Bioactive natural products of fungi from marine and terrestrial habitats

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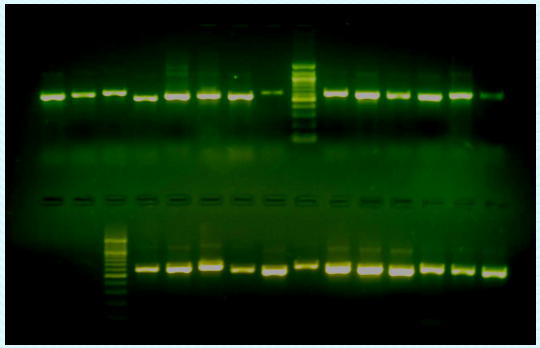
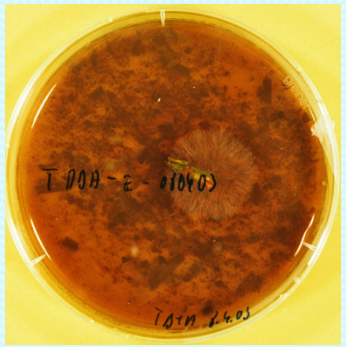
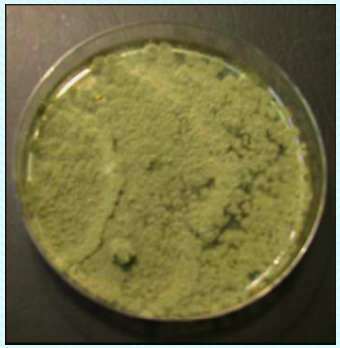






WHY MICROORGANISMS ?

- Over 120 of the most important medicines (Penicillins, Cyclosporin, Adriamycine,... 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 address some important questions:

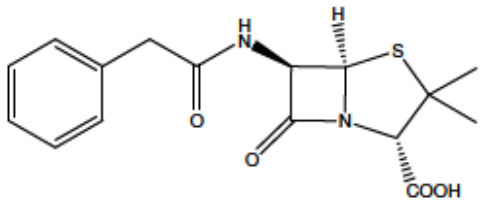
-Which evolutionary pressures led to gene clustering?,

-Why closely related species produce different profiles of secondary metabolites?,

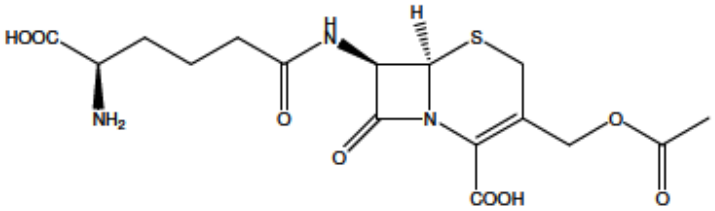
-whether fungal genomics will accelerate the discovery of new secondary metabolites of potential biological activity ?

Secondary metabolites from fungi

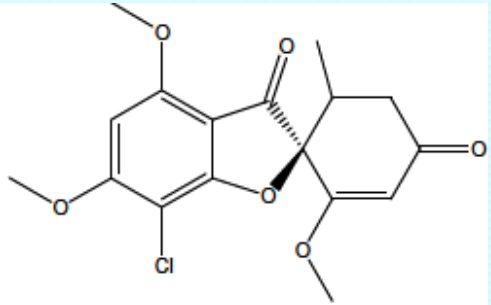
Microorganisms as a source for natural products



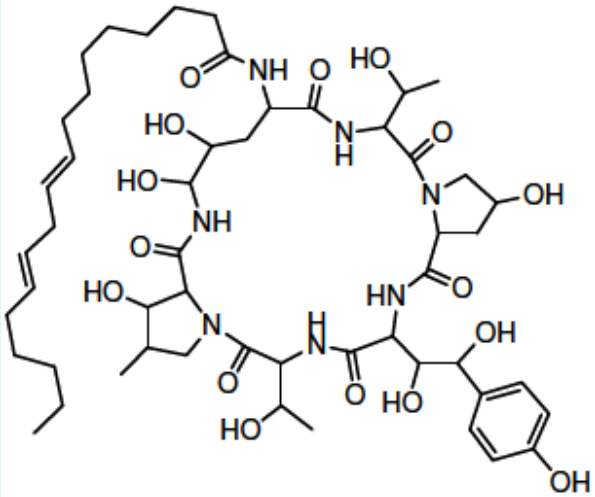
Penicillin G



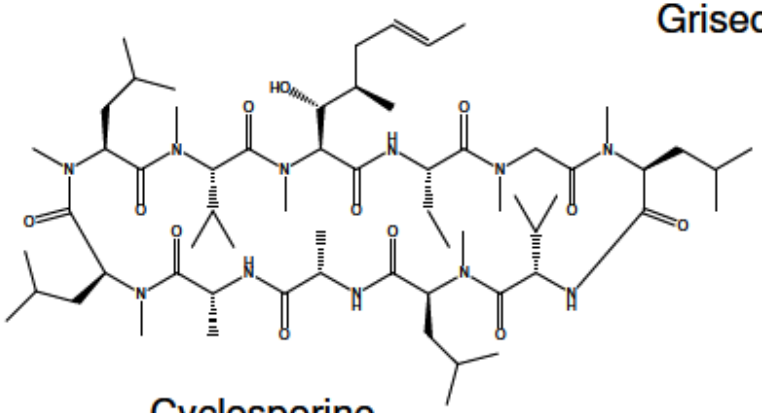
Cephalosporin C



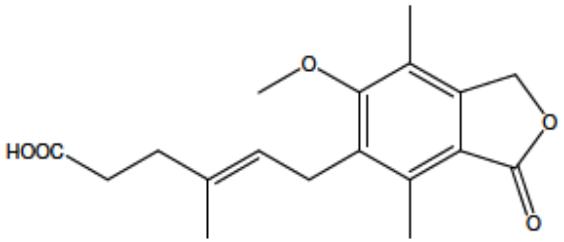
Griseofulvin



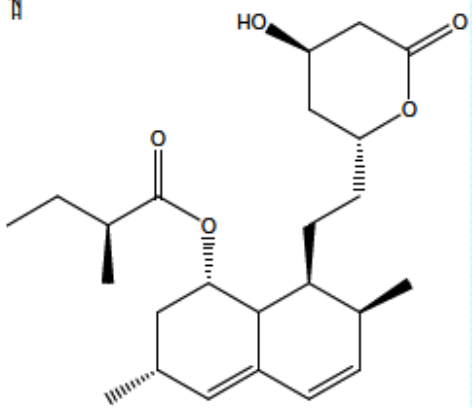
Echinocandin B



Cyclosporine

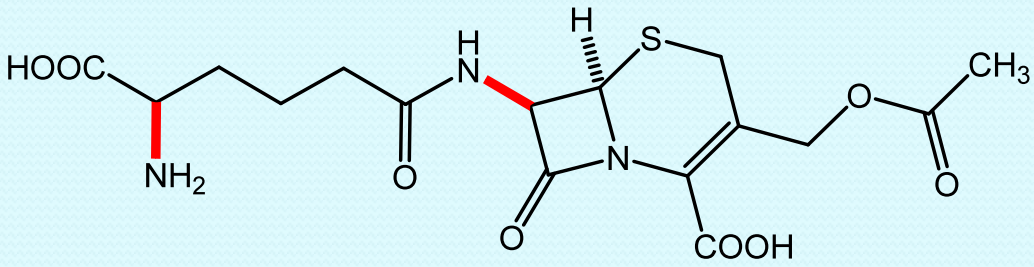


Mycophenolic acid



Lovastatin

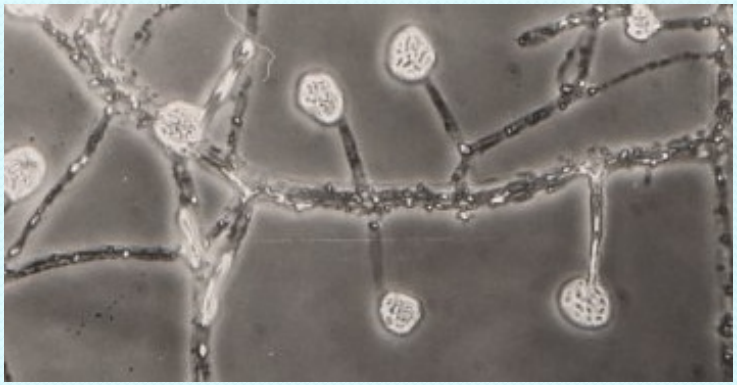
Secondary metabolites from marine-derived fungi

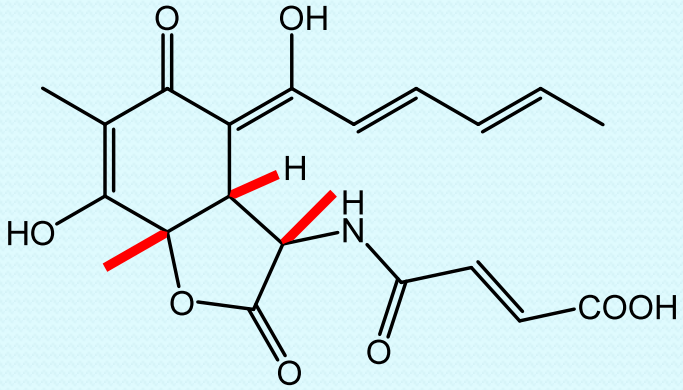


Cephalosporin C

Acremonium chrysogenum

first natural product of a marine-derived fungus (1946)





Sorbicillactone A

Penicillium chrysogenum

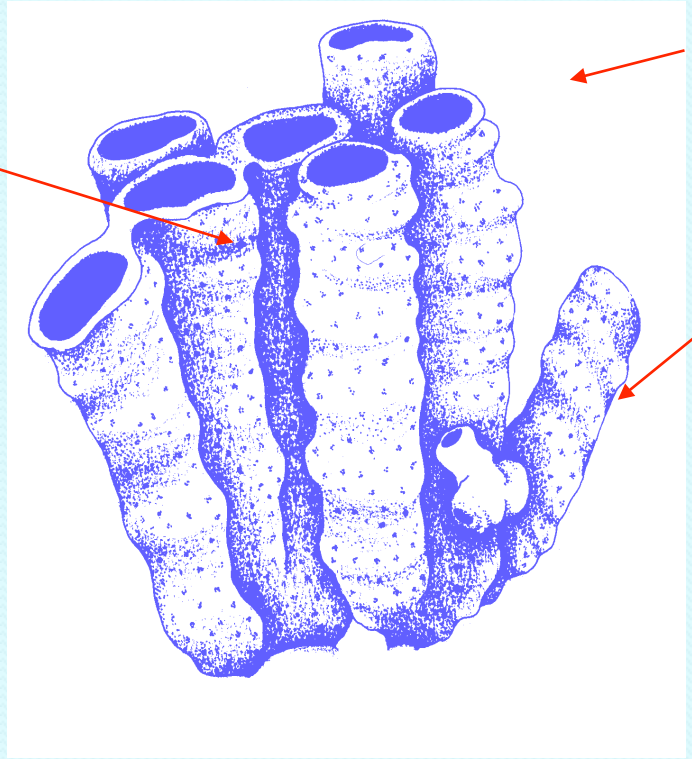
from sponges belonging to the genus *Ircinia* (2003)

Isolation of microorganisms from marine sponges

cut with a
sterile blade

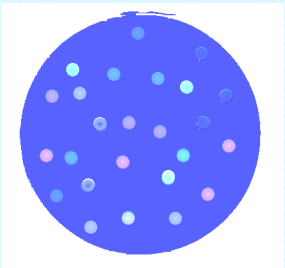
transfer a piece
of the inner part
of the sponge
onto an agar plate

streak over the plate

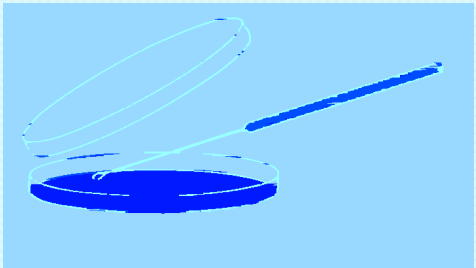


control #1
(surrounding
sea water)

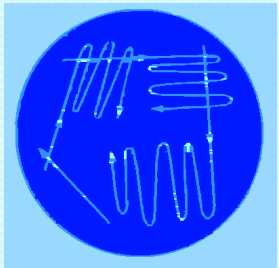
control #2
(sponge
surface)



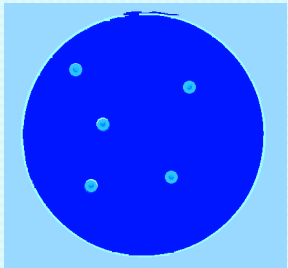
primary isolates
(mixed cultures)



pick single colonies;
inoculate new agar plates



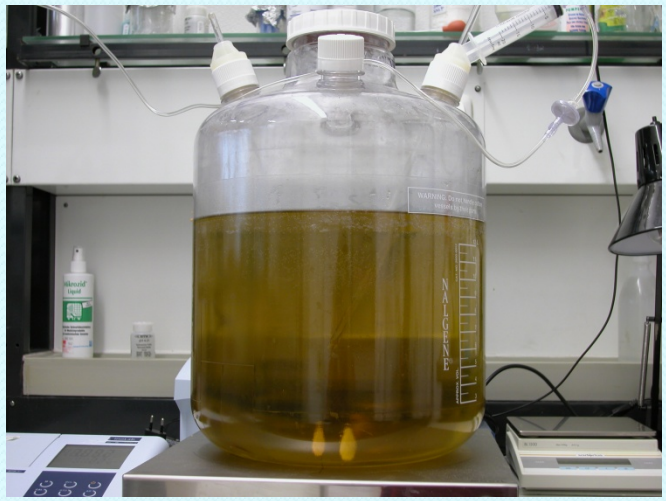
”dilute” the colony



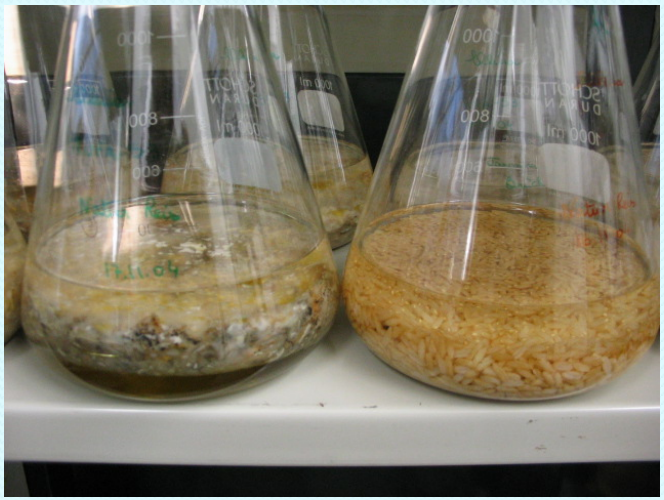
pure culture

Fermentation of fungal strains

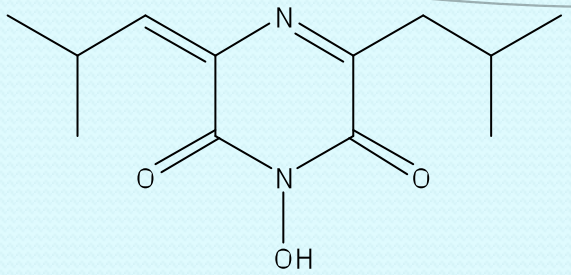
static culture in Erlenmeyer flasks



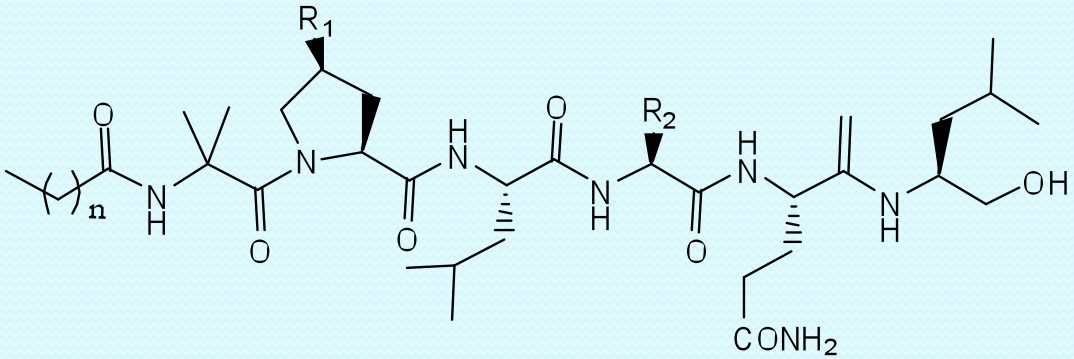
liquid culture in fermenter (5 L scale)



solid-state fermentation (rice-based medium)



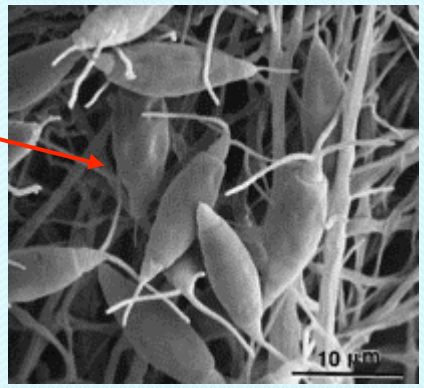
Flutimide isolated from
Delitschia confertaspora

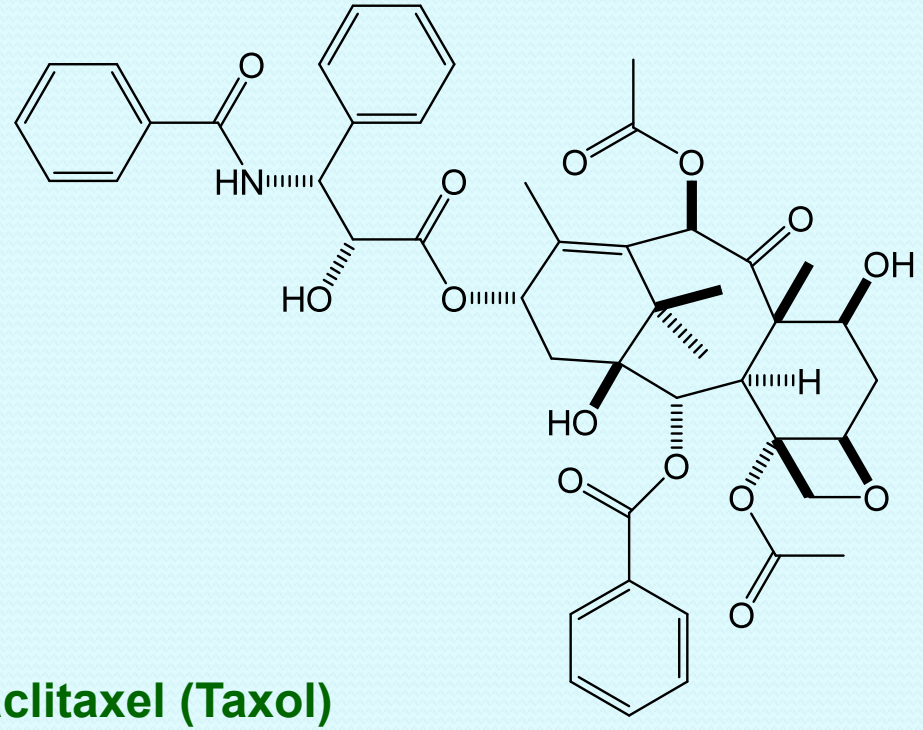


Halovir A R_1= OH, R_2= $CHMe_2$, n=12
Halovir B R_1= OH, R_2= Me, n=12
Halovir C R_1= H, R_2= $CHMe_2$, n=12
Halovir D R_1= OH, R_2= $CHMe_2$, n=10
Halovir E R_1= H, R_2= $CHMe_2$, n=10

Fungal metabolites with antiviral activity isolated from *Scytalidium* sp.

Secondary metabolites from endophytic fungi

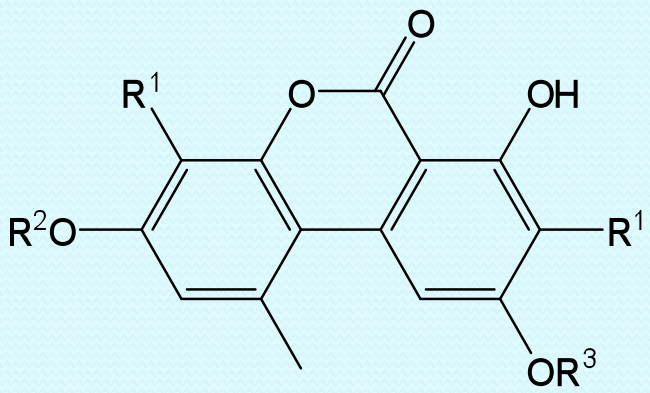


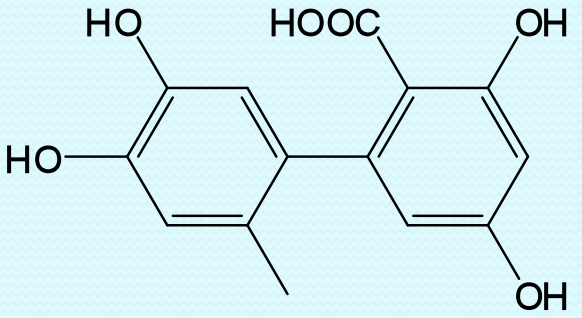


Paclitaxel (Taxol)

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*

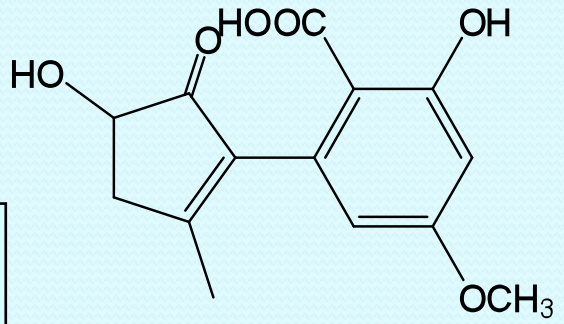




demethylaltenusin

	R^1	R^2	R^3
alternariol sulphate	H	H	SO_3^-
alternariol methyl ether sulphate	H	SO_3^-	CH_3
hydroxyalternariol methyl ether	OH	H	CH_3

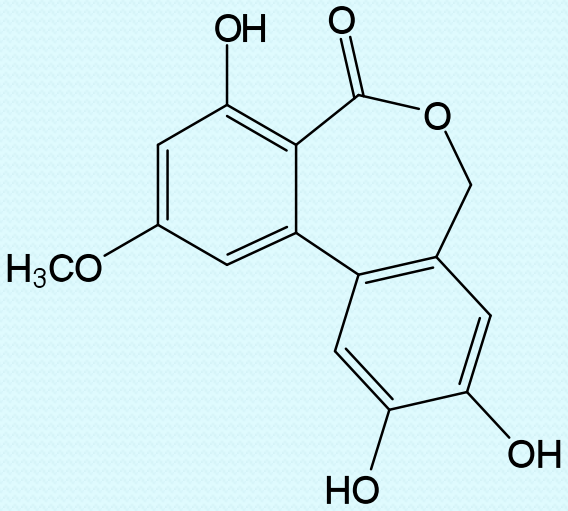
cytotoxic activity *
- EC_{50} 6.6 µM vs. L 5178 Y (alternariol sulphate)
- EC_{50} 6.2 µM vs. L 5178 Y (demethylaltenusin)



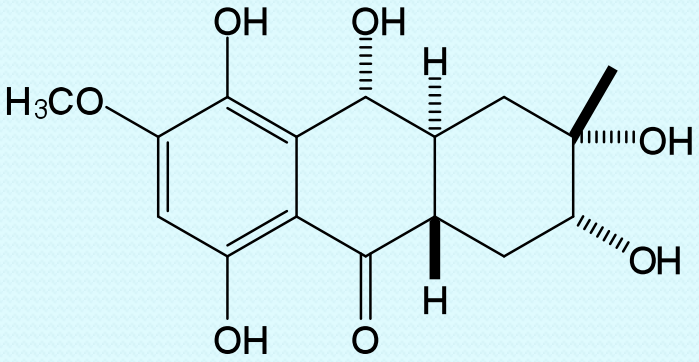
talaric acid

* data provided by
Prof. W. E. G. Müller, Mainz

Secondary metabolites from endophytic *Alternaria* sp. from the Egyptian plant *Polygonum senegalense*



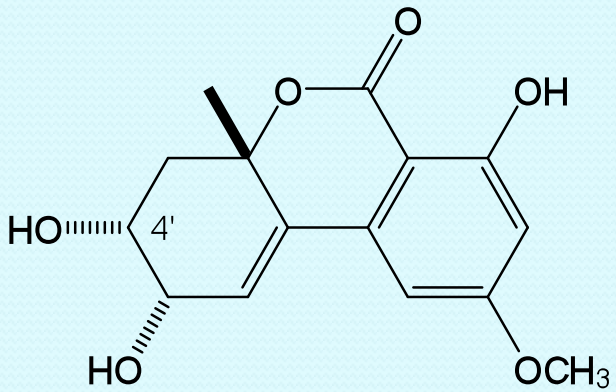
alterlactone



5-hydroxytetrahydroaltersolanol B

antibacterial activity towards

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

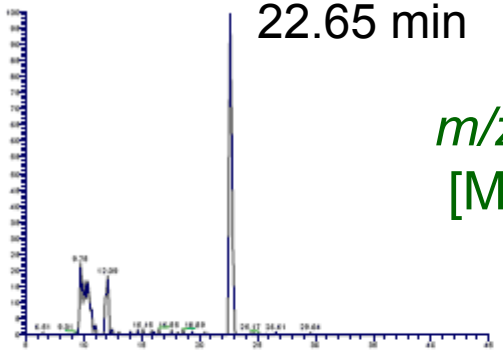


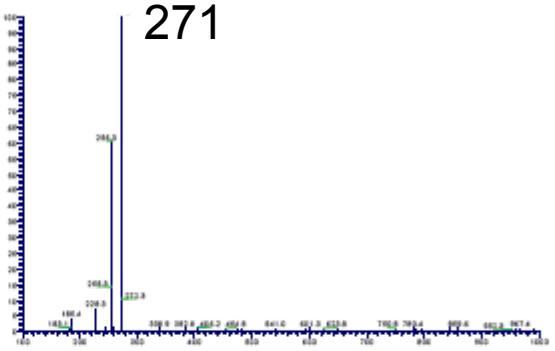
4'-epialtenuene

* data provided by
Dr. U. Hentschel, Würzburg

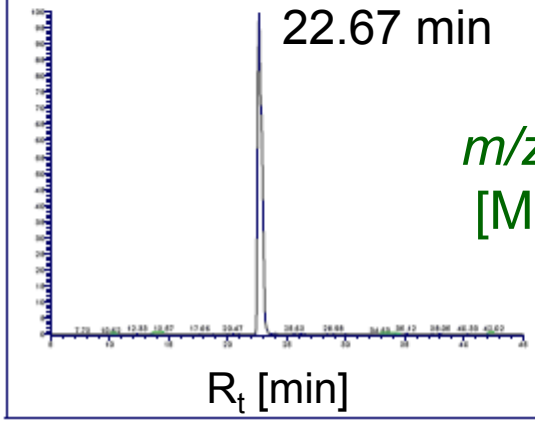
Detection of secondary metabolites produced by endophytic fungi in host plant extracts

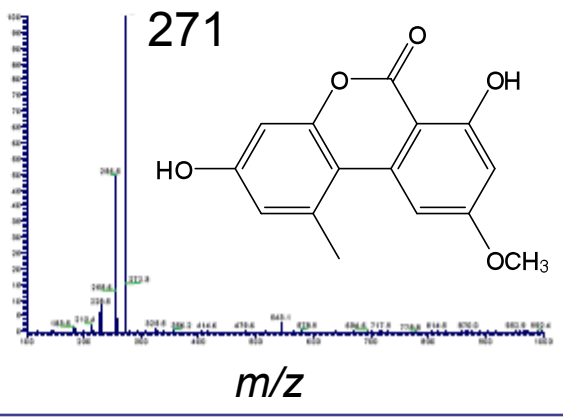
A) extract of *Polygonum senegalense*

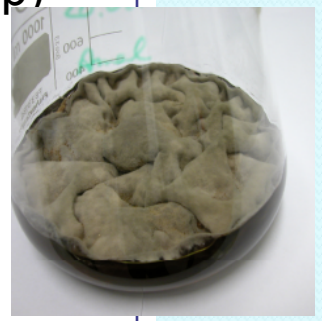




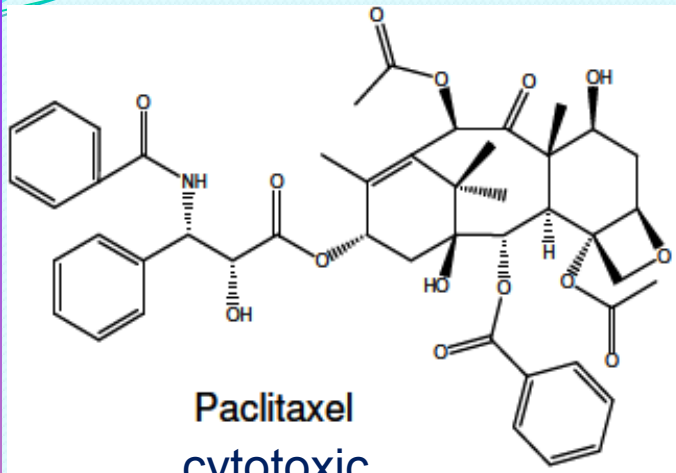
B) alternariol monomethylether (isolated from alternaria spp)





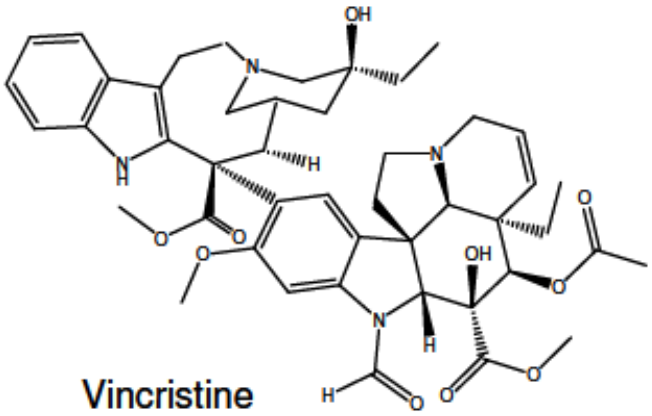


shown:LC-Ms extracted ion chromatogram (left), full MS (right)



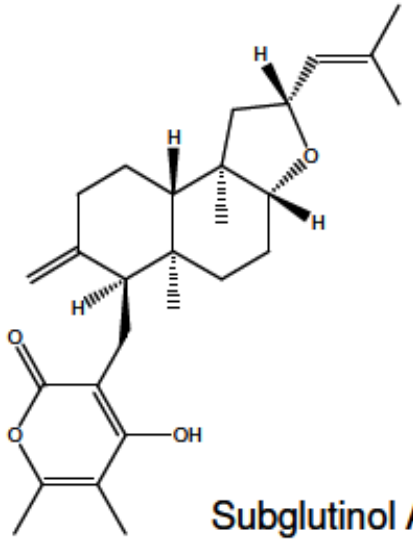
Paclitaxel

cytotoxic



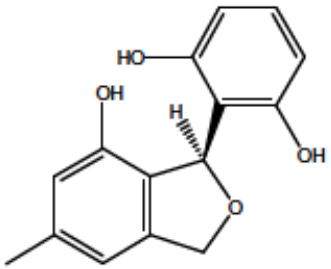
Vincristine

cytotoxic



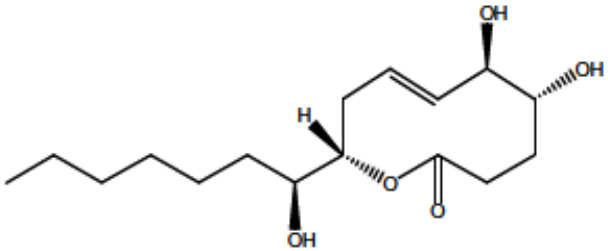
Subglutinol A

immunosuppressive



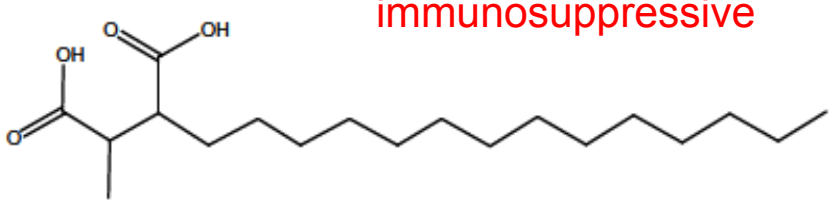
Pestacin

antioxidant



Microcarpalide

cytotoxic



Chaetomellic acid A

cytotoxic

Fungal metabolites with anticancer, immunosuppressive and antioxidant activities

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.

A. Kelecom, *Anais da Academia Brasileira de Ciencias* 2002, 74(1), 151-170.

R. A. MacLeod, The question of the existence of specific marine bacteria. *Bacterial Rev.* 1965, 29, 9-23.

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.