Bioactive natural products of fungi from marine and terrestrial habitats

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WHY MICROORGANISMS ?
Over 120 of the most important medicines (Penicillins, Cyclosporin, Adriamycin, ... etc.) are obtained from terrestrial microorganisms.

- Expected enormous biodiversity of microorganisms.
- Constitute the ultimate ‘Undiscovered’ frontier for the search of marine natural products.
- Supplying sufficient materials.
Endophytic fungi inhabit such abiotope. In the course of the last 12 years, about 6500 endophytic fungi were isolated from herbaceous plants and trees, screened for their biological activities, their metabolites and have isolated and determined the structures of the biologically active compounds.
The isolated metabolites originated from different biosynthetic pathways: isoprenoid, polyketide, amino acid derivatives, and belonged to diverse structural groups: terpenoids, steroids, xanthones, quinones, phenols, isocumarines, benzopyranones, etc.
The potential role of the endophyte and its biologically active metabolites in its association with its host has been studied. The fungal endophytes possess the exoenzymes necessary to colonize their hosts.
The fungal endophyte–plant host interaction is characterized by a finely tuned equilibrium between fungal virulence and plant defense. If this balance is disturbed by either a decrease in plant defense or an increase in fungal virulence, disease develops. Many groups of fungi in different biotopes are waiting to be exploited.
Since natural products are adapted to a specific function in nature, the search for novel secondary metabolites should concentrate on organisms that inhabit novel biotopes.
This addresses some important questions:

- Which evolutionary pressures led to gene clustering?
- Why closely related species produce different profiles of secondary metabolites?
- Whether fungal genomics will accelerate the discovery of new secondary metabolites of potential biological activity?
Secondary metabolites from fungi
Microorganisms as a source for natural products

- Penicillin G
- Cephalosporin C
- Griseofulvin
- Echinocandin B
- Cyclosporine
- Mycophenolic acid
- Lovastatin
Secondary metabolites from marine-derived fungi

Cephalosporin C
*Acremonium chrysogenum*
first natural product of a marine-derived fungus (1946)

Sorbicillactone A
*Penicillium chrysogenum*
from sponges belonging to the genus *Ircinia* (2003)
Isolation of microorganisms from marine sponges

1. Cut with a sterile blade
2. Transfer a piece of the inner part of the sponge onto an agar plate
3. Streak over the plate
4. Primary isolates (mixed cultures)
5. Pick single colonies; inoculate new agar plates
6. "Dilute" the colony
7. Pure culture

Control #1 (surrounding sea water)
Control #2 (sponge surface)
Fermentation of fungal strains

static culture in Erlenmeyer flasks
liquid culture in fermenter (5 L scale)
solid-state fermentation (rice-based medium)
Flutimide isolated from
*Delitschia confertaspora*

**Halovir A** $R_1 = \text{OH}, R_2 = \text{CHMe}_2, n = 12$

**Halovir B** $R_1 = \text{OH}, R_2 = \text{Me}, n = 12$

**Halovir C** $R_1 = \text{H}, R_2 = \text{CHMe}_2, n = 12$

**Halovir D** $R_1 = \text{OH}, R_2 = \text{CHMe}_2, n = 10$

**Halovir E** $R_1 = \text{H}, R_2 = \text{CHMe}_2, n = 10$

Fungal metabolites with antiviral activity isolated from
*Scythalidium* sp.
Secondary metabolites from endophytic fungi

Paclitaxel (Taxol)
originally isolated from the Pacific yew tree, *Taxus brevifolia*, but later also reported from endophytic fungi, including *Taxomyces andreanae* and *Pestalotiopsis microspora* (1993)
Cytotoxic metabolites from endophytic fungi *Alternaria* sp. from the Egyptian plant *Polygonum senegalense*

alternariol sulphate
alternariol methyl ether sulphate
hydroxyalternariol methyl ether

cytotoxic activity *
- $EC_{50} = 6.6$ µM vs. L 5178 Y (alternariol sulphate)
- $EC_{50} = 6.2$ µM vs. L 5178 Y (demethylaltenusin)

* data provided by Prof. W. E. G. Müller, Mainz
Secondary metabolites from endophytic *Alternaria* sp.
from the Egyptian plant *Polygonum senegalense*

antibacterial activity towards
- *Bacillus subtilis*
- biofilm forming strains of *Staphylococcus epidermis* (MIC 100 µg/mL) *

* data provided by
Dr. U. Hentschel, Würzburg
Detection of secondary metabolites produced by endophytic fungi in host plant extracts

A) extract of *Polygonum senegalense*

- **R\text{t} [\text{min}]**
  - 22.65 min
- **m/z 271**
  - [M+H]\(^+\)

B) alternariol monomethylether (isolated from alternaria spp)

- **R\text{t} [\text{min}]**
  - 22.67 min
- **m/z 271**
  - [M+H]\(^+\)

shown: LC-Ms extracted ion chromatogram (left), full MS (right)
Fungal metabolites with anticancer, immunosuppressive and antioxidant activities

Paclitaxel: cytotoxic
Vincristine: cytotoxic
Subglutinol A: immunosuppressive
Pestacin: antioxidant
Microcarpalide: cytotoxic
Chaetomelic acid A: cytotoxic
Unexpected Problems

- Taxonomy of marine bacteria and marine fungi is very poorly defined.
- Technical problems that arise in culturing marine microorganisms.
- Metabolic changes may occur probably due to partially unsatisfied micronutrients in culture medium.
- High unpredictability of expected results.

General goals of work on microorganisms

- The first goal of the studies on microorganisms (as bacteria, fungi...etc.) to prove which are the true sources of isolated metabolites, either the host or their associated microorganisms.

- It should be possible to obtain reasonable amounts of valuable substances through large-scale production by culture or fermentation.

- A random search may afford unexpected new metabolites that might eventually be endowed with interesting pharmacological properties.