ARE NATURAL PRODUCTS ISOLATED FROM PLANTS, PRODUCTS OF EPI- AND/OR ENDO-PHYTIC MICROBIAL INTERACTIONS WITH / WITHIN THE HOST?

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Richard G. Powell Symposium

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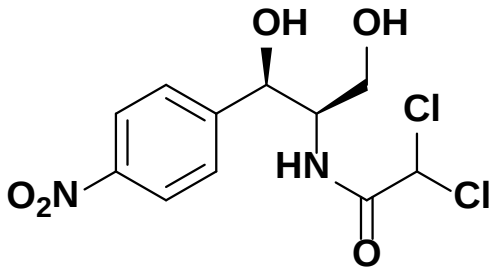
Monday, 27JUL15

Introduction I

The title to this talk is a "trifle long" but hopefully the talk will not be!

By way of introduction, in the Marine area, the systematic investigation of which from a secondary metabolite aspect, has really only occurred in the last 40 or so years, it became apparent early on, that bioactive compounds (or close relatives that were known from terrestrial sources) were being reported.

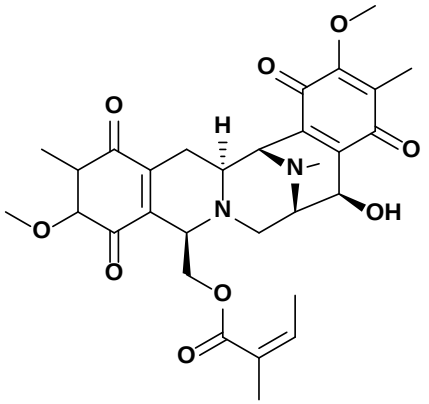


Chloramphenicol

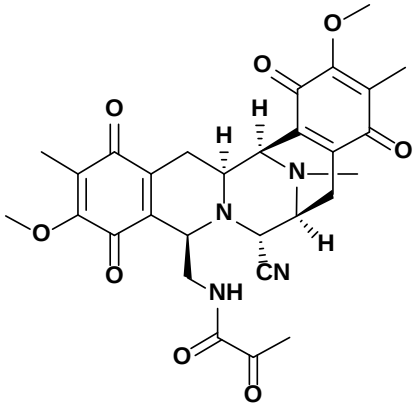
Price et al, JA, 1981

Introduction II

Then in 1982, Frincke and Faulkner identified the renieramycins (saframycin-like) from Eastern Pacific sponges of the Genus Reniera.



1. Renieramycin A



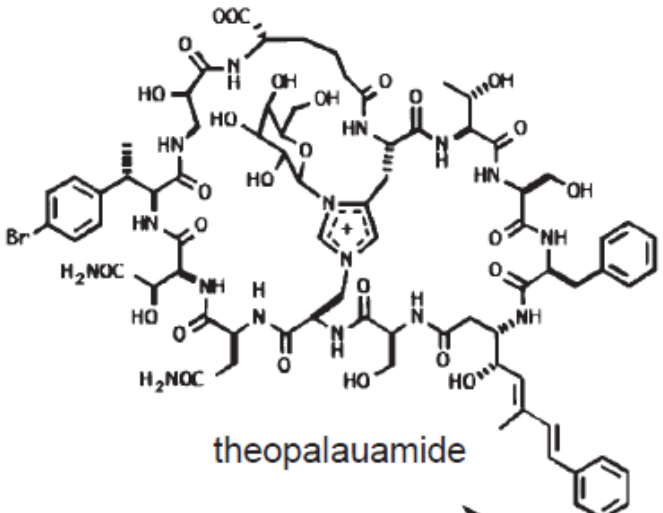
2. Saframycin A

The saframycins had been reported as antibiotics by Arai in 1977 from the terrestrial microbe S. lavendulae, and as antitumor agents in 1980.

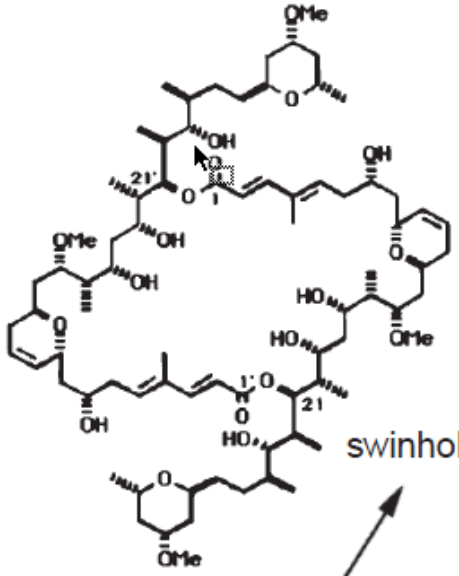
So do sponges and other invertebrates contain microbes that are the source?

Early Evidence for Cryptic Microbial Production

Paluan chemotype of
Theonella swinhoei

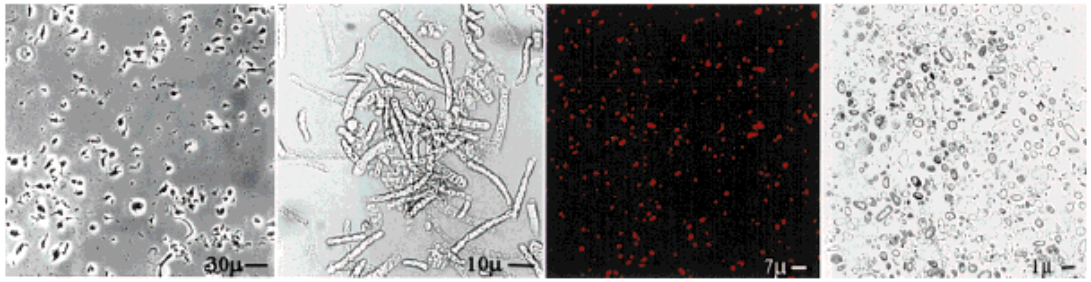


theopalauamide



swinholide A

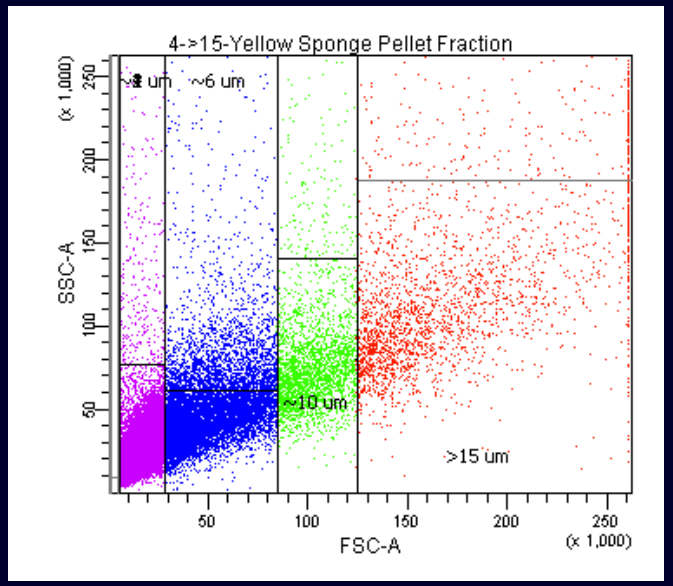
Micrographs of purified cell types. Associated chemistry shown above

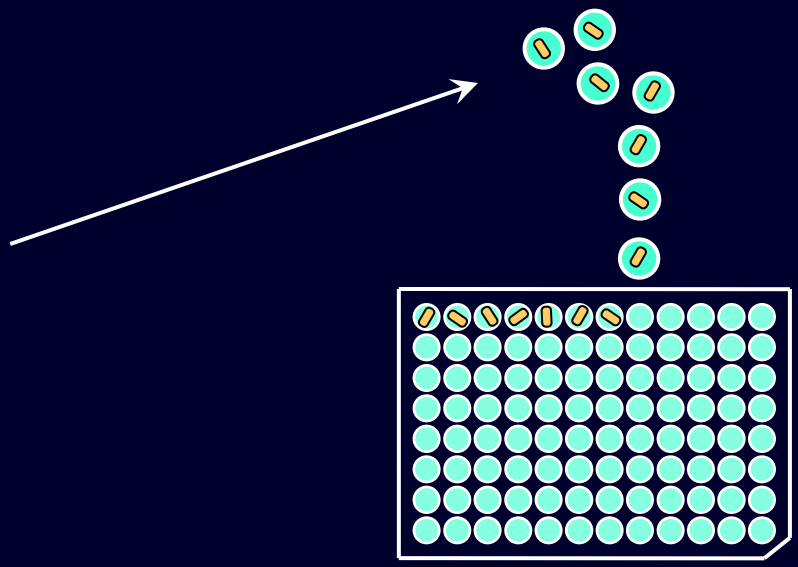


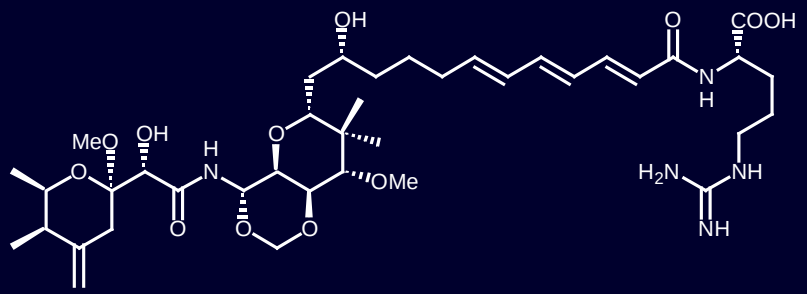
Sponge cells | **"Entotheonella palauensis"** | **Unicellular cyanobacteria** | **Unicellular bacteria**

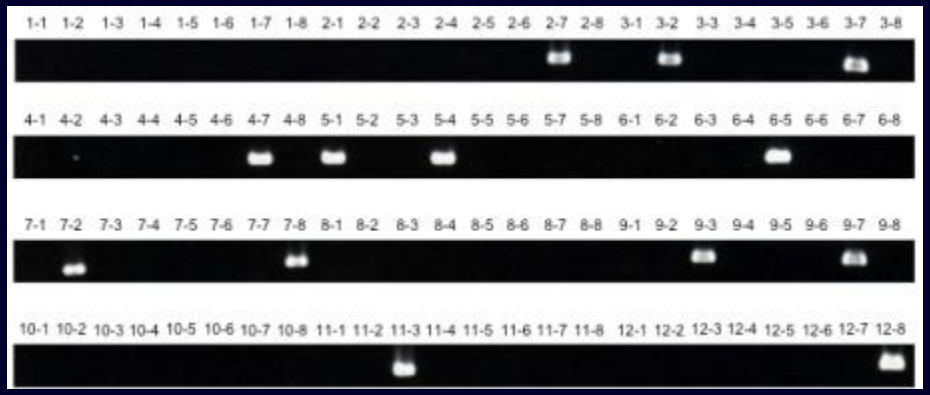
Courtesy of Joern Piel

Schmidt, Faulkner, Haygood et al., *Mar. Biol.* (2000) 136, 969
Reviewed in Bewley, Faulkner, *Angew. Chem. Int Ed.* (1998) 37, 2162



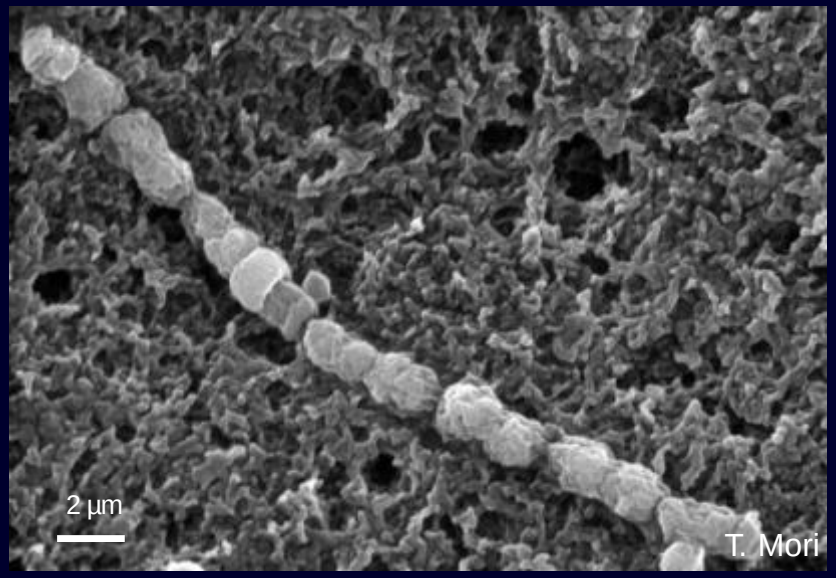


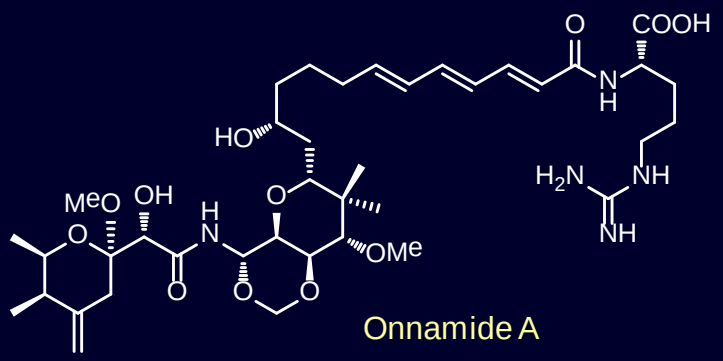




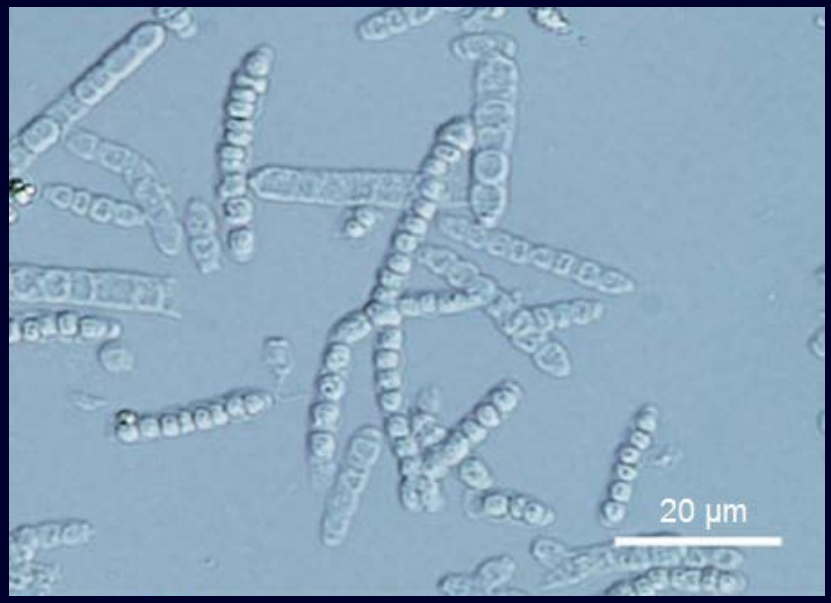
Courtesy of Joern Piel

The Putative Onnamide Producer Is a Member of the Candidate Genus “Entotheonella”





Onnamide A



Courtesy of Joern Piel

M. Wilson *et al.*, *Nature* 2014

Marine Microbial Involvement Proven

So the answer in the case of the marine environment was shown to be a "resounding YES" from the recent work of Joern Piel's group, where 31 of the 32 then known bioactive compounds from T. swinhoei (yellow morph) were produced by an as yet uncultured bacterium in the sponge.

In the plant kingdom, the reports of endophytic and epiphytic microbes (often fungi) being present in / on plants has been well documented, but the idea that " some plant secondary metabolites" may have a microbial connection, has been "resisted" by significant numbers of NP plant chemists over the years.

Let me now see if I can at least make people consider this idea

Why Plant-Derived Secondary Metabolites ?

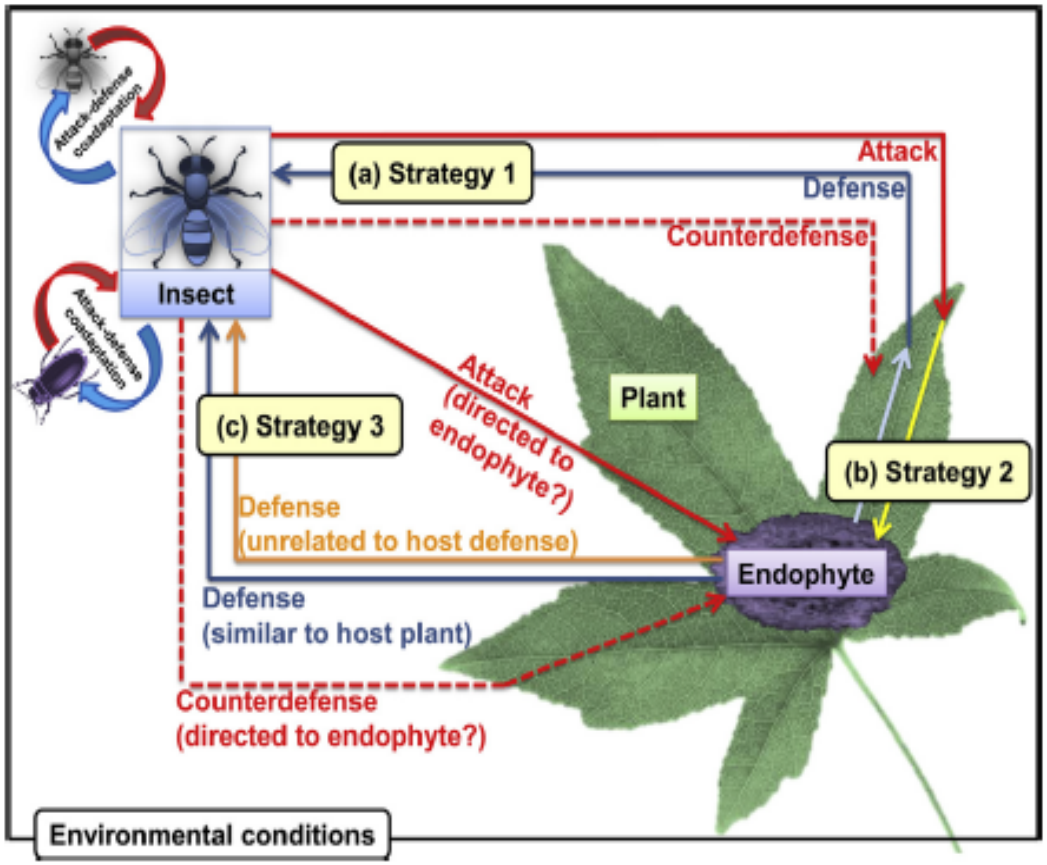


Fig. 2. Schematic representation of the different attack-defense-counterdefense strategies linking insects with plants and endophytes producing the same compounds. (a–c) Three different strategies.

Assumption: Plant-derived secondary metabolites are protective agents

Kusari et al, Phytochem, 2013

Plant – Microbe Axis I

In the case of higher plants, frequently metabolites are reported at one location but not from another even though the genus/species are identical.

Over the last (perhaps) 21+ years, there have been a number of reports in the literature that have invoked endophytic fungi as being involved in the production of plant-sourced 2° metabolites of interest as AT agents.

The first was the report on taxol® production by Taxomyces by Stierle and Strobel. This was followed over the years by reports on the vincas, podophyllotoxin and camptothecin.

All were very low level producers and were often considered to be artifacts.

Plant Microbe Axis II

We recently cultured a novel Taxol-producing fungus living inside *T. x media* wood and identified it as *Paraconiothyrium* strain SSM001 (Soliman et al., 2011). We demonstrated that SSM001 could produce Taxol independently of plant tissues following two cycles of *in vitro* hyphal tip transfer and inoculation into liquid media where the fungus grew >1000-fold prior to peak Taxol production, all in the absence of any plant tissues or extracts (Soliman et al., 2011). The fungus was localized to the living, nutrient-rich vascular tissues that radially traverse wood, known as wood medullary rays.

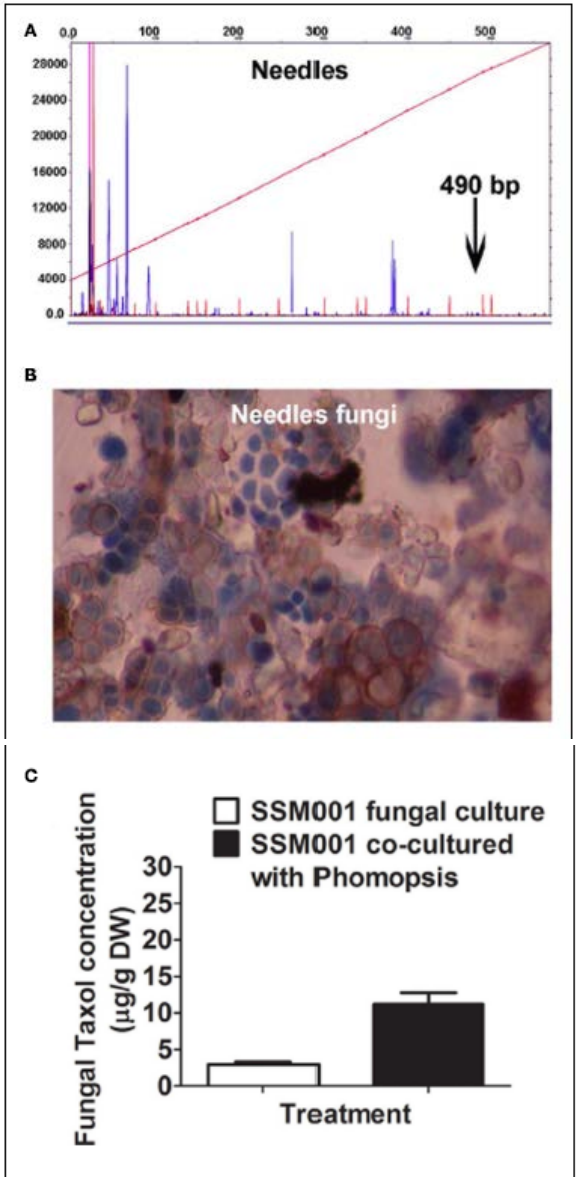


FIGURE 3 | Co-habitating fungi present in *Taxus x media* needles stimulate Taxol production from *Paraconiothyrium* SSM001 fungi. (A) tRFLP analysis of DNA pooled from *Taxus* needles showing several fungal peaks, none of which corresponded to SSM001. **(B)** Detection of different fungal spores from *T. x media* needles. **(C)** Effect of co-culturing of pure *Phomopsis* fungus isolated from *Taxus* needles with SSM001 in liquid culture.

Soliman & Raizada, Front. Micro 2013

Recent Relevant Comments on Fungal Taxol

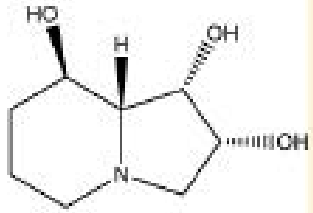
The reported revival of Taxol production as well as the stimulatory / inductive effect of host plant components on Taxol production by some endophytic fungi point towards a need-based Taxol production scenario.

Soliman and Raizada, working with Paraconiothyrium sp. isolated from T. x media, recently obtained results in agreement with such a hypothesis . The fungus showed higher Taxol production upon treatment with the host bark extracts and more importantly, when co-cultured with one or more endophytic fungi not capable of taxane production but isolated from the same host plant bark.

The production of Taxol by endophytic fungi might thus represent a means to thwart attack by invading fungi to keep plants healthy for an unhindered access to their apoplastic space

Kusari et al, Trends Biotech, 2014

Swainsonine I



Swainsonine

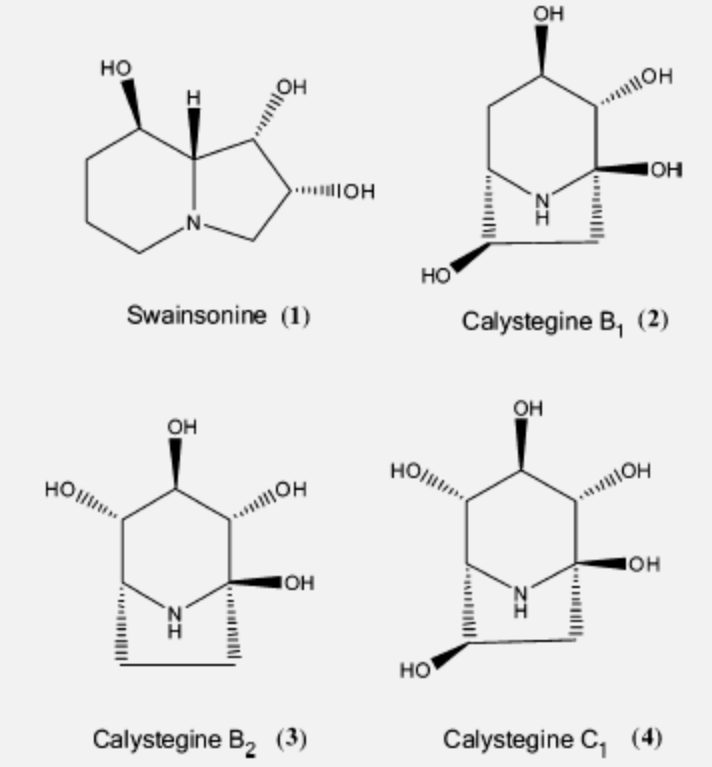
Astragalus, Oxytropis species in the Americas, Asia and Australia do not produce swainsonine but it is actually produced by an Undifilum species previously cultured from locoweeds in North America and Asia.

This fungal endophyte is actually vertically transmitted via the seed.

Grum et al, JNP 2013

Swainsonine II

Although swainsonine is produced by endophytic fungi, the same fungus does not produce the glycosidase inhibitors known as the calystegines, Treatment of the seeds with fungicides abolished the production of swainsonine but the calystegine levels were unchanged.



Cook et al, JAFC 2013

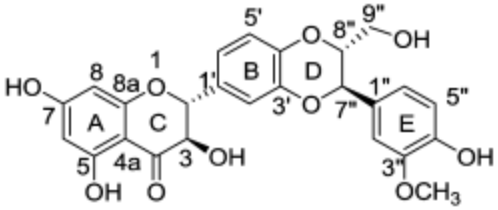
Plant Microbe Axis III

There is a recent paper from the Proksch group (Aly, Debbab & Proksch, Pharmazie, 2013, 68, 499-505) , that gives information as to the methods that can be used to increase "yields" in fermentations of endophytic fungi, and also gives an excellent "catalogue of molecules" obtained by fermentation.

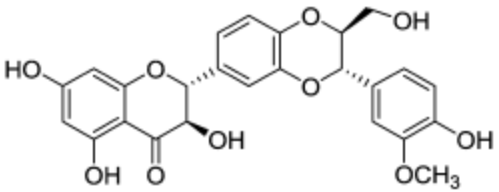
As an "old antibiotiker" these brought back memories of what we used to use to increase yields in the 1970s (and earlier) prior to genetic manipulations; the preferred route today.

That people have read /absorbed information from those days is shown by the work in the next slide.

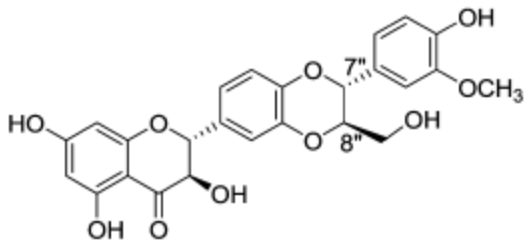
Silybin (Milk Thistle)



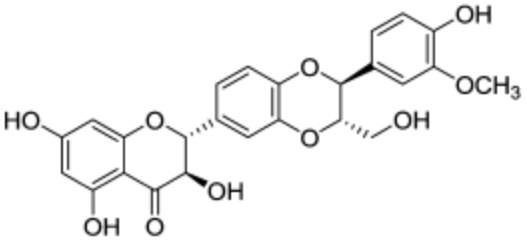
Silybin A (**1**)



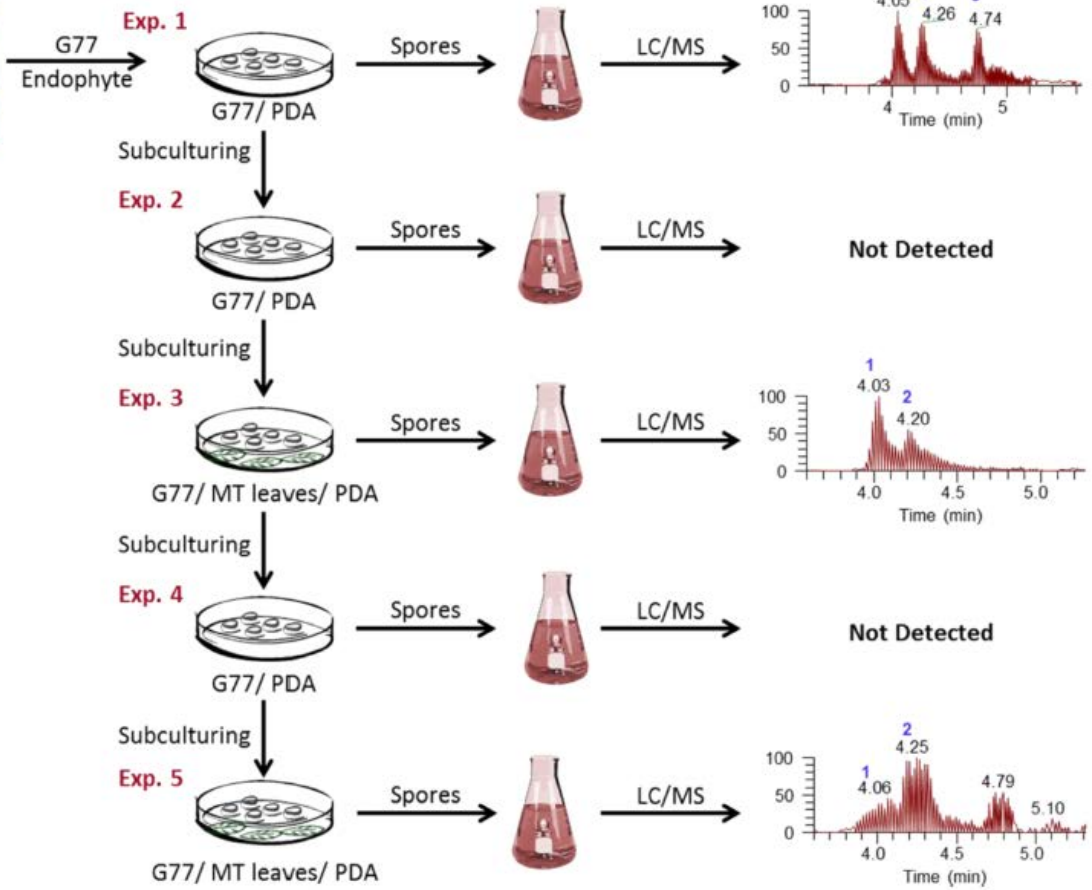
Silybin B (**2**)



Isosilybin A (**3**)



Isosilybin B (**4**)



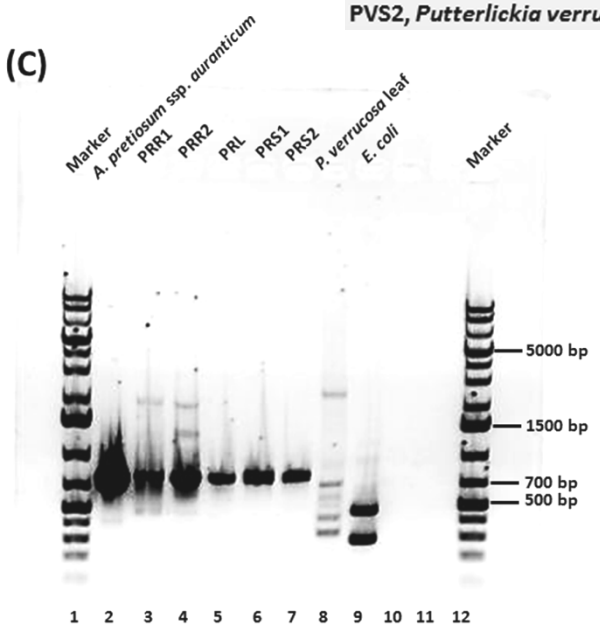
Aspergillus iizukae

El-Elimat et al, JNP, 2014

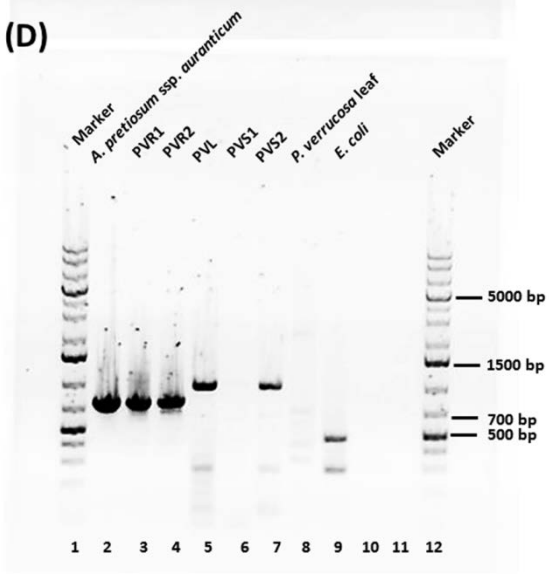
Maytansine Produced by Root Endophytes

This was further confirmed by the presence of AHBA synthase genes in the root endophytic communities. Finally, MALDI-imaging-HRMS was used to demonstrate that maytansine produced by the endophytes is typically accumulated mainly in the root cortex of both plants. Our study thus reveals that maytansine is actually a biosynthetic product of root associated endophytic microorganisms.

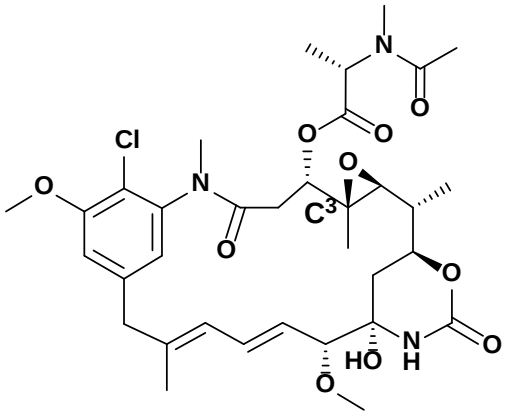
Index:
PVR1, *Putterlickia verrucosa* – primary root endophytic community
PVR2, *Putterlickia verrucosa* – secondary root endophytic community
PVL, *Putterlickia verrucosa* – leaf endophytic community
PVS1, *Putterlickia verrucosa* – thick (brown) stem endophytic community
PVS2, *Putterlickia verrucosa* – tender (greenish) twigs endophytic community



AHBA Synthase (755 bp)



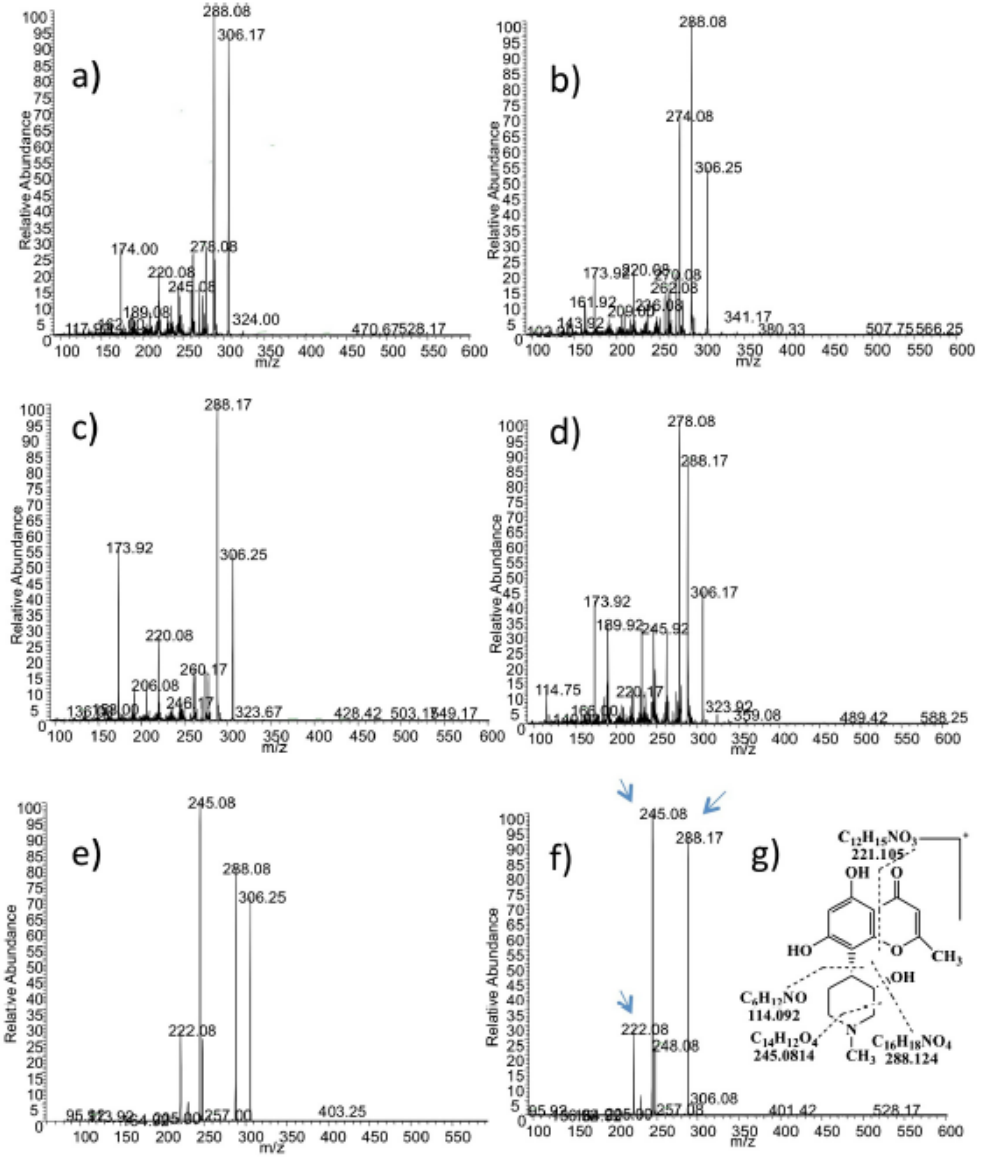
Purified AHBA Synthase (755 bp)

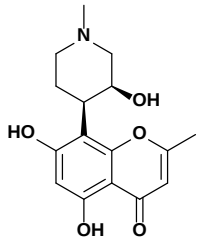


Maytansine

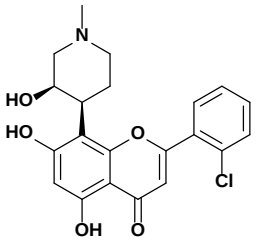
Kusari et al, JNP, 2014

Rohutikine I

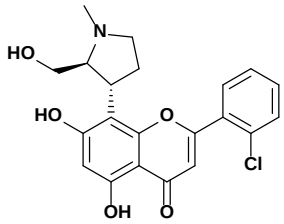




Rohutikine



Flavopiridol



P276-00

Clinical Trials

Mohanna Kumara et al, Phytomed, 2014

Fig. 2. LC–MS/MS spectra of (a) *Fusarium solani* MTCC 11385, (b) *Gibberella fujikuroi* MTCC 11382, (c) *Fusarium oxysporum* MTCC 11383, (d) *Fusarium oxysporum* MTCC 11384, (e) stem bark extract of host plant *D. binectariferum*, (f) reference rohitukine, arrows in the figure indicates the respective daughter ions and (g) ion fragmentation of rohitukine.

Rohutikine II

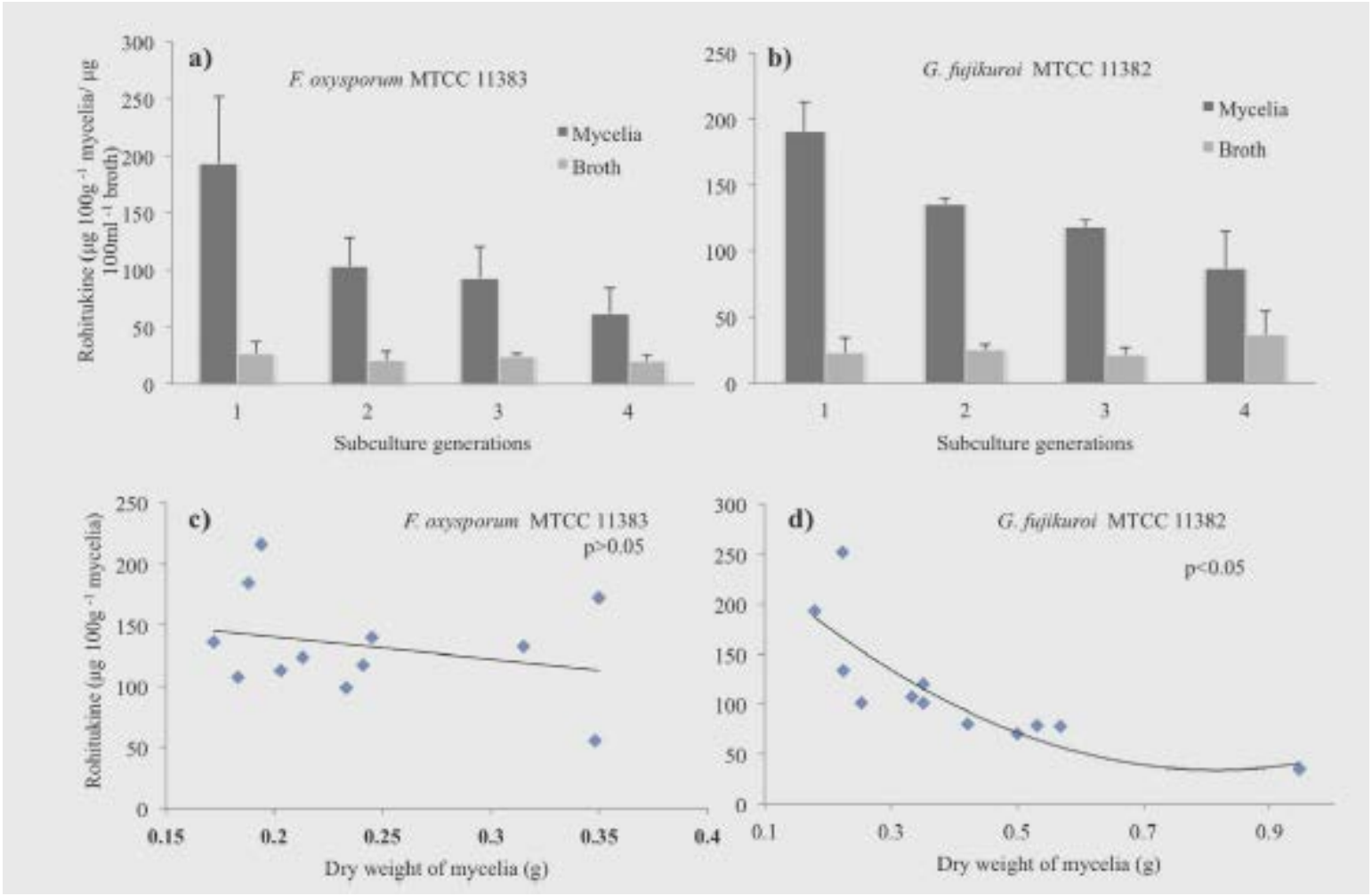
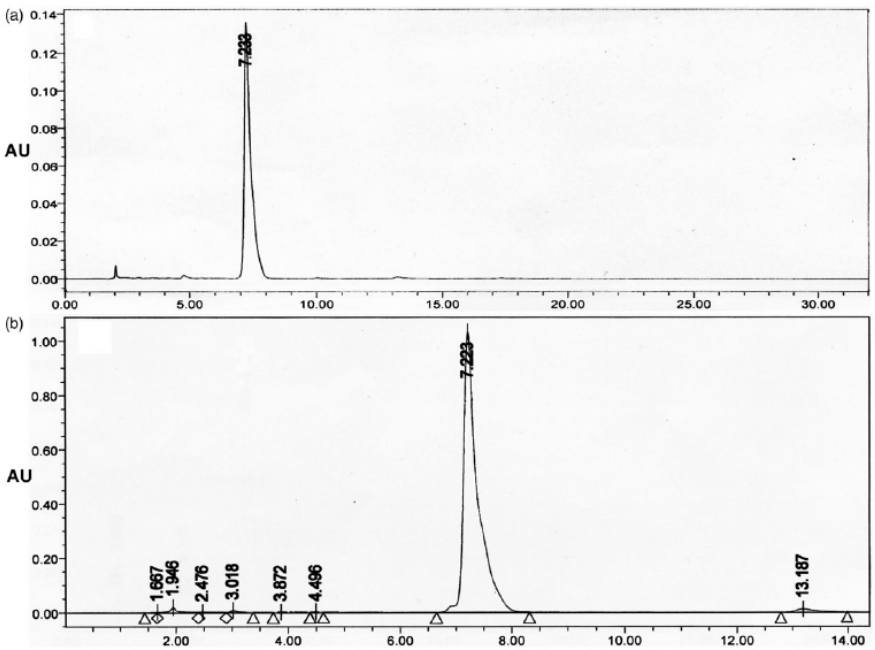
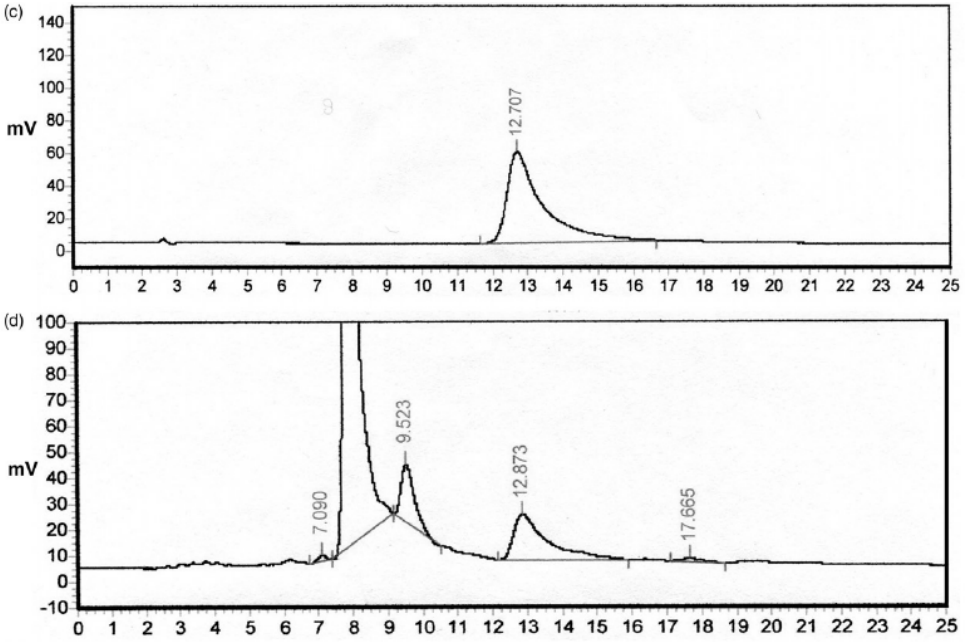


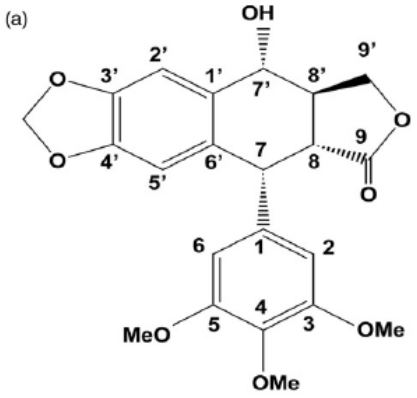
Fig. 3. Rohitukine yields in *F. oxysporum* MTCC 11383 (a) and *G. fujikuroi* MTCC 11382 (b) at first, second, third and fourth subculture generations. Relationship between rohitukine content and mycelial dry weight of endophytic fungi *F. oxysporum* MTCC 11383 (c) and *G. fujikuroi* MTCC 11382 (d).

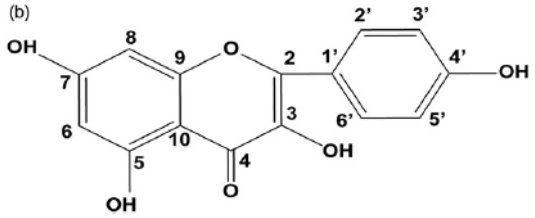
Mohanna Kumara et al, Phytomed, 2014

Podophyllotoxin & Kaempferol from Mucor fragilis; an endophyte of Sinopodophyllum hexandrum









NMR match as well

Huang et al, Pharm Biol, 2014

Pederin-Like Material from a Lichen I

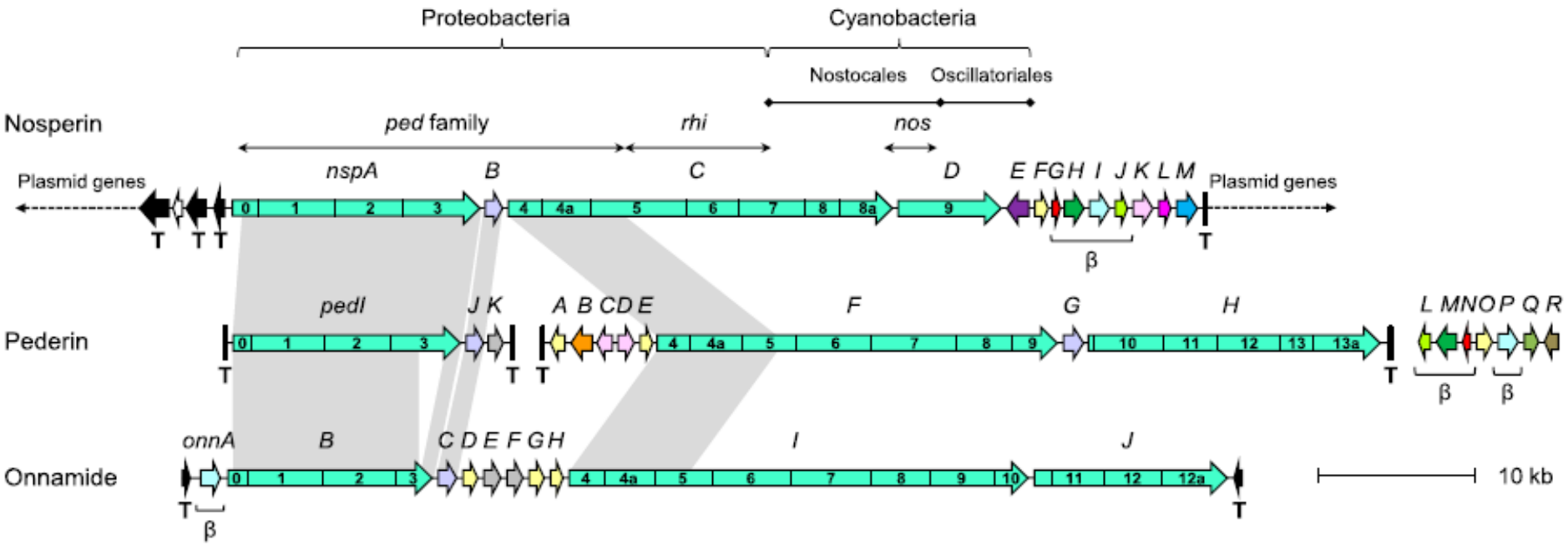


Fig. 3. Nosperin biosynthetic gene cluster *nsp* and flanking regions. Microsynteny and homology with pederin and onnamide biosynthetic gene clusters are indicated in gray. Similarity of *nsp* to other PKS biosynthetic gene clusters is indicated by double-headed arrows. Numbers denote individual modules. Genes with similar proposed functions (Table 1) are indicated with identical colors. β, genes involved in β-branch formation; T, transposon. See *SI Appendix*, Figs. S15 and S16 for details of regions flanking the *nsp* locus.

Nostoc cyanophyte in the lichen Peltigera membranacea

Kampa et al, PNAS, 2013

Pederin-Like Material from a Lichen II

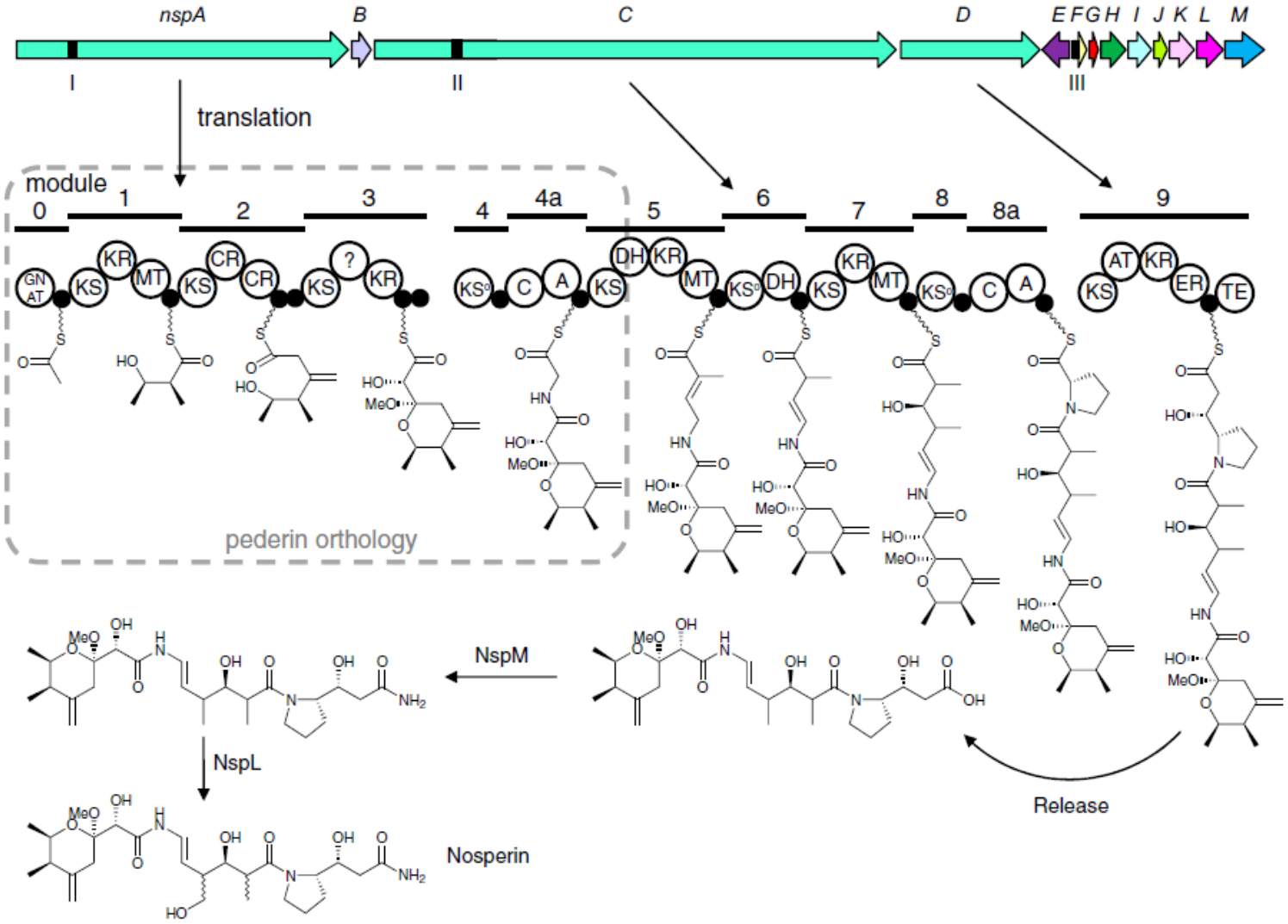


Fig. 4. The *nsp* gene cluster, deduced architecture of the PKS proteins NspA, NspC, and NspD, and proposed biosynthesis of nosperin. GNAT, GCN5-related *N*-acetyltransferase family (20); KS, β-ketoacyl synthase; KR, ketoreductase; MT, *C*-methyltransferase; CR, crotonase superfamily (also known as enoyl-CoA hydratase) (30); KS^0, nonelongating KS; C, nonribosomal peptide synthetase (NRPS) condensation domain; A, NRPS adenylation domain; DH, dehydratase; AT, acyltransferase; ER, enoyl reductase; TE, thioesterase; ?, unknown. Small black circles symbolize acyl and peptidyl carrier proteins. The positions of amplicons used for the *nsp* screening are shown with black boxes and roman numerals.

Kampa et al, PNAS, 2013

Terpene Synthases in Bacteria

But you had always taught that terpenes were products of the Eukaryota?

"Terpenes are generally considered to be plant or fungal metabolites, although a small number of odoriferous terpenes of bacterial origin have been known for many years. (Think Geosmin djn)

Recently, extensive bacterial genome sequencing and bioinformatic analysis of deduced bacterial proteins using a profile based on a hidden Markov model have revealed 262 distinct predicted terpene synthases.

Although many of these presumptive terpene synthase genes seem to be silent in their parent microorganisms, controlled expression of these genes in an engineered heterologous Streptomyces host has made it possible to identify the biochemical function of the encoded terpene synthases.

Genes encoding such terpene synthases have been shown to be widely distributed in bacteria and represent a fertile source for discovery of new natural products"

Yamada et al, PNAS 2015

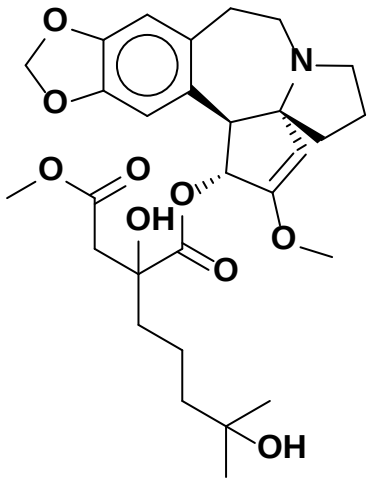
Is "Homoharringtonine" the Next One?

Searching Google Scholar revealed this paper from 2012

Optimization of Homoharringtonine Fermentation Conditions for Alternaria tenuissima CH1307, an Endophytical Fungus of Cephalotaxus mannii Hook. f.

Y. LIU, S .LIU, Y. LI, C. LI - Journal of Tropical Organisms, 2012, 3:236-242

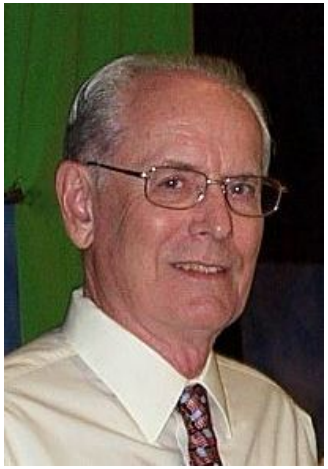
Homoharringtonine (HHRT) was produced from Alternaria tenuissima CH1307, an endophytical fungus of Cephalotaxus mannii Hook. f through fermentation.

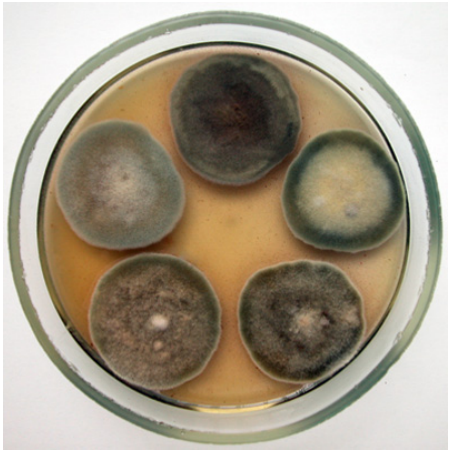


Homoharringtonine

Homoharringtonine was approved by the FDA in October 2012, almost 40 years after its first isolation.

So this is the final question





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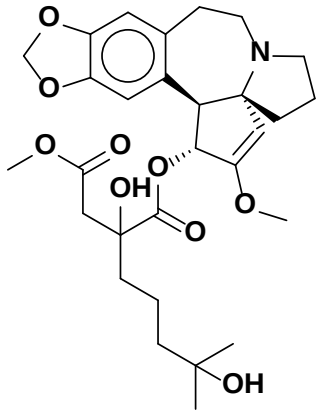
So please tell me



Homoharringtonine

who is my real Dad?

Many Thanks For Your Attention