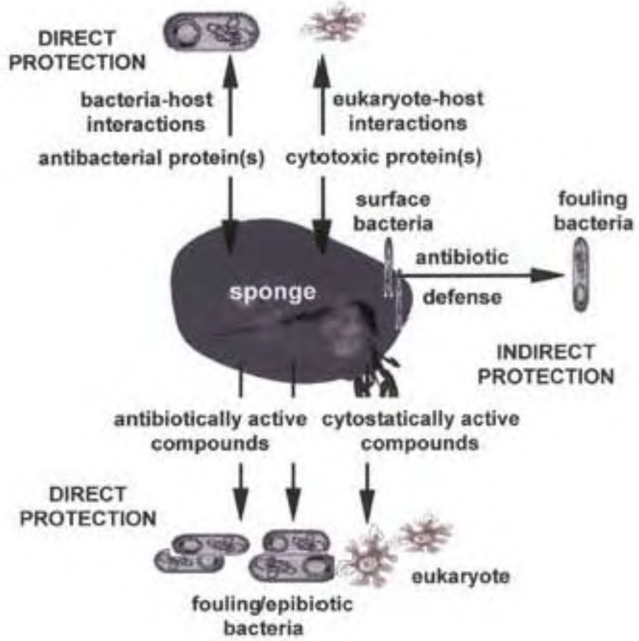


Figure 3. Different defense strategies of the sponge against invading bacteria and eukaryotes. First, direct protection by producing secondary metabolites and second, indirect protection with the help of associated bacteria, which produce bioactive metabolites (modified after Thakur *et al.*[43]).

As far as the laboratory culture of sponge cells is concerned, the first successful approach to show that sponge cells can proliferate and grow *in vitro* (primmorphs) was recently begun with the demosponge *S. domuncula*[49,50]. Primmorphs are a special form of *in vitro* cell culture that allows the formation of three-dimensionally organized aggregates comprising proliferating and differentiating cells (Figure 4). The first successful production of a sponge secondary metabolite in a bioreactor was achieved with primmorphs from *Dysidea avara* that produced avarol (a potent antiviral, antitumour and anti-inflammatory compound)[51]. These data indicate that the primmorph system is useful for an *in vitro* production of sponge secondary metabolites and warrants further studies on this approach. There are several groups, which have been working on monolayer cell culture of sponge for at least 10 years yielding some interesting results[52,53]. Some progress has also been made with culturing sponges in semi-controlled systems and completely controlled systems[54]. However, this work is in its infancy. There is a long history of sponge aquaculture research but until recently, all efforts in this direction were concentrated with bath sponge production. Most recent reports showed that the sponge aquaculture could be an alternative approach for the production of bioactive metabolites[55].

Molecular biology and evolution

Understanding of genome properties and functions is a fundamental task in modern bioscience. Molecular biology has a major role in many aspects of marine biotechnology. Genome studies of different commercially important fish are related to biotechnology. Also the genome analysis of marine microorganisms facilitates the use of genes for cell factories and bio-indicator strains as well as identification of new drug targets. Analysis of genome organization of marine sponges is a novel approach and it led to the elucidation of selected genes and gene arrangements that exist in gene clusters (e.g. receptor tyrosine kinase cluster and the allograft inflammatory factor cluster). Most of these studies were performed with the sponges *S. domuncula* and *Geodia cydonium* (Demospongiae), *Aphrocallistes vastus* (Hexactinellida) and *Sycon raphanus* (Calcarea)[56–58]. Work is in progress to identify, analyse and express arrays of genes present in the sponge genome, which encode a chain of enzymes involved in the

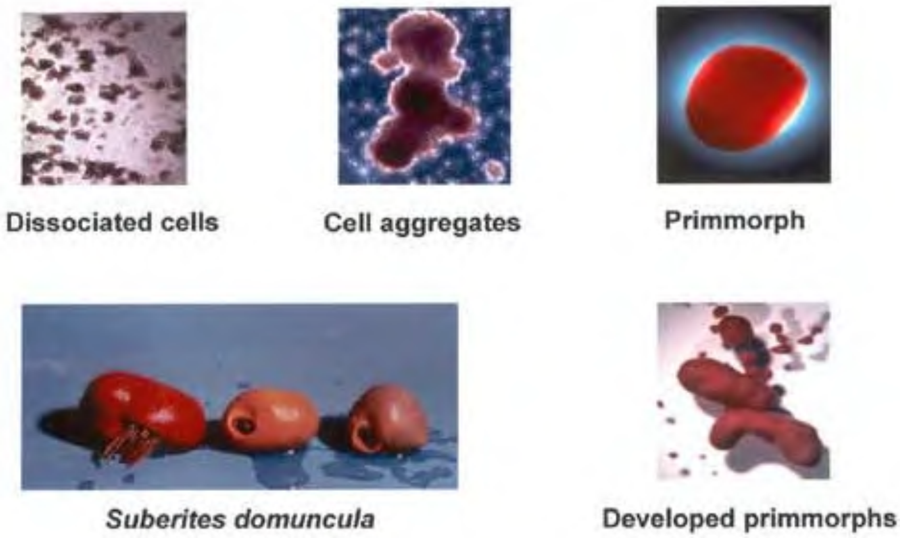


Figure 4. Development of sponge (*S. domuncula*) primmorphs from dissociated cells.

synthesis of bioactive compounds. With the help of molecular biological techniques, efforts are also underway to synthesize desired compounds in genetically engineered organisms by transferring biosynthetic gene clusters from sponge to bacteria (our group is currently working in this field).

Sponges (Porifera) form the basis of the metazoan kingdom and have been reported to be the evolutionarily most ancient phylum still extant. Hence, as living fossils, they are the taxon closest related to the hypothetical ancestor of all metazoa, the Urmetazoa[58] (Figure 5). This evolutionary finding became obvious after analysing sponge genetic diversity and their genomes[59]. Until recently, it was still unclear whether sponges are provided with a defined body plan. Only after the cloning, expression and functional studies of characteristic metazoan genes has it been proved that the sponges are not merely recognized cell aggregates, but are already complex animals provided with a defined body plan. Sponge evolution (approximately 600–800 Mya) was also calculated from the cDNA sequence similarities among galectin molecules among the metazoans[60,61]. Only few years ago, who would have expected that the majority of sponge proteins would be found to be significantly more similar to human sequences than to those from *C. elegans*[62]? The analysis of the sponge genome allows us to pinpoint the evolutionary mechanism which occurred approximately 600–800 Mya.

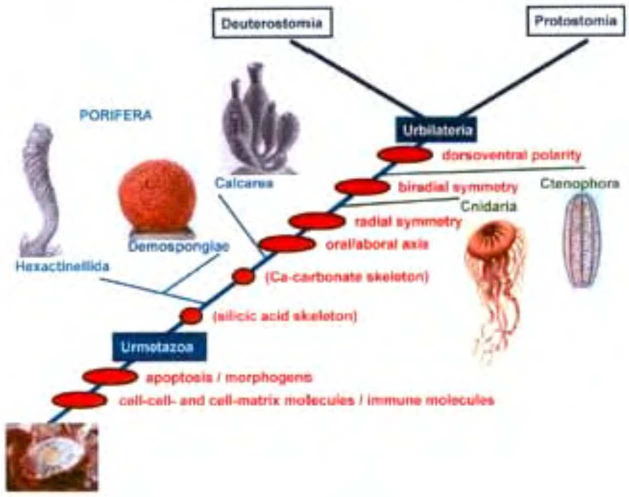


Figure 5. Molecular evolution. Phylogenetic relationship of the metazoan taxa which evolved from the hypothetical ancestor of all metazoan phyla, the Urmetazoa. The major evolutionary novelties which had been attributed to the Urmetazoa were those molecules which mediate apoptosis, control morphogenesis, the immune molecules and primarily the cell-adhesion molecules. The first class/phylum which emerged had been the Archaeocyatha which however became extinct. Then the siliceous sponges with the two classes Hexactinellida and Demospongiae emerged and finally the Calcarea, which possess a calcareous skeleton. These three classes of Porifera are living fossils that provide a reservoir for molecular biological studies. The Calcarea are very likely a sister group of the Cnidaria. From the latter phylum the Ctenophora evolved which comprise not only an oral/aboral polarity but also a biradial symmetry. Finally, the Urbilateria emerged from which the Protostomia and the Deuterostomia originated.

Immune system

Immune systems are uniquely present in every kingdom of life. The immune systems protect animals against attacking organisms and also hinder malformation during morphogenetic growth and aging. Until recently nobody believed that sponges having only a simple body structure have immune systems. This status changed after the first nucleotide (nt) sequences coding for immune and immune-related molecules were isolated from sponges[63]. These molecules include G-protein linked transmembrane recep-

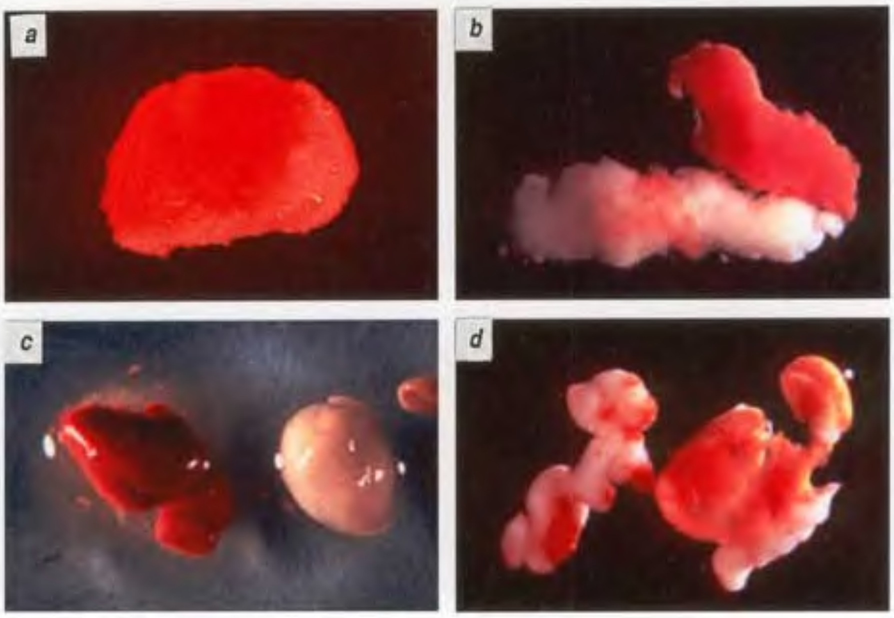


Figure 6. Identification of molecules involved in self/self and self/non-self recognition in *S. domuncula*. Mixed sponge cell reaction. Small aggregates from the same individual (autogeneic MSCR) or from different individuals (allogeneic MSCR) were placed together and cultured for two days. In the autogeneic MSCR the aggregates formed 1–2 mm large clumps after 2 days (***a***), while in the allogeneic MSCR the aggregates sort out and form individual clumps, as can be seen from the different colours of the aggregates, originating from white and red individuals (***b***) additional five days later primmorphs are formed (***c***). In one series of experiments, allogeneic MSCR was performed with aggregates which were incubated in the presence of 20 ng/ml of FK506; during the period of two days the cells formed mixed aggregates (***d***) Magnification: × 10 (***a***, ***b*** and ***d***), and × 20 (***c***).

tors and transmembrane adhesion molecules, which were isolated mainly from *G. cydonium* and *S. domuncula*. Very successfully the autogeneic- and the allogeneic mixed sponge cell reaction (MSCR) system was applied for the first time to identify distinct factors in sponges *in vitro* (Figure 6).

It was found that sponges are provided with elements of the mammalian innate immune system, such as molecules containing scavenger receptor cysteine-rich domains. Latest investigations showed that in sponge (*S. domuncula*), lectin and lyso-PAF (platelet-activating factor) act as antimicrobial molecules involved in immune defense against bacterial invaders[64,65]. These findings revealed that sponges are already provided with a series of elements used in higher vertebrates for both the innate and adaptive immune recognition[63]. In future this simple sponge could become an ideal model to study complex immune systems in different organisms including human being.

Conclusion

This review highlights the biotechnological significance of marine sponges with the help of four different classical approaches, which comprise chemistry, microbiology, cell biology and molecular biology. Efforts of marine natural product researchers to tap the fascinating chemical diversity in sponges have explored novel potential drugs. The success of this classical approach is highlighted by the leading position of sponge-originated compounds that are already in the market or in the stages of clinical trials. The microbiological approach elucidates the key role, played by sponge-associated microorganisms in drug discovery. These valuable findings regarded sponges as 'microbiological fermenters' that hold an untapped potential for natural product discovery. Cell biology provides us with new perspectives on the ecology and biology of the cells, leading to the cultivation of sponges and ultimately to a sustainable use of marine resources. Finally, the molecular biological approach provides the basis for the establishment of Porifera as model organisms for the understanding of the metazoan body plan, immune systems and diseases. With the advanced molecular biological tools, target-oriented screens have become available that will accelerate the quest for new sponge-derived drugs. This review impressively illustrates the power of interdisciplinary approaches in the exploration of biotechnological implications of marine organisms.

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