Marine Genetic Resources: A Source of New Drugs
The Experience of the Biotechnology Sector

Biodiversity and Genetic Resources of the Deep Sea

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Converting Marine Biodiversity into New Drugs.

The process of Drug Discovery

MNP Real Examples: Solving the problem of supply

Case studies: Yondelis, Bryostatin 1, Kahaladide F

The Future of Marine Natural Products.

Genomic exploration
Converting Marine Biodiversity into New Drugs.
The process of Drug Discovery
Global Pharmaceutical Market

Major areas of research in human health:
- CV: Cardiovascular
- CNS: Central Nervous System
- Anti-infectives
- GI: Gastro Intestinal
- Cancer
- ODA: Obesity, diabetes, arteriosclerosis.

The oncology sector is expected to grow to $60b in 2008.

New medicines are needed, with Selective & Specific Mechanisms of Action

Bio-Active Molecules
Sources of Bioactive Molecules

Natural and natural-derived drugs form 50% of *US Market Approvals* from 1981-2002. And more than ¾ in oncology!


**BioDiversity**

**Bio-Active Molecule**

**Combinatorial Chemistry** *(High Throughput Screening)*

**Target-based Design**

MEDICINE

TARGET
Bioactive Molecule ≠ Drug-like Molecule

*In vitro* activity does not guarantee *in vivo* activity or good drug-like properties.
Advantages of Marine Biodiversity

- Competition for Survival & Environmental pressure
  - Biodiversity
  - Defence, Attack, Signalling
  - Chemical diversity
  - Potential new Drugs

Sea has higher biodiversity than land
High Biodiversity = High chemical diversity

Opportunity: Marine exploration for pharmaceutical purposes < 20 years old.

0.01% of terrestrial samples show anti-tumor potential vs 1% of marine samples*

* US National Cancer Institute (NCI) Estimates
Which are the most promising MARINE organisms as a source of metabolites for application in human health?

**Macro-Organisms (mainly invertebrates)**
- Wide range of chemical defenses
- Industrial supply

**Micro-Organisms**
- Abundance > 99% non-cultivable
- Industrial supply

**Environmental DNA (Metagenomes)**
- Directly to the genes
- Heterologous expression

- Sponges, tunicates, corals, bryozoans, mollusks
- Actinomycetes, fungi, dinoflagellates, cyanobacteria
- From non-cultivable organisms (symbionts)
Numbers of MARINE NATURAL PRODUCTS for the period 1965--2005

Marine Metabolites

Sources of MARINE NATURAL PRODUCTS for the period 1965--2003

- Sponges: 31%
- Corals: 24%
- Ascidians: 6%
- Mollusks: 6%
- Microorganisms: 15%
- Green algae: 4%
- Brown algae: 5%
- Red algae: 1%
- Others: 8%

Citations of Marine Natural Products for source for the period 2001-2005
as percentage of the totals of 1965-2005


Marine Biodiversity
Biodiversity to Bioactive Molecules

BioDiversity
- Macro-Organisms
- Micro-Organisms
- Environmental DNA

Drug Discovery
- Extraction & Bio-Assay
- Guided Purification

Bioactive Molecule
- Chemical structure
- In Vitro Activity
- Intellectual property
- Small Quantity

Supply
- Bioactive molecules
  < 10^{-6} \% wet weight of marine organisms!

Synthesis, Fermentation, Biotechnology
Bioactive ≠ Drug-like Molecule

Bioactive Molecule

Drug-like Molecule

Medicine

Ready for Clinical Trials

Demonstrated Quality, Safety & Efficacy

Reaches Site-of-Action in Man (with required concentration, duration & safety)

Modify Structure, Drug Delivery Technology, Clinical Protocol

15-20 years
The Drug Development Process

**BioDiversity**
- 10,000

**Bioactive Molecule**
- 100

**Drug-like Molecule**
- 5 Failures

**Oncology Medicine**
- 1

**Discovery**
- 2-10 years

**Pre-clinical**
- 3.4 years

**Clinical Studies & Approval**
- 9.6 years

**Market**
- 11 years

Yondelis (ET-743): 15 years
- From Structural Determination

Paclitaxel (TAXOL): > 20 years
- (Bioactive molecule) to FDA approval

**Cost ~ $802 mill.**
- Only 2 in 5 Marketed Drugs recover costs

* Di Massi 2003
Marine Natural Products: Solving the Problem of Supply
Case studies: Yondelis, Bryostatin 1, Kahaladide F
Past: Some examples of Commercial marine drugs

• Early 1900s. Kainic acid (Red alga) was used as antihelmintic

• 1950s. Spongothymidine and spongouridine (Sponge) from these, Ara-C and Ara-A were synthetized. Then, AZT

  Ara-C: Antitumor. 1972
  Ara-A: Antiviral
  AZT: Antiviral (HIV) GSK

• 1965. Cephalosporin (Marine fungi) antibiotic

• 1969. Reported Prostaglandins from Caribbean gorgonians.

• 1970s. Shark cartilage oil (squalamine) inhibits angiogenesis.

• 1980s. Entered in Oncology Clinical Trials Didemnin B, isolated from a Caribbean ascidian (tunicate Trididemnum solidum). NCI
Current Commercial Marine Drugs

**Chronic pain (analgesic)**
Approved by FDA in 2004
**Prialt®.** - Elan Pharm.

**Cancer (soft tissue Sarcoma)**
Positive Opinion by EMEA in 2007
Currently in Phase III ovarian cancer (J&J/PhM)
**Yondelis®.** - PharmaMar

**Antiinflamatory (skin care lotions)**
Yearly Income > 750,000$
**Resilience®.** Estée Lauder
<table>
<thead>
<tr>
<th>MoA</th>
<th>Compound</th>
<th>Source</th>
<th>Company</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Inducer</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Protein C Kinase Inhibitor</td>
<td><strong>Bryostatin-1</strong></td>
<td>Bryozoan/ Symbiont</td>
<td>NCI/ Bristol Myers</td>
<td>Wang et al., (1998) Biochem Pharmacol</td>
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<tr>
<td>Microtubule Interfering agents</td>
<td><strong>Dolastatin 10</strong></td>
<td>Sea Slug</td>
<td>NCI/ Knoll</td>
<td>Jordan et al., (2005) Mol Cancer Ther</td>
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<tr>
<td></td>
<td><strong>Discodermolide</strong></td>
<td>Sponge</td>
<td></td>
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<tr>
<td></td>
<td><strong>Halichondrins</strong></td>
<td>Sponge</td>
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</table>

... and 12 more compounds....
### Marine Drugs

#### Other than antitumors


<table>
<thead>
<tr>
<th>Condition</th>
<th>Source</th>
<th>Sponge</th>
<th>Mollusk</th>
<th>Alga</th>
<th>Coral</th>
<th>Microorganism</th>
<th>Others</th>
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<tr>
<td>Alzheimer’s</td>
<td>GTS-21 (DMBX)</td>
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<tr>
<td>AntiPsoriatic</td>
<td>Manoalide</td>
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<td>AntiAsthematic</td>
<td>IPL-512,601</td>
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<td>Pain</td>
<td>Contulakin G</td>
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<tr>
<td>AntiInflamatory</td>
<td>Several compounds</td>
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<tr>
<td>AntiBacterial</td>
<td>Several compounds</td>
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<tr>
<td>AntiFungal</td>
<td>Several compounds</td>
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<td>AntiViral</td>
<td>Several compounds</td>
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<td>AntiMalarial</td>
<td>Manzamines</td>
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<td>AntiTuberculosis</td>
<td>Elisapterosin B</td>
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<td>AntiCoagulant</td>
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<tr>
<td>AntiPlatelet</td>
<td>Eryloside F</td>
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<tr>
<td>Inmmuno-suppr.</td>
<td>Theonellapectolides</td>
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<tr>
<td>Nervous system</td>
<td>Conantokins G, R</td>
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</tr>
</tbody>
</table>
"With the enormous potential for discovery, development and marketing of novel marine bioproducts comes the obligation to develop methods by which these products can be supplied in a way that will not disrupt the ecosystem or deplete the resource..."

Shirley A. Pomponi, President & CEO
Harbor Branch Oceanographic Institute. FLORIDA, USA
The majority of Bioactive Compounds represent \(<10^{-6}\) wet weight of the marine invertebrate:

1,000 g wet sponge \(\rightarrow\) 0.1 g active compound
The supply of MNP.
Case Study: Yondelis

**Ascidian** *Ecteinascidia turbinata*

1 Kg biomass → 1 mg (0.001 g) Compound

3 Alternatives to Manufacture

- Total Chemical Synthesis
- Mariculture
- Hemi-synthesis

Chemical structure of ET-743 (Yondelis)
The supply of MNP. Case Study: Yondelis

Total Chemical Synthesis


> 50 chemical steps. Not cost-effective

Mariculture


1 gram drug substance from 1 metric Ton of the frozen ascidian

Hemi-synthesis

Cuevas et al, Org. Lett.. 2000, 2, 2545

Bacterial fermentation + Chemical modifications

Current Industrial Process for manufacturing Yondelis under GMP

Safracin B, produced by Pseudomonas fluorescens

ET-743 (Yondelis)
The supply of MNP.
Other examples

Bryostatin 1

Bryostatin 1 is currently in Oncology Clinical Phase II

NCI & Bristol Mayers

Bryozoan Bugula neritina

1 Kg biomass → 10 mg (0.01 g) Compound

Chemical structure of Bryostatin 1

3 Alternatives for Manufacture

- Total Chemical Synthesis
- Mariculture
- Biotechnology

0.5 m² panel (1.5 Kg) B. neritina
After 5 months growing in the sea

(*) A bacterial symbiont harboring the PKS gene cluster of Bryostatin has been identified. In the future, Could be possible to manufacture Bryostatin 1 by fermentation

Kahaladide F is currently in Oncology Clinical Phase II

**PharmaMar**

**Sea hare** *Elysia rufescrens*

**Algal diet** *Bryopsis sp.*

**Chemical structure of kahaladide F**

3 Alternatives for Manufacture

- Total Chemical Synthesis
- Mariculture
- Biotechnology / Fermentation

In 2005, M. Hamman & R. Hill have reported two marine bacteria Vibrio sps. as producers of this compound

WO2005/042720 (2005) PCT
The Future of the Marine Natural Products. Past, Present and Future

Jacques Cousteau, 1947
The knowledge of Marine Genetic Resources is advancing rapidly due to

**New technology of diving**

**New scientific technologies (Biotechnology)**

- Genomics (DNA sequencers & Bioinformatics)
- Proteomics (selection & validation of targets)
- Metabolomics (structural elucidation as NMR)

**Industrial applications**

- Pharmaceuticals
- Enzymes
- Cosmetics
- Others

*PharmaMar’s divers, 2007*
More than 18,000 marine metabolites registered in MarinLit
(Marine Literature Database. Vpc 14.3, Jun, 2007)

About 30 marine compounds in Human Clinical Trials
Cancer, Anti-inflammatory, Anti-infectives, Pain, etc.

Classical view of **BioDiversity** has been radically changed with the advent of **molecular tools**

ALL plants, animals and fungi

Eucarya

Monera

Plantae

Fungi

Animalia

Protista

Bacteria

Archaea

Eucarya
New Frontiers: The close FUTURE

INNOVATIVE STRATEGIES OF MARINE BIOPROSPECTION

Is this paradigm true?

New Habitat

New GENETIC Diversity

New CHEMICAL Diversity

New MEDICINES

DEEP-SEA

Metagenomes

Apart of some corals and sponges from the deep-sea reporting interesting bioactive molecules, Bamboo corals (-1,000m) are currently being studied as bone substitutive (*orthopedic implants*) due to its efficient calcarean structures (*using a gorgonian protein*)


**Salinosporamide A** isolated from the new genera *Salinospora* (actinomyceye), collected at depths of more 1,000m is being developing in clinical trials for cancer


The major part of the Genetic Resources (as abundance of DNA) in the Earth are located in the water-column and sediments.
# The marine environment

## Number of prokaryotes in aquatic habitats

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Volume ml</th>
<th>Cells/ml $\times 10^5$</th>
<th>Total no. of cells $\times 10^{26}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Marine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continental shelf</td>
<td>$2.03 \times 10^{20}$</td>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>Open ocean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water, upper 200 m</td>
<td>$7.2 \times 10^{22}$</td>
<td>5</td>
<td>360</td>
</tr>
<tr>
<td>Water, below 200 m</td>
<td>$1.3 \times 10^{24}$</td>
<td>0.5</td>
<td>650</td>
</tr>
<tr>
<td>Sediment, 0-10 cm</td>
<td>$3.6 \times 10^{19}$</td>
<td>4600</td>
<td>170</td>
</tr>
<tr>
<td><strong>Fresh</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lakes</td>
<td>$1.25 \times 10^{20}$</td>
<td>10</td>
<td>1.3</td>
</tr>
<tr>
<td>Rivers</td>
<td>$1.2 \times 10^{18}$</td>
<td>10</td>
<td>0.012</td>
</tr>
<tr>
<td>Saline lakes</td>
<td>$1.04 \times 10^{20}$</td>
<td>10</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>1180</td>
</tr>
</tbody>
</table>

From Whitman et al 1998
Archaeal dominance in the mesopelagic zone of the Pacific Ocean

Markus B. Kanner, Edward F. DeLong & David M. Karl

% of DAPI Count

Depth (m)

Bacteria

Group 1 Archaea

% of DAPI Count
More than 99% of the microbial community remains to be explored. The known 1% have been reporting the large majority of antibiotic compounds.

$1 \times 10^{10}$ (One billion) microbial cells per liter of sea-water.

There are more microbes in one gram of ocean sediment than there are humans alive today!

One sponge contains up to $10^{12}$ uncultivable cells.

That is, a sponge contains up to $1,3 \times 10^{13}$ bp prokaryotic DNA. Considering 5% of these prokaryotes are bioactive compounds type $\text{PKS/NRPS}^*$ producers, then, each sponge may potentially have $1,3 \times 10^6$ genes involved in the biosynthesis of pharmacological interesting compounds.

*Jenke-Kodama et al., (2007) Mol Biology and Evolution*

$\text{PKS/NRPS}^*$: Genes involved in the production of secondary metabolites:

PKS: Poly Ketide Syntethases  NRPS: Non Ribosomal Peptide Syntethases
Metagenomes Vs Classical Bioprospection

Metagenomic Approach: Study of environmental DNA (or genomic DNA)

Bioactive compounds
(Peptides and polyketides)

NRPS / PKS enzymes

DNA
(genomic, environmental)

Marine samples
(Organisms, sediments, Water column)

Analysis of DNA sequences of the global community
Metagenomes: Biotechnology applied to Marine Studies

Sampling

Isolation of DNA

Cloning DNA

Metagenomic libraries

Screening

Functional screening

Sequence screening

Pharmaceuticals

Enzymes

Phylogenetic diversity


FROM FUNCTIONAL GENOMICS TO NATURAL PRODUCTS OF MARINE MICROORGANISMS

Marine Functional Genomics
New Drugs
Enzymes
Metagenomics

June 21-24, 2006
Greifswald, Germany
Alfried-Krupp-Wissenschaftskolleg

Scope of the Conference

During evolution the extreme marine environmental conditions have produced adaptation strategies in marine microorganisms that are different from their terrestrial counterparts. This involves new natural products that are different from known structures of terrestrial organisms. However, the exploration of new drugs, enzymes or biochemical capabilities of marine origin is difficult. Less than 1% of marine microorganisms can be cultivated so far, and for a negligible number of these known marine microorganisms genetic tools are available. Metagenomics, genome sequencing and the techniques of functional genomics make it possible to visualize potential metabolic and biochemical capabilities of even unculturable marine cells. The conference will present recent results of these new research fields and discuss the potential of molecular methods for the discovery of new natural compounds from marine microorganisms.
Material ??? Transfer today
Genetic transmission

1. Expedition
2. Sampling
3. Genomic library
4. Screening
5. Sequence positive clones
6. Data transmission
7. Synthetise genes
8. Cloning into vector and transforming Bacteria
9. Fermentation
10. Pharmaceutical compound
BioDiversity yields very interesting, highly potent BioActive Molecules.

Biotechnology and Chemical Synthesis are necessary to convert these to medicines.

New habitats (Deep-sea) and innovative genetic explorations (metagenomes) may lead to new clinical candidates in the near future.

Today, it is possible to synthesize genetic products from marine genomic resources collected overseas.

BioDiversity survival is essential for business survival.
"In one drop of water are found all the secrets of the oceans"

Kahlil Gilbran
1883-1931