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Production of anti-cancer bryostatins by the bryozoan *Bugula neritina*

Objectives

- Study the biological and environmental traits of the Bryozoan *Bugula neritina.*
- Know how and by which manner the bryostatins are produced.
- Understand the anti-cancer effect of the bryostatins and pathways through which they act.
- Analyze the biomedical development of bryostatin based drugs from a historical point of view.
- Inform about the current clinical status and the possible ways forward.

Bugula neritina

Bugula neritina belongs to the bryozoans, a phylum of aquatic invertebrate animals, mostly marine. *B.neritina* is a sessile filter-feeder organism that sieve food particles using a retractable lophohore. It is a colonial organism that reproduces both sexually and asexually.

In 1982 Pettit G. *et al* discovered bryostatins as a compound by using an antineoplastic bioassay-guided fraction approach. *B.neritina* was designated to be its main source.

A few years later, bacterial extracellular symbionts located in the pallial sinus of the larvae of *B.neritina* were described. Subsequently, it has been suggested that bacterial symbionts most likely play a role in bryostatin production (Anthoni. U *et al*.,1990).

Biological function of bryostatins

Bryostatins provide chemical defense for *B. neritina* larvae (Fig **4**).

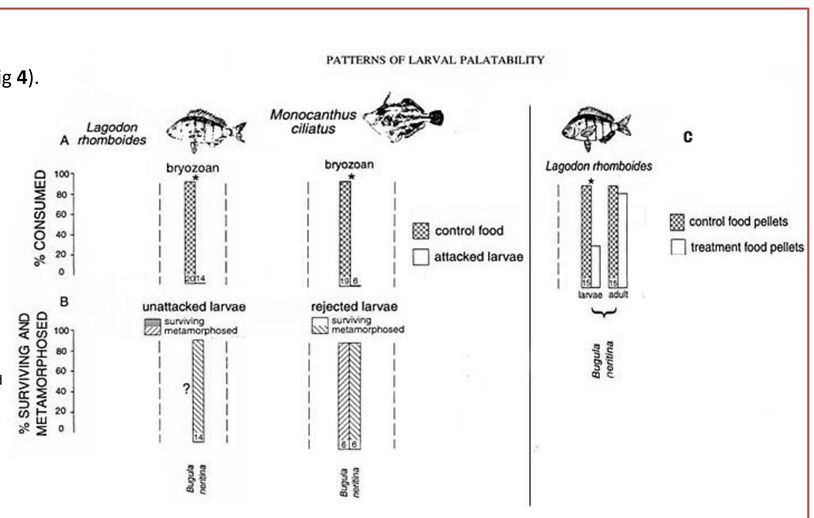


Figure 4, Extracted and modified from (Lindquist N & Hai ME.,1996) :

(A) The percentage of bryozoan larvae consumed after attack.

(B) The percentage of larvae surviving and metamorphosing 1-3 days after being mouthed and rejected.

(C) Consumption of control food pellets and treatment food pellets containing Bugula neritina.

E.Sertula symbiothic growth

In 1997 through the use of amplification, cloning and sequencing of the PKS-1 gene complex it was proved that bryostatins are bacterial secondary metabolites (S.K Davidson & M.G Haygood., 1997). The bacterial symbiont was characterized as gamma-proteobacterium and received the name of ' Candidatus endobugula sertula'.

Additionally, It was shown that antibiotic treatments which reduced '*E. sertula*' density in *B. neritina* adult colonies, decreased bryostatin concentration in *B.neritina* and lowered the expression of *bryA,* a portion of the gene cluster (Fig **1**) (S.K Davidson et al.,2001).

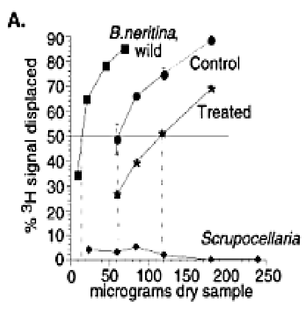
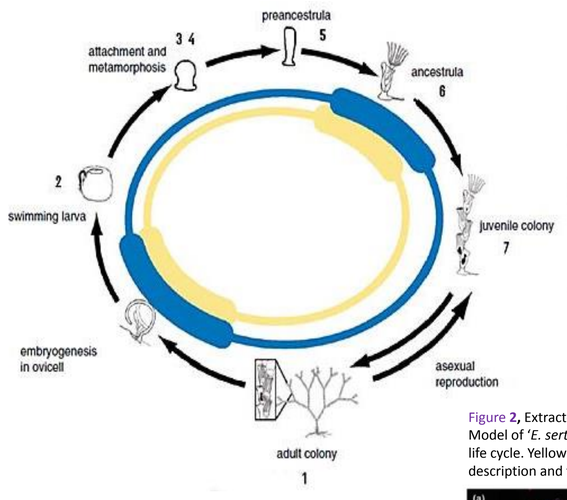


Figure 1, Extracted from (Davidson SK et al. 2001): Bryostatin activity.

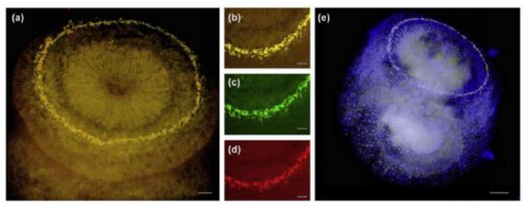
A novel bryostatin detection method based on its ability to bind mammalian protein kinase C allowed to determine where '*E. sertula*' and the bryostatins are located within *B. neritina* throughout the already known life cycle (K.H Sharp *et al*.,2007) (Fig **2**).



Stage number[a]	Time (h)[b]	Morphological description
1	—	Adult colony with ovicells and rhizoids
2	0	Ciliated, free-swimming larvae
3	1.25	Attached larva; pallial epithelium evagination toward oral surface
4	2	Elongation into upright pillar; internalization of apical disc and larval pallial epithelium
5	48	Further elongation; formation of chitinous cuticle; initiation of histogenic differentiation
6	96	Lophophore functional; bud for second autozooid starting to develop

Figure 2, Extracted and modified from (K.H Sharp *et al*.,2007) : On the left side: Model of '*E. sertula'* proliferation and bryostatin levels in *B. neritina* throughout the life cycle. Yellow 'E. sertula'; blue, bryostatin. On the right side: Morphological description and timeline of each stage of the lifecycle.

Although the bacteria are restricted to the pallial sinus, the bryostatins are transmitted throughout the entire larva (Fig **3**).



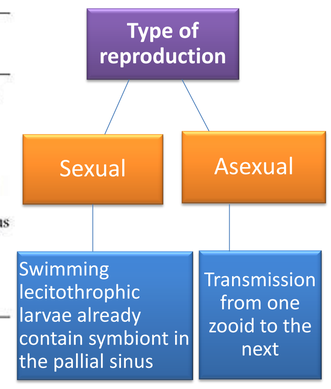
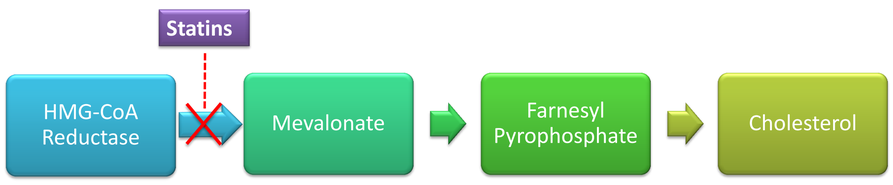


Figure 3: Extracted from (Amaro E et al.,2010): 'Candidatus Endobugula sertula' and bryostatin localization in Bugula neritina larvae. Bacteria in the pallial sinus of a Bugula neritina larva, as shown by simultaneous fluorescence in situ hybridization (FISH) with two probes.

Statins and cancer

As its name suggests, bryostatins pertain to a bigger group of drugs named statins. The statins are proteins that mainly act inhibiting the HMG-CoA reductase, which ultimately implies a reduction of the cholesterol in the blood (Figure **4**).



Simplified Cholesterol synthesis pathway , the red cross indicates where the process is stopped due to the statin activity.

Cholesterol acts as a fundamental structural component of mammalian cell membranes and it's essential for cellular proliferation. Statins inhibit the production of endogenous cholesterol and block protein prenylation. Therefore statin use may influence by reducing cell proliferation and migration, properties that are associated to anti-cancer activity.

The ultimate test that has supported this theory was a study executed crossing the data of The Danish Civil Registration System with the Danish Registry of Medical Products.

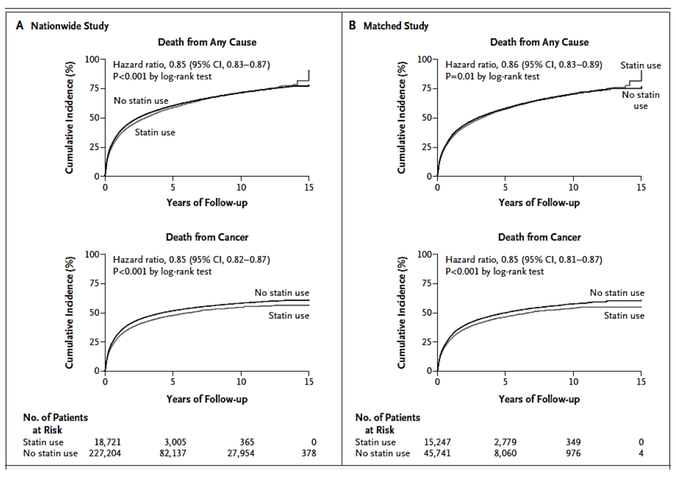


Figure 5, Extracted from (Nielsen SF & Nodestgaard BG.,2012): Regular Statin Use and Cumulative Incidence of Death from Any Cause and Death from Cancer, According to time. This includes patients who received a diagnosis of cancer during the period from 1995 through 2007 and followed them until the end of 2009. Patients younger than 40 years were excluded due to the improbability to take statin treatments.

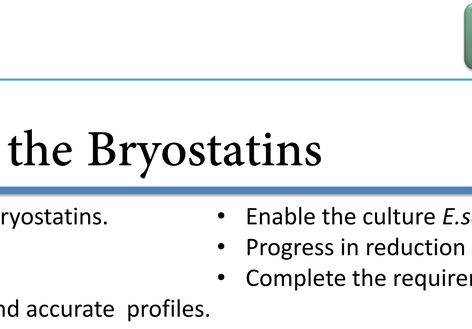
The ultimate test that has supported this theory was a study realized crossing the data of The Danish Civil Registration System with the Danish Registry of Medical Products (Nielsen SF & Nodestgaard BG.,2012).

The results led to the conclusion that The cumulative incidence of death from any cause since the date of the cancer diagnosis was lower among statin users (Fig **5**).

Bryostatins

Bryostatins are a family of protein Kinase C modulators which are found mainly in *B. neritina* and are isolated at a yield of 10^{-7} % of the wet weight of an adult colony.

From 1982 until nowadays, up to 20 different bryostatins have been discovered. All of them share a common structure: a macrolactone core with three tetrahydropyran rings. They predominantly differ in their substituents at C-7 and C-20 positions (Fig **6**).

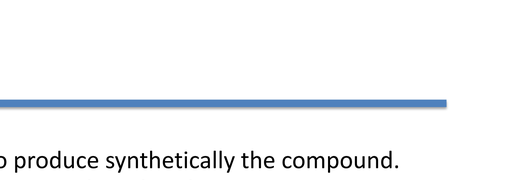


Bryostatin	R^1	R^2
"0"	OH	H
1	d	a
2	OH	a
3	d	a
4	c	d
5	c	d
6	e	d
7	d	d
8	e	e
9	d	e
10	c	H
11	d	H
12	e	a
13	e	H
14	c	OH
15	d	b
16	c	H
17	c	H
18	c	H
19	c	e
20	c	H

Figure 6: Extracted from (Amaro E *et al*.,2010): structures of bryostatins '0'-20 ('bryostatin 0' is the hypothetical precursor to all the known bryostatins).

The conclusions of the latest genetic trials propose 'bryostatin 0' as the hypothetical compound that acts as the precursor of the rest of bryostatins.'Bryostatin 0' would be symbiotically produced whereas the rest of bryostatins would be the result of diverse processes (Amaro E *et al*.,2010).

- Abiotic processes
- Host enzymatic modifications
- Other microbial activity



Bryostatins have the capability to bind the diacylglycerol binding site of the C-1 regulatory domain of the protein Kinase C. Most of the pharmacological effects of this molecules are attributed to this interaction(Nelson TJ & Alkon DL., 2009).

Clinical status

More than thirty phase I and II clinical trials have been carried out for several cancer types but neither of them have obtained the required results to enable a phase III clinical trial as a cancer treatment yet.

The major areas where bryostatins are highlighted are:

1. Promising applications in central nervous system (Alzheimer, depression, stroke etc.).
2. Antineoplastic effect.
3. Proposed as possible therapy for human immunodeficiency virus (HIV).
4. Enhancer of cytotoxic agents .

Despite all those virtues, the poor availability of bryostatins keeps hindering its clinical development.

Future of the Bryostatins

- More thorough research of the sibling species that produce different strains of bryostatins.
- Identify the biomarkers that regulate the biosynthetic process.
- Implementation of combined therapies with other targeted agents.
- Increase the research effort around analogues production in order to get safe and accurate profiles.
- Enable the culture *E.sertula.*
- Progress in reduction of the steps needed to produce synthetically the compound.
- Complete the requirements to advance to phase III clinical trial.

References : Amaro E, Trinidade-Silva, Lim-Fong GE, Sharp KH and Haygood MG (2010). Bryostatins : biological context and biotechnological prospects. Pharmaceutical biotechnology. DOI 10.1016/j.copbio.2010.09.018. Anthoni, U., P. H. Nielsen, M. Perieira, and C. Christophersen. 1990. Bryozoan secondary metabolites: a chemotaxonomical challenge. Comp. Biochem. Physiol. 96B:431–437. Davidson SK, Allen SW, Lim GE, Anderson CM, Haygood MG. (2001). Evidence for the biosynthesis of bryostatins by the bacterial symbiont 'Candidatus Endobugula sertula' of the bryozoan Bugula neritina. Appl Environ Microbiol 67: 4531–4537. Haygood MG and Davidson SK, Small-Subunit rRNA Genes and In Situ Hybridization with Oligonucleotides Specific for the Bacterial Symbionts in the Larvae of the Bryozoan Bugula neritina and Proposal of " Candidatus Endobugula sertula ", Applied And Environmental Microbiology, Nov.1997 , p. 4612-4616. Lindquist N, Hay ME. Palatability and chemical defense of marine invertebrate larvae. Ecol Monogr 1996 66: 431–450. Nelson TJ, Alkon DL: Neuroprotective versus tumorigenic protein kinase C activators. Trends Biochem Sci 2009, 34:136-145. Nielsen SF, Ph.D., Nodestgaard BG.(2012) Statin Use and Reduced Cancer-Related Mortality. The New England Journal of Medicine. 367:1792-802. Pettit G, Herald C, Doubek D, Herald D, Arnold E, Clardy J: Isolation and structure of bryostatin 1. J Am Chem Soc 1982, 104:6846-6848. Sharp KH, Davidson SK and Haygood MG. (2007) Localization of 'Candidatus Endobugula sertula' and the bryostatins thoughout the life cycle of the bryozoan Bugula neritina. The ISME journal(2007) 1, 693-702.